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Role of starch structure in texture of winter squash (*Cucurbita maxima* D.) fruit and starch functional properties

by

David Graham Stevenson

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Food Science and Technology

Program of Study Committee:
Jay-lin Jane, Major Professor
Pamela White
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Iowa State University

Ames, Iowa

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David Stevenson

has met the dissertation requirements of Iowa State University

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ABSTRACT

Starch structural and functional properties of 13 winter squash (*Cucurbita maxima* D.) cultivars were investigated at harvest and after 5 or 10 weeks storage at 12°C. Texture profile analysis was carried out on winter squash fruit steamed at 6 different cooking times from 0 to 20 minutes, and for all three storage times. Buttercup squash cultivars had very high proportion of dry matter as starch (50-60%), whereas Halloween squash often accumulate no starch. Squash starches were B-type, with long amylopectin branch chain-lengths, low amylopectin polydispersity, gelatinization temperature of 60-65°C, and narrow range of gelatinization temperatures. Starch pastes had high peak and final viscosity, and high setback. Hardness of fruit was similar after 10 weeks storage, fracturability increased during storage and springiness of squash fruit decreased during storage. Correlations among squash starch structural and functional properties, and fruit textural attributes were observed. Starch content was positively correlated to fruit hardness and fracturability when raw and negatively correlated at long-duration cooking times. Springiness of raw and cooked fruit was negatively correlated to starch content. Apparent amylose content correlated negatively to hardness and fracturability of squash fruit, but absolute amylose content correlated positively to hardness and fracturability. Hardness and fracturability of squash fruit was consistently correlated to short (DP $\leq$ 12) and long (DP $\geq$ 37) amylopectin branch chain-lengths and negatively correlated to intermediate amylopectin branch chain-lengths (DP 13-36), regardless of storage time. Starch structural and functional properties, and textural attributes all varied among seasons. Ultrasound was transmitted through raw and cooked squash fruit as novel method of measuring texture. Ultrasonic velocity (UV) was slower than air for raw fruit, and despite softening during cooking, UV increased as squash were

steamed. Light microscopy analysis showed fruit with fastest UV had cells engorged with gelatinized starch, whereas fruit with slower UV had no starch or other material in their cells. Zapallo fruit had dramatic variation in starch accumulation and could be used as model system for starch biosynthesis. Apple starches were also studied and were C-type with long amylopectin branch chains and low paste breakdown.

GENERAL INTRODUCTION

Introduction

Starch is debatably the most abundant carbohydrate on Earth. Starch is abundant in leaves and stems of green plants, and their associated storage organs such as roots, tubers, fruits and seeds. Starch serves as the chemical energy from sunlight energy during photosynthesis. Starch is globally the predominant food reserve, providing typically 65 to 75% of the calories consumed by humans worldwide. Starch is biosynthesized in plastids and is also found in amyloplasts, which are plastids specifically for storage of starch in the form of semi-crystalline granules.

Starch is a homopolymer comprised solely of D-Glucose. Starch is divided into three structural categories: (1) amylose that is essentially linear comprising of largely α-1→4 linkages of D-Glucose monomers, (2) amylopectin that has 95% of its linkages α-1→4 of D-Glucose and 5% of linkages are D-Glucose monomers linked α-1→6, (3) D-Glucose polymers with both α-1→4 and α-1→6 linkages that have properties in common with both amylose and amylopectin and are known as the intermediate material. Amylopectin molecules are considered the largest naturally occurring molecules in the world. Amylopectin is the predominant polymer of starch, typically comprising 70-85% of total starch by weight for nonmutant plants. Mutant plants have been discovered including waxy varieties that have starches with very low levels of amylose, and high-amylose varieties, such as amylomaize VII which has 40% absolute amylose.

Starches and their modifications have an enormous number of food and non-food industrial uses. Food industrial applications include adhesion, binding, clouding, dusting, encapsulation, fat substitute, film-formation, foam strengthening, antistaling, gelling, glazing,

moisture retention, stabilizing, texturizing and thickening. Typical modifications of starches to improve food applications include hydroxypropylation, acid hydrolysis phosphorylation, phosphate ester crosslinking, octenylsuccinylation, acetylation and pregelatinization.

The most important use of starch in food industry is to alter the rheological properties of the food in order to meet the textural sensory attributes demanded by consumers. Starch can produce various textural attributes ranging from smooth to grainy and from cohesive to gelled. Particle size of starch granules influences graininess texture, but also starch dispersibility. Flow properties of starch pastes influence the viscous textural parameters of the final food product. Starch gelling agents can provide a range of clarities from dull to clear paste depending on botanical source of starch used.

To further improve the utilization of starches in the food industry, it is important to understand how starch structural properties influence starch functional properties that impart various textural attributes to the final food product. The objectives of this study are to investigate the role of starch structural and functional properties in the texture of raw and cooked winter squash, and to determine the starch structural components that influence functional properties. I expect this research to greatly advance the knowledge of textural attributes of winter squash and also the starch structural and functional properties of squash starches, and to contribute to the understanding of the role of starch structure and functional properties in the texture of foods.

Dissertation Organization

This dissertation consists of ten papers. The first paper, "Structural and Physicochemical Characteristics of Winter Squash (*Cucurbita maxima* D.) Fruit Starches at Harvest" and the tenth paper, "Structural and Functional Properties of Apple (*Malus domestica* Borkh) Fruit Starch" will be submitted to *Carbohydrate Polymers* for publication. The second paper, "Role of Starch Structure in Texture of Squash and Starch Functional Properties. I. Structural Properties of Starch Extracted From Winter Squash Fruit (*Cucurbita maxima* D.) at Harvest and After Storage", the third paper, "Role of Starch Structure in Texture of Squash and Starch Functional Properties. II. Functional Properties of Starch Extracted From Winter Squash Fruit (*Cucurbita maxima* D.) at Harvest and After Storage", the fourth paper, "Role of Starch Structure in Texture of Squash and Starch Functional Properties. III. Texture of Raw and Cooked Winter Squash (*Cucurbita maxima* D.) Fruit at Harvest and After Storage", the fifth paper "Role of Starch Structure in Texture of Squash and Starch Functional Properties. IV. Correlations Among Starch Structure, Starch Functionality and Texture of Winter Squash (*Cucurbita maxima* D.) From Fruit at Harvest and After Storage", and the sixth paper "Role of Starch Structure in Texture of Squash and Starch Functional Properties. V. Transmission of Ultrasound and Microscopic Observations of Winter Squash (*Cucurbita maxima* D.) Fruit to Examine Texture and Correlations with Starch and Cell Walls", will all be submitted to *Journal of Agricultural and Food Chemistry* for publication as five serial papers. The seventh paper "Seasonal Variation in Winter Squash (*Cucurbita maxima* D.) fruit. I. Variation in Starch Structural and Functional Properties" and the eighth paper "Seasonal Variation in Winter Squash (*Cucurbita maxima* D.) Fruit. II. Variation in Texture of Raw and Cooked Fruit" will be submitted to the New

Zealand Journal of Crop and Horticultural Science. The ninth paper "Variation in Agronomic Traits, Starch Structural Properties, Starch Functional Properties and Textural Attributes of *Cucurbita maxima* D. cv. Zapallo Macre Winter Squash Fruit" will be submitted to the Journal of the Science and Food Agriculture. The ten papers are preceded by a General Introduction and a Literature Review and followed by a General Conclusion, Appendices and Acknowledgements.

LITERATURE REVIEW

STARCH STRUCTURAL PROPERTIES

Amylopectin structure

One of the earliest models for starch granules is the trichitic model proposed by Meyer (1895, cited by French 1972) but this model did not fit amylopectin behavior well because the model assumes regular branched structure with inner chains of about 8 glucose units, which becomes space limiting after a few tiers (Fig. 1). Viscosity measurements of amylopectin indicated highly asymmetric or highly hydrated molecules and after β-amylase hydrolysis, limit dextrins of amylopectin were highly asymmetric indicating amylopectin is asymmetric.

Hydrolysis of amylopectin with α-amylase showed chains varied in length and a modified trichitic model was developed (Fig. 1). This model allowed indefinite expansion from the reducing end of molecule, with branching occurring as space permits (cited by French 1972) and was the basis for the cluster models.

Staudinger and Husemann (1937) proposed a comb-like model for amylopectin structure that consisted of one C-chain and all A-chains, with no B-chains, that was quickly dismissed (Fig. 1). Haworth (1939) observed that methylated derivatives of starch gave rise, upon hydrolysis, to an end-group of tetramethyl glucose corresponding to a chain-length of 24-30 α-glucose residues. The glucose unit farthest removed in the chain could not be shown to have a reducing group and it was proposed that starch consisted of a large number of aggregates with repeating unit of 24-30 glucose residues. Later Haworth established that amylopectin had about 30 glucose units in a polymer chain by the same glucosidic link and

one glucose unit had a different polymeric link, adjoining to another identical chain, allowing amylopectin structure to have A-, B- and C-chains (Fig. 1).

Meyer et al. (1940) degraded amylopectin with β-amylase and subsequently α-1→6 glucosidase in a stepwise manner and proposed a bush-like structure deducted from decreased yield of products on repeated hydrolysis (Fig. 2).

Meyer structure was redrawn by French (1964) to consist of equal proportion of A- and B-chains and Whelan modified proposed amylopectin structure further, which consisted of only half the B-chains carrying A-chains, and half the B-chains having their nonreducing termini inside the molecule and not at surface (Gunja-Smith et al. 1970). One year preceding, the first cluster model was proposed by Nikuni (1969) in which amylopectin clusters were spaced apart. French (1972) proposed a more compact, racemic cluster hypothesis of amylopectin structure (Fig. 2), in which such a pattern could originate if parts of amylopectin molecule crystallize during growth, thus sterically blocking off that portion from further chain elongation. This molecular pattern would have alternating crystalline and amorphous regions, with dimensions of crystalline domain approximately the average outer chain-length of amylopectin.

Gel filtration and enzymatic studies (β-amylase and pullulanase) of lintnerized potato starch confirmed and further enhanced the racemic cluster model proposed by French (Robin et al. 1974) (Fig. 3). This model is able to account for the linear populations of A and B which appear after debranching native starch; the long chains obtained in excluded volume after debranching or β-amylase treatment of native starch; the debranching of β-limit dextrins during lintnerization; and the formation of acid-resistant populations with chain-length 15-

25. The cluster model by French (1972) and Robin et al. (1974) was revised again by Manners and Matheson (1981) to alter the A:B-chain ratio (Fig. 3).

The now largely accepted cluster model for amylopectin has been further revised by Hizukuri (1986), who used gel permeation chromatography and debranching to develop a cluster structure which comprised of A-chains that carry no chains and B-chains that carry A- or other B-chains (Fig. 3). B-chains were also divided into B1, which like A-chains, are found in a single cluster, whereas, B2, B3 and B4 chains extend into 2, 3 and 4 clusters, respectively. Bertoft (1991) proposed an amylopectin structure consisting of unit clusters built up of A- and B1a-chains interconnected regularly by longer B-chains (Fig. 4).

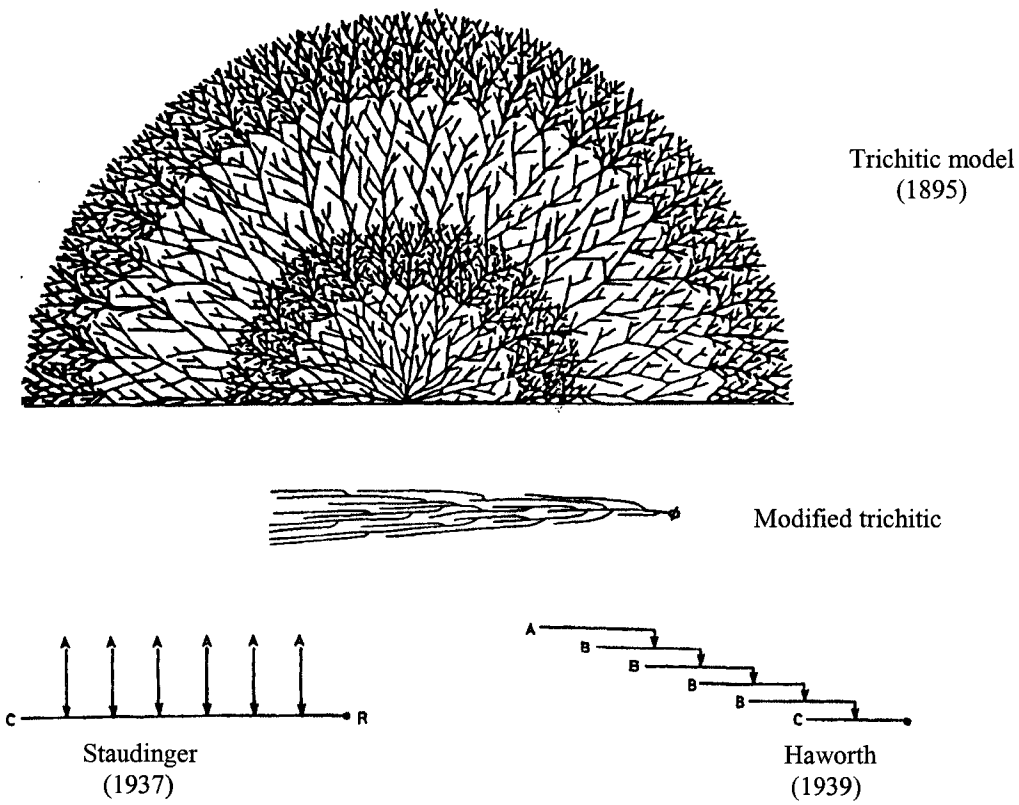
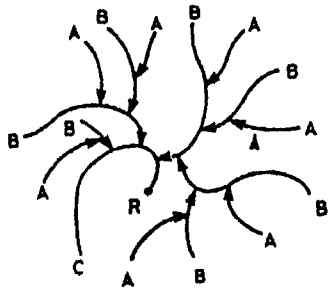
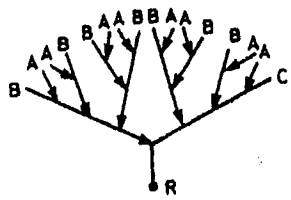


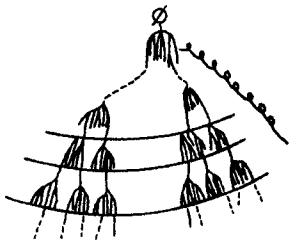
Figure 1. Some early proposed amylopectin structures.



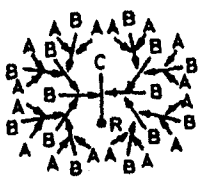
Meyer
(1940)



Redrawn
Meyer



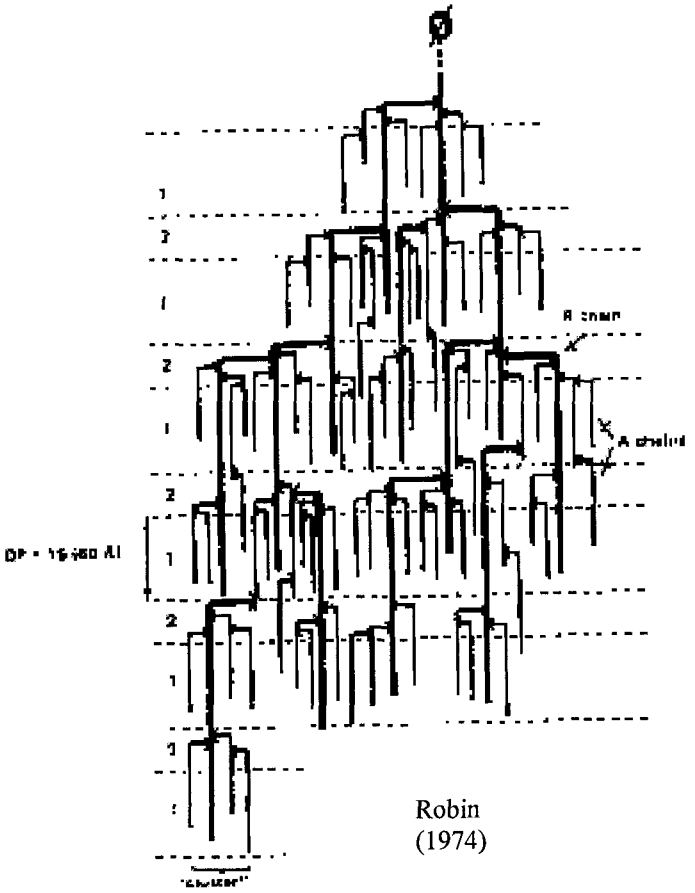
Nikuni
(1969)



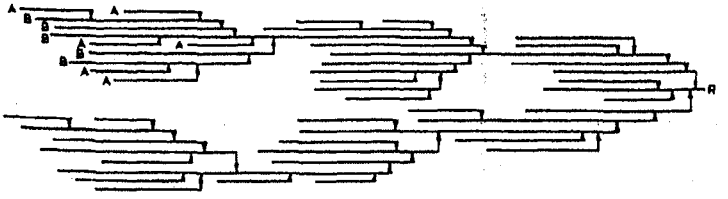
Whelan
(1970)

French
(1972)

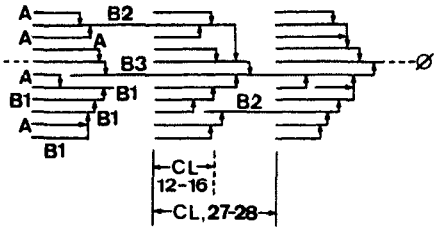
Figure 2. Proposed models of amylopectin structure, 1940s to early 1970s.



Robin
(1974)

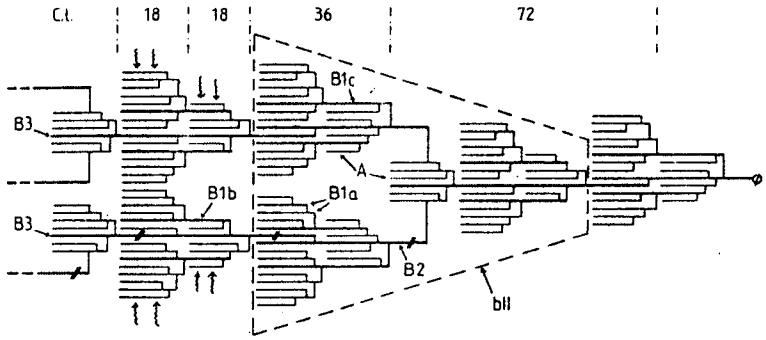


Manners
(1981)



Hizukuri
(1986)

Figure 3. Further advancements in proposed models of amylopectin structure.



Bertoft
(1991)

Figure 4. Latest modification to proposed amylopectin structure.

Peat et al. (1956) proposed an amylopectin structure consisted of three types of chains, A, B and C. In the type A-chain, only the reducing end glucose unit is involved in α-1→6 glucosidic linkage. B-chains are linked at their reducing end to another B- or to a C-chain while additionally having one or more chains as branches, and C-chains carry only reducing group in the molecule.

Peat et al. (1952, 1956) prepared β-dextrins of amylopectin and debranched with R-enzyme yielding A-chain derived products of maltose and maltotriose, and yielding B-chain derived products of maltohexaose and higher maltosaccharides. Proportion of A-chains was initially calculated at 5.3% (1952), but then later corrected to 12.8% (1956). Calculation of A-chain proportion enabled evaluation of three possible amylopectin models proposed by Haworth, Staudinger and Meyer (see Fig. 1 and 2). Peat assumed the β-dextrin had a degree of polymerization of 3,000, which would provide a maltose and maltotriose yield of 0.083% for Haworth structure and 25% for Staudinger structure. Therefore Peat favored the Meyer structure with its multiple and random branching.

Increasing acceptance of amylopectin models based on Meyer's, such as proposed by Robin et al. (1974), led to investigating the ratio of A- to B-chains. Majority of revisions proposed for the fine structure of amylopectin have concentrated on altering the ratio of A- to B-chains. Many models have focused on amylopectin structure of waxy starches and been extrapolated for nonwaxy amylopectin, but this creates problems as the A:B-chain ratio has been demonstrated to differ for waxy and nonwaxy amylopectin (Marshall & Whelan 1974). Additionally, initial measurements of A:B-chain ratio were based on comparison of reducing power generated when amylopectin β-limit dextrin is digested with isoamylase and/or pullulanase, and then measuring the ratio of maltotriose to maltose, and the ratio of both saccharides combined to maltohexaose and higher maltosaccharides. However, there are errors with the measurement of reducing power and isoamylase concentration has been shown to affect A:B-chain ratio (Manners & Matheson 1981). Errors also arise if the β-limit dextrin is not free of maltose (Manners 1989a) or if the amylopectin is high in phosphorus (Hizukuri & Maehara 1990).

A:B-chain ratio has been reported by many researchers for amylopectins from many different botanical sources as summarized in Table 1, although it should be considered that higher growing temperatures have been shown to result in a slight decrease in the A:B-chain ratio for rice (Asaoka et al. 1985). A:B-chain ratio as high as 2.6 has been reported (Marshall & Whelan 1974), but Atwell et al. (1980) suggests this was due to experimental errors in the measurement of reducing power. Yuan et al. (1993) compared A:B-chain ratios calculated from debranched native amylopectins and debranched β-limit dextrins and found *ae wx* to be in good agreement, but *wx* and *du wx* were in poor agreement, with greater than double magnitude difference. It is speculated that the higher A:B-chain ratios reported by Hizukuri

(1986) compared with other researchers is because of shorter B1 chains present in low-molecular weight fraction were erroneously considered A-chains. Yuan et al. (1993) underestimated the A:B-chain ratio of *ae wx* maize amylopectin from debranched native amylopectin compared to debranched β-limit dextrin, and this may be explained by the presence of a population of unusually long A-chains in intermediate molecular weight fraction of debranched native amylopectins that were erroneously considered as B-chains.

Hizukuri & Maehara (1990) classified B-chains into Ba-chains that carry at least one A-chain, and Bb-chains that carry no A-chains, but instead bear one or more B-chain(s). Longer Ba-chains carried an increased number of A-chains which may imply random branching in outermost layers of amylopectin molecules. Average amylopectin branch chain-length for starches from various botanical sources is shown in Table 2.

Table 1. Ratio of A-chains to B-chains in amylopectins from various botanical sources.

Starch	A:B ratio	Starch	A:B ratio
ae maize	1.2[o], 1.7[g]	rye	1.8[h]
ae wx maize	1.2[no]	*sul* maize	1.0[g]
du maize	1.3[g]	*su2* maize	1.5[g]
du wx maize	0.9-2.5[n]	tapioca	1.5[e*]
kuzu	0.9[e]	triticale	2.1[h]
maize (normal)	1.2-1.3[cgo], 1.7[i]	waxy maize	1.0[b], 1.3[dglo], 1.1-2.3[n], 2.6[i]
mango	1.2[k]	waxy rice	1.2[o], 1.5[aj], 2.2[e]
potato	0.8[e], 1.1[c], 1.[3b], 1.45[p]	waxy sorghum	1.2[b], 2.6[i]
rice	1.5[adi]	wheat	0.7[m], 1.3[f], 1.5-1.9[h]

a = Asaoka et al. (1985), b = Bathgate & Manners (1966), c = Bender et al. (1982), d = Enevoldsen & Juliano (1988), e = Hizukuri (1986), f = Hizukuri & Maehara (1990), g = Inouchi et al. (1987), h = Lii & Lineback (1977), i = Marshall & Whelan (1974), j = Umeki & Yamamoto (1977), k = Würsch & Hood (1981), l = Yamada & Taki (1977), m = You et al. (1999), n = Yuan et al. (1993), o = Yun & Matheson (1993), p = Zhu & Bertoft (1996), * = Hizukuri reports tapioca A:B ratio to be 0.89 but is later found to be an error and should be 1.5, as explained by Yuan et al. (1993).

Table 2. Average amylopectin branch chain-length of starches from various botanical sources.

Starch	Average Amylopectin Chain-length (DP_n)
Acha	18-21[11]
Adzuki bean	25-26[9]
Amaranth, waxy	21.8[22]

Table 2. (continued)

Starch	Average Amylopectin Chain-length (DP_n)
Arrowroot	20[1,3]
Banana	26.4[22]
Barley, commercial	22.1[22]
Barley, waxy	24.2[15]
Barley cv. Glacier	26.6[15]
Barley cv. High amylose Glacier	25.5[15]
Barley cv. Hull-less Glacier	24.5[15]
Barley cv. W.B. Merlin	24.2[15]
Bracken	22[3]
Canna	28.9[22], 44[7]
Chestnut	23[1]
Chickpea (garbanzo)	22[9], 26.0[13]
Chinese taro	23.4[22]
Ebiimo (yam)	20[3]
Faba bean	21[9]
Gingko	24.2[16]
Gladiolus	27[7]
Iburu	20-21[11]
Iris	25[7]
Koimo (yam)	19[3]
Kuzu	20.5[17], 21[3], 26[7,8]
Lentil	20[9]
Lily	23.6[19], 24[3], 34[7]
Lotus root	22[1,3], 25.4[22], 30[7]
Maize, normal	19[3], 22[1,10], 24.4[16,22], 28[7]
Maize, amylomaize V	28.9[22]
Maize, amylomaize VII	30.7[22], 32[1], 44[7]
Maize, waxy	20[6], 23.5[22], 24[7]
Maize *ae wx*	29.5[22]
Maize *du wx*	23.1[22]
Millet, cattail	21.5[22]
Millet, finger	20.7[13]
Mungbean	23[9], 24.8[22]
Nagaimo (yam)	24[3], 28[7]
Navy bean	22[9]
Potato, commercial	23[1,3,18], 24[10], 28.6[12], 29.4[22], 34[7], 35[8]
Potato, waxy	25.8[12]
Red kidney bean	20[9], 22-23[4]
Rice, commercial	22.7[22]
Rice, sweet	21.6[22]
Rice, waxy	18[1], 18.8[22], 19[9], 23[7], 24[8]
Rice cv. Akihikari	19[2]
Rice cv. Cypress	20.5[5]

Table 2. (continued)

Starch	Average Amylopectin Chain-length (DP_n)
Rice cv. Indica	21[10], 28[7]
Rice cv. IR32	21[1,3]
Rice cv. IR36	21[1]
Rice cv. IR42	22[1]
Rice cv. IR48	20[1]
Rice cv. IR64	20[1]
Rice cv. Japonica	19[10], 25[7]
Rice cv. Koshihikari	20[1]
Rice cv. Sasanishiki	19[1,3]
Sago	22[1]
Smooth pea	22[9], 22-24.2[14]
Sweet potato, commercial	21[3], 21.3-22.4[20], 26.3[12], 29[7]
Sweet potato cv. Koganesengan	22[1]
Sweet potato cv. Norin	21[1], 30[7]
Takenokoimo (yam)	20[3]
Tamba	20[11]
Tapioca	21[3], 26[7,8], 27.6[22]
Taro (satoimo)	25[7]
Tulip	30[7]
Water chestnut	22[1], 26.7[22]
Wheat, commercial	19[10], 22[3], 22.7[22], 23.5[21], 25[7]
Wheat, waxy	24.4[21]
Wheat, ASW	13[1]
Wheat cv. Centura	24.9[21]
Wheat cv. Chihoku	20[1]
Wheat cv. Horoshiri	21[1]
Wheat cv. Kanto 107	24.2[21]
Wrinkled pea	34[9,14]
Yam	24-26[1], 25.8[12]

1 = Hizukuri (1993), 2 = Tako & Hizukuri (2000), 3 = Suzuki (1993), 4 = Yoshida et al. (2003), 5 = Wang et al. (2002), 6 = Cheetham & Tao (1997), 7 = Hizukuri (1985), 8 = Hizukuri (1986), 9 = Biliaderis et al. (1981), 10 = Hizukuri (1988), 11 = Jideani et al. (1996), 12 = McPherson & Jane (1999), 13 = Madhusudhan & Tharanathan (1996), 14 = Ratnayake et al. (2002), 15 = Song & Jane (2000), 16 = Spence & Jane (1999), 17 = Suzuki et al. (1981), 18 = Suzuki et al. (1994), 19 = Takeda et al. (1983), 20 = Takeda et al. (1986), 21 = Yoo & Jane (2002), 22 = Jane et al. (1999)

Amylose structure

Generally amylose is the minor component of starch, and is typically 20-25% of the starch fraction. Amylose content of starch from various botanical sources is shown in Table 3. Exceptions to this are waxy starches which ranged from waxy maize and waxy rice with no amylose (Jane et al. 1999) to waxy barley with 9% amylose (Song & Jane 2000). Some

starches have also been reported to contain unusually high levels of amylose, such as 70% amylose for wrinkled pea (Banks et al. 1974, Colonna & Mercier 1984) and HYLON VII maize (Mercier 1973). Recently, a low-amylopectin maize starch was developed with 90% amylose (Shi et al. 1998). Proportion of amylose in starch was found to increase during development of normal and *ae* maize kernels, but decrease for double mutant, *ae/wx* (Yun & Matheson 1992). Determination of amylose content of starches is an extremely important problem since most research bases analysis on iodine affinity (IA) of the whole starch (apparent amylose), ignoring contribution to IA by amylopectin fraction (absolute amylose). For example, HYLON VII maize starch is reported to have 70% apparent amylose, but after IA for amylopectin fraction is subtracted, absolute amylose content is 40% (Jane et al. 1999).

Amylose was initially found to be a linear molecule because only one reducing and nonreducing terminal residue was found (Meyer et al. 1940), and hydrolysis of methylated amylose enabled the determination that amylose was linked by α-1→4 glucosidic linkages with a number-average degree of polymerization (DP_n) of 300-400 (Hassid & McCready 1943). Potter and Hassid (1948) investigated molecular weight of amylose from five different botanical sources and found a range from 100,000 to 210,000. Kerr and Cleveland (1952) observed amyloses with a few nonreducing terminals per molecule and concluded that amylose had a few branches, with relatively long branches in potato compared to corn and tapioca. Further support for branching in amylose came from Kjølberg and Manners (1963), and Curá et al. (1995), who showed that the linkages preventing complete amylose degradation by β-amylase are α-1→6 glucosidic. Later amylose from corn was found to have a α-1→6 glucosidic branch link every 300 D-glucose residues, whereas in potato amylose, a branch link was present every 400 D-glucose residues (Misaki et al. 1967).

Using advanced technology of low-angle laser-light scattering , Hizukuri & Takagi (1984) determined amylose had considerably larger molecular weight, ranging from 518,000 to 1,080,000, depending on botanical source, with corresponding DP_n of 3,220 to 6,680. Rice amylose DP_n was found to be shorter than other amyloses (980-1,100), with average chain-lengths of 250-370 and β-amylolysis of 73-84%, indicating slightly branched molecules (Takeda et al. 1986). Hizukuri et al. (1989) found similar characteristics for rice amylose, but regarded branched amylose as an intermediate, third component of starch. Using gel permeation chromatography, maize and rice amylose were separated into three subfractions with similar properties for each fraction of both amyloses, including highest molecular weight amylose being highly branched (Takeda et al. 1992). The authors description of highly branched is misleading since the average chain-length of the side chains of high-molecular weight amylose of maize was DP_n 275-520 with chains ranging from DP_n 200-2,000, and then reported side chains up to DP_n 5,000 using amylose labeled at reducing terminal with sodium [^{3}H]borohydride (Takeda et al. 1992a, Takeda et al. 1993). Observations such as this create uncertainty as to whether these higher molecular weight amyloses should be considered instead as intermediate material or amylopectin.

It is now known that amylose contains more than 2-3 branches as reported in 1940s, with Takeda et al. (1989a) reporting 17-19 chains in an average sago amylose molecule and Shibanuma et al. (1994) reporting 13-19 chains for wheat amylose. Fewer chains (≤ 8) on average were reported for kuzu, tapioca and potato starches (Takeda et al. 1994) and just 6 chains reported for rice (Takeda et al. 1989b) and for three African cereal grains (Jideani et al. 1996). β-amylolysis of maize and rice amylose produced small and large β-limit dextrins, suggesting that branch linkages were located randomly on chains (Takeda et al. 1992).

Concurrent hydrolysis of amylose with β-amylase and pullulanase gave 98-100% conversion into maltose, providing further evidence that all branch linkages were α-1→6 (Takeda et al. 1984). Combination of all enzymatic hydrolysis studies of amylose have led to the consensus that amylose molecules are branched, with various chain-lengths and broad distributions of molecular weight, and only a minor proportion is truly linear. Reported values for amylose chain-length for starch from various botanical sources is shown in Table 3.

Amylose typically exists in the double helix form, with single helices of six glucose units per turn only obtainable when in the form of inclusion complex with solvents such as dimethyl sulfoxide (French and Zobel 1967), *n*-butanol (Rundle & Edwards 1943, Helbert & Chanzy 1994), in *n*-pentanol (Helbert & Chanzy 1994) or in dry or hydrated state upon removal of organic solvents entrapped in these complexes (Zaslow et al. 1974, Zugenmaier & Sarko 1976, Rappenecker & Zugenmaier 1981).

Molecular modeling based on X-ray diffraction and computed lipophilicity profiles revealed double helical A-form amylose to be compact structure with an irregular distribution of hydrophilic and hydrophobic regions over the entire outer surface, with the interior of the double helix being inaccessible even for small molecules (Immel & Lichtenthaler 2000). Amylose also forms single helix complex with iodine (Bluhm & Zugenmaier 1981, Murdoch 1992), in which the hydrophobic channel of single-helical amylose serves as a well-ordered matrix for incorporation and alignment of the iodine-iodide species to form a linear polyiodide chain in nearly perfect steric fit and in full complementarily of hydrophobic regions of guest and host (Immel & Lichtenthaler 2000).

Table 3. Amylose content (% by weight) and number-average degree of polymerization (DP_n) of starches from various botanical sources. All analysis is based on iodine titiration and values in blue denote absolute amylose content were determined, black values denote apparent amylose content.

Starch	Amylose (%)	DP_n
Acha	18.7[32]	1,040[32]
Achira (canna)	23.8[86]	
Adzuki bean	19.5[4]; 21.2[22]	1,800[22]
Aegilops sp.	20-34[67]	
Amaranth	3.9-5.7[9]; 7.8[13]	
Amaranth, waxy	3.4[47]	
Arrowroot	25.6[78]	2,840[61]
Banana cv. Cavendish	19.5[83]	
Banana cv. Criollo	87.0[84]	
Banana cv. Macho	18.0[84]	
Banana cv. Nandigobe	8.5[112]	
Banana cv. Valery	40.7[82]	
Barley, commercial	23.6[47]; 23.7-24.2[25]; 25-26[22,45,46]; 27.5[15]; 29.5[22]	1,275[45]; 1,700[22]
Barley, hull-less, h.amylose	33.9-38.6[88]	
Barley, hull-less, normal	22.5-24.6[88]	
Barley, hull-less, waxy	0[88]	
Barley, waxy	5.6-6.7[111]	
Barley cv GlacierPentlandfd	37.0-40.9[111]	
Barley cv. Golden Prairie	24.9-28.0[111]	
Barley cv. Triumph	24.7-27.5[111]	
Black bean	20.1-22.2[115]	
Bracken	19.7[78]	
Buckwheat	21-26[12]; 21-27[71]	
Canna	22.7[47]	1,380[61]
Chestnut		1,690[61]
Chickpea (Garbanzo bean)	18.9[4]; 30.4-31.3[115]; 35.9[60]; 39[35]	
Chinese taro	13.8[47]	
Ebiimo (yam)	14.5[78]	
Enset	29[7]	
Faba bean	19.6[4]	
Ginger	26[56]	
Gingko	24[18]	
Green gram	30[34]	
High-amylose barley	41.5[25]; 44.7[17]	
Iburu	19.6[32]	1,120[32]
Jack bean	37.5[121]	

Table 3. (continued)

Starch	Amylose (%)	DP_n
Koimo (yam)	16.6[78]	
Kuzu	20.0[28]; 20.8[78]; 21.0[19]	1,540[49,61]
Lentil	19.6[4]; 34.1-37.4[115]	
Lily	25.0[78]; 25-26.8[20]	2,310[20,49,61]
Lima bean	32.7[3]	
Lotus root	15.9[61,78]	4,200[61]
Maize, Amylomaize V	27.3[47]; 36.1[61]; 50[58]	
Maize, Amylomaize VII	40.2[47]; 58.6[61]; 72[58]; 75.5[119]	640[53]; 690[61]
Maize, normal	17.4[18]; 19-25[22,42]; 21.0[55]; 21.4[61]; 21.6[88]; 22.4[31]; 22.5[47]; 23.5-25[14,69]; 26.7[63]; 28[79,118]; 29[58,131]	730[53]; 800[134]; 930[132]; 990[61]
Maize, waxy	0[47,58,88,131]; 1-5[42]	
Maize cv. A632	26.8[114]	
Maize cv. A632 *du*	29.0[114]	
Maize cv. A632 *su2*	29.5[114]	
Maize *ae*	33[131]; 46.0[63]	
Maize *ae bt1*	32.4[63]	
Maize *ae du1*	47[131]; 57.3[63]	
Maize *ae du wx*	0[131]	
Maize *ae du su wx*	0[131]	
Maize *ae su*	28[131]	
Maize *ae su wx*	0[131]	
Maize *ae wx*	0[131]	
Maize cv. B73	24.8[59]	
Maize *bt1*	24.9[63]	
Maize *bt2*	24.7[63]	
Maize *du1*	30.5[63]; 55[131]	
Maize *du su*	70[131]	
Maize *du1 su1*	34.5[63]	
Maize *du su wx*	0[131]	
Maize *du wx*	0[131]	
Maize *h*	28.1[63]	
Maize *h sh2*	26.4[63]	
Maize *h wx*	0[63]	
Maize cv. Hz85	25.8[114]	
Maize cv. Hz85 *du*	37.0[114]	
Maize cv. Hz85 *su2*	43.2[114]	
Maize cv. Oh43	24.7[114]	
Maize cv. Oh43 *du*	35.7[114]	
Maize cv. Oh43 *su2*	36.7[114]	
Maize *sh2*	30.1[63]	
Maize *sh2 bt1*	27.7[63]	
Maize *sh2 wx*	0[63]	

Table 3. (continued)

Starch	Amylose (%)	DP_n
Maize *su*	65[131]	
Maize *su1*	31.2[63]	
Maize *su wx*	0[131]	
Maize *wx du1*	0[63]	
Mango	34[27]	
Millet, cattail	15.3[47]	
Millet, foxtail	22[35]; 14-31[64]; 15.9-27.1[70]	
Millet, foxtail, waxy	0.0[64]; 1.8-2.8[70]	
Mungbean	19.4[4]; 26.4[122]; 30.7[47]	
Nagaimo (yam)	21.3[78]	
Navy bean	18.5[4]; 23.8-25[115]; 32.1[123]; 37.8[75]	
Oat	22.1-26.6[24]	
Oat cv. Alymer	16.9[81]	
Oat cv. Antoine	17.0[81]	
Oat cv. Baton	20.1[81]	
Oat cv. Borrus	20.6[125]	
Oat cv. Erbgraf	21.0[125]	
Oat cv. Erich	22.0[125]	
Oat cv. Ernie	18.7[81]	
Oat cv. Francis	18.4[81]	
Oat cv. Gosline	19.5[81]	
Oat cv. Pendragon	20.5[125]	
Oat cv. Pitol	20.1[125]	
Oat cv. Selma	19.7[125]	
Okenia	26.1[79]	
Oxalis (oca)	18.4[86]	
Pea, green	34.2[123]	
Pinto bean	29.5-30.1[115]; 32.2[123]; 37[75]	
Potato, commercial	16.9[47]; 18.3[11]; 19.3[55,56]; 19.8[78]; 20.0[69]; 20.5[28]; 29.4-29.7[87]; 25.2-31.2[106]; 46.4[119]	2190[135]; 3,000[134]; 4,450[53]; 4,900[132]; 4,920[49,61]
Potato cv. Mainechip	22.7[75]	
Quinoa	7.1[22]; 11.2[13]	900[22]
Red kidney bean	20.0[4]; 27[85]	1080[85]
Rice, commercial	7-11[43]; 17-18.3[30]; 20.0-21.1[38,80]; 20.5[47]; 29[8]	920-1,200[30,38,50,52,53]
Rice, high amylose	35.2[95]	
Rice, sweet	2.1[47]	
Rice, waxy	0[47]; 0.0[95]; 0.3[103]; 0.9[62]	
Rice cv. Akihikari	20.7[66]	
Rice cv. Arbor red	23.7[90]; 24.3[91]	
Rice cv. Aya	13.8[92]; 17.6[57]	
Rice cv. Basmati 370	23.9[77]; 25.4[54,89]	

Table 3. (continued)

Starch	Amylose (%)	DP_n
Rice cv. Calrose	14.5[100]; 16.7[103]	
Rice cv. Changlei	17.6[54]; 19.2[77,91]; 19.4[90]; 19.7[89]	
Rice cv. Chucheongbyeo	20.8[73,105]	730[105]; 860[73]
Rice cv. Chukanbohon	6.0[92]	
Rice cv. Co32	26.5[91]; 26.8[54]; 28.1[89]	
Rice cv. Cypress	18.6[116]; 20.1[103]; 21.5[74]	
Rice cv. Dalian	24.8[95]	
Rice cv. Dawn	22.6[100]	
Rice cv. Della	20.4[103]; 21.4[74,100]	
Rice cv. Dongfan	27.0[95]	
Rice cv. Doongara	22.4[100]	
Rice cv. Habataki	14.4[92]; 19.2[57]	
Rice cv. Hakuchoumochi	0.0[57]	
Rice cv. Himenomochi	0.0[124]	
Rice cv. Hokkaido	18.0[65]	1100[50]
Rice cv. Hokuriku	15.4[68]; 16.9[93]; 27.3[92]	
Rice cv. Hoshiyutaka	24.1[68,93]; 25.0[92]; 26.4[57,107]	
Rice cv. Huwan	26.2[95]	
Rice cv. Indica KSS7	25.9[62]	1000[132]
Rice cv. Indica TCS10	11.4[62]	
Rice cv. Intan	25.6[91]; 26.0[89]	
Rice cv. IR8	7.8[96]; 27.6[76]; 28.1[89]	
Rice cv. IR32	18.5[61,65,78]	1000[61]; 1040[50]
Rice cv. IR36	17.0[61,65];	920[50,61]
Rice cv. IR42	15.5[61,65]	980[50,61]
Rice cv. IR48	17.0[61]	930[50,61]
Rice cv. IR64	18.3[61]; 21.5[74,100]	1000[61]; 1020[50]
Rice cv. IR72	24.5[74,100]	
Rice cv. Japonica TC189	14.1[62]	1000[132]
Rice cv. Japonica TG9	12.7[62]	
Rice cv. Jasmine	15.0[103]	
Rice cv. Jaya	27.0[77]; 27.7[89]; 28.2[91]; 29.4[54]	
Rice cv. Jhona 20	28.9[89]; 29.9[54]	
Rice cv. Jingyu	22.7-24.0[95]	
Rice cv. Jiran	14.3[95]	
Rice cv. Kanto	16.3[93]	
Rice cv. Kinuhikari	18.5[57]	
Rice cv. Koshihikari	16.0[74,100]; 16.3[92,107]; 17.6[57]; 16.7[61,65]	
Rice cv. Langi	18.0[100]	
Rice cv. Madhu	28.9[89]	
Rice cv. Mars	12.4[103]; 14.0[74,100]	
Rice cv. Maybelle	20.1[103]	

Table 3. (continued)

Starch	Amylose (%)	DP_n
Rice cv. Milky Queen	7.3[68]; 9.3[107]	
Rice cv. Mochiminori	0.5[57]; 1.8[107]	
Rice cv. Nampungbyeo	23.5[104]	1010[105]
Rice cv. Namyoungbyeo	18.8[73,105]	930[73]
Rice cv. Nipponbare	16.1[74,100]; 16.6[68]; 17.1[93]; 17.3[92]; 18.2[107]; 18.5[57]; 25.6[124]	
Rice cv. Ochikara	15.2[92]	
Rice cv. Purple puttu	4.2[90]; 5.2[91]	
Rice cv. Rexmont	23.6[74,100]	
Rice cv. Rojolele	26.2[89]	
Rice cv. Saikai	21.0[57]	
Rice cv. Samgangbyeo	19.6[73,105]	900[105]; 1000[73]
Rice cv. Sari-queen	17.4[92]	
Rice cv. Sasanishiki	17.5[61,65,78]	1100[50,61]
Rice *shr*	2.4[104]	
Rice cv. Snow Pearl	3.3[68]	
Rice cv. Sukunandi	24.3[54]; 24.5[90,91]; 24.8[89]	
Rice cv. Taichung	0.9[74]	
Rice cv. TaichungN1 T(N)1	24.9[100]; 27.8[77]; 28.2[54,91]; 28.4[76]; 28.6[89]; 28.8[90]; 30.7[108]	
Rice cv. Taim	24.3[74,100]	
Rice cv. Tainung	18.5[108]	
Rice cv. Tamjinbyeo	20.4[73,105]	740[105]; 800[73]
Rice cv. Tomohikari	20.9[92]	
Rice cv. Tongjinbyeo	21.0[73,105]	750[105]; 790[73]
Rice cv. Toro	14.7[103]; 15.3[100]	
Rice cv. Whachungbyeo	22.0[104]	
Rice cv Whachungchalbyeo	3.3[104]	
Rice cv. Whachung *du1*	6.3[104]	
Rice cv. Yongjubyeo	19.5[73,105]	970[73]; 1000[105]
Rice cv. Yumetoiro	26.2[68]; 27.1[93]; 29.2[107]	
Sago	24-31[1,36]; 24.3[61]; 24.4[69]	2,490[51,61]; 4,490[61]
Smooth pea	18.8[4]; 33.2[29]; 30.0-35.8[115]; 30-43[5]; 44.5-48.8[60]	1350[133]
Sorghum	18.6-22.5[97]; 19.2-28.8[109]	
Squash, winter (pumpkin)	18-21[26]	
Sweet potato	16.2-17.3[10,21]; 14.2-24.3[71]; 18.2[78]; 20.5-25.5[6,11]; 27.6[16]	4,100[49,61]; 3,400-4,400[21]
Sweet potato cv. Adams 3	27.1[101]	
Sweet potato cv. Bataan	17.8[101]	
Sweetpotato cv. Beauregard	24.4[94]	
Sweet potato cv. Binicol	15.3[101]	
Sweet potato cv. Chiba	21.5[128]	

Table 3. (continued)

Starch	Amylose (%)	DP_n
Sweet potato cv. Gunmi	25.0[126]	
Sweet potato cv. Jewel	23.0[94]	
Sweet potato cv. Junmi	25.0[126]	
Sweetpotato Koganesengan		4100[21]
Swet potato cv. Kagoshima	22.0[128]	
Sweetpotato Minamiyutaka	17.2[21]; 25[127]	4400[21]
Sweet potato cv. Norin		3400[21]
Sweetpotato cv Tamayutaka	22[127]	
Sweetpotato cv Tres colores	23.6[101]	
Takenokoimo (yam)	15.1[78]	
Tamba	19.8[32]	1,420[32]
Tapioca	14.9[78]; 17.2[55]; 17.8[47]; 20.0[28]; 25.1[129]; 28[2]; 37[56]	2,660[49,61,131]; 3000[134]
Taro	7-10[41]	
Tef	27.2-28.8[118]	
Waterchestnut	16.0[47]; 23[37]; 23.3[61]	800[48,61]
Waxy amaranth	3.4[47]	
Waxy barley	0[45]; 4.2-5.8[25]; 9.1[17]	
Waxy potato	0[11]	
Wheat, commercial	12.6[80]; 18.3-20[44]; 19.1[55]; 20.5[28]; 21.6[61,98]; 22-27[23,33]; 23.2[113]; 25.0[78]; 25.8[47]; 26.6[130]; 29.9[129]	800[134]; 1,030[53]; 830-1,570[33]; 1180[61]; 1300[132]
Wheat, durum	26.2-28.5[23]	
Wheat, waxy	0.1[130]; 0.7[117]; 0.8-0.9[44]	
Wheat cv. Centura	26.6[130]	
Wheat cv. Chikugoizumi	21.6[110]	
Wheat cv. Condor	33.3[72]	
Wheat cv. Fillmore	20.9[102]	
Wheat cv. Freedom	23.0[102]	
Wheat cv. Geneva	22.3[102]	
Wheat cv. Insignia	28.8[72]	
Wheat cv. Kanto 107	26.2[130]	
Wheat cv. Meering	29.9[99]	
Wheat cv. Nishihonami	21.6[110]	
Wheat cv. Norin	25.8[110]; 27.8[117]	
Wheat cv. Pioneer 2550/5	22.3[102]	
Wheat cv. Rosella	27.9[99]; 30.2[72]	
Wheat cv. Sakai	23.3[110]	
Wheat cv. Westeern white		1570[61]
White carrot	4.0[86]	
Wrinkled pea	19.8[4]; 70.9[29]; 71-82[39]	1050[133]
Yam	17.7[11]	2000[61]
Yam bean	11.6-23.7[113]	

Table 3. (continued)

Starch	Amylose (%)	DP_n
Yucca	17.0[120]	

1 = Ahmad et al. (1999), 2 = Atichokudomchai et al. (2001), 3 = Betancur et al. (2001), 4 = Biliaderis et al. (1981), 5 = Davydova et al. (1995), 6 = Garcia & Walter (1998), 7 = Gebre-Mariam & Schmidt (1996), 8 = Hoover et al. (1996), 9 = Hoover et al. (1998), 10 = Katayama et al. (2002), 11 = McPherson & Jane (1999), 12 = Qian & Kuhn (1999a), 13 = Qian & Kuhn (1999b), 14 = Sahai & Jackson (1996), 15 = Schulman et al. (1995), 16 = Shin & Ahn (1983), 17 = Song & Jane (2000), 18 = Spence & Jane (1999), 19 = Suzuki et al. (1981), 20 = Takeda et al. (1983), 21 = Takeda et al. (1986), 22 = Tang et al. (2002), 23 = Vansteelandt & Delcour (1999), 24 = Wang & White (1994a), 25 = You & Izydorczyk (2002), 26 = Sugimoto et al. (1998a), 27 = Würsch & Hood (1981), 28 = Takeda et al. (1984), 29 = Colonna & Mercier (1984), 30 = Takeda et al. (1989a), 31 = Yun & Matheson (1992), 32 = Jideani et al. (1996), 33 = Shibanuma et al. (1994), 34 = Madhusudhan et al. (1996a), 35 = Madhusudhan et al. (1996b), 36 = Takeda et al. (1989b), 37 = Hizukuri et al. (1988), 38 = Takeda & Hizukuri (1986), 39 = Banks et al. (1974), 40 = Kalistratova et al. (1999), 41 = Kinjo & Fukuba (1978), 42= Matveev et al. (2001), 43 = Ramesh et al. (1999c), 44 = Sasaki et al. (2000), 45 = Yoshimoto et al. (2002), 46 = Yuryev et al. (1998), 47 = Jane et al. (1999), 48 = Hizukuri et al. (1988), 49 = Hizukuri & Takagi (1984), 50 = Hizukuri et al. (1989), 51 = Takeda et al. (1989c), 52 = Takeda et al. (1993), 53 = Takeda et al. (1992), 54 = Ramesh et al. (1999b), 55 = Inaba et al. (1994), 56 = Blennow et al. (2001), 57 = Yoshii et al. (1997), 58 = Mun et al. (1998), 59 = Yamin et al. (1999), 60 = Czuchajowska et al. (1998), 61 = Hizukuri (1993), 62 = Lin et al. (2001), 63 = Wang et al. (1993a) , 64 = Inouchi et al. (1993), 65 = Takeda et al. (1987), 66 = Tako & Hizukuri (2000), 67 = Stoddard & Sarker (2000), 68 = Kuno et al. (2000), 69 = Hamanishi et al. (2000), 70 = Fujita et al. (1996), 71 = Noda et al. (1998), 72 = Wootton et al. (1998), 73 = Kang et al. (1995), 74 = Meullenet et al. (2000), 75 = Kim et al. (1996), 76 = Bhattacharya et al. (1972), 77 = Sowbhagya & Bhattacharya (2001), 78 = Suzuki (1993), 79 = González-Reyes et al. (2003), 80 = Aarathi et al. (2003), 81 = Hoover et al. (2003), 82 = Waliszewski et al. (2003), 83 = Ling et al. (1982) 84, Bello-Pérez et al. (1999), 85 = Yoshida et al. (2003), 86 = Santacruz et al. (2003), 87 = Liu et al. (2003), 88 = Li et al. (2001a), 89 = Reddy et al. (1994), 90 = Sandhya Rani & Bhattacharya (1989a), 91 = Sowbhagya et al. (1991), 92 = Takahashi et al. (1998), 93 = Takahashi et al. (2000), 94 = Walter et al. (2000), 95 = Yao et al. (2002), 96 = Sodhi & Singh (2003), 97 = Aboubacar & Hamaker (2000), 98 = Akashi et al. (1999), 99 = Black et al. (2000), 100 = Champagne et al. (1999), 101 = Collado & Corke (1997), 102 = Gaines et al. (2000), 103 = Kadan et al. (1997), 104 = Kang & Han (2001), 105 = Kang et al. (1994), 106 = Kaur et al. (2002), 107 = Kohyama et al. (1998), 108 = Lai (2001), 109 = Lee et al. (2001), 110 = Noda et al. (2001), 111 = Kiseleva et al. (2003), 112 = Lehmann et al. (2002), 113 = Forsyth et al. (2002), 114 = Li & Corke (1999), 115 = Hoover & Ratnayake (2002), 116 = Wang et al. (2002), 117 = Sasaki et al. (2002), 118 = Bultosa et al. (2002), 119 = Jiang & Liu (2002), 120 = Swinkels (1985b), 121 = Betancur & Chel (1997), 122= Gálvez & Resurrección (1993), 123 = Gujska et al. (1994), 124 = Singh et al. (2000), 125 = Tester & Karkalas (1996), 126 = Seog et al. (1987), 127 = Shiotahi et al. (1991), 128 = Kitada et al. (1988), 129 = Del Rosario & Pontiveros (1983), 130 = Yoo & Jane (2002), 131 = Yeh et al. (1981), 132 = Hizukuri (1988), 133 = Ratnayake et al. (2002), 134 = Swinkels (1985a), 135= Suzuki et al. (1994)

Intermediate Material

Separation of starch components using solvent complexes revealed a fraction with structural properties that could not be easily classified as amylose or amylopectin. Typically this unknown fraction was included with amylose, inflating its true content. This new starch fraction became known as the intermediate starch material and was first investigated in detail by Wolff et al. (1955), who discovered its presence in amylomaize, and confirmed speculation of its presence by other researchers (Kerr & Trubell 1943, Schoch 1945, Whistler & Hilbert 1945, Hodge et al. 1948, Lansky et al. 1949).

Whistler & Doane (1961) reported intermediate fraction to consist 4.5-8.7% of total starch. This fraction gave a deep-blue color with iodine, but lower blue values than for amylose, and had a maximum absorbance between values for amylopectin and amylose.

Intermediate fraction was less branched with only 64% of the branching frequency of amylopectin and higher β-amylolysis limit.

Explanations for the anomalous properties of intermediate fraction began to be proposed. One theory was that high-amylose starches have an imbalance of synthesizing enzymes resulting in a structurally homogeneous amylopectin produced with greater than normal amylopectin (Wolff et al. 1955, Montgomery et al. 1964, Erlander et al. 1965). Another theory is intermediate material is a mixture of normal amylopectin and degraded amylose, with the quantity of contaminating amylose unable to complex with *n*-butanol (Greenwood & MacKenzie 1966, Adkins & Greenwood 1966). This theory was later amended to have low molecular weight amylose present (Banks & Greenwood 1968).

Development of gel chromatography packing materials has allowed separation of starch components based on molecular size. Yamada and Taki (1976) did not observe any intermediate material in maize starches fractionated using agarose gel but they dissolved starch in chilled perchloric acid which may have been too severe. High-amylose maize starches, fractionated using Sephadex, were shown to contain higher proportions of intermediate material than normal maize starches (Ikawa et al. 1978, Yeh et al. 1981). Colonna & Mercier (1984), using Sephacryl S-200, reported 18.9% of wrinkled pea starch was intermediate material that had low molecular weight and intrinsic viscosity, with high polydispersity. Isoamylase debranching gave products with average chain-length of 29, with two distinct chain populations after separation, with DP values of 15 and 45. Rice starches were shown to contain 3-11% intermediate fraction (Yano et al. 1985, Asaoka et al. 1986). Intermediate fraction was reported to be < 1.6% of total starch for normal maize and all other maize mutants except amylose extender (Inouchi et al. 1987).

Baba et al. (1987) reported the occurrence of abnormal amylopectin and intermediate material in amylomaize. Abnormal amylopectin possessed less highly branched structure with long inner and outer branches, whereas intermediate material has lower molecular weight than normal amylose and high iodine binding capacity despite low λ_{max} for iodine staining. Amylomaize VII was found to contain 55% intermediate material, contributing to the apparently high amylose content.

In the last decade and half, research has continued to characterize intermediate material but overall little advances in understanding the synthesis, structure, biological role and functionality have been made. Fine structure of intermediate starch material in rice was studied by Takeda et al. (1989b), who determined number-average DP of 930-1,200, average chain-length of 380-450 and molar ratio of branched to unbranched molecules was 1:2 to 1:3. Intermediate material had six branches per molecule on average, similar to that reported for amylomaize (Baba & Arai 1984), which may be poorly branched amylose. Low molecular weight intermediate material in wrinkled pea starch has been proposed to be built-up of cluster units with same principal architecture and sizes similar to high molecular weight branched material, but with a different mode of interconnection (Bertoft et al. 1993). In recent years, focus has been on intermediate material in mutant maize starches. Intermediate material was reported to contribute greatly to iodine affinity measurements in *ae* maize but not for *du1*, *bt1* and *ae du1* (Wang et al. 1993b). This result implied maize mutants differed in their intermediate material structure and it was later shown that *ae* intermediate material has long B-chains present (Kasemsuwan et al. 1995, Tziotis 2001).

Starch Granule

Starch is laid down in all higher plants in form of birefringent, semi-crystalline granules. At the center of the starch granule is the original growing point, known as the hilum, which is usually less organized than the rest of the granule (Blanshard 1987), and typically is not the geometric center of the granule.

The final shape of starch granules is characteristic for different species of plants, and an extensive variety in morphology has been reported (Jane et al. 1994) and some of the variety in morphology is shown in Fig. 5. Characteristics of starch granules from a range of botanical sources are shown in Table 4. Cellular organization of synthetic apparatus is considered the primary factor determining final granule topography. However, striving to minimize surface free energy may also influence shape. Granular curvature will enable closer cluster packing in crystalline layer, reducing specific surface free energy to compensate for increased surface-to-volume ratio (Larsson 1991). Shape of pea starch granules from lines containing mutations at either *r* or *rug5* loci, encoding a starch branching enzyme and a soluble starch synthase, differ significantly from non-mutant granules. These mutations also cause changes in granular structure and Maltese cross is no longer observed (Hedley et al. 2002).

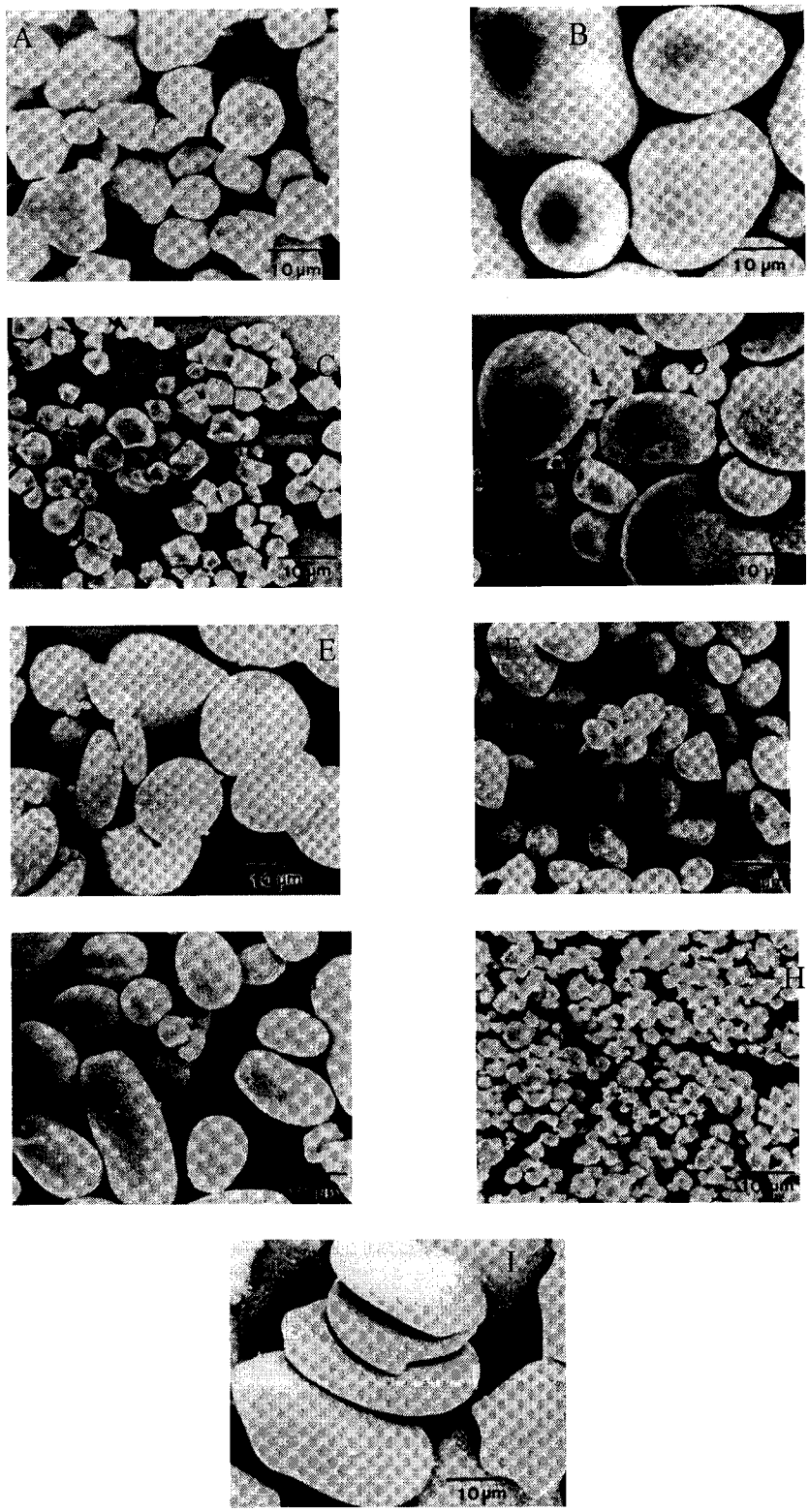


Figure 5. SEM images are starch granules from maize (A) and potato (B), rice (C), wheat (D), barley (E), acorn (F) avocado (G), parsnip (H) and shoti (I) (source: Jane et al. 1994).

Very little is known about the arrangement of amylose and amylopectin molecules in starch granules. Trichitic model (Meyer 1895) was first to show concentric ring-like organization of amylopectin molecules (see Fig. 1), but was dismissed due to overcrowding of molecules as granules enlarged (French 1972). Nikuni (1969) proposed the unitary theory of starch where all molecules in starch granule may be covalently bound. Both amylose and amylopectin are incorporated with the appearance of concentric ring structure. However, this model was also dismissed because molecular weight obtained by this model greatly exceeded that determined by physicochemical methods. Lineback (1984) modified Nikuni model to incorporate the concept of a double helix of an outer chain of amylopectin. Amylose existed in a random or helical configuration without binding to an amylopectin molecule. Kainuma (1980) and French (1984) proposed possible arrangement of amylopectin clusters in waxy starch granules based on transmission electron microscopy observations and analyses of branching pattern of amylopectin and structure of Nägeli amylodextrin. In this model, starch molecules are aligned perpendicular to the growth ring. Combining data from three-dimensional tomographic reconstructions and electron diffraction, Oostergetel & van Bruggen (1993) proposed a model for semi-crystalline structure and arrangement of amylopectin molecules in potato starch. In this model, helices form a continuous, regular crystalline network. Linear segments form double helices which are crystallized into 5 nm thick lamellae, alternating with amorphous layers in which the α-1$\rightarrow$4, α-1$\rightarrow$6 branch points are located. Since neighboring helices interpenetrate each other, crystalline lamellae form a continuous super helical structure. Recently, computer-simulated models have implied that certain internal chain-lengths will impede the formation of parallel structures and thus

crystalline amylopectin (O'Sullivan & Pérez 1999), supporting earlier findings (Pfannemüller 1987).

Granule crystallinity is associated with the amylopectin component (Veregin et al. 1986). Regions of amylopectin double helix formation fall within the crystalline lamellae, whilst the amylopectin branch points lie in both the amorphous and crystalline lamellae, depending on whether starch is A- or B-type (Jane et al. 1997). Branch points in amylopectin are not randomly distributed along the molecule, but are clustered and the interjacent linear segments form thin (~ 5 nm) crystalline lamellae domains (Oostergetel & van Bruggen 1993). These domains are visible in transmission electron micrographs of starch granule thin sections (Kassenbeck 1978) or fragments (Yamaguchi et al. 1979, Oostergetel & van Bruggen 1989) and were initially believed to have a regular spacing of ~ 10 nm (Blanshard et al. 1984, Oostergetel & van Bruggen 1989), but later was revised to ~ 9 nm (Jenkins et al. 1993), and a crystalline lamellae diameter of ~ 18 nm (Oostergetel & van Bruggen 1993). Crystalline lamellae are present in two main directions with a relative angle of 25° (Oostergetel & van Bruggen 1993). Cluster models for amylopectin structure (French 1972, Zobel 1988, Manners 1989b) do not explain why crystalline lamellae are seen locally in essentially two different orientations.

Growth rings are often seen in granules, and amylopectin and amylose are arranged radially with their molecular axes aligned perpendicular to the growth rings and to the granule surface (Kainuma 1988, Waigh et al. 1997). Growth rings are alternating ring structures consisting of amorphous and crystalline regions (French 1984). It is postulated that crystalline smectic lamellar periodicity (Yamaguchi et al. 1979), which is more conducive at longer spacer lengths (Waigh et al. 1998), is due to antagonistic effect of

entropy of amylopectin backbone and the ordering of helices (Waigh et al. 1999). Formation of distinct rings results from both the apposition of new material on the granule and the action of swelling, with largest rings towards outer edge of granule where swelling is greatest. Growth rings themselves may be broken into smaller "blocklets" due to creation of radial canals on swelling that break up the rings. Blocklets, structures that are visible optically (size ~ 1μm) have been reported to be artifacts due to separation of layers during preparation of starch for analysis (Badenhuizen 1959).

Gallant et al. (1997) revived the blocklet concept, describing organization of amylopectin lamellae into spherical blocklets, with diameters from 20-500 nm depending on botanical origin and location within granule. Scanning electron microscopy studies showed wheat starch has small blocklets (~ 25 nm) in the semi-crystalline shells, and larger 80-120 nm blocklets in the hard crystalline layers (Baker et al. 2001). Potato starch shows much larger blocklets of 200-500 nm. Atomic force microscopy revealed wheat starch has small surface protrusions of 10-50 nm, whereas larger spherical protrusions (200-500 nm) were observed on surface of potato starch granules (Ohtani et al. 2000, Baker et al. 2001). Topographic and phase images of ~ 100 nm thick sections showed mostly no indication of growth ring structure (Baker et al. 2001), consistent with early electron microscope observations of sections through maize endosperm where less than 15% of starch granules showed growth rings (Whistler & Thornburg 1957, Badenhuizen 1959). However, Baker et al. (2001) did report towards outer region of corn starch granules the presence of growth rings spaced by 450 nm, and some slightly finer rings with widths of 100-200 nm (Baker et al. 2001). Both ring sizes are well within the normal range of 150-700 nm observed for corn starch (Mussulman & Wagoner 1968).

The precise structural role played by amylose is unclear. It is likely that a large portion is found within amorphous growth ring, with only small amounts associated with semi-crystalline growth ring (Montgomery & Senti 1958). It has been suggested that some amylose co-crystallizes with amylopectin within crystalline lamella (Blanshard 1987, Kasemsuwan & Jane 1994, Gérard et al. 2002). Amylose may also form inclusion complexes with any lipids present internally within starch granule (Morrison 1988, Morgan et al. 1995).

Studies of waxy, normal and high-amylose maize starch found all three had combined size of crystalline and amorphous lamellae of 9 nm (Jenkins et al. 1993) but the fraction of this repeat distance allocated to crystalline lamellae was observed to be strongly influenced by the amylose content of starch (Jenkins & Donald 1995). Size of crystalline lamellae increased with increasing amylose content for the three maize starches studied. Additionally, amylose content has been reported to increase, particularly at the periphery, as granules enlarge (Jane & Shen 1993, Takeda et al. 1999, Pan & Jane 2000), and larger starch granules have been reported to have greater degree of crystallinity (Franco et al. 1998). A new interesting theory has been proposed by Kitahara et al. (2002), who demonstrated, using sweet potato callus as a model system, that amylopectin molecular synthesis was completed before the appearance of any amylose in starch granules.

The relationship between amylose content and crystalline lamellae size is at first sight, counter-intuitive. Amylopectin is known to be responsible for granular crystallinity, yet reducing amylopectin content has the effect of increasing the crystalline region size. However, using chromatographic techniques to analyze enzymatic debranched amylopectin, average A-chain of maize and waxy maize comprise about 18 glucose units (Hizukuri et al.

1983, Hizukuri 1985, Takeda et al. 1988), whilst amylomaize has longer A-chains comprising around 20 glucose units (Hizukuri 1985, Takeda, C. et al. 1993). Assuming that the two residues nearest the branch point can not participate in double helix formation due to steric constraints (Umeki & Kainuma 1981) and using molecular structures derived by Imberty et al. (1991), these numbers of glucose units correspond to crystallize amylopectin sections of length around 5.7 nm for maize and waxy maize, and 6.2 nm for amylomaize (Jenkins & Donald 1995). However, Umeki & Kainuma (1981) used acid to etch away the amorphous regions of waxy maize starch granules, and calculated a crystallizing chain-length of 5.0 nm. Therefore the difference suggests estimated lengths are an overestimate, or etching destroys part of amylopectin chain within crystals.

As amylose content is increased, the difference between length of crystalline lamellae and crystallizing amylopectin A-chain, also increases (Jenkins & Donald 1995). It appears amylose is acting to disrupt the packing of amylopectin double helices within the crystalline lamellae. Jenkins & Donald (1995), propose two possible mechanisms, one involving amylose co-crystallizing with amylopectin, pulling amylopectin chains out of register, and other mechanism involves amylose chains oriented transverse to the lamellar stack, penetrating the amorphous lamellae and introducing disorder. Additionally, Jenkins & Donald (1995) observed electron density decreases as amylose content increased, with the change probably associated with a reduction in crystalline lamellae electron density, and/or an increase in amorphous lamellae electron density. Improved packing array of amylopectin chains would have greater density than a disrupted array. Therefore most of the observed change in electron density is probably due to a reduction of crystalline lamellar density with increasing amylose content.

X-ray diffraction of humidified starch powders gives distinctive patterns for A- and B-type starches, as well as amylose-lipid complexes. Amylose-lipid complex changes the intensity ratio in the scattering region $2\theta = 19\text{-}23°$, and peaks are found at $2\theta = 7.4°$, 13.0° and 20.5° (Gernat et al. 1993).

Two different X-ray diffraction patterns can be obtained for starch according to botanical source. A-type is predominantly found in cereals, and B-type predominantly in root and tuber starches, although this is only a rough generalization. Using electron diffraction of single crystals, X-ray powder patterns and X-ray fiber diffraction, a three-dimensional structure of crystalline part of A-starch was determined in which the unit cell contains 12 glucose residues located in two left-handed, parallel-stranded double helices packed in a parallel fashion with four water molecules located between the helices (Imberty et al. 1987, Imberty et al. 1988a, Baker et al. 2001). Chains are crystallized in a monoclinic lattice. Three-dimensional structure of B-starch was determined to have a unit cell containing 12 glucose residues in two left-handed, parallel-stranded double helices packed in a parallel register with 36 water molecules located between these helices (Imberty et al. 1988b, Baker et al. 2001), and was therefore less dense than A-starch (Bogracheva et al. 1999). Chains are crystallized in a hexagonal space group. For both A- and B-starch, primary hydroxyl groups exist in *gauche-gauche* conformation and no intramolecular hydrogen bonding occurs. Within double helix, interstrand stabilization is achieved without steric conflict through the occurrence of O(2)...O(6) type hydrogen bonds, with two such bonds per glucose residue (Imberty et al. 1988a, Imberty et al. 1988b). However hydrogen bonding is not the only force stabilizing double helices, as van der Waals interactions still contribute majority of the stabilizing. Inner dimension of a double helix cavity is 0.35 nm in

diameter which does not allow any water molecules to pack internally (Imberty et al. 1988a). For B-starch, hydration is 27% (w/w), in which half the water molecules are tightly bound to double helices and remainder form a complex network centered around the six fold screw axis of the unit cell (Wu & Sarko 1977, Imberty et al. 1988b). *gauche-gauche* orientation was found to be consistently better than other models in terms of low values of crystallographic R factor and energy criteria. For each strand, conformation at the α-1→4 glycosidic linkage is in a low-energy conformation and the association of two such strands in a double-helical fashion exhibits further energy stabilization through interchain hydrogen bonding (Imberty et al. 1988b). Transition form B-starch to A-starch can be accomplished by rearrangement of the pairs of double helices (Imberty et al. 1991), typically involving heat-moisture treatments (Perera et al. 1997, Jacobs & Delcour 1998). Many authors have stated that water molecules play an important role in establishing the crystalline organization of B-starch. The effect of water acting as a plasticizer, inducing an alignment of starch molecules was suggested by French (1984). In contrast, development of an ordered water structure has been postulated, in which water molecules are arranged either as systematic water bridges (Eisenhaber & Schulz 1992) or every string of six helical macromolecular chains encloses a cylindrical cavity filled with water structured like cubic ice (Cleven et al. 1978).

Despite the large presence of water in B-type starch, a greater volume of water is found in amorphous regions of granules (Jenkins & Donald 1996). Additionally, water content within amorphous lamellae and amorphous background is similar for B-type starches, but in A-type starches, amorphous lamellae have a lower density and therefore a more open structure, which will therefore be more easily penetrated by water, compared to the

amorphous background (Donald et al. 2001). One possible explanation for this qualitative difference in amorphous region properties is based on environmental growth conditions of cereal and tuber starches. A-type cereal starches develop above ground, experiencing cyclical light and darkness, whilst potato starch is formed in constant darkness. Cereal starches are believed to form one semi-crystalline and one amorphous growth ring in a single day, in periods of light and darkness, respectively (Buttrose 1962). However, transmission electron microscopy studies have shown that growth rings are still present in potato starch granules grown in constant light or darkness. Recently, Tang et al. (2000) attempted to study the behavior of water in different regions of starch granules using NMR but interpretations are difficult as it is challenging to identify correctly the different populations of protons.

A third type of starch crystal packing, involving both A- and B-type crystallinity present has been observed for some starches. Smooth pea starch is reported to be C-type with proportion of A and B polymorphs about 56 and 44%, respectively (Gernat et al. 1990, Gernat et al. 1993, Cairns et al. 1997). X-ray diffraction from C-type starch was demonstrated to be similar to diffraction pattern constructed by combining diffraction patterns from crystalline portions of A- and B-type starches (Cairns et al. 1997).

Hydrolysis, involving α- and β-amylase, of two double maize mutant starches of A-crystalline (*wxdu*) and B-crystalline type (*aewx*) isolated clusters and all branching zones of clusters (BZC). Size exclusion chromatography combined with enzymatic analysis revealed that A-type clusters were larger ($DP_n > 80$) and contained more, but shorter, chains than B-type clusters (Gerard et al. 2000). BZC of A-type starch were also larger, but with shorter distance between the branching points than in B-type BZC. A-type clusters had a densely packed structure and B-type had a poorly branched structure. Gerard et al. (2000) also found

distance between two α-1→6 linkages and the branching density inside each cluster are determining factors for development of crystallinity in starch granules. Clusters composed of numerous short chains and characterized by a short distance between successive chains within the amylopectin molecule produce densely packed structures, and appear to crystallize into A allomorphic type. Conversely, clusters composed with fewer but longer chains and BZC with a long distance between branching points produce a poorly packed structure and subsequently crystallize into B allomorphic type.

Some starch granules of corn, sorghum and millet have been reported to have small pores randomly distributed over their surfaces and wheat, rye and barley starches have pores along the equatorial groove (Fannon et al. 1992a, Fannon et al. 1992b). Later these pores were reported to be openings to serpentine, radial, tube-like channels that penetrate into the granule interior, connecting the central cavity to external environment (Fannon et al. 1993, Huber & BeMiller 1997, Huber & BeMiller 2000). Pores were absent in many other starches studied. Pores were reported as normal, real, anatomical features of native granules and were not artifacts produced by isolation, preparation or observation techniques. Although Fannon proposes that pores affect the pattern by amylases, it is still contentious whether the pores are in fact the product of amylase attack during experimental procedures, and variation in susceptibility of granule surface to amylase attack, which would create the sporadic distribution of surface pores observed, has been reported (Evers & McDermott 1970, Fuwa et al. 1977, Fuwa et al. 1979).

Table 4. Granule size and morphology of starches from various botanical sources.

Starch	Granule diameter (μm)	Shape Descriptors
Acorn	5-20[1], 12[37]	Dome-shaped[1]
Adzuki bean	15-100[30]	
Ae 1-5108 maize	5-15[1]	Round[1], smooth surface[1]

Table 4. (continued)

Starch	Granule diameter (μm)	Shape Descriptors
Ae waxy maize	5-15[1]	Irregular[1], rough-edged[1]
Aegilops	4-27[38]	Bimodal[38], unimodal[38]
Algae, red	1.7-3.4[34]	Spherical[34], ovoid[34], discoid[34], shallow-bowl[34]
Amaranth	0.5-2[1]; 1-2[19]; 0.75-1.5[15]	Polygonal[1,15,19], smooth surface[15]
Amylomaize-5	10-15[1]	Smooth[1], irregular[1], rod-shaped[1]
Amylomaize-7	6-15[1]	Smooth[1], irregular[1], rod-shaped[1]
Arrowroot	8-30[1]	Smooth[1], oval[1], round[1],
Avocado	10-27[1]	Egg- and barrel-shaped[1], smooth[1]
Babassu	8-15[1]	Dome- and egg-shaped[1], hemispherical[1]
Banana	20-50[5]; 15-45[1]	Oval[5] elongated[5,45], irregular[1,45], discoid[1], flat[45], smooth[45]
Barley	2-3 and 10-50[29,30]; 2-3 and 15-32[1]	Bimodal[1,29], oval[29], round[29], disk-shaped[1], dumb-bell shaped[36]
Barley, hull-less *wx*	4.5-15.6[36]	Large lenticular with pinholes, equatorial grooves and furrows[36], small irregular[36], angular[36], polyhedral[36]
Barley, high amylose	5.2-13.2[36]	
Bean, green	10-45[1]	Smooth[1], oval[1]
Bitter yam	4-5 and 9[11]	Bimodal[11], rounded[11]
Black bean	7-30[41]	Round[41], irregular[41], elliptical[41]; oval[41]
Buckwheat	2-6[18]; 5-10[1], 17[37]	Spherical[18], oval[18], polygonal[1,18]
Canna (achira)	30-100[1]; 35-101[22]	Smooth[1], ellipsoidal[1], spherical[1]
Cattail millet	3-15[1]	Irregular[1], polygonal[1]
Chickpea (garbanzo)	9-30[41], 10-27[1]	Irregular[1], indented cut at one end[1]
Chinese taro	1-4[1]	Irregular[1], polygonal[1]
Cocoyam	0.05-1.05[42] and 0.3-1.8[42]	Small oval[42], kidney-shaped[42]
Cow cockle	0.5-2[1]	Irregular[1], polygonal[1]
Cow pea	10-35[1]	Ellipsoidal[1]
Dasheen	0.5-3[1]	Irregular[1], polygonal[1]
Diffenbachia	15-80[1]	Disk-shaped[1], irregular[1], rod-shaped[1], submarine[1]
Du waxy maize	5-15[1]	Irregular[1], smooth[1]
Enset	20-70[12]	Angular[12], elliptical[12]
Ginger	15-60[1]	Disk-shaped[1]
Gingko	5-20[25]	Spherical[25], oval[25], smooth surface[25]
Grass pea seed	15-33[4]	Oval[4]
Huaishan	19-29[47]	Spherical[47]
Jicama	25-30[1]	Round[1], polygonal[1], irregular[1]

Table 4. (continued)

Starch	Granule diameter (μm)	Shape Descriptors
Kiwifruit	6-8[44]	Spherical[44], irregular[44], polygonal[44]
Kuzu	5-30[26]	Spherical[26], hemispherical[26], polygonal[26]
Lentil	3-30[24]; 7-28[41], 10-20[1]	Ovoid[24,41], spherical[24], smooth[1], indents[1], elliptical[41], irregular[41]
Lily	18-30[28]; 20-70[1]	Slender[28], ovoid[28], elliptical[28], polygonal[28]
Lima bean	10-45[1]; 10-52[7]	Oval[7], smooth[1], ellipsoid[1],
Lotus root	10-50[1]	Oval[1], round[1]
Maize, normal	2-32[21]; 5-20[1], 5-30[43]	Irregular[1], polyhedric[1], sharp-edged[1]
Maize, waxy	5-18[1]	Irregular[1], polygonal[1], rough-edged[1]
Maize *ae*	4-11[46]	
Maize *ae bt1*	3-10[46]	
Maize *ae du1*	4-14[46]	
Maize *bt1*	4-9[46]	
Maize *bt2*	6-19[46]	
Maize *du1*	4-11[46]	
Maize *du1 su1*	3-12[46]	
Maize *h*	8-22[46]	
Maize *h sh2*	6-23[46]	
Maize *h wx*	3-20[46]	
Maize *sh2*	2-9[46]	
Maize *sh2 bt1*	3-9[46]	
Maize *sh2 wx*	4-16[46]	
Maize *su1*	2-10[46]	
Maize *wx du1*	4-19[46]	
Matai	6-16[47]	Spherical[47]
Mungbean	10-27[1], 22[37]	Oval[1],irregular[1], pronounced indents[1]
Narcissus, white	5-40[1]	Smooth[1], irregular[1]
Navy bean	8-32[41]	Round[41], irregular[41], elliptical[41], oval[41]
Oat	4-5[31]; 2-15[1], 3.8-10.5[39]	Compound[39], clustered[39], Polyhedral[1,31], polygonal[39], irregular[1,31]
Oxalis	22-55[22]; < 7 and 10-50[9]	Compound[9], assymetrical[9]
Parsnip	1-6[1]	Irregular[1], polygonal[1]
Pea, green, smooth	10-45[1]; 14-32[41], 22-30[10]; 2-40[20]	Spherical[10,20], oval[10,20,41], irregular[10,41], discoid[1], elliptical[41]
Pea, wrinkled	10-40[20]	Compound[20], 4-6 granules in a ring[20]

Table 4. (continued)

Starch	Granule diameter (μm)	Shape Descriptors
Peijbaye	3-10[1]	Round[1], oval[1], hemispherical[1]
Pigweed	1-2[1]	Irregular[1], polygonal[1]
Pineapple stem	3-10[1]	Compound[1], irregular[1], multifaceted[1]
Pinto bean	6-32[41], 10-27[1]	Smooth[1], spherical[1], round[41], elliptical[41], oval[41], irregular[41]
Potato	15-75[1]; 40-60[27]; 12-70[16]	Rounded[16], oval[16], smooth[1]
Quinoa	<1[3]; 1-2[19]; 0.3-2[30]	Polygonal[19]
Rice	3-8[1]; 3-5[8]; 2-8[14]	Compound[1,8], polyhedral[1,8,14], ellipsoid[8]
Rye	2-3 and 22-36[1]	Bimodal[1], Spherical[1], disk-shaped[1]
Sago	20-50[1]; 20-40[2]	Oval[2]
Shoti	24-60[1]	Smooth[1], Disk-shaped[1], indented curves[1], lenticular[1]
Sorghum	5 and 10-30[1]	Bimodal[1], spherical[1], lenticular[1]
Sweet corn, immature	1-2 and 5 and 10-12[1]	Trimodal[1], round[1]
Sweet potato	5-25[1]; 4-15[16]; 8-11[17]; 14-34[23]; 2-42[32]	Angular[16], spherical[17,23], polygonal[1,17,23]
Tapioca	5-22[6]; 5-25[1]; 5-20[11]	Smooth[1], irregular[1], rounded[11]
Tef	2-6[43]	Compound[43], polygonal[43], cubic[43]
Triticali	5 and 22-36[1]	Bimodal[1], spherical[1], lenticular[1]
Water chestnut	10-30[13]; 5-27[1]	Round[13], elliptical[13], polygonal[1]
Water yam	31-35[11]	Ellipsoid[11], rounded[11]
Waxy potato	12-72[16]	Rounded[16], oval[16]
Waxy rice	3-8[1]	Compound[1], irregular[1], polygonal[1]
Wheat, commercial	2-3 and 22-36[1]; 2-5 and 18-33[35]	Bimodal[35], spherical[35], disk-shaped[35]
Wheat, durum	2-8 and 10-40[33]	Bimodal[33], spherical[33], lenticular[33]
White yam	31-35[11]	Polyhedral[11]
White carrot	7-23[22]	
Yam	4-20[16]	Angular[16]
Yam bean	4-35[40]	Spherical[40], irregular[40]
Yellow yam	10-50[11]	Polyhedral[11], ellipsoid[11]
Yucca	5-25[1]	Dome-shaped[1]

1 = Jane et al. (1994), 2 = Ahmad et al. (1999), 3 = Lorenz (1990), 4 = Akalu et al. (1998), 5 = Bello-Pérez et al. (2000), 6 = Atichokudomchai et al. (2001), 7 = Betancur et al. (2001), 8 = Champagne (1996), 9 = Cortella & Pochettino (1995), 10 = Davydova et al. (1995), 11 = Farhat et al. (1999), 12 = Gebre-Mariam & Schmidt (1996), 13 = Hizukuri et al. (1988), 14 = Hoover et al. (1996), 15 = Hoover et al. (1998), 16 = McPherson & Jane (1999), 17 = Noda et al. (1995), 18 = Qian & Kuhn (1999a), 19 = Qian & Kuhn (1999b), 20 = Ratnayake et al. (2002), 21 = Sahai & Jackson (1996), 22 = Santacruz et al. (2002), 23 = Shin & Ahn (1983), 24 = Sotomayor et al. (1999), 25 = Spence & Jane (1999), 26 = Suzuki et al. (1981), 27 = Suzuki et al. (1994), 28 = Takeda et al. (1983), 29 = Tang et al. (2000), 30 = Tang et al. (2002), 31 = Tester & Karkalas (1996), 32 = Tian et al. (1991), 33 = Vansteelandt & Delcour (1999), 34 = Yu et al. (2002), 35 = Yoo & Jane (2002), 36 = Li et al. (2001a), 37 = Cho & Kim (2000), 38 = Stoddard & Sarker (2000), 39 = Hoover et al. (2003), 40 = Forsyth et al. (2002), 41 = Hoover & Ratnayake (2002), 42 = Sefa-Dedeh & Sackey (2002), 43 = Bultosa et al. (2002), 44 = Sugimoto et al. (1988), 45 = Fuwa et al. (1979), 46 = Wang et al. (1993c), 47 = Yu et al. (1999)

Phosphorus Content

Incorporation of phosphorus into starch is considered to proceed concurrently with starch synthesis (Nielsen et al. 1994), and recently speculated to be produced by starch-branching enzymes utilizing phosphorylated α-1→4 glucans (Viskø-Nielsen et al. 1998). Although initial step remains unknown, glucose-6-phosphate has been suggested as a precursor (Wischmann et al. 1999). Starch contains three types of phosphorus, esterified phosphates, phospholipids and inorganic phosphorus, all in small quantities, but amounts vary depending on botanical source as illustrated in Table 5. Phosphate monoester groups are found exclusively in amylopectin at a frequency of one atom of phosphorus per 220-250 anhydroglucose units (AGU) (Schoch 1942, Gracza 1965, Abe et al. 1982). Potato amylopectin contains the greatest amount of phosphate monoesters (0.085-0.09 %, dry basis), with all other starches considerably lower (0.021% or less) (Jane et al. 1996, Kasemsuwan & Jane 1996). Root and tuber starch phosphorus contents consisted almost entirely of phosphate monoesters (Kasemsuwan & Jane 1996). Cereal starches contain mainly phospholipids, typically greater than 0.048%, however maize is considerably lower (0.016%). Waxy starches contain very low levels of phosphorus (Jane et al. 1996). Jacobsen et al. (1998) demonstrated that phosphorus content of potato starch increased by supplying developing tubers with phosphorus fertilizer.

Posternak (1935, 1951) determined phosphate groups were located at C-6 primary hydroxyl group, and later this was confirmed to be present proportionally at two-thirds, along with one-third occurrence of phosphate at C-3 and trace amounts at C-2 (Hizukuri et al. 1970, Tabata & Hizukuri 1971, Lim & Seib 1993b). Phosphorylation at C-3 was independent of potato variety, while substantial variation at C-6 was observed, suggesting

different regulation at the two phosphate sites (Muhrbeck & Tellier 1991). Bay-Smidt et al. 1994 found the level of C-6 bound phosphorus per milligram of potato starch increased 50% from the cortex towards the pith. Phosphate monoesters were not found on non-reducing terminals of α-1$\rightarrow$4 linked unit chains (Tabata et al. 1978). Average chain-length of phosphorylated potato starch is approximately DP 40, indicating these phosphates link mainly to long B-chains (Takeda & Hizukuri 1982). Phosphate monoesters are located a minimum of nine anhydroglucose units inward from a branch point (Takeda & Hizukuri 1982). Decrease in organic phosphate and increase in inorganic phosphate during storage of autoclaved starch has been observed (Sugimoto & Goto 1966).

Glucoamylase has been used to study in further detail the location of phosphate monoesters because of its inability to bypass phosphorylated AGU, with the C-3 phosphate group more obstructive than C-6 (Takeda et al. 1983). Exhaustive digestion of potato starch (one phosphorus atom per 209 AGU) with glucoamylase yielded 17% γ-dextrin with one phosphorus per 36 AGU and average chain-length of 14. Since phosphorus in potato starch was concentrated in the 17% γ-dextrin, it was concluded that either few amylopectin molecules are highly phosphorylated or phosphate monoesters are concentrated locally within starch granule (Abe et al. 1982). Additionally, Jane & Shen (1993) determined by stepwise chemical gelatinization that phosphorus was densely located in the core of potato starch granules together with amylopectin.

Lipid content of starch from *Triticeae* comprise predominantly of lysophospholipids, particularly lysophosphatidyl-choline, -ethanolamine, -serine, -inositol, and –glycerol, respectively (Morrison 1988). Starches from other cereals have much higher proportions of free fatty acids. The most predominant fatty acid of all cereal starches is linoleic acid, except

for rice and rye where palmitic and oleic acid are the major component, respectively (Morrison 1988). Lysophospholipid content of wheat starch has been shown to decrease with granule size but increase with granule maturity. Starch deposited on both A- and B-granules at successive development stages contained higher proportions of lysophospholipids than initial deposits. A close relationship was established between amylose and lysophospholipid contents, but this relationship was different for A- and B-granules. Due to the oblate and lenticular shape of wheat A-granules, gradients of starch and lysophospholipids occur along outer parts of any radius from the hilum. The role lysophospholipids play in starch synthesis was concluded to be different in the two types of wheat starch granules (Morrison & Gadan 1987).

Table 5. Phosphorus content (% dry starch weight) of starches from various botanical sources.

Starch	Monoester-Phosphate	Phospholipid
Normal maize		0.016[1,4]-0.02[2,16,17]
Amaranth	0.13-0.46[14]	
Wheat	0.01[1]	0.053[1]-0.059[4,17]
Rice	0.013[1]	0.032[5]-0.048[1]
Oat		0.056[1]
Millet		0.058[1]
Barley		0.022-0.06[15]
Waxy maize	0.002[1], 0.01[17]	
Waxy rice	0.003[1]	
Waxy potato	0.069[13]	
High-amylose maize	0.013[1]	0.014[1]
Potato	0.05-0.06[8], 0.07[6,13], 0.08[17], 0.089[1]-0.16[3], 0.37-0.97[18]	
Sweet potato	0.011[1], 0.012-0.014[19], 0.02[13]	
Tapioca	0.008[1], 0.01[2,17], 0.007[10]-0.012[9]	
Arrowroot	0.02[2]-0.021[1]	
Mungbean	0.011[1]	
Navy bean	0.004[6]	
Pinto bean	0.004[6]	
Kidney bean	0.017[7]	

Table 5. (continued)

Starch	Monoester-Phosphate	Phospholipid
Green pea	0.004[1]	
Ginger	0.045[11]	
Gingko	0.006[16]	
Water chestnut	0.03[12]	
Yam	0.012[13]	
Sago	0.01-0.015[2]	

References 1 = Jane et al. (1996), 2 = Posternak (1935), 3 = Muhrbeck & Tellier (1991), 4 = Gracza (1965), 5 = Hizukuri et al. (1983), 6 = Kim et al. (1996), 7 = Yoshida et al. (2003), 8 = Liu et al. (2003), 9 = Rickard et al. (1991), 10 = Soni et al. (1985), 11 = Jyothi et al. (2003), 12 = Hizukuri et al. (1988), 13 = McPherson & Jane (1999), 14 = Pérez et al. (1993), 15 = Song & Jane (2000), 16 = Spence & Jane (1999), 17 = Swinkels (1985a), 18 = Suzuki et al. (1994), 19 = Takeda et al. (1986)

STARCH FUNCTIONAL PROPERTIES

Gelatinization

Gelatinization is frequently described as the range of irreversible events occurring when starch is heated in water. While heating is the most common method of starch gelatinization, technically gelatinization can occur without additional heat when starches are dispersed in solutions possessing crystal destructing properties. Non-thermal methods of gelatinization include use of alkali, dimethyl sulfoxide, cations and water structure breaking compounds. Alkali such as NaOH and KOH interact with the proton of the anhydroglucose hydroxyl group, giving starch a negative charge that exerts a repelling force on starch polymer chains, causing the double helix to be pulled apart, thereby gelatinizing starch (Maher 1983, Kim et al. 1984). Dimethyl sulfoxide acts as a hydrogen bond acceptor to pull apart double helix. Cations that carry high positive density charge, such as $CaCl_2$ (Jane & Shen 1993, Koch & Jane 2000) and LiCl (Ahmad & Williams 1999, Pan & Jane 2000), can cause an exothermic reaction and heat released will melt starch crystallites. Water structure breaking compounds such as KIO_3 and NaSCN have large volume but low charge density that breaks hydrogen bonds in water to enhance solubilization and gelatinization of starch at room temperature.

Not all combinations of starch, water and temperature result in gelatinization. There is a minimum level of water content and a certain temperature that has to be reached. Additionally, despite the name "gelatinization", not all combinations of water and heat that result in gelatinization, will result in gel formation. Gelatinization temperature is always a temperature range. For a single granule in excess water, the gelatinization temperature range might be 1-2°C, whereas an entire population of starch granules can range from 5-15°C (Evans & Haisman 1982, Liu & Lelievre 1993). Gelatinization temperatures and enthalpy change of gelatinization for starches from various botanical sources is listed in Table 6.

Many factors affect gelatinization temperature and gelatinization temperature range, with the most critical being water content. Water is present in both amorphous and crystalline regions of starch granules (Eliasson et al. 1987). Water acts as a plasticizer for the starch crystallites (Donovan 1979) and X-ray diffraction pattern disappears if starch is completely dried (French 1984). Presence of water will decrease glass transition temperature (T_g) and as a consequence, decrease temperature that crystallites melt. Although starch granules are built up of polymers that are hydrophilic, the starch granule is not soluble in water due to its semi-crystalline structure and hydrogen bonding between hydroxyl groups of starch polymers. A proportion of water, typically 0.25-0.4 g water/g starch will not freeze, even at temperatures below -50°C (Wootton et al. 1974, Leung & Steinberg 1979, Eliasson 1985). When dry starch granules are placed in water, a small amount of water is absorbed which is an exothermic process (French 1984). As temperature increases, amount of absorbed water by starch granules increases. Until the onset gelatinization temperature (T_o), water uptake by starch granules is reversible.

In polarized light, ungelatinized starch granules show a distinctive birefringence pattern called the "Maltese Cross" (Fitt & Snyder 1984). Birefringence begins to disappear as T_o is reached, indicating order is beginning to be lost in starch granule, but not necessarily crystallinity. Gelatinization temperature range can be determined by following loss of birefringence in excess water (Moss 1976). At low water content (< 8%), birefringence was not destroyed by heating wheat starch to 232°C (Burt & Russell 1983).

Loss of crystallinity occurs in two steps: at first loss occurs at a very low rate, but then at a temperature specific to each starch, the rate increases dramatically (Svensson & Eliasson 1995). Starch gelatinization is an endothermic process with enthalpy values typically 10-19 J/g. Some researchers suggest that melting of starch crystallites is preceded by glass transition (Zeleznak & Hoseney 1987, Slade & Levine 1988). For application of starches for food industries, functional properties of starch events occurring after melting of crystalline structure, such as water-holding capacity or rheological properties are important. When starches are heated in excess water, granules can swell to a similar degree in all directions, such as maize or potato (Williams & Bowler 1982) or swell unevenly, such as for wheat and barley (Bowler et al. 1980). Starch granule swelling typically results in over double expansion in granule diameter (Ziegler et al. 1993). Swelling of starch begins at T_o but continues to much higher temperatures than conclusion gelatinization temperature (T_c) (Tester & Morrison 1990). Swelling is not affected by presoaking before heating, but increases with water/starch ratios up to 25 mL water/g starch. Swelling increases with defatting for the majority of starches (Tester & Morrison 1990), but for oat and pigeon pea starches, defatting decreases swelling (Doublier et al. 1987, Hoover & Vasanthan 1992, Hoover et al. 1993). Increased shearing will increase swelling (Doublier 1987), but severe

shearing will cause fragmentation of granules (Svegmark & Hermansson 1991). Higher heating rates result in greater swelling ratio of cereal starches (Ellis et al. 1989). Waxy starch varieties have higher gel volumes than higher amylose starch varieties (Colonna & Mercier 1985, Tester & Morrison 1990).

During heating, and at the same time as absorption of water, amylose is leached from starch granules, and to a lesser extent amylopectin is leached depending on starch and conditions (Doublier 1981, Ellis et al. 1988, Tester & Morrison 1990, Svegmark & Hermansson 1991). Lipids are not leached from starch granules during gelatinization (Tester & Morrison 1990). At low temperatures (50-70°C), leached material from starch granules comprises entirely of amylose, but as temperature increases above 70°C, the leached material increases in molecular weight and is more branched (Ellis & Ring 1985, Doublier 1987, Prentice & Stark 1992). For some starches such as oat, leached amylose forms a network structure around granules (Autio 1990). If starch contains a proportion of enzymatically or mechanically damaged starch then amylopectin of low molecular weight can be preferentially leached (Craig & Stark 1984). For some starches such as maize, most of the amylose is solubilized before leaching of amylopectin starts (Doublier 1981) whereas in oat starch, coleaching of amylose and amylopectin occur (Doublier et al. 1987, Hoover & Vasanthan 1992). A proportion of amylose frequently remains inside starch granule and is never leached during heating (Ellis et al. 1988).

If the water content is lower than that required for gelatinization but the temperature is at least sufficient for gelatinization, then starch is exposed to heat-moisture treatment (Kulp & Lorenz 1981, Lorenz & Kulp 1981a). If samples are subsequently gelatinized, their properties will have been altered such as increase in T_o and T_c, broader gelatinization

temperature range, decreased swelling power and decreased solubility (Kulp & Lorenz 1981, Donovan et al. 1983, Hoover & Vasanthan 1994). Heat-moisture treatment causes a change in type of crystallinity from less stable polymorphs (that exhibit B- or C-pattern of X-ray diffraction) to most stable form (A-pattern) (Lorenz & Kulp 1982, Donovan et al. 1983).

If the water content is high enough for gelatinization but temperature is too low, conditions might be suitable for annealing, a process that improves crystallinity. Temperature to achieve annealing (T_A) must be below T_o but above T_g, otherwise system is too rigid. In this temperature range, least perfect crystallites melt and molecules will crystallize on other more perfect crystals. Gelatinization temperature range moves to higher temperatures and becomes narrower (Gough & Pybus 1971, Knutson 1990, Larsson & Eliasson 1991, Seow & Teo 1993, Hoover & Vasanthan 1994). Annealing has been reported to occur as early as 24-72 hours of steeping cereal grains (Lorenz & Kulp 1978, 1981b, 1984, Knutson 1990, Larsson & Eliasson 1991) with the important parameter not being time but difference between T_o and T_A. If T_o-T_A is 20-25°C or more then no annealing occurs, whereas a 5°C difference can cause substantial increase in T_o (Larsson & Eliasson 1991). Effect of annealing decreases with increasing amylose content and there is no leaching of amylose during annealing (Knutson 1990). Granule swelling usually decreases due to annealing (Lorenz & Kulp 1984).

Table 6. Onset gelatinization temperature (T_o), peak gelatinization temperature (T_p), conclusion gelatinization temperature (T_c) and enthalpy change of gelatinization (ΔH) for starches from various botanical sources.

Starch	T_o	T_p	T_c	ΔH
Achira (canna)	56.8^{25}	61.2^{25}	67.7^{25}	15.7^{25}
Amaranth, commercial	66.3^{52}	74.5^{52}	86.9^{52}	2.6^{52}
Amaranth, waxy	66.7^{59}	70.2^{59}	75.2^{59}	16.3^{59}
Amaranth cv. African	69.8^{45}	76.3^{45}	84.1^{45}	12.3^{45}
Amaranth cv. Mexican	68.7^{45}	78.5^{45}	87.0^{45}	12.5^{45}

Table 6. (continued)

Starch	T_o	T_p	T_c	ΔH
Arrowroot	49.5-61.5[20]			
Banana cv. Criollo	71.4[37]	75.0[37]	80.4[37]	14.8[37]
Banana cv. Macho	69.6[37]	74.5[37]	81.6[37]	13.0[37]
Banana cv. Nandigobe	69.5[27]	73.3[27]	78.7[27]	10.0[27]
Banana cv. Valery	69.5[22]	74.6[22]	81.2[22]	5.2[22]
Barley cv. CDC Alamo (hull-less waxy)	54.5[1]	61.8[1]	74.5[1]	12.6[1]
Barley cv. CDC Candle (hull-less waxy)	55.4[1]	61.9[1]	73.8[1]	13.1[1]
Barley cv. CDC Dawn	52.0[1]	58.1[1]	72.5[1]	12.7[1]
Barley, high amylose	53.2[1]	62.0[1]	74.4[1]	12.0[1]
Barley,normal,compound	50.1[1]	59.9[1]	72.0[1]	13.5[1]
Barley cv. Glacier	55.0[54]	59.0[54]		9.2[54]
Barelycv Glacier hi AM	55.5[54]	62.8[54]		7.7[54]
Barley hull-less Glacier	56.5[54]	63.2[54]		7.3[54]
Barley cv. Glacier Pentld	61.3[26]			
Barley cv. Golden Prom.	57.9[26]			
Barley cv. Phoenix	53.1[1]	59.1[1]	71.0[1]	12.8[1]
Barley cv. Triumph	59.0[26]			
Barley cv. W.B. Merlin	55.4[54]	60.3[54]		13.0[54]
Barley, waxy	56.1[1]; 60.0[26]	62.1[1]	75.8[1]	13.1[1]
Barley, waxy,compound	50.5[1]	64.5[1]	74.5[1]	9.6[1]
Bitter yam	78.1[41]	81.3[41]	86.4[41]	
Black bean	62.0-66.9[30]	69.9-76.5[30]	82.8-84.2[30]	12.5[30]
Bracken	59.5-62.5[20]			
Buckwheat	51.5-62.3[18]	57.2-66.7[18]		9.4-13.9[18]
Canna, green leaf	59.3[59]	65.4[59]	80.3[59]	15.5[59]
Chick pea (garbanzo)	59.5[30]	64.7-67.7[30]	71.1-78.2[30]	9.7-12.4[30]
Chinese taro	67.3[59]	72.9[59]	79.8[59]	15.0[59]
Cocoyam	74[51]	78[51]	87[51]	4.06-[51]
Dioscorea	64.2[42]	68.2[42]	74.8[42]	19.2[42]
Ebiimo (yam)	71.0[20]			
Enset	61.8[43]	65.2[43]	71.7[43]	21.6[43]
Ginkgo	60.8[55]	67.1[55]	78.7[55]	14.6[55]
Grass pea seed	61.3[36]			10.9[36]
Kiwifruit cv. Abbot	57.9[34]	66.7[34]	70.6[34]	3.9[34]
Kiwifruit cv. Bruno	59.9[34]	63.7[34]	68.3[34]	3.4[34]
Kiwifruit cv. Hayward	62.4[34]	65.2[34]	69.5[34]	4.3[34]
Koimo (yam)	64.5[20]			
Kuzu	59.0-66.5[20]			
Lentil	60.7-63.0[30]	66.1-69.6[30]	76.1-78.7[30]	13.0[30]
Lily	56.0-59.5[20]			
Lima bean	75[38]		87[38]	

Table 6. (continued)

Starch	T_o	T_p	T_c	ΔH
Lotus root	58.5-62.0[20]; 60.6[59]	66.2[59]	71.1[59]	13.5[59]
Maize, normal	59.8[1]; 60.0[23]; 63.2[43]; 64.1[59]; 65.0[32]; 65.6[55]; 67.4[35]; 68[47]	66.9[1,23]; 69.0[43]; 69.4[59]; 70.5[55]; 71[47]; 73.0[32]	74.9[59]; 75.2[43]; 77[47]; 77.2[23]; 77.8[1]; 78.5[55]; 80[32]	9.7[23]; 12.4[35,59]; 13.8[55]; 15.5[1]; 16.2[43]
Maize, amylomaize V	71.0[59]	81.3[59]	112.6[59]	19.5[59]
Maize, amylomaize VII	70.6[59]		129.4[59]	16.2[59]
Maize, waxy	60.6[1]; 63.5-67.5[20]; 64.2[59]; 67[47]; 68.1[35]	67.1[1]; 69.2[59]; 75[47]	74.6[59]; 78.1[1]; 80[47]	13.3[1]; 13.9[35]; 15.4[59]
Maize cv. A632	67.9[29]	72.2[29]	78.2[29]	12.0[29]
Maize cv. A632 *du*	67.7[29]	72.6[29]	79.4[29]	9.1[29]
Maize cv. A632 *su2*	58.7[29]	63.9[29]	70.4[29]	3.4[29]
Maize *ae bt1*	63.7[13]			2.0[13]
Maize *ae du1*	65.6[13]			1.2[13]
Maize *ae wx*	71.5[59]	81.0[59]	97.2[59]	22.0[59]
Maize cv. B73	65.3[11]; 67.2[2]	71.1[2]		11.6[2]; 13.3[11]
Maize *du*	67[47]	71[47]	76[47]	
Maize *du1 su1*	62.3[13]			1.7[13]
Maize *du wx*	66.1[59]	74.2[59]	80.5[59]	15.6[59]
Maize cv. Hz85	69.9[29]	74.0[29]	80.5[29]	12.3[29]
Maize cv. Hz85 *du*	69.6[29]	74.3[29]	79.7[29]	9.1[29]
Maize cv. Hz85 *su2*	59.0[29]	62.6[29]	68.6[29]	5.2[29]
Maize cv. Mo17	65.0[3]; 66.2[2]	70.8[2]		12.6[2]; 13.8[3]
Maize cv. Oh43	71.1[29]	75.0[29]	81.7[29]	12.6[29]
Maize cv. Oh43 *du*	70.4[29]	74.0[29]	79.9[29]	12.0[29]
Maize cv. Oh43 *su2*	57.0[29]	61.8[29]	67.5[29]	4.8[29]
Maize *su2*	54[47]	58[47]	64[47]	
Millet, cattail	67.1[59]	71.7[59]	75.6[59]	14.4[59]
Millet, foxtail	64-69[15]	70-77[15]	76-84[15]	10-18[15]
Mungbean	60.0[59]	65.3[59]	71.5[59]	11.4[59]
Nagaimo (yam)	60.0-65.0[20]			
Navy bean	65.8[30]	74.7[30]	84.9[30]	13.4[30]
Oat, commercial	61[46]	66[46]	73[46]	10.4[46]
Oat cv. Alymer	57.0[21]	61.5[21]	69.0[21]	13.7[21]
Oat cv. Antoine	58.5[21]	63.5[21]	70.5[21]	13.2[21]
Oat cv. Baton	63.0[21]	64.5[21]	72.0[21]	12.9[21]
Oat cv. Borrus	46.7[56]	59.5[56]	73.0[56]	9.2[56]

Table 6. (continued)

Starch	T_o	T_p	T_c	ΔH
Oat cv. Erbgraf	46.3[56]	56.8[56]	68.7[56]	8.1[56]
Oat cv. Erich	45.6[56]	58.5[56]	69.2[56]	8.1[56]
Oat cv. Ernie	56.0[21]	59.5[21]	65.5[21]	14.6[21]
Oat cv. Francis	60.0[21]	63.5[21]	70.5[21]	13.5[21]
Oat cv. Gosline	63.5[21]	66.0[21]	74.0[21]	12.4[21]
Oat cv. Pendragon	47.3[56]	58.2[56]	72.3[56]	9.5[56]
Oat cv. Pitol	44.8[56]	57.1[56]	73.7[56]	9.0[56]
Oat cv. Selma	44.7[56]	56.2[56]	72.0[56]	9.0[56]
Oxalis (oca)	50.2[25]	55.9[25]	63.3[25]	14.6[25]
Pea, green	56.1[35]			9.5[35]
Peruvian carrot	56[51]	60[51]	73[51]	4.2[51]
Pigeon pea	64[60]	71[60]	77[60]	
Pinto bean	72.3[30]	75.3[30]	80.8[30]	15.8[30]
Potato, commercial	58.7[43]; 59.8[41]; 60.0-61.6[8]; 60.8[49]; 61.0-62.5[20]; 63.1[35]	62.6[43]; 63.4-64.6[8]; 64.3[41]; 65.2[49]	67.4-68.9[8]; 68.1[43]; 69.3[41]; 70.6[49]	11.9-12.2[8]; 17.3[49]; 17.8[35]; 19.8[43]
Potato, waxy	62.5[49]	66.6[49]	70.2[49]	18.2[49]
Quinoa	59.9[52]	64.5[52]	71.0[52]	1.7[52]
Red kidney bean	61.5[24]	66.8[24]	90.8[24]	15.4[24]
Rice, commercial	68.5[7]; 70.3[59]	75.7[7]; 76.2[59]	80.2[59]; 84.0[7]	6.3[7]; 13.2[59]
Rice, sweet	58.6[59]	64.7[59]	71.4[59]	13.4[59]
Rice, waxy	56.9[59]; 58.0[20]	63.2[59]	70.3[59]	15.4[59]
Rice cv. Cypress	72.1[31]	77.6[31]		14.1[31]
Rice cv. Hokuriku	68.4[16]			
Rice cv. IR5	69.0[7]	76.5[7]	87.8[7]	8.5[7]
Rice cv. IR8	66.3[4]	69.7[4]	74.1[4]	8.6[4]
Rice cv. IR28	62.6[7]	68.0[7]	76.5[7]	3.6[7]
Rice cv. IR32	69.0[20]; 70.3[7]	77.2[7]	88.2[7]	9.2[7]
Rice cv. IR42	59.5[7]	66.7[7]	78.7[7]	5.6[7]
Rice cv. Milky Queen	70.5[16]			
Rice cv. Nipponbare	68.9[16]			
Rice cv. Sassnidhiki	58.5[20]			
Rice cv. Snow Pearl	67.1[16]			
Rice cv. Tainung	61.9[9]	70.1[9]		7.4[9]
Rice cv. T(N)1	67.3[9]	74.1[9]		8.1[9]
Rice cv. Yumetoiro	66.4[16]			
Sago	64.1[17]; 64.4[50]; 69.4-70.1[35]	70.0[17]; 70.4[50]	76.7[17]; 80.4[50]	5.4[50]; 14.3[17]; 15.1-16.7[35]
Smooth pea	55-61[53]; 60-63[40]; 61.3[30]	60-68[53]; 67.2[30]	73.4-74.5[30]; 75-80[53]	10.8-13.8[30]; 14-23[40,53]

Table 6. (continued)

Starch	T_o	T_p	T_c	ΔH
Sorghum		67.3-68.8[5]		7.7-9.5[5]
Soybean	73[39]		81[39]	
Squash, buttercup	62.1[33]	65.9[33]	74.1[33]	13.8[33]
Squash, butternut	63.3[33]	67.3[33]	74.6[33]	12.6[33]
Sweetpotato, commercial	55.7-73.1[18]; 57.9[49]; 63.5-71.0[20]	61.3-77.6[18]; 63.1[49]	71.9[49]	12.7-16.8[18]; 13.5[49]
Sweetpotatocv Kanto116	39.0[48]	46.9[48]	64.8[48]	8.8[48]
Sweetpot Koganesengan	59.9[48]; 72.6[14]	67.3[48]; 77.2[14]	82.2[48]; 89.5[14]	12.4[48]; 13.1[14]
Sweet potato cv. Kyushu	64.6[48]; 74.3[14]	71.2[48]; 78.4[14]	84.8[48]; 90.1[14]	13.7[48]; 13.9[14]
Sweet potato cv. Norin	72.9[14]	78.0[14]	89.8[14]	14.1[14]
Sweetpotat Shirosatsuma	71.6[14]	76.6[14]	89.7[14]	13.2[14]
Tapioca	52.0-58.0[20]; 64.1[41]; 64.3[59]; 66.3[35]	68.3[59]; 69.0[41]	74.4[59]; 76.4[41]	14.7[59]; 15.1[35]
Takenokoimo (yam)	71.5[20]			
Tef	67.0[32]	73.5[32]	80.0[32]	
Water chestnut	58.7[59]; 62[44]	70.1[59]	82.8[59]	13.6[59]
Water yam	76.5[41]	78.8[41]	81.9[41]	
Wheat, commercial	51.0-58.5[20]; 54.9[58]; 57[46,59]	58.9[58]; 62[46,59]; 62.4-63.7[6,28]	63.8[58]; 66.2[59]; 67[46]	6.1-7.5[6]; 10.6[58,59]; 11.5[46]
Wheat, durum	50.7[57]	56.3[57]	62.7[57]	13.5[57]
Wheat, waxy	55.6[12]; 55.7[58]	61.4[58]; 63.5[12]	67.6[58]; 73.5[12]	10.3[12]; 13.6[58]
Wheat, wild	51.9[12]	60.8[12]	71.4[12]	9.2[12]
Wheat cv. Centura	55.6[58]	59.1[58]	63.1[58]	10.7[58]
Wheat cv. Chikugoizumi		62.1[10]		12.3[10]
Wheat cv. Condor	60[19]	66[19]	75[19]	11.3[19]
Wheat cv. Insignia	58[19]	63[19]	71[19]	11.3[19]
Wheat cv. Kanto 107	57.5[58]	62.1[58]	67.0[58]	11.8[58]
Wheat cv. Nishihonami		62.6[10]		12.3[10]
Wheat cv. Norin		59.4[10]		11.0[10]
Wheat cv. Rosella	61[19]	66[19]	74[19]	12.4[19]
Wheat cv. Saikai		60.1[10]		11.1[10]
White carrot	53.8[25]	60.1[25]	65.9[25]	17.6[25]
White yam	71.5[41]	74.8[41]	80.5[41]	
Yam	64.6[49]	70.9[49]	77.8[49]	13.3[49]
Yam bean		58.8-68.5[28]		9.1-14.2[28]
Yellow yam	69.4[41]	72.9[41]	76.7[41]	

1 = Li et al. (2001b), 2 = Ji et al. (2003), 3 = Seetharaman et al. (2001), 4 = Sodhi & Singh (2002), 5 = Beta & Corke (2001), 6 = Akashi et al. (1999), 7 = Bhattacharya et al. (1999), 8 = Kaur et al. (2002), 9 = Lai (2001), 10 = Noda et al. (2001), 11 = Yamin et al. (1999), 12 = Demeke et al. (1999), 13 = Wang et al. (1993a), 14 = Noda et al. (1993), 15 = Inouchi et al. (1993), 16 = Kuno et al. (2000), 17 = Hamanishi et al. (2000), 18 = Noda et al. (1998), 19 = Wootton et al. (1998), 20 = Suzuki (1993), 21 = Hoover et al. (2003), 22 = Waliszewski et al. (2003), 23 = Fiedorowicz & Rębilas (2002), 24 = Yoshida et al. (2003), 25 = Santacruz et al. (2003), 26 = Kiseleva et al. (2003), 27 = Lehmann et al. (2002), 28 = Forsyth et al. (2002), 29 = Li & Corke (1999), 30 = Hoover & Ratnayake (2002), 31 = Wang et al. (2002), 32 = Bultosa et al. (2002), 33 = Sugimoto et al. (1998a), 34 = Sugimoto et al. (1988), 35 = Ahmad et al. (1999), 36 = Akalu et al. (1998), 37 = Bello-Pérez et al. (2000), 38 = Betancur et al. (2001), 39 = Hoover et al. (1991), 40 = Davydova et al. (1995), 41 = Farhat et al. (1999), 42 = Gebre-Mariam & Schmidt (1998), 43 = Gebre-Mariam & Schmidt (1996), 44 = Hizukuri et al. (1988), 45 = Hoover et al. (1998), 46 = Hoover & Vasanthan (1992), 47 = Inouchi et al. (1984), 48 = Katayama et al. (2002), 49 = McPherson & Jane (1999), 50 = Maaurf et al. (2001), 51 = Pérez et al. (1998), 52 = Qian & Kuhn (1999a), 53 = Ratnayake et al. (2002), 54 = Song & Jane (2000), 55 = Spence & Jane (1999), 56 = Tester & Karkalas (1996), 57 = Vansteelandt & Delcour (1999), 58 = Yoo & Jane (2002), 59 = Jane et al. (1999), 60 = Hoover et al. (1993)

Starch Retrogradation

Changes that occur in gelatinized starch, from initially an amorphous to more ordered or crystalline state, are termed retrogradation. Changes occur because gelatinized starch is not in thermodynamic equilibrium. Rheological properties change during retrogradation such as increased firmness and rigidity. Loss of water-holding capacity and restoration of crystallinity increase on aging of gelatinized starch. These processes exert a major and usually unacceptable influence on texture of foods rich in starch. Water content together with storage temperature are very important because they control the rate and extent of retrogradation. Lipids and surfactants interfere with retrogradation process. Amylose gel crystallization reaches a limit after 2 days (Miles et al. 1985b), whereas amylopectin gels increase slowly in crystallinity with time approaching a limit after 30-40 days (Ring et al. 1987). Heating whole starch retrograded gels at 90°C reduced crystallinity by 70%, whereas amylose gel crystallinity was reduced by only 25% (Miles et al. 1985a) and amylopectin gels are fully reversible on heating (Ring 1985), indicating residual crystallinity of starch gels after heating is solely due to amylose fraction. Crystallinity of amylose fraction can be seen as an endothermic peak at 145-153°C (Eberstein et al. 1980, Sievert & Würsch 1993). Amylose reassociates very quickly after heating to 180°C, as an exothermic peak appears immediately after heating that is not observed for waxy starches (Sievert & Würsch 1993).

Gels with 75-90% amylose content resulted in high values for melting enthalpy (Gudmundsson & Eliasson 1990), resulting in possibility of cocrystallization in relation to retrogradation (Russell 1987). Schierbaum et al. (1986) found that linear segments of amylopectin and amylose can interact in solution.

Retrogradation is greatly affected by storage temperature. Starch gels with 45-50% water at low temperature, but above T_g, increases retrogradation compared to storage at room temperature. Storage at freezing temperatures virtually inhibits recrystallization (Colwell et al. 1969, Eliasson 1985). Higher temperatures (30-40°C) reduce retrogradation (Colwell et al. 1969). Crystallites formed from storage at low temperature (4-5°C) are less perfect (have lower T_c) than crystallites formed at higher storage temperature (Gidley 1985, Nakazawa et al. 1985).

A three-step mechanism for retrogradation has been proposed consisting of initial nucleation (junction point of two or more molecules), followed by crystal growth/propagation and crystal perfection (Slade & Levine 1987). Within the range T_g-T_c, both nucleation and propagation exhibit an exponential dependence on temperature. Nucleation rate increases with decreasing temperature down to T_g, while propagation rate increases with increasing temperature up to T_c. This explains why crystallization occurs at low temperatures but only a limited degree at temperatures above 30°C. For longer storage periods, retrogradation is maximal at a temperature midway between T_g and T_c, since nucleation and propagation are both at moderate rates. Both normal and waxy starch retrogradation follow the proposed three-step mechanism as retrogradation rate increased during 48 hours period with decreasing temperatures from 1-25°C, whereas amylose gels

stored at 6°C did not develop a staling endotherm (Biliaderis & Zawistowski 1990, Teo & Seow 1992, Wu & Eads 1993).

Retrogradation is sensitive to water content in starch gels. Crystallization during aging only occurs if starch concentration is between 10 and 80%, with maximum crystallization occurs at 50-55% starch concentration (Longton & LeGrys 1981, Eliasson 1983, Zeleznak & Hoseney 1986, Teo & Seow 1992). Retrogradation is only dependent on the amount of water present during aging and not on the amount of water present during gelatinization (Zeleznak & Hoseney 1986). Solutes, such as sugars, reduce retrogradation of starch gels due to their anti-plasticizing effect compared to water alone (Slade & Levine 1987), thereby reducing mobility of starch chains in the amorphous matrix by increasing T_g.

Retrogradation rate is greatly affected by botanical source of starch, which is not entirely due to differences in amylose contents (Orford et al. 1987, Kalichevsky et al. 1990, Gudmundsson & Eliasson 1991, 1992). There is some debate whether retrogradation rate increases with higher amylose content, especially since amylopectin is considered responsible for long-term retrogradation. The debate is largely due to observations that some waxy starches retrograde slowly, while pea and potato starches with high amylose content retrograde to a greater extent (Roulet et al. 1990, Chang & Liu 1991, Teo & Seow 1992). Starches from different botanical sources differ in their amylopectin branch chain-length distribution. For retrogradation to occur, minimum chain-length requirement for aggregation is 8-10 glucose units and chains with less than 15 glucose units have been shown to not participate in crystallization (Robin et al. 1974, Gidley & Bulpin 1987, Ring et al. 1987). Short amylopectin chains (6-9 glucose units) have been shown to inhibit or retard retrogradation (Krüsi von & Neukom 1984, Levine & Slade 1986). B-type starches, with

longer average branch chain-length distribution than A-type, on average have faster retrogradation rates than A-type starches such as nonmutant cereal starches and chain-length has been attributed to this difference (Suzuki et al. 1985, Orford et al. 1987, Kalichevsky et al. 1990).

Pasting Properties

Heating of starch granules in excess water results in granule swelling and granules are disrupted if shear is applied. Starch pasting properties are frequently measured by studying viscosity changes during a programmed heating and cooling cycle. Brabender Viscoamylograph and Rapid Visco-Analyser are the typical instruments used to measure starch pasting properties. Changes in viscosity of starches during cooking give an indication of the stability during processing, while changes occurring during cooling may give an indication of the consistency of the final product. Starch paste is a viscous mass consisting of a continuous phase of solubilized amylose and/or amylopectin and a discontinuous phase of granule remnants. Pasting of granules occurs because as temperature of starch-water suspension increases, molecules within granule vibrate and rotate violently causing intermolecular hydrogen bonds to break and are replaced with hydrogen bonds to water molecules, producing more extensive hydration. Starch molecules become sheathed in layers of water molecules that plasticize them and allow them to move more freely. Granule become fragile as swelling increases and are easily broken by stirring, resulting in a decrease in viscosity. By time peak viscosity is reached, some granules have ruptured and fragmented due to shear forces. With continued stirring, more granules disintegrate, causing greater decrease in viscosity. Clarity of starch suspensions improves as granules swell and fragment.

Breakdown refers to the drop in viscosity of the starch paste at the end of the cooking with reference to the peak viscosity. On cooling, some starch molecules begin to reassociate, forming a precipitate or gel and an increase in paste opacity. This change in viscosity after cooling is referred to setback and is also measured in reference to peak viscosity, based on increase in viscosity from that at breakdown.

Structure of starch is important in determining pasting properties. Low-amylose starches have been reported to have lower peak viscosity, breakdown and setback than starches with normal amylose contents (Noda et al. 2003). In contrast, reduced amylose content of durum wheat and rice has been reported to increase peak viscosity and breakdown (Sharma et al. 2002, Yasui et al. 2002). However, radiation-induced mutants of rice that had similar amylose contents to normal rice showed different pasting properties (Wu et al. 2002). Waxy cereal starches typically have higher peak viscosity than their normal counterparts (Jane et al. 1999, Abdel-Aal et al. 2002), but waxy potato has lower peak viscosity due to presence of phosphate monoesters (Jane et al. 1999). Starches with longer amylopectin branch chain-lengths have higher peak viscosity and lower pasting temperature (Franco et al. 2002). Pasting properties have been shown to vary within different kernels of the same plant, with chalky rice kernels having higher peak and breakdown viscosity, but lower pasting temperature, setback and final viscosity compared to translucent kernels (Patindol & Wang 2003).

Various other factors can influence starch pasting properties. Pasting properties of corn starch have been shown to be influenced by maturity with starch from immature corn having higher peak and trough viscosities, and lower pasting temperature (Jennings et al. 2002). Addition to starch of protein or free fatty acids alone inhibits formation of cooling

stage viscosity, but combination of all three results in prominent viscosity observed (Zhang & Hamaker 2003). Peak viscosity and breakdown decrease when starch is aged by storage at 37°C for 16 months (Zhou et al. 2003).

Tuber and root starches tend to have weaker intermolecular bonding and gelatinize easily to produce high-viscosity pastes that thin rapidly with moderate shear because their granules are highly swollen and break easily. Clarity of starch pastes differs greatly depending on botanical source. Craig et al. (1989) reported light transmittance of potato, tapioca, wheat, waxy corn, corn and high amylose corn to be 96, 73, 62, 61, 41 and 5% respectively. Increasing starch concentration results in increased paste clarity (Bello-Pérez & Paredes-López 1996). Starches that possess phosphate monoesters typically produce a very clear viscous paste because repulsion between electronegative groups prevents starch molecules from hydrogen bonding to each other, collapsing, and ultimately retrograding (Craig et al. 1989, Lim & Seib 1993a, Liu et al. 2000, Sitohy & Ramadan 2001). Thus, phosphate monoesters help keep starch molecules fully hydrated, promoting light transmittance and decreasing whiteness of starch pastes. Absence of amylose in potato starch has been shown to improve paste clarity (Visser et al. 1998). Addition of sugars greatly increases paste clarity of cereal starches (Craig et al. 1989). Addition of fatty acids decreases paste clarity, with unsaturated fats reducing clarity the most due to their greater intermolecular interactions with starch (Swinkels 1985a, Craig et al. 1989, Bello-Pérez et al. 1998). Modification of starch structure is regularly employed by industry to improve clarity of starch pastes (Rutenberg & Solarek 1984). Pasting properties of starches from various botanical sources is shown in Table 7.

Table 7. Pasting properties of starches from various botanical sources. Peak viscosity, final viscosity, breakdown and setback units are in Rapid Visco-Analyser Units (RVU) and pasting temperature units is °C.

Starch	Peak Viscosity	Final Viscosity	Breakdown	Setback	Pasting Temp.
Acorn	150[1]	196[1]	17[1]	63[1]	
Amaranth	139[19]	108[19]	73[19]	42[19]	71.7[19]
Banana	250[21]	272[21]	56[21]	78[21]	74.0[21]
Barley cv. Glacier	77[20]	106[20]		61[20]	90.8[20]
Barley highAM	5[20]	23[20]		24[20]	
Barl. hiAM hull-less	5[20]	16[20]		18[20]	
Barley cvWB.Merlin	221[20]	102[20]		32[20]	61.8[20]
Buckwheat	160[1]	109[1]	45[1]	75[1]	
Chinese taro	171[21]	161[21]	83[21]	73[21]	73.1[21]
Ginger	205[2]	166[2]			
Lotus root	307[21]	138[21]	223[21]	54[21]	67.4[21]
Maize, normal	313[16]	344[16]	129[16]	161[16]	74.1[16]; 82.0[21]
Maize, waxy	205[21]	100[21]	121[21]	16[21]	69.5[21]
Maize cv. A632	196[14]	281[14]	69[14]	154[14]	
Maize cv. A632 du	100[14]	77[14]	39[14]	17[14]	
Maize cv. A632 su2	13[14]	14[14]	0[14]	1[14]	
Maize *ae wx*	162[21]	190[21]	12[21]	40[21]	83.2[21]
Maize cv. B73	171[4]	213[4]	59[4]	100[4]	
Maize *du wx*	109[21]	99[21]	32[21]	22[21]	75.7[21]
Maize cv. Hz85	162[14]	220[14]	45[14]	102[14]	
Maize cv. Hz85 du	64[14]	41[14]	35[14]	12[14]	
Maize cv. Hz85 su2	26[14]	39[14]	0[14]	3[14]	
Maize cv. Oh43	129[14]	165[14]	40[14]	76[14]	
Maize cv. Oh43 du	128[14]	85[14]	71[14]	28[14]	
Maize cv. Oh43 su2	45[14]	48[14]	3[14]	6[14]	
Millet, cattail	201[21]	208[21]	121[21]	128[21]	74.2[21]
Mungbean	162[1]; 186[21]	203[1]; 363[21]	20[1]; 25[21]	62[1]; 202[21]	73.821
Potato	106[2]; 282-425[21]	238-274[2]; 116-161[21]	217-330[21]		62.7-66.4[21]
Quinoa	346[19]	416[19]	75[19]	145[19]	66.8[19]
Rice, commercial	232[9]; 113[21]	160[21]	17[21]; 87[9]	64[21]; 105[9]	79.9[21]
Rice, sweet	219[21]	128[21]	119[21]	28[21]	64.6[21]
Rice, waxy	205[21]	100[21]	121[21]	16[21]	64.1[21]
Rice cv. Cypress	240[15]	245[15]	112[15]	115[15]	
Rice cv. Dellrose	246[3]		130[3]	-15[3]	
Rice cv. Hokuriku	160[5]	141[5]	96[5]	77[5]	80.6[5]
Rice cv. IR5	96[9]		28[9]	53[9]	
Rice cv. IR28	143[9]		30[9]	106[9]	
Rice cv. IR32	128[9]		48[9]	62[9]	

Table 7. (continued)

Starch	Peak Viscosity	Final Viscosity	Breakdown	Setback	Pasting Temp.
Rice cv. IR36	269[3]		83[3]	69[3]	
Rice cv. Jasmine	260[3]		140[3]	-42[3]	
Rice cv. Jodon	137[3]		58[3]	36[3]	
Rice cv. Mars	257[3]		131[3]	-32[3]	
Rice cv MilkyQueen	203[5]	145[5]	85[5]	27[5]	94.6[5]
Rice cv. Nipponbare	166[5]	142[5]	96[5]	72[5]	85.7[5]
Rice cv. Rexmont	251[3]		97[3]	92[3]	
Rice cv. Snow Pearl	244[5]	122[5]	149[5]	27[5]	77.5[5]
Rice cv. Tainung	520[12]	474[12]	190[12]	244[12]	
Rice cv. T(N)1	616[12]	632[12]	306[12]	322[12]	
Rice cv. Toro	276[3]		158[3]	-14[3]	
Rice cv. Yumetoiro	118[5]	155[5]	21[5]	58[5]	93.3[5]
Sago					72.5-74[17]
Sorghum	50[2]	57[2]			
Sorghum cv. Kasvik.	314[8]		197[8]	130[8]	89.2[8]
Sorghum cv. Katand.	319[8]		203[8]	121[8]	84.9[8]
Sorghum cv. Mukad.	307[8]		204[8]	118[8]	89.2[8]
Sorghum cv. Mutode	339[8]		220[8]	123[8]	83.4[8]
Sweetpotato Kanto					52.6[18]
Sweetpotato Kogan.					70.7[18]
Sweetpotato Kyushu					73.6[18]
Tapioca	130[2]; 173[21]	107[21]; 126[2]	112[21]	46[21]	67.6[21]
Tef	269[16]	292[16]	79[16]	101[16]	74.0[16]
Water chestnut	61[21]	27[21]	45[21]	11[21]	74.3[21]
Wheat, commercial	267[7]	403[7]	43[7]	179[7]	88.6[21]
Wheat, A-granules	341[7]	435[7]	147[7]	241[7]	
Wheat, B-granules	193[7]	295[7]	63[7]	165[7]	
Wheat cv Chikugoiz.	330[13]		53[13]	116[13]	68.9[13]
Wheat cv. Condor	103[6]	167[6]	8[6]	71[6]	
Wheat cv. Fillmore	422[11]	355[11]	216[11]	149[11]	
Wheat cv. Freedom	396[11]	406[11]	175[11]	185[11]	
Wheat cv. Geneva	394[11]	433[11]	181[11]	220[11]	
Wheat cv. Insignia	230[6]	281[6]	63[6]	113[6]	
Wheat cv. Meering	198[10]	273[10]	21[10]	78[10]	
Wheat cv Nishihona.	338[13]		55[13]	139[13]	79.9[13]
Wheat cv. Norin	307[13]		61[13]	122[13]	82.8[13]
Wheat cv. Pioneer	421[11]	445[11]	173[11]	197[11]	
Wheat cv. Rosella	197[6]; 278[10]	279[6]; 321[10]	30[6]; 50[10]	90[10]; 112[6]	
Wheat cv. Saikai	326[13]		52[13]	124[13]	80.8[13]

1 = Cho & Kim (2000), 2 = Blennow et al. (2001), 3 = Shu et al. (1998), 4 = Yamin et al. (1999), 5 = Kuno et al. (2000), 6 = Wootton et al. (1998), 7 = Shinde et al. (2003), 8 = Beta & Corke (2001), 9 = Bhattacharya et al. (1999), 10 = Black et al. (2000), 11 = Gaines et al. (2000), 12 = Lai (2001), 13 = Noda et al. (2001), 14 = Li & Corke (1999), 15 = Wang et al. (2002), 16 = Bultosa et al. (2002), 17 = Ahmad et al. (1999), 18 = Katayama et al. (2002), 19 = Qian & Kuhn (1999a), 20 = Song & Jane (2000), 21 = Jane et al. (1999)

Fruit Starches

Numerous types of fruit accumulate starch during development. However, because few fruit have high levels of starch when consumed, characterization of starches in fruit has received little attention. Of all fruit starches, banana starch has received the most attention. Scanning electron micrographs have shown banana starch granules are long, large, irregularly-shaped, smooth granules that have some resemblance to banana fruit shape (Fuwa et al. 1979, Jane et al. 1994). Green banana starch exhibits C-type X-ray diffraction pattern, has onset gelatinization temperature of 68.6°C, narrow gelatinization temperature range of 7.5°C, enthalpy change of gelatinization of 17.2 J/g, pasting temperature of 74°C, peak viscosity of 250 rapid viscoanlyser units (RVU), final viscosity of 272 RVU, setback of 78 RVU and average amylopectin branch chain-length of DP 26.4 (Jane et al. 1997, 1999).

Kiwifruit starch was reported to have mixture of polyhedral and dome-shaped granules that were predominantly 6-8 μm (Sugimoto et al. 1988). Kiwifruit starch exhibited B-type X-ray diffraction pattern, apparent amylose content of 15-18% and onset gelatinization temperature of 62-63°C. Apple starch characteristics have been researched, but carbohydrate analytical techniques have advanced considerably since (Potter et al. 1949). Apple starch was reported to have 25-27% apparent amylose content. Degradation of apple amylose with β-amylase yielded 90% maltose, whereas amylopectin hydrolysis ceased when 63.5% was degraded to maltose. Average apple amylose and amylopectin molecule were determined to consist of 560 and 4,200 glucose residues, respectively, corresponding to an amylopectin molecular weight of 1.2×10^6. Average apple amylopectin branch chain-length was determined to be DP 24.

Squash Fruit Starches

In the last 25 years, just three studies, the first ever, have emerged characterizing starch from fruit of any squash, pumpkin, melon, gourd, cucumber or zucchini from the Cucurbitaceae plant family. One study characterized starch from *Cucurbita* squash cultivars grown in Moldavia (Kakhana & Ludnikova 1981). Their study found Moldavia Spanish squash cultivar accumulated 12% of its fresh weight as starch. Moldavia Spanish cultivar starch granules were reported to be spherical and not compound, with a diameter ranging from 7 to 20 μm, and half of the granules with diameters 9 to 12 μm. Apparent amylose content was 21% and ashed starch was reported to contain 0.085% phosphorus. During 3 hour degradation at 35°C, one gram of squash starch was hydrolyzed to 383 mg and 213 mg of glucose by α- and β-amylase, respectively.

Two studies reported starch characteristics of two winter squash cultivars, a buttercup squash Ebisu, and a butternut squash, Kogiku, during development up to horticultural maturity (Sugimoto et al. 1998a), and the starch characteristics of Kogiku during storage (Sugimoto et al. 1998b). Average starch granular size of Ebisu and Kogiku was 6.5 to 8.0 μm at harvest for both cultivars. X-ray diffraction studies showed both squash starches had B-type pattern. Apparent amylose content of Ebisu and Kogiku at harvest was 21-22% and 17%, respectively. Onset gelatinization temperature of Ebisu and Kogiku starches from fruit at harvest was 62.1°C and 62.6°C, respectively, and enthalpy change of gelatinization was about 14 J/g for both. Peak viscosity, breakdown and final viscosity, in Brabender units, was 810, 460 and 590 respectively for Ebisu starch at harvest, and 735, 445 and 495, respectively, for Kogiku starch at harvest. Storage of Kogiku fruit for 1-2 months at room temperature

resulted in greater decrease in starch content than storage at 5°C. Amylose contents of the starches increased during storage. Starch granules suffered enzymic attack during fruit storage and extracted starch was more susceptible to hog pancreatin than starches extracted from fruit during development.

FOOD TEXTURE MEASUREMENTS

Texture Profile Analysis

Texture Profile Analysis (TPA) is an imitative test utilized by many food technologists because of its ability to provide standardized tests which are difficult to achieve when using human subjects. TPA was developed at General Foods in 1960s (Szczesniak et al. 1963). The first TPA that did not involve sensory panelists used a force-deformation instrument called the General Foods Texturometer (Rosenthal 1999). In the present day, an Instron Universal Testing Machine or TAXT2 texture analyser is commonly employed. Szczesniak et al. (1963) established a variety of textural terms using sensory panels and later a two-cycle compression test was developed, attempting to simulate the textural attributes perceived by human subjects. A diagram representing the parameters that are measured instrumentally by TPA is shown in Fig. 6 (Szczesniak et al. 1963). Hardness is calculated from the height of the "first bite", or compression curve (A_1). Fracturability (brittleness) is the force that material fractures significantly on first bite. Springiness (elasticity) is defined as the distance that the food recovered its height during the time that elapsed between the end of the first bite and the start of the second bite (BC). The ratio of the positive force areas under the first and second compression (A_2/A_1) is defined as cohesiveness. Adhesiveness is defined as the negative force area of the first bite (A_3), representing the work required to pull

compression probe away from sample. Gumminess and chewiness are derived by calculating the measured textural parameters. Gumminess is the product of hardness x cohesiveness, and chewiness is the product of gumminess x springiness.

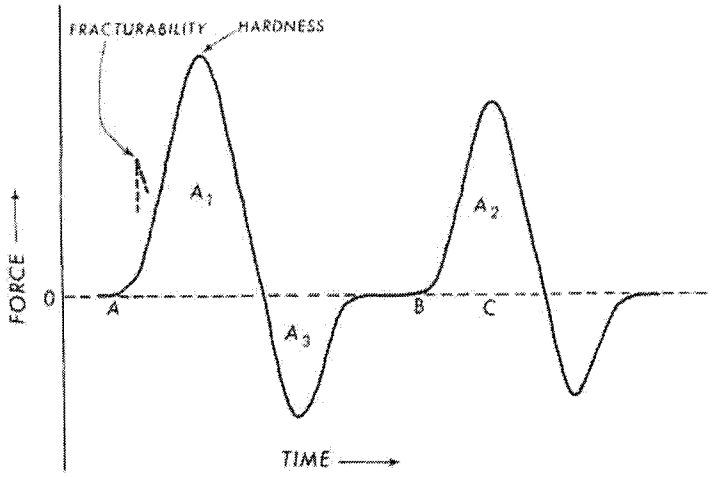


Figure 6. A typical Texture Profile Analysis curve using the Instron Universal Testing Machine.

Texture of Squash Fruit

Texture of squash fruit has been investigated utilizing sensory panels. Sensory attributes of three buttercup cultivars grown in New Zealand were investigated, with a development hybrid, rated by sensory panelists to have more desirable dry, mealy texture than the cultivars Delica and Kaboten (Hurst et al. 1995). Tropical pumpkin fruit flesh was found to range in texture from fine, pasty, smooth, silky mouth feel to watery, fibrous and stringy (Daniel et al. 1995). Non-buttercup squash had more acceptable texture than buttercup squash soon after harvest, but as storage progressed, buttercup squash texture was rated more favorably by sensory panelists (Merrow & Hopp 1961). The most thorough textural analysis of squash utilizing a sensory panel was carried out by Corrigan et al. (2001) and Cumarasamy et al. (2002). In their studies, high-starch buttercup squash cultivars were

found to have higher brittleness and cohesiveness than low-starch squash cultivars. High-starch cultivars had significantly smaller particle size mouth feel, and were significantly drier, crumblier, harder, more gummy, more adhesive and had fewer fibers compared to low-starch cultivars.

In addition to sensory panels, Instron and other instrumental compression tests have been used to determine mechanical properties of squash fruit. These data have been related to textural attributes perceived by human senses. The first study to implement use of the Instron Universal Testing Machine studied 'Dixie" hybrid summer squash and fruit hardness, brittleness, gumminess, chewiness, cohesiveness and elasticity all increased during storage, although storage was only a maximum of nine days (Smittle et al. 1980). Firmness of raw 'Delica' buttercup squash at harvest was reported to be 70-80 N by two studies (Harvey et al. 1997, Ratnayake et al. 1999). Firmness of uncooked and cooked buttercup squash showed similar patterns of change over storage with decreasing fracturability, hardness and gumminess, and similar springiness, chewiness and cohesiveness during storage (Ratnayake et al. 1999). Cooked buttercup squash has been reported to exhibit an abrupt failure of fruit samples when compression is applied (Corrigan et al. 2001). Differences were observed between squash cultivars for hardness, springiness, cohesiveness and gumminess of cooked fruit (Corrigan et al. 2001, Cumarasamy et al. 2002).

Role of Starch in Texture of Squash Fruit

Differences observed in textural attributes of raw and cooked squash fruit had led researchers to investigate the determinants of texture. The earliest study to propose that starch may play a role in texture of squash observed total solids and acid-hydrolyzable polysaccharides were

higher in cooked squash that was more viscous and had drier texture (Culpepper & Moon 1945). Study of six winter squash cultivars during storage found no distinction between cultivars until 10 weeks storage, where textural attributes varied due to differences in sugar to starch ratio (Merrow & Hopp 1961). In a separate study, a sensory panel's preferences for cooked squash were positively correlated to starch ($r = 0.94$) and negatively correlated to pectin, hemicellulose and cellulose content (Smittle et al. 1980). Positive correlations were observed between starch content and smoothness or pastiness of cooked tropical pumpkin fruit (Daniel et al. 1995). Squash with significantly higher starch content have been reported to have significantly drier texture (Hurst et al. 1995).

In most recent studies, significant correlations were observed between starch content of squash fruit, and all textural attributes rated by sensory panelists (Corrigan et al. 2001, Cumarasamy et al. 2002). Mouthfeel-type correlations, such as adhesiveness, particle size, mouthfeel, moistness and fibrousness, were higher than correlations involving mechanical parameters such as hardness, brittleness and cohesiveness. Similar to a study by Hurst et al. (1995), starch content was negatively correlated to the sensory parameter, moistness of squash fruit which has previously been proposed to be caused by partially hydrolyzed starch molecules (Szczesniak & Ilker 1988).

Squash Texture and Cell Wall Polysaccharides

Only two studies have investigated the role of cell wall polysaccharides in the texture of raw and cooked squash fruit. In the first study, sensory panelists squash texture preferences were negatively correlated to water-soluble pectin, Calgon-soluble pectin (which indicate low-methoxyl pectinates), hemicellulose and cellulose, but were positively correlated to

protopectin content (Smittle et al. 1980). In the second study, cell walls of buttercup squash were found to be composed of 34% pectin, 26% hemicelluloses, and 39% cellulose on a dry weight basis (Ratnayake et al. 1999). Unfortunately subsequent studies opted to publish research on squash textural attributes and cell wall polysaccharides in separate papers with no attempt made to correlate the two factors (Ratnayake 2000, Ratnayake et al. 2003). However, one can educe from these studies that total cell wall yields were found to be unaltered after 2 months storage, but then decrease for some squash cultivars after 3 months storage. Textural analysis showed parallel changes in failure force, failure strain and deformation of raw squash fruit to that observed for cell wall yields. However not all textural attributes correlated well with changes in cell wall yields as some cultivars showed significant decreases in modulus of deformability and failure stress at early storage times when no changes in cell wall yields were observed.

Starch Content and Texture

Endogenous starch content has been implicated in the texture of many foods. Starch content has been reported to contribute to both harder cooked grain texture (Lee et al. 2001) and softer cooked plantain texture (Qi et al. 2000). Stickiness and gumminess of potatoes was found to be related to the amount of starch that exudes from ruptured cells (Reeve 1977).

Mealiness textural attribute has often been linked to starch content and the behavior of starch within cells. Non-cooked potatoes have been observed to fracture through cells leaving starch granules intact, whereas cooked potatoes fracture between cells preferentially alongside the cell walls (Marle van et al. 1992). Firm-cooking potato cultivars had fracture planes with a generally flat appearance and large intercellular contacts were visible. Mealy

potato cultivars have rougher fracture planes and intercellular contacts were small (Marle van et al. 1992). Mealiness of potatoes has been linked to higher starch content (Sweetman 1936, Wright et al. 1936, Haddock & Blood 1939, Clark et al. 1940, Smith & Nash 1940, Kirkpatrick 1953, Bettelheim & Sterling 1955, Shewfelt et al. 1955, Unrau & Nylund 1957, Reeve 1977, Sterling & Aldridge 1977, Marle van et al. 1992, McComber et al. 1994). Mealy potatoes form a starch gel, which will be rigid if the starch concentration exceeds 30% (Ring 1985), resulting in round cells with more turgid appearance due to high starch swelling pressure (Jarvis et al. 1992) and more resistance to forces exerted during fracturing (Marle van et al. 1992). Differences in behavior of various starches during heating have been proposed to be more significant to the texture of cooked potatoes than differences in the amounts of starch (Mica & Brod 1985, McComber et al. 1994). Cooked potatoes with higher mealiness scores have lower compression forces and greater amount of starch leached (Kaur et al. 2002).

Mealy potatoes have been reported to have cells engorged with sponge-like gelatinized starch and little void space between cell wall and gelatinized starch (Marle van et al. 1992). However, other studies found all cultivars of potatoes have cells not completely engorged with gelatinized starch (Moledina et al. 1978, Huang et al. 1990) and it was speculated that microscopy techniques resulted in shrinkage of gelatinized starch. The lack of complete cell engorgement of gelatinized starch was also observed by McComber et al. (1994) who found striking differences in the amount of gelatinized starch filling the cells of cooked potatoes from different cultivars. Mealy potatoes were completely engorged with gelatinized starch whereas waxy potatoes only have 30-50% of cells filled. Gelatinized starch filling each mealy cell better retains water, providing the dry characteristic. Steamed

sweet potatoes produce a coarser structure of gelatinized starch than when boiled (Valetudie et al. 1999).

Moisture content has been found to be critical in influencing the role of starch in texture. In corn, a minimum moisture content of 35% was found to be necessary for gelatinization of starch (Cabrera et al. 1984) and high-moisture vegetables, such as taro with 87% water, had no lag phase for onset of gelatinization (Njintang & Mbofung 2003).

Protein content can interact with starch to influence texture. Softer-textured wheats were found to have larger starch granules than harder-textured wheats (Black et al. 2000). Smaller granules had larger surface area available for noncovalent bonding with the endosperm protein matrix and may also pack more efficiently, producing harder endosperm (Glenn et al. 1992, Bechtel et al. 1993, Zayas et al. 1994, Bechtel & Wilson 1997, Gaines et al. 2000). Therefore, surface properties of starch granules might be more important than components within granule in determining grain hardness. Additionally, starch granule surface has been found to contribute to elastic and sticky texture of pasta (Cunin et al. 1995). More protein in grain has been hypothesized to form a thicker barrier around starch granules, thus slowing water uptake (Juliano et al. 1965, Chakrabarthy et al. 1972, Yanase et al. 1984), resulting in slower gelatinization and retarding swelling of granules, thus leading to more water lost as steam during cooking. Two friabilin components, puroindoline a and b have been found to affect wheat grain texture with the former contributing to soft texture and the latter contributing to harder texture (Corona et al. 2001). Absence of granule-bound starch synthase protein of wheat produces udon noodles of superior soft-textured quality (Briney et al. 1998).

Amylose and Texture

Amylose content has been reported to contribute to hard texture of rice (Kurasawa et al. 1969, Manohar Kumar et al. 1976, Lorenz et al. 1978, Perez & Juliano 1979, Bhattacharya et al. 1982, Desphande & Bhattacharya 1982, Sandhya Rani & Bhattacharya 1985, Sowbhagya et al. 1987, Sandhya Rani & Bhattacharya 1989a, Ong & Blanshard 1995a, 1995b, Kohyama et al. 1998, Takahashi et al. 1998, Bhattacharya et al. 1999, Champagne et al. 1999, Ramesh et al. 1999a, Qi et al. 2000, Ramesh et al. 2000, Takahashi et al. 2000), rice-based fries (Kadan et al. 1997), wheat (Black et al. 2000, Noda et al. 2001) and wheat gels (Gaines et al. 2000). However, there have been a few studies that report softer texture with increasing amylose content in rice (Sowbhagya et al. 1991, Lai 2001) and kernels of wheat (Gaines et al. 2000). A mechanism for how amylose contributes hardness to foods was shown by Mestres et al. (1988), who showed for rice that amylose crystallites, when gluten was absent, helped create a continuous network by strongly linking to one another by junction zones. However, this finding does not explain how amylose contributes to hardness of wheat grains observed for some studies (Black et al. 2000, Noda et al. 2001). Hardness measured by Instron, but not by sensory panels, was found to be positively correlated to molecular weight of amylose (Ong & Blanshard 1995b).

Stickiness of foods has often been linked to low amylose content (Bhattacharya & Sowbhagya 1979, Bhattacharya et al. 1982, Sandhya Rani & Bhattacharya 1985, Unnikrishnan & Bhattacharya 1987, Sowbhagya et al. 1991, Windham et al. 1997, Perdon et al. 1999, Rousset et al. 1999, Takahashi et al. 2000). However, other studies found stickiness was associated with higher amylose contents (Sowbhagya et al. 1987, Ong & Blanshard

1995a, Kohyama et al. 1998). Stickiness of cooked rice can be enhanced by protease removal of albumins and globulins from grain (Watanabe 1993).

Rice with higher amylose content has been reported to have greater chewiness (Bhattacharya et al. 1999, Kang & Han 2001). Masticatory behavior of rice with different amylose contents was evaluated using electromyography of masticatory muscles (Kohyama et al. 1998). Masticatory behavior was more related to adhesiveness and stickiness of rice than to the hardness. Number of chews and masticatory time, total duration of mastication and total muscle activities were greater in cooked rice with a high amylose content, which showed low adhesiveness and stickiness. Cooked rice with a high amylose content was masticated with high masseter muscle activities. Ratio of jaw-opening muscle activity to the preceding jaw-closing muscle activity was low in high-amylose varieties, which corresponded to the ratio of stickiness to hardness using texturometer.

Gumminess has also been associated with amylose content. High gumminess was correlated with high amylose content (Kadan et al. 1997, Bhattacharya et al. 1999), while another study reported the opposite relationship (Lai 2001). Elasticity in foods has been shown to be negatively correlated to amylose content (Sowbhagya et al. 1987, Akashi et al. 1999, Noda et al. 2001). Other textural parameters positively correlated to amylose content include adhesiveness (Windham et al. 1997, Takahashi et al. 2000), flakiness (Bhattacharya et al. 1982), tensile strength (Bhattacharya et al. 1999) and cohesiveness of mass (Champagne et al. 1999), and negatively correlated to smoothness (Noda et al. 2001).

A correlation has been established between intercellular adhesion and amylose content (Linehan & Hughes 1969). During cooking, amylose leaches out through weakened cell walls and acts as a cementing material between the cell walls, leading to increased

intercellular adhesion. A positive correlation between hardness or stickiness of rice and the amount of leached amylose has been observed (Ong & Blanshard 1995b).

Amylopectin and Texture

During the last 25 years, hot-water insoluble amylose content has been propounded as the key determinant of varietal differences in the texture of cooked rice (Bhattacharya & Sowbhagya 1979, Sandhya Rani & Bhattacharya 1985, Sowbhagya et al. 1987, Unnikrishnan & Bhattacharya 1995), only to be later discovered to in fact be long-chain amylopectins rather than amylose (Ramesh et al. 1999a). Soluble amylose content showed no relationship to rice texture, indicating amylose plays little role in texture (Reddy et al. 1993). Long-chain amylopectin has been reported to be correlated to hardness (Reddy et al. 1993, Takahashi 1993, Ong & Blanshard 1995a, 1995b, Ramesh et al. 1999a, Takahashi et al. 2000). Specifically, Ong & Blanshard (1995a, 1995b) report hardness is positively correlated to the proportion of amylopectin branch chain-lengths of DP 92-98 and negatively correlated to DP 10-11, 18 and 22-25. Hizukuri et al. (1989) had previously reported viscosity of rice was affected especially by relative proportion of longest B-chain amylopectin branches (DP 70-90), and shortest A-chains (DP 15-17), despite the former chains being present in small concentrations. The longest amylopectin chains (DP 92-98) were not found in leached starches, and could interact with other components in rice, with the resultant complexes being retained in cooked grain and inhibit softening (Ong & Blanshard 1995b).

Rheological and microscopic studies (Sandhya Rani & Bhattacharya 1985, 1989a, 1995) and studies on viscoelastic properties of rice (Reddy et al. 1994) have provided some indication of how amylopectin structure affects hardness of rice. High long-chain

amylopectin rice is rigid with elastic properties and strong starch granules which resist swelling as well as disintegration when heated under shear (Reddy et al. 1993). Short-chain amylopectin has weak, deformable and fragile starch granules that swell and tend to breakdown easily under heat and shear. The proportion and relative external disposition of long B-chains of rice amylopectin was related positively to both strength of granule and hardness of cooked rice. Rice texture has been correlated not only to long amylopectin B-chain content, but also to long chains of entire starch, therefore proposing that amylose participated in intermolecular interactions affecting rigidity of granule and indirectly the texture of rice (Takeda et al. 1989b, Ramesh et al. 1999a). Since amylopectin component is the primary contributor to starch crystallinity (Hizukuri et al. 1983, French 1984, Hizukuri 1985, Eliasson et al. 1987, Zobel 1988), a greater content and more external disposition of long B-chains of long-chain amylopectin could make starch granules strong and rigid through intermolecular interactions (Reddy et al 1993). Paucity of long and external chains in short-chain amylopectin would be expected to render its starch granules weak and fragile due to a low degree of intermolecular interlocking. Rigid and strong starch granules in turn would be expected to render cooked rice hard, non-sticky and dry, because starch would not leach out of intact granules. Conversely, fragile granules would tend to disintegrate on cooking, rendering cooked rice soft, sticky and moist.

Wheat starch digested by α-amylase produced good textured noodles when a small proportion of oligosaccharides with DP 5 or greater were present, while wheat starch producing poor textured noodles had much greater amounts of these larger oligosaccharides (Batey et al. 1997). Because oligosaccharides of greater than DP 4 have at least one branch

point, α-amylase digestion studies indicate an amylopectin structure with relatively few branch points close together provides good noodle texture.

Stickiness has been both positively (Ong & Blanshard 1995a) and negatively (Takeda et al. 1989b, Takahashi et al. 2000) correlated with long-chain amylopectin branches, positively correlated to intermediate branch chain-lengths (DP 26-30) (Aboubacar & Hamaker 2000), negatively correlated to short-chain amylopectin branches (Takahashi et al. 2000), and positively correlated to ratio of short to long amylopectin branch chains (Takahashi et al. 2000).

Starch Thermal Properties and Texture

Higher onset gelatinization temperature (T_o), which can often result in a low degree of starch gelatinization in cooked grains, has been reported to maintain hardness (McComber et al. 1988, Arai & Watanabe 1994, Zeng et al. 1997). Shear stress of cooked sweet potato has also been correlated to T_o (Walter et al. 2000). In potatoes, mealy texture has been shown to result from lower gelatinization temperatures, higher gelatinization temperature range, higher enthalpy change of gelatinization (ΔH) and higher retrogradation rate (McComber et al. 1988, McComber et al. 1994, Kaur et al. 2002). Peak gelatinization temperature has been reported to be positively correlated to softness of white-salted wheat noodles and elasticity of sorghum noodles (Beta & Corke 2001, Noda et al. 2001).

Hardness of rice was found to be positively correlated to ΔH (Ong & Blanshard 1995b), and white-salted noodle hardness was positively correlated to ΔH of amylose-lipid complex (Noda et al. 2001). Decreased elasticity and smoothness of the noodles was also reported for higher ΔH of amylose-lipid complex. Starch retrogradation has been shown to

be correlated to firmness of rice, but its relationship with stickiness was dependent on cultivar and storage temperature (Perdon et al. 1999). Sticky rice has been observed to have a well-developed network of gelatinized starch covering the surface of every grain (Matsuda et al. 1989). There has been some speculation that the retrogradation behavior of starch within whole plant tissue may differ from the purified form (McComber et al. 1994), but recently retrogradation of rice starch was observed to be similar in both purified and cooked grain form (Yao et al. 2002).

Starch Pasting Properties and Texture

Hardness has been correlated positively to setback (Yun et al. 1997, Limpisut & Jindal 2002), final viscosity (Yun et al. 1997, Noda et al. 2001, Limpisut & Jindal 2002), pasting temperature (Ong & Blanshard 1995b, Collado & Corke 1997, Limpisut & Jindal 2002) and negatively correlated to peak viscosity (Moss 1980, Endo et al. 1988, Ebata & Hirasawa 1989, Crosbie 1991, Crosbie et al. 1992, Baik et al. 1994, Limpisut & Jindal 2002) and breakdown (Oda et al. 1980, Baik et al. 1994, Sandhya Rani & Bhattacharya 1995, Champagne et al. 1999, Ramesh et al. 1999b, Limpisut & Jindal 2002). Inverse relationship between peak viscosity and hardness may be due to amylose-lipid complex, which has been shown to harden grain surface and lower viscosity (Yamada et al. 1995). Springiness has been reported to be correlated positively to peak viscosity (Akashi et al. 1999, Beta & Corke 2001, Noda et al. 2001), final viscosity (Yun et al. 1997, Beta & Corke 2001, Limpisut & Jindal 2002), setback (Ong & Blanshard 1995b, Yun et al. 1997, Limpisut & Jindal 2002) and correlated negatively to breakdown (Limpisut & Jindal 2002, Bhattacharya et al. 1999). Adhesiveness was reported to be negatively related to pasting temperature, setback and final

viscosity, and positively related to peak viscosity and breakdown, with the complete opposite relationships reported for cohesiveness (Limpisut & Jindal 2002), although Collado & Corke (1997) did report a positive relationship between pasting temperature and adhesiveness. Cohesiveness was correlated positively to pasting temperature, final viscosity and setback (Champagne et al. 1999, Limpisut & Jindal 2002) and negatively correlated to breakdown (Limpisut & Jindal 2002). Other textural parameters reported to be related to starch pasting properties are smoothness (Noda et al. 2001), stickiness (Juliano et al. 1964, El-Saied et al. 1979), slickness and cohesiveness of mass (Champagne et al. 1999).

Breakdown of starch paste is considered a fundamental attribute of rice textural quality. Starch granules of high-amylose rice are more rigid and better reinforced so they resist disintegration and hence result in a stable paste, while granules of low-amylose rice are more fragile and susceptible to disintegration, resulting in an unstable paste (Sandhya Rani & Bhattacharya 1995). Therefore, the amount and structure of amylose may be responsible for granule strength, and granular rigidity/fragility may be the root cause of differences in eating quality of rice.

Amylose Effect on Pasting Properties

Peak viscosity and breakdown of starch pastes has been reported to be negatively correlated to amylose content by many researchers (Sandhya Rani & Bhattacharya 1989b, Kusutani et al. 1992, Wang et al. 1993b, Reddy et al. 1994, Sandhya Rani & Bhattacharya 1995, Kitahara et al. 1996, Matsue 1996, Collado & Corke 1997, Yoshii et al. 1997, Zeng et al. 1997, Ogata & Matsue 1998, Bhattacharya et al. 1999, Jane et al. 1999, Aboubacar & Hamaker 2000, Araki et al. 2000, Collado et al. 2000, Kuno et al. 2000, Song & Jane 2000,

Aslam-Sagar et al. 2001, Beta & Corke 2001, Blennow et al. 2001, Li et al. 2001). However, Sandhya Rani & Bhattacharya (1985) and Taylor et al. (1997) reported higher peak viscosity with higher amylose content. The explanation for this discrepancy is that low-amylose rice starch heated at 60°C, 75°C, and 95°C with less than 7% starch concentration, has higher paste viscosity than high-amylose rice but at 95°C and greater than 7% starch concentration, high-amylose rice has higher paste viscosity (Sandhya Rani & Bhattacharya 1989b). This also explains why A-type wheat starch granules that have higher amylose content (Kulp 1973, Meredith 1981, Soulaka & Morrison 1985, Peng et al. 1999) are positively correlated to pasting attributes (Shinde et al. 2003). Additionally, amylose chain-length could explain discrepancies since peak viscosity has been reported to be positively correlated to amylose chain-length (Shibanuma et al. 1996). Pasting temperature has been reported to be positively correlated (Li et al. 2001, Noda et al. 2001) and negatively correlated (Seetharaman et al. 2001) to amylose content. Setback and final viscosity are positively correlated to amylose content (Taylor et al. 1997, Sasaki et al. 2000).

Amylose Effect on Thermal Properties

Several researchers have previously reported higher apparent amylose content to be correlated to lower T_o (Inouchi et al 1993, Visser et al. 1997, Demeke et al. 1999). However, other researchers have reported a positive relationship between T_o and apparent amylose content (Asaoka et al. 1994, Wang & White 1994b) and many report no relationship between apparent amylose content and all gelatinization temperatures and enthalpy changes (Noda et al. 1993, Kim et al. 1995, Moorthy et al. 1996, Mun et al. 1998, Noda et al. 1998, Wootton et al. 1998, Nakamura et al. 2002). Since apparent amylose measurement includes iodine

affinity of amylopectin fraction, long-chain amylopectins may play a critical role in thermal properties of starches and explain the discrepancies found in literature for the influence of amylose content on starch thermal properties. A negative correlation has been reported between ΔH and apparent amylose content (Kosson et al. 1994, Fujita et al. 1996, Czuchajowska et al. 1998, 1999, Sasaki et al 2000), but a positive relationship between ΔH of amylose-lipid complex and apparent amylose (Villwock et al. 1999).

Amylopectin Effect on Pasting Properties

Short amylopectin branch chain-lengths proportion (DP 6-12) of wheat starch has been shown to be positively correlated to setback and pasting temperature (Noda et al. 2001), while long amylopectin branch chains have been positively correlated to peak viscosity (Shibanuma et al. 1996). However, for hull-less barley starch, no relationship was observed for short-chain amylopectins, but intermediate amylopectin branch chain-lengths (DP 18-34) were positively correlated to peak viscosity and breakdown (Li et al. 2001). Long chains of rice amylopectin have been reported to be negatively correlated to breakdown, while short amylopectin chains were positively correlated (Han & Hamaker 2001). Frequency ratio of short A-chains to intermediate glucose chains (B-chains), and the ratio of B-chains to long C-chains, and the relative frequencies of A- or B-chains were found to be closely associated with breakdown and setback of rice starch pastes (Choi et al. 1999). Corn starches with short amylopectin branch chain-lengths were reported to have high pasting temperature, and low peak viscosity and breakdown (Mua & Jackson 1997).

Amylopectin Effect on Thermal Properties

Proportion of short amylopectin branch chain-lengths (DP 6-12) has been shown to be negatively correlated to T_p, ΔH and ΔH of amylose-lipid complex (Noda et al. 2001). However, another study has shown short amylopectin branch chains (DP 5-17) are positively correlated to T_o and negatively correlated to range of gelatinization temperature, and vice versa for long amylopectin branch chain-lengths (DP $\geq$ 35) and the average amylopectin branch chain-length (Li et al. 2001). Intermediate amylopectin branch chain-lengths (DP 18-34) are positively correlated to T_p and T_c. Ji et al. (2003) reports corn starches with a lower onset gelatinization temperature had lower degree of intermediate amylopectin branch chain-lengths (DP 15-24) and a higher degree of short amylopectin branch chain-lengths (DP 6-12). Increasing ΔH with increasing amylopectin chain-length has also been observed (Tang et al. 2001). Jane et al. (1999) studied starches from 21 different botanical sources, finding starches with short average amylopectin branch chain-lengths and high proportion of chain-lengths DP 6-12, or amylopectins with high content of phosphate monoester groups, had lower gelatinization temperatures.

Relationship Between Starch Thermal and Pasting Properties

Negative correlations between starch thermal and pasting properties far outweigh any positive correlations observed for rice and wheat starches. Setback has been reported to be negatively correlated to T_p (Noda et al. 2001) T_c and ΔH (Bhattacharya et al. 1999). Peak time was negatively correlated to T_o, T_p, T_c and ΔH (Bhattacharya et al. 1999). Negative correlations have also been observed between pasting temperature and T_p, and also between ΔH and peak viscosity (Noda et al. 2001). The only positive relationship observed is higher

pasting temperature of wheat starch with higher ΔH of amylose-lipid complex (Noda et al. 2001).

Studies of physicochemical properties of hull-less barley found positive relationships between starch thermal and pasting properties (Li et al. 2001). T_p and T_c were both positively correlated to peak viscosity and breakdown. Studies of corn starch also found predominantly more positive relationships between thermal and pasting properties. T_o of native and retrograded starch was positively correlated to peak viscosity, final viscosity and setback, while pasting temperature was negatively correlated to native T_o and range of retrograded gelatinization temperature (Seetharaman et al. 2001).

ULTRASOUND AS TOOL FOR TEXTURE MEASUREMENTS

Ultrasound in food science

In the last two decades, ultrasound has emerged as a promising new technology that will enable fresh produce wholesalers and food processors to nondestructively gain information about the composition of food products. Recently, potential applications of ultrasound in food science have been demonstrated including rheological determination of tomato concentrates (Dogan et al. 2003), extraction of compounds from food for liquid chromatography with elimination of solvent extraction (Furusawa 2003, Luo et al. 2003), inactivation of *Salmonella* (Alvarez et al. 2003), fat content of meat (Youssao et al. 2003), fat content of chocolate (Saggin & Coupland 2002), rapid freezing of potatoes (Li & Sun 2002), accelerated aging of wine (Chang & Chen 2003), texture of cheese (Cho et al. 2003) and detection of glass or metal in bottled beverages (Zhao et al. 2003). Ultrasound application research in the food sciences is gaining momentum in Europe, but to present date just one

study, that demonstrated hollow heart disorder of potatoes could be detected by ultrasound, in the United States has attempted to utilize ultrasound (Cheng & Haugh 1994).

Ultrasonics for evaluation of fruits and vegetables

Ultrasound technology is gaining interest for nondestructive quality assessment of fruit and vegetables (Javanaud 1988, Self et al. 1992, Mizrach et al. 1994, Mulet et al. 2003). Low-frequency ultrasonics with relatively high excitation voltages have been employed for analysis of vegetative tissue because higher frequencies result in high attenuation of ultrasound signal, making interpretation difficult (Self & Wainwright 1993, Povey 1998). Parameters most commonly measured by low-frequency ultrasonics include velocity, attenuation and acoustic impedance of the propagation medium (Mulet et al. 1999). The measured parameter depends on the physical properties of the propagation medium, such as elastic modulus, density and microstructure. Low-frequency ultrasonics is a non-destructive technique because at low intensities the pressure and temperature gradients produced by an ultrasonic wave are small, thereby passing through plant material without altering the fundamental physical and chemical properties.

The cause of high attenuation when transmitting ultrasound through plant tissue has been investigated with suggestion that presence of air in intercellular spaces retards ultrasonic waves (Povey 1989). Further evidence has been provided by measurements of attenuation in potato parenchyma observing decreased attenuation with immersion time in water and decrease further when tissue was degassed (Sarkar & Wolfe 1983). The mechanism by which air has dominant effect on attenuation in plant tissues is likely to be scattering of ultrasonic waves (Povey 1989). Scattering occurs in heterogeneous materials in

which the physical properties of components are different (cellular material and air). Large differences in compressibilities of different components, such as when one is air, result in likelihood of resonant scattering (Miller 1979, Gaunaurd & Uberall 1981). At resonance, attenuation reaches a maximum and can be so large that it becomes impracticable to make transmission measurements. At frequencies below resonance, velocity is lower than that of the continuous phase (water) and in some systems can be lower than that of the dispersed phase (air). Therefore, ultrasonic velocity of some fruits and vegetables has been reported to be even slower than air (330 m s^{-1}) (Hayes & Chingon 1982).

Low frequency ultrasonics has been used to evaluate texture of raw and cooked carrots (Nielsen & Martens 1997, Nielsen et al. 1998). During the first two minutes of cooking, ultrasonic velocity decreased which may be due to disintegration of cell membranes, cell walls and thus a loss of turgor pressure. Extended heating resulted in increased ultrasonic velocity and attenuation decreased as a result of changes in mechanical properties and air and water content of the carrot tissue. Intercellular spaces grew in number and size with prolonged heating, resulting in low adhesion between neighboring cells. Further cooking ruptures cell walls causing cellular contents into air-filled intercellular spaces, resulting in more uniform ultrasonic signal and reduced acoustic impedance.

Ultrasound has been used to nondestructively evaluate quality of avocados, mangoes and melons. Ultrasonic studies with mangoes established that ultrasonic attenuation could be used to accurately measure the sugar content, acidity, total solids and softening process of fruit (Mizrach et al. 1997, Mizrach et al. 1999b). Attenuation of ultrasound signals increased during storage, and was correlated to firmness of avocado fruit during storage (Mizrach et al. 1996, Mizrach & Flitsanov 1999, Mizrach et al. 1999a). Ripening processes, dry weight and

oil content of avocado fruit were also correlated to ultrasonic parameters (Mizrach & Flitsanov 1998, 1999). Subsequent studies showed that ultrasound could accurately measure avocado firmness and composition of fruit stored at various temperatures (Flitsanov et al. 2000, Mizrach et al. 2000). During ripening of avocado fruit, volume fraction of intercellular spaces tended to decrease and ultrasonic velocity decreased from 350 to 200 m s^{-1} during a 12-day period of ripening (Self et al. 1994). Attenuation was found to have potential for identifying internal fruit quality of melons, but wave propagation velocity was found to be a poor predictor (Mizrach et al. 1991).

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CHAPTER 1. STRUCTURAL AND PHYSICOCHEMICAL CHARACTERISTICS OF WINTER SQUASH (*Cucurbita maxima* D.) FRUIT STARCHES AT HARVEST

A paper to be submitted to Carbohydrate Polymers

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Abstract

Structural and physicochemical properties were studied from fruit (pepo) of seven winter squash cultivars (*Cucurbita maxima* D.). Squash starches exhibited B-type X-ray diffraction patterns and had continuous granule size distribution with most granule diameters either 1.5-2.5 μm, 6-8 μm, or 11-13 μm. Squash starches had high iodine affinities for amylopectin and had low absolute amylose contents. Squash starch amylopectins had weight-average molecular weights ranging from 2.03-3.22 x 10^8 g/mol, gyration radii of 294-337 nm, and unusually low polydispersity. Isoamylase-debranched amylopectins showed average chain-length varied from DP 26.5 to 28.1. Starch from squash cultivars had onset gelatinization temperature ranging from 60.6°C to 63.5°C. Change in enthalpy of gelatinization (ΔH) ranged from 15.9 to 17.4 J/g, measured by using differential scanning calorimetry. Retrogradation rate for squash starches ranged from 41% to 55% after 7 d at 4°C, and ΔH was high (6.5 to 9.5 J/g). Squash starch pastes, measured by using Rapid Visco-Analyser, had high peak viscosity (174-233 RVU), final viscosity (193-244 RVU) and setback (79-100 RVU), with pasting temperature ranging from 65.6°C to 68.8°C.

Keywords: Starch, starch structure starch function, amylose, amylopectin, winter squash, pumpkin, cucurbit, polydispersity

1. Introduction

Starch is the major carbohydrate in plant storage organs. Starch structures and physicochemical properties have been characterized for storage organs of many plant families. However, Cucurbitaceae, which includes squash and pumpkins, have had little published research of fruit (pepo) starch characteristics.

Sugimoto et al. (1998a), investigated fruit starch properties of one cultivar each of *Cucurbita maxima* D. and *C. moschata* D. during development. Squash starch was shown to exhibit a B-type x-ray pattern, granule size ranging from 2.9 to 8.8 μm, amylose content ranging from 14-23%, and an onset gelatinization temperature of 62-66°C.

Starch granules were found to have a larger diameter range (3-35 μm) than reported by Sugimoto et al. (1998a) for the same *C. maxima* D. cultivar (Yoshida, 1989). Starch content and granule size variation between external and internal cellular layers of the fruit were also observed.

Few cultivars of squash have their starch properties characterized. Additionally, to our knowledge, there has been no report of starch retrogradation, phosphorus content of starch, absolute amylose content, and amylopectin structures such as molecular size, gyration radii, and amylopectin branch chain-length distribution, in any *Cucurbita* L. sp. starch. In this paper, we thoroughly characterize the structures and physicochemical properties of fruit starch isolated from seven cultivars of *Cucurbita maxima* D., grown in the same year, at

same location and using identical procedures. Structures and physicochemical properties will be related to their textural and eating qualities in Chapter 5.

2. Materials and methods

2.1 Plant Material

Seven squash cultivars were planted in summer of 1998 at an Iowa State University farm site 1.7 miles south of Ames, Iowa (geographical location 41° 58' 57.5" N, 93° 38' 22.9"), in a completely randomized block (3.05 m x 3.05 m blocks) with 18 replicates (4 plants/replicate). Normal crop husbandry was followed as required. Climatic conditions during the 1998 season can be found in Chapter 7. Five replicates of each cultivar were randomly selected for analysis of starch characteristics. Squash cultivars studied were three buttercups (Delica, Kurijiman and Sweet Mama), one Halloween-type (Prizewinner), one Hubbard-type (Scarlet Warren), one Crown-type (Whangaparoa Crown) and one Native American Indian squash (Lakota) (Coyne, Reiser, Sutton, & Graham, 1995). Seeds were purchased for Kurijiman, Scarlet Warren and Whangaparoa Crown from Webling and Stewart Ltd., Petone, New Zealand, for Delica from Yates New Zealand Ltd., Onehunga, New Zealand, for Prizewinner from King's Seeds, Auckland, New Zealand, for Sweet Mama from Henry Field Seed & Nursery Co., Shenandoah, IA, and for Lakota from W. Atlee Burpee & Co., Warminster, PA. Squash fruit maturity was adjudged when stalks became woody (Hawthorne, 1990), and this stage had been previously shown to have the highest starch content (Irving, Hurst, & Ragg, 1997).

2.2 Starch Isolation

Starch was isolated from squash fruit by using the method reported by Badenhuizen (1964) with slight modifications (Kasemsuwan, Jane, Schnable, Stinar, & Robertson, 1995). Two fruit per replicate, peeled and deseeded, were used for starch isolation. Squash pulp was ground in 0.01 M $HgCl_2$ and then filtered through 106 μm mesh. Filtrate was washed with 10% toluene in 0.1 M NaCl, washed three times with distilled water, twice with ethanol, and then recovered by filtration using Whatman No. 4 filter paper. Purified starch cake was dried in a convection oven at 35°C for 24 h.

2.3 Starch Granule Morphology by Scanning Electron Microscopy

Starch granules, spread on silver tape and mounted on a brass disk, were coated with gold/palladium (60/40) for all five replicates of each cultivar. Sample images were observed at 1500x magnification under a scanning electron microscope (JOEL model 1850, Tokyo, Japan) following the method of Jane, Kasemsuwan, Leas, Zobel, & Robyt (1994).

2.4 Crystalline Structure by X-ray Diffractometry

Crystallinity of starch granules was studied using X-ray diffractometry. X-ray diffraction patterns were obtained with copper, Kα radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI). Analysis was conducted following procedure of Song & Jane (2000). Degree of crystallinity was calculated based on method of Hayakawa, Tanaka, Nakamura, Endo, & Hoshino (1997). The following equation was used to determine percent crystallinity:

$$\text{Crystallinity (\%)} = A_c/(A_c + A_a) \times 100$$

where A_c = crystalline area on the X-ray diffractogram and A_a = amorphous area on the X-ray diffractogram.

2.5 Molecular Weight Distribution and Gyration Radius of Amylopectin by High-Performance Size-Exclusion Chromatography (HPSEC)

Weight-average molecular weight and z-average gyration radius of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). Starch samples, duplicate measurements of each replicate, were prepared as described by Yoo & Jane (2002a). The HPSEC system consisted of a HP 1050 series isocratic pump (Hewlett Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., Tokyo, Japan) were used. Operating conditions and data analysis are described by Yoo & Jane (2002b).

2.6 Phosphorus content

Phosphorus content was determined by the method described by Smith & Caruso (1964) except that five grams of starch was used and all glassware was soaked 24 h in 0.625% sodium molybdate (w/v) in 1.75 N H_2SO_4:10% ascorbic acid (w/v) mixture (4:1) to remove residual phosphorus. Duplicate analysis of each replicate was conducted.

2.7 Absolute Amylose Contents by Potentiometric Autotitration and Concanavalin A precipitation

Absolute amylose content of starch was determined following the procedure of Lu, Jane, Keeling, & Singletary (1996). Analysis was based on iodine affinities of defatted whole starch and amylopectin fraction using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). Starch samples were defatted using a 90% dimethyl sulfoxide (DMSO) solution, followed by alcohol precipitation. Amylose content of defatted starch samples was also determined by treating starch samples with concanavalin A as described by Yun & Matheson (1990) and measuring glucose content, by the glucose oxidase method, of the α-amylase and amyloglucosidase hydrolyzed, non-concanavalin A-complexed fraction using amylose/amylopectin assay kit from Megazyme International Ireland Ltd. (Wicklow, Ireland). Determination of amylose content, by both methods, was duplicated for each squash cultivar replicate.

2.8 Amylopectin Branch Chain-Length Distribution by High-Performance Anion-Exchange Chromatography (HPAEC) and by HPSEC

Amylopectin was fractionated by complexing amylose with n-butanol as described by Schoch (1942). Amylopectin (10 mg/mL) was defatted in boiling 90% DMSO for 1 h, followed by stirring for 24 h and then debranched using isoamylase (EC 3.2.1.68 from *Pseudomonas amyloderamosa*) (EN102, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) as described by Jane & Chen (1992). Branch chain-length distribution of amylopectin was determined using an HPAEC system (Dionex-300, Sunnyvale, CA) equipped with an amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, A-7255, Sigma

Chemical Co., St Louis, MO) post-column, on-line reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Wong & Jane, 1997a). PA-100 anion exchange analytical column (250 x 4 mm, Dionex, Sunnyvale, CA) and a guard column were used for separating debranched amylopectin samples. Gradient profile of eluents and operating conditions were described previously (McPherson & Jane, 1999). Branch chain-length distribution of amylopectin was also analyzed to determine extra-long branch-chains by using a HPSEC equipped with a RI detector. Operating conditions have been described earlier (McPherson & Jane, 1999), except flow rate was 0.6 mL/min, analytical column used for analysis was Shodex OH pak SB-803HQ (Showa Denko K.K., Tokyo, Japan) and sample concentration was 0.8 mg/mL. HPAEC-ENZ-PAD and HPSEC analysis was duplicated for each replicate of each cultivar.

2.9 Thermal Properties by Differential Scanning Calorimetry (DSC)

Thermal properties of starch were determined using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) (Jane et al. 1999). Approximately 2 mg of starch was weighed in an aluminum pan, mixed with 6 mg of deionized water and sealed. The sample was allowed to equilibrate for 2 hr and scanned at a rate of 10°C/min over a temperature range of 10-100°C. An empty pan was used as the reference. The rate of starch retrogradation was determined using the same gelatinized samples, stored at 4°C for 7 d, and analyzed using DSC as described previously (White, Abbas, & Johnson 1989). Analysis of all thermal properties were carried out in triplicate for each replicate of each cultivar.

2.10 Pasting Properties by Rapid Visco-Analyser (RVA)

Starch pasting properties were analyzed using a Rapid Visco-Analyser (RVA-4, Newport Scientific, Sydney, Australia) (Jane et al. 1999). Starch suspension (8%, w/w), in duplicate for each replicate of each cultivar, was prepared by weighing starch (2.24 g, dry starch basis (dsb)) into a RVA canister and making up the total weight to 28 g with distilled water. The starch suspension was equilibrated at 30°C for 1 min, heated at a rate of 6.0°C/min to 95°C, maintained at that temperature for 5.5 min, and then cooled to 50°C at a rate of 6.0°C/min. Constant paddle rotating speed (160 rpm) was used throughout entire analysis.

2.11 Data analysis

All statistical significance tests were calculated by using SAS (SAS Institute Inc. 1999) and applying Tukey difference test (Ramsey & Schafer, 1996).

3. Results and discussion

3.1 Starch granule morphology

Scanning electron micrographs show that all squash cultivar starches have a continuous granule size distribution, but with a preponderance of granules ranging from 1.5-2.5 μm, 6-8 μm or 11-13 μm in diameter (Fig. 1). Squash starches exhibited a mixture of spherical and polyhedral granules with some dome-shaped granules that suggest that some squash granules are compound. Dome-shaped granules tended to have larger diameters on average, which would be expected for compound granules. Yoshida (1989) reported that starch granules in the endocarp region of squash fruit flesh have compound starch granules, but the remainder of the fruit is absent of compound granules. Compound starch granules are not common

among all botanical sources currently investigated, with their occurrence reported only in oat (Jane et al. 1994), oxalis (Cortella & Pochettino 1995), wrinkled pea (Ratnayake et al. 2002), rice (Jane et al. 1994, Champagne 1996), waxy rice (Jane et al. 1994), pineapple (Jane et al. 1994) and some hull-less barley lines (Li et al. 2001a). Squash starch granules had many surface indentations. Large granules of Whangaparoa Crown, Lakota, Prizewinner and Sweet Mama had low incidence of indentations, whereas Delica, Kurijiman and Warren Scarlet frequently had indentations. Whangaparoa Crown and Prizewinner had a high incidence of indentations in medium-size starch granules. Granule indentations could be due to non-uniform growth within starch granules or collision of spherulites as granules expand since some squash starch granules are compound. Sugimoto et al. (1998a) reported two cultivars of squash starch to have low size variation and average granule size of 7.0-7.5 μm for starch collected from fruit at harvest. In contrast, additional research showed a scanning electron micrograph of squash starch displaying a wider granule size distribution that is similar in dimensions to what we observed (Sugimoto, Yamashita, Suzuki, Morishita, & Fuwa, 1998b). Yoshida (1989) reported starch granule diameters up to 35 μm, but we did not observe granule dimensions above 14 μm.

3.2 Crystalline structure

Squash starches all exhibited typical B-type X-ray diffraction patterns (Fig. 2), with a strong peak at $2\theta = 17.2°$, another peak at $2\theta = 5.6°$, and a split peak at $2\theta = 22\text{-}24°$. Prizewinner, Warren Scarlet, Whangaparoa Crown and Kurijiman all had an additional peak at $2\theta = 28.2°$ that was not observed in other three cultivars. Percentage crystallinity of Delica, Kurijiman, Lakota, Prizewinner, Sweet Mama, Warren Scarlet and Whangaparoa Crown squash

starches, calculated based on X-ray diffractograms, was 35.0, 55.7, 43.9, 48.0, 45.7, 54.0 and 42.9 respectively. The three buttercup cultivar squash starches, Delica, Kurijiman and Sweet Mama, showed different degrees of crystallinity. Percentage crystallinity of some squash starches is higher than other native starches, such as 20-28% reported for a variety of A- and B-type starches (Cooke & Gidley, 1992), 25 to 44% reported for a range of wheat starches (Fujita, Yamamoto, Sugimoto, Morita, & Yamamori, 1998), 12 to 18% reported for different wheat starches (Yoo & Jane, 2002a), and 38% reported for normal maize (Keppel, 2001).

3.3 Amylose content

Iodine affinities for defatted whole starch and the corresponding apparent amylose contents were significantly different for the squash cultivars, with Kurijiman higher in apparent amylose than Lakota (Table 1). Absolute amylose content, calculated by subtracting iodine affinity for amylopectin fraction from the defatted whole starch, significantly differed between the squash cultivars. Iodine affinities of the amylopectin fraction were high relative to most native starches, resulting in absolute amylose content of all squash starches substantially lower than apparent amylose content. Iodine affinity of amylopectin fraction for Prizewinner was significantly higher than for Lakota. Absolute amylose content of the squash cultivars was significantly different but the conservative Tukey statistical test detected no significant difference among individual cultivars. However, cultivars could be divided into two groups, those with lower absolute amylose content, Prizewinner, Sweet Mama and Lakota, compared to other four cultivars. High iodine affinities for amylopectin fraction indicate squash starches have amylopectins with considerable proportion of long branch chains.

Concanavalin A underestimated the amylose content for all squash cultivars but in general the relativeness among cultivars was similar to that of absolute amylose content. The underestimation of amylose content using concanavalin A could be due to rapid retrogradation of amylose. Differences in amylose content among squash cultivars using concanavalin A were not significant, but there was still some weak evidence to suggest variable amylose content. Concanavalin A, and particularly apparent amylose, appear to be unsuitable methods for measuring amylose content of squash starches, but for other starches, good agreement between concanavalin A and iodine potentiometric titration methods has been reported (Gibson, Solah, & McCleary, 1997).

3.4 Phosphorus content

Phosphorus content of starch can have great influence on the functional properties. Apart from potato, most B-type starches have phosphorus content lower than normal cereal starches. Squash starches phosphorus content ranged from 0.022% for Whangaparoa Crown to 0.026% for Lakota (dry weight basis, w/w), and cultivars were not significantly different. Phosphorus content of all squash starches was higher than reported for all B-type starches, except potato, but was lower than all normal cereal starches except maize (Lim , Kasemsuwan, & Jane, 1994; Jane, Kasemsuwan, Chen, & Juliano, 1996; Kasemsuwan & Jane, 1996).

3.5 Amylopectin molecular weight and size

Average molecular weight, polydispersity, and gyration radius of squash starches are shown in Table 2. Squash starch weight-average molecular weight (M_w) ranged from 2.03 to 3.22 x 10^8 g/mol for the seven squash cultivars, with no significant differences. Squash amylopectin M_w is smaller than normal maize and rice (Yoo & Jane, 2002b) but comparable to wheat (Yoo & Jane, 2002a) and larger than barley, tapioca and potato starches (Yoo & Jane, 2002b). A distinctive characteristic of squash amylopectins was their low polydispersity (M_w/M_n), particularly in Lakota, Prizewinner, Whangaparoa Crown and Sweet Mama cultivars. Polydispersity of squash amylopectin is substantially lower than amylopectin from other native starches (Table 3), and it is remarkably uniform in size for biological molecules of such large magnitude. Although there is no clear trend, amylopectin polydispersities less than two were found for only A-type starches with the exception of potato (1.79). Therefore it is surprising that the B-type squash amylopectin would have such low polydispersity.

Gyration radius was not significantly different for the squash cultivars indicating that their spatial arrangement of amylopectin chains within molecules may be similar. Prizewinner and Whangaparoa Crown amylopectins were considerably denser than other squash cultivars but differences were not significant. Squash amylopectin densities were lower than most cereal starches, but higher than tuber and root starches (Yoo & Jane, 2002b). Gyration radius and density of amylopectin for squash starch is different from all other B-type starches, except for *ae wx* maize amylopectin which had M_w, gyration radius and density similar to squash (Yoo & Jane, 2002b).

3.6 Debranched amylopectin chain distribution

HPAEC-ENZ-PAD chromatograms of each squash cultivar with standard deviation of each individual DP is shown in Fig. 3a and 3b. Chromatograms show that all squash starches have peak chain-length at DP 13-14 and the error associated with quantifying individual DP is low.

Isoamylase-debranched squash amylopectin revealed the chain-length distribution (Table 4). Average amylopectin chain-length varied from DP 26.5-28.1 for the squash cultivars and was not significantly different. However, proportion of short amylopectin chains was significantly different. The greatest observable difference in squash amylopectin chain-length distribution was chains with DP <12. Lakota had significantly less DP 6-12 amylopectin chains than all other cultivars. Prizewinner had significantly more DP 6-9 amylopectin chains than Delica and Lakota. Lakota had significantly greater proportion of DP 13-24 than Sweet Mama, Delica and Warren Scarlet, and significantly greater proportion of DP 25-36 than Kurijiman, Whangaparoa Crown and Prizewinner. Warren Scarlet also had greater proportion of DP 25-36 than Prizewinner. Long amylopectin chains (DP ≥ 37) were not significantly different between the squash cultivars. Squash amylopectin average branch chain-length is longer than most A-type starches such as barley, rice and normal maize, comparable to tapioca and C-type starches, but shorter than all other B-type starches (Jane et al. 1999).

Iodine affinity of the amylopectin fraction reflects branch chain-length distribution. Proportion of long amylopectin chains (DP ≥ 37) of squash starches was comparable to all other B-type starches (Jane et al. 1999), thus explaining the high iodine affinity of the amylopectin fraction.

3.7 Thermal properties

Thermal properties of native squash starches are shown in Table 5. Three buttercup squash cultivars, Delica, Kurijiman and Sweet Mama, showed some consistence in starch properties that differed from other types of squash starch. The three buttercup squash starches displayed onset gelatinization temperature (T_o), which was significantly higher than Prizewinner and Whangaparoa Crown. In contrary, Whangaparoa Crown had significantly lower peak gelatinization temperature (T_p) than the three buttercup cultivars and Lakota, and had significantly lower conclusion gelatinization temperature (T_c) than all other cultivars except Delica. The range of gelatinization temperature (T_c-T_o) (ROG) is very low compared with other starches . Starch from three buttercup squash cultivars had significantly lower range of gelatinization than Prizewinner and Warren Scarlet, and to our knowledge, is amongst the lowest reported for any starch, with water yam (*Dioscorea alata*) the only starch lower with a ROG of 5.4°C (Farhat, Oguntona, & Neale, 1999) and barley the next closest, with a ROG of 6.6°C (Jane et al. 1999). The low ROG could be due to the low polydispersity of amylopectins that squash starches exhibit, causing a sharp melting peak of the relatively uniform amylopectins. Biliaderis, Maurice, and Vose (1980) suggested that the greater the degree of amylopectin branching, the wider the melting temperature range, but in our study we found no correlations between T_o-T_c and amylopectin branch-chain distribution. Enthalpy change of gelatinization for all squash starches was similar to other B-type starches (Jane et al. 1999), and there were no significant differences between cultivars.

Sugimoto et al. (1998a) studied thermal properties of two squash starches, in which one was *Cucurbita maxima* cv. Ebisu, which is the Japanese name for Delica. They reported an T_o of 62.1°C for Ebisu, lower than 63.4°C that we report for Delica, and a T_c of 74.1°C,

much higher than 69.7°C we report for Delica. Therefore their range of gelatinization (12°C) is almost twice what we report.

Squash starches, retrograded for 7 d at 4°C, showed no significant differences for T_o, T_p and T_c (Table 6). Despite no significant differences in enthalpy change of gelatinization for native starches, retrograded squash starches were significantly different, with Lakota and Sweet Mama retrograded starch having higher enthalpy change of gelatinization than Prizewinner. Enthalpy change of gelatinization of retrograded starch for squash is higher than all other native starches, except green banana (Jane et al. 1999). Percentage retrogradation of Lakota, Sweet Mama and Warren Scarlet starches were significantly higher than Prizewinner.

3.8 Pasting properties

Pasting properties of squash starches are shown in Table 7. Peak viscosity was the only pasting parameter that was significantly different for squash starches, with Lakota and Prizewinner peak viscosity being higher than Warren Scarlet, Delica and Whangaparoa Crown. Pasting temperature of squash starches was only 4-6°C higher than T_o, which could be attributed to the absence of phospholipids and low amylose content, conducive for granule breakdown during swelling. Final viscosity of greater than 200 RVU for squash starches is considerably higher than A-type starches, but typical for B-type starches (Jane et al. 1999). Setback for all squash starches is higher than for most starches, with cattail millet, mungbean and green leaf canna starches the only exceptions (Jane et al. 1999). High setback indicates that amylose may be retrograding very rapidly, and this may explain why concanavalin A method underestimates the amylose content of squash starches.

Squash starch pasting profiles are compared with normal maize and potato starch in Fig.4. Whangaparoa Crown and Delica tended to resemble a normal maize pasting profile with slightly higher peak viscosity, breakdown, setback and final viscosity. Lakota pasting profile showed some resemblance to potato starch, with peak viscosity high relative to other squash starches, and pasting temperature and setback similar to normal maize.

3.9 Presence of long amylopectin chains

The high iodine affinity of squash starch amylopectin fraction could be due to extremely long branch chains present in amylopectins, which were unable to be detected by HPAEC-ENZ-PAD. To investigate this, squash amylopectin was debranched using isoamylase and molecular size was characterized using HPSEC. The HPSEC chromatograms showed two distinct regions of low and high molecular weight chains (Table 8). Peak I represents the longer amylopectin chains and their proportion of the total amylopectin chains for the squash cultivars Lakota, Prizewinner, Sweet Mama and Warren Scarlet were significantly higher than the proportion of long amylopectin chains of Kurijiman. This result is not in agreement with the HPAEC analysis (Table 4). No amylopectin chains longer than detection limit (DP $\approx$ 80) of HPAEC-ENZ-PAD were observed, which is consistent with other B-type starches. The proportion of longer amylopectin chains is greater than the 17 % reported for tapioca starch (Wong & Jane, 1997b), and 33 % reported for corn (Jideani, Takeda, & Hizukuri, 1996) or wheat starch (Yoo & Jane 2002a).

3.10 Correlations to amylose content

Correlation coefficients among selected squash starch structural and functional properties are shown in Table 9. Correlation coefficients are mentioned in the text when not included in Table 9. Apparent amylose content of squash starches was correlated to amylopectin chains of length DP 6-9 ($r = 0.74$, $P = 0.05$), DP 6-12 ($r = 0.78$, $P = 0.04$), DP 13-24, DP 25-36, onset gelatinization temperature of retrograded starch (T_{oR}) ($r = 0.73$, $P = 0.05$), and pasting properties of peak viscosity, breakdown, final viscosity and setback. Iodine affinity of amylopectin fraction was correlated to amylopectin chains of length DP 6-9 ($r = 0.77$, $P = 0.04$), DP 6-12 ($r = 0.74$, $P = 0.05$), and DP 25-36 ($r = 0.77$, $P = 0.04$). Absolute amylose content was correlated to amylose content measured by concanavalin A, and pasting properties of peak viscosity, breakdown and final viscosity. Amylose content measured by concanavalin A was correlated to final viscosity, setback and long amylopectin chains measured by HPSEC.

Apparent amylose content correlated positively to short amylopectin branch chain-lengths and negatively correlated to intermediate amylopectin branch chain-lengths found in our study contrasts previous findings of apparent amylose content positively correlated to amylopectin branch chain-length for barley (Salomonsson & Sundberg, 1994, Li, Vasanthan, Rossnagel, & Hoover, 2001a) and maize (Cheetham & Tao, 1997). The negative relationship observed between squash apparent amylose content and peak or final viscosity is in agreement with many other studies (Wang, White, & Pollak, 1993; Kusutani, Asanuma, Kogure, Seki, Hirata, & Yanagihara, 1993; Reddy, Subramanian, Ali, & Bhattacharya, 1994; Kitahara et al. 1996; Collado & Corke, 1997; Yoshii, Arisaka, Jou, & Hayakawa, 1997; Jane et al. 1999; Kuno, Kainuma, & Takahashi, 2000; Blennow, Bay-Smidt, & Bauer, 2001).

3.11 Correlations to Amylopectin Molecular Weight and Size

Squash starch M_w was correlated to polydispersity, R_z, average amylopectin branch chain-length, amylopectin branch chains of DP $\geq$ 37, proportion of long amylopectin chains (DP $\geq$ 26) measured by HPSEC, range of gelatinization temperature (ROG), final viscosity and setback. Polydispersity was correlated to setback and proportion of long amylopectin chains measured by HPSEC. R_z was correlated to proportion of amylopectin chain-lengths of DP 25-36, proportion of long amylopectin chains measured by HPSEC, ROG and setback. Amylopectin density was correlated to T_o, T_p (r = -0.77, P = 0.04) and pasting temperature.

Squash amylopectins with higher M_w have wider gyration radius most likely because of a greater number of clusters per molecule rather than different cluster structure as shown previously for various starches (Takeda, Shibahara, & Hanashiro, 2003). Negative correlation observed between M_w and average amylopectin branch chain-length or proportion of DP $\geq$ 37 is surprising, and contrasts findings by Mua and Jackson (1997) and Lu, Chen, & Lii (1997) who both reported higher molecular weight for amylopectins with longer chain-lengths, and You, Fiedorowicz, & Lim (1999) who reported similar chain-length distribution for different molecular-weight amylopectins. Correlations indicate that squash cultivar starches with narrow ROG possess a greater proportion of low amylopectin molecular weight with smaller R_z.

3.12 Correlations to Amylopectin Branch Chain-Length Distribution

Average squash cultivar amylopectin branch chain-length was correlated to T_o, ROG and ΔH. Short branch chains of amylopectin were only correlated to amylose content. Amylopectin branch chain-lengths of DP 13-24 were correlated to peak viscosity and breakdown, and

chain-lengths of DP 25-36 were correlated to T_{cR} ($r = 0.86$, $P = 0.01$), percent retrogradation ($r = 0.77$, $P = 0.04$) and R_z. Long amylopectin branch chains (DP $\geq$ 37) were correlated to ROG, breakdown, setback and M_w.

Fine structure of amylopectin correlated to pasting properties has previously been reported. Long amylopectin chains were found to be negatively correlated to breakdown and conversely, short amylopectin chains were positively correlated (Han & Hamaker, 2001). Peak viscosity positively correlated to intermediate amylopectin branch chains has been previously reported for barley amylopectins of DP 18-34 length (Li, Vasanthan, Rossnagel, & Hoover, 2001b). However, increasing peak viscosity with increasing amylopectin branch chain-length has also been reported (Shibanuma, Takeda, & Hizukuri, 1996; Sasaki & Matsuki, 1998). Results imply that long amylopectin chains contribute greatly to breakdown of swollen granules and viscosity after gelatinization, and this finding has recently been found for apple starches (Chapter 10). T_o correlated to amylopectin average branch chain-length has been reported previously (Jane et al. 1999, Li et al. 2001b). Higher squash average amylopectin branch chain-lengths resulted in higher ΔH, which has been reported previously for barley starches (Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001). Negative correlation that we observed between amylopectin average branch chain-length and ROG contrasts findings by Li et al. (2001b) who reported positive correlation between long amylopectin chains and ROG and negative correlation for short amylopectin chains. Correlations between retrograded starch and amylopectin branch chain-length distribution were observed only for DP 25-36, which is expected since high proportion of short amylopectin chains (DP < 12) has been shown to inhibit retrogradation (Wűrsch & Gumy, 1994; Lu, Chen, & Lii, 1997).

3.13 Correlations to Starch Thermal and Pasting Properties

In addition to previously mentioned correlations involving thermal properties, To, Tp and ΔH were correlated to pasting temperature ($r = 0.86$, $P = 0.01$ for T_p) and ROG was correlated to ΔH. All correlations involving pasting properties have been previously mentioned except both final viscosity and setback were correlated to proportion of long amylopectin branch chains measured by HPSEC.

4. Conclusion

Seven cultivars of squash starches had continuous granule size distribution, displayed B-type X-ray diffraction patterns, consisted of low amylose (12.9-18.2%), and moderate phosphorus content (0.022-0.026%). All squash starches had long amylopectin branch chain-lengths, but Lakota had relatively fewer short and long chains. Distinctive attributes of squash starches were low polydispersity and range of gelatinization, and high retrogradation rate. Squash starches have high peak and final viscosity, very high setback, and pasting temperature was only 4-5°C higher than onset gelatinization temperature.

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Table 1

Iodine affinities, apparent amylose, absolute amylose contents, and amylose content measured using concanavalin A (Con A) for squash fruit defatted starches.[♦]

Cultivar	Iodine Affinity		Apparent amylose content (%)[*]	Absolute amylose content (%)[#]	Amylose (%) content measured by Con A[♣]
	whole starch	amylopectin fraction			
Delica	5.51[ab]	1.89[ab]	27.8[ab]	18.2	12.4
Kurijiman	5.63[a]	2.35[ab]	28.3[a]	16.5	12.6
Lakota	4.45[b]	1.65[b]	22.3[b]	14.0	10.2
Prizewinner	5.31[ab]	2.73[a]	26.4[ab]	12.9	7.9
Sweet Mama	5.02[ab]	2.33[ab]	24.9[ab]	13.2	9.1
Warren Scarlet	5.41[ab]	1.84[ab]	27.1[ab]	17.9	11.4
Whangaparoa Crown	5.48[ab]	1.96[ab]	27.9[ab]	18.0	11.8
	$P = 0.03$[♠]	$P = 0.02$	$P = 0.03$	$P = 0.007$[♥]	$P = 0.07$

[*] Apparent amylose contents were averaged from two analyses for each of five replicates.; Values were calculated from dividing iodine affinity by a factor of 0.199.

[#] Absolute amylose contents were averaged from two analyses for each of five replicates.; Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.

[♣] Amylose contents measured by concanavalin A were averaged from two analyses for each of five replicates.

[♦] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♠] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

[♥] Conservative Tukey test was unable to detect differences between individual cultivars.

Table 2

Average amylopectin molecular weight, polydispersity, gyration radius and density of squash fruit starches.*

Cultivar#	M_w x 10^8 (g/mol)♣	Polydispersity (M_w)	R_z (nm)♦	ρ (g/mol/nm^3)♠
Delica	2.41	1.44	310	10.7
Kurijiman	2.03	1.81	294	10.6
Lakota	3.16	1.21	324	10.8
Prizewinner	3.22	1.23	317	12.2
Sweet Mama	2.54	1.30	311	10.6
Warren Scarlet	2.91	1.35	337	10.1
Whangaparoa Crown	2.70	1.29	304	12.0
	$P = 0.12$☼	$P = 0.27$	$P = 0.11$	$P = 0.15$

* Data were obtained from two injections of all five replicates.

Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; Freshly prepared starch aqueous solution (100 µL; 0.8 mg/mL) was injected to HPSEC system.

♣ *weight*-average molecular weight.

♦ *z*-average radius of gyration.

♠ Density is equal to M_w/R_z^3.

☼ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 3

Polydispersity and crystallinity of amylopectins from various botanical sources.

Starch	Polydispersity (M_w/M_n)*	Crystallinity#
Normal maize	1.60	A
Waxy maize	1.57	A
du waxy maize	1.96	A
su1 maize	2.05	A
sh2 maize	11.16	A
Barley	1.66	A
Wheat	1.82	A
Waxy wheat	1.68	A
Sweet rice	6.08	A
Waxy rice	3.28	A
Cattail millet	4.02	A
Tapioca	2.12	A
Mungbean	1.47	A
Chinese taro	5.42	A
Amaranth	3.22	A
Waxy amaranth	4.36	A
Hylon V maize	2.65	B
Hylon VII maize	3.78	B
ae waxy maize	2.03	B
Potato	1.79	B
Green leaf canna	2.24	B
Sweet potato	2.45	C
Waterchestnut	4.17	C
Green banana	2.11	C
Lotus root	3.47	C

* Data were obtained of at least two injections.

Based on X-ray diffraction patterns reported by Jane, Wong & McPherson (1997) and Jane et al. (1999).

Table 4

Branch chain-length distributions of squash fruit amylopectins[*#].

Cultivar	Peak DP		Average	Percent distribution					Highest
	I	II	CL	DP 6-9	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37	Detectable DP
Delica	13	50	28.1	5.0^{b}	14.9^{a}	40.6^{b}	14.7^{abc}	29.3	72
Kurijiman	13	48	27.4	5.4^{ab}	15.6^{a}	41.0^{ab}	14.2^{bc}	28.4	76
Lakota	14	49	27.0	3.8^{c}	12.6^{b}	44.7^{a}	16.5^{a}	25.4	69
Prizewinner	13	49	26.5	6.1^{a}	16.7^{a}	41.9^{ab}	14.1^{c}	26.2	76
Sweet Mama	13	48	27.9	5.3^{ab}	15.2^{a}	40.1^{b}	15.0^{abc}	28.8	74
Warren Scarlet	14	49	26.9	5.4^{ab}	15.4^{a}	40.4^{b}	16.0^{ab}	27.1	68
Whangaparoa Crown	13	49	27.2	5.5^{ab}	15.9^{a}	41.2^{ab}	14.6^{bc}	27.3	72
			$P = 0.26^{\clubsuit}$	$P < 0.0001$	$P < 0.0001$	$P = 0.01$	$P = 0.003$	$P = 0.13$	

[*] Grouping of degree of polymerization (DP) numbers followed that of Hanashiro et al. (1996).

[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 5

Thermal properties of native squash fruit starches.

Cultivar*	Native starch				
	T_o (°C)[#♦]	T_p (°C)	T_c (°C)	Range (°C)[♣]	ΔH (J/g)
Delica	63.4[a]	66.5[a]	69.7[ab]	6.3[b]	17.3
Kurijiman	63.5[a]	66.5[a]	69.8[a]	6.3[b]	16.9
Lakota	62.9[ab]	66.4[a]	70.4[a]	7.5[ab]	16.8
Prizewinner	60.9[bc]	65.0[ab]	69.7[a]	8.8[a]	15.9
Sweet Mama	63.5[a]	66.2[a]	69.8[a]	6.3[b]	17.4
Warren Scarlet	61.7[abc]	65.7[ab]	70.4[a]	8.7[a]	16.4
Whangaparoa Crown	60.6[c]	64.0[b]	67.7[b]	7.1[ab]	16.3
	$P = 0.0001$[♠]	$P = 0.001$	$P = 0.006$	$P < 0.0001$	$P = 0.26$

Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.

[#] Values were calculated from three analyses for each of five replicates.

[♣] Range of gelatinization is equal to T_c-T_o.

[♦] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column .

[♠] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 6

Thermal properties of retrograded squash fruit starches[#].

Cultivar[*]	Native starch				
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	% retrogradation
Delica	36.3	54.0	64.5	8.1^{ab}	46.6^{ab}
Kurijiman	36.6	54.5	64.8	7.8^{ab}	45.8^{ab}
Lakota	35.2	53.3	65.4	8.9^{a}	53.3^{a}
Prizewinner	36.6	54.8	64.3	6.5^{b}	40.8^{b}
Sweet Mama	35.1	52.4	65.0	9.5^{a}	54.7^{a}
Warren Scarlet	36.0	52.6	65.5	8.8^{ab}	53.4^{a}
Whangaparoa Crown	35.6	53.3	65.0	8.2^{ab}	49.8^{ab}
	$P = 0.25$[♣]	$P = 0.31$	$P = 0.59$	$P = 0.008$	$P = 0.002$

[*] Same starch samples after gelatinization (see Table 5) were left for 7 days at 4°C and rescan using DSC.
[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.
[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 7

Pasting properties of squash fruit starches measured by Rapid Visco-Analyzer.

Cultivar[*]	Peak Viscosity[#♣]	Breakdown[#]	Final Viscosity[#]	Setback[#]	Pasting Temperature (°C)
Delica	174.8[b]	52.2	206.9	84.3	68.8
Kurijiman	179.2[ab]	63.1	195.3	79.2	67.8
Lakota	232.8[a]	88.7	243.8	99.7	67.2
Prizewinner	225.1[a]	76.7	244.3	95.8	66.5
Sweet Mama	205.2[ab]	66.6	231.6	93.0	68.4
Warren Scarlet	176.8[b]	52.6	217.5	93.2	68.0
Whangaparoa Crown	173.7[b]	65.4	193.1	84.6	65.6
	$P = 0.01$[♦]	$P = 0.26$	$P = 0.49$	$P = 0.70$	$P = 0.16$

[*] 8% (w/w) starch suspension.

[#] Viscosity measured in Rapid Visco-Analyzer units (RVU), 1 RVU = 12 centipoise.

[♣] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♦] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 8

Squash fruit isoamylase-debranched amylopectins measured using HPSEC[♣].

Cultivar	Peak I (%)[*#]	Peak II (%)
Delica	42.4[ab]	57.6[ab]
Kurijiman	38.7[b]	61.3[b]
Lakota	46.7[a]	53.3[a]
Prizewinner	47.1[a]	52.9[a]
Sweet Mama	47.3[a]	52.7[a]
Warren Scarlet	47.3[a]	52.7[a]
Whangaparoa Crown	43.0[ab]	57.0[ab]
	$P < 0.0001$[♦]	

Peak I and II represent long (DP > 26) and short (DP < 26) amylopectin chains respectively.
Values represent proportion of long and short amylopectin chains.
[#] Values were obtained from two analyses for each of five replicates.
[♣] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.
[♦] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 9

Correlation coefficients (r x 100) for selected squash starch structural and functional properties.

	Ap_A	Ab_A	A_{con}	M_w	P_{Mw}	R_z	DS	CL	DP_{13-24}	DP_{25-36}	$DP_{\geq 37}$	$DP_{\geq 26}$	T_o	ROG	ΔH	PV	BK	FV	SB
Ap_A	100																		
Ab_A	68	100																	
A_{con}	55	89**	100																
M_w	-58	-44	-68	100															
P_{Mw}	59	39	65	-86**	100														
R_z	-44	-8	-33	73*	-61	100													
DS	11	-25	-41	38	-38	-29	100												
CL	17	28	43	-72*	34	-44	-44	100											
DP_{13-24}	-72*	-39	-24	57	-36	22	21	-46	100										
DP_{25-36}	-69	-1	0	46	-42	74*	-53	-13	49	100									
$DP_{\geq 37}$	57	39	46	-85**	56	-52	-36	87**	-80*	-46	100								
$DP_{\geq 26}$	-64	-53	-76*	82*	-86**	80*	3	-38	20	53	-50	100							
T_o	-21	-9	26	-59	49	-25	-72*	72*	-6	14	53	-29	100						
ROG	-14	-17	-47	81*	-51	74*	27	-89**	25	25	-74*	62	-72*	100					
ΔH	-15	5	31	-61	32	-28	-64	92**	-23	13	69	-23	91**	-84*	100				
PV	-84*	-90**	-82*	68	-55	30	27	-44	72*	28	-67	60	-1	34	-17	100			
BK	-78*	-75*	-59	58	-44	6	41	47	87**	25	-75*	34	-11	21	-25	89**	100		
FV	-80*	-83*	-87**	76*	-66	57	9	-40	51	39	-58	81*	-1	48	-14	92**	66	100	
SB	-83*	-65	-76*	88**	-81*	77*	5	-49	55	62	-71	92**	-19	61	-25	81*	62	93**	100
PT	-2	8	22	-45	35	11	-83*	66	-37	18	62	-4	83*	-40	78*	-19	-47	3	-10

Ap_A = apparent amylose content, Ab_A = absolute amylose content, A_{con} = amylose content measured by concanavalin A, M_w = weight-average amylopectin molecular weight, P_{Mw} = polydispersity (M_w/M_n), R_z = gyration radius, DS = density, CL = average amylopectin branch chain-length, DP_{13-24} = proportion of amylopectin branch chain-lengths DP 13-24, DP_{25-36} = proportion of amylopectin branch chain-lengths DP 25-36, $DP_{\geq 37}$ = proportion of amylopectin branch chain-lengths DP ≥ 37, $DP_{\geq 26}$ = proportion of amylopectin branch chain-lengths DP ≥ 26 measured by HPSEC, T_o = onset gelatinization temperature, ROG = range of gelatinization temperature, ΔH = enthalpy change of gelatinization, PV = peak viscosity, BK = breakdown, FV = final viscosity, SB = setback, PT = pasting temperature. * = 0.05 and ** = 0.01 level of significance.

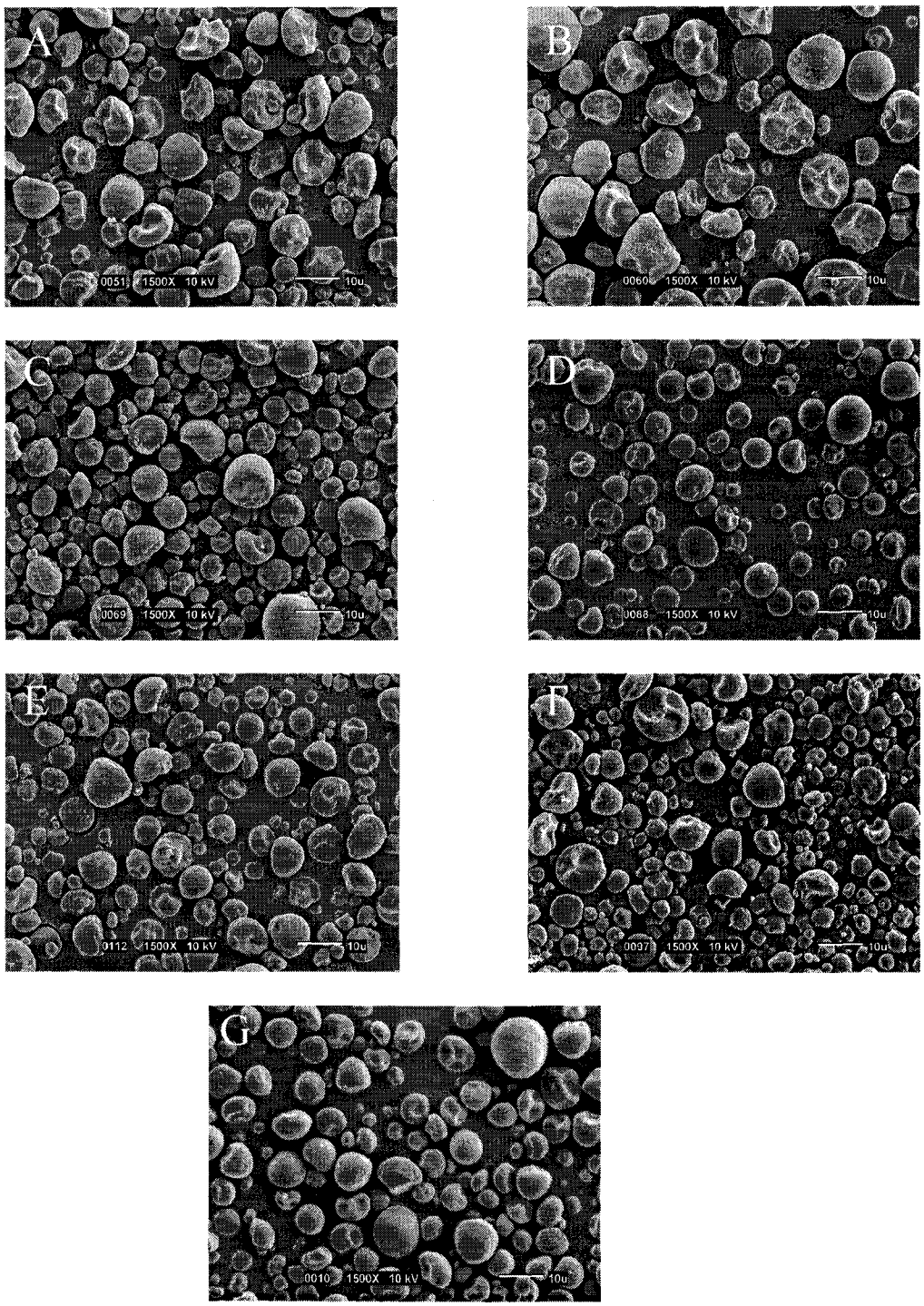

Figure 1. Scanning electron micrographs of Delica (A), Kurijiman (B), Lakota (C), Prizewinner (D), Sweet Mama (E), Warren Scarlet (F) and Whangaparoa Crown (G) squash fruit starches (scale bar = 10 μm).

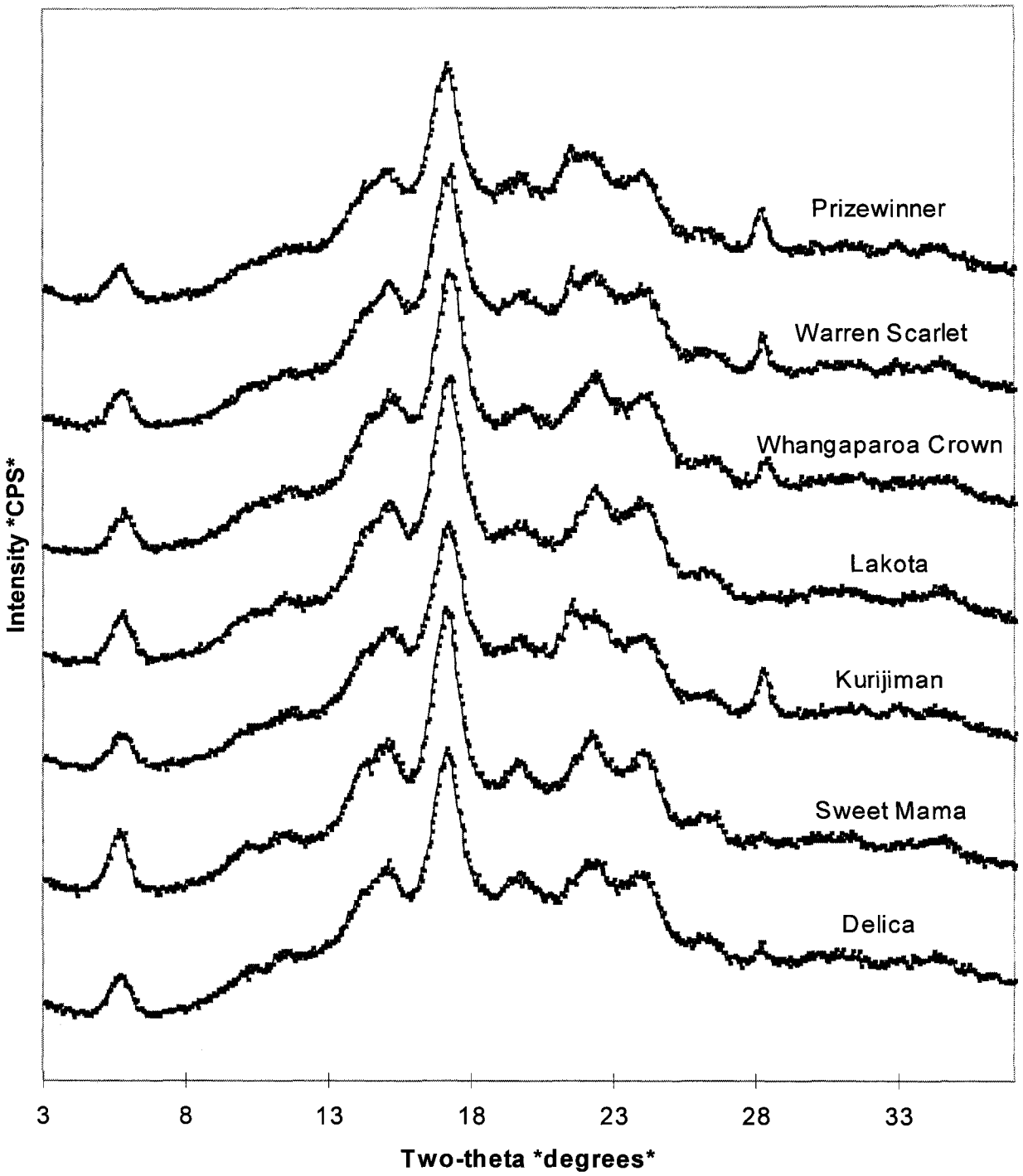

Fig. 2. X-ray diffraction patterns of Delica, Kurijiman, Lakota, Prizewinner, Sweet Mama, Warren Scarlet and Whangaparoa Crown squash fruit starches.

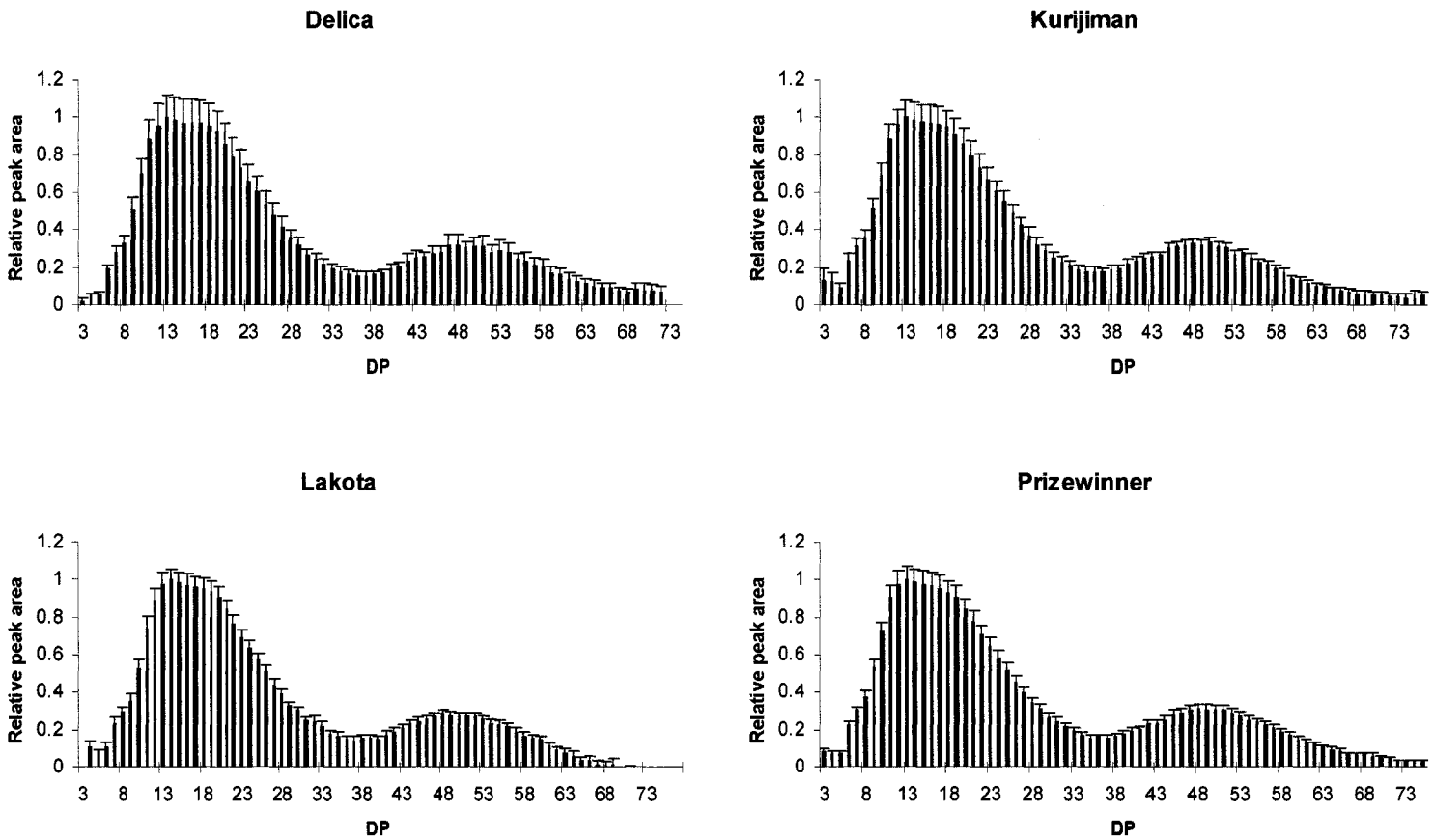

Fig. 3a. Relative peak area distributions of Delica, Kurijiman, Lakota and Prizewinner squash fruit amylopectins analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of five replicates. DP = Degree of polymerization.

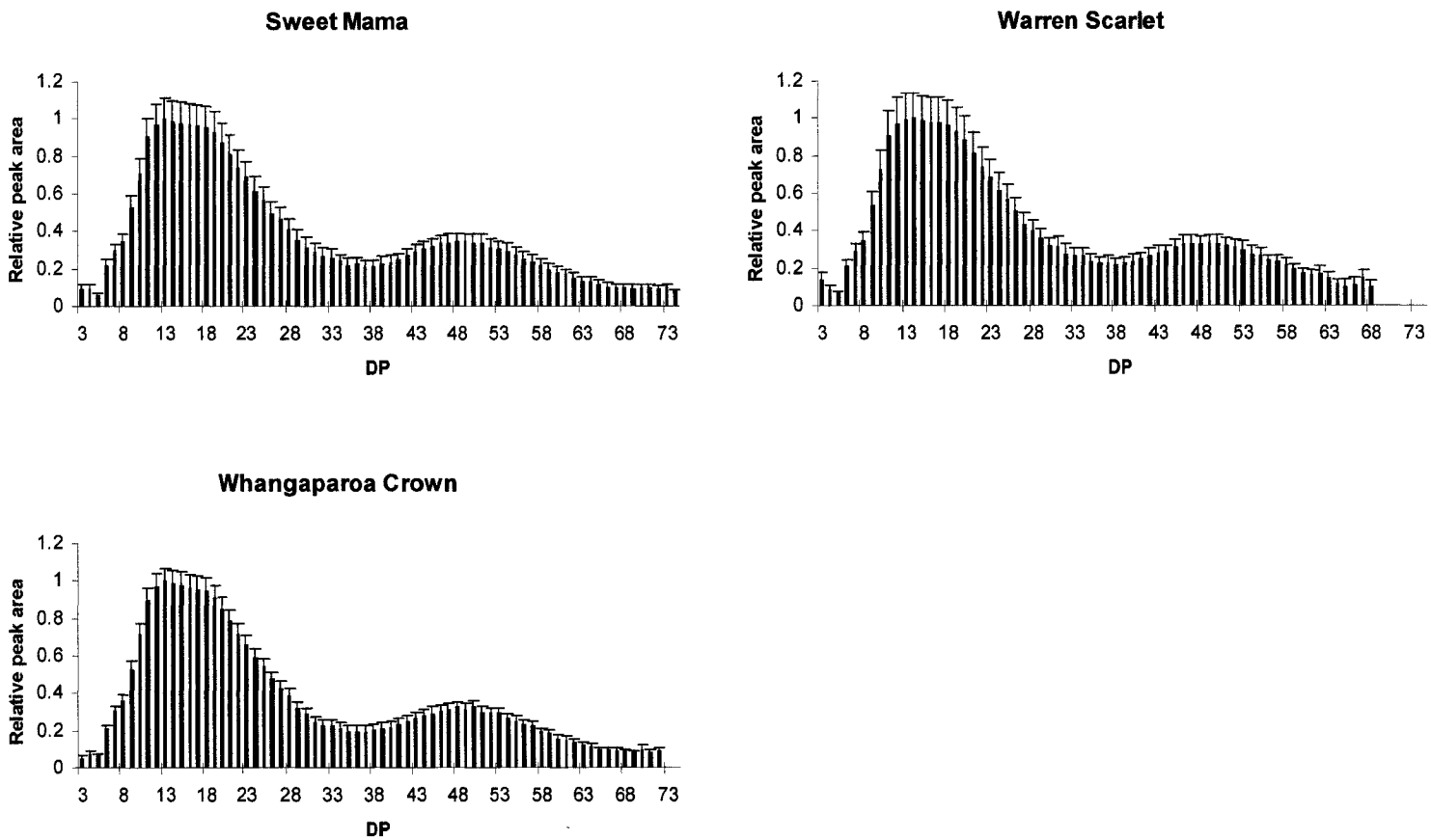

Fig. 3b. Relative peak area distributions of Sweet Mama, Warren Scarlet and Whangaparoa Crown squash fruit amylopectins analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of five replicates. DP = Degree of polymerization.

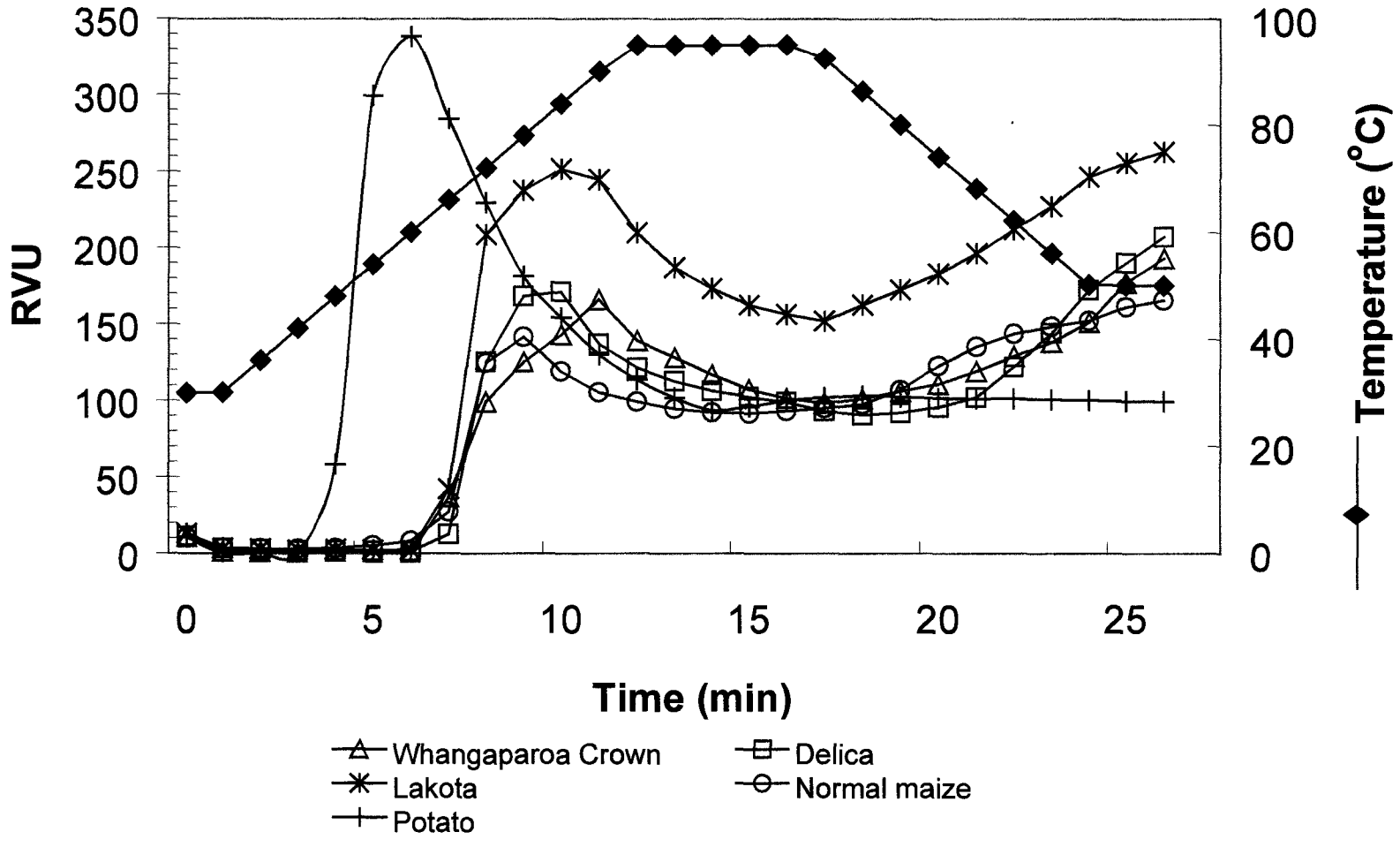

Fig. 4. Rapid Visco-Analyser pasting profiles of Whangaparoa Crown, Delica and Lakota squash fruit starch compared with normal maize and potato starches (8.0% dsb, w/w).

CHAPTER 2. ROLE OF STARCH STRUCTURE IN TEXTURE OF SQUASH AND STARCH FUNCTIONAL PROPERTIES. I. STRUCTURAL PROPERTIES OF STARCH EXTRACTED FROM WINTER SQUASH FRUIT (*Cucurbita maxima* D.) AT HARVEST AND AFTER STORAGE.

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ABSTRACT

Starch from fruit of twelve winter squash cultivars (*Cucurbita maxima* D.) was extracted at harvest, 5 or 10 weeks storage. Squash cultivars could be classified into three groups based on harvest starch dry weight content: one cultivar < 1%, six cultivars between 11 and 18%, and five cultivars, all buttercups or related crosses, had > 50%. Starch granules exhibited continuous size distribution, with most granule diameters 2-11 μm. After storage, the proportion of small granules decreased and some cultivars had granule remnants. Squash starches displayed B-type X-ray patterns and high iodine affinities for whole and amylopectin fraction at harvest and after storage. Absolute amylose content ranged from 10.8-21.1% at harvest and tended to decrease after 10 weeks storage. Average amylopectin molecular weight (M_w) at harvest ranged from 2.83-5.52 x 10^8 g/mol. M_w increased after 5 weeks storage, suggesting that amylases prioritized hydrolyzing smaller amylopectin. Amylopectin polydispersity was extremely low after 5 and 10 weeks storage.

KEYWORDS: Starch structure; winter squash; buttercup squash; pumpkin; *Cucurbita maxima*; postharvest; storage

INTRODUCTION

Starch is the abundant storage carbohydrate in plants. Starch structural and functional properties have been studied extensively from a wide variety of botanical sources. However starches from the plant family Cucurbitaceae have largely been ignored, and in particular, *Cucurbita*, the genus primarily comprising of squash and pumpkins, has received little attention. Hurst et al. (1) investigating three buttercup squash cultivars (*Cucurbita maxima* D.) reported considerably high starch levels with 58-66% of dry matter consisting of starch. Corrigan et al. (2) classified squash cultivars into two groups according to their starch contents. High-starch group consisted entirely of buttercup squash cultivars, whereas the low-starch group included cultivars such as Crown, Hubbard and Scarlet Warren.

Sugimoto et al. (3) investigated starch characteristics of two squash, one *C. maxima* D., and one *C. moschata* D. during fruit (pepo) development. Squash starch was shown to exhibit B-type X-ray diffraction pattern, granule diameter ranging from 2.9-8.8 μm and amylose content ranging from 14-23%.

Starch granule characteristics have been studied from squash fruit grown in New Zealand, Mexico and Japan (4). Starch granule diameters were reported to have much wider range (3-35 μm) than reported by Sugimoto et al. (3). Starch was also reported to be located primarily in specific cellular layers closer to the squash fruit exterior.

Recently, we have characterized the starch structural properties from fruit of seven *C. maxima* squash cultivars (Chapter 1). Squash starches exhibited B-type X-ray diffraction patterns and had continuous granule size distribution. Squash starches had low absolute amylose contents and small amylopectins (2-3 x 10^8) with very low polydispersity and

average chain-lengths of DP 27-28. Onset gelatinization temperature (T_o) of squash starches ranged from 60.6°C to 63.5°C, and enthalpy of gelatinization (ΔH) was very high for both native and retrograded starch. Squash starches were shown to have high peak viscosity, final viscosity and setback, and pasting temperature ranging from 65.6°C to 68.8°C.

In our present study, we investigate storage effect on structural properties of twelve winter squash fruit starches, belonging to the species *Cucurbita maxima*, extracted from fruit at harvest and after 5 or 10 weeks of storage. We will correlate the starch structural properties (Chapter 5) with research to be published later on the starch functional properties (Chapter 3), and the textural attributes of raw and cooked squash fruit (Chapter 4).

MATERIALS AND METHODS

Plant Material. Twelve squash cultivars were planted in summer, 2000, at an Iowa State University farm site 1.7 miles south of Ames, Iowa (geographical location 41° 58' 57.5" N, 93° 38' 22.9"), in a randomized complete block (8.23 m x 3.05 m blocks) with 3 replicates (36 plants/replicate). Normal crop husbandry was followed as required. Climatic conditions during the 2000 growing season can be found in Chapter 7. Squash cultivars studied were four buttercups (Cha Cha, Delica, Kurijiman and Sweet Mama), one cross between a buttercup, Green Delicious and a non-buttercup, Table Queen (Hyvita), two Halloween-type (Big Max and Rouge Vif D'Etampes), one Hubbard-type (Warren Scarlet, also known as Red Warren), one Crown-type (Whangaparoa Crown), one Native American Indian squash (Lakota), and two non-commercially developed squash, one from Burkina Faso (Yogorou) and one from the Bolivian/Peruvian border (Zapallo Macre). Seeds were purchased for Kurijiman, Warren Scarlet and Whangaparoa Crown from Webling and

Stewart Ltd., Petone, New Zealand, for Delica from Yates New Zealand Ltd., Onehunga, New Zealand, for Sweet Mama from Henry Field Seed & Nursery Co., Shenandoah, IA, for Lakota and Big Max from W. Atlee Burpee & Co., Warminster, PA, for Rouge Vif D'Etampes from J.W. Jung Seed Co., Randolph, WI, and for Cha Cha from Johnny's Select Seeds Co., Winslow, ME. Hyvita was received as a gift from Dr Henry Munger, Department of Plant Breeding, Cornell Univeristy, Ithaca, NY. Yogorou and Zapallo Macre were both obtained from the USDA, ARS Plant Genetic Resources Unit, Cornell University, Geneva, NY with the accession numbers being PI 490352 and PI 298818, respectively. Squash fruit maturity was adjudged when stalks became woody (5), and this stage had been previously shown to have the highest starch content (6). Squash fruit were stored at 12°C and low humidity for 5 or 10 weeks as these conditions have been previously determined to be optimum for squash (7).

Starch Isolation and Quantification, and Water Content. Starch was isolated from squash fruit using method reported by Badenhuizen (8) with slight modification (9) and further modification in this study. Four randomly selected fruit per replicate, peeled and deseeded, were used for starch isolation. Squash fruit was ground through a meat grinder ("The Butcher Shop", item#402, Krups North America Inc., Peoria, IL), due to its hardness, and immediately blended in 0.3% (w/v) sodium metabisulfite and then filtered through 106 μm mesh. Filtrate was washed with 10% toluene in 0.1 M NaCl, and this step was found to be critical in obtaining high starch yields. It was observed that during toluene/salt washes, considerable amount of starch is removed with the lipid/protein toluene layer. Toluene/salt waste removed after each wash was collected and allowed to stand for at least 12 h, in which

time further starch had formed a sediment. Removal of starch in toluene/salt waste may simulate losses of starch in commercial extraction, but our objectives were to investigate role of starch structure in functional properties of a food system in which starch was not being extracted. Therefore it was important for meaningful analysis to extract most of the endogenous starch. Collection of starch sediment from toluene/salt wash was repeated until little starch was obtained, which ranged from 5-20 times. Starch yield from the toluene/salt waste that is usually discarded ranged from 8-50% of the total starch yield and was almost always at least 15%. We did not investigate whether the starch obtained from toluene/salt waste differed in granule size, shape or physicochemical properties from the remainder. Toluene/salt washed starch was washed three times with distilled water, twice with ethanol, and then recovered by filtration using Whatman No. 4 filter paper. Purified starch cake was dried in a convection oven at 35°C for 48 h. Starch yields varied due to cultivar and storage time, therefore results presented in this study are for the cultivar x storage treatments in which sufficient starch was present to conduct analysis. Water content of squash fruit, with skins and seeds removed, was determined by freeze-drying. Total starch content of freeze-dried squash fruit powders, measured in duplicate, was determined using total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), based on AOAC method 996.11, AACC method 76.13 and ICC standard method No. 168, in which fruit powders are hydrolyzed with α-amylase and amyloglucosidase, and subsequent glucose content determined using glucose oxidase-peroxidase. Internal standards of corn starch were added to samples to check quantitation and recovery of starch.

Starch Granule Morphology by Scanning Electron Microscopy. Starch granules, spread on silver tape and mounted on a brass disk, were coated with gold/palladium (60/40) for each cultivar. Sample images were observed at 1500x magnification under a scanning electron microscope (JOEL model 1850, Tokyo, Japan) following the method of Jane et al. (10).

Crystalline Structure by X-ray Diffractometry. Crystallinity of starch granules was studied using X-ray diffractometry. X-ray diffraction patterns were obtained with copper, Kα radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI). Analysis was conducted following procedure of Song and Jane (11). Degree of crystallinity was calculated based on method of Hayakawa et al. (12). The following equation was used to determine percent crystallinity:

$$\text{Crystallinity (\%)} = A_c/(A_c + A_a) \times 100$$

where A_c = crystalline area on the X-ray diffractogram and A_a = amorphous area on the X-ray diffractogram.

Molecular Weight Distribution and Gyration Radius of Amylopectin by High-Performance Size-Exclusion Chromatography (HPSEC). Weight-average molecular weight and *z*-average gyration radius of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). Starch samples, duplicate measurements of each replicate for all cultivars and storage times, were prepared as described by Yoo and Jane (13). The HPSEC system consisted of a HP 1050 series isocratic

pump (Hewlett Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., Tokyo, Japan) were used. Operating conditions and data analysis are described by Yoo and Jane (14), except flow rate was 0.4 mL/min and sample concentration was 0.8 mg/mL.

Absolute Amylose Contents by Potentiometric Autotitration. Absolute amylose content of starch was determined following the procedure of Lu et al. (15). Analysis was based on iodine affinities of defatted whole starch and amylopectin fraction using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). Starch samples were defatted using a 90% dimethyl sulfoxide (DMSO) solution, followed by alcohol precipitation. Determination of amylose content was duplicated for each squash cultivar replicate at each storage time.

Amylopectin Branch Chain-Length Distribution by High-Performance Anion-Exchange Chromatography (HPAEC) and by HPSEC. Amylopectin was fractionated by complexing amylose with *n*-butanol as described by Schoch (16). Amylopectin (2 mg/mL) was defatted in boiling 90% DMSO for 1 h, followed by stirring for 24 h and then debranched using isoamylase (EC 3.2.1.68 from *Pseudomonas amyloderamosa*) (EN102, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) as described by Jane and Chen (17). Branch chain-length distribution of amylopectin was determined using an HPAEC

system (Dionex-300, Sunnyvale, CA) equipped with an amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, A-7255, Sigma Chemical Co., St Louis, MO) post-column, on-line reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (18). PA-100 anion exchange analytical column (250 x 4 mm, Dionex, Sunnyvale, CA) and a guard column were used for separating debranched amylopectin samples. Gradient profile of eluents and operating conditions were described previously (19). Branch chain-length distribution of amylopectin was also analyzed to determine extra-long branch-chains by using a HPSEC equipped with a RI detector. Operating conditions have been described earlier (19), except flow rate was 0.4 mL/min, analytical column used for analysis was Shodex OH pak SB-803HQ (Showa Denko K.K., Tokyo, Japan) and sample concentration was 0.8 mg/mL. HPAEC-ENZ-PAD and HPSEC analysis were quadruplicated for the former and duplicated for latter analysis for each replicate of each cultivar at each storage time.

Data analysis. All statistical significance tests were calculated using SAS (20) and applying Tukey difference test (21) at the 5% level of significance.

RESULTS AND DISCUSSION

Water and Starch Content. Water content of squash fruit (excluding seeds and skins) was significantly different between cultivars at harvest and after both storage times ($P < 0.0001$ for all) (**Table 1**). Squash cultivars that had fruit with greater than 88% water at harvest, had no or slight increases in water content after 10 weeks of storage. The four buttercup squash cultivars, plus the closely related buttercup-cross (Hyvita) had the lowest water content at harvest, and Cha Cha, Hyvita and Sweet Mama had the greatest increase in water content during storage. Fruit from some squash cultivars had very little dry matter,

with fruit from the five cultivars that had above 91% water content after 10 weeks of storage having much heavier fruit weight than the lighter buttercups that were all below 86% water (based on obvious differences in fruit weight during handling of fruit and not from weighing on a balance).

Water content of squash fruit has been reported previously. Corrigan et al. (2) reported similar findings of water content for Warren Scarlet and Delica, but considerably lower water content was reported for Kurijiman, Sweet Mama and Whangaparoa Crown. Hurst et al. (1) reported lower water content (76.5%) for Delica. Despite Corrigan et al. (2) finding higher dry matter content for three squash cultivars, their reported starch content of the two buttercup cultivars was substantially lower than what we report in our study.

Starch content of squash fruit (excluding seeds and skins) between the cultivars was significantly different at harvest and after both storage times ($P < 0.0001$ for all). At harvest, squash cultivars could be classified into three distinct groups based on their starch content: (i) squash cultivars with less than one percent (dry weight basis (db)) starch (Big Max), (ii) squash cultivars with a medium percentage (11-18% db) of starch (Lakota, Rouge Vif D'Etampes, Warren Scarlet, Whangaparoa Crown, Yogorou and Zapallo Macre) and (iii) squash cultivars with high percentage (> 50% db) of starch, which consisted of the four buttercups and closely related Hyvita (Cha Cha, Delica, Hyvita, Kurijiman and Sweet Mama).

Starch contents for Delica, Kurijiman, Sweet Mama, Warren Scarlet and Whangaparoa Crown have been previously reported for squash grown in New Zealand (2), in which only Whangaparoa Crown had starch content comparable to the levels we report (16.3% db). The starch contents reported for Warren Scarlet (3.3% db), Delica (13.4% db),

Kurijiman (41.4% db) and Sweet Mama (41.2% db), were all substantially lower than what we found. This is surprising considering Wilhelm et al. (22) has shown that starch synthase enzymes are down regulated when temperature exceeds 25°C, and Iowa experiences considerably higher durations above 25°C relative to Levin, New Zealand. In four years of growing squash, we have not observed Delica with such low starch content as reported by Corrigan et al. (2), and we suspect there were unfavorable environmental conditions during their growth. Further support of this is provided by Hurst et al (1) who reported Delica, grown at same research center as that reported by Corrigan et al. (2), had 61% of its dry matter as starch, which is in agreement with our results.

Additional explanation for the large differences in starch content reported for the same cultivar could be due to high variability in starch content of individual squash fruit. In our study, we observed large variation in starch content for Warren Scarlet, Yogorou and Zapallo Macre. This large variation meant that pooled variance analysis was ineffective in determining all significant differences between squash cultivars for starch content. For example, at harvest, the three replicates of Rouge Vif D'Etampes and Big Max ranged from 14.2-14.7% and 0.7-0.9%, respectively, yet they were not significantly different due to the contribution to pooled variance from the highly variable starch content for the replicates of Warren Scarlet and Yogorou. This necessitated use of the Friedman two way analysis of variance by rank test (23) in an attempt to establish significant differences in starch content between cultivars, but this test could not establish any new differences among cultivars.

Granule Morphology. Squash starches exhibited continuous granule size distribution. Squash granules were primarily 1.5-3 μm, 4.5-6.5 μm or 9-12 μm in diameter

(**Figure 1a** and **1b**), except Rouge Vif D'Etampes which had few granules above 8 μm in diameter. A greater proportion of large starch granules were spherical, whereas medium and small size granules tended to be irregular. Many large starch granules were dome-shaped with one side flattened, often with a small indentation, and this may indicate the presence of compound granules. Larger indentations were mainly observed on medium size granules (5-8 μm in diameter). Granule indentations are usually attributed to collision of expanding spherulites (24), which would occur for compound starch granules. The endocarp region of squash fruit flesh has been reported to have compound starch granules present (4).

Previous study of starch from two squash cultivars during development and at harvest, including Ebisu (close genetic relative to Delica), found average granule size to be 7.0-7.5 μm, with low size variation (3). However, these researchers studying these same squash during storage presented scanning electron micrographs that do exhibit wider variation in starch granule size distribution with similar ranges in granule diameters as we report (25). The greater variation in granule size distribution that we observed for squash starch, extracted from fruit at harvest, compared with that of Sugimoto et al. (3), could be due to different guidelines used to adjudge harvest maturity. Yoshida (4) reported starch granule diameters up to 35 μm for squash grown in New Zealand, Mexico and Japan, but we rarely observed granules wider than 15 μm and no granules exceeded 18 μm.

Squash cultivars with high levels of starch after 5 and 10 weeks storage (buttercups plus Hyvita) showed little increase in partially degraded starch granules (**Figure 2**). However there was an overall increase in average starch granule size after 5 and 10 weeks, because of a reduction in granules less than 3 μm in diameter and increasing abundance of granules with diameters of 6-9 μm. Squash cultivars that had rapid starch degradation after 5

weeks of storage showed extensive granule degradation, with many granules being "bowl-shaped" (**Figure 2C**).

Crystalline Structure. Squash starches all exhibited typical B-type X-ray diffraction patterns (**Figure 3**), with a strong peak at $2\theta = 17.2°$, another peak at $2\theta = 5.6°$, and a split peak at $2\theta = 22\text{-}24°$. Kurijiman , Warren Scarlet and Whangaparoa Crown were previously reported to have an additional peak at $2\theta = 28.2°$ (5), but this peak was not observed in any of the squash cultivars in this study. Percentage crystallinity of starch extracted from fruits at harvest for Cha Cha, Delica, Hyvita, Kurijiman, Lakota, Rouge Vif D'Etampes, Sweet Mama, Warren Scarlet, Whangaparoa Crown, Yogorou and Zapallo Macre, calculated based on X-ray diffractograms, was 43.2, 40.7, 38.4, 40.0, 48.2, 40.1, 41.3, 41.0, 35.7, 41.1, and 41.4 respectively. After 5 weeks storage, percentage crystallinity for Cha Cha, Delica, Hyvita, Kurijiman, Lakota and Sweet Mama was 40.0, 39.0, 36.5, 40.8, 33.9 and 39.0 respectively, and after 10 weeks storage, for Cha Cha, Delica and Kurijiman, percent crystallinity was 40.1, 47.7 and 36.7 respectively. Starch percent crystallinity decreased for most squash cultivars after 5 weeks storage.

Amylose Content. Iodine affinities for defatted whole starch and the corresponding apparent amylose contents were not significantly different at harvest, but were significant at both 5 and 10 weeks storage ($P < 0.0001$) (**Table 3, 4** and **5**). Apparent amylose content was higher for the high-starch squash cultivars at both 5 and 10 weeks storage (**Table 4** and **5**). Iodine affinities of both whole starch and amylopectin fraction, from fruit at harvest, in this study were considerably higher than reported previously for squash (Chapter 1), and this is close to the highest values reported for native starches (26). Differences could be seasonal effects as Tester et al. (27) has shown wheat grown at 4°C higher had apparent amylose

contents five percent higher (i.e. from 26 to 31%), and Araki et al. (28) found 3% difference in wheat apparent amylose contents between two consecutive seasons. Additionally, cereal starches have received extensive breeding improvements over many centuries to eliminate genetic variation due to climate and other environmental conditions, whereas squash have received little attention from plant breeders. Furthermore, apparent amylose is a measure of two components, so variation in amylopectin branch chain-length distribution could also be contributing to the variation. Absolute amylose contents reported in this study were similar to the previous study, except for Sweet Mama (Chapter 1), suggesting differences in proportion of long-chain amylopectins are contributing to variation in apparent amylose content. Absolute amylose contents of high-starch cultivars were higher than remaining cultivars at harvest and 5 weeks storage. Sweet Mama and Kurijiman had substantially higher absolute amylose contents after 5 weeks storage that was not observed in any other cultivar. Since amylose content has been shown to be higher in larger diameter granules (29), Sweet Mama and Kurijiman may have had more small granules degraded after 5 weeks storage relative to other squash cultivars.

Amylopectin Molecular Weight and Size. Average molecular weight, polydispersity, and gyration radius of squash amylopectins, from fruit at harvest, are shown in **Table 6**. All squash starches at harvest had amylopectins of greater molecular weight than reported previously for squash amylopectins (Chapter 1), but amylopectin polydispersity was slightly higher in this study. Average molecular weight, polydispersity, gyration radius and density of amylopectins for the squash cultivars were not significantly different.

After 5 weeks storage, average amylopectin molecular weight increased for all 8 squash cultivars except Lakota (**Table 7**), implying that during storage of squash fruit, there

is selective degradation of smaller amylopectin molecules. Polydispersity of amylopectins, after five weeks storage, decreased to values lower than reported previously for any other starches (Chapter 1), and the uniformity of squash fruit amylopectin is remarkable for biological molecules of such magnitude. The increased uniformity in amylopectin weight is most likely due to the selective degradation of smaller amylopectin molecules, possibly because squash amylases were unable to hydrolyze a high proportion of high-molecular weight amylopectins located primarily in larger diameter granules. Decrease in number of small granules (< 3μm) observed after 5 or 10 weeks storage (**Figure 1** and **2**) suggests that amylopectins of small molecular weight are predominantly in the smaller starch granules. Larger-weight amylopectins would proportionally be less degraded because they are in larger size granules which the amylases did not degrade. If this scenario is true, we could not determine if squash amylases are impeded from entering large granules to degrade high molecular weight amylopectins just as effectively as low molecular weight amylopectins, or whether high molecular weight amylopectins contribute to the resistance of the granule, making it difficult for amylases to tunnel into the hilum. Similar size distribution of amylopectins in all starch granule sizes has been reported (30). Li et al. (29) has reported higher average amylopectin branch chain-length in smaller granules, but that does not necessarily translate to higher molecular weight, and in contrast, Tang et al. (31) reported higher average amylopectin branch chain-length in larger granules.

Average molecular weight and density were higher, and polydispersity was lower for squash amylopectins after 10 weeks storage compared to that at harvest (**Table 8**). Gyration radius of squash starches after 10 weeks storage, like at harvest, were not significantly different despite significant differences observed after 5 weeks storage. Gyration radius

increased for all squash amylopectins during storage, except Lakota and Sweet Mama, providing further support that smaller amylopectins are preferentially degraded after harvest. Squash starch gyration radius was narrower than reported previously for all other B-type starches, except *ae wx* maize which was comparable in size (14).

Debranched Amylopectin Chain Distribution. Amylopectin branch chain-length distribution of squash starches, from fruit at harvest, is shown in **Table 9**. Squash starches exhibited typical B-type starch characteristics of high proportion of long amylopectin chains (DP ≥ 37) and average amylopectin branch chain-length. For fruit at harvest, the only significant difference observed among the squash cultivars was average amylopectin branch chain-length ($P = 0.002$), with branch chains of Warren Scarlet significantly shorter than Cha Cha, Delica, Sweet Mama, Whangaparoa Crown and Yogorou. Chromatograms of amylopectin branch chain-length distribution for the 12 squash cultivars are shown in **Figure 4a**, **4b** and **4c** also illustrate the lack of long amylopectin chains for Warren Scarlet. Most squash cultivars have similar amylopectin chain-length distribution chromatograms, but the two non-commercially available squash had starches with distinct chromatograms. The peculiar chromatogram of Zapallo Macre is due to high variability in fruit size, shape and color of this cultivar, in which all fruit had starch, and further on, amylopectin, extracted separately, and fruit also had variability in amylopectin size distribution which when combined results in the unusual size distribution. Yogorou amylopectins had relatively high amounts of intermediate chain-lengths compared to all other squash cultivars except Zapallo Macre, with no anomalous circumstances to explain this difference.

After 5 weeks storage, proportion of amylopectin branch chain-lengths of DP ≥ 37 decreased for all squash cultivars, except Kurijiman, and proportion of very short chains (DP

6-9) increased for all cultivars, except Hyvita (**Table 10**). For amylopectin from fruit stored 5 weeks, Kurijiman had significantly higher proportion of amylopectin branch chain-lengths of DP ≥ 37 and lower proportion of DP 13-24 than Hyvita. Average amylopectin branch chain-length for Kurijiman was significantly longer than Delica, Hyvita and Zapallo Macre ($P = 0.004$). Chromatograms of amylopectin branch chain-length distribution for most squash cultivars is similar after 5 weeks storage and chromatogram of Zapallo Macre now has normal appearance (**Figure 5a** and **5b**). All squash cultivars, except Hyvita, had higher proportion of amylopectin branch chain-lengths of DP ≥ 37 after 10 weeks storage (**Table 11**) than at harvest.

For fruit stored 10 weeks, Cha Cha and Delica had significantly higher proportion of amylopectin branch chain-lengths of DP ≥ 37 than Hyvita ($P = 0.02$). Kurijiman and Delica had significantly lower proportion of amylopectin branch chain-lengths of DP 25-36 than Hyvita ($P = 0.01$). Cha Cha had significantly lower proportion of amylopectin branch chain-lengths of DP 6-12 than Hyvita ($P = 0.04$). Average amylopectin branch chain-length of Cha Cha and Sweet Mama was significantly longer than Hyvita ($P = 0.01$). Amylopectin branch-chain length distribution separated, to some extent, the buttercup-cross Hyvita from the buttercup cultivars. Chromatograms of amylopectin branch chain-length distribution for fruit stored 10 weeks, show the greater proportion of longer amylopectin chain-lengths compared to at harvest, and Cha Cha and Hyvita have a slight shoulder at DP 25-33 relative to the chromatograms of amylopectins from fruit at harvest (**Figure 6a** and **6b**). Amylopectin average molecular weight increased and amylopectin polydispersity decreased after 10 weeks storage, most likely attributed to higher proportion of long amylopectin branch chains.

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Table 1

Water content (%) of squash fruit at harvest and after 5 or 10 weeks storage at 12°C*.

Cultivar	Harvest#	5 weeks	10 weeks
Big Max	94.2[a]	94.9[a]	95.5[a]
Cha Cha	71.8[d]	76.6[e]	77.4[e]
Delica	80.1[c]	79.1[de]	81.5[de]
Hyvita	84.4[bc]	87.1[bc]	88.6[bc]
Kurijiman	79.0[cd]	78.7[de]	81.8[de]
Lakota	89.5[ab]	90.3[ab]	90.8[ab]
Rouge Vif D'Etampes	94.3[a]	93.7[a]	95.2[a]
Sweet Mama	79.4[c]	82.6[cd]	85.9[cd]
Warren Scarlet	88.9[ab]	90.4[ab]	91.1[ab]
Whangaparoa Crown	91.0[ab]	93.5[a]	92.4[ab]
Yogorou	92.3[a]	92.2[ab]	91.2[ab]
Zapallo Macre	88.4[ab]	90.5[ab]	89.3[bc]
	$P < 0.0001$♣	$P < 0.0001$	$P < 0.0001$

Water contents were averaged from three replicates.

*Values with different letters denote cultivar differences at the 5% level of significance for each comparison between cultivars in the respective column.

♣ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 2

Starch content (% dry weight) of squash fruit at harvest and after 5 or 10 weeks storage at 12°C*.

Cultivar	Harvest#	5 weeks	10 weeks
Big Max	0.8^{b}	0.2^{c}	0.3^{d}
Cha Cha	61.2^{a}	33.3^{a}	34.9^{a}
Delica	56.7^{a}	33.0^{a}	21.0^{bc}
Hyvita	54.4^{a}	12.5^{bc}	6.5^{d}
Kurijiman	55.2^{a}	34.3^{a}	24.7^{ab}
Lakota	17.6^{b}	7.0^{c}	2.8^{d}
Rouge Vif D'Etampes	14.5^{b}	0.6^{c}	0.6^{d}
Sweet Mama	52.3^{a}	20.2^{b}	10.2^{cd}
Warren Scarlet	17.4^{b}	2.7^{c}	1.1^{d}
Whangaparoa Crown	14.2^{b}	0.8^{c}	1.7^{d}
Yogorou	11.3^{b}	0.3^{c}	1.2^{d}
Zapallo Macre	14.7^{b}	3.6^{c}	0.9^{d}
	$P < 0.0001$♣	$P < 0.0001$	$P < 0.0001$

Starch contents were averaged from two duplicates of each of three replicates.

*Values with different letters denote cultivar differences at the 5% level of significance for each comparison between cultivars in the respective column.

♣ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 3

Iodine affinities, apparent amylose and absolute amylose contents for squash fruit defatted starches at harvest.[♦]

Cultivar	Iodine Affinity		Apparent amylose	Absolute amylose
	whole starch	amylopectin fraction	content (%)[*]	content (%)[#]
Cha Cha	7.50	3.88^{abc}	37.7	18.2^{abc}
Delica	7.27	3.89^{abc}	36.5	17.0^{abc}
Hyvita	6.70	3.68^{bc}	33.7	18.3^{abc}
Kurijiman	6.89	2.92^{c}	34.6	20.0^{ab}
Lakota	6.44	3.84^{abc}	32.4	13.1^{bc}
Rouge Vif D'Etampes	6.91	4.76^{a}	34.7	10.8^{c}
Sweet Mama	7.71	3.51^{ab}	38.7	21.1^{a}
Warren Scarlet	7.37	4.05^{bc}	37.0	16.7^{abc}
Whangaparoa Crown	7.16	3.78^{bc}	36.0	17.0^{abc}
Yogorou	7.44	4.09^{ab}	37.4	16.8^{abc}
Zapallo Macre	6.88	3.75^{bc}	34.6	15.7^{abc}
	$P = 0.09$[♠]	$P = 0.0006$	$P = 0.09$	$P = 0.008$

Apparent amylose contents were averaged from two analyses for each of three replicates.; Values were calculated from dividing iodine affinity by a factor of 0.199.

[#] Absolute amylose contents were averaged from two analyses for each of three replicates.; Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.

[♦] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♠] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 4

Iodine affinities, apparent amylose and absolute amylose contents for squash fruit defatted starches extracted from fruit stored for 5 weeks.[♦]

Cultivar	Iodine Affinity		Apparent amylose content (%)[*]	Absolute amylose content (%)[#]
	whole starch	amylopectin fraction		
Cha Cha	7.43^{a}	4.12^{a}	37.3^{a}	16.6^{cd}
Delica	6.99^{a}	3.27^{abc}	35.1^{a}	18.7^{bc}
Hyvita	6.54^{ab}	4.02^{a}	32.9^{ab}	12.7^{cde}
Kurijiman	7.04^{a}	1.58^{d}	35.4^{a}	27.5^{a}
Lakota	4.94^{cd}	2.29^{bcd}	24.8^{cd}	13.3^{cde}
Sweet Mama	6.35^{ab}	1.24^{d}	31.9^{ab}	25.6^{ab}
Warren Scarlet	4.08^{d}	1.78^{cd}	20.5^{d}	11.5^{de}
Zapallo Macre	5.52^{bc}	3.79^{ab}	27.7^{bc}	8.7^{e}
	$P = < 0.0001$[♠]	$P = < 0.0001$	$P = < 0.0001$	$P = < 0.0001$

Apparent amylose contents were averaged from two analyses for each of three replicates.; Values were calculated from dividing iodine affinity by a factor of 0.199.

[#] Absolute amylose contents were averaged from two analyses for each of three replicates.; Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.

[♦] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♠] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 5

Iodine affinities, apparent amylose and absolute amylose contents for squash fruit defatted starches extracted from fruit after 10 weeks storage.[♦]

Cultivar	Iodine Affinity		Apparent amylose	Absolute amylose
	whole starch	amylopectin fraction	content (%)[*]	content (%)[#]
Cha Cha	7.49[a]	4.04[a]	37.6[a]	17.3[ab]
Delica	6.06[cd]	3.35[ab]	30.5[cd]	13.6[ab]
Hyvita	6.62[bc]	3.92[ab]	33.3[bc]	13.6[ab]
Kurijiman	6.78[b]	2.85[b]	34.1[b]	19.7[a]
Sweet Mama	5.82[d]	3.56[ab]	29.2[d]	11.4[b]
Zapallo Macre	4.25[e]	3.00[ab]	21.3[e]	15.1[ab]
	$P = < 0.0001$[♠]	$P = 0.02$	$P = < 0.0001$	$P = 0.02$

Apparent amylose contents were averaged from two analyses for each of three replicates.; Values were calculated from dividing iodine affinity by a factor of 0.199.

[#] Absolute amylose contents were averaged from two analyses for each of three replicates.; Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.

[♦] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♠] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 6

Average amylopectin molecular weight, polydispersity, gyration radius and density of squash fruit starches extracted from fruit at harvest.*

Cultivar#	M_w x 10^8 (g/mol)♣	Polydispersity (M_w)	R_z (nm)♦	ρ (g/mol/nm^3)♠
Cha Cha	3.46	1.38	309	11.7
Delica	2.83	1.95	297	10.8
Hyvita	4.00	1.51	318	12.4
Kurijiman	3.05	1.82	299	11.4
Lakota	5.52	1.82	349	13.0
Rouge Vif D'Etampes	3.48	1.51	304	12.4
Sweet Mama	4.15	1.43	329	11.7
Warren Scarlet	3.73	1.54	317	11.7
Whangaparoa Crown	3.88	1.41	313	12.7
Yogorou	3.28	1.69	310	11.0
Zapallo Macre	3.37	1.95	311	11.2
	$P = 0.82$☼	$P = 0.75$	$P = 0.64$	$P = 0.84$

* Data were obtained from two injections of all three replicates.

\# Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; Freshly prepared starch aqueous solution (100 µL; 0.8 mg/mL) was injected to HPSEC system.

♣ *weight*-average molecular weight.

♦ *z*-average radius of gyration.

♠ Density is equal to M_w/R_z^3.

☼ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 7

Average amylopectin molecular weight, polydispersity, gyration radius and density of squash fruit starches extracted from fruit after 5 weeks of storage.[*♥]

Cultivar[#]	M_w x 10^8 (g/mol)[♣]	Polydispersity (M_w)	R_z (nm)[♦]	ρ (g/mol/nm^3)[♠]
Cha Cha	4.07^{bc}	1.18	314^{bc}	13.2^{ab}
Delica	4.00^{bc}	1.24	316^{bc}	12.6^{b}
Hyvita	5.31^{a}	1.26	333^{a}	14.3^{a}
Kurijiman	3.72^{bc}	1.26	313^{bc}	12.2^{bc}
Lakota	3.53^{c}	1.20	321^{ab}	10.7^{c}
Sweet Mama	4.38^{b}	1.18	323^{ab}	12.9^{ab}
Warren Scarlet	4.57^{ab}	1.17	321^{ab}	13.8^{ab}
Zapallo Macre	3.46^{c}	1.42	303^{c}	12.5^{b}
	$P = < 0.0001$[☼]	$P = 0.23$	$P = 0.0001$	$P = < 0.0001$

[*] Data were obtained from two injections of all three replicates.

[#] Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; Freshly prepared starch aqueous solution (100 μL; 0.8 mg/mL) was injected to HPSEC system.

[♣] *weight*-average molecular weight.

[♦] *z*-average radius of gyration.

[♠] Density is equal to M_w/R_z^3.

[♥] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[☼] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 8

Average amylopectin molecular weight, polydispersity, gyration radius and density of squash fruit starches extracted from fruit after 10 weeks of storage.[*♥]

Cultivar[#]	M_w x 10^8 (g/mol)[♣]	Polydispersity (M_w)	R_z (nm)[♦]	ρ (g/mol/nm^3)[♠]
Cha Cha	3.74	1.32[b]	315	12.0
Delica	4.01	1.29[b]	320	12.2
Hyvita	4.51	1.40[b]	324	13.3
Kurijiman	4.27	1.16[b]	319	13.2
Sweet Mama	4.52	1.26[b]	321	13.7
Zapallo Macre	4.67	1.73[a]	326	13.5
	$P = 0.50$[☼]	$P = 0.001$	$P = 0.35$	$P = 0.52$

[*] Data were obtained from two injections of all three replicates.

[#] Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; Freshly prepared starch aqueous solution (100 µL; 0.8 mg/mL) was injected to HPSEC system.

[♣] *weight*-average molecular weight.

[♦] *z*-average radius of gyration.

[♠] Density is equal to M_w/R_z^3.

[♥] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[☼] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 9

Branch chain-length distributions of squash fruit amylopectins purified from starch extracted from fruit at harvest[*#♦].

Cultivar	Peak DP		Average	Percent distribution				
	I	II	CL	DP 6-9	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37
Cha Cha	12.9	49.4	28.8[a]	4.9	14.1	38.4	17.1	30.2
Delica	12.9	48.9	28.0[a]	5.5	15.3	39.5	16.0	28.9
Hyvita	12.8	48.4	26.9[ab]	5.8	16.1	39.9	17.4	26.3
Kurijiman	13.2	49.5	27.3[ab]	5.4	15.4	40.5	16.6	27.1
Lakota	12.8	49.3	27.3[ab]	5.7	15.6	39.5	16.9	27.4
Rouge Vif D'Etampes	13.1	49.4	27.1[ab]	5.3	14.9	40.1	17.5	28.0
Sweet Mama	13.2	49.0	28.3[a]	4.9	14.4	39.5	16.5	29.3
Warren Scarlet	12.7	49.0	25.7[b]	6.1	16.7	41.0	16.7	25.6
Whangaparoa Crown	12.7	48.2	28.3[a]	6.0	15.9	38.2	16.4	29.1
Yogorou	12.9	49.3	28.1[a]	6.1	15.9	38.1	17.2	28.2
Zapallo Macre	13.6	49.2	28.5[a]	5.2	14.7	39.4	16.7	28.7
	$P = 0.08$[♣]	$P = 0.23$	$P = 0.002$	$P = 0.27$	$P = 0.13$	$P = 0.15$	$P = 0.91$	$P = 0.08$

[*] Grouping of degree of polymerization (DP) numbers followed that of Hanashiro et al. (32).

[♦] Values comprise of four injections for all three replicates.

[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 10

Branch chain-length distributions of squash fruit amylopectins purified from starch extracted from fruit after 5 weeks of storage[*#♦].

Cultivar	Peak DP		Average	Percent distribution				
	I	II	CL	DP 6-9	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37
Cha Cha	13.3	48.8	28.3^{ab}	5.9	15.5	38.9^{ab}	16.4	28.7^{ab}
Delica	12.8	49.0	26.1^{b}	6.6	17.2	41.2^{ab}	16.4	24.6^{ab}
Hyvita	13.2	49.0	26.0^{b}	5.8	16.1	42.2^{a}	17.1	23.8^{b}
Kurijiman	13.3	49.3	28.6^{a}	5.8	14.7	36.5^{b}	17.9	30.2^{a}
Lakota	13.0	49.5	26.4^{ab}	6.8	16.9	41.1^{ab}	15.9	25.6^{ab}
Sweet Mama	12.8	49.3	26.9^{ab}	6.2	16.0	40.0^{ab}	16.8	27.5^{ab}
Warren Scarlet	13.0	49.5	26.5^{ab}	6.4	16.5	40.8^{ab}	16.5	25.6^{ab}
Zapallo Macre	12.5	49.0	26.0^{b}	5.7	15.9	41.3^{ab}	15.6	26.6^{ab}
	$P = 0.20^{\clubsuit}$	$P = 0.98$	$P = 0.004$	$P = 0.78$	$P = 0.39$	$P = 0.04$	$P = 0.76$	$P = 0.03$

[*] Grouping of degree of polymerization (DP) numbers followed that of Hanashiro et al. (32).

[♦] Values comprise of four injections for all three replicates.

[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 11

Branch chain-length distributions of squash fruit amylopectins purified from starch extracted from fruit after 10 weeks of storage[*#♦].

Cultivar	Peak DP		Average	Percent distribution				
	I	II	CL	DP 6-9	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37
Cha Cha	13.7[ab]	49.0	29.7[a]	5.3	13.6[b]	36.8	16.7[ab]	30.9[a]
Delica	14.5[a]	49.3	28.3[ab]	5.8	15.0[ab]	37.3	16.0[b]	30.8[a]
Hyvita	13.0[b]	49.0	25.2[b]	5.9	16.5[a]	41.6	17.8[a]	23.8[b]
Kurijiman	13.0[b]	50.5	28.1[ab]	5.6	15.1[ab]	39.1	16.1[b]	29.1[ab]
Sweet Mama	13.3[ab]	48.3	28.6[a]	5.0	14.4[ab]	39.2	16.5[ab]	29.5[ab]
Zapallo Macre	13.5[ab]	48.0	28.0[ab]	5.5	14.5[ab]	38.5	17.4[ab]	29.0[ab]
	$P = 0.03$[♣]	$P = 0.18$	$P = 0.01$	$P = 0.29$	$P = 0.04$	$P = 0.15$	$P = 0.01$	$P = 0.02$

[*] Grouping of degree of polymerization (DP) numbers followed that of Hanashiro et al. (32).

[♦] Values comprise of four injections for all three replicates.

[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 12

Squash fruit isoamylase-debranched amylopectins, from starch extracted from fruit at harvest and after 5 or 10 weeks storage, measured using HPSEC[♣].

Cultivar	Harvest		5 weeks		10 weeks	
	Peak I (%)[*#]	Peak II (%)	Peak I (%)	Peak II (%)	Peak I (%)	Peak II (%)
Cha Cha	50.9	49.1	47.3	52.7	46.7	53.3
Delica	53.6	46.4	50.2	49.8	48.0	52.0
Hyvita	40.4	59.6	52.1	47.9	44.6	55.4
Kurijiman	46.3	53.7	44.4	55.6	42.5	57.5
Lakota	54.9	45.1	48.0	52.0		
Rouge Vif D'Etampes	52.7	47.3				
Sweet Mama	47.9	52.1	45.6	54.4	49.0	51.0
Warren Scarlet	45.2	54.8	40.9	59.1		
Whangaparoa Crown	55.7	44.3				
Yogorou	55.2	44.8				
Zapallo Macre	49.4	50.6	51.1	48.9	55.3	44.7
	$P = 0.54$[♦]		$P = 0.22$		$P = 0.42$	

Peak I and II represent long (DP > 26) and short (DP < 26) amylopectin chains respectively. Values represent proportion of long and short amylopectin chains.

[#] Values were obtained from two analyses for each of three replicates.

[♣] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♦] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

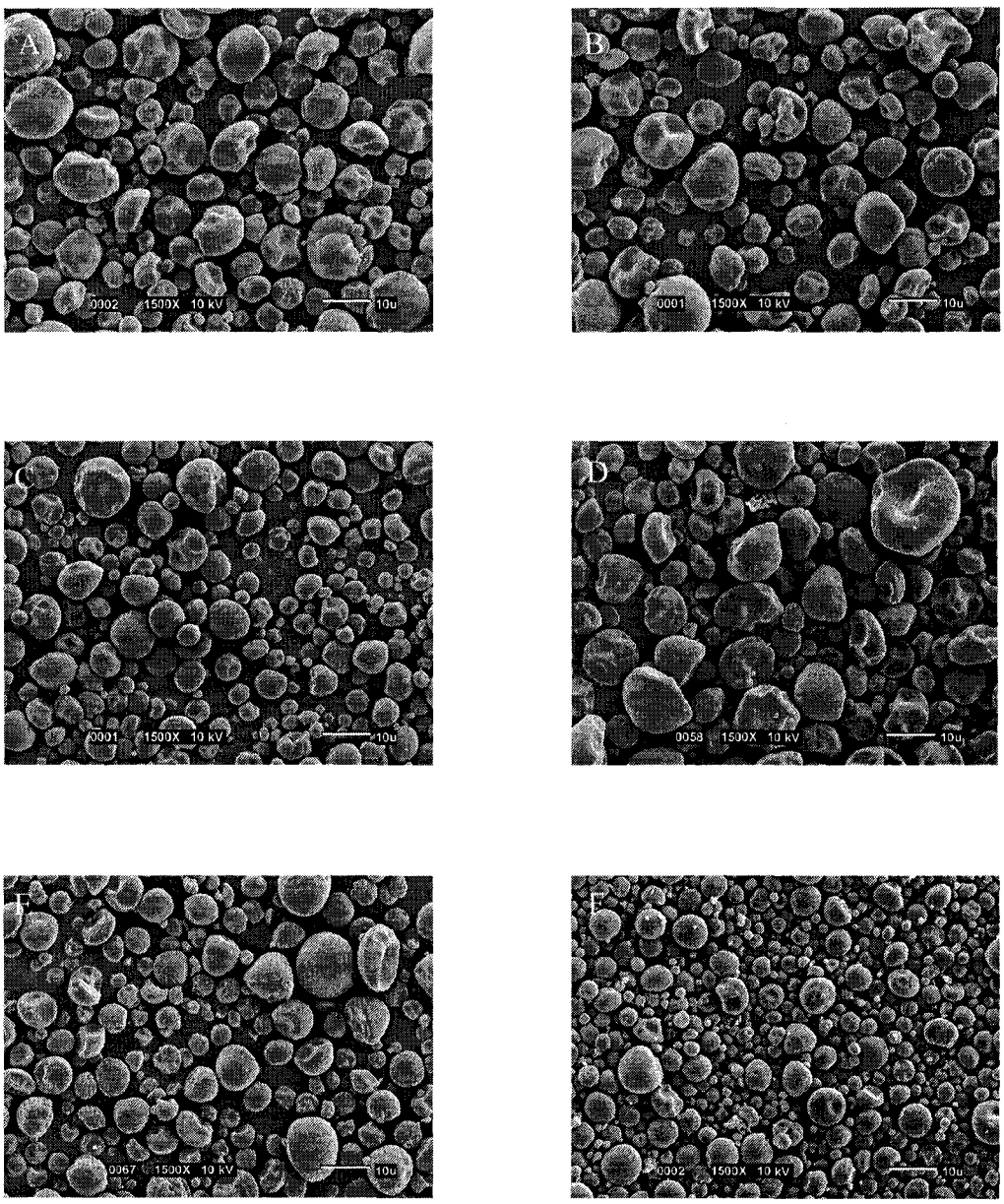

Figure 1a. Scanning electron micrographs of Cha Cha (A), Delica (B), Hyvita (C), Kurijiman (D), Lakota (E) and Rouge Vif D'Etampes (F) squash fruit starches, extracted at harvest (scale bar = 10 μm).

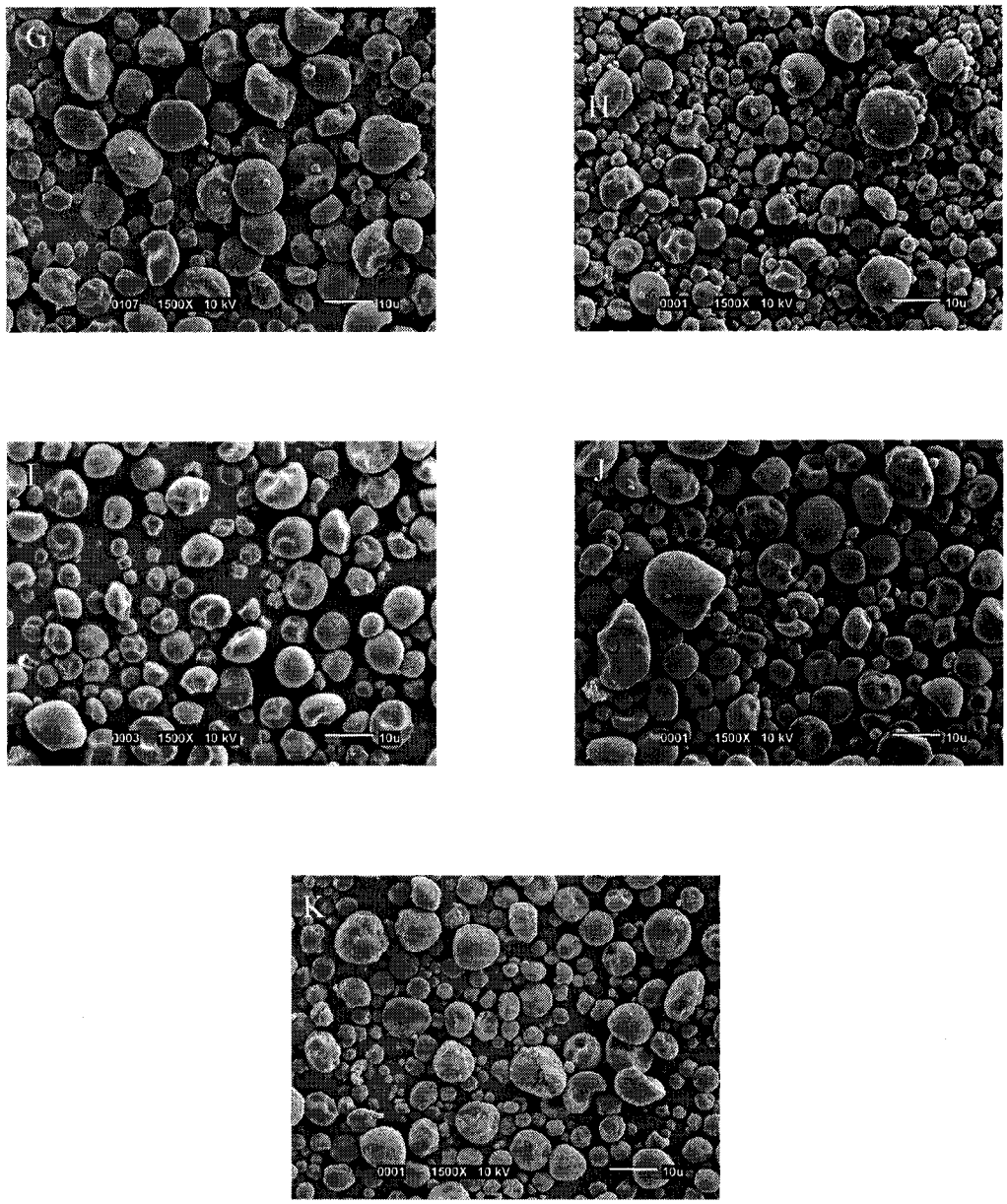

Figure 1b. Scanning electron micrographs of Sweet Mama (G), Warren Scarlet (H), Whangaparoa Crown (I), Yogorou (J) and Zapallo Macre (K) squash fruit starches, extracted at harvest (scale bar = 10 μm).

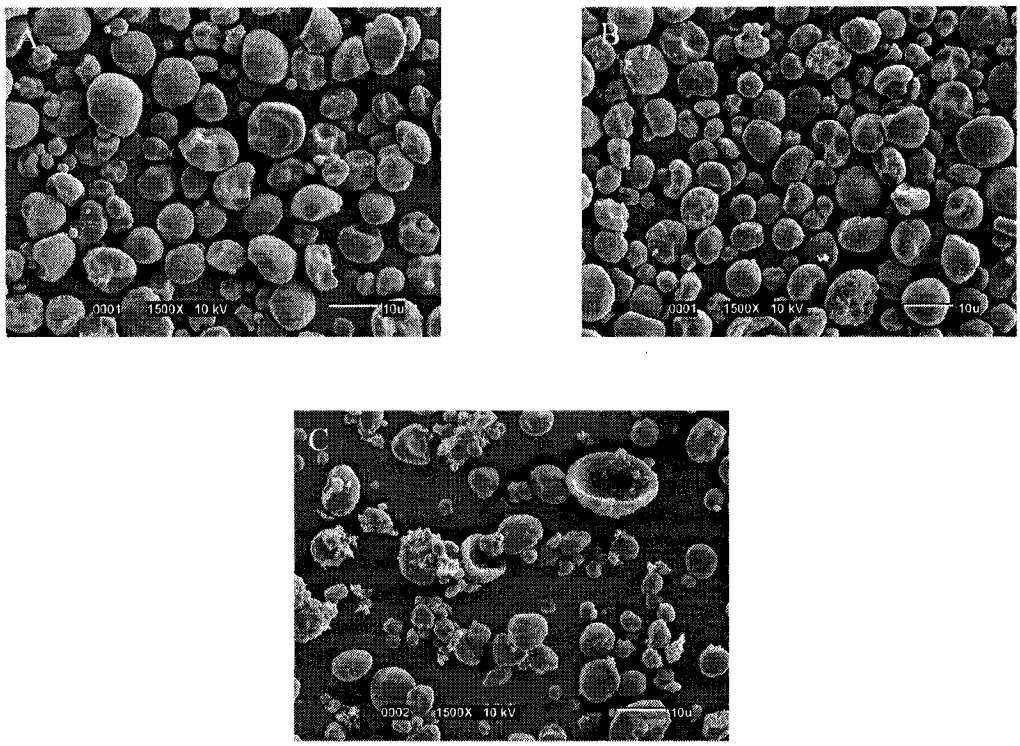

Figure 2. Scanning electron micrographs of squash starch extracted from fruit of Delica after 5 (A) and 10 weeks (B) of storage, and Warren Scarlet (C) after 5 weeks of storage (scale bar = 10 μm).

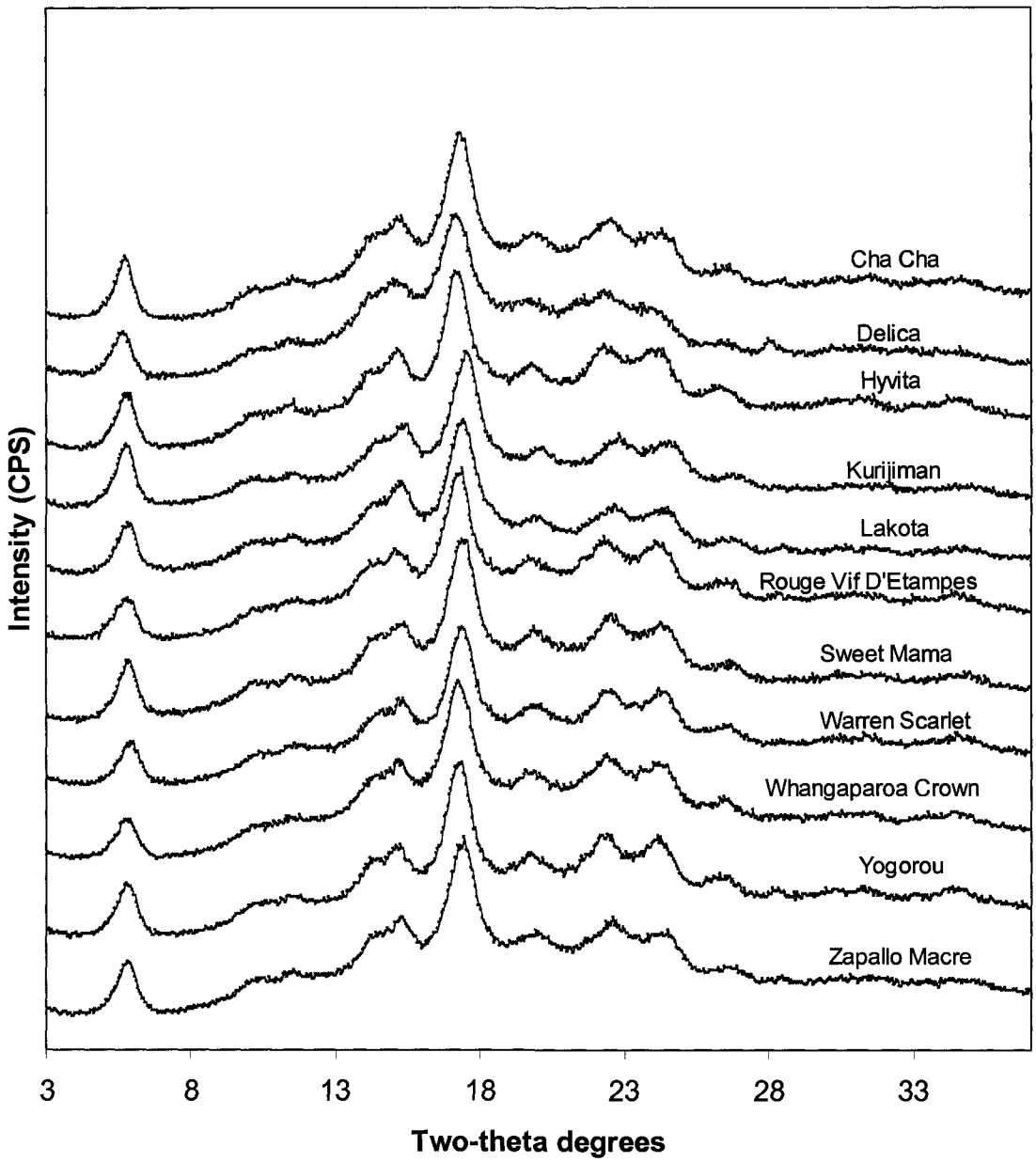

Figure 3. X-ray diffraction patterns of Cha Cha, Delica, Hyvita, Kurijiman, Lakota, Rouge Vif D'Etampes, Sweet Mama, Warren Scarlet, Whangaparoa Crown, Yogorou and Zapallo Macre squash fruit starches.

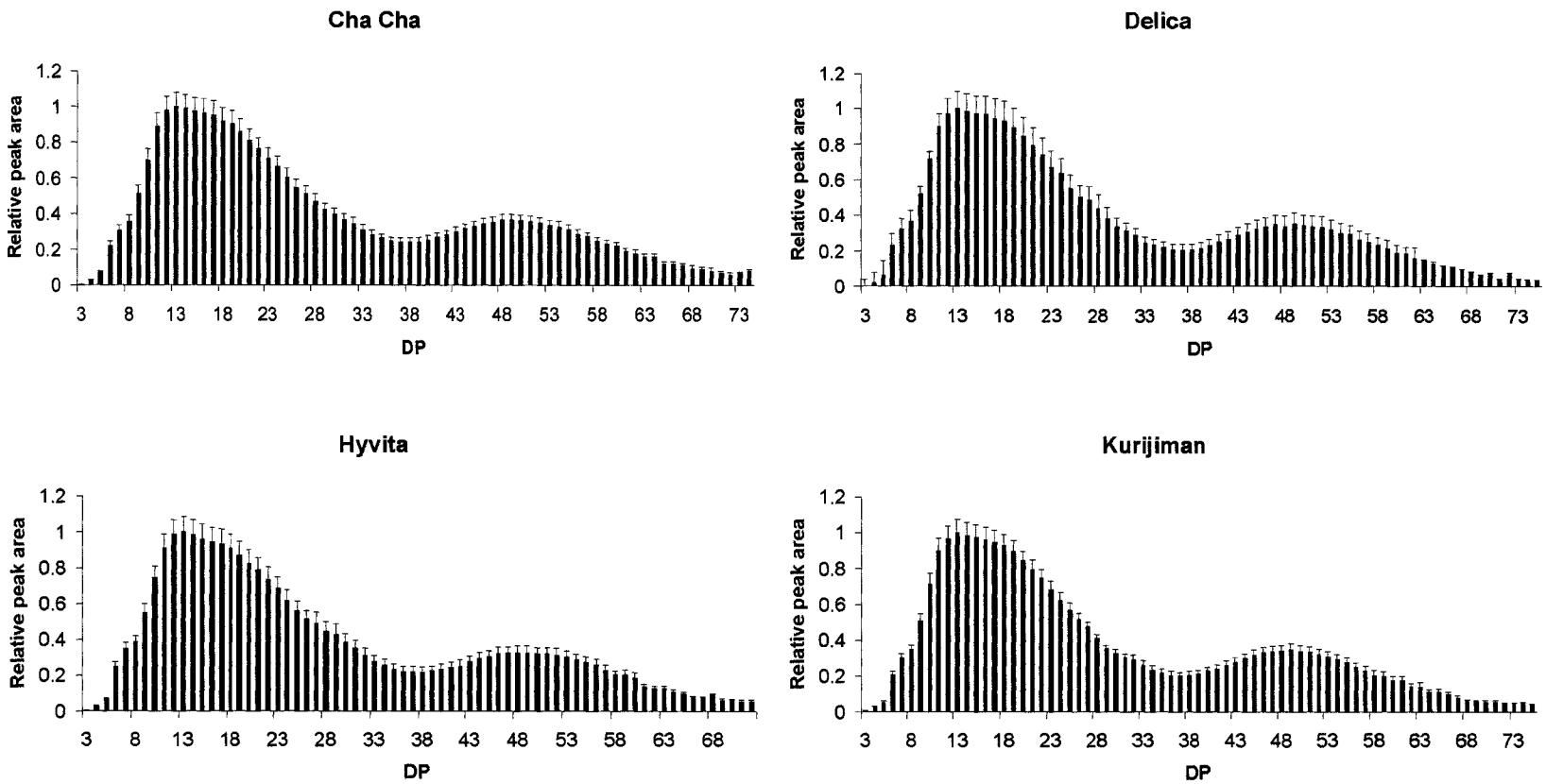

Figure 4a. Relative peak area distributions of Cha Cha, Delica, Hyvita and Kurijiman squash amylopectin branch chain-lengths, from fruit at harvest, analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from four analyses of three replicates. DP =Degree of polymerization.

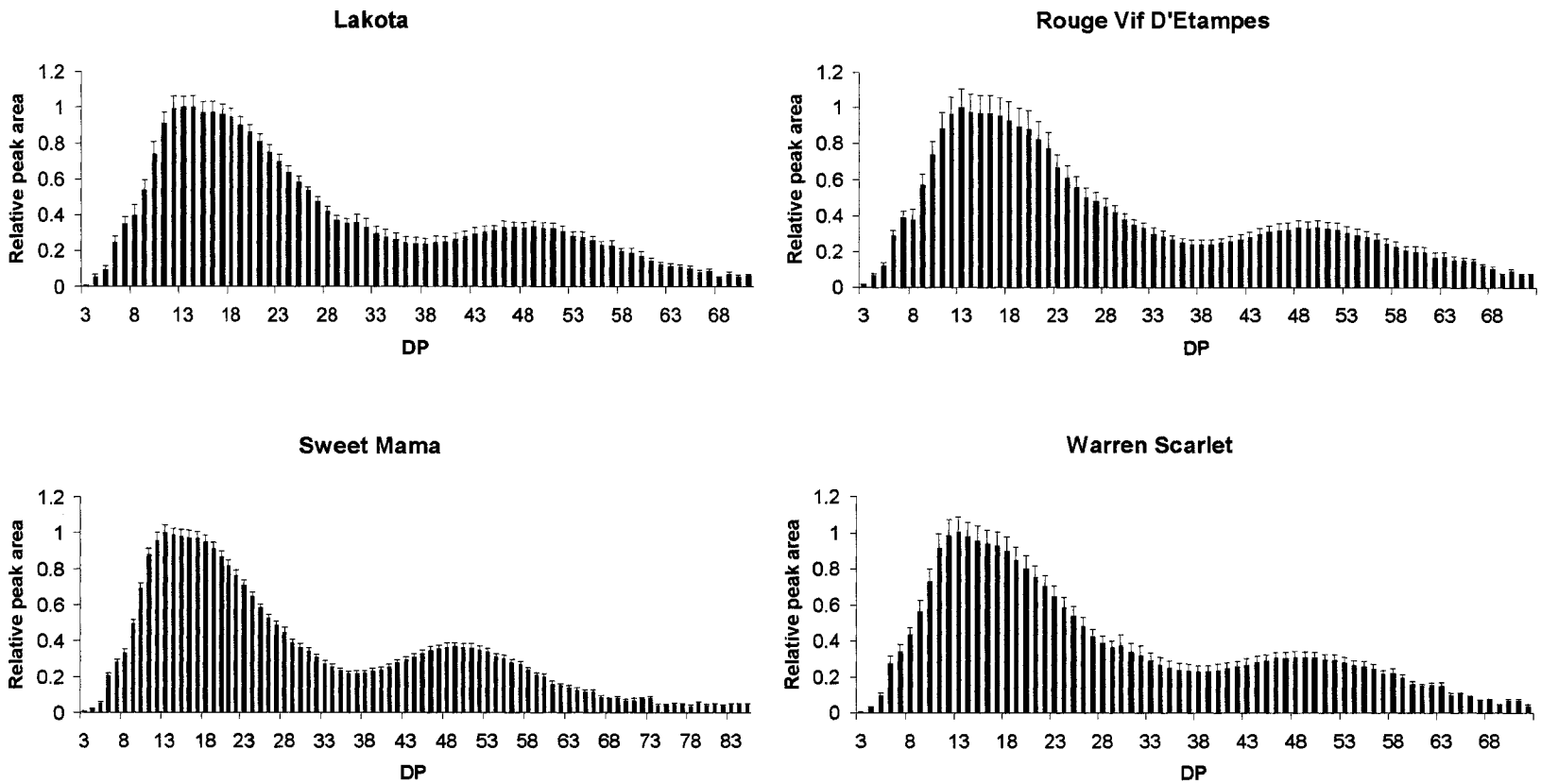

Figure 4b. Relative peak area distributions of Lakota, Rouge Vif D'Etampes, Sweet Mama and Warren Scarlet squash amylopectin branch chain-lengths, from fruit at harvest, analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from four analyses of three replicates. DP =Degree of polymerization.

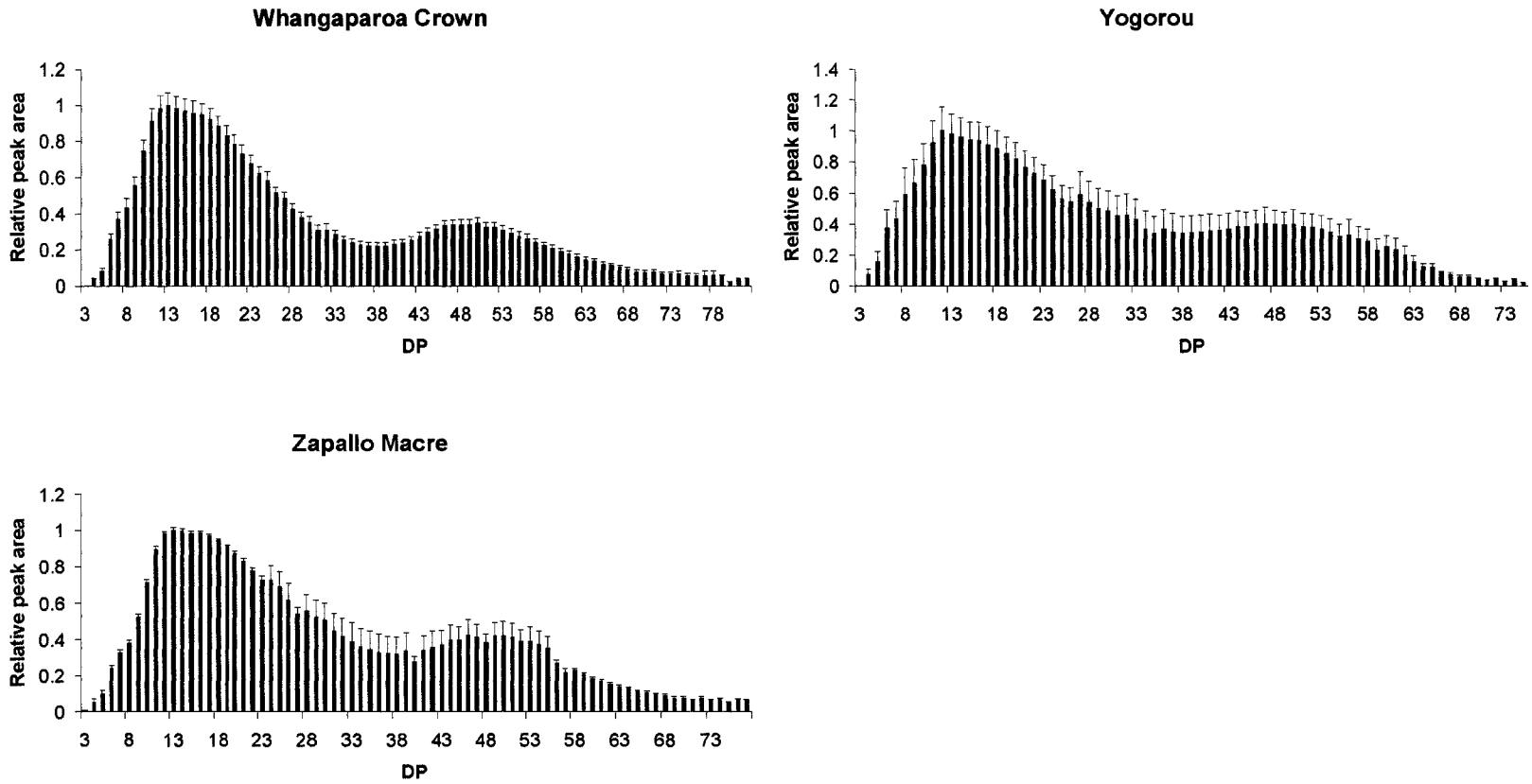

Figure 4c. Relative peak area distributions of Whangaparoa Crown, Yogorou and Zapallo Macre squash amylopectin branch chain-lengths, from fruit at harvest, analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from four analyses of three replicates. DP =Degree of polymerization.

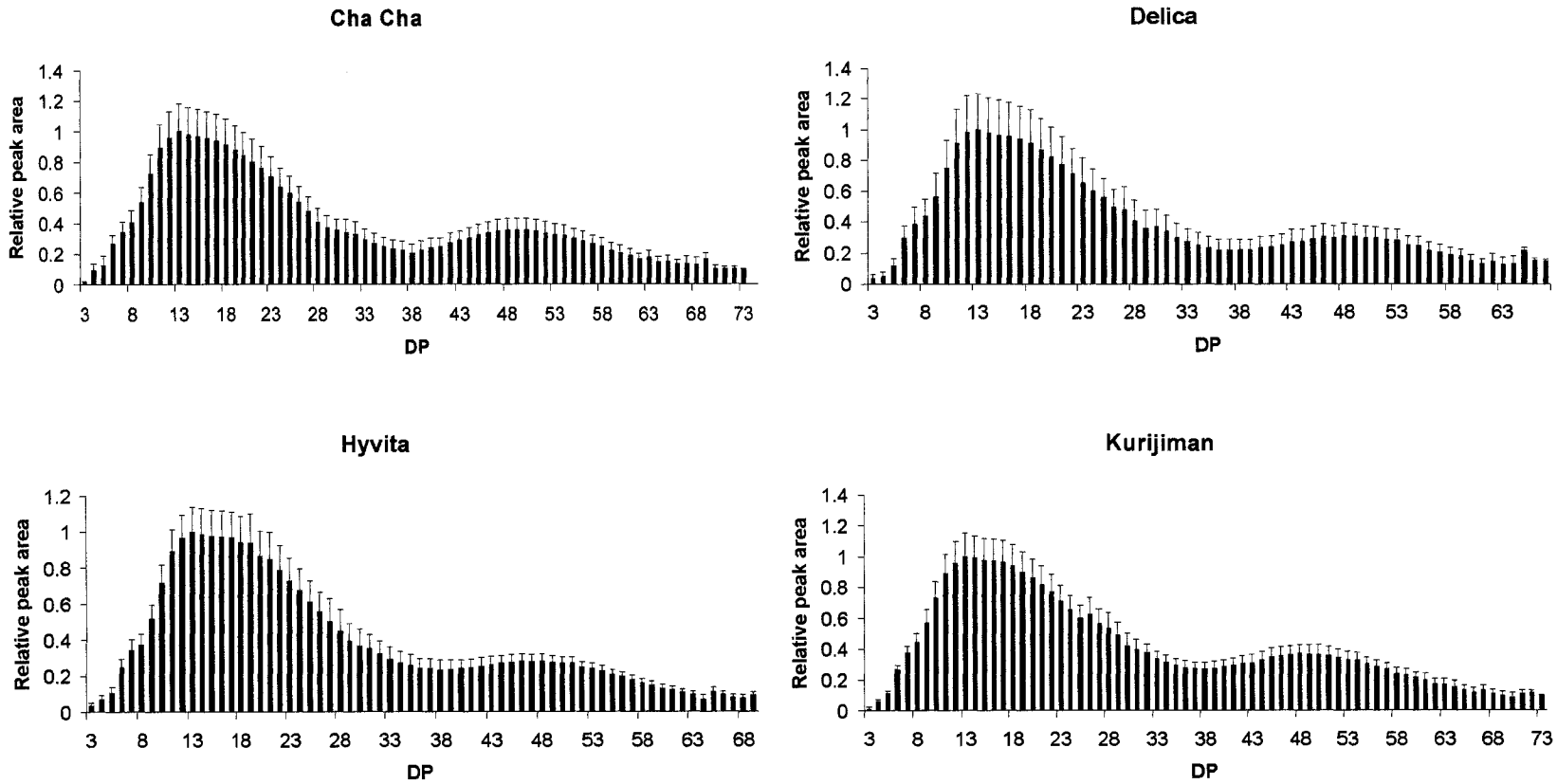

Figure 5a. Relative peak area distributions of Cha Cha, Delica, Hyvita and Kurijiman squash fruit amylopectin branch chain-lengths, from fruit stored 5 weeks, analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from four analyses of three replicates. DP =Degree of polymerization.

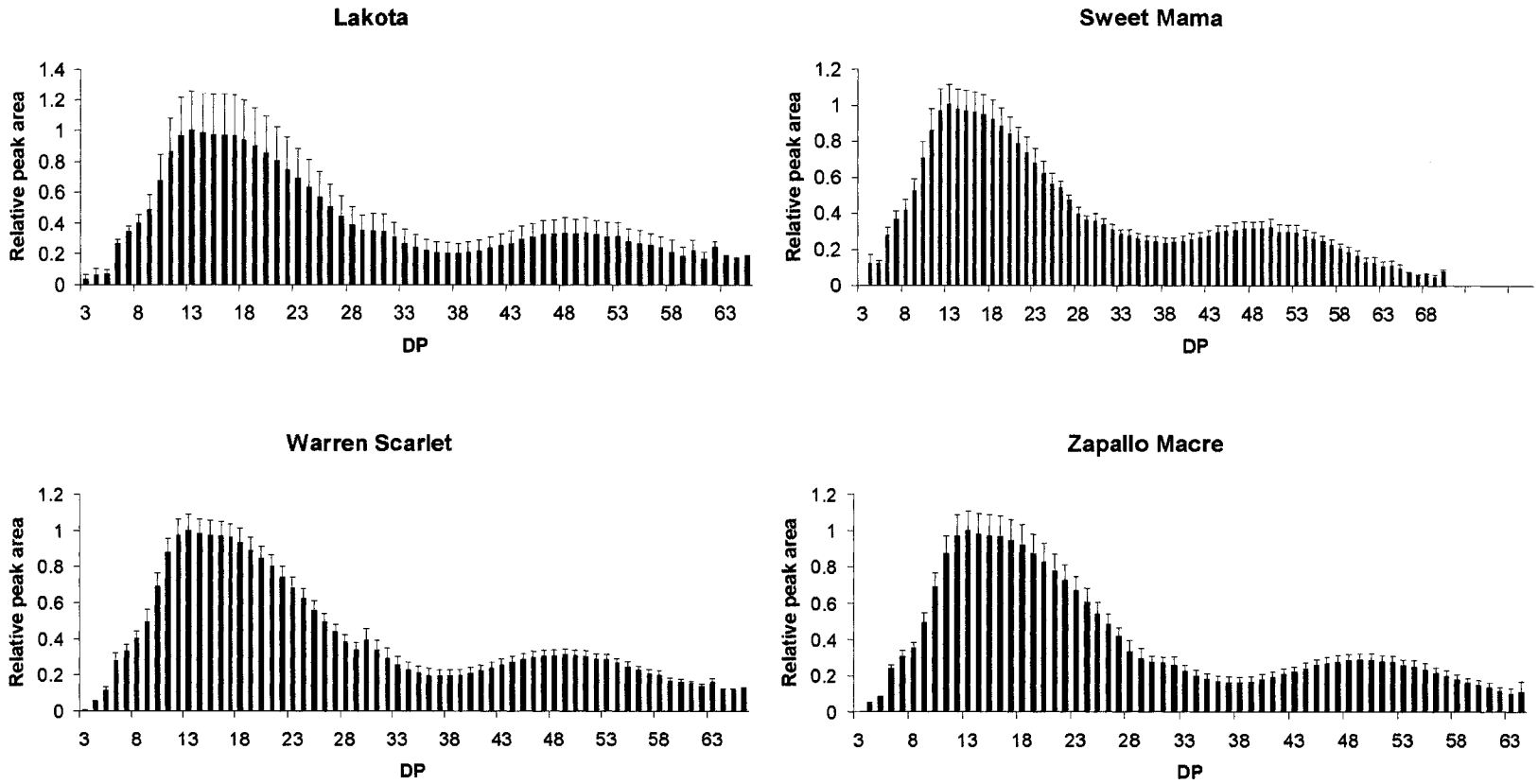

Figure 5b. Relative peak area distributions of Lakota, Sweet Mama, Warren Scarlet and Zapallo Macre squash fruit amylopectin branch chain-lengths, from fruit stored 5 weeks, analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from four analyses of three replicates. DP =Degree of polymerization.

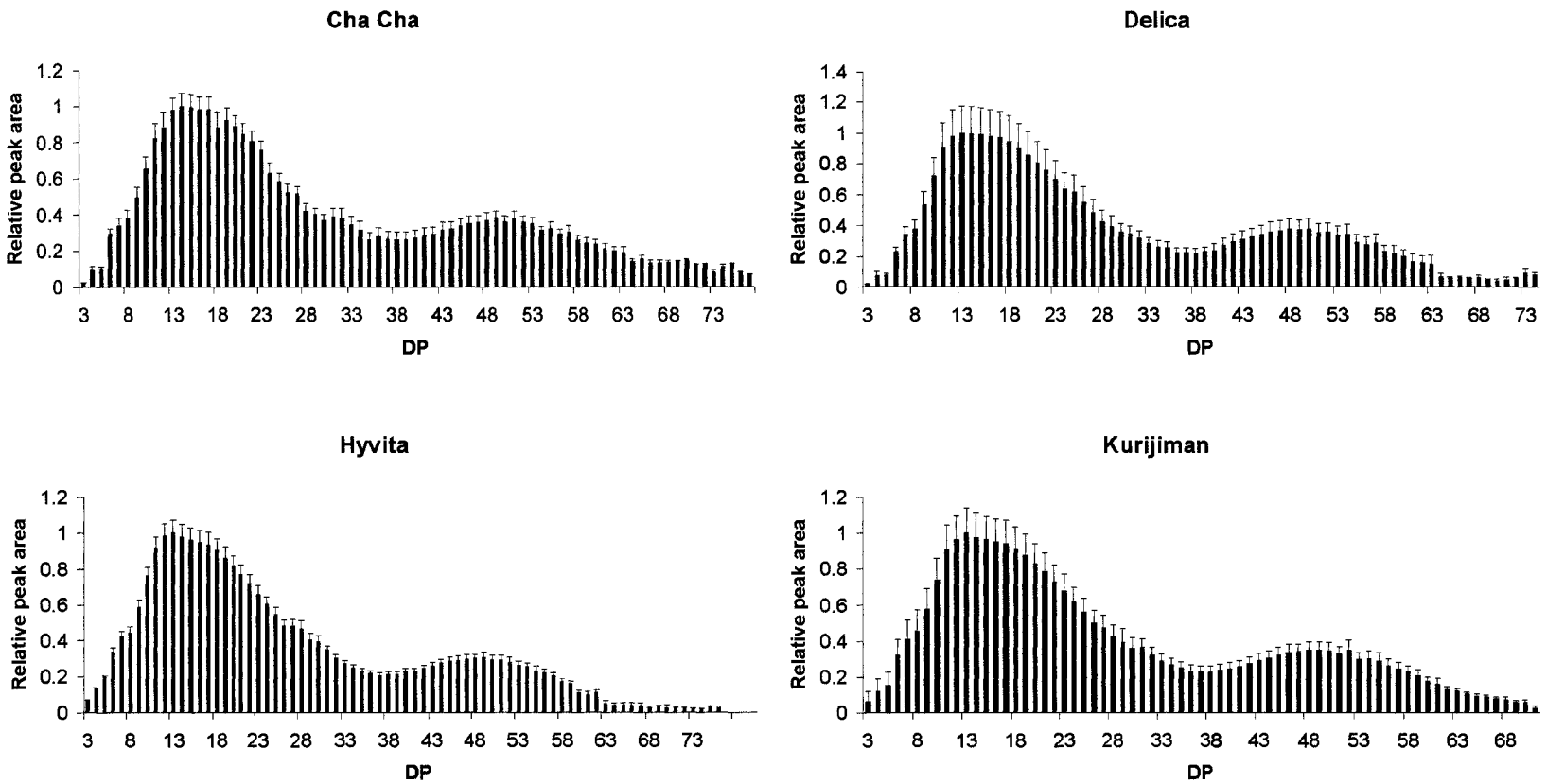

Figure 6a. Relative peak area distributions of Cha Cha, Delica, Hyvita and Kurijiman squash fruit amylopectin branch chain-lengths, from fruit stored 10 weeks, analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from four analyses of three replicates. DP =Degree of polymerization.

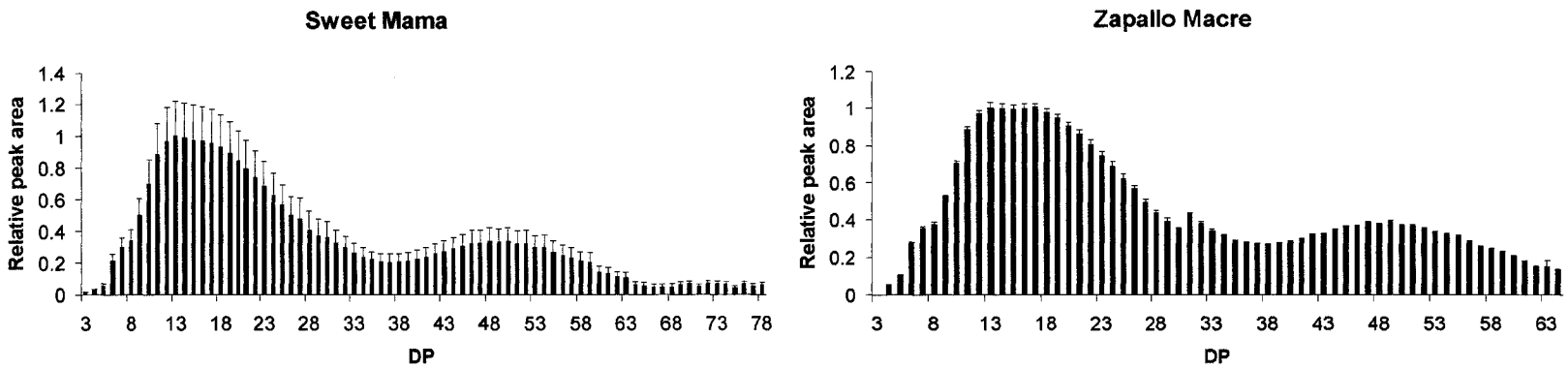

Figure 6b. Relative peak area distributions of Sweet Mama and Zapallo Macre squash fruit amylopectin branch chain-lengths, from fruit stored 10 weeks, analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from four analyses of three replicates. DP =Degree of polymerization.

CHAPTER 3. ROLE OF STARCH STRUCTURE IN TEXTURE OF SQUASH AND STARCH FUNCTIONAL PROPERTIES. II. FUNCTIONAL PROPERTIES OF STARCH EXTRACTED FROM WINTER SQUASH FRUIT (*Cucurbita maxima* D.) AT HARVEST AND AFTER STORAGE.

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ABSTRACT

Starch was isolated from fruit of twelve winter squash cultivars at harvest, 5 or 10 weeks storage and starch functional properties were investigated. Gelatinization temperature of onset (T_o) and conclusion (T_c), range of gelatinization temperature (ROG) and enthalpy change of gelatinization (ΔH) , measured by using differential scanning calorimetry, were significantly different among the squash cultivars at all storage times. Starch, at harvest, had T_o from 61.2°C-65.0°C, T_c from 67.8°C-72.1°C, ROG from 5.7°C-9.0°C, and ΔH from 14.2-17.8 J/g. After 5 and 10 weeks storage, starch typically had lower T_o, T_c, ΔH and higher ROG. Starch, at harvest, retrograded 7 d at 4°C had T_o about 36.5°C, T_c from 65.1°C-68.5°C and ΔH was high from 7.5 to 9.8 J/g. Starch pastes from fruit at harvest, measured by using Rapid Visco-Analyser, had high peak viscosity (from 179-224 rapid viscoanalyser units (RVU)), final viscosity (163-268 RVU), setback (60-108 RVU) and pasting temperature from 66°C-74°C. Starches, at harvest, exhibited strong gels with firmness 1 d at 4°C from 16.1-26.7 g.

KEYWORDS: Starch; winter squash; buttercup squash; pumpkin; *Cucurbita maxima*; postharvest; storage

INTRODUCTION

Starch is the major storage carbohydrate in plants. Starch has been extensively studied from many botanical sources, but starches in squash and pumpkins have received little attention. Evaluation of commercial potential of novel starches requires investigation of the starch functional properties. Functional properties of many cereal starches have been well characterized, but little research has focused on the functional properties of fruit starches from the plant family Cucurbitaceae, which includes winter squash.

Sugimoto et al. (1) studied starch functional properties of two winter squash cultivars, *Cucurbita maxima* cv. Ebisu (close genetic relative to Delica) and *C. moschata* cv. Kogiku. For starch obtained from squash fruit at harvest, Ebisu had onset gelatinization temperature (T_o) of 62.1°C, peak gelatinization temperature (T_p) of 65.9°C and conclusion gelatinization temperature (T_c) of 74.1°C. T_o, T_p and T_c for Kogiku were 62.6°C, 67.3°C and 75.3°C, respectively. Enthalpy change of gelatinization was 13.8 and 12.6 joules per gram for Ebisu and Kogiku, respectively. Brabender amylography was used to show peak viscosity of Ebisu and Kogiku was 810 and 735 Brabender units (BU), final viscosity was 590 and 495 BU and breakdown was 460 and 445 BU, respectively. Functional properties of starches from these squash after storage were not studied.

In a study involving seven *Cucurbita maxima* squash cultivars, we have shown that squash starch, extracted from fruit at harvest, had T_o ranging from 60.6°C to 63.5°C, T_c ranging from 67.7°C to 70.4°C, and ΔH ranging from 15.9 to 17.4 J/g (Chapter 1).

Retrogradation rate of squash starches, after 7 d at 4°C, ranged from 41% to 55%. Squash starches, relative to native starches from other botanical sources, had high peak viscosity (ranging from 174 to 233 rapid visco-analyser units (RVU)), final viscosity (193 to 244 RVU), setback (79 to 100 RVU), and pasting temperature ranged from 65.6°C to 68.8°C.

In our present study, we investigate the functional properties of winter squash fruit starches, belonging to species *Cucurbita maxima*, extracted from fruit at harvest and after 5 or 10 weeks of storage. In a future publication, we will correlate (Chapter 5) the starch functional properties with results of the starch structural properties (Chapter 2), and the textural attributes of raw and cooked squash fruit (Chapter 4).

MATERIALS AND METHODS

Plant Material and Starch Isolation. Starches used in this study were the same starch samples described previously (Chapter 2). Therefore, all procedures of squash cultivation, harvesting, storage and starch isolation were identical.

Thermal Properties by Differential Scanning Calorimetry (DSC). Thermal properties of starch were determined using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) (2). Approximately 2 mg of starch was weighed in an aluminum pan, mixed with 6 mg of deionized water and sealed. Sample was allowed to equilibrate for 2 h and scanned at a rate of 10°C/min over a temperature range of 10-100°C. An empty pan was used as the reference. Rate of starch retrogradation was determined using the same gelatinized samples, stored at 4°C for 7 d, and analyzed using DSC as described previously

(3). All thermal properties were carried out in triplicate for each replicate of each cultivar at each storage time.

Pasting Properties by Rapid Visco-Analyser (RVA) and Gel Properties. Starch pasting properties were analyzed using a Rapid Visco-Analyser (RVA-4, Newport Scientific, Sydney, Australia) (2). Starch suspension (8%, w/w), in duplicate for each replicate of each cultivar at each storage time, was prepared by weighing starch (2.24 g, dry starch basis (dsb)) into a RVA canister and making up the total weight to 28 g with distilled water. Starch suspension was equilibrated at 30°C for 1 min, heated at a rate of 6.0°C/min to 95°C, maintained at that temperature for 5.5 min, and then cooled to 50°C at a rate of 6.0°C/min. Constant paddle rotating speed (160 rpm) was used throughout entire analysis. Immediately after the completion of the RVA sample run, the spindle was removed, and the canister was wrapped in several layers of Saran® wrap, to minimize dehydration, and placed in a refrigerator at 4°C. After 1 or 7 d, canisters were removed from the refrigerator, equilibrated to room temperature, and gel firmness and stickiness were measured using a Stable Micro Systems TAXT2*i* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with Texture Expert for Windows software (v 1.22). Each gel was measured five times by using a 4 mm diameter cylindrical stainless steel punch probe (TA54). Pretest speed was 2.0 mm/s, and gels were compressed at a test speed of 0.9 mm/s and a penetration test distance of 7.5 mm. Peak force of the curve was reported as the firmness of gels and stickiness of gels was defined as the negative load portion of the curve as described previously (4).

Statistical analysis. All statistical significance tests were calculated using SAS (5) and applying Tukey difference test (6) at the 5% level of significance.

RESULTS AND DISCUSSION

Thermal properties of Native Starch. Onset gelatinization temperatures (T_o) of squash starches, extracted from fruit at harvest, ranged from 61.2°C to 65.0°C, and there were significant differences between cultivars ($P < 0.0001$) (**Table 1**). The four buttercup cultivars (Cha Cha, Delica, Kurijiman and Sweet Mama) had T_o significantly higher than the closely related, high-starch content, buttercup-cross, Hyvita, and also the medium-starch content squash cultivars, Warren Scarlet, Whangaparoa Crown and Zapallo Macre. T_o of Kurijiman was also significantly higher than Lakota. Buttercup cultivars having a higher T_o than other squash cultivars was reported previously (2).

Peak gelatinization temperatures (T_p) of squash starches, from fruit at harvest, ranged from 64.2°C to 67.9°C, with significant differences found between cultivars (**Table 1**). Hyvita T_p was significantly lower than the four buttercup cultivars. Rouge Vif D'Etampes T_p was significantly higher than that of Hyvita, Warren Scarlet, Whangaparoa Crown and Zapallo Macre. Conclusion gelatinization temperature (T_c) of starch was also significantly different for the squash cultivars ($P < 0.0001$), with Lakota and Rouge Vif D'Etampes having higher T_c than Hyvita, Whangaparoa Crown and Zapallo Macre (**Table 1**).

The range of gelatinization temperature (ROG) (T_c-T_o) of squash starch isolated from fruit at harvest is very low compared with other starches, and significant differences were observed between cultivars ($P < 0.0001$) (**Table 1**). Previous findings (2) reported three buttercup cultivars to have ROG of 6.3°C, and in our present study the four buttercup cultivars had ROGs of 6.4°C or less, with ROG of 5.7°C for Kurijiman. Despite T_o for Hyvita distinctly lower than the high-starch content buttercup squash, ROG of 6.5°C for

Hyvita was similar to buttercup cultivars. Two medium-starch content cultivars, Whangaparoa Crown and Yogorou, had ROGs (6.7°C and 6.2°C, respectively) comparable to buttercup cultivars. Medium-starch content cultivars having similar ROGs to buttercup cultivars were not found in previous study (2). To our knowledge, only water yam starch (*Dioscorea alata*) with a ROG of 5.4°C is lower than some of the ROG of squash cultivar starches found in this study (7). Sugimoto et al. (1) studied thermal properties of starches from two winter squash, including the buttercup *Cucurbita maxima* cv. Ebisu that is a close genetic relative to Delica. T_o for Ebisu was reported to be 62.1°C, which is lower than the 63.8°C for Delica, and T_c for Ebisu was 74.1°C, compared with 70.1°C for Delica. ROG of 12°C for Ebisu is much wider than the ROG for Delica (6.3°C) reported in this study. The ROG of (12°C) reported for Ebisu is substantially wider than all the cultivars that we have investigated (5.7-9.0°C).

Enthalpy change of gelatinization (ΔH) for squash starches, extracted from fruit at harvest, was significantly different between cultivars ($P = 0.0003$) (**Table 1**). Cha Cha, Lakota and Sweet Mama ΔH were significantly higher than Warren Scarlet and Zapallo Macre. There was no clear trend for ΔH of the medium- and high-starch content squash cultivars, but there is some suggestion that buttercup cultivars are higher. The high ΔH reported for squash starches in this study is comparable with previous findings (2), and is higher than all other native starches, except green banana starch (4). However, Sugimoto et al. (1) reported ΔH for squash starches that were about 20% lower than what we observed.

T_o of squash starches extracted from fruit after 5 weeks storage was lower for all cultivars except Zapallo Macre, and significant differences were observed between

cultivars ($P = 0.004$) (**Table 2**). Most squash starches T_o decreased by about 3°C, probably due to increased crystal defects, possibly due to enzymic attack. Significant differences between squash cultivars were also observed for T_p and T_c for starch from fruit after 5 weeks storage. Zapallo Macre T_p was significantly higher than Delica, Hyvita and Warren Scarlet, and T_c was significantly higher than all cultivars except Lakota and Sweet Mama. ΔH of starch from fruit stored 5 weeks decreased for all squash cultivars and was significantly different ($P = 0.0005$). Squash cultivar ROG of starch from fruit after 5 weeks storage was significantly different ($P = 0.0009$). After 5 weeks storage, the high-starch content squash cultivars showed differences in ROG. Cha Cha, Delica and Kurijiman all had ROGs < 6.6°C after 5 weeks of storage, similar to ROG at harvest. Hyvita and Sweet Mama ROGs were 8.8°C and 7.4°C, respectively, considerably higher than at harvest.

Squash starch, extracted from fruit after 10 weeks storage, showed no change in T_o, T_p and T_c compared with starch extracted from fruit after 5 weeks storage (**Table 3**). T_o, T_p and T_c were all significantly different for the starch of squash cultivars after 10 weeks storage, primarily because Zapallo Macre went against the overall trend and had increases in T_o, T_p and T_c during storage. Zapallo Macre starch, from fruit stored 10 weeks had significantly lower ΔH than all other squash cultivars. ROGs for all squash cultivars after 10 weeks storage were similar to that at the harvest, except for Sweet Mama which was significantly higher than most cultivars.

Thermal Properties of Retrograded Starch. Thermal properties of starch isolated from squash fruit at harvest and retrograded for 7 d at 4°C, are shown in **Table 4**. Thermal transition onset temperature (T_{oR}) of the retrograded starch was not significantly different

among the squash cultivars as the range in T_{oR} was 35.6°C to 37.5°C. Significant differences were observed for peak temperature (T_{pR}) and conclusion (T_{cR}) of retrograded squash starches ($P = 0.001$ for both). Rouge Vif D'Etampes T_{pR} was significantly higher than Delica, Hyvita, Lakota and Whangaparoa Crown at harvest. Enthalpy change of the thermal transition (ΔH_R) for all retrograded squash starches was higher than that of starches from other botanical sources (4). Percentage retrogradation of squash starch from fruit at harvest was similar for all cultivars, except Kurijiman that had significantly lower percentage retrogradation than did Rouge Vif D'Etampes. For starch isolated from squash fruit at harvest, T_{oR}, T_{pR}, T_{cR}, ΔH_R and percent retrogradation that we report are very similar to previous findings (2).

After 5 weeks storage of fruit, the general ranges of values for T_{oR}, T_{pR} and T_{cR} of retrograded starches remain unchanged compared with that of starches isolated at harvest, but T_{oR} is now significantly different ($P = 0.005$) among the squash cultivars and T_{pR} is no longer significant but there is still strong suggestion of cultivar differences ($P = 0.06$) (**Table 5**). T_{oR} of Sweet Mama starch at 5 weeks storage was significantly lower than T_{oR} for Delica, Warren Scarlet and Zapallo Macre. ΔH_R of retrograded starch after 5 weeks storage decreased for all squash cultivars, except Kurijiman that remained unchanged. Hyvita had significantly higher ΔH_R than Lakota and Warren Scarlet after 5 weeks storage. Delica, Kurijiman and Sweet Mama had significantly higher ΔH_R than Warren Scarlet after 5 weeks storage. Because of the variation in starch retrogradation processes, the range of thermal transition of retrograded starch (ROG_R) is rarely studied. Although no significant differences in ROG_R between squash cultivars were observed for retrograded starch from fruit at harvest,

ROG_R of retrograded starch from fruit after 5 weeks storage was significantly different (P = 0.01), with ROG_R of Hyvita and Sweet Mama higher than Warren Scarlet.

T_{oR} of retrograded squash starch, extracted from fruit after 10 weeks storage, was either the same or higher than from fruit at 5 weeks of storage (**Table 6**). ΔH_R of retrograded starch isolated from 10-week stored fruit for all squash cultivars, except Hyvita, was lower than retrograded starch from fruit at harvest. This is likely related to the structure of starch attacked by amylases. Percentage retrogradation also declined for all squash cultivars except Hyvita that had significantly higher retrogradation percentage than Delica and Zapallo Macre.

Pasting Properties. Pasting properties of squash fruit starches, extracted at harvest, are shown in **Table 7**. Pasting profiles of selected squash cultivar starches, extracted from fruit at harvest, are shown in **Figure 1**. Peak viscosity for squash starches, from fruit at harvest, was high relative to native starch from other botanical sources (4), and ranged from 178 to 224 Rapid Visco-Analyzer units (RVU). Peak viscosity was not significantly different for the squash cultivars, in contrast to findings reported previously (2). Despite lack of significant difference for squash starch peak viscosity and breakdown, Cha Cha had a significantly higher trough than Delica. Final viscosity of squash starches, from fruit at harvest, was not significantly different, but ranged from 163 to 268 RVU. Squash starches, from fruit at harvest, have very high setback compared with starches from other botanical sources (4), with three cultivars, Cha Cha, Sweet Mama and Whangaparoa Crown all exceeding 100 RVU, which is rarely observed for native starches. Setback was not significantly different for squash starches at harvest. High setback with no significant differences has been reported previously for squash starches (2). Pasting temperature of

squash cultivar starches, from fruit at harvest, was significantly different ($P = 0.002$). Warren Scarlet pasting temperature was considerably higher than all other squash cultivars except Lakota and Rouge Vif D'Etampes. Pasting temperature of squash starch, from fruit at harvest, is similar to previous studies of squash starch (2).

Five weeks storage of squash fruit resulted in starches from the squash cultivars that differed significantly in every pasting parameter except pasting temperature (**Table 8**). Pasting profiles of Cha Cha and Delica starches, obtained from fruit stored 5 or 10 weeks, is shown in **Figure 2**. There was no consistent trend in changes in peak viscosity after 5 weeks storage, but Kurijiman had significantly higher peak viscosity than Hyvita, and all squash cultivars were significantly higher than Lakota. Lakota had relatively low peak viscosity at harvest as well, which is in contrast with that reported by Stevenson et al. (2) in which Lakota had the highest peak viscosity. The trough for Cha Cha was significantly higher than all other cultivars, and Lakota had significantly lower trough than all cultivars except Hyvita. Breakdown of starch paste, for Kurijiman starch extracted from fruit after 5 weeks storage, was significantly greater than Cha Cha and Lakota. Breakdown for Delica and Sweet Mama was also significantly higher than Lakota. Final viscosity of starch, from fruit stored for 5 weeks, showed dramatic differences for Lakota that had a final paste viscosity of 132 RVU which was significantly lower than all other squash cultivars, having final viscosity exceeding 200 RVU. Cha Cha in particular had very high final viscosity (295 RVU). Setback was high for all squash starches, from fruit stored 5 weeks, except for Lakota which had setback that was significantly lower than Cha Cha and Hyvita. Pasting temperature decreased for all cultivars after 5 weeks storage, except Hyvita which remained constant and Cha Cha that increased.

All pasting property parameters of squash starches, extracted from fruit after 10 weeks storage, were significantly different between cultivars (**Table 9**). Zapallo Macre had significantly lower peak viscosity, trough, breakdown, final viscosity and setback than all other squash cultivars. Cha Cha had significantly higher trough and lower breakdown than Sweet Mama. The four buttercup cultivars were similar in peak viscosity, final viscosity, setback and pasting temperature for starch obtained from fruit stored for 10 weeks. Pasting profiles of selected starches, obtained from fruit after 10 weeks storage, are shown in **Figure 2.**

Gel Properties. Squash starches, isolated from fruit at harvest, developed strong gels, but no significant differences between cultivars were observed either for firmness or stickiness of gels formed at 4°C for either 1 or 7 d (**Table 10** and **11**). However, after squash fruit have been stored for 5 or 10 weeks, significant differences in gel firmness were observed. Cha Cha starch gels were significantly firmer than Lakota after both 1 and 7 d at 4°C for fruit stored 5 weeks, and significantly firmer than all other squash cultivars for fruit stored for 10 weeks ($P \leq 0.02$). Hyvita gels, formed from starch obtained from fruit after 5 weeks storage, had significantly higher stickiness than Delica after 1 d at 4°C ($P = 0.02$). No other significant differences were observed for stickiness for squash starches obtained from fruit at either 5 or 10 weeks storage. Firmness of starch gels for all buttercups, except Sweet Mama, increased with fruit storage time. For starches obtained from fruit at harvest, firmness of Sweet Mama gels and stickiness of Rouge Vif D'Etampes gels were considerably higher than any other cultivar.

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Table 1

Thermal properties of native squash starches isolated from fruit at harvest.

Cultivar*	T_o (°C)#♦	T_p (°C)	T_c (°C)	Range (°C)♣	ΔH (J/g)
Cha Cha	64.4^{ab}	67.2^{abc}	70.4^{ab}	6.0^{d}	17.8^{a}
Delica	63.8^{ab}	66.7^{abcd}	70.1^{abc}	6.3^{cd}	16.5^{abc}
Hyvita	61.3^{c}	64.2^{e}	67.8^{c}	6.5^{bcd}	15.8^{abc}
Kurijiman	65.0^{a}	67.7^{a}	70.7^{ab}	5.7^{d}	17.2^{ab}
Lakota	62.9^{bc}	67.6^{ab}	71.8^{a}	8.9^{a}	17.7^{a}
Rouge Vif D'Etampes	64.4^{ab}	67.9^{a}	72.1^{a}	7.7^{abc}	16.4^{abc}
Sweet Mama	64.4^{ab}	67.5^{abc}	70.8^{ab}	6.4^{bcd}	17.7^{a}
Warren Scarlet	61.2^{c}	65.8^{bcde}	70.2^{abc}	9.0^{a}	14.8^{bc}
Whangaparoa Crown	61.8^{c}	64.9^{de}	68.4^{bc}	6.7^{bcd}	16.3^{abc}
Yogorou	64.2^{ab}	67.3^{abc}	70.4^{ab}	6.2^{cd}	16.8^{ab}
Zapallo Macre	61.2^{c}	65.6^{cde}	69.3^{bc}	8.1^{ab}	14.2^{c}
	$P < 0.0001$♠	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0003$

Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.

Values were calculated from three analyses for each of three replicates.

♣ Range of gelatinization is equal to T_c-T_o.

♦ Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♠ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 2

Thermal properties of native squash starches isolated from fruit after 5 weeks of storage.

Cultivar*	T_o (°C)#♦	T_p (°C)	T_c (°C)	Range (°C)♣	ΔH (J/g)
Cha Cha	60.8^{abc}	64.1^{abc}	67.4^{bcd}	6.6^{cd}	14.9^{a}
Delica	60.6^{abc}	63.4^{bc}	66.7^{cd}	6.1^{d}	15.8^{a}
Hyvita	58.7^{bc}	63.2^{bc}	67.5^{bcd}	8.8^{a}	14.4^{a}
Kurijiman	60.9^{abc}	64.0^{abc}	67.5^{bcd}	6.6^{bcd}	15.0^{a}
Lakota	61.7^{ab}	65.7^{ab}	69.6^{ab}	7.9^{abcd}	12.6^{ab}
Sweet Mama	61.6^{ab}	65.0^{ab}	69.0^{abc}	7.4^{abcd}	14.6^{a}
Warren Scarlet	57.5^{c}	61.5^{c}	65.9^{d}	8.4^{ab}	9.8^{b}
Zapallo Macre	62.6^{a}	66.9^{a}	70.6^{a}	8.0^{abc}	13.4^{a}
	$P = 0.004$♠	$P = 0.0006$	$P = 0.0003$	$P = 0.0009$	$P = 0.0005$

Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.

Values were calculated from three analyses for each of three replicates.

♣ Range of gelatinization is equal to T_c-T_o.

♦ Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♠ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 3

Thermal properties of starch isolated from squash fruit after 10 weeks of storage.

Cultivar*	T_o (°C)#♦	T_p (°C)	T_c (°C)	Range (°C)♣	ΔH (J/g)
Cha Cha	60.9^b	64.9^b	68.2^{ab}	7.2^{ab}	15.3^a
Delica	60.9^b	64.2^b	67.8^b	6.9^b	15.6^a
Hyvita	60.8^b	63.8^b	67.4^b	6.7^b	15.3^a
Kurijiman	61.4^b	64.5^b	67.7^b	6.4^b	15.9^a
Sweet Mama	60.0^b	64.8^b	68.6^{ab}	8.6^a	13.8^a
Zapallo Macre	63.3^a	66.5^a	69.4^a	6.1^b	10.0^b
	$P = 0.0003$♠	$P = 0.004$	$P = 0.04$	$P = 0.002$	$P < 0.0001$

Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.

Values were calculated from three analyses for each of three replicates.

♣ Range of gelatinization is equal to T_c-T_o.

♦ Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♠ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 4

Thermal properties of starch isolated from squash fruit at harvest[#] and retrograded.

Cultivar[*]	T_o (°C)	T_p (°C)	T_c (°C)	Range (°C)	ΔH (J/g)	% retrogradation
Cha Cha	37.0	56.3^{ab}	65.9^{b}	28.9	8.8^{ab}	49.3^{ab}
Delica	35.7	53.9^{b}	65.4^{b}	29.6	9.3^{ab}	56.2^{ab}
Hyvita	36.4	54.1^{b}	65.3^{b}	28.9	8.1^{ab}	51.1^{ab}
Kurijiman	37.5	54.5^{ab}	65.4^{b}	27.9	7.5^{b}	43.5^{b}
Lakota	36.8	53.5^{b}	65.1^{b}	28.3	8.3^{ab}	47.2^{ab}
Rouge Vif D'Etampes	37.4	57.3^{a}	68.5^{a}	31.1	9.8^{a}	59.8^{a}
Sweet Mama	36.2	54.4^{ab}	65.9^{b}	29.7	9.0^{ab}	51.2^{ab}
Warren Scarlet	36.4	56.6^{ab}	66.1^{b}	29.7	8.1^{ab}	55.6^{ab}
Whangaparoa Crown	35.6	54.1^{b}	65.1^{b}	29.4	8.3^{ab}	51.1^{ab}
Yogorou	36.7	56.0^{ab}	65.9^{b}	29.2	7.8^{ab}	46.3^{ab}
Zapallo Macre	36.0	56.0^{ab}	65.4^{b}	29.4	7.8^{ab}	53.8^{ab}
	$P = 0.57^{\clubsuit}$	$P = 0.001$	$P = 0.001$	$P = 0.20$	$P = 0.02$	$P = 0.05$

[*] Same starch samples after gelatinization (see Table 1) were left for 7 days at 4°C and rescan using DSC.

[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 5

Thermal properties of starch isolated from squash fruit stored for 5 weeks and retrograded[#].

Cultivar[*]	T_o (°C)	T_p (°C)	T_c (°C)	Range (°C)	ΔH (J/g)	% retrogradation
Cha Cha	36.3^{ab}	55.9	65.1	28.8^{ab}	6.3^{abc}	42.3
Delica	37.6^{a}	56.3	65.0	27.4^{ab}	7.6^{ab}	48.2
Hyvita	35.0^{ab}	53.3	65.7	30.6^{a}	7.9^{a}	53.9
Kurijiman	35.7^{ab}	54.7	64.7	29.0^{ab}	7.5^{ab}	49.6
Lakota	36.4^{ab}	55.6	65.2	28.7^{ab}	5.6^{bc}	43.9
Sweet Mama	34.0^{b}	54.1	65.3	31.3^{a}	7.3^{ab}	50.3
Warren Scarlet	37.8^{a}	56.8	64.0	26.3^{b}	5.0^{c}	52.0
Zapallo Macre	37.7^{a}	54.4	65.2	27.5^{ab}	6.9^{abc}	51.8
	$P = 0.005$[♣]	$P = 0.06$	$P = 0.46$	$P = 0.01$	$P = 0.002$	$P = 0.07$

[*] Same starch samples after gelatinization (see Table 2) were left for 7 days at 4°C and rescan using DSC.

[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 6

Thermal properties of starch, isolated from squash fruit stored for 10 weeks and retrograded[#].

Cultivar[*]	T_o (°C)	T_p (°C)	T_c (°C)	Range (°C)	ΔH (J/g)	% retrogradation
Cha Cha	37.3	56.7	66.3	28.9	7.0^{ab}	45.9^{ab}
Delica	37.6	54.7	64.1	26.4	5.7^{abc}	35.8^{b}
Hyvita	37.2	55.7	66.7	29.5	8.0^{a}	52.2^{a}
Kurijiman	36.2	54.0	64.8	28.6	6.2^{ab}	38.8^{ab}
Sweet Mama	38.5	56.1	65.8	27.3	5.6^{bc}	40.4^{ab}
Zapallo Macre	37.6	54.6	64.3	26.6	3.6^{c}	35.6^{b}
	$P = 0.30$[♣]	$P = 0.19$	$P = 0.14$	$P = 0.15$	$P = 0.001$	$P = 0.02$

[*] Same starch samples after gelatinization (see Table 3) were left for 7 days at 4°C and rescan using DSC.
[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.
[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 7

Pasting properties of squash fruit starches, extracted at harvest, measured by Rapid Visco-Analyzer.

Cultivar*	Peak Viscosity#♣	Trough#	Breakdown#	Final Viscosity#	Setback#	Peak Time (min)	Pasting Temperature (°C)
Cha Cha	224.4	160.5[a]	63.7	268.3	107.8	11.2	67.7[b]
Delica	178.7	103.5[b]	75.3	163.1	59.6	10.2	66.1[b]
Hyvita	192.3	126.3[ab]	66.1	218.3	92.0	10.9	66.0[b]
Kurijiman	187.0	115.8[ab]	71.2	190.9	75.0	10.0	66.9[b]
Lakota	184.0	113.9[ab]	70.1	190.5	76.6	10.4	68.6[ab]
Rouge Vif D'Etampes	181.1	131.0[ab]	50.1	215.6	84.6	11.4	69.2[ab]
Sweet Mama	207.1	152.8[ab]	54.3	256.8	104.0	11.5	68.0[b]
Warren Scarlet	184.2	148.6[ab]	35.5	236.1	87.5	11.9	73.8[a]
Whangaparoa Crown	221.0	139.3[ab]	81.7	246.7	107.5	10.8	65.9[b]
Yogorou	187.0	124.5[ab]	62.6	223.1	98.6	11.1	66.9[b]
Zapallo Macre	211.8	141.8[ab]	70.0	238.0	96.3	11.1	67.0[b]
	$P = 0.42$♦	$P = 0.04$	$P = 0.28$	$P = 0.13$	$P = 0.38$	$P = 0.06$	$P = 0.002$

* 8% (w/w) starch suspension measured in duplicate for all three replicates.

Viscosity measured in Rapid Visco-Analyzer units (RVU), 1 RVU = 12 centipoise.

♣ Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 8

Pasting properties of squash fruit starches, extracted after 5 weeks of storage, measured by Rapid Visco-Analyzer.

Cultivar[*♠]	Peak Viscosity[#♣]	Trough[#]	Breakdown[#]	Final Viscosity[#]	Setback[#]	Peak Time (min)	Pasting Temperature (°C)
Cha Cha	214.6^{ab}	187.2^{a}	27.4^{c}	294.9^{a}	107.7^{a}	12.2^{a}	72.6
Delica	221.0^{ab}	143.2^{b}	77.8^{ab}	224.0^{a}	80.8^{ab}	11.0^{ab}	65.6
Hyvita	177.2^{bc}	119.4^{bc}	57.8^{abc}	217.5^{a}	98.1^{a}	10.9^{b}	66.3
Kurijiman	243.0^{a}	148.8^{b}	94.2^{a}	236.1^{a}	87.3^{ab}	10.9^{b}	64.5
Lakota	136.5^{c}	97.0^{c}	39.5^{bc}	132.0^{b}	35.0^{b}	10.6^{b}	66.8
Sweet Mama	204.0^{ab}	131.1^{b}	72.9^{ab}	218.4^{a}	87.3^{ab}	11.1^{ab}	66.1
	$P = 0.001$[♦]	$P = < 0.0001$	$P = 0.002$	$P = 0.0006$	$P = 0.01$	$P = 0.02$	$P = 0.09$

[*] 8% (w/w) starch suspension measured in duplicate for all three replicates.

[#] Viscosity measured in Rapid Visco-Analyzer units (RVU), 1 RVU = 12 centipoise.

[♣] Values with different letters denote differences at the 5% level of significance.

[♦] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

[♠] Zapallo Macre is missing because insufficient starch yield for fruit randomly selected at 5 weeks storage for each comparison between cultivars in the respective column.

Table 9

Pasting properties of squash fruit starches, extracted after 10 weeks of storage, measured by Rapid Visco-Analyzer.

Cultivar*	Peak Viscosity#♣	Trough#	Breakdown#	Final Viscosity#	Setback#	Peak Time (min)	Pasting Temperature (°C)
Cha Cha	187.9^{a}	169.1^{a}	19.0^{bc}	274.9^{a}	105.8^{a}	12.4^{a}	69.8^{b}
Delica	197.1^{a}	151.2^{ab}	45.9^{a}	235.2^{a}	84.0^{a}	11.8^{ab}	68.6^{b}
Kurijiman	190.4^{a}	150.1^{ab}	40.3^{ab}	240.8^{a}	90.8^{a}	11.9^{ab}	70.2^{b}
Sweet Mama	185.5^{a}	127.7^{b}	57.8^{a}	222.1^{a}	94.5^{a}	11.2^{b}	67.2^{b}
Zapallo Macre	71.0^{b}	65.0^{c}	6.0^{c}	104.9^{b}	39.9^{b}	11.3^{b}	82.4^{a}
	$P = < 0.001$♦	$P = < 0.0001$	$P = 0.0002$	$P = < 0.0001$	$P = 0.005$	$P = 0.01$	$P = < 0.0001$

* 8% (w/w) starch suspension measured in duplicate for all three replicates.

Viscosity measured in Rapid Visco-Analyzer units (RVU), 1 RVU = 12 centipoise.

♣ Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 10

Gel firmness (g) from starches extracted from fruit at harvest and after 5 or 10 weeks storage, heated under RVA temperature profile and stored at 4°C for 1 or 7 d[#♣].

Cultivar	Harvest		5 weeks		10 weeks	
	1 day	7 days	1 day	7 days	1 day	7 days
Cha Cha	20.0	27.5	22.6[a]	35.3[a]	25.4[a]	41.8[a]
Delica	16.1	20.1	13.5[ab]	21.3[ab]	14.4[b]	23.4[b]
Hyvita	18.4	25.7	15.9[ab]	24.3[ab]		
Kurijiman	18.3	23.2	15.5[ab]	24.9[ab]	17.3[b]	24.4[b]
Lakota	21.7	27.5	8.4[b]	12.3[b]		
Rouge Vif D'Etampes	19.4	27.7				
Sweet Mama	26.7	37.2	15.7[ab]	23.8[ab]	12.1[b]	15.0[b]
Warren Scarlet	16.4	28.8				
Whangaparoa Crown	17.7	23.1				
Yogorou	22.1	29.8				
Zapallo Macre	17.3	20.2				
	$P = 0.53$[♦]	$P = 0.51$	$P = 0.02$	$P = 0.02$	$P = 0.0009$	$P = 0.001$

[#] Values were obtained from five measurements for each of three replicates.

[♣] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♦] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 11

Gel stickiness (g/s) from starches extracted from fruit at harvest and after 5 or 10 weeks storage, heated under RVA temperature profile and stored at 4°C for 1 or 7 d[#♣].

Cultivar	Harvest		5 weeks		10 weeks	
	1 day	7 days	1 day	7 days	1 day	7 days
Cha Cha	9.8	15.4	5.9[ab]	10.4	10.1	9.3
Delica	11.5	15.7	2.8[b]	13.2	6.2	13.1
Hyvita	9.2	11.3	9.6[a]	14.3		
Kurijiman	12.4	18.4	3.5[ab]	10.7	6.5	12.4
Lakota	13.3	18.6	8.7[ab]	14.6		
Rouge Vif D'Etampes	21.0	23.4				
Sweet Mama	8.8	18.2	8.9[ab]	14.2	7.6	8.1
Warren Scarlet	12.3	19.1				
Whangaparoa Crown	7.4	14.1				
Yogorou	16.5	14.8				
Zapallo Macre	11.7	17.0				
	$P = 0.20$[♦]	$P = 0.41$	$P = 0.02$	$P = 0.46$	$P = 0.09$	$P = 0.28$

[#] Values were obtained from five measurements for each of three replicates.

[♣] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♦] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

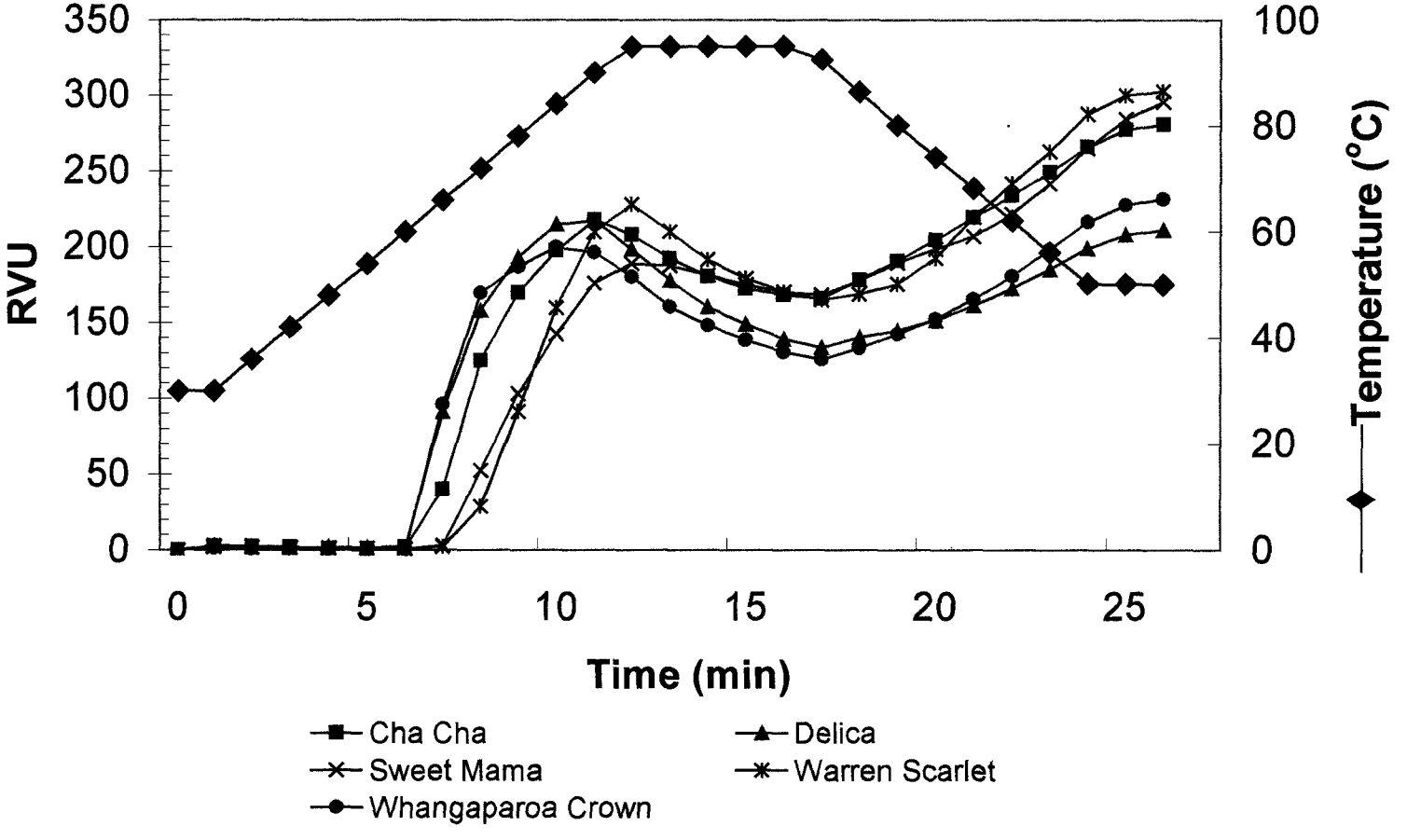

Figure 1. Rapid Visco-Analyser pasting profiles of Cha Cha, Delica, Sweet Mama, Warren Scarlet and Whangaparoa Crown squash fruit starches extracted at harvest (8.0% dsb, w/w).

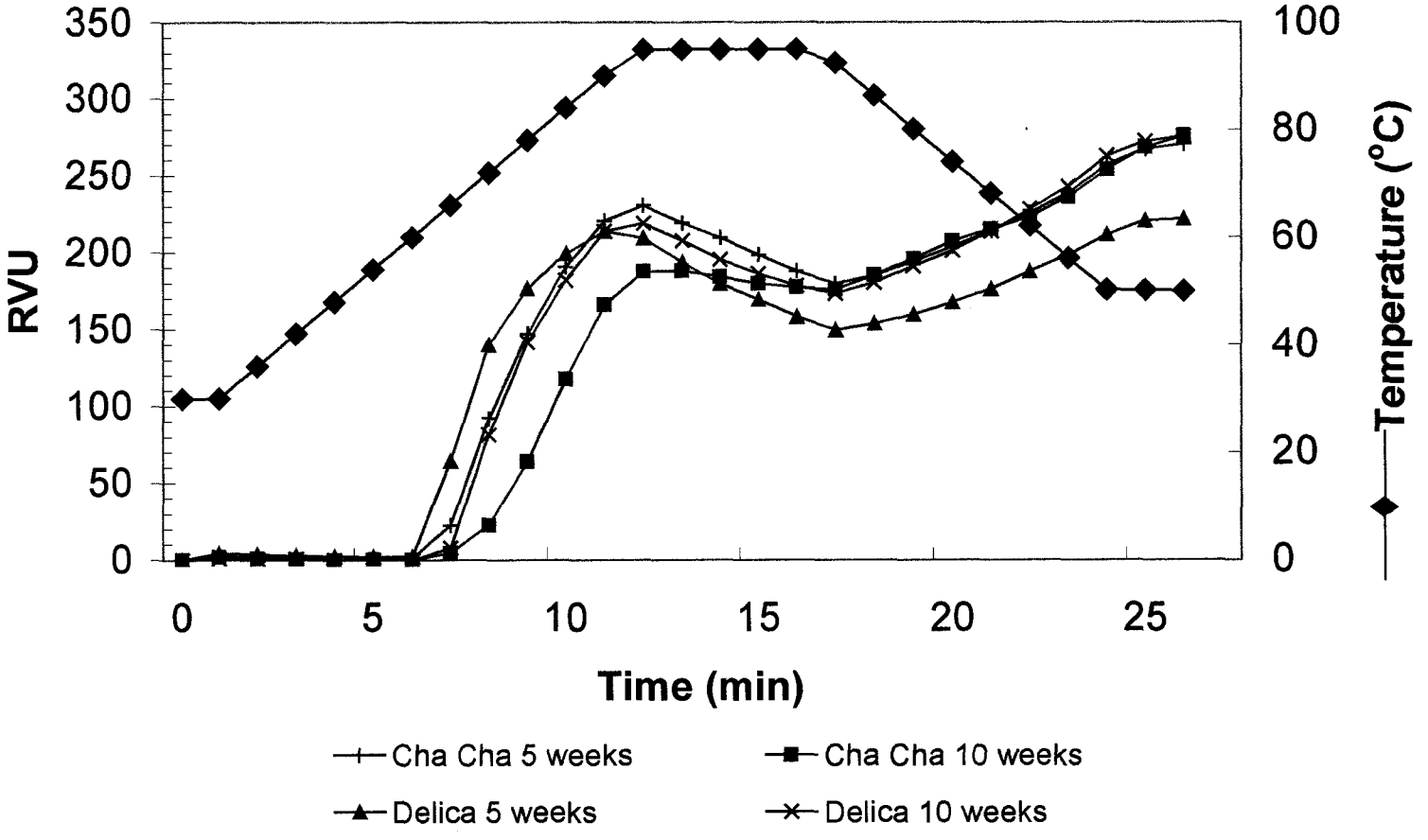

Figure 2. Rapid Visco-Analyser pasting profiles of Cha Cha and Delica squash starches extracted from fruit after 5 or 10 weeks of storage (8.0% dsb, w/w).

CHAPTER 4. ROLE OF STARCH STRUCTURE IN THE TEXTURE OF SQUASH AND STARCH FUNCTIONAL PROPERTIES. III. TEXTURE OF RAW AND COOKED WINTER SQUASH (*Cucurbita maxima D.*) FRUIT AT HARVEST AND AFTER STORAGE.

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ABSTRACT

Twelve winter squash cultivars (*Cucurbita maxima* D.), at harvest and 5 or 10 weeks storage, had fruit steamed 0, 2, 5, 10, 15 or 20 min and two-cycle compression was used to measure textural parameters, including hardness, fracturability and springiness. At harvest, raw fruit was harder for high-starch buttercup cultivars compared to low-starch Halloween-type. Hardness of fruit decreased with steaming time, and significant differences were observed among cultivars at almost all steaming times/storage combinations. After storage, many cultivars had increased hardness and fracturability of fruit, both raw and steamed 2 or 5 min. Buttercup cultivars had high fracturability when raw, low fracturability when steamed 20 min. Springiness increased with steaming time, with Halloween-type squash high and buttercup cultivars low. A high-starch cultivar, Hyvita, differed in textural parameters from buttercups. Results suggest that starch increases hardness and fracturability, but decreases springiness of raw fruit, whereas starch decreases hardness and fracturability of cooked fruit.

KEYWORDS: Texture; sensory; buttercup squash; winter squash; pumpkin; cucurbits; postharvest; storage

INTRODUCTION

Buttercup squash (*Cucurbita maxima* D.) is an important export crop for countries such as New Zealand, with the lucrative Japanese market the major focus. Japanese consumers are particular about sensory attributes of foods, and for buttercup squash, texture is an important component of quality, with geographical preferences ranging from dry, mealy to moist texture reported within Japan (1, 2).

In last ten years, considerable amount of research has attempted to understand the determinants of texture in winter squash. Sensory evaluation of eleven tropical pumpkin (*C. moschata* D.) cultigens found that fruit flesh texture varied from smooth to a fibrous mouthfeel (3). Pumpkins rated as fibrous tended to break into thin spaghetti-like particles when crushed. Cultigens were also described as being either watery, with a loose particle mouthfeel, or pasty, with a sticky mouthfeel.

Corrigan et al. (4) investigated the texture of four low- (all non-buttercups), and four high-starch content (all buttercups) *C. maxima* D. winter squash cultivars by using sensory evaluation and Instron Universal Testing Machine measurements after 4-5 and 9-10 weeks storage. For Instron Universal Testing Machine measurements, the low-starch cultivar, Warren Scarlet was softer than the other seven cultivars. The buttercup cultivar, Delica, had higher springiness than other buttercups after storage. Significant differences were found in cohesiveness, but all squash cultivars had very low cohesiveness. Sensory panelists rated high-starch buttercup cultivars to have similar brittleness, cohesiveness and hardness, but lower particle size, moistness and fibers relative to low-starch cultivars.

In order to meet Japanese consumer preferences, it is important to understand the determinants of squash texture. Daniel (3) found a high positive relationship between starch content and pastiness or smoothness of cooked pumpkin. Corrigan (4) reported high correlations between starch content and the sensory panel textural attributes of brittleness, cohesiveness, hardness, adhesiveness, particle size, mouthfeel and moistness, but only found starch content correlated to gumminess by using the Instron Universal Testing Machine. In our study, using the Instron Universal Testing Machine, we measured raw and steamed fruit flesh textural attributes of twelve *C. maxima* winter squash cultivars, obtained at harvest and after 5 or 10 weeks storage. From the same fruit as textural analysis, we have previously isolated starch and have characterized structural features (Chapter 2) and functional properties (Chapter 3). In a following publication, we will discuss the correlations between textural attributes of raw and cooked squash and starch structural and functional properties, at harvest and after storage.

MATERIALS AND METHODS

Plant Material. Twelve squash cultivars studied were fruit from the same completely randomized block planting described previously with the same cultivation, harvesting and storage procedures (Chapter 2). Squash cultivars studied were four buttercups (Cha Cha, Delica, Kurijiman and Sweet Mama), one cross of buttercup and Green Delicious (Hyvita), two Halloween-type (Big Max and Rouge Vif D'Etampes), one Hubbard-type (Warren Scarlet), one Crown-type (Whangaparoa Crown), one Native American Indian squash (Lakota) (5), and two non-commercially developed squash, one from Burkina Faso (Yogorou) and one from the Bolivian/Peruvian border (Zapallo Macre).

Steaming of Squash Fruit and Texture Analysis. Four fruit, from each of the three replicates for all 12 squash cultivars, were randomly selected as described previously (Chapter 2). All fruit skins were marked into quarters, and one quarter was randomly selected for texture analysis. From this quarter, six 3-cm wide at the equator, longitudinal segments were used for texture analysis. Depending on fruit size, allocation of segments for texture may be greater than one quarter of the fruit's circumference. Therefore, approximately two-thirds to three-quarters was randomly allocated for isolation of starch and the remainder for textural analysis, and the groundspot (part of squash fruit with skin discoloration due to contact with ground) was not excluded from the textural analysis. Squash fruit longitudinal segments, with skins remaining, were randomly selected to be steamed in a 10-cup size rice steamer (Zojirushi America Corporation, Commerce, CA, model NHS18) for 0, 2, 5, 10, 15 or 20 min. The plane of the squash fruit skin was perpendicular to the water surface, so that the pieces of fruit did not impede heat flow. After each segment was steamed for its specified time, a 20 mm diameter apple corer (Oxo brand, BASF Corp., Mount Olive, NJ) with a recessed cutting edge, preventing further compression, was used to immediately remove a fruit cylinder, cut from the direction of seed cavity to skin. The fruit cylinder was then placed in a metal cylinder with cut grooves 12 mm apart, allowing a sliced fruit cylinder to have flat surfaces with height of 12 mm and width of 20 mm, and skin excluded. Sliced fruit cylinders were placed on the base plate of the Instron Universal Testing Machine (Instron Corp., Canton, MA) with the side closest to skin against the base plate. The texture profile analysis was started exactly 40 seconds after removal of squash fruit from the steamer. Texture profile analysis of squash cylinders involved a two-

cycle compression, with 75% compression of their original height, using an Instron Universal Testing Machine. Compression, using the 57 mm compression anvil (Instron part 2830-009), was at a crosshead speed of 30 mm/min. Measurements of hardness, fracturability, adhesiveness, cohesiveness, gumminess and springiness were made by using Series 12 software (Instron Corp., Canton, MA) based on calculations described by Szczesniak (6) and Bourne (7). Hardness was defined as the maximum force of the first compression cycle that was not associated with the first fracture, unless the sample experienced first fracture at end of the first compression. Fracturability is the force at which material fractures in the first peak (compression force decreases). Springiness is the height that food recovers during the time that elapses between end of the first compression peak and start of the second compression peak. Adhesiveness is the negative force area between end of the first compression peak and start of the second compression peak. Cohesiveness is the ratio of positive force area during the second compression to that during the first compression. Gumminess is calculated as hardness x cohesiveness.

Statistical Analysis. All statistical significance tests were calculated using SAS (8) and applying Tukey difference test (9) at the 5% level of significance.

RESULTS AND DISCUSSION

Squash Fruit Hardness at Harvest. Hardness of steamed squash fruit, at harvest, is shown in **Table 1**. Comparing with other fruits and vegetables, uncooked squash fruit is very hard at harvest (10). Differences in hardness of uncooked squash fruit among cultivars were highly significant ($P < 0.0001$). The four buttercup squash cultivars, which have high starch contents (Chapter 2), had hardness when raw greater than 850 N and were significantly

harder than all other cultivars except Lakota. Hardness at harvest of the raw high-starch squash cultivars separated the closely related buttercup-cross, Hyvita, from the buttercup cultivars, as raw Hyvita's hardness was significantly lower, only 512 N. The two Halloween-type squash had the softest raw fruit, and both were significantly softer than all other cultivars except Hyvita.

Most squash cultivars steamed for 2 min experienced some reduction in hardness, and trends in significant differences among cultivars were similar to raw fruit (**Table 1**). After 5 min steaming, the range in hardness of squash fruit among cultivars was reduced with only Lakota and Sweet Mama being significantly harder than Big Max, Hyvita and Rouge Vif D'Etampes. Ten min of steaming resulted in greater variability in hardness measurements for squash fruit. The only significant difference observed was that Whangaparoa Crown and Yogorou were harder than all other cultivars, but differences among cultivars were still highly significant ($P < 0.0001$). The greatest magnitude of difference among hardness of squash cultivars was observed after 10 min steaming, as Yogorou hardness was about 7.5 times higher than Hyvita. Hardness of squash fruit after 15 min steaming ranged from 14 to 91 N for the squash cultivars, but there were still significant differences ($P = 0.005$). Yogorou fruit steamed for 15 min was significantly harder than all other cultivars except Big Max, Cha Cha, Lakota and Whangaparoa Crown. Despite the difference in hardness between cultivar extremities being only 25 N (range 9-34 N) after 20 min steaming, squash cultivar hardness was still significantly different ($P = 0.001$). The high-starch content Cha Cha fruit, steamed for 20 min, was significantly harder than the high-starch content Hyvita and Big Max, Zapallo Macre and Rouge Vif D'Etampes. Kurijiman and Yogorou fruit steamed for 20 min were also significantly harder than Rouge Vif D'Etampes.

Squash Fruit Hardness After Storage. Hardness of raw and cooked squash fruit, stored for 5 weeks, is shown in **Table 2**. Squash cultivars were significantly different for hardness of uncooked fruit ($P < 0.0001$). After 5 weeks storage, greater differences in the hardness of the high-starch content squash cultivars occurred. The two high-starch content buttercups, Cha Cha, and Kurijiman, were both significantly harder than the high-starch content Hyvita and Sweet Mama. Sweet Mama and Delica were also significantly harder than Hyvita. The lower hardness of Sweet Mama after 5 weeks storage could be due to its greater decrease in starch content, relative to the other buttercup cultivars (Chapter 2). Kurijiman raw fruit was harder than all other cultivars except Cha Cha and Delica. Both Halloween-type squash cultivar's uncooked fruit were soft with Big Max significantly softer than all cultivars except Hyvita and Rouge Vif D'Etampes.

After just 2 min steaming, hardness of some 5-week stored medium-starch content squash cultivars, Lakota, Warren Scarlet and Yogorou, were not significantly different from that of the four buttercup cultivars (**Table 2**), but significant differences among cultivars were observed ($P < 0.0001$). Although not significantly different, hardness of 5-week stored Sweet Mama squash steamed 2 min was considerably lower than the three other buttercups. This could be attributed to its fast starch degradation. Halloween-type squash cultivars were softest after 2 min steaming but were not significantly different from Hyvita, Whangaparoa Crown and Zapallo Macre. Considerable changes in relative hardness of the squash cultivars occurred after 5 min steaming compared with that of 2 min. The high-starch content buttercup, Cha Cha, was significantly softer than the other three buttercups after 5 min steaming. Only Lakota and Warren Scarlet, two non-buttercup cultivars, were significantly harder than the two Halloween-type cultivars. We have previously observed Lakota, stored

for 5 weeks, to have the greatest hardness after 5 min steaming for squash grown in 1998 (unpublished data). For squash stored 5 weeks, steaming for 10 min resulted in large magnitudes of difference in hardness with Whangaparoa Crown ten times harder than two buttercups, Cha Cha and Sweet Mama. Additionally Whangaparoa Crown was harder than all cultivars except Lakota and Yogorou. After 15 min steaming, Whangaparoa Crown was still the hardest but was only significantly harder than Hyvita and Rouge Vif D'Etampes. The two Halloween-type cultivars were the softest when raw, were harder than several cultivars after 10 min steaming but were the softest cultivars again after 20 min steaming. Despite similarity in low-starch content after 5 weeks storage (Chapter 2), Yogorou was hardest, and Big Max and Rouge Vif D'Etampes were softest, after 20 min steaming. The three high-starch content (> 33% DW) buttercup cultivars after 5 weeks storage, Cha Cha, Delica and Kurijiman, had almost parallel changes in hardness throughout steaming.

Hardness of steamed squash fruit, stored for 10 weeks is shown in **Table 3**. Surprisingly, no overall trend of fruit softening was observed during the 10 weeks storage as most cultivar's hardness of raw fruit increased or remained constant. Although the four raw buttercup cultivars, which all had greater than ten percent of dry matter as starch after 10 weeks storage, were considerably harder than other squash cultivars, combined they were only significantly harder than the two Halloween-type cultivars. Cha Cha raw fruit was also significantly harder than Hyvita, Lakota, Whangaparoa Crown, Yogorou and Zapallo Macre, which all had significantly lower starch content (Chapter 2). After steaming for 2 min, the Halloween-type cultivars that had the lowest starch content (Chapter 2) were softer than all other cultivars except Hyvita. Similar to the trend observed after 5 weeks storage, squash fruit stored for 10 weeks had considerable changes in relative hardness between 2 and 5 min

steaming. Cha Cha, which was the hardest when raw, was not significantly harder than the two Halloween-type cultivars after 5 min steaming, and was softer than Lakota, Warren Scarlet and Zapallo Macre. Whangaparoa Crown, stored for 10 weeks and steamed for 10 min, was eleven times harder than Cha Cha, and was also significantly harder than Delica, Hyvita, and Kurijiman. There were no significant differences in hardness for squash fruit, after being stored for 10 weeks and steamed for 15 or 20 min, which was not observed for fruit at harvest or stored for 5 weeks (**Table 1** and **2**).

Our results for hardness are difficult to compare with that of Corrigan et al. (4) because in the latter study, squash cultivars were steamed for just one time per cultivar, but this cooking time varied depending on cultivar, because tenderness was used as a marker to determine cooking time. Comparing with our results, the range in hardness among the eight cultivars studied by Corrigan et al. (4) is narrow, most likely because cooking all fruit until tender minimized intrinsic hardness variation. Corrigan et al. (4) also reports that Warren Scarlet had significantly lower hardness than all other cultivars, particularly buttercups, but we did not observe Warren Scarlet being softer than buttercups at any of the later steaming times (**Table 2** and **3**). Corrigan et al. (4) reports Delica and Sweet Mama fruit hardness, after storage, to be significantly softer than Kurijiman, but in our findings there are no significant differences in hardness among any of the buttercup cultivars, at the later steaming times. Additionally Corrigan et al. (4) uses just one fruit per cultivar for Instron Universal Testing Machine and sensory panel analysis, resulting in no measure of variation for the textural parameters, whereas in our study, textural parameters for each cultivar at each storage time and steaming time consists of measurements from twelve fruit.

Squash Fruit Fracturability at Harvest. Fracturability of squash fruit, at harvest and steamed up to 20 min, is shown in **Table 4**. Fracturability of raw fruit was significantly different among cultivars ($P < 0.0001$) and all squash cultivars fractured. For raw fruit, four buttercup cultivars required greater force to fracture than did other cultivars with Cha Cha and Sweet Mama significantly higher than Big Max, Hyvita, Rouge Vif D'Etampes, Warren Scarlet and Zapallo Macre. Similar to hardness, Halloween-type cultivars had the lowest force required to fracture squash fruit. Hyvita, while not significantly different from all buttercup cultivars, required a considerably lower force to fracture and this was another distinctive textural attribute that separated this cultivar from the other high starch content cultivars. After 2 min steaming of harvest fruit, differences among squash cultivars in fracturability was less than that observed for hardness, with the only significant difference ($P < 0.0001$) observed being two Halloween-type cultivars that required less force to fracture than Cha Cha, Delica, Lakota, Sweet Mama and Whangaparoa Crown. After 5 min steaming, Lakota, from fruit at harvest, required significantly higher force to fracture than Big Max, Cha Cha, Delica, Hyvita, Kurijiman and Rouge Vif D'Etampes, similar to hardness. Lakota and Whangaparoa Crown fruit at harvest, steamed for 10 min, required substantially greater force to fracture than other cultivars. A similar trend was observed for fruit steamed 15 min, but force required to fracture was greatly reduced for all cultivars except Yogorou. After 20 min steaming, squash, from fruit at harvest, fractured typically between 3-5 N, but Yogorou fracturing upon 11 N force was significantly higher than all cultivars except Lakota and Whangaparoa Crown.

Squash Fruit Fracturability After Storage. After 5 weeks storage, raw squash fruit showed a similar trend to hardness with the four buttercup cultivars requiring the greatest

force to fracture, and was significantly higher than Big Max, Hyvita and Rouge Vif D'Etampes ($P < 0.0001$) (**Table 5**). Halloween-type squash required the least force to fracture for fruit uncooked and steamed 2 min. After 5 min steaming, the biggest change was the large reduction in force required to fracture Cha Cha fruit, although this was only significantly lower than the non buttercups, Lakota, Warren Scarlet, Whangaparoa Crown and Yogorou. Lakota fruit after 5 weeks storage, like that of at harvest, required considerably greater force to fracture for fruit steamed 10 min, but unlike that at harvest, fracturability was still much higher than other cultivars after 15 min steaming. Fruit, stored for 5 weeks, fractured under low force after 20 min steaming, but Lakota, Whangaparoa Crown and Yogorou fractured under significantly higher force than all other cultivars except Warren Scarlet ($P < 0.0001$).

Fracturability of squash fruit stored for 10 weeks and steamed up to 20 min is shown in **Table 6**. Halloween-type cultivars, raw, required the least amount of force to fracture, which was significantly lower than Cha Cha, Delica, Kurijiman, Lakota and Whangaparoa Crown. Despite this, force required to fracture raw Halloween-type squash increased during storage. This trend was also observed by all other cultivars except Sweet Mama, Hyvita and Cha Cha. This phenomenon is difficult to explain. Starch within cells of uncooked fruit would be expected to provide some support to the cell walls when compression is applied, requiring greater force to find a plane of weakness in the cell-wall matrix. However all squash cultivars, except Big Max, experienced substantial degradation of starch during storage, therefore not explaining why force required to fracture for the three storage times remained constant. Additionally, Big Max had little starch to lose during storage and therefore this theory does not explain its increase in force required to fracture. Squash, like

all fruit, contain disproportionately greater amounts of pectin (11) than other plant tissues, but function of pectin in fruit is to allow rapid fruit softening (12), making it edible. Therefore a substantial loss of starch, creating a greater proportion of pectin, would be expected to decrease hardness and the force required to fracture squash fruit. An increase in proportion of non-pectic cell-wall polysaccharides (NPCWP) is unlikely to cause increased hardness and resistance to fracture since Big Max's dry matter, at harvest, is predominantly NPCWP and has the softest fruit (**Table 1**) with nearly the lowest fracturability (**Table 4**).

Eight squash cultivars had higher fracturability after 2 min steaming for fruit stored 10 weeks compared with 5 weeks storage. Lakota, stored for 10 weeks, had significantly higher fracturability after 2 min steaming than Big Max, Cha Cha, Hyvita, Rouge Vif D'Etampes and Warren Scarlet. Lakota, Whangaparoa Crown, Yogorou and Zapallo Macre, with low starch contents after 10 weeks storage (< 3% DW), all had very high forces required for fruit fracture (> 430 N), when steamed for 5 min, relative to other cultivars, and all four cultivar's fracturability were significantly higher than the high-starch content (> 10% DW) buttercup cultivars. The same four low-starch content squash cultivars had higher fracturability after 10 min steaming than other cultivars, although only Lakota and Whangaparoa Crown were significantly different from the other eight cultivars. Although Cha Cha fractured at 17 times less force than Zapallo Macre when fruit were steamed for 15 min, high variability at this cooking time meant that no significant differences were observed. After 20 min steaming, for fruit stored 10 weeks, buttercup squash cultivars had fracturability between 2 and 4 N, significantly lower than Yogorou. Fracturability of cooked squash by Corrigan et al. (4) was not reported because some cultivars did not fracture, therefore comparison with our results is not possible.

Squash Fruit Springiness at Harvest. Springiness of squash fruit, at harvest, steamed up to 20 min, is shown in **Table 7**. Springiness of raw fruit, at harvest, was significantly different among squash cultivars ($P = 0.004$), with Big Max, Lakota and Whangaparoa Crown more springy than Cha Cha. Raw buttercup squash fruit tended to be less springy than other cultivars at harvest. After 2 and 5 min steaming, the only significant difference ($P = 0.05$) was the highest starch content squash, Cha Cha, was less springy than the lowest starch content squash, Big Max. For fruit at harvest, greater numbers of cultivar differences for springiness were observed for squash steamed 10 to 20 min. For most squash cultivars, springiness increased with increasing steaming time. Lakota was most springy after 10 min steaming and springiness decreased considerably thereafter. After 10 min steaming, for fruit at harvest, the five high-starch content cultivars had the lowest springiness, with Big Max and Rouge Vif D'Etampes significantly springier than Cha Cha, Delica, Hyvita and Sweet Mama. Big Max, with very little fruit starch content, steamed for 15 min, had significantly higher springiness than the high starch containing Delica, Hyvita and Sweet Mama, and was also springier than Warren Scarlet. The two Halloween-type squash, after 20 min steaming, had the highest springiness.

Squash Fruit Springiness After Storage. Squash cultivars differed significantly for springiness of raw fruit, stored for 5 weeks ($P = 0.002$) (**Table 8**). Kurijiman, uncooked, was significantly less springy than Big Max, Cha Cha, Rouge Vif D'Etampes and Whangaparoa Crown, and Kurijiman, after 5 weeks storage, had the lowest springiness of all cultivars at all steaming times. Similar to fruit at harvest, Lakota stored for 5 weeks had highest springiness after 10 min steaming and then reduced considerably thereafter. The four high-starch content buttercup cultivars, stored for 5 weeks, were significantly less springy than the low-starch

content Rouge Vif D'Etampes and Whangaparoa Crown ($P < 0.0001$). After 20 min steaming of fruit stored for 5 weeks, the two Halloween-type cultivars, plus Whangaparoa Crown, were considerably springier than other cultivars with the low-starch content, and springiness of Rouge Vif D'Etampes was significantly higher than the high-starch content Kurijiman and Sweet Mama.

After 10 weeks storage, raw fruit of Halloween-type cultivars had higher springiness than other cultivars, but the only significant difference was that Big Max was springier than Warren Scarlet ($P = 0.04$) (**Table 9**). No significant differences in springiness were observed after 2 min steaming for fruit stored 10 weeks, but after 5 min steaming, springiness increased markedly for four cultivars, Rouge Vif D'Etampes, Sweet Mama, Whangaparoa Crown and Yogorou, which were all significantly springier than Warren Scarlet ($P = 0.01$). Four low-starch content cultivars, Big Max, Lakota, Rouge Vif D'Etampes and Whangaparoa Crown, stored for 10 weeks, had significantly higher springiness than the high-starch content Cha Cha and Delica after 10 min steaming ($P = 0.0003$). Rouge Vif D'Etampes's springiness increased considerably between 10 and 15 min steaming, and was significantly higher than Cha Cha, Delica, Kurijiman and Warren Scarlet ($P < 0.0001$). After 20 min steaming of fruit stored for 10 weeks, Halloween-type cultivars were significantly springier than Warren Scarlet ($P = 0.009$).

Corrigan et al. (4) reported the buttercups Delica and Kaboten, after 5 weeks storage, to have significantly greater springiness than other buttercups, but in our study, there was no significant difference between Delica and other buttercup cultivars for springiness at the later steaming times (**Table 8**). Once again, variation in cooking time, and the absence of accounting for variation among fruit of the same cultivar due to the use of just one fruit per

cultivar by Corrigan et al. (4) could easily lead to findings that differ from our study which attempted to account for individual cultivar fruit variation by sampling four fruit from each of three replicate blocks.

Adhesiveness, Cohesiveness and Gumminess of Squash Fruit. Squash fruit were found to lack any adhesiveness for all cultivars, at all storage times and all cooking times. Despite finding significant differences in sensory panel ratings of squash cultivar adhesiveness, Corrigan et al. (4) did not comment on adhesiveness measurements using the Instron Universal Testing Machine. Although Corrigan et al. (4) reported significant differences among cultivars for cohesiveness, their values and our own values were so low that we did not report results as we conclude that cohesiveness is not measured well using an Instron Universal Testing Machine. Significant differences in cohesiveness among squash cultivars, observed using sensory panels (4), also suggests measurements of cohesiveness by Instron Universal Testing Machine are ineffective. Since gumminess is the product of cohesiveness and hardness, and cohesiveness was almost zero, we did not attempt to calculate gumminess.

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Table 1

Hardness (N) of squash fruit, at harvest, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	312^{ef}	312^{f}	250^{b}	137^{b}	48^{ab}	15^{bc}
Cha Cha	1083^{a}	940^{a}	380^{ab}	63^{b}	43^{ab}	34^{a}
Delica	872^{b}	867^{ab}	316^{ab}	49^{b}	27^{b}	19^{abc}
Hyvita	512^{de}	506^{ef}	244^{b}	42^{b}	22^{b}	15^{bc}
Kurijiman	909^{ab}	789^{abcd}	345^{ab}	60^{b}	33^{b}	28^{ab}
Lakota	754^{bc}	680^{abcde}	529^{a}	148^{b}	51^{ab}	23^{abc}
Rouge Vif D'Etampes	304^{f}	346^{f}	245^{b}	73^{b}	14^{b}	9^{c}
Sweet Mama	853^{b}	801^{abc}	510^{a}	56^{b}	24^{b}	17^{abc}
Warren Scarlet	549^{cd}	535^{cdef}	354^{ab}	123^{b}	30^{b}	17^{abc}
Whangaparoa Crown	581^{cd}	528^{def}	351^{ab}	293^{a}	46^{ab}	21^{abc}
Yogorou	595^{cd}	653^{bcde}	429^{ab}	313^{a}	91^{a}	31^{ab}
Zapallo Macre	541^{d}	554^{cdef}	382^{ab}	95^{b}	23^{b}	15^{bc}
	$P < 0.0001$♦	$P < 0.0001$	$P = 0.008$	$P < 0.0001$	$P = 0.005$	$P = 0.001$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 2

Hardness (N) of squash fruit, after 5 weeks of storage, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	291^{g}	282^{f}	239^{cd}	186^{bcd}	84^{ab}	13^{bc}
Cha Cha	968^{ab}	868^{a}	256^{cd}	41^{d}	31^{ab}	22^{abc}
Delica	943^{abc}	850^{ab}	418^{abcd}	57^{cd}	30^{ab}	23^{abc}
Hyvita	455^{efg}	416^{def}	242^{cd}	53^{d}	18^{b}	14^{bc}
Kurijiman	1012^{a}	873^{a}	404^{abcd}	66^{cd}	31^{ab}	23^{abc}
Lakota	769^{bcd}	763^{abc}	594^{a}	266^{abc}	73^{ab}	26^{ab}
Rouge Vif D'Etampes	348^{fg}	327^{ef}	199^{d}	96^{cd}	19^{b}	11^{c}
Sweet Mama	730^{cd}	640^{abcd}	397^{abcd}	43^{d}	22^{ab}	15^{bc}
Warren Scarlet	625^{de}	633^{abcd}	494^{ab}	197^{bcd}	43^{ab}	19^{abc}
Whangaparoa Crown	554^{def}	591^{bcde}	448^{abc}	423^{a}	94^{a}	23^{abc}
Yogorou	681^{de}	612^{abcd}	430^{abc}	377^{ab}	78^{ab}	32^{a}
Zapallo Macre	569^{def}	517^{cdef}	362^{bcd}	136^{cd}	36^{ab}	17^{bc}
	$P < 0.0001$♦	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.003$	$P = 0.0003$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 3

Hardness (N) of squash fruit, after 10 weeks of storage, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	361^{ef}	381^{cd}	317^{abc}	156^{ab}	30	14
Cha Cha	949^{a}	762^{a}	204^{c}	28^{b}	24	21
Delica	862^{abc}	778^{a}	361^{abc}	54^{b}	24	17
Hyvita	500^{def}	448^{bcd}	232^{bc}	44^{b}	23	12
Kurijiman	898^{ab}	833^{a}	371^{abc}	42^{b}	21	16
Lakota	659^{bcd}	687^{ab}	503^{ab}	177^{ab}	72	24
Rouge Vif D'Etampes	318^{f}	316^{d}	201^{c}	89^{ab}	55	9
Sweet Mama	672^{abcd}	663^{ab}	355^{abc}	75^{ab}	26	16
Warren Scarlet	740^{abcd}	689^{ab}	521^{a}	150^{ab}	36	28
Whangaparoa Crown	617^{cde}	595^{abc}	457^{abc}	323^{a}	165	33
Yogorou	614^{cde}	614^{abc}	410^{abc}	190^{ab}	69	27
Zapallo Macre	594^{cdef}	631^{abc}	496^{ab}	206^{ab}	118	56
	$P < 0.0001$♦	$P < 0.0001$	$P = 0.001$	$P = 0.01$	$P = 0.35$	$P = 0.12$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 4

Fracturability (N) of squash fruit, at harvest, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	255^{cd}	239^{b}	140^{b}	12^{b}	7^{bc}	5^{b}
Cha Cha	862^{a}	659^{a}	163^{b}	17^{b}	6^{c}	4^{b}
Delica	745^{ab}	594^{a}	134^{b}	14^{b}	7^{bc}	3^{b}
Hyvita	489^{bcd}	416^{ab}	147^{b}	15^{b}	7^{c}	3^{b}
Kurijiman	735^{ab}	490^{ab}	167^{b}	14^{b}	5^{c}	4^{b}
Lakota	703^{ab}	709^{a}	508^{a}	108^{ab}	16^{a}	8^{ab}
Rouge Vif D'Etampes	252^{d}	247^{b}	173^{b}	29^{b}	4^{c}	3^{b}
Sweet Mama	795^{a}	738^{a}	363^{ab}	19^{b}	6^{c}	3^{b}
Warren Scarlet	517^{bc}	425^{ab}	252^{ab}	14^{b}	8^{bc}	4^{b}
Whangaparoa Crown	646^{ab}	577^{a}	360^{ab}	154^{a}	14^{ab}	7^{ab}
Yogorou	633^{ab}	563^{ab}	320^{ab}	19^{b}	16^{a}	11^{a}
Zapallo Macre	493^{bcd}	423^{ab}	254^{ab}	30^{b}	8^{bc}	3^{b}
	$P < 0.0001$♦	$P < 0.0001$	$P = 0.002$	$P = 0.0004$	$P < 0.0001$	$P = 0.001$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 5

Fracturability (N) of squash fruit, after 5 weeks of storage, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	273^{c}	273^{cd}	142^{bc}	17^{b}	14^{ab}	3^{c}
Cha Cha	873^{a}	698^{ab}	22^{c}	14^{b}	6^{b}	4^{c}
Delica	829^{a}	635^{abcd}	194^{bc}	15^{b}	6^{b}	3^{c}
Hyvita	513^{bc}	418^{bcd}	128^{bc}	15^{b}	6^{b}	4^{c}
Kurijiman	898^{a}	710^{ab}	273^{bc}	18^{b}	7^{b}	3^{c}
Lakota	806^{ab}	821^{a}	641^{a}	279^{a}	42^{a}	11^{ab}
Rouge Vif D'Etampes	264^{c}	260^{d}	125^{bc}	34^{b}	7^{b}	4^{c}
Sweet Mama	852^{a}	657^{abc}	204^{bc}	17^{b}	7^{b}	3^{c}
Warren Scarlet	693^{ab}	509^{abcd}	400^{ab}	87^{b}	14^{ab}	6^{bc}
Whangaparoa Crown	660^{ab}	605^{abcd}	407^{ab}	131^{ab}	17^{ab}	10^{ab}
Yogorou	684^{ab}	556^{abcd}	428^{ab}	16^{b}	18^{ab}	13^{a}
Zapallo Macre	591^{ab}	538^{abcd}	237^{bc}	40^{b}	13^{ab}	4^{c}
	$P < 0.0001$♦	$P = 0.0004$	$P < 0.0001$	$P = 0.0002$	$P = 0.02$	$P < 0.0001$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 6

Fracturability (N) of squash fruit, after 10 weeks of storage, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	337^{de}	344^{cd}	254^{abcd}	21^{b}	10	5^{abcd}
Cha Cha	861^{abc}	459^{bcd}	21^{d}	13^{b}	4	2^{cd}
Delica	922^{ab}	622^{abc}	184^{bcd}	16^{b}	6	2^{d}
Hyvita	464^{cde}	388^{cd}	99^{d}	20^{b}	8	2^{cd}
Kurijiman	1003^{a}	753^{ab}	84^{d}	17^{b}	6	4^{bcd}
Lakota	872^{ab}	865^{a}	438^{abc}	266^{a}	28	11^{abc}
Rouge Vif D'Etampes	289^{e}	264^{d}	83^{d}	33^{b}	5	3^{bcd}
Sweet Mama	699^{abcd}	619^{abc}	81^{d}	23^{b}	8	3^{bcd}
Warren Scarlet	595^{bcde}	527^{bcd}	118^{cd}	21^{b}	11	6^{abcd}
Whangaparoa Crown	798^{abc}	721^{ab}	535^{a}	229^{a}	14	12^{ab}
Yogorou	695^{abcd}	609^{abc}	431^{abc}	162^{ab}	30	14^{a}
Zapallo Macre	621^{abcde}	558^{abcd}	455^{ab}	98^{ab}	69	10^{abcd}
	$P < 0.0001$♦	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.31$	$P = 0.0001$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 7

Springiness (mm) of squash fruit, at harvest, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	12.74^{a}	12.72^{a}	13.00^{a}	13.31^{a}	13.32^{a}	13.30^{ab}
Cha Cha	11.88^{b}	12.10^{b}	12.30^{b}	12.67^{bcd}	13.04^{abc}	13.07^{abcd}
Delica	12.20^{ab}	12.27^{ab}	12.52^{ab}	12.59^{cd}	12.79^{bcd}	12.97^{abcd}
Hyvita	12.31^{ab}	12.34^{ab}	12.72^{ab}	12.22^{c}	12.44^{d}	12.94^{abcd}
Kurijiman	12.14^{ab}	12.27^{ab}	12.60^{ab}	12.71^{abcd}	12.93^{abcd}	12.97^{abcd}
Lakota	12.53^{a}	12.50^{ab}	12.84^{ab}	13.20^{ab}	13.09^{abc}	12.86^{bcd}
Rouge Vif D'Etampes	12.42^{ab}	12.62^{ab}	12.89^{ab}	13.30^{a}	13.27^{ab}	13.34^{a}
Sweet Mama	12.45^{ab}	12.34^{ab}	12.71^{ab}	12.58^{cd}	12.72^{cd}	13.09^{abc}
Warren Scarlet	12.35^{ab}	12.32^{ab}	12.72^{ab}	12.76^{abcd}	12.43^{d}	12.62^{d}
Whangaparoa Crown	12.57^{a}	12.65^{ab}	12.92^{ab}	13.22^{ab}	13.07^{abc}	13.11^{abc}
Yogorou	12.17^{ab}	12.17^{ab}	12.78^{ab}	13.02^{abc}	12.83^{abcd}	12.80^{cd}
Zapallo Macre	12.35^{ab}	12.45^{ab}	12.68^{ab}	12.99^{abc}	12.93^{abcd}	13.00^{abcd}
	$P = 0.004$♦	$P = 0.05$	$P = 0.05$	$P < 0.0001$	$P < 0.0001$	$P = 0.0007$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 8

Springiness (mm) of squash fruit, after 5 weeks of storage, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	12.68^{a}	12.72^{a}	12.80^{ab}	13.14^{ab}	13.12^{ab}	13.18^{ab}
Cha Cha	12.43^{a}	12.57^{ab}	12.68^{ab}	12.74^{bc}	12.87^{abc}	12.97^{ab}
Delica	12.19^{ab}	12.31^{ab}	12.60^{ab}	12.48^{cd}	12.40^{cde}	12.53^{ab}
Hyvita	12.41^{ab}	12.49^{ab}	12.95^{a}	12.87^{abc}	12.62^{bcde}	12.77^{ab}
Kurijiman	11.71^{b}	11.98^{b}	12.32^{b}	12.12^{d}	12.05^{e}	12.36^{b}
Lakota	12.32^{ab}	12.25^{ab}	12.70^{ab}	13.19^{ab}	12.99^{abc}	12.78^{ab}
Rouge Vif D'Etampes	12.54^{a}	12.54^{ab}	12.87^{ab}	13.20^{a}	13.27^{a}	13.27^{a}
Sweet Mama	12.06^{ab}	12.33^{ab}	12.53^{ab}	12.48^{cd}	12.21^{de}	12.43^{b}
Warren Scarlet	11.98^{ab}	12.07^{b}	12.39^{ab}	12.87^{abc}	12.48^{cde}	12.46^{ab}
Whangaparoa Crown	12.60^{a}	12.56^{ab}	12.91^{a}	13.27^{a}	13.14^{ab}	13.13^{ab}
Yogorou	12.16^{ab}	12.06^{b}	12.70^{ab}	12.97^{ab}	12.83^{abcd}	12.71^{ab}
Zapallo Macre	12.17^{ab}	12.29^{ab}	12.63^{ab}	12.91^{abc}	12.89^{abc}	12.80^{ab}
	$P = 0.002$♦	$P = 0.003$	$P = 0.009$	$P < 0.0001$	$P < 0.0001$	$P = 0.005$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 9

Springiness (mm) of squash fruit, after 10 weeks of storage, steamed for 0 to 20 minutes*.

Cultivar	Steaming Time (min)					
	0#	2	5	10	15	20
Big Max	12.55[a]	12.61	12.57[ab]	13.03[a]	13.09[ab]	13.18[a]
Cha Cha	11.97[ab]	12.29	12.55[ab]	12.36[bc]	12.33[bc]	12.49[ab]
Delica	11.84[ab]	12.08	12.50[ab]	12.27[c]	11.87[c]	12.32[ab]
Hyvita	12.19[ab]	12.28	12.76[a]	12.58[abc]	12.49[abc]	12.80[ab]
Kurijiman	11.86[ab]	12.13	12.61[ab]	12.69[abc]	12.30[bc]	12.46[ab]
Lakota	11.78[ab]	11.93	12.47[ab]	13.05[a]	13.00[ab]	13.03[ab]
Rouge Vif D'Etampes	12.44[ab]	12.27	12.92[a]	13.08[a]	13.21[a]	13.26[a]
Sweet Mama	12.37[ab]	12.28	12.90[a]	12.83[abc]	12.49[abc]	12.52[ab]
Warren Scarlet	11.72[b]	11.97	11.90[b]	12.77[abc]	12.28[bc]	12.07[b]
Whangaparoa Crown	12.32[ab]	12.35	12.75[a]	13.02[a]	12.96[ab]	12.88[ab]
Yogorou	12.34[ab]	12.39	12.72[a]	12.94[ab]	12.78[ab]	12.76[ab]
Zapallo Macre	12.23[ab]	12.17	12.58[ab]	12.88[ab]	12.93[ab]	12.82[ab]
	$P = 0.04$♦	$P = 0.44$	$P = 0.01$	$P = 0.0003$	$P < 0.0001$	$P = 0.009$

* Values are from four fruit for each of three replicates.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

CHAPTER 5. ROLE OF STARCH STRUCTURE IN TEXTURE OF SQUASH AND STARCH FUNCTIONAL PROPERTIES. IV. CORRELATIONS AMONG STARCH STRUCTURE, STARCH FUNCTIONALITY AND TEXTURE OF WINTER SQUASH (*Cucurbita maxima* D.) FROM FRUIT AT HARVEST AND AFTER STORAGE.

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ABSTRACT

Correlations among starch structure, starch functionality and raw or cooked texture of winter squash fruit were investigated. Correlations were dependent on fruit storage time and cooking time. Both water and starch content of squash were correlated to hardness, fracturability and springiness. Apparent amylose correlated negatively to hardness and fracturability, but absolute amylose correlated positively to hardness and fracturability, suggesting long-chain amylopectins play a role in texture. Amylopectin molecular weight and polydispersity correlated positively to hardness. Hardness and fracturability of squash fruit was always correlated positively to short (DP $\leq$ 12) and very long (DP $\geq$ 37) amylopectin branch chain-lengths and negatively correlated to intermediate amylopectin branch chain-lengths (DP 13-36), regardless of storage time. Hardness was negatively correlated to gelatinization temperatures. Paste viscosity was negatively correlated to hardness and fracturability, while breakdown was positively correlated to springiness. Amylose was positively correlated to paste viscosity. Higher molecular weight amylopectin, with lower polydispersity, results in lower gelatinization temperatures.

KEYWORDS: Starch structure; starch function; squash; pumpkin; cucurbits; texture; correlation; postharvest; storage

INTRODUCTION

Winter squash is an important export crop, for countries such as New Zealand, with the lucrative Japanese market receiving the greatest attention. Japanese consumers vary in their textural preference of cooked winter squash, depending on geographical location (1, 2). In order to meet Japanese consumer demands, the textural determinants of cooked winter squash need to be unraveled. Winter squash have previously been reported to have starch content above 60% of the dry matter (3), leading to the postulate that starch properties could be influencing winter squash texture.

Few studies have investigated the role of starch properties in texture of fruit and vegetable crops. Widespread opinion in the scientific community is that fruit and vegetables soften solely due to demethylation of pectin, resulting in pectin degradation and a loss of middle lamellae that holds the cellulose/hemicellulose cell wall matrix together (4, 5). Starch is typically considered to have no involvement in fruit and vegetable texture or is overlooked when investigating textural determinants. Literature on the role of potato composition in cooked texture is useful for comparisons due to its high proportion of dry matter as starch. Steamed potatoes have been shown to fracture between cells preferentially alongside the cell wall, with the degree of intercellular spaces determining texture (6). Subsequent studies showed mealy potatoes had more cell wall material per unit cell surface area than nonmealy potatoes (7). Further studies of cooked potato microstructure showed that swelling of gelatinized starch had no influence on texture, but destruction of cell walls resulted in

softening (8). Recently evidence of starch playing a role in cooked potato texture has emerged. Modeling starch gelatinization kinetics in potatoes showed that starch gelatinization is completed at early cooking times and contributes to texture during gelatinization process (10). Studies of cooked sweet potato found no evidence that textural properties resulted from cell wall structure, but instead found that enzymic degradation of starch induced textural changes (10). Starch content has been implicated in cooked potato crisp texture (11) and another study found starch content was a powerful variable that encompassed the major part of potato attributes affecting texture (12). A combination of starch swelling and cell wall degradation resulting in soggy potato texture has been reported (13).

Currently the main agricultural commodity in which a well established relationship between starch structure or functionality and cooked texture has been made is rice. Greatest emphasis has been the relationship between amylopectin fine structure and cooked rice texture. Proportion of long amylopectin B-chains, and proportion of these chains in the exterior regions, carrying non-reducing ends, was found to be strongly related to cooked rice texture (14, 15, 16). Low-amylose and waxy rice were found to provide hard and sticky texture (17, 18). Rice starch pasting properties have also been found to be highly correlated with textural parameters (19, 20).

The objectives of this study are to determine the role of starch structure in the texture of raw and cooked winter squash fruit, the role of starch functionality in the texture of raw and cooked winter squash fruit and the role of squash starch structure in starch functional properties. We have previously studied extensively, from winter squash fruit at harvest, and stored for 5 or 10 weeks, the starch structural properties (Chapter 2), starch functional

properties (Chapter 3), and textural attributes of raw and cooked fruit (Chapter 4). In this paper we investigate the correlations among starch structure, starch functionality and textural attributes of winter squash fruit.

MATERIALS AND METHODS

Plant Material, Starch Extraction, Starch Structural Analysis, Starch Functional Analysis. Twelve winter squash (*Cucurbita maxima* D.) cultivars (3 replicates/cultivar), described previously (Chapter 2) were cultivated and had starch extracted from fruit at harvest, and after 5 or 10 weeks storage, as described previously (Chapter 2). Starch structural analysis included starch content, apparent and absolute amylose content, amylopectin molecular weight, amylopectin gyration radii, amylopectin branch chain-length distribution and percent crystallinity. All methods of starch structural analysis have been described previously (Chapter 2). Starch functional analysis included thermal properties of native and retrograded starch, pasting properties and gel firmness and stickiness. All methods of starch functional analysis have been described previously (Chapter 3).

Squash Fruit Texture Measurements. Textural parameters measured were hardness, fracturability and springiness of squash fruit from all 12 cultivars, for all three replicates, at each of the three storage times. Fruit textural measurements were made on raw, and fruit steamed for five different times between 2 and 20 min as described previously (Chapter 4).

Correlation Analysis. Starch Structural, starch functional and squash fruit textural attributes were correlated using SAS (21) and the PROC CORR function specifying use of the Pearson correlation coefficient. A 5% level of statistical significance was used to discriminate correlations of importance. Means of the twelve squash cultivars were used for the

correlations. Since not all squash cultivars yielded sufficient levels of starch to conduct all starch structural and functional analyses, the number of squash cultivar means (n) correlated varied depending on parameters correlated, as follows: $n = 12$ for all correlations involving entirely textural parameters, water or starch content; $n = 11$ for any correlation involving, from fruit at harvest, starch DSC thermal analysis, iodine affinities of starch, apparent amylose content, absolute amylose content, isoamylase-debranched amylopectin chain-length measured using high-performance anion-exchange chroamtatography (HPAEC), isoamylase-debranched amylopectin chain-length measured using high-performance size-exclusion chromatography (HPSEC), amylopectin molecular size and polydispersity measured using HPSEC, gyration radii, percent crystallinity, gel firmness, gel stickiness, and all starch RVA pasting analysis; $n = 8$ for any correlation involving, from fruit stored for 5 weeks, DSC thermal analysis, iodine affinities, apparent amylose content, absolute amylose content, isoamylase-debranched amylopectin chain-length measured using HPAEC, isoamylase-debranched amylopectin chain-length measured using HPSEC, amylopectin molecular size and polydispersity measured using HPSEC and gyration radii; $n = 6$ for any correlation involving, from fruit stored 5 weeks, starch percent crystallinity and RVA pasting properties, and any correlation involving, from fruit stored 10 weeks, DSC thermal properties, iodine affinities, apparent amylose content, absolute amylose content, isoamylase-debranched amylopectin chain-length measured using HPAEC, isoamylase-debranched amylopectin chain-length measured using HPSEC, amylopectin molecular size and polydispersity measured using HPSEC and gyration radii; $n = 5$ for any correlation involving, from fruit stored 5 weeks, gel firmness and gel stickiness; $n = 4$ for any correlation involving, from fruit

stored 10 weeks, gel firmness, gel stickiness and RVA pasting properties; $n = 3$ for any correlation involving, from fruit stored 10 weeks, starch percent crystallinity.

RESULTS AND DISCUSSION

Correlation coefficients among selected starch structural, starch functional and fruit textural properties of squash are shown in **Tables 1-12**. Correlation coefficients are mentioned in the text when not included in **Tables 1-12**. Correlation **Table 3** and **7** consist of only texture correlations that are separated to reduce the number of columns in **Tables 4, 5, 8** and **9**.

Effects of Water/Dry Matter Percentage on Fruit Texture. Water and dry matter content of squash fruit at harvest, and both storage times, among the cultivars, were significantly correlated to the hardness, fracturability and springiness of raw and cooked squash fruit. For squash cultivar fruit at harvest, water content of raw fruit was significantly correlated to hardness ($r = -0.90$, $P < 0.0001$), fracturability ($r = -0.78$, $P = 0.003$) and springiness ($r = 0.72$, $P = 0.008$). Harvest fruit of the squash cultivars, steamed for 2 or 10 min, had water content also significantly correlated to hardness ($r = -0.87$ and 0.57, $P = 0.0003$ and 0.05, respectively) and springiness ($r = 0.72$, $P = 0.009$ for both). Water content of squash cultivar fruit steamed for 5 min were not significantly correlated to hardness or fracturability at harvest or both storage times. However springiness was strongly correlated to water/dry matter content after 5 min steaming for fruit at harvest only ($r = 0.90$, $P < 0.0001$). The lack of relationship for the squash cultivars between water/dry matter content and hardness after 5 min steaming, but then a significant relationship after 10 min steaming, with a change from negative to positive, suggests that the 5 min steaming time could be a transition stage in

which dry matter components contributing to hardness are converted to products eliciting softer texture.

Raw squash cultivar water content, from fruit after 5 and 10 weeks storage, was significantly correlated to hardness (r = -0.85 and -0.88, P = 0.005 and 0.0002 respectively) and fracturability (r = -0.74 and -0.72, P = 0.006 and 0.008 respectively), but not springiness. Fracturability of steamed squash fruit, from cultivars at both 5 or 10 weeks storage, was not significantly correlated to water content, but hardness was significantly correlated to water content, for both storage times, after steaming for 2 min (r = -0.78, P = 0.003 for both) and 10 min (r = 0.67 and 0.62, P = 0.02 and 0.03 respectively).

For squash cultivar fruit stored for 5 or 10 weeks, springiness was only significantly correlated to water content at the later cooking times of 10 min (r = 0.86, P = 0.0005 for both), 15 min (r = 0.70 and 0.79, P = 0.01 and 0.002 respectively) and 20 min (r = 0.57 and 0.63, P = 0.05 and 0.03 respectively). Increasing springiness with decreasing dry matter percentage for the squash cultivars is surprising as in the later steaming times, starch would be expected to form a paste exhibiting viscoelastic rheological properties contributing to fruit springiness. An overall lack of dry matter in squash fruit, primarily due to lack of starch accumulation, may mean that a greater proportion of the dry matter is pectin, providing viscoelastic characteristics.

Effects of Starch Content on Fruit Texture. Starch content, from fruit at harvest and 5 or 10 weeks storage, was significantly correlated to hardness of raw fruit (**Table 1**, **4** and **8**), and fruit steamed for 2 min (r = 0.76, 0.75 and 0.64; P = 0.004, 0.005 and 0.02 respectively) or 10 min (**Table 1**, **4** and **8**). Qi et al. (22) reported starch in fruit to contribute to firmer texture of raw fruit but to softening during starch gelatinization, and Lee et al. (23) reported a

positive correlation between starch content and hardness of sorghum grain. Increased fruit hardness with increasing starch content was very obvious when processing fruit for starch extraction. Highly significant positive relationships between starch content and raw or 2 min steamed fruit indicates that ungelatinized starch granules contribute greatly to hardness of fruit. The strong lack of any relationship between starch content and hardness of squash fruit steamed for 5 min, for fruit at harvest or both storage times ($r < 0.04$ for all), suggests this is a transition cooking stage in which considerable amount of starch has gelatinized, and the significant negative correlation between starch content and fruit steamed for 10 min indicates most starch has gelatinized and the paste formed contributes to softer fruit than fruit with dry matter predominantly consisting of cell walls. Lack of contribution to texture once gelatinization is completed has been reported (10).

Starch content of fruit, at harvest and after 5 or 10 weeks storage, was significantly correlated to fracturability of raw fruit (**Table 1, 4** and **8**) but not correlated when steamed. The increased force required to fracture raw squash fruit that has greater starch content is most likely because each cell to some extent is supported internally by starch granules within, and this theory has been previously postulated (24). If the fracture plane is through cell walls, then lack of involvement of starch in fracturability, even after 2 min cooking, could be due to decreased support from starch granules undergoing early phases of gelatinization. If the fracture plane included starch granules then the lack of involvement of starch in fracturability of steamed squash during cooking, could be because as squash fruit is heated, starch granules begin to absorb water, reducing glass transition temperature (25), and resulting in starch moving away from the brittle glassy region to the less brittle ductile region (26). Since squash fruit fractured at all steaming times, steamed fruit most likely fractured

because some cell walls separated due to pectin breakdown in the middle lamellae, as has been reported for potatoes (6). Distension of cell walls due to starch swelling has also been reported to contribute to fracturability of fruit and vegetable tissues (6) but other studies have observed starch swelling without cell wall breakdown (27). Binner et al. (10) has enlightened this debate by demonstrating that potatoes cooked at 100°C were softer and experienced swollen starch distending cell walls causing separation, whereas potatoes cooked at 70°C were firmer because there was no pectin demethylation and starch breakdown produced oligomers that could escape from the cells. In our studies we observe a mixture of cells with swollen starch distended that may or may not have caused cell separation (Chapter 6).

The effect of starch content on springiness of steamed squash was dependent on storage time. Starch content of squash fruit at harvest was significantly correlated to springiness for raw fruit and fruit steamed for 2, 5 or 10 min (r = -0.66, -0.67, -0.80 and -0.84; P = 0.02, 0.02, 0.002 and 0.0005 respectively). However, fruit stored for 5 or 10 weeks resulted in significant correlations between starch content and springiness only for fruit steamed 10 or 15 min (**Table 4** and **8**). As mentioned previously, decreased starch content resulting in increased springiness implies that other dry matter components are more viscoelastic. Pectin is the most likely component since pectin is well known to be at higher concentrations in fruit and has been reported to be 10% of dry matter in pumpkins (28). Cell wall swelling and sliding in fruit tissues during ripening, due to pectin solubilization, increases viscoelastic properties of cell walls (29, 30), but fruits which ripen to a fracturable texture, which squash possess (Chapter 4), do not exhibit cell wall swelling (30). Additionally, an absence of any differences in total cell wall polysaccharide content for low-

and high-starch winter squash after two months storage was reported (31). For lack of pectin solubilization, and absence of cell wall swelling, to explain greater springiness of low-starch squash cultivar fruit during cooking, starch could be interacting with pectin to reduce springiness. We have shown, using light microscopy, that winter squash in this study have pectic strands and starch interacting (32), which may restrict cell wall sliding.

Starch Granule Size Distribution and Fruit Texture. For fruit at harvest, we did not observe any differences in starch granule shape or size distribution (Chapter 2) that we could attribute to textural differences in the squash fruit. Despite scanning electron microscopy images showing some squash starches had amylase-hydrolyzed and other damaged granules after 5 weeks storage, there was no clear trend between this and textural attributes. There was some suggestion that squash with a high proportion of starch hydrolyzed after 5 weeks storage may be less hard when raw and more firm after 10 min steaming compared to squash cultivars which had few hydrolyzed or damaged starch granules after 5 weeks storage (Chapter 2, Chapter 4). A lack of relationship between starch granule size and texture has been reported previously (15, 32). However, Gaines et al. (33) reported wheats with larger starch granules had softer texture, and in contrast, Seetharaman et al. (34) reported that larger corn starch granules resulted in firmer gels. In some resemblance to our findings for squash fruit with a greater proportion of hydrolyzed and damaged starch, increased hardness of baked wheat tandoori roti was observed with increasing percentage of damaged starch (35).

Effect of Amylose on Fruit Texture. Hardness and fracturability of raw squash fruit, at harvest, was significantly correlated to iodine affinity of the amylopectin fraction (r = -0.59 and -0.61; P = 0.05 and 0.04 respectively) and absolute amylose content (**Table 1**). Absolute amylose was also correlated to hardness of fruit, at harvest, steamed for 2 min (r = 0.60, P =

0.05). After 5 weeks of storage, hardness was not correlated to iodine affinity of amylopectin fraction or absolute amylose content. However, hardness of fruit stored 5 weeks, and steamed for 10 min, was correlated to apparent amylose content (**Table 4**). Fracturability was correlated to absolute amylose content for raw fruit after 5 weeks storage (**Table 4**), and apparent amylose content of fruit, stored for 5 weeks and steamed for 5 min (r = -0.75, P = 0.03). After 10 weeks storage, apparent amylose content of squash fruit steamed for 5, 10, 15 or 20 min was significantly correlated to hardness and fracturability (**Table 8**). For fruit stored 10 weeks, hardness after 5 min and fracturability after 2 min steaming, were significantly correlated to iodine affinity of amylopectin fraction (r = -0.87 and -0.82, P = 0.03 and 0.04 respectively), but not correlated to absolute amylose content.

Harvest fruit springiness was largely unrelated to amylose content with the only correlations observed being springiness of fruit steamed 10 min and absolute amylose content (**Table 1**). No correlations between springiness and amylose content were observed for fruit stored 10 weeks, but many correlations were observed for fruit stored 5 weeks. For fruit stored 5 weeks, iodine affinity of the amylopectin fraction was significantly correlated to springiness of fruit steamed 0, 2, 5 and 20 min (r = 0.75, 0.74, 0.73 and 0.82; P = 0.03, 0.04, 0.04, and 0.01 respectively). Absolute amylose of the same fruit was significantly correlated to springiness of fruit steamed at the two times that were not correlated for iodine affinity of amylopectin fraction, 10 and 15 min (**Table 4**).

Overall, results suggest high levels of apparent amylose could result in lower hardness and fracturability, but high levels of absolute amylose could result in harder fruit, with more force required to fracture and less springiness. Since apparent amylose incorporates absolute amylose and long-chain amylopectins capable of complexing iodine to

form blue color (36), long-chain amylopectins may play some role in textural attributes of squash fruit. Proportion of long amylopectin chains ($DP_n > 92$), but not intermediate length, has been reported to critically control texture of cooked rice (15). Comparisons to other literature for absolute amylose content effects on texture are difficult since few researchers measure absolute amylose, and those that do are typically not focused on texture of foods. In contrast to our findings, apparent amylose content has been found to be positively correlated to hardness in rice (15, 16, 18, 37, 38, 39), rice-based fries (40), bananas and plantain (22), starch gels from cereals (33, 41) and legumes (42). The only exception was Gaines et al. (41) who reported that apparent amylose content contributed to a softer kernel texture. Explanation of why most researchers findings for apparent amylose agree with our findings for absolute amylose could be that all their research was on cereals or legumes with A-type crystal patterns, which have shorter amylopectin branch chain-lengths than B-type starches (43), such as squash (Chapter 2). Therefore the overestimation in amylose content by the apparent amylose method is small for A-type starches, compared to the high iodine affinities for amylopectin fraction of B-type squash cultivar starches (Chapter 2).

Effect of Amylopectin Molecular Size on Fruit Texture. Effect of amylopectin molecular size on squash fruit texture depended on fruit storage time, with few correlations observed for fruit at harvest or after 5 weeks storage, but many correlations observed for fruit stored 10 weeks. For fruit at harvest, hardness of fruit steamed for 5 min was correlated to *z*-average amylopectin molecular weight (M_z) and gyration radius based on M_z (R_z) (r = 0.60 and 0.70; P = 0.05 and 0.02 respectively). Additionally, fracturability of fruit steamed for 5 min was correlated to number-average amylopectin molecular weight (M_n) and weight-average amylopectin molecular weight (M_w) (r = 0.73 and 0.79; P = 0.01 and 0.004 respectively).

There were no other correlations observed between textural and amylopectin molecular size parameters, for raw and steamed fruit at harvest. For fruit stored 5 weeks, only correlations observed were fracturability of fruit steamed for 2 min was correlated to gyration radius based on M_n (R_n) and gyration radius based on M_w (R_w) (r = -0.70 and -0.76; P = 0.05 and 0.03 respectively), and hardness of fruit steamed for 20 min was correlated to R_w (r = -0.71, P = 0.05).

We report previously that amylopectin molecular weight increased after 10 weeks storage, with decreased polydispersity (Chapter 2), and this increased size and uniformity may have a profound effect on texture of squash. Weight-average (polyM$_w$) and z-average (polyM$_z$) polydispersity of squash amylopectin stored for 10 weeks were highly correlated to hardness of fruit steamed for 10, 15 and 20 min (**Table 8**). PolyM$_w$ and polyM$_z$ of squash stored for 10 weeks were highly correlated to fracturability of fruit steamed for 5, 10, 15 and 20 min (**Table 8**). Hardness of raw fruit, stored for 10 weeks was correlated to M_w, M_z and R_z (**Table 8**). M_z was also correlated to hardness of fruit steamed for 10 or 15 min, fracturability of fruit steamed for 5, 10 or 15 min, and springiness of fruit steamed for 10, 15 or 20 min (**Table 8**). Springiness of fruit, stored 10 weeks and steamed for 10 min was also correlated to M_w (**Table 8**).

Increase in average amylopectin molecular size during storage influencing textural attributes has not been reported previously in literature. To our knowledge there have been no previous reports of amylopectin molecular size increasing with storage, and authors who studied amylopectin size and its effects on texture, debranched their molecular weight fractionated amylopectin and reported the effects of debranched fractions of low and high

amylopectin molecular weight on texture (16, 44), thereby making comparison with our results difficult.

Effect of Amylopectin Branch Chain-Length on Fruit Texture. Isoamylase-debranched amylopectin separated by HPAEC-ENZ-PAD revealed many correlations with squash fruit texture. Surprisingly, the second degree of polymerization peak (DPII), a parameter that usually receives little attention, had the most correlations with fruit texture, but all correlations were observed for stored fruit. For squash fruit at harvest, proportion of amylopectin branch chain-lengths of DP 3-6 was correlated to hardness of fruit steamed for 10 ($r = 0.69$, $P = 0.02$) or 15 min ($r = 0.64$, $P = 0.03$), fracturability of fruit steamed 15 ($r = 0.79$, $P = 0.003$) or 20 min ($r = 0.77$, $P = 0.004$) and springiness of fruit steamed for 20 min ($r = -0.64$, $P = 0.03$). The slightly longer, but still very short amylopectin branch chain-lengths of DP 6-9, had their proportion from fruit at harvest, correlated to similar textural attributes as DP 3-6, with correlations for hardness of fruit steamed 10 min ($r = 0.66$, $P = 0.03$), fracturability of fruit steamed 15 ($r = 0.61$, $P = 0.04$) or 20 min ($r = 0.60$, $P = 0.05$) and springiness of fruit steamed for 20 min ($r = -0.60$, $P = 0.05$). For fruit at harvest, proportion of amylopectin branch chain-lengths of DP 6-12 and DP $\geq$ 37 had just one correlation with springiness of fruit steamed 20 min ($r = -0.66$ and 0.59, $P = 0.03$ and 0.05 respectively) and no correlations were observed between textural attributes and proportion of amylopectin branch chain-lengths of DP 25-36. Proportion of amylopectin branch chain-lengths of DP 13-24 was similar to DP 3-6 with hardness correlated to fruit steamed 10 ($r = -0.61$, $P = 0.04$) or 15 min ($r = -0.64$, $P = 0.03$) and fracturability of fruit steamed 15 or 20 min ($r = -0.59$, $P = 0.05$ for both). Average amylopectin branch chain-length was not correlated to any textural attribute of fruit at harvest, largely due to our results indicating that

very short amylopectin branch chains (DP ≤ 9) and intermediate chain-lengths (DP 13-24) are the major contributors to texture.

Squash fruit stored for 5 weeks had many correlations between DPII and textural attributes including hardness and fracturability of fruit steamed for 5 or 10 min (**Table 4**), and springiness of fruit steamed for 2 min (r = -0.74, P = 0.04). Very short amylopectin branch chain-lengths (DP 3-6), from fruit stored 5 weeks, were not correlated to squash texture. Proportion of short amylopectin branch chain-lengths (DP 6-9) was correlated to hardness and fracturability of fruit steamed 5 min, and fracturability of fruit steamed 10 min (**Table 4**). Intermediate amylopectin branch chain-lengths (DP 13-24) proportion from fruit stored 5 weeks was correlated to hardness and fracturability of raw fruit (**Table 4**), hardness of fruit steamed 2 min (r = -0.67, P = 0.05), and springiness of fruit steamed 5 or 10 min (**Table 4**). Proportion of longer amylopectin branch chain-lengths (DP 25-36) from fruit stored 5 weeks was correlated to springiness of fruit steamed 10 or 15 min (**Table 4**) and no correlations were observed with long amylopectin branch chain-lengths (DP ≥ 37). Average amylopectin branch chain-length, from fruit stored 5 weeks, was correlated to hardness and fracturability of raw fruit (**Table 4**) and hardness of fruit steamed for 2 min (r = 0.67, P = 0.05).

DPII from fruit stored 10 weeks was only correlated to springiness of raw fruit (r = -0.85, P = 0.03). No correlations were observed between textural attributes and proportion of short amylopectin branch chain-lengths (DP ≤ 12). Proportion of intermediate amylopectin branch chain-lengths (DP 13-24), from fruit stored 10 weeks, was correlated to hardness of raw fruit and fruit steamed for 2 min (r = -0.79, P = 0.05 for both). Long amylopectin branch chain-lengths (DP 25-36) proportion was correlated to hardness and fracturability of raw fruit

(**Table 8**) and fruit steamed for 2 min (r = -0.90 and -0.79, P = 0.01 and 0.05 respectively), and springiness of fruit steamed 20 min (**Table 8**). Proportion of very long amylopectin branch chain-lengths (DP ≥ 37) from fruit stored 10 weeks, was correlated to hardness and fracturability of raw fruit (**Table 8**) and hardness of fruit steamed for 2 min (r = 0.86, P = 0.03). Average amylopectin branch chain-length, from fruit stored 10 weeks, was correlated to hardness of raw fruit (**Table 8**) and fruit steamed for 2 min (r = 0.81, P = 0.04). Our results indicate that intermediate amylopectin branch chain-lengths (DP 13-36) are the only chain-lengths that influence textural attributes at both harvest and after storage. Influence of short amylopectin branch chain-lengths on textural attributes of squash fruit seemed to diminish as storage progressed. Hardness and fracturability of squash fruit was always correlated positively with short (DP ≤ 12) and very long (DP ≥ 37) amylopectin branch chain-lengths and negatively correlated to intermediate amylopectin branch chain-lengths (DP 13-36), regardless of storage time.

Isoamylase-debranched amylopectin separated on size-exclusion chromatography yielded long-chain amylopectin fraction (DP ≥ 26) and short-chain amylopectin fraction (DP < 26), which were correlated to textural attributes of squash fruit, largely dependent on storage time. Hardness of fruit, at harvest, steamed for 10 min was positively correlated to percentage of long-chain amylopectin (r = 0.60, P = 0.05) and negatively correlated to percentage of short-chain amylopectin. Fractruability of fruit, at harvest, steamed for 15 or 20 min was correlated to percentage of long-chain amylopectin (r = 0.61 and 0.66; P = 0.05 and 0.03 respectively). Springiness of harvest fruit, steamed for 10 or 15 min was correlated to long-chain amylopectin fraction (r = 0.76 and 0.72; P = 0.007 and 0.01). These findings contradict those found using HPAEC-ENZ-PAD, which found no correlations between

textural attributes and very long chain-lengths (DP $\geq$ 25), and correlations between short amylopectin chains and hardness or fracturability were negative. HPAEC-ENZ-PAD is limited to measuring amylopectin branch chain maximum length of about 70-80 DP, whereas the long amylopectin fraction from HPSEC method is incorporating amylopectins of longer chain-lengths which may explain the differences we observe, and suggest that it is the extremely long chain-lengths that contribute to hardness of squash fruit.

For fruit stored for 5 weeks, the only correlation observed between long-chain amylopectin fraction measured by HPSEC and texture was springiness after 5 min steaming ($r = 0.80$, $P = 0.02$). More correlations were observed for fruit stored 10 weeks. Long-chain amylopectin fraction, from fruit stored 10 weeks, was correlated to hardness of fruit after steaming for 10, 15 or 20 min and correlated to fracturability of fruit after steaming for 5, 10 or 15 min (**Table 8**). For fruit stored 10 weeks, HPSEC and HPAEC-ENZ-PAD both found long amylopectin branch chains to have positive correlation with hardness and fracturability, but the cooking times that significant correlations are observed differs.

The mechanism of how long-chain amylopectin contributes to hardness remains somewhat unknown. Squash starch amylopectins have very high iodine affinities, which is known to be directly proportional to long B-chains and inversely to short B-chains (45, 46), and these long B-chains have been shown to have uninterrupted external unbranched portions (13). Rheological and microscopic studies by Sandhya Rani and Bhattacharya (20, 46, 47, 48) on the viscoelastic properties of rice pastes have shown amylopectin with long B-chains have rigid, elastic and strong starch granules which resist swelling and disintegration when heated in water under shear. Conversely, rice starches with short B-chains have weak, deformable and fragile starch granules that tend to break down under same conditions.

Ramesh et al. (16) proposes that long amylopectin chains have greater propensity to participate in intermolecular interactions, thereby affecting rigidity of starch granules and indirectly affecting texture. Interactions of long amylopectin chains have also been suggested to form complexes that are retained during cooking rather than leached, allowing hardness to be maintained (49). In agreement with our findings, many researchers have reported long-chain amylopectins contribute to a firmer texture of rice (14, 15, 16, 49, 50, 51, 52, 53, 54, 55), rice bread (56), corn starch gels (57) and sweet potatoes (58). Only report to contrast these findings was by Wang and Wang (59) who reported hardness of rice was correlated to the short A and B1 amylopectin chains.

Effect of Starch Thermal Properties on Fruit Texture. The relationship between thermal properties of squash starches and squash fruit textural attributes depended on fruit storage time. Very few correlations were observed between starch thermal properties and fruit texture, for fruit at harvest. Springiness was not correlated to starch thermal properties from fruit at harvest. Hardness of fruit, at harvest, steamed for 15 min was correlated to percent retrogradation (%re) (r = -0.61, P = 0.05), and fruit steamed for 20 min was correlated to %re and range of gelatinization of retrograded starch (ROG_R) (r = -0.77 and -0.65; P = 0.005 and 0.03 respectively). Fracturability of raw fruit, at harvest, was correlated to conclusion temperature of retrograded starch (T_{cR}), ROG_R and %re (r = -0.63, -0.61 and -0.61; P = 0.04, 0.05 and 0.05 respectively). Fracturability of fruit steamed 2 min was also correlated to T_{cR} and change in enthalpy of gelatinization (ΔH) (r = -0.60 and 0.63; P = 0.05 and 0.04 respectively).

After 5 weeks storage, an increase in correlations between starch thermal properties and texture were observed. ROG and %re were correlated to hardness of raw (**Table 5**) and

fruit steamed 2 min (r = -0.87 and -0.75; P = 0.005 and 0.03 respectively), with the latter thermal property also correlated to hardness after 20 min steaming (**Table 5**). Hardness of fruit steamed for 10 min was also correlated to ΔH (**Table 5**) and ΔH of retrograded starch (ΔH_R) (r = -0.79, P = 0.02), with the latter thermal property also correlated to steamed fruit after 15 min (r = -0.74, P = 0.04). Fracturability of raw fruit, stored 5 weeks, was correlated to ROG and %re (**Table 5**), with the latter thermal property also correlated to fracturability of fruit steamed for 2 min (r = -0.83, P = 0.01). Springiness was only correlated with T_{cR} after fruit was steamed for 2 (r = 0.69, P = 0.05) or 5 min (r = 0.78, P = 0.02).

Squash fruit stored for 10 weeks had an increase in number of correlations between starch thermal properties and texture, with most correlations involving the five thermal properties of onset gelatinization temperature (T_o), peak gelatinization temperature (T_p), conclusion gelatinization temperature (T_c), ΔH and ΔH_R. Hardness of squash fruit, stored 10 weeks and steamed for 10, 15 or 20 min, was correlated to T_o, T_p, T_c, ΔH and ΔH_R (**Table 9**). Both ΔH_R and T_{cR} were correlated to fruit hardness after 5 min steaming (r = -0.93 and -0.83; P = 0.007 and 0.04 respectively). Fracturability of fruit, stored 10 weeks, and steamed for 10, 15 or 20 min was correlated to T_o (r = 0.88, T_p and ΔH (**Table 9**). Fracturability was correlated to T_c at 10 and 15 min steaming (**Table 9**). Significant correlations were also observed between fracturability of fruit steamed 5 min and T_o, ΔH and ΔH_R (**Table 9**). Springiness was only correlated to ΔH and T_{cR} after 2 min steaming (r = 0.88 and 0.91; P = 0.02 and 0.01 respectively), and the former thermal property after 15 min steaming (r = -0.81, P = 0.05).

Previous reports of starch thermal properties correlating with texture exist, but most found just one parameter that was related. The most common trend observed is increasing

onset gelatinization temperature (T_o) correlates to increasing hardness of rice (60), bread (61), corn starch gels (34), potatoes (62) and sweet potatoes (63). These reports are in agreement with squash cultivar fruit, stored for 10 weeks, and steamed 10 to 20 min, which had increased hardness and fracturability with higher T_o, T_p and T_c. However Tan et al. (64) reported a decrease in hardness with increasing T_o for rice, and Ong and Blanshard (65) found gelatinization temperature was not correlated to texture of cooked rice. Percent retrogradation was also reported to be positively correlated to firmness of cooked rice (66, 67). Higher ΔH of the amylose-lipid complex has been reported to increase springiness (68), but no valid comparison can be made with our study since squash starches did not exhibit an amylose-lipid complex. In our study, ΔH and ΔH_R were both negatively correlated to hardness and fracturability of steamed squash fruit. This is difficult to explain since if hardness of cooked fruit was due to amylopectin with long B-chains (14), then ΔH would also be high (43).

Effect of Starch Pasting Properties on Fruit Texture. Depending on storage time, starch pasting properties were correlated to texture of squash, but unlike for starch thermal properties, most correlations involving pasting properties were for fruit stored 5 weeks. No correlations between harvest squash fruit starch pasting properties and textural parameters were observed. For fruit stored 10 weeks, hardness of fruit steamed for 10 min was correlated to trough (r = -0.97, P = 0.03), breakdown (**Table 9**) and peak time (r = -0.99, P = 0.01). Fracturability of fruit stored for 10 weeks was correlated to setback after 5 min steaming (r = -0.97, P = 0.03), trough after 10 min steaming (r = -0.95, P = 0.05), and peak viscosity after 20 min steaming (**Table 9**). Springiness was not correlated to starch pasting properties for fruit stored 10 weeks.

Squash fruit stored for 5 weeks had greatest number of correlations between starch pasting properties and texture. For fruit stored 5 weeks, hardness after 5 or 10 min steaming was correlated to final viscosity and setback (**Table 5**), with the latter pasting property also correlated to hardness after 15 min steaming ($r = 0.90$, $P = 0.02$). Fracturability of fruit stored 5 weeks and steamed for 10, 15 or 20 min was correlated to peak viscosity, final viscosity and setback (**Table 5**), with the latter two pasting properties also correlated after 5 min steaming ($r = -0.92$ and -0.98; $P = 0.01$ and 0.0006 respectively). Springiness was correlated to breakdown for raw and fruit steamed for 10, 15 or 20 min (**Table 5**). Springiness was also correlated to peak viscosity after 10 min steaming and pasting temperature after 20 min steaming (**Table 5**).

From our results, the main trends observed is at various cooking times for peak viscosity, final viscosity and setback to have a negative relationship with squash fruit fracturability, for final viscosity and setback to have a negative relationship with fruit hardness, and for breakdown to have a negative relationship with fruit springiness. A negative relationship between peak viscosity or breakdown and hardness has been previously reported in cooked rice (69, 70), wheat noodles (71), cereal gels (33, 72, 73) and beans (74). However final viscosity has also been reported to be positively correlated to hardness (70), and Lee et al. (23) reported no relationship between sorghum grain hardness and starch pasting properties. Limpisut and Jindal (70) also reported that hardness was positively correlated to setback, and springiness was negatively correlated to peak viscosity, and positively correlated to setback, final viscosity and pasting temperature, with our findings only in agreement with the latter pasting property. However, springiness correlated positively to peak viscosity has been reported for sorghum noodles (75).

Squash starches long amylopectin B-chains (Chapter 2), may be primarily positioned externally (14), which is conducive to forming intermolecular interactions, which help hold granules together when heated, thereby aiding swelling and resulting in high peak viscosity (Chapter 3). The intermolecular interactions have been speculated to be retained within cells during cooking (15), creating strong, rigid starch granules (14) that would be expected to maintain hardness and reduce starch granule breakdown. One explanation for decreased hardness with increased peak viscosity is that starch with high peak viscosity may have swelled sufficiently to distend cell walls, causing breakdown of cell wall matrix and a softer texture. We have observed swollen gelatinized starch engorged the majority of cell volume in high-starch squash cultivars (Chapter 6).

Effect of Starch Gel Properties on Fruit Texture. Very few correlations were observed between squash starch gel properties and fruit texture, regardless of storage time. Hardness of fruit, at harvest and steamed 5 min, was correlated to gel firmness after gel was stored for 1 d ($r = 0.68$, $P = 0.02$), and raw fruit fracturability was correlated to stickiness of gels stored for 1 d ($r = -0.60$, $P = 0.05$). For fruit stored 5 weeks, hardness of raw fruit was correlated to gel stickiness after 1 d ($r = -0.84$, $P = 0.04$), and hardness and fracturability of fruit steamed 5 min was correlated to firmness of gels stored 1 d ($r = -0.86$ and -0.90; $P = 0.03$ and 0.02 respectively). Hardness of fruit stored 10 weeks and steamed for 5 min was correlated to firmness of gels stored 1 d ($r = -0.96$, $P = 0.04$). Fracturability of fruit stored 10 weeks and steamed for 15 min was correlated to firmness of gels stored 7 d, and springiness of fruit stored 10 weeks and steamed 2 min was correlated to stickiness of gels stored 7 d ($r = -0.97$, $P = 0.03$ for both). Numerous studies have investigated the rheological properties of starch gels but few studies have attempted to correlate this with textural properties of the plant

tissue the starch was extracted from, thereby making comparison with our results difficult. Our results disagreed, for fruit at harvest, and agreed, for fruit after 5 or 10 weeks storage, with Gaines et al. (33) who reported harder starch gels were derived from cereals with softer kernels.

Effect of Starch Crystallinity on Texture and Starch Structural and Functional Properties. Correlations were observed among starch crystallinity percent and texture or starch functional properties for fruit at harvest and 5 weeks storage, but no correlations were observed after 10 weeks storage. For fruit at harvest, starch percent crystallinity was correlated to T_c ($r = 0.64$, $P = 0.03$), M_z ($r = 0.63$, $P = 0.04$), R_z ($r = 0.60$, $P = 0.05$) and the only textural attribute was hardness after 5 min steaming ($r = 0.62$, $P = 0.04$). Starch percent crystallinity after 5 weeks storage was correlated to apparent amylose content ($r = 0.89$, $P = 0.02$), the thermal property, ΔH ($r = 0.84$, $P = 0.04$), and the pasting properties, peak viscosity ($r = 0.98$, $P = 0.0007$), trough ($r = 0.83$, $P = 0.04$) and final viscosity ($r = 0.84$, $P = 0.04$). Starch percent crystallinity, from fruit stored 5 weeks, was also correlated to the textural attributes of hardness, springiness and fracturability of fruit steamed 10 min ($r = -0.81$, -0.88 and -0.82; $P = 0.05$, 0.02 and 0.05 respectively) and the latter textural attribute was also correlated after 15 or 20 min steaming ($r = -0.83$, $P = 0.05$ for both).

In general, our results suggest increased crystallinity results in higher gelatinization temperatures, higher ΔH, higher paste viscosity and reflects higher apparent amylose content. Crystallinity has been reported to be correlated positively with gelatinization temperatures (65, 76, 77), peak viscosity (78), and ΔH (76, 79). In contrast to our findings, other studies have reported apparent amylose content to be negatively correlated to crystallinity (80, 81, 82).

Decreases we observed in hardness and fracturability of squash fruit, stored 5 weeks, after 10 min steaming, most likely reflect the transformation from hard crystalline starch granules to non-crystalline swollen gelatinized starch. Decreased springiness with increasing crystallinity, for fruit steamed 10 min, could reflect melted crystallites distending as one large gelatinized starch mass, rupturing cell walls and reducing cell sliding and swelling, thereby reducing viscoelastic properties of the cell wall matrix (29, 30). Comparisons with our findings are difficult as few studies have focused on effects of crystallinity on texture. Most studies have focused on bread staling in which starch crystallinity has been reported to be correlated to bread firmness (83, 84), but other studies indicate stronger factors have been reported to contribute to bread firmness than starch crystallinity (85, 86, 87). However, studies of bread firmness are difficult to compare with squash fruit hardness since the former is caused by starch retrogradation. Three studies are in contrast to our findings by reporting cooked cereal grains have increased firmness with increasing starch crystallinity (88, 89, 90).

Correlations Between Squash Starch Amylose Content and Thermal Properties. Iodine affinity of amylopectin fraction of squash starch, from fruit at harvest, was correlated to T_{pR}, T_{cR}, ΔH_R, ROG_R and %re (r = 0.60, 0.72, 0.60, 0.79, and 0.70; P = 0.05, 0.01, 0.05, 0.004 and 0.02 respectively). Absolute amylose content, from fruit at harvest, was correlated to ROG (**Table 2**). Apparent amylose content of fruit, from 5 and 10 weeks storage, was correlated to ΔH and ΔH_R (**Table 10**). Iodine affinity of amylopectin fraction from starches of fruit stored 10 weeks was correlated to T_{pR} and T_{cR} (r = 0.91 and 0.82; P = 0.01 and 0.05 respectively), and absolute amylose content was correlated to T_{oR} (r = -0.88, P = 0.02).

Our results show some trend for higher T_o with increasing absolute amylose content and higher ΔH with increasing apparent amylose content. Several researchers have

previously reported higher apparent amylose content to be correlated to lower T_o (91, 92, 93). However, other researchers have reported a positive relationship between T_o and apparent amylose content (94, 95) and many report no relationship between apparent amylose content and all gelatinization temperatures and enthalpy changes (96, 97, 98, 99, 100, 101, 102). Since we also observed some correlations between iodine affinity of amylopectin fraction and starch thermal properties, long-chain amylopectins may play a critical role in thermal properties of starches and explain the discrepancies found in literature for the influence of amylose content on starch thermal properties. A negative correlation has been reported between ΔH and apparent amylose content (42, 103, 104, 105), and this disagreement with our findings may be because long-chain squash amylopectins could be providing a significant contribution to ΔH.

Correlations Between Squash Starch Amylose Content and Pasting Properties. No correlations between amylose content and pasting properties were observed for starch extracted from fruit at harvest, but correlations were observed after 5 and 10 weeks storage. Apparent amylose content was correlated to trough ($r = 0.89$, $P = 0.02$), peak viscosity, final viscosity and setback (**Table 6**) for starch from fruit after 5 weeks storage. For fruit stored 10 weeks, only breakdown and final viscosity were correlated to apparent amylose content (**Table 6**), but trough ($r = 0.97$, $P = 0.03$), breakdown (**Table 6**), final viscosity (**Table 6**) and peak time ($r = 0.98$, $P = 0.02$) were all correlated to absolute amylose content.

Overall our results indicate a positive relationship between amylose content and peak or final viscosity, and a negative relationship between amylose content and breakdown. This is in disagreement with many studies that reported a negative relationship between apparent amylose content and peak or final viscosity (43, 53, 106, 107, 108, 109, 110, 111 112, 113,

114, 115, 116, 117, 118). However, several other studies are in agreement with our findings of a positive relationship between apparent amylose content and peak or final viscosity (95, 105, 119, 120, 121, 122, 123, 124), and one study reported no relationship between amylose and pasting properties (129). Relationship between amylose content and breakdown is also conflicting with some studies reporting a positive relationship (20, 106, 122, 124) and other studies reporting, as we found, a negative relationship (53, 107, 109, 111, 112, 126, 127).

Correlations Between Squash Starch Amylopectin Molecular Size and Thermal Properties. No correlations were observed between squash starch amylopectin molecular size and thermal properties, for fruit at harvest, but correlations were observed at 5 and 10 weeks storage. T_o was correlated to M_n, M_w and M_z of starch from fruit after 5 weeks storage (r = -0.79, -0.76 and -0.76; P = 0.02, 0.03 and 0.03 respectively), and correlated to polyM_z after 10 weeks storage (r = 0.82, P = 0.05). ΔH, after fruit were stored for 10 weeks, was correlated to polyM_z, polyM_w and M_z (r = -0.91, -0.88 and -0.81; P = 0.01, 0.02 and 0.05 respectively). T_p, of starch from fruit after 5 weeks storage, was correlated to M_n (r = -0.74, P = 0.04).

Our results suggest that higher molecular weight amylopectin with higher degree of uniformity have lower T_o. It would be expected that this phenomenon is largely due to the increased uniformity since larger molecules, with no change in polydispersity, would be expected to melt at higher temperatures. However, our results also suggest that starch with lower polydispersity require greater energy to melt crystals, indicating that increased uniformity is not the cause of lower T_o when amylopectin molecular size increases during storage of fruit. Although reports of correlations between amylopectin chain-length and thermal properties are common, few authors have commented on amylopectin molecular size

and thermal properties, with the only study we found reporting ΔH was independent of amylopectin molecular weight, in which they also reported that polydispersity was similar (44).

Correlations Between Squash Starch Amylopectin Molecular Size and Pasting Properties. Unlike correlations for starch thermal properties, some parameters of amylopectin molecular size were correlated to pasting properties of starch from fruit at harvest, and after 10 weeks storage, but not after 5 weeks storage. Trough, final viscosity and setback of starch from fruit at harvest were correlated to polyM_w (r = -0.69, -0.73, and -0.70; P = 0.02, 0.01, and 0.02 respectively) and gyration radii based on M_n (R_n) (r = 0.66, 0.70 and 0.68; P = 0.03, 0.02, and 0.02 respectively). For fruit stored 10 weeks, trough and peak time were correlated to M_n (r = -0.99 and -0.98; P = 0.009 and 0.02 respectively), pasting temperature was correlated to R_n (r = -0.99, P = 0.01) and final viscosity was correlated to gyration radius based on M_w (R_w) (r = -0.97, P = 0.03).

There was no consistent trend between amylopectin molecular size and pasting properties, for starch from fruit at various storage times. For example, final viscosity was positively correlated to gyration radius for starch from fruit at harvest, but negatively correlated for starch from fruit stored 10 weeks. Higher peak viscosity with higher amylopectin molecular weight has been reported (57, 128, 129, 130), but one study reported viscosity of starch pastes was correlated to amylopectin branch chain-length but not molecular size (131).

Correlations Between Squash Starch Amylopectin Branch Chain-Length Distribution and Thermal Properties. Squash isoamylase-debranched amylopectin branch chain-length distribution, measured by HPAEC-ENZ-PAD was correlated to starch thermal properties.

DPII, for amylopectin from fruit at harvest, was correlated to T_o ($r = 0.62$, $P = 0.04$), T_p ($r = 0.84$, $P = 0.0006$), T_c ($r = 0.80$, $P = 0.001$) and T_{oR} ($r = 0.74$, $P = 0.009$). Only correlation observed with amylopectin chain-length categories, for fruit at harvest, was proportion of amylopectin branch chain-lengths of DP 25-36 was correlated to T_{oR} ($r = 0.62$, $P = 0.04$). No correlations were observed, for fruit stored 5 weeks, between starch thermal properties and amylopectin branch chain-length distribution. Similar findings were observed for fruit stored 10 weeks except DPII was correlated to ΔH ($r = 0.76$, $P = 0.05$) and T_{oR} ($r = -0.84$, $P = 0.03$). Overall, measurements of amylopectin branch chain-lengths by HPAEC-ENZ-PAD do not correlate well with starch thermal properties.

Squash isoamylase-debranched amylopectin chain-length distribution, measured by HPSEC, for starch from fruit at harvest, was not correlated to starch thermal properties, but correlations were observed for starch from fruit after storage. T_{cR} of starch from fruit stored 5 weeks was correlated to long ($r = 0.83$, $P = 0.01$) and short ($r = -0.83$) amylopectin branch-chains. For fruit stored 10 weeks, long-chain amylopectin was correlated to T_p ($r = 0.86$, $P = 0.03$), T_c ($r = 0.91$, $P = 0.01$), ΔH ($r = -0.92$, $P = 0.009$) and ΔH_R ($r = -0.82$, $P = 0.04$). Positive relationship between long-chain amylopectins and gelatinization temperatures that we found for squash disagrees with the findings by Li et al. (132) for barley. Negative relationship found between ΔH and long amylopectin branch chain-lengths disagrees with findings from corn starch (44).

Correlations Between Squash Starch Amylopectin Branch Chain-Length Distribution and Pasting or Gel Properties. Amylopectin branch chain-length distribution, measured by HPAEC-ENZ-PAD, was correlated to pasting properties of squash starches. Short amylopectin branch chain-lengths (DP $\leq$ 12) were not correlated with pasting properties, for

starch from fruit at harvest. Intermediate amylopectin branch chain-lengths (DP 13-24) proportion was correlated to peak viscosity and pasting temperature (r = -0.59 and 0.59; P = 0.05 and 0.05 respectively). Long amylopectin branch chain-lengths (DP 25-36) were not correlated to pasting properties, but very long amylopectin branch chain-lengths (DP $\geq$ 37) proportion was correlated to peak viscosity (**Table 2**). Average amylopectin branch chain-length, from fruit at harvest, was correlated to peak viscosity and pasting temperature (**Table 2**). For fruit stored 5 weeks, only short amylopectin branch chain-length proportion (DP 6-9) was correlated to any pasting property, that of setback (r = -0.83, P = 0.008). However DPII was correlated to final viscosity (r = -0.81, P = 0.01), setback (r = -0.80, P = 0.02) and peak time (r = -0.76, P = 0.05). No correlations were observed between amylopectin branch chain-length distribution and pasting properties of starch from fruit stored 10 weeks.

Stickiness of gels, stored 1 or 7 d, from starch extracted from fruit at harvest, was correlated to DPII (r = -0.62, P = 0.04 for both). Stickiness of gels, stored 7 d, from fruit stored 5 weeks, was correlated to proportion of DP 12-24 amylopectin branch chain-lengths (r = 0.81, P = 0.01) and average amylopectin branch chain-length (**Table 6**). Short amylopectin branch chain-lengths, DP 6-9 and DP 6-12 were both highly correlated to gel stickiness after 1 and 7 d storage, respectively (r = -0.98 and 0.98, P = 0.001 and 0.001 respectively). Gel stickiness was also correlated to long amylopectin branch chain-lengths (DP 25-36) after 1 or 7 d, for fruit stored 10 weeks (r = 0.93 and -0.90, P = 0.007 and 0.01 respectively). Average amylopectin branch chain-length, from fruit stored 10 weeks, was correlated to gel stickiness after 1 d storage (r = 0.98, P = 0.001). Greater stickiness with higher proportion of amylopectin in starch has previously been reported (32, 133, 134) but

the fine structure of amylopectin was not investigated. No correlations were observed between amylopectin branch chain-length distribution and gel firmness.

The positive correlation for very long amylopectin branch chain-lengths proportion (DP $\geq$ 37) and peak viscosity, for fruit at harvest, is in agreement with reports for rice (132, 135) and wheat (130, 136) starches. Studies have reported a negative relationship between long amylopectin chains and breakdown (55, 132, 135) in which it is proposed that long amylopectin chains may restrain the collapse of starch granules by cross linking amylopectin molecules (55). However, in our study we did not observe any correlations between amylopectin branch chain-lengths and breakdown.

Conclusion. The presence of starch in squash fruit contributes to greater hardness and fracturability when raw, but lower hardness and fracturability at later cooking stages, presumably due to transition of hard semi-crystalline starch granules to gelatinized starch paste. Higher springiness observed for squash with low starch content suggests starch is restricting cell wall sliding that provides viscoelastic properties. Correlations observed between hardness or fracturability and apparent amylose were negative, whereas for absolute amylose correlations they were positive, suggesting that amylopectin branch chain-lengths influence texture. Further evidence was provided by establishing correlations between hardness or fracturability and short (DP $\leq$ 12) and long (DP $\geq$ 37) amylopectin branch chains. Both starch pasting and thermal properties had strong correlations to squash fruit texture. Amylose content tended to influence squash starch pasting properties, whereas fine structure of amylopectin influenced starch thermal properties.

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Table 1

Correlation coefficients (r x 100) for selected squash textural and starch structural properties from fruit at harvest.

	H_{rw}	H_{10}	F_{rw}	S_{rw}	S_{10}	S_{20}	Ab_A	M_w	CL	$D_{6\text{-}12}$	$D_{\geq 37}$
H_{rw}	100										
H_{10}	-27	100									
F_{rw}	95***	-4	100								
S_{rw}	-68**	25	-58*	100							
S_{10}	-50	56*	-47	56*	100						
S_{20}	-28	-18	-40	33	39	100					
Ab_A	60*	-16	66*	-36	-72**	-22	100				
M_w	-7	12	1	59*	22	-14	-25	100			
CL	46	17	52	-27	9	41	26	-20	100		
$D_{6\text{-}12}$	-45	43	-30	32	-1	-66*	-9	14	-71**	100	
$D_{\geq 37}$	45	8	47	-24	17	59*	14	-20	94***	-79**	100
St	77**	-64*	66*	-66*	-84***	-9	66*	-25	18	-38	20

Abbreviations: H_{rw} = hardness raw, H_{10} = hardness 10 min steaming, F_{rw} = fracturability raw, S_{rw} = springiness raw, S_{10} = springiness 10 min steaming, S_{20} = springiness 20 min steaming, Ab_A = absolute amylose, M_w = weight-average amylopectin molecular weight, CL = average amylopectin chain-length, $D_{6\text{-}12}$ = proportion of amylopectin branch chain-lengths DP6-12, $D_{\geq 37}$ = proportion of amylopectin branch chain-lengths DP ≥37 and St = starch content.
* = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 2

Correlation coefficients (r x 100) for selected squash starch structural and functional properties from fruit at harvest.

	Ab_A	PD	CL	$D_{\geq 37}$	RG	PV	BK	FV	SB	PT	GF
Ab_A	100										
PD	-17	100									
CL	26	1	100								
$D_{\geq 37}$	14	-13	94***	100							
RG	-64*	17	-50	-46	100						
PV	36	-46	70**	65*	-26	100					
BK	12	36	56	43	-43	30	100				
FV	27	-73**	36	35	-3	80***	-30	100			
SB	26	-70**	44	38	-15	81***	-9	95***	100		
PT	-26	-19	-63*	-49	69*	29	-87***	18	-5	100	
GF	21	-59*	-8	2	1	2	-60*	47	42	38	100
GS	-50	8	-30	-9	47	-36	-52	-11	-28	59*	24

Abbreviations: Ab_A = absolute amylose, PD = polydispersity, CL = average amylopectin chain-length, $D_{\geq 37}$ = proportion of amylopectin branch chain-lengths DP ≥37, RG = range of gelatinization temperature, PV = peak viscosity, BK = breakdown, FV = final viscosity, SB = setback, PT = pasting temperature, GF = gel firmness 7 d storage and GS = gel stickiness 7 d storage. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 3

Correlation coefficients (r x 100) for selected squash textural attributes from fruit stored 5 weeks.

	H_{rw}	H_5	H_{10}	H_{20}	F_{rw}	F_5	F_{10}	F_{15}	F_{20}	S_{rw}	S_5	S_{10}	S_{15}
H_{rw}	100												
H_5	44	100											
H_{10}	-25	50	100										
H_{20}	61*	66*	54	100									
F_{rw}	93***	61*	-10	62*	100								
F_5	11	90***	72**	60*	28	100							
F_{10}	2	70**	51	29	17	81**	100						
F_{15}	-2	68**	62*	44	9	85***	91***	100					
F_{20}	-1	62*	91***	73**	12	82**	61*	74**	100				
S_{rw}	-64*	-45	26	-34	-66*	-23	13	13	11	100			
S_5	-64*	-43	28	-25	-62*	-15	11	11	23	74**	100		
S_{10}	-72**	-6	62*	-12	-64*	49	48	51	51	79**	74**	100	
S_{15}	-64*	-28	48	-15	-67**	21	31	36	37	86***	76**	92***	100
S_{20}	-66*	-53	29	-34	-73**	-19	8	10	11	92***	80**	79**	93***

Abbreviations: H_{rw} = hardness raw, H_5 = hardness 5 min steaming, H_{10} = hardness 10 min steaming, H_{20} = hardness 20 min steaming, F_{rw} = fracturability raw, F_5 = fracturability 5 min steaming, F_{10} = fracturability 10 min steaming, F_{15} = fracturability 15 min steaming, F_{20} = fracturability 20 min steaming, S_{rw} = springiness raw, S_5 = fracturability 5 min steaming, S_{10} = springiness 10 min steaming, S_{15} = springiness 15 min steaming and S_{20} = springiness 20 min. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 4

Correlation coefficients (r x 100) for selected squash textural and starch structural properties from fruit stored 5 weeks.

	Ap_A	Ab_A	M_w	R_z	CL	DP_{6-9}	DP_{13-24}	DP_{25-36}	DPII	St
H_{rw}	55	67*	-31	-38	73*	18	-74*	29	-10	83**
H_5	-64	3	-41	1	-16	82*	2	-21	86**	-3
H_{10}	-86**	-54	-35	-5	-35	56	30	-52	69*	-67*
F_{rw}	38	74*	-47	-24	69*	33	-72*	25	16	69**
F_5	-75*	-20	-38	6	-27	67*	14	-27	85**	-39
F_{10}	-63	-34	-37	11	-24	67*	24	-45	63	-32
S_5	16	-49	39	43	-48	-7	67*	-31	-48	-47
S_{10}	-63	-86**	4	17	-58	30	72*	-75*	12	-84**
S_{15}	-29	-80*	-22	-15	-34	10	52	-80*	-24	-67*
S_{20}	11	-62	-6	-10	-12	-18	36	-57	-57	-51
Ap_A	100	57	4	-7	51	-42	43	46	-72*	89**
Ab_A		100	-7	5	64	-5	-74*	72*	12	74*
M_w			100	82**	-22	-16	36	39	10	-10
R_z				100	-24	25	35	40	24	-13
CL					100	-34	-95**	57	-8	67*
DP_{6-9}						100	31	-33	54	-13
DP_{13-24}							100	-62	15	-64
DP_{25-36}								100	10	51
DPII									100	-44

Abbreviations: H_{rw} = hardness raw, H_5 = hardness 5 min steaming, H_{10} = hardness 10 min steaming, F_{rw} = fracturability raw, F_5 = fracturability 5 min steaming, F_{10} = fracturability 10 min steaming, S_5 = springiness 5 min steaming, S_{10} = springiness 10 min steaming, S_{15} = springiness 15 min, S_{20} = springiness 20 min, Ap_A = apparent amylose, Ab_A = absolute amylose, M_w = weight-average amylopectin molecular weight, R_z = gyration radius, CL = average amylopectin chain-length, DP_{6-9} = proportion of amylopectin branch chains DP 6-9, DP_{13-24} = proportion of amylopectin branch chains DP 13-24, DP_{25-36} = proportion of amylopectin branch chain-lengths DP 25-36, DPII = second DP peak, RG = range of gelatinization temperature and St = starch content. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 5

Correlation coefficients (r x 100) for selected squash textural and starch functional properties from fruit stored 5 weeks.

	ΔH	RG	%re	PV	BK	FV	SB	PT	GS
H_{rw}	59	-93***	-68*	62	18	37	3	17	-79**
H_5	-50	4	-22	-43	10	-81*	-95**	-42	5
H_{10}	-77*	46	-14	-78*	-36	-85**	-94***	-11	-18
H_{20}	4	-57	-75*	0	-14	-19	-52	11	-79*
F_{rw}	34	-81**	-68*	46	15	20	-13	13	-61
F_{10}	-50	31	-40	-82**	-42	-83**	-93***	-5	2
F_{15}	-44	30	-41	-81*	-42	-83*	-93**	-5	-50
F_{20}	-51	32	-41	-82**	-47	-80**	-90***	0	-47
S_{rw}	11	22	-34	-60	-85**	-2	1	64	24
S_{10}	-57	67*	-13	-93***	-82**	-52	-49	37	36
S_{15}	-26	33	-40	-74	-94**	-24	-33	65	-11
S_{20}	7	17	-44	-50	-95***	12	5	82*	57
ΔH	100	-67*	-21	89**	51	75*	73*	-4	-24
RG		100	55	-73*	-28	-49	-20	-14	62
%re			100	15	64	-6	29	-67*	73*
PV				100	59	77*	68*	-5	-72*
BK					100	0	10	-83*	19
FV						100	92***	53	-80**
SB							100	35	-69*
PT								100	-45

Abbreviations: H_{rw} = hardness raw, H_5 = hardness 5 min steaming, H_{10} = hardness 10 min steaming, H_{20} = hardness 20 min steaming, F_{rw} = fracturability raw, F_{10} = fracturability 10 min steaming, F_{15} = fracturability 15 min steaming, F_{20} = fracturability 20 min steaming, S_{rw} = springiness raw, S_{10} = springiness 10 min steaming, S_{15} = springiness 15 min steaming, S_{20} = springiness 20 min, ΔH = enthalpy change of gelatinization, RG = range of gelatinization temperature, PV = peak viscosity, BK = breakdown, FV = final viscosity, SB = setback, PT = pasting temperature and GS = gel stickiness 7 d storage. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 6

Correlation coefficients (r x 100) for selected squash starch structural and functional properties from fruit stored 5 weeks.

	Ap_A	Ab_A	R_z	CL	$DP_{\geq 37}$	ΔH	RG	ΔH_R	PV	BK	FV	SB	PT	GF
Ap_A	100													
Ab_A	57	100												
R_z	-7	5	100											
CL	51	64	-24	100										
$DP_{\geq 37}$	38	62	-50	-83^{**}	100									
ΔH	94^{***}	56	-9	29	24	100								
RG	-68^{*}	-62	45	-56	-51	-67^{*}	100							
ΔH_R	75^{*}	45	8	0	1	84^{**}	-28	100						
PV	88^{**}	73^{*}	-58	61	59	89^{**}	-73^{*}	56	100					
BK	24	74^{*}	-13	6	18	51	-28	72^{*}	59	100				
FV	95^{***}	29	-36	59	46	75^{*}	-49	35	77^{*}	0	100			
SB	90^{***}	24	0	38	26	73^{*}	-20	60	68^{*}	10	92^{***}	100		
PT	29	-39	-21	33	19	-4	-14	-50	-5	-83^{**}	53	35	100	
GF	66^{*}	-25	-32	63	47	-26	-15	-91^{**}	5	-82^{**}	96^{***}	88^{**}	93^{***}	100
GS	-89^{**}	-23	81^{**}	-90^{***}	-78^{*}	-24	62	69^{*}	-72^{*}	19	-80^{**}	-37	-45	-67^{*}

Abbreviations: Ap_A = apparent amylose Ab_A = absolute amylose, R_z = gyration radius, CL = average amylopectin chain-length, $D_{\geq 37}$ = proportion of amylopectin branch chain-lengths DP ≥37, ΔH = enthalpy change of gelatinization, RG = range of gelatinization temperature, ΔH_R = enthalpy change of retrograded thermal transition, PV = peak viscosity, BK = breakdown, FV = final viscosity, SB = setback, PT = pasting temperature, GF = gel firmness 7 d storage and GS = gel stickiness 7 d storage. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 7

Correlation coefficients (r x100) of selected squash textural attributes from fruit stored 10 weeks.

	H_{rw}	H_5	H_{10}	H_{15}	H_{20}	F_{rw}	F_5	F_{10}	F_{15}	F_{20}	S_{rw}	S_{10}	S_{15}
H_{rw}	100												
H_5	40	100											
H_{10}	-31	66*	100										
H_{15}	-22	51	90***	100									
H_{20}	11	68**	64*	71**	100								
F_{rw}	89***	34	-6	5	17	100							
F_5	-22	66*	90***	85***	66*	12	100						
F_{10}	-11	58*	77**	75**	44	26	86***	100					
F_{15}	-17	57*	51	57*	88***	0	68**	44	100				
F_{20}	-17	67*	85***	75**	64*	11	91***	85***	66*	100			
S_{rw}	-76**	-35	29	26	-5	-68**	19	0	10	14	100		
S_{10}	-71**	4	66*	55	18	-45	51	55	37	62*	50	100	
S_{15}	-79**	-37	58*	34	19	-56*	29	50	16	53	65*	89***	100
S_{20}	-83***	31	29	48	-9	-64*	57*	21	32	21	81**	62*	85***

Abbreviations: H_{rw} = hardness raw, H_5 = hardness 5 min steaming, H_{10} = hardness 10 min steaming, H_{15} = hardness 15 min steaming, H_{20} = hardness 20 min steaming, F_{rw} = fracturability raw, F_5 = fracturability 5 min steaming, F_{10} = fracturability 10 min steaming, F_{15} = fracturability 15 min steaming, S_{10} = springiness 10 min steaming and S_{20} = springiness 20 min steaming. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 8

Correlation coefficients (r x 100) for selected squash textural and starch structural properties from fruit stored 10 weeks.

	Ap_A	M_w	M_z	PD_w	PD_z	R_z	CL	DP_{25-36}	$D_{\geq 37}$	$DP_{\geq 26}$	St
H_{rw}	55	-85*	-83*	-58	-58	-89**	76*	-82*	78*	-36	80**
H_5	-88**	57	66	47	53	58	15	-12	25	65	-42
H_{10}	-94**	64	84*	88**	91**	73	2	39	4	89*	-67*
H_{15}	-86*	53	79*	92**	95**	65	4	45	4	87*	-49
H_{20}	-80*	41	68	89**	91**	52	18	37	17	87*	-28
F_{rw}	37	-66	-67	-61	-58	-72	69	-92**	76*	-34	63*
F_5	-92**	55	-79*	87*	89**	75*	-7	33	2	83*	-56*
F_{10}	-89**	61	84*	91**	94**	71	-2	47	-2	86*	-43
F_{15}	-87*	57	82*	92**	95**	69	-1	46	-1	84*	-41
F_{20}	-79*	58	79*	80*	85*	62	3	37	1	71	-56*
S_{10}	-62	86*	80*	39	45	61	-14	34	-25	40	-80**
S_{15}	-57	70	81*	77*	76*	60	-18	71	-33	57	-69*
S_{20}	-46	70	81*	77*	77*	74	-59	95**	-69	40	-47
Ap_A	100	-72	-84*	-75*	-79*	-81*	7	-27	-1	-86*	80*
M_w		100	93**	49	54	90**	-55	53	-56	39	-94**
M_z			100	76	80*	94**	-46	64	-47	61	-93**
PD_w				100	100***	70	-18	69	-19	83*	-68
PD_z					100	72	-17	67	-17	84*	-65
R_z						100	-64	63	-58	53	-97**
CL							100	-66	96**	24	59
DP_{25-36}								100	-76*	29	-63
$D_{\geq 37}$									100	26	56
$DP_{\geq 26}$										100	-55

Abbreviations: H_{rw} = hardness raw, H_5 = hardness 5 min steaming, H_{10} = hardness 10 min steaming, H_{15} = hardness 15 min steaming, H_{20} = hardness 20 min steaming, F_{rw} = fracturability raw, F_5 = fracturability 5 min steaming, F_{10} = fracturability 10 min steaming, F_{15} = fracturability 15 min steaming, S_{10} = springiness 10 min steaming, S_{20} = springiness 20 min, Ap_A = apparent amylose, M_w = weight-average amylopectin molecular weight, M_z = z-average amylopectin molecular weight, PD = polydispersity, R_z = gyration radius, CL = average amylopectin chain-length, DP_{25-36} = proportion of amylopectin branch chain-lengths DP 25-36, $D_{\geq 37}$ = proportion of amylopectin branch chain-lengths DP $\geq$ 37, $DP_{\geq 26}$ = proportion of amylopectin branch chain-lengths DP $\geq$ 26 and St = starch content. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 9

Correlation coefficients (r x 100) for selected squash textural and starch functional properties from fruit stored 10 weeks.

	T_o	T_p	T_c	ΔH	ΔH_r	PV	BK	FV	PT	GF	GS
H_{10}	82*	89*	86*	-97***	-86*	-14	97**	-94*	-89*	-92*	-26
H_{15}	90**	92**	83*	-96**	-80*	-69	41	-31	-82*	-17	-79
H_{20}	91**	96**	86*	-93**	-81*	-19	-55	63	-12	88*	-44
F_{rw}	-10	-12	-18	45	-9	36	-23	28	-30	32	88*
F_5	87*	78	70	-86*	-82*	84*	56	-61	-24	-54	69
F_{10}	88**	90*	83*	-96***	-80*	-37	88*	-88*	-57	-90*	-41
F_{15}	91**	90*	81*	-95**	-79*	-17	79*	-83*	-29	-97**	-12
F_{20}	93**	89*	77*	-89**	-77*	-95*	18	-14	-21	-17	12
S_{rw}	-3	35	53	-60	-20	-78	53	-50	-39	-42	-88*
T_o	100	79*	59	-74	-67	67	-72	66	76	47	77
T_p		100	95**	-92**	-84*	-57	-16	27	-49	31	-94*
T_c			100	-92*	-86*	-59	25	-15	-78	-25	-94*
ΔH				100	80*	76	-60	-52	76	47	84
ΔH_r					100	-13	-57	50	90*	95*	-17
PV						100	1	-8	20	-1	100**
BK							100	-99**	-76	-100**	-1
FV								100	69	100**	-6
PT									100	97**	26
GF										100	-4
GS											100

Abbreviations: H_{10} = hardness 10 min steaming, H_{15} = hardness 15 min steaming, H_{20} = hardness 20 min steaming, F_{rw} = fracturability raw, F_5 = fracturability 5 min steaming, F_{10} = fracturability 10 min steaming, F_{20} = fracturability 20 min steaming, S_{rw} = springiness raw, T_o = onset gelatinization temperature, T_p = peak gelatinization temperature, T_c = conclusion gelatinization temperature, ΔH = enthalpy change of gelatinization, ΔH_r = retrogradation enthalpy change of gelatinization, PV = peak viscosity, BK = breakdown, FV = final viscosity, PT = pasting temperature, GF = gel firmness 7 d storage and GS = gel stickiness 7 d storage. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

Table 10

Correlation coefficients (r x 100) for selected squash starch structural and functional properties from fruit stored 10 weeks.

	Ap_A	Ab_A	PD	M_w	R_z	ΔH	ΔH_r	BK	FV	PT
Ap_A	100									
Ab_A	39	100								
PD	-75*	-20	100							
M_w	-72	-40	49	100						
R_z	-81*	-44	70	90**	100					
ΔH	89**	24	-88**	-62	-67	100				
ΔH_r	87*	-88**	-56	-39	-42	80*	100			
BK	-98**	-99**	-41	76	68	-60	-57	100		
FV	98**	99**	32	-79	-75	-52	50	-99**	100	
PT	78	70	89*	-31	-6	76	90*	-76	69	100
GF	93*	58	44	-91*	-99**	47	95**	-100**	100**	69

Abbreviations: Ap_A = apparent amylose Ab_A = absolute amylose, PD = polydispersity, M_w = weight-average amylopectin molecular weight, R_z = gyration radius, ΔH = enthalpy change of gelatinization, ΔH_r = retrograded enthalpy change of gelatinization, PV = peak viscosity, BK = breakdown, FV = final viscosity, PT = pasting temperature and GF = gel firmness 7 d storage. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

CHAPTER 6. ROLE OF STARCH STRUCTURE IN TEXTURE OF SQUASH AND STARCH FUNCTIONAL PROPERTIES. V. TRANSMISSION OF ULTRASOUND AND MICROSCOPIC OBSERVATIONS OF WINTER SQUASH (*Cucurbita maxima* D.) FRUIT TO EXAMINE TEXTURE AND CORRELATIONS WITH STARCH AND CELL WALLS.

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ABSTRACT

Twelve winter squash cultivars had fruit stored 7.5 weeks and low-frequency ultrasound transmitted through fruit raw and steamed 10 or 20 min, with significant differences in ultrasonic velocity (UV) between squash cultivars for fruit raw and steamed 10 min. UV through raw squash fruit was comparable or slower than air, ranging from 190-362 m s^{-1}. UV increased after 10 min steaming and the five high-starch squash cultivars had the fastest UV. Despite fruit becoming at least 20 times softer after 20 min steaming, UV increased ranging from 1,950-2,800 m s^{-1}. Light micrographs show that high-starch squash cultivars steamed 10 min have cells engorged 50-100% of volume with gelatinized starch, which 7 low-starch cultivars do not possess, suggesting the swollen gelatinized starch mass contributes to the higher UV. Fruit cell wall rupturing depended on cultivar and cooking time. Light micrographs indicate that starch and cell walls contribute to texture, but an

additional factor also contributes to texture. UV seemed related to the behavior of starch within cells and cell wall structure.

KEYWORDS: Ultrasonic velocity, ultrasound, nondestructive evaluation, winter squash, pumpkin, cucurbits, texture, starch, cell walls, light microscopy, postharvest, storage.

INTRODUCTION

Texture of cooked winter squash is an important aspect in determining consumer preference (1, 2). Previous studies have measured changes in squash mechanical properties after cooking using an Instron Universal Testing Machine or TAXT2 texture analyser (3, 4, Chapter 4). Application of nondestructive, low-frequency ultrasound waves for measuring textural properties of plant tissues has been reported (5, 6, 7). The use of ultrasound for measurement of plant tissue, other than wood, is rare, but there is increasing interest in utilizing ultrasonic velocity measurements of fruit and vegetables to determine textural attributes.

Ultrasonic velocities through fruit and vegetable tissues are extremely slow compared with plastics or metals (8) and vary according to species and cultivars within species (9). Low-frequency ultrasound, below 50 kHz, has been found to be optimal for measuring ultrasonic transmission through plant tissue, as higher frequencies result in high attenuation and difficulty interpreting signals (10). Additionally, at low-frequency ultrasonication, pressure and temperature gradients produced by ultrasonic waves are small, passing through plant tissues without altering physical and chemical properties, and therefore are nondestructive. Thickness of fruit and vegetable tissue samples used in ultrasonic evaluation has necessitated pulse through-transmission rather than pulse-echo techniques to avoid high

attenuation. Ultrasonic velocity depends on the elastic modulus and density of the tissue with most variation between tissues explained by elastic modulus (9). Quasi-static modulus, measured by Instron Universal Testing Machine, does not directly relate to ultrasonic velocity, but is related to the dynamic modulus that ultrasonic velocity does depend on.

Air content of plant tissues has also been found to be an important factor influencing ultrasonic velocity (9). It is proposed that air content has an indirect effect on velocity through an effect on elastic modulus, in which greater air content could reduce area of cell to cell contact, resulting in decreased elastic modulus and velocity.

Low-frequency ultrasonics has been used for textural measurements of cooked carrots (8). Raw carrots had a damped oscillation pulse that changed shape and decreased amplitude after just 1 min cooking. During further cooking, ultrasonic signals became more complex, echoes developed and amplitude increased. Raw carrot tissue was composed of polyhedral cells with few intercellular spaces, but after heating, irregular cell shapes emerged with the development of intercellular cavities between separated middle lamellas. Despite decreasing firmness during extended heating, ultrasonic velocity increased and attenuation (α) decreased, which may be due to exclusion of air from the tissue, the simultaneous release of cell contents into the intercellular spaces, or the absorption of water.

Ultrasonic velocity was measured in tangential and radial sections of apple parenchyma, which was significantly higher in the latter orientation (11). Storage modulus of apple tissue was also found to be higher for radial sections, corresponding to ultrasonic velocity. Apple cortical cells were found to be radially elongated and intercellular air spaces were arranged in radial columns. Studies on bananas have shown intercellular spaces decrease during ripening resulting in increased ultrasonic velocity (12).

Recently, ultrasound has been applied to avocado harvestable maturity and softening during storage. Analysis of attenuation of ultrasonic signals yielded good linear fits indicating ultrasound was a good nondestructive method for measuring dry weight content, thereby providing a useful maturity index tool (13, 14) and good for measuring firmness during storage (15). However, high variation in ultrasonic measurements between individual fruit was observed (16).

In this study we measure the ultrasonic velocity of twelve winter squash cultivars fruit, raw and cooked. We fixed samples from same raw and cooked fruit sections, and used light microscopy to observe, at the cellular level, changes in starch and cell wall morphology to explain differences in ultrasonic velocity we observed. We also relate ultrasonic velocity measurements and light microscopy observations to textural attributes of raw and cooked squash measured previously using an Instron Universal Testing Machine (Chapter 4), and fruit composition, such as starch content that was measured previously (Chapter 2).

MATERIALS AND METHODS

Plant Material. Twelve winter squash cultivars (*Cucurbita maxima* D.) were used for ultrasonic velocity studies. Experimental layout of squash plantings, cultivation, harvest, storage procedures and source of seeds have been described previously (Chapter 2). Twelve winter squash cultivars included four buttercup squash (Cha Cha, Delica, Kurijiman and Sweet Mama), one closely-related buttercup cross (Hyvita), two Halloween-type squash (Big Max and Rouge Vif D'Etampes), one native American Indian squash (Lakota), one Hubbard-type squash (Warren Scarlet), one Crown-type squash (Whangaparoa Crown) and two non-commercial squash obtained from seed germplasm center (Yogorou and Zapallo Macre).

Ultrasonic Velocity Measurements. Three replicates of all twelve squash cultivars were measured for transmission speed of ultrasound. One fruit per replicate was randomly selected from fruit stored for 7.5 weeks at 12°C. A region of the fruit was randomly selected, as described previously (Chapter 4), and from this region, three, 3 cm wide at the equator, longitudinal segments were removed. Squash fruit were steamed as described previously (Chapter 4) for 0, 10 or 20 min.

After each segment was steamed for its appropriate time, a 20 mm diameter apple corer (Oxo brand, BASF Corp., Mount Olive, NJ) with recessed cutting edge, preventing further compression, was used to immediately procure a fruit cylinder, cut from the direction of seed cavity to skin, that was gently placed inside a sizing disk that was made of rigid plastic having a central hole into which the sample was slid. A razor knife was used to slice through the cylindrical cored squash sample protruding from either end of the sizing disk, thus producing a sample of known thickness. Three sizing disks were employed, allowing the preparation of samples that measured 10.5, 8.7, and 6.9 mm long to be obtained. The squash fruit region immediately under the skin, and the pulpy, fibrous area near the interior of specimen were avoided. Sliced fruit cylinders were then gently slid out of the sizing disk and placed on face of one 100 kHz transducer (GE Panametrics, Atlanta, GA). A thin foam plastic wafer, having a cutout for the squash sample was then slid down around the squash sample, allowing the cored sample to be centered with middle of transducer. A Perspex™ collar was used to align the transducers applied, and the second transducer was slid down into the collar. While active elements in transducers were covered by the squash sample, it was determined that a foamed plastic wafer was needed to defeat transmission of signal attributes that obfuscated the real signal's appearance on the oscilloscope. Transducers were

connected to a Panametrics 5058 PR high-voltage pulser-receiver (GE Panametrics, Atlanta, GA) to generate and receive ultrasonic signals through squash samples. Transducers measured 44.5 mm in diameter, while active piezoelectric element within these housings was actually 15.9 mm in diameter, thus the 20 mm diameter squash cylinder was sufficiently large enough to cover the useful portion of ultrasonic signal. The pulse-receiver was connected to a LeCroy 9310L digital oscilloscope (LeCroy Corp., Chestnut Ridge, NY) to permit viewing and acquisition of ultrasonic signals produced in this experiment. Typically, the weight of transducers was sufficient to make viable contact between the send/receive transducers and squash sample, although a small amount of ultrasonic coupling gel was used to wet transducer faces to ensure good contact. Attention was focused on careful sizing of squash samples in the rigid rings to avoid warping the sample's dimensions and disturbing internal structure. Although error could have occurred during this process, care was taken to prevent damage to the specimen, and replicate measurements were always made at various squash thicknesses to minimize this effect, with values markedly different from others discarded. Raw samples generally showed good behavior during the sizing process, exhibiting little undesirable deformation during slicing operation. More care was necessitated in preparing cooked samples, with consistency of some samples making velocity measurements more challenging.

The waveforms collected from the digital oscilloscope were transferred to computer as text files consisting of X, Y pairs, or the collection of time/amplitude values. A common feature of the various waveforms, the first significant positive peak, was chosen as the point of comparison between various samples and zero. Zero length was represented by time-of-flight for the case where the two transducers were in contact with one another. Time-of-

flight for a given length of a particular cultivar was the difference between first significant positive peak for that sample's waveform and the same feature on the zero waveform. Depending on sample material, including cultivar and whether sample was in raw or cooked state, waveforms obtained presented a range of indications of the first positive peak. Some signals were unambiguous, showing very clearly the sinusoidal waveform desired. Others required manipulation of the scale upon which data was plotted to permit a reasonable assessment of transit time of the signal.

Squash Fruit Preparation for Light Microscopy. After each longitudinal fruit segment was steamed for its appropriate time and a fruit cylinder had been removed for ultrasonic velocity measurements, a section (1 cm wide, traversing entire length from seed cavity to skin exterior) of the slice 2 cm away from the fruit cylinder was immediately removed and used for light microscopy sample preparation. The section was trimmed so that it was still 1 cm wide, but 0.5 cm long that included the fruit flesh that was half way between seed cavity and skin exterior. This 1 x 0.5 cm section was gently sliced into 2 x 2.5 mm pieces and placed immediately into a fixative consisting of 2% (v/v) paraformaldehyde (made from 16% formaldehyde solution, electron microscopy (EM) grade) and 3% (v/v) glutaraldehyde (made from 70% glutaraldehyde, EM grade) in 0.05 M sodium cacodylate buffer, pH 7.2, with all three reagents obtained from Electron Microscopy Sciences, Fort Washington, PA. Squash fruit samples then underwent dehydration process consisting of deionized water rinse to remove fixative, followed by 50% ethanol for 30 min, 70% ethanol for 30 min, 95% ethanol for 30 min, 0.5% (w/v) eosin stain in 95% ethanol for 3 min, 2 x 100% ethanol for 30 min, ethanol:xylene (1:1) for 1 h, 100 % xylene for 30 min, and 100% xylene for 3 h. Ethanol and xylene were obtained from Fisher Scientific, Pittsburgh, PA. Squash fruit pieces then had

one-fifth of xylene removed and replaced with paraffin (Paraplast X-tra tissue embedding medium, 52°C melting point, Fisher Scientific, Pittsburgh, PA) and placed at 60°C. Paraffin infiltration was continued four more times, with a greater amount of the xylene/paraffin medium removed with each progressive infiltration step, until squash pieces were in 100% paraffin. Squash pieces in paraffin were then poured into an aluminum pan and allowed to harden by cooling. Paraffin-embedded squash pieces were sectioned on a rotary microtome (Spencer "820" microtome, American Optical Co., Buffalo, NY), with a section thickness of 9 μm. Paraffin ribbons were divided and strips consisting of serial sections were placed on glass microscope slides.

Staining of Paraffin Sections for Light Microscopy. Paraffin was removed from squash fruit sections by two washes, for 2 min, in 100% xylene, followed by 2 min washes in xylene:ethanol (1:1) and 100% ethanol. Sections were further hydrated in 2 min steps of 95% ethanol, 70% ethanol, 50% ethanol and deionized water. Five staining procedures were used as follows: (1) 2% (w/v) hematoxylin in deionized water for 15 min and counterstained with 1% (w/v) safranin O in 50% ethanol for 24 h (HS); (2) 2% (w/v) hematoxylin in deionized water for 15 min and counterstained with 0.15% (w/v) fast green in 95% ethanol for 10 s (HF); (3) 1% (w/v) safranin O in 50% ethanol for 24 h and counterstained with 0.5% (w/v) chlorazol black E in 100% ethanol for 15 min (SC); (4) 0.2% (w/v) ruthenium red, made fresh in deionized water, for 20 min; (5) 1% (w/v) I_2, 1% (w/v) KI in deionized water for 15 min. All stains were purchased from Sigma Chemical Co., St Louis, MO. For the first four staining procedures, after staining, slides were dehydrated on 2 min step increments of increasing ethanol concentration, followed by ethanol:xylene (1:1), and then two washes in 100% xylene before permanently mounted. Observations for iodine stained squash sections

were carried out immediately after staining since dehydration steps required for permanent mounting remove iodine stain.

Light Microscopy of Stained Squash Sections. Slides of stained squash sections were observed under an Olympus BX40 compound light microscope (Leeds Precision Instruments, Minneapolis, MN) and images were captured on a Zeiss Axiocam MRc color digital camera (Carl Zeiss Microscopy, Göttingen, Germany).

RESULTS AND DISCUSSION

Ultrasonic Velocity Transmitted Through Raw Squash Fruit. Ultrasonic velocity transmitted through raw squash fruit ranged from 193 to 362 m s^{-1} (**Table 1**). Velocity measurements are extremely slow relative to other materials and are comparable or slower than ultrasonic velocity through air (330 m s^{-1}). Ultrasonic velocity transmitted through papaya fruit has also been reported to be slower than air (17). Significant differences in ultrasonic velocity were observed between the raw fruit of squash cultivars, with velocity transmitted through Kurijiman and Lakota faster than Big Max and Hyvita ($P = 0.0006$). Although raw Lakota fruit had the fastest velocity, the four buttercup cultivars, Cha Cha, Delica, Kurijiman and Sweet Mama, which all have high starch content, were the next fastest. Big Max, which had considerably slower ultrasonic velocity than all other squash cultivars, accumulated very little starch or dry matter. Hyvita was an exception, with high starch content, but substantially slower velocity. The ultrasonic signal showed high attenuation, which has been attributed to presence of air in the intercellular spaces which scatter ultrasonic waves (18). Intercellular air spaces, which are larger in raw fruit and vegetable tissues than cooked ones, are thought to cause the slow ultrasonic velocity, but the

heterogeneous composition of cells, including cellular contents, cell walls and air spaces may all contribute to the slow velocity. In particular, the cell wall, a composite of cellulose, hemicelluloses and pectin, may refract and reflect ultrasound, retarding its transmission.

Ultrasonic Velocity Transmitted Through Cooked Squash Fruit. Ultrasonic velocity of cooked fruit increased, relative to raw, for all squash cultivars (**Table 1**). Ultrasonic velocity was significantly different depending on cooking time ($P < 0.0001$), and there was also a highly significant cultivar*cooking time interaction ($P = 0.006$). All buttercup squash cultivars had large increases in ultrasonic velocity after fruit was steamed for 10 min, resulting in buttercup squash cultivars with the fastest velocities (all above 1,750 m s^{-1}). The squash cultivar with the next highest ultrasonic velocity, Hyvita, is closely related to buttercups, since it is a cross between a buttercup and a non-buttercup cultivar, Green Delicious. Squash cultivars with very slow ultrasonic velocity, Big Max, Rouge Vif D'Etampes, Whangaparoa Crown, Yogorou and Zapallo Macre, were Halloween-type or other squash which after 7.5 weeks storage had very low starch content (< 2% of dry weight (18)). Significant differences ($P = 0.0002$) in ultrasonic velocities were observed between fruit of the squash cultivars steamed for 10 min, with velocity of Kurijiman and Sweet Mama faster than Big Max, Yogorou and Zapallo Macre. Additionally velocity of Cha Cha and Delica was faster than Big Max and Zapallo Macre. After 20 min steaming, ultrasonic velocity transmitted through squash fruit was faster than that after 10 min, but was not significantly different between the cultivars. Buttercup squash were four of the five fastest samples. Squash fruit velocity increased after cooking, despite fruit hardness becoming considerably softer.

Increasing ultrasonic velocity with cooking time has previously been reported for boiled carrots (8). Raw carrots velocity was 420 m s^{-1}, decreased to 300 m s^{-1} after boiling for 2 min and then increased to be 1,150 m s^{-1} after 10 min steaming and 1,350 m s^{-1} after 15 min steaming. Velocities for raw and cooked carrots are comparable to the velocities we observe for squash fruit.

Light Micrographs In Relation to Instron Universal Testing Machine Texture Measurements. Four buttercup cultivars, stored for 5 or 10 weeks, all had relatively high hardness compared to all squash cultivars when raw (Chapter 4). Light micrographs of raw fruit stored for 7.5 weeks show the four buttercup cultivars had parenchyma cells containing starch granules (**Figure 1B**, **1C**, **1E** and **1H**) that were absent in most other squash cultivars, and is reflected in starch content previously shown (Chapter 2). Halloween-type squash cultivars, which had considerably lower hardness when raw than all other squash cultivars (Chapter 4), had relatively large parenchyma cells, with no starch granules (**Figure 1A** and **1G**), confirming starch content analysis shown previously (Chapter 2). Hyvita, a close relative to buttercup squash, also had parenchyma cells containing starch granules (**Figure 1D**), but was significantly softer than all four buttercups after 5 weeks storage, and all except Sweet Mama after 10 weeks storage (Chapter 4). Degradation of cell walls could not be attributed to differences in hardness of raw fruit, after 5 or 10 weeks storage, because Kurijiman and Sweet Mama that were both relatively firm (Chapter 4) showed considerable breakdown of some cells to form very large intercellular spaces (**Figure 1E** and **1H**), whereas cultivars such as Yogorou had significantly lower hardness (Chapter 4) but less cell wall degradation (**Figure 1K**), and Whangaparoa Crown had similar cell wall degradation (**Figure 1J**), but was significantly softer (Chapter 4). We were concerned that fixation

methods could have caused cell wall damage. Sections were collected from squash fruit that were grown in the subsequent year, also after 7.5 weeks storage. The light micrographs of the sections showed similar patterns of cell wall degradation. While we can not discount that the fixative solution used could be inappropriate for squash fruit preservation, the fixative is commonly used for many plant tissues. Therefore we feel the cell wall degradation observed for some squash cultivars is part of the natural phenomenon of fruit senescence (19).

Fracturability measurements of raw squash fruit, stored for 5 or 10 weeks, showed that buttercup squash cultivar fruit typically fractured at a higher force than low-starch cultivars, especially Halloween-type (Chapter 4). Light micrographs of raw fruit stored for 7.5 weeks, showed the buttercups all have small parenchyma cells with starch granules present (**Figure 1B**, **1C**, **1E** and **1H**), whereas Halloween-type had larger cell sizes with some cell wall degradation, and no starch granules present (**Figure 1A** and **1G**). Lakota, stored for 7.5 weeks, based on starch content after 5 and 10 weeks storage, had low starch content compared to Hyvita, but required nearly twice the force to fracture raw fruit (Chapter 4). Light micrographs of Lakota and Hyvita (**Figure 1D** and **1F**) do not appear to clearly explain the differences we observe in fracturability, but Lakota has thicker cell walls that may be a contributor. Additionally, especially Lakota and Whangaparoa Crown, but also Yogorou and Zapallo Macre, had considerably higher fracturability compared to other squash cultivars after 10 weeks storage and 10 min steaming, but light micrographs do not seem to explain this observation as Lakota and Yogorou have maintained a high proportion of cellular integrity (**Figure 2F** and **2K**), whereas Whangaparoa Crown and Zapallo Macre have extensive cell wall breakdown (**Figure 2J** and **2L**). All four cultivars do have one thing in common, a lack of starch granules after 7.5 weeks storage, but Big Max, Rouge Vif

D'Etampes and Warren Scarlet also lack presence of starch granules (**Figure 2A**, **2G** and **2I**), but had fracturability similar to high-starch cultivars. After 20 min steaming of fruit stored 5 or 10 weeks, Yogorou had highest fracturability and was the only cultivar which still showed high proportion of cellular integrity maintained (**Figure 3K**). High-starch cultivars steamed for 20 min have gelatinized starch interacting with cell walls (**Figure 3B**, **3C**, **3D**, **3E** and **3H**), which when compressed may readily fracture, explaining the low fracturability observed (Chapter 4).

The two Halloween-type squash cultivars, stored for 5 or 10 weeks, had the highest springiness when raw, and light micrographs show these two cultivars had the lowest level of cellular organization (**Figure 1A** and **1G**), in which cell sliding is conducive, which has previously been reported to result in greater viscoelastic properties (22, 23). However after 10 min steaming, Lakota has considerably high springiness but does not show the same level of cell wall destruction (**Figure 2F**) that other high springiness cultivar fruit exhibit (**Figure 2A**, **2G** and **2J**). After 10 weeks storage and steamed for 20 min, Big Max, Lakota and Rouge Vif D'Etampes had highest springiness and all three cultivars, after 7.5 weeks storage, had weak staining intensity of cell wall matrix compared to other cultivars (**Figure 3A**, **3F** and **3G**). Low staining intensity, which included ruthenium red staining, a stain specific for pectin, may indicate greater pectin breakdown, increasing viscoelastic properties of cell walls.

After squash fruit was steamed for 10 min, the four buttercup cultivars and closely related Hyvita, from fruit stored 7.5 weeks, all had a mass of aggregated gelatinized starch filling 50 to 100 percent of cell volume (**Figure 2B**, **2C**, **2D**, **2E** and **2H**). Light micrographs of Cha Cha fruit represent iodine staining of the high-starch cultivars, demonstrating how the

small starch granules of raw fruit (**Figure 4A**), swelled substantially after 10 min steaming (**Figure 4B**), and swollen gelatinized mass appeared, on average, to reduce in size after 20 min steaming (**Figure 4C**). The five high-starch squash cultivars, stored for 5 or 10 weeks and steamed for 10 min, had hardness ranging from 28 to 75 N (Chapter 4). There was little evidence that engorgement of gelatinized starch had distended to cause cell wall breakdown after 10 min steaming since all had majority of cell wall matrix intact, except for Sweet Mama which already had some cell wall breakdown when raw. The two Halloween-type cultivars had similar size cells after 10 min steaming compared to raw (**Figure 1A**, **1G**, **2A** and **2G**). Whangaparoa Crown also had little change in cell size or degree of degradation after 10 min steaming compared with raw fruit (**Figure 1J** and **2J**), but Warren Scarlet and Zapallo Macre both had larger cell sizes or extensive cell wall breakdown (**Figure 1I**, **1L**, **2I** and **2L**). Halloween-type cultivars and the latter three cultivars mentioned, which all had larger cell size or air spaces compared to high-starch cultivars, were considerably harder than high-starch cultivars, after 10 min steaming, with hardness ranging from 89 to 323 N for fruit stored 10 weeks (Chapter 4). Relatively high hardness of Lakota fruit, after 5 or 10 weeks storage and steamed 10 min (Chapter 4), is difficult to explain because although Lakota cells lacked presence of starch granules that were the attribute of softer fruit texture, the cell walls were still largely intact, unlike all other squash cultivars with firm texture. Low-starch squash cultivars that have cells with high amounts of free water may maintain turgor pressure more effectively than high-starch squash cultivars that have gelatinized starch entrapping large quantities of water, which may have sponge-like texture plus reduced turgor pressure of cells. Higher maintenance of turgor pressure has been shown to reduce softening rate (22, 23, 24).

Yogorou, which had the firmest fruit (32 N), stored for 5 weeks and steamed for 20 min (Chapter 4), had most cell walls still intact (**Figure 3K**). Delica, after 20 min steaming of fruit stored 7.5 weeks, had most cells completely engorged with gelatinized starch, but cell walls were only partly ruptured (**Figure 3C**). The high-starch squash cultivars, Hyvita and Kurijiman, also had considerable amount of cells engorged with gelatinized starch but there was considerably greater extent of cell wall rupturing (**Figure 3D** and **3E**). Two other high-starch squash cultivars, Cha Cha and Sweet Mama, had aggregations of gelatinized starch with extensive rupturing of cell walls, and parts of cell wall were either entrapped or adhered to the gelatinized starch (**Figure 3B** and **3H**). All low-starch squash cultivars except Yogorou, stored for 7.5 weeks and steamed for 20 min, had some degree of cell wall breakage, but cell wall matrix was generally more intact (**Figure 3A, 3F, 3G, 3I, 3J** and **3L**) compared with high-starch cultivars.

Light Micrographs in Relation to Ultrasonic Velocity. Light micrographs reveal that starch content of squash cultivar fruit plays an important role influencing ultrasonic velocity transmitted through fruit tissue. The four buttercup cultivars, with considerably high ultrasonic velocity when raw and stored 7.5 weeks, all have an abundance of cells with starch granules (**Figure 1B, 1C, 1E** and **1H**). However, Hyvita also had large proportion of cells with starch granules but had relatively slow velocity (**Figure 1D**). Big Max, which accumulated very little starch and had no starch granules after 7.5 weeks storage, had the slowest ultrasonic velocity (**Figure 1A**). Although the trend between ultrasonic velocity and starch content of fruit is not entirely clear-cut for raw fruit, steamed fruit for 10 min showed a very clear trend. The five high-starch squash cultivars, which had the five highest ultrasonic velocities after 10 min steaming, all had cells engorged with aggregations of gelatinized

starch, which occupied 50 to 100 percent of cell volume (**Figure 2B**, **2C**, **2D**, **2E** and **2H**). Engorgement of gelatinized starch within cells is not observed in the seven other squash cultivars which all had slower velocities (**Figure 2A**, **2F**, **2G**, **2I**, **2J**, **2K** and **2L**). The trend of high-starch cultivars having higher ultrasonic velocity is not quite as clear after 20 min steaming, but buttercup cultivars still had four of the five fastest velocities. These four buttercup cultivars after 20 min steaming still showed engorgement of cells with gelatinized starch (**Figure 3B**, **3C**, **3E** and **3H**), but Hyvita had relatively slow ultrasonic velocity and also exhibited cells engorged with gelatinized starch (**Figure 3D**).

After 7.5 weeks storage, raw squash fruit from the cultivars varied from tissues with large cell wall breakdown where cellular contents were dispersed under most likely low turgor pressure and few visible air spaces, to tissues with most cell wall matrix maintained and some intercellular air spaces present (**Figure 1**). High-starch cultivars, had a greater proportion of cells intact after 10 min steaming (**Figure 2B**, **2C**, **2D**, **2E** and **2H**) compared with low-starch Halloween-type cultivars (**Figure 2A** and **2G**), but the associated differences in intercellular spaces could not be attributed to observed differences in ultrasonic velocity because other low-starch squash cultivars had high level of cell wall integrity (**Figure 2F** and **2K**) but did not have relatively high ultrasonic velocity. After fruit were steamed for 20 min, there were no significant differences in ultrasonic velocity between the squash cultivars, and light micrographs showed squash tissues with diverse characteristics including having most cell wall matrix intact (**Figure 3K**), having most cell walls degraded with lack of cell organization (**Figure 3A**, **3F**, **3G**, **3J** and **3L**), and tissues with large aggregates of gelatinized starch interacting with cell walls that varied from high (**Figure 3C**) to low (**Figure 3B**) level of cell wall organization.

Squash fruit samples were also stained with ruthenium red which is considered by many to be very specific for pectin (25). Cha Cha, used as a representative of the high-starch cultivars, showed strong ruthenium red staining when raw indicating an abundance of pectin in middle lamellae of cell walls (**Figure 5A**). After 10 min steaming, there is a large decrease in staining intensity of cell walls indicating a substantial decrease in pectins (**Figure 5B**). During this time, starch has gelatinized into one swollen mass, and pectin breakdown could be due to distension of gelatinized starch rupturing cell walls or the temperature from steaming could have degraded pectin. Large reduction in pectin staining occurred after 20 min steaming and some pectic fragments of cell walls are entrapped, adhered or complexing with gelatinized starch (**Figure 5C**). Ultrasonic velocity of all buttercup cultivars was above 1,750 m s^{-1} after 10 min steaming, and 2,680 m s^{-1} after 20 min steaming (**Table 1**). All buttercup cultivars had some cell wall breakdown after 10 min and extensive cell wall breakdown after 20 min, and at both cooking times, swollen gelatinized starch was very evident. Yogorou, a low-starch cultivar, showed little change in ruthenium red staining for raw and cooked squash fruit, indicating a low degree of pectin breakdown (**Figure 5D**, **5E** and **5F**). Yogorou's ultrasonic velocity for fruit steamed 10 and 20 min was 696 m s^{-1} and 2,068 m s^{-1}, respectively (**Table 1**). There was no significant difference between hardness of all 10-week stored fruit steamed for 20 min, but for 5-weeks stored fruit, Yogorou was the hardest, and was significantly firmer than two high-starch cultivars, Hyvita and Sweet Mama (Chapter 4). Therefore maintained cell wall integrity, because of lower level of pectin breakdown and cell separation, could result in harder texture. Cell wall breakdown resulted in increased ultrasonic velocity, and this may be attributed to cellulose, hemicelluloses or pectin in the cell wall matrix reflecting and refracting ultrasound, retarding its transmission.

Alternatively, aggregates of gelatinized starch mass may reduce cell structure heterogeneity, creating greater volume of solid material, allowing ultrasound to be transmitted more effectively. Scattering of ultrasonic waves by plant cells due to their heterogeneous makeup has been reported (18). Magnified images of iodine-stained gelatinized starch reveal a coarse, but uniform exterior (**Figure 4D**), which may allow ultrasound to transmit faster than cells containing free water.

Correlations Between Instron Universal Testing Machine Textural Measurements and Ultrasonic Velocity. Almost all correlations observed between Instron Universal Testing Machine textural measurements of fruit stored for 5 or 10 weeks and ultrasonic velocity of fruit stored 7.5 weeks were for raw fruit and fruit steamed for 10 min. Only correlations observed for fruit steamed 20 min was fracturability of fruit, stored for 5 weeks and steamed 20 min (r = -0.64, P = 0.03) and stored for 10 weeks and steamed 20 min (r = -0.69, P = 0.009). Fruit stored for 5 weeks, raw or steamed for 2 or 5 min, was correlated to ultrasonic velocity of raw fruit for hardness (r = 0.82, 0.84 and 0.68; P = < 0.0001, 0.0005 and 0.01 respectively) and springiness (r = -0.68, -0.67 and -0.66; P = 0.01, 0.02 and 0.02 respectively). Fracturability of fruit stored 5 weeks, raw or steamed for 2 min, was correlated to ultrasonic velocity of raw fruit (r = 0.81 and 0.84; P = 0.001 and 0.0005 respectively). Ultrasonic velocity of raw fruit, was correlated to the fruit textural parameters, stored 10 weeks and raw or steamed for 2 min, of hardness (r = 0.71 and 0.80; P = 0.009 and 0.0002 respectively), fracturability (r = 0.81 and 0.76; P = 0.0001 and 0.004 respectively) and springiness (r = -0.72 and -0.76; P = 0.008 and 0.004 respectively). Springiness of fruit, stored 5 or 10 weeks and steamed 20 min was also correlated to ultrasonic velocity of raw fruit (r = -0.64 and -0.61; P = 0.03 and 0.04 respectively).

Ultrasonic velocity of fruit steamed 10 min had the most correlations with Instron Universal Testing Machine textural measurements. Ultrasonic velocity of fruit steamed 10 min was correlated to hardness of fruit raw and steamed 2, 10, or 15 min that was stored for 5 (r = 0.72, 0.68, -0.58 and -0.56; P = 0.008, 0.01, 0.05 and 0.05 respectively) and 10 weeks (r = 0.68, 0.59, -0.71, -0.60; P = 0.01, 0.04, 0.009 and 0.04 respectively). Fracturability of fruit, stored 5 weeks and raw or steamed 2 min, was correlated to ultrasonic velocity of fruit steamed 10 min (r = 0.70 and 0.56; P = 0.01 and 0.05 respectively). Fruit stored 10 weeks and raw or steamed for 5, 15, or 20 min, had fracturability correlated to ultrasonic velocity of fruit steamed 10 min (r = 0.57, -0.67, -0.60 and -0.64; P = 0.05, 0.02, 0.04 and 0.03 respectively). Ultrasonic velocity of fruit steamed 10 min was correlated to springiness of fruit steamed 10, 15 or 20 min that was stored for 5 (r = -0.75, -0.75 and -0.63; P = 0.005, 0.005 and 0.03 respectively) or 10 weeks (r = -0.64, -0.74 and -0.64; P = 0.03, 0.006 and 0.03 respectively). Springiness of raw fruit that was stored 10 weeks was also correlated to ultrasonic velocity of fruit steamed 10 min (r = -0.57, P = 0.05).

Highly positive correlation was previously reported between compressive Young's modulus and ultrasonic velocity of boiled carrots (8). This finding is interesting compared with our findings since we found significant correlations between hardness of squash fruit and ultrasonic velocity but whether correlation was positive or negative depended on cooking time. Hardness of squash fruit raw or steamed for 2 or 5 min was positively correlated to ultrasonic velocity, but hardness of fruit steamed for 15 or 20 min was negatively correlated to ultrasonic velocity.

Correlations Between Starch Characteristics and Ultrasonic Velocity. Correlations were made between ultrasonic velocity measured from fruit stored 7.5 weeks and characteristics of

starch extracted from different fruit, that were stored 5 or 10 weeks, but grown in same completely randomized block research plot. Majority of correlations observed between ultrasonic velocity and starch characteristics were for fruit steamed for 10 min. Ultrasonic velocity through raw fruit was correlated to weight-average amylopectin molecular weight (M_w) and *z*-average amylopectin molecular weight (M_z) of fruit stored 5 weeks (r = -0.73 and -0.77; P = 0.04 and 0.02 respectively). Squash steamed for 20 min also had ultrasonic velocity correlated to M_w of fruit stored 5 weeks (r = -0.69, P = 0.05) and percent starch crystallinity for fruit stored 5 weeks (r = 0.79, P = 0.05). Peak viscosity, trough and final viscosity pasting properties of starch from fruit after 5 weeks storage were correlated to ultrasonic velocity (r = 0.82, 0.85 and 0.79; P = 0.04, 0.03 and 0.05 respectively).

Starch content of fruit stored 5 or 10 weeks was correlated to ultrasonic velocity of fruit steamed 10 min (r = 0.84 and 0.74; P = 0.0003 and 0.006 respectively), confirming microscopic observations. Water content of same fruit was correlated to ultrasonic velocity of fruit steamed 10 min (r = -0.86 and -0.77; P = < 0.0001 and 0.0008 respectively). Water content has been previously reported to be positively correlated to ultrasonic velocity of raw avocado (26), apple and potato (27). Dry matter content was previously reported to be negatively correlated to ultrasonic velocity in boiled carrots (8). This finding contradicts our results as squash with greater starch content, and resulting dry matter (Chapter 2), had faster ultrasonic velocity. However, carrots do not have starch granules present and after boiling for 15 min, a high degree of cell wall integrity remained in the carrots. Studies using the high-starch accumulating parsnip, belonging to same plant family as carrots (Umbelliferae), could be useful in determining if starch plays a role in ultrasonic velocity measurements and textural attributes of vegetables. Ultrasonic velocity, of fruit steamed 10 min, was correlated

to starch thermal properties, onset and peak gelatinization temperature (T_o and T_p), and change in enthalpy of gelatinization (ΔH) from fruit stored 10 weeks (r = -0.88, -0.79 and 0.89; P = 0.02, 0.05 and 0.02 respectively). Ultrasonic velocity was correlated to polydispersity, based on M_w and M_z (polyM_w and polyM_z) of amylopectins from squash cultivar fruit after 5 (r = -0.71 and -0.80; P = 0.05 and 0.01 respectively) and 10 weeks storage (r = -0.98 and -0.99; P = 0.0005 and 0.0002 respectively). Number-average amylopectin molecular weight (M_n) and M_z of fruit stored 10 weeks was correlated to ultrasonic velocity (r = -0.79; P = 0.05 for both). Gyration radii of amylopectin, based on M_n, from fruit stored 10 weeks, was correlated to ultrasonic velocity (r = 0.90, P = 0.01). Similar to fruit steamed 20 min, crystallinity of fruit stored 5 weeks was correlated to ultrasonic velocity of fruit steamed 10 min (r = 0.93, P = 0.008). Proportion of long amylopectin chains (DP $\geq$ 26) from fruit stored 10 weeks, measured using high-performance size-exclusion chromatography, was correlated to ultrasonic velocity of fruit steamed 10 min (r = -0.82, P = 0.04). Absolute amylose content of fruit stored 5 weeks and apparent amylose content of fruit stored 10 weeks were both correlated to ultrasonic velocity of fruit steamed 10 min (r = 0.83 and 0.80; P = 0.004 and 0.03 respectively).

Conclusions. Ultrasound has been demonstrated as a potential nondestructive tool for the evaluation of squash fruit texture. Ultrasound velocity was frequently slower than air for raw fruit, and velocity increased dramatically upon cooking. High-starch squash cultivars had the fastest ultrasonic velocity after 10 min steaming and this coincided with the presence of large aggregates of gelatinized starch that were not present in the low-starch cultivars which had slower ultrasonic velocity. All cultivars softened substantially during 20 min steaming, but light micrographs showed some cultivars had cell wall rupturing during cooking, while

others had cell walls still intact. Some cultivars lacked any presence of starch yet all cultivars had significant changes in textural attributes during cooking. Ultrasonic transmission studies and light micrographs, along with previous studies (Chapter 2, Chapter 4) indicate that starch structure, starch functionality and cell walls contribute to the texture of raw and cooked squash at harvest and during storage, but an additional factor, possibly turgor pressure, is also playing a role in determining squash texture.

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Table 1

Ultrasonic velocity (m s^{-1}) transmitted through raw and cooked winter squash fruit flesh*.

	Cooking Time (min)		
Cultivar	0#	10#	20
Big Max	193[c]	434[c]	2712
Cha Cha	296[abc]	1829[ab]	2795
Delica	329[ab]	1758[ab]	2740
Hyvita	218[bc]	1562[abc]	2107
Kurijiman	356[a]	2065[a]	2713
Lakota	362[a]	1345[abc]	1949
Rouge Vif D'Etampes	265[abc]	915[abc]	2421
Sweet Mama	317[ab]	1996[a]	2684
Warren Scarlet	296[abc]	1308[abc]	2067
Whangaparoa Crown	266[abc]	854[abc]	1966
Yogorou	279[abc]	696[bc]	2068
Zapallo Macre	280[abc]	356[c]	2628
	$P = 0.0006$♦	$P = 0.0002$	$P = 0.47$

* Velocity measurements are from three replicates for all cultivars.

Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

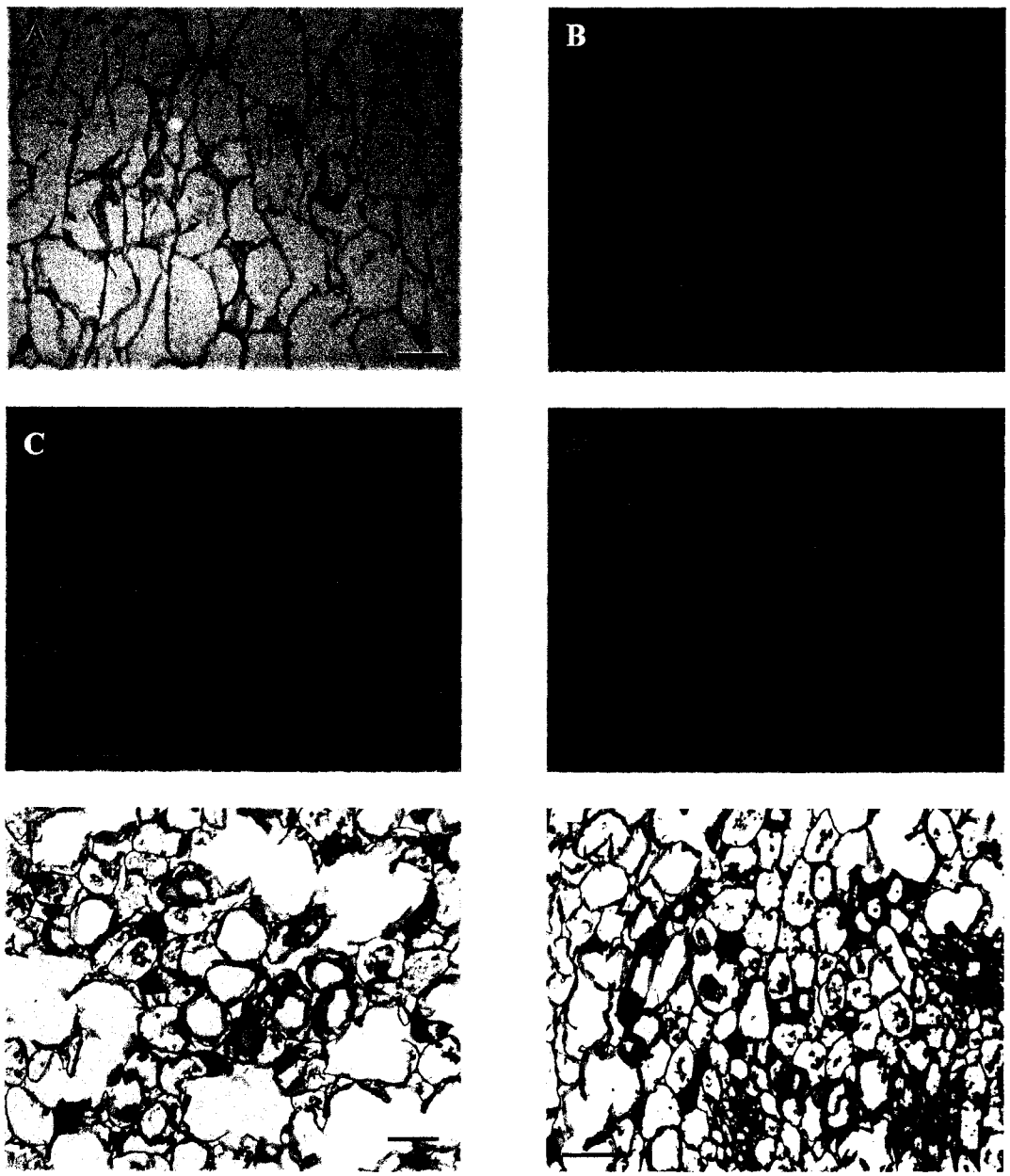

Figure 1. Representative light micrographs of Big Max stained with HF (A), Cha Cha stained with HF (B), Delica stained with HF (C), Hyvita stained with HS (D), Kurijiman stained with HS (E) and Lakota stained with SC (F) raw winter squash fruit flesh stored for 7.5 weeks. Scale bar = 100 μm and magnification is 33x for all images.

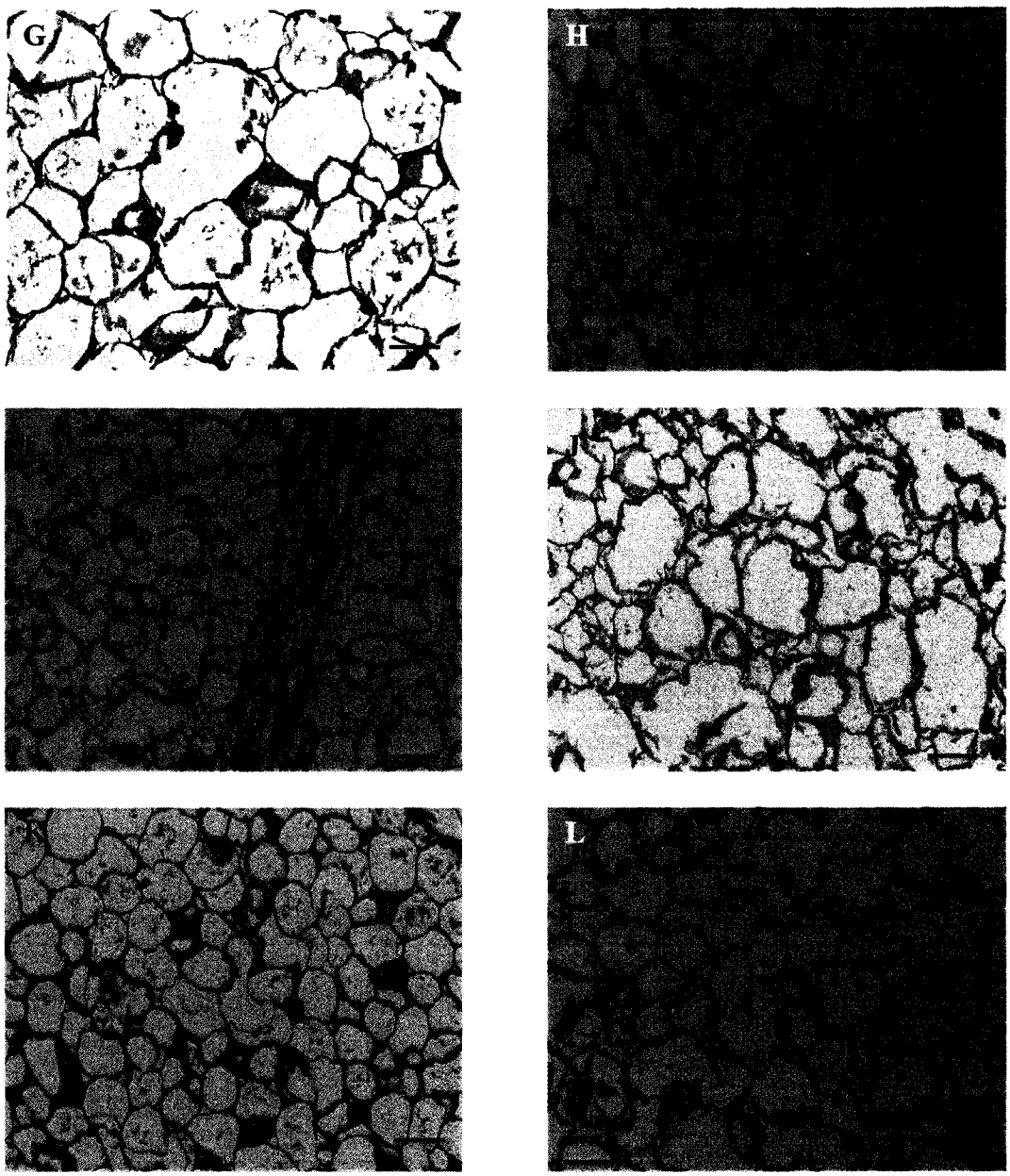

Figure 1. continued. Representative light micrographs of Rouge Vif D'Etampes stained with HF (G), Sweet Mama stained with HF (H), Warren Scarlet stained with HF (I), Whangaparoa Crown stained with SC (J), Yogorou stained with HS (K) and Zapallo Macre stained with SC (L) raw squash fruit flesh stored for 7.5 weeks. Scale bar = 100 μm, and magnification is 33x for all images.

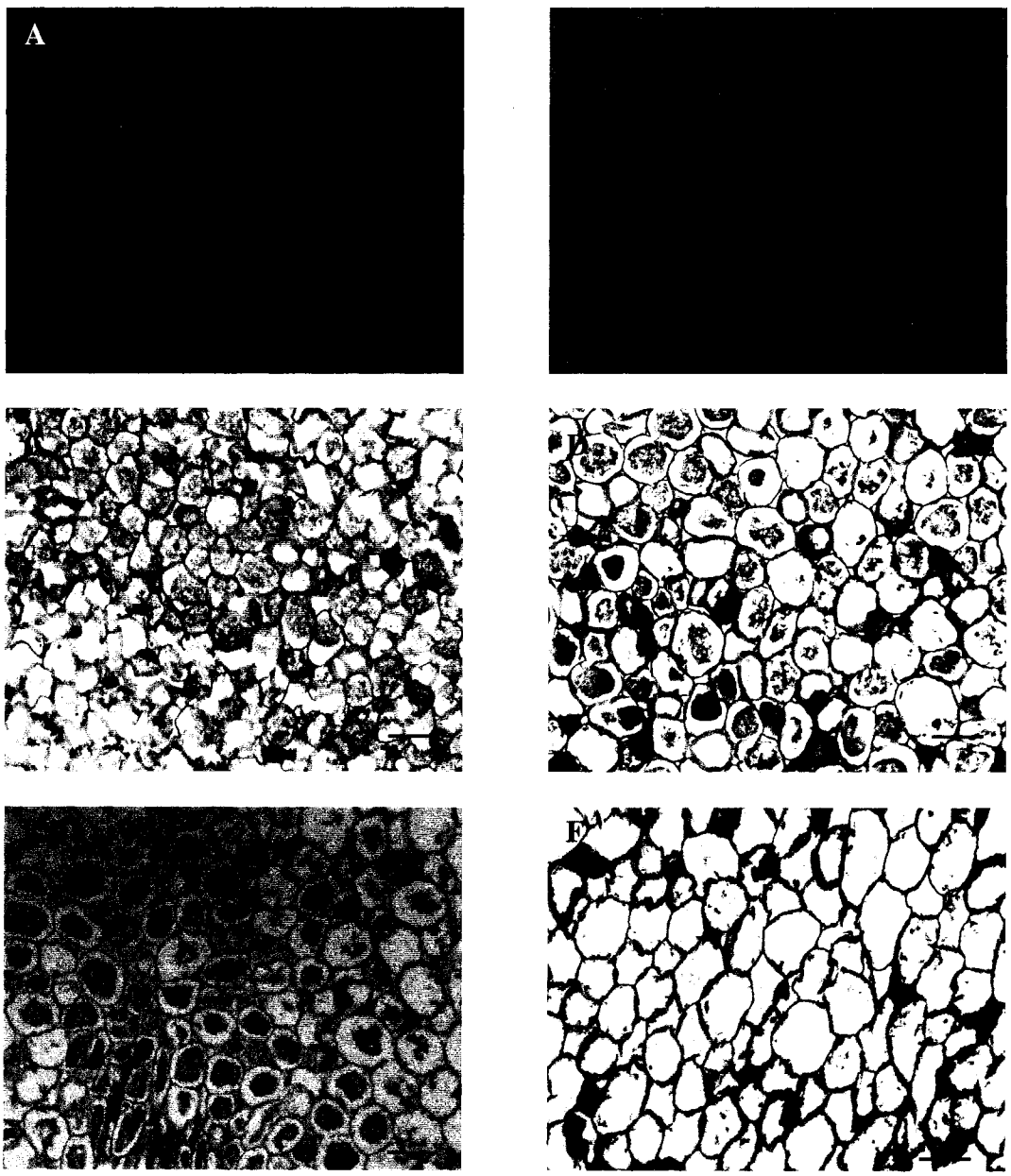

Figure 2. Representative light micrographs of Big Max stained with SC (A), Cha Cha stained with HS (B), Delica stained with SC (C), Hyvita stained with HS (D), Kurijiman stained with HF (E) and Lakota stained with SC (F) winter squash fruit flesh steamed for 10 minutes. Scale bar = 100 μm, and magnification is 33x for all images.

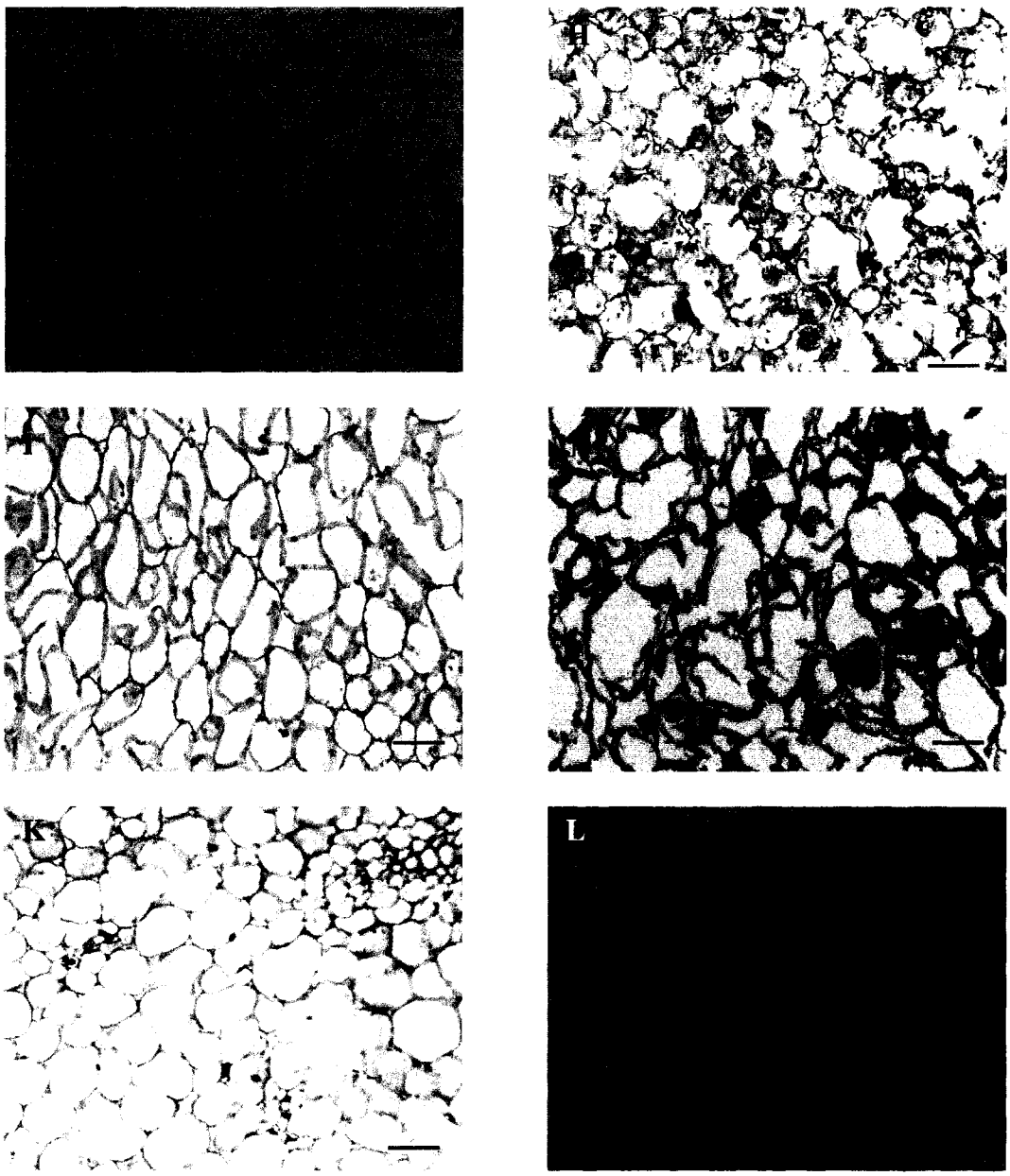

Figure 2. continued. Representative light micrographs of Rouge Vif D'Etampes stained with SC (G), Sweet Mama stained with SC (H), Warren Scarlet stained with HF (I), Whangaparoa Crown stained with SC (J), Yogorou stained with HF (K) and Zapallo Macre stained with HS (L) squash fruit steamed for 10 minutes. Scale bar = 100 μm.

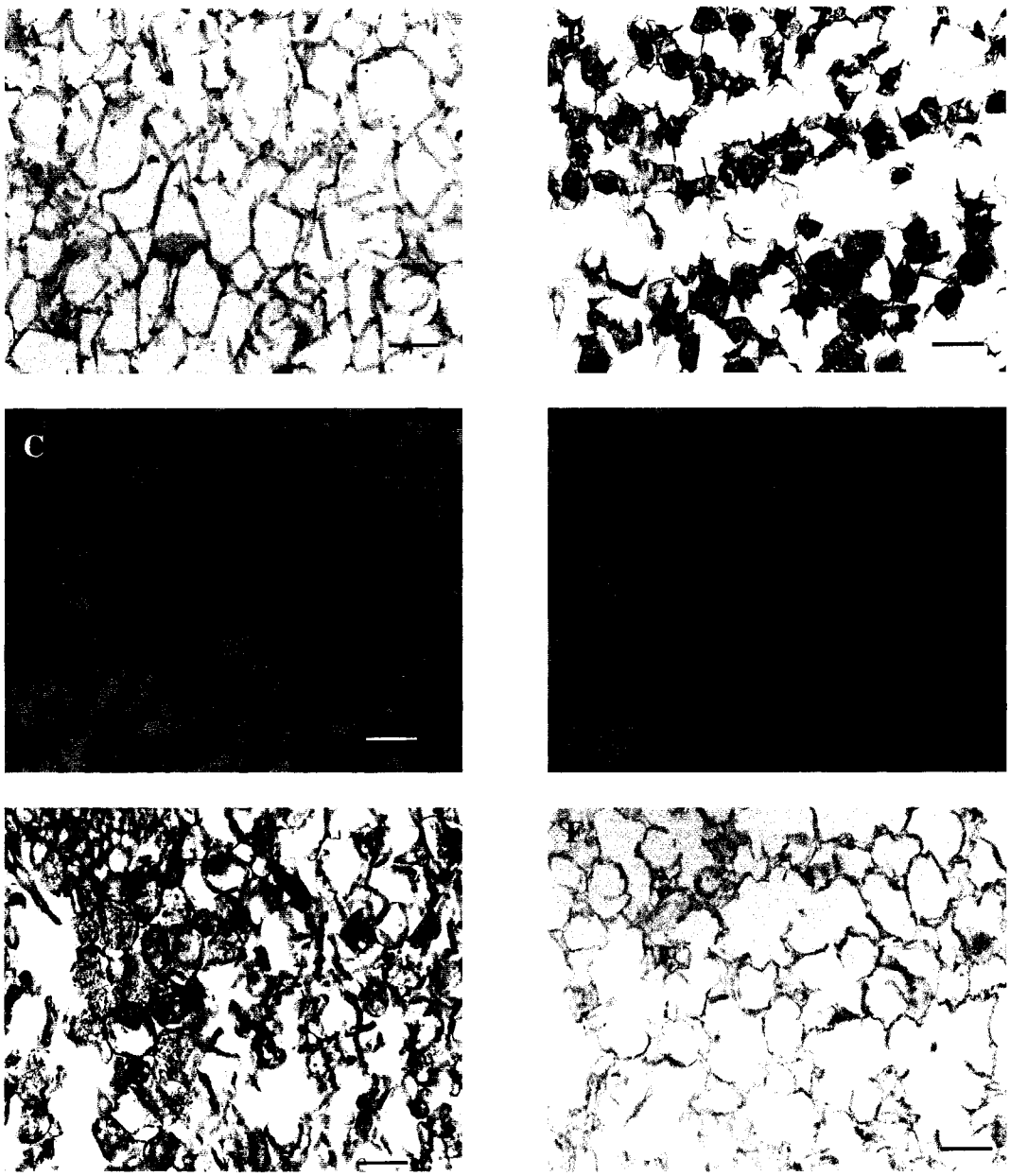

Figure 3. Representative light micrographs of Big Max stained with SC (A), Cha Cha stained with HS (B), Delica stained with SC (C), Hyvita stained with SC (D), Kurijiman stained with SC (E) and Lakota stained with HF (F) winter squash fruit flesh steamed for 20 minutes. Scale bar = 100 μm, and magnification is 33x for all images.

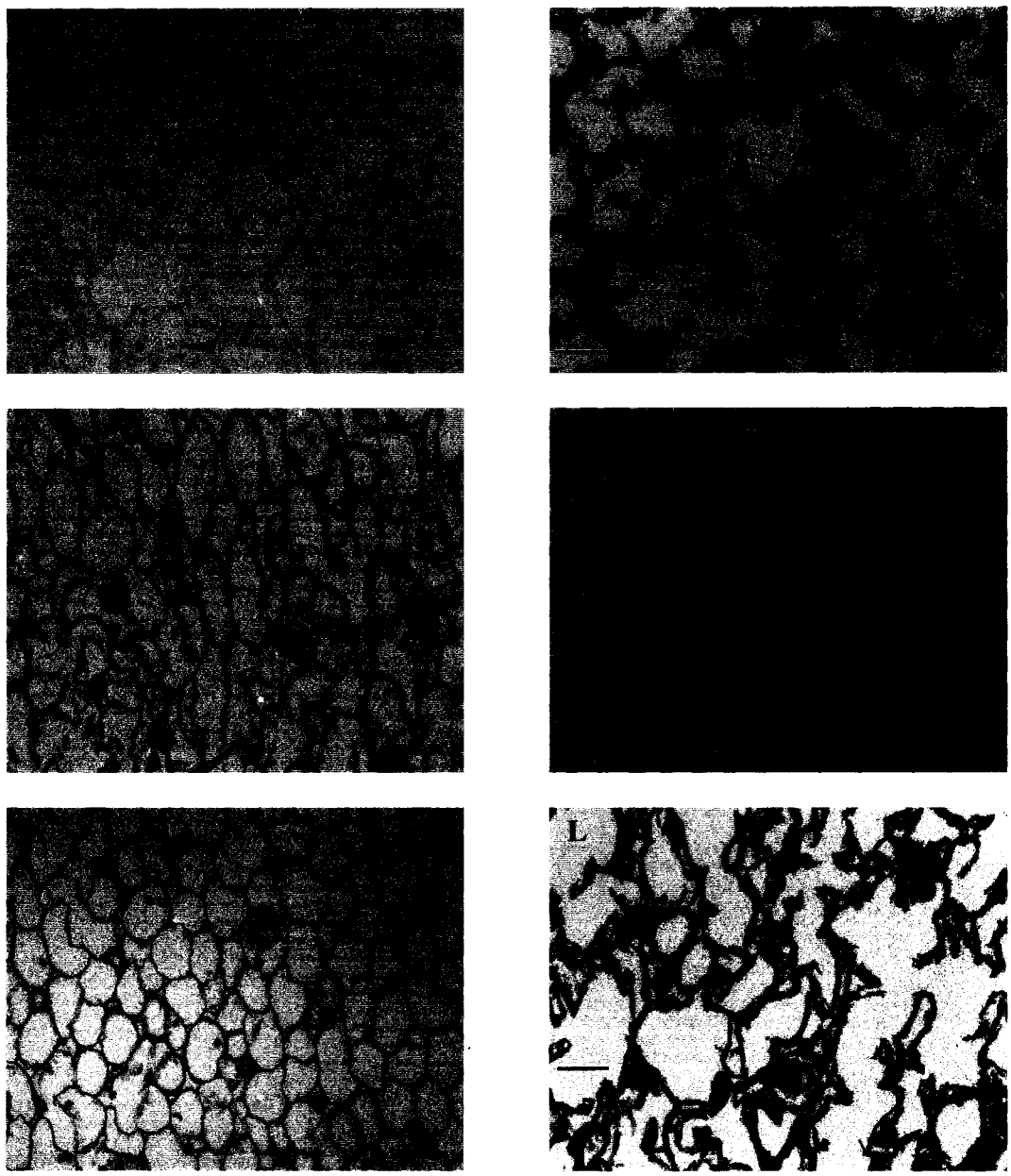

Figure 3. continued. Representative light micrographs of Rouge Vif D'Etampes stained with HF (G), Sweet Mama stained with SC (H), Warren Scarlet stained with HF (I), Whangaparoa Crown stained with SC (J), Yogorou stained with HF (K) and Zapallo Macre stained with SC (L) squash fruit steamed for 20 minutes. Scale bar = 100 μm.

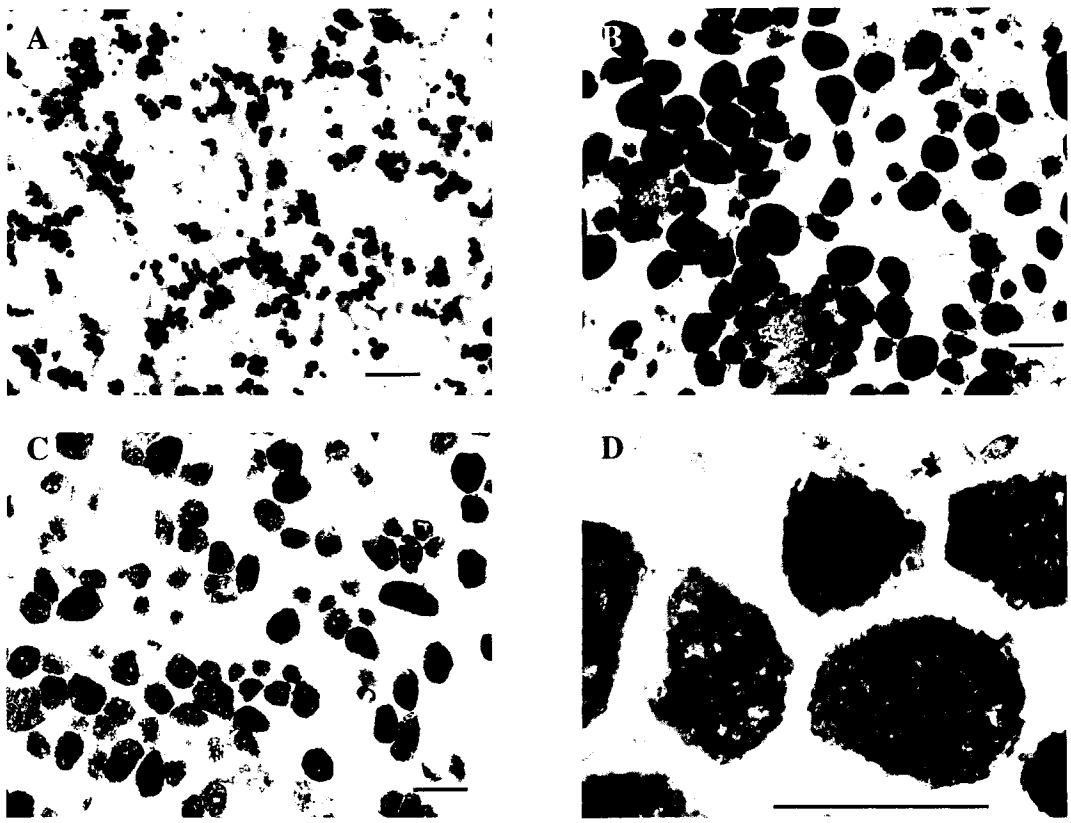

Figure 4. Representative light micrographs, stained with iodine/potassium iodide, of Cha Cha steamed for 0 (A), 10 (B) and 20 (C) minutes at 33x magnification. Representative light micrograph of buttercup squash after 10 minutes steaming is shown for iodine stained Kurijiman section at 134x magnification (D). Scale bar = 100 μm.

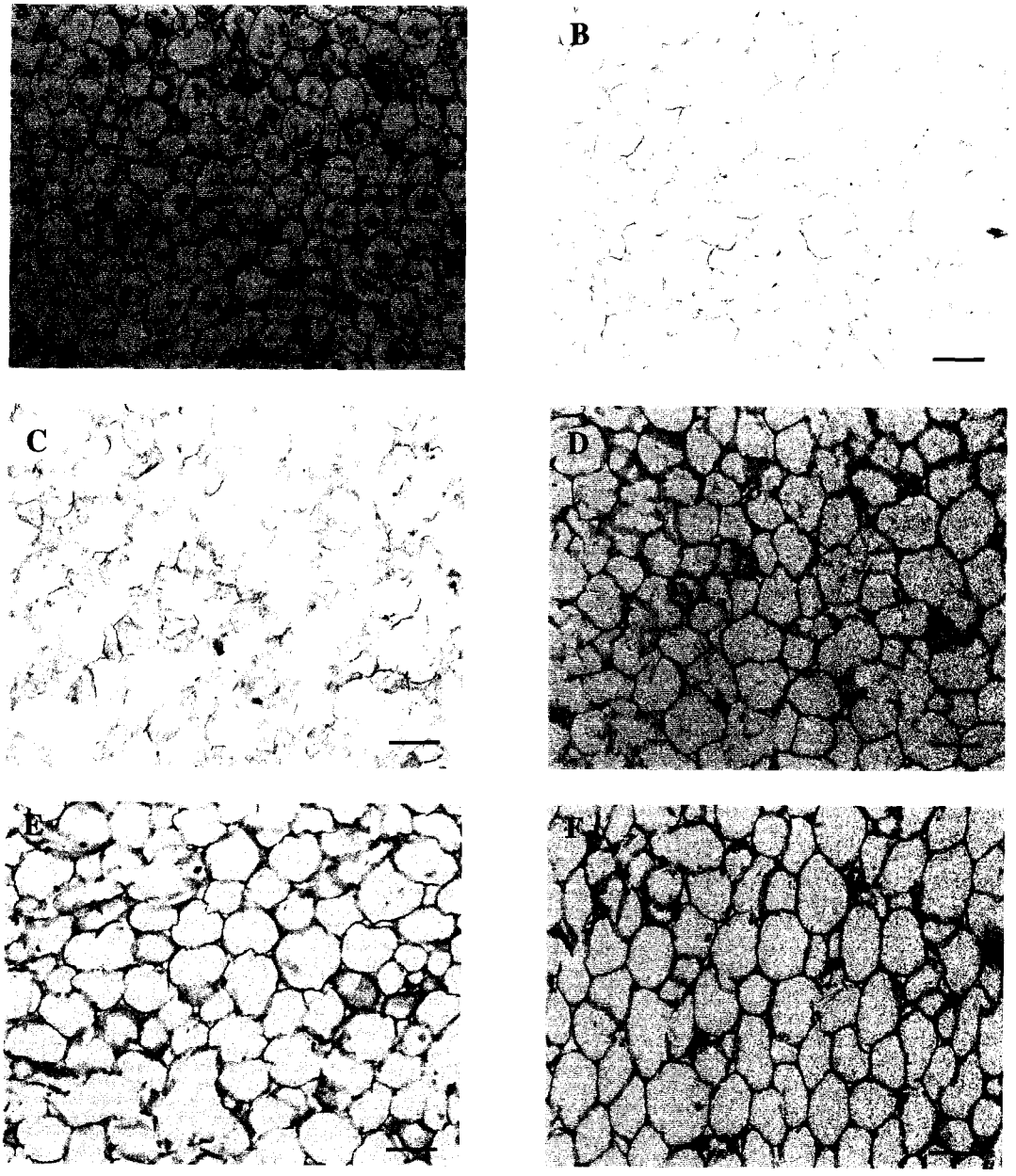

Figure 5. Representative light micrographs, stained with ruthenium red, of the winter squash cultivars Cha Cha steamed for 0 (A), 10 (B) and 20 (C) minutes, and Yogorou steamed for 0 (D), 10 (E), and 20 (F) minutes. Scale bar = 100 μm and magnification is 33x for all images.

CHAPTER 7. SEASONAL VARIATION IN WINTER SQUASH (*Cucurbita maxima* D.) FRUIT. I. VARIATION IN STARCH STRUCTURAL AND FUNCTIONAL PROPERTIES.

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Abstract Nine winter squash cultivars were grown for 2, 3 or 4 years at Ames, Iowa, and starch was extracted from fruit at harvest. Seasonal variation in starch structural and functional characteristics was investigated. There was large seasonal variation in starch content of fruit. All starch structural properties, including amylose content, average amylopectin molecular weight, amylopectin polydispersity, amylopectin gyration radius and amylopectin branch chain-length distribution varied between seasons. Starch functional properties, including gelatinisation temperatures and enthalpy change of native starch, thermal transition temperatures, and enthalpy of retrograded starch and percent retrogradation; pasting properties such as peak viscosity, breakdown, final viscosity, setback and pasting temperature; gel firmness and stickiness, all varied between seasons. Only differences that could be attributed to climatic effects are starch content, amylose content, amylopectin molecular weight and amylopectin branch chain-length distribution which showed consistent variation for all cultivars between seasons.

Keywords winter squash; buttercup squash, *Cucurbita maxima*; starch; amylose; amylopectin; seasonal variation;

INTRODUCTION

Winter squash are an important export crop for many countries, including New Zealand. Quality of winter squash is important in gaining access to lucrative markets. Knowledge of squash fruit composition can help determine factors important for improving quality. Some fruit from squash cultivars have been previously shown to contain moderate to high levels of starch (Hurst et al. 1995, Irving et al. 1997, Sugimoto et al. 1998, Corrigan et al. 2001, Curamasamy et al. 2002, Kang et al. 2002, Chapter 2). Understanding the starch structural and functional properties can provide information about the determinants of texture in winter squash fruit (Chapter 5).

Seasonal variation in squash fruit composition can adversely influence texture and other quality attributes. Therefore, to better manage the dispatch of high quality squash to export markets, knowledge on seasonal variation in starch content, structure and function is important. Currently there is no information on how starch characteristics in squash fruit vary between growing seasons.

Despite the extensive research on starch, publications on seasonal variation of starch structural and functional properties are not common for any crop, and typically report variation of just two seasons. Additionally these publications on seasonal variation have focused on just a few crops, particularly wheat (Tester et al. 1995, Lin & Czuchajowska 1997, Araki et al. 1999), maize (Campbell et al. 1995), rice (Ayers et al. 1997), cassava (Defloor et al. 1995) and sweet potatoes (Noda et al. 1998). In our study, we present results

of seasonal variation in starch structural and functional properties grown four seasons in Iowa.

MATERIALS AND METHODS

Climatic data

Daily mean, maximum and minimum temperatures for Ames between the primary squash cultivation period of May 20 to September 30, from 1998 to 2001 was obtained from Wunderground.com historical weather database.

Plant material

In total, nine winter squash (*Cucurbita maxima* D.) cultivars were grown for at least two seasons between 1998 and 2001 in Iowa. Squash cultivars studied were four buttercups (Cha Cha, Delica, Kurijiman and Sweet Mama), one cross between a buttercup, Green Delicious, and a non-buttercup, Table Queen (Hyvita), one Halloween-type (Rouge Vif D'Etampes), one Hubbard-type (Warren Scarlet, also known as Red Warren), one Crown-type (Whangaparoa Crown) and one Native American Indian squash (Lakota). In 1998, winter squash cultivars Delica, Kurijiman, Lakota, Sweet Mama, Warren Scarlet and Whangaparoa Crown were grown as part of seven cultivars in a completely randomized block at an Iowa State University farm site that is 1.7 miles south of Ames, Iowa (geographical location 41° 58' 57.5" N, 93° 38' 22.9"). In 1999, at an adjacent field, winter squash cultivars Delica,

Hyvita, Kurijiman, Lakota, Sweet Mama, Warren Scarlet and Whangaparoa Crown were grown as part of eight cultivars in a randomized complete block. Starch of Whangaparoa Crown grown in 1999 was not extracted and only water and total starch content are reported for this cultivar for the 1999 season. In 2000, on the same field as for 1998, winter squash cultivars Cha Cha, Delica, Hyvita, Kurijiman, Lakota, Rouge Vif D'Etampes, Sweet Mama, Warren Scarlet and Whangaparoa Crown were grown as part of twelve cultivars in a randomized complete block. In 2001, at a pumpkin farm located at Gilbert, Iowa, four miles north of Ames, Iowa, winter squash cultivars Cha Cha, Delica, Rouge Vif D'Etampes and Sweet Mama were grown as part of eight cultivars in a randomized complete block. In 1998, eighteen replicates were planted of each cultivar, in which five replicates were randomly selected for all analysis. In subsequent years, three replicates for all squash cultivars were planted and analysed. Seeds were purchased for Kurijiman, Warren Scarlet and Whangaparoa Crown from Webling and Stewart Ltd., Petone, New Zealand, for Delica from Yates New Zealand Ltd., Onehunga, New Zealand, for Sweet Mama from Henry Field Seed & Nursery Co., Shenandoah, IA, USA, for Lakota from W. Atlee Burpee & Co., Warminster, PA, USA, for Rouge Vif D'Etampes from J.W. Jung Seed Co., Randolph, WI, USA, and for Cha Cha from Johnny's Select Seeds Co., Winslow, ME, USA. Hyvita was received as a gift from Dr Henry Munger, Department of Plant Breeding, Cornell University, Ithaca, NY, USA. Squash fruit maturity was adjudged when stalks became woody (Hawthorne 1990), and this stage had been previously shown to have the highest starch content (Irving et al. 1997).

Starch isolation and quantification, and water content

Starch was extracted from fruit using procedure described previously (Chapter 2). In 1998, 1999 and 2001, starch was extracted from two fruit per replicate, and in 2000 four fruit per replicate were used. Water content of fruit was determined by freeze-drying. Total starch content of freeze-dried squash fruit powders, measured in duplicate, was determined using total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), that is described in more detail by Stevenson & Jane (Chapter 2).

Amylopectin molecular weight distribution and gyration radius

Weight-average molecular weight and *z*-average gyration radius of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI) as described previously (Chapter 2).

Apparent and absolute amylose content

Apparent and absolute amylose content of starch was determined following the procedure of Lu et al. (16). Analysis was based on iodine affinities of defatted whole starch and amylopectin fraction described previously using a potentiometric autotitrator (Chapter 2).

Amylopectin branch chain-length distribution

Amylopectin branch chain-length distribution was measured using high-performance anion-exchange chromatography equipped with an amyloglucosidase post-column on-line reactor and pulsed amperometric detector. Fractionated amylopectin was debranched with isoamlyase as described previously (Chapter 2).

Starch thermal properties

Thermal properties of native and retrograded starch were determined using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) as described previously (Chapter 2).

Starch pasting and gel properties

Starch pasting properties were analysed using a rapid visco-analyser (RVA-4, Newport Scientific, Sydney, Australia) as described previously (Chapter 2). Firmness and stickiness of squash starch gels after 1 or 7 d storage at 4°C was measured as described previously (Chapter 2).

RESULTS AND DISCUSSION

Ames seasonal climatic variance

Average daily mean, maximum and minimum temperatures and rainfall of about 15-16 day periods is shown in Table 1. The 1998 season was characterised by high rainfall in late May and June, but a dry late July and early August and warm temperatures late in growing season provided good conditions for squash fruit production. The 1999 season was much drier in initial months, and temperatures in mid to late July, when flowering was at its fullest, were very hot, causing problems for pollination. Late September of 1999 was very cool with one frost shortening the growing season. The 2000 season, when all squash cultivars were grown, was characterised by a warmer first month of growing season and predominantly warmer days later in growing season, resulting in the most productive yields. A frost in late September of 2000 shortened the growing season. High-rainfall in late May and associated substantially cooler temperatures slowed growth at first, but a hot two-month spell from mid-June to mid-August, where temperatures were frequently above 30°C, resulted in good squash fruit production for 2001 season. Growing season of 2001, like 1998, was not curtailed early by frost.

Water and starch content

Water and starch content of the squash cultivars grown over four seasons is shown in Table 2. Buttercup squash had lower water content and higher starch content than other squash cultivars. Changes in water content of fruit appeared to be unrelated to the amount of starch accumulated. Squash fruit accumulated high levels of starch and is in similar range to the 50-66% starch content, on dry weight basis, reported by Hurst et al. (1995) for three buttercup squash cultivars grown in New Zealand. Squash fruit in the 1999 and 2001 seasons

had substantially more starch than fruit grown in 2000. Although starch accumulation during squash fruit development has been reported to be fairly progressive, a greater proportion of starch is accumulated in the first 20 days after pollination (Irving et al. 1997, Kang et al. 2002), which typically was the last half of July. The 1999 and 2001 seasons had much higher average daily mean, maximum and minimum temperatures during last half of July than the 2000 season, which may explain the greater amount of starch accumulated by harvest. Wilhelm et al. reports that starch synthase enzymes in cereal grains are down regulated when temperature exceeds 25°C, but the overall high levels of starch accumulated in squash fruit for all three seasons, suggests starch synthesising enzymes in squash are active at much greater temperatures. Significant variation in starch content between different years has been reported for wheat (Lin & Czuchajowska 1997). In contrast to our results, no difference in starch content was observed for barley grown in two consecutive seasons (Oscarsson et al. 1998) and minor differences in starch content has been reported for wheat grown in different seasons and soil moisture status (Coles et al. 1997).

Amylose content

Seasonal variation in iodine affinity of amylopectin fraction and amylose content of squash fruit starches is shown in Table 3. Iodine affinity of amylopectin fraction reflects the proportion of amylopectin molecules with sufficiently long enough chain-lengths to complex iodine, exhibiting blue colour, and contributes to the apparent amylose content which measures iodine affinity of the whole starch. Absolute amylose content subtracts the iodine affinity of the amylopectin fraction from that of the whole starch and therefore measures the

true amylose content. Large seasonal variation was observed for iodine affinity of the amylopectin fraction with 1999 and 2000 considerably higher than 1998 and 2001, suggesting that amylopectin branch chain-lengths of squash starches are shorter in the 1998 and 2001 season. Apparent amylose content of squash starch in the last three seasons is high relative to most other starches (Jane et al. 1999), and was substantially higher than in 1998. Absolute amylose content of all four cultivars grown in 2001 was higher than their respective absolute amylose content observed in other seasons. Sugimoto et al. (1998) has shown that amylose content of buttercup squash fruit increases the greatest between 15 and 40 days after flowering, which generally was the month of August for squash grown in Iowa. Average minimum daily temperature in the last half of August of 1998 was warmer than the three other seasons, which may have caused a reduction in synthesis of long-chain amylopectin molecules that contribute to higher apparent amylose content. Seasonal differences in amylose content of squash fruit is in agreement with other studies. Apparent amylose content of wheat starch has been shown to increase 5% (i.e. 26 to 31%) when grown at temperatures 4°C warmer (Tester et al. 1995), and other studies of starches from 78 wheat lines grown in consecutive seasons observed, on average, a 3% difference between seasons in apparent amylose content and that allelic differences at *Wx*-B1 locus, that is influenced by environment, causes variation in apparent amylose content (Miura et al. 1994, Araki et al. 1999). The same *Wx* gene has been found to contribute to variation of amylose content of rice starch grown in different environments (Ayers et al. 1997). A highly significant ($P < 0.0001$) difference between years was reported for winter wheat apparent amylose content (Lin & Czuchajowska 1997). Amylose content of potatoes has been reported to be significantly influenced by temperature with heated glasshouse grown potatoes having starch

with higher amylose content than field-grown potatoes (Cottrell et al. 1995). Another study reported no difference in apparent amylose content of sweet potatoes grown in consecutive years (Noda et al. 1998).

Amylopectin molecular weight, polydispersity and gyration radius

Seasonal variation in amylopectin molecular weight, polydispersity and gyration radius of squash starches is shown in Table 4. Differences in weight-average amylopectin molecular weight were observed between the seasons but the variation was dependent on cultivar. However, no squash grown in 1998 had large amylopectin molecules relative to the other seasons. Polydispersity of amylopectin molecules was higher for most squash cultivars in 1999 season compared with 1998. Polydispersity of all buttercup squash cultivars was not similar in any individual season. Gyration radius measures the size of amylopectin molecules in respect to their spatial arrangement. Amylopectin gyration radius of squash cultivars showed much clearer trend with all cultivars grown in 1999 having wider amylopectin molecules compared with squash grown in 1998 and 2000 seasons. Sugimoto et al. (1998) reports average amylopectin molecular weight, from buttercup squash starch, increases rapidly during the first 22 days after flowering (8 to 9 times), reaching a maximum at 26 days after flowering and then sharply declines up to 42 days after flowering (8 to 9 times) before doubling in average molecular weight in the last 6 days of development prior to harvest (48 days after flowering). Based on Sugimoto's results, it is unclear whether the hot temperatures experienced in the first 22 days after flowering (late July to early August) in the 1999 season contributed to the higher molecular weight and gyration radius of amylopectin

molecules, or whether the climatic conditions experienced in the final week prior to harvest are critical. If the week prior to harvest is the developmental stage that determines amylopectin molecular characteristics, then seasonal variation is expected since harvest date, based on stems becoming woody, would have some associated variation with respect to days after flowering. There have been no previous reports in the literature about seasonal variation in amylopectin molecular weight, polydispersity and gyration radius for any crop.

Amylopectin branch chain-length distribution

Seasonal variation in amylopectin branch chain-length distribution of squash starches is shown in Table 5 and 6. The proportion of short amylopectin branch chain-lengths (DP 6-12) was different for some cultivars between seasons but for all cultivars, except Lakota, seasonal variation in short chains was small and some cultivars showed almost no variation. Proportion of short to intermediate amylopectin branch chain-lengths (DP 13-24) was lower for all squash cultivars grown in 2001 compared with 2000 season. Overall, greater proportion of DP 13-24 amylopectin branch chain-lengths was observed for starch from squash grown in 1998. Intermediate amylopectin branch chain-lengths (DP 25-36) showed the opposite trend to DP 13-24 chain-lengths as squash grown in 2001 season had higher intermediate chain-lengths than squash grown in 2000, and 1998 season tended to have the lowest proportion of intermediate amylopectin branch chain-lengths. Long amylopectin branch chain-lengths proportion was higher for starch from squash fruit grown in 2000 season compared with 2001. Apart from Hyvita that did not differ between 1999 and 2000 season, all other squash cultivars had higher proportion of long amylopectin branch chain-

lengths in 1999 season. The high proportion of long amylopectin branch chain-lengths in the 1999 season, relative to other seasons, is reflected also in the average branch chain-length. Since Sugimoto et al. (1998) has shown the largest amylopectin molecules are produced in the first 22 days after flowering, the hot temperatures in late July of 1999 may have resulted in faster metabolic rates producing amylopectins with larger molecular weights and greater proportion of longer chains. However, until an extensive study is conducted on amylopectin molecular weight during fruit development, we will not know whether the large decline in average amylopectin molecular weight occurring 22-42 days after flowering reported by Sugimoto et al. (1998) is due to large amylopectin molecules being degraded or a large increase in synthesis of relatively small amylopectin molecules. There has been no previous report in literature of variation in amylopectin branch chain-length distribution in different growing seasons for any starch.

Starch thermal properties

Onset (T_o), peak (T_p) and conclusion (T_c) gelatinisation temperature seasonal variation of native squash starches is shown in Table 7. Range of gelatinisation temperature (ROG) and enthalpy change of gelatinisation (ΔH) seasonal variation of native squash starches is shown in Table 8. Starch from fruit of buttercup squash cultivars had lower T_o, T_p and T_c grown in 1999 season than for other three seasons. Additionally, T_o of squash starches in 2001 season was lower than 2000 season, but T_p and T_c only varied for some cultivars. ROG of squash starches is very low relative to starches from other botanical sources (Jane et al. 1999). ROG of starch from buttercup squash cultivars varied considerably between seasons, particularly

for Cha Cha and Sweet Mama. There was no consistent trend among the squash cultivars for ROG variation between seasons. Seasonal variation in ΔH of squash starches was also observed for Hyvita, Rouge Vif D'Etampes, Sweet Mama and Warren Scarlet, but not for the other cultivars. Variation in thermal properties is unlikely to be attributed to degree of crystallinity because variation in gelatinisation temperatures does not correspond with the variation in ΔH. A 3°C increase in temperature has been shown to reduce ΔH of wheat starch from 17 to 13 J/g (Tester et al. 1995), and Cottrell et al. (1995) reported glasshouse grown potatoes had starch with lower ΔH and higher gelatinisation temperatures. Significant variation in all gelatinisation parameters except ΔH of maize starches between seasons has been postulated to be due to large differences in growing temperature and rainfall (Campbell et al. 1995). Seasonal differences in gelatinisation temperatures and ΔH of cassava starch from many genotypes has been reported and similar to our findings, variation was not consistent for each genotype between seasons (Defloor et al. 1995).

Thermal transition onset (T_{oR}), peak (T_{pR}) and conclusion (T_{cR}) temperature seasonal variation of retrograded squash starches is shown in Table 9. Enthalpy change of the thermal transition (ΔH_R) and retrogradation percentage variation across the different seasons is shown in Table 10. Similar to native starch, T_{oR}, T_{cR} and ΔH_R of retrograded starch varied for many squash cultivars between seasons but the variation was not consistent for each cultivar. T_{pR} varied between seasons for Cha Cha, Rouge Vif D'Etampes and Warren Scarlet. Whangaparoa Crown retrograded starch did not vary between seasons for any parameter measured and only thermal measurement that did vary between seasons was T_o of native starch. Percentage retrogradation of the squash starches showed much greater variation

between seasons than measurements of gelatinisation temperatures and enthalpy changes, and variation was not consistent between seasons for each cultivar. There has been just one previous report in literature of seasonal variation of retrograded starch for maize grown over two seasons (Campbell et al. 1995).

Starch pasting properties

Seasonal variation in the starch pasting properties of peak viscosity, breakdown and final viscosity of the squash starches is shown in Table 11, and the pasting properties of setback and pasting temperature are shown in Table 12. In the 2001 season, Cha Cha and Delica both had higher peak viscosity, breakdown, final viscosity and setback than 2000 season, with the complete opposite trend observed for Sweet Mama. Pasting temperature of the three buttercup squash starches in 2001 was not different from 2000 season. Variation in peak viscosity, breakdown, final viscosity, setback and pasting temperature was observed between seasons but differences were not consistent for each cultivar between seasons. Only Hyvita and Warren Scarlet showed no seasonal variation in peak viscosity, whereas Cha Cha, Hyvita and Kurijiman did not vary in breakdown between seasons. Warren Scarlet was the only squash starch paste that did not show any seasonal variation in final viscosity and setback, and only Cha Cha and Whangaparoa Crown showed no seasonal effects on starch pasting temperature. Viscosity of starch pastes has been reported to vary for barley and wheat grown over three seasons (Fastnaught et al. 1996, Udall et al. 1999).

Gel properties

Seasonal variation in gel firmness and stickiness of the squash starch gels is shown in Table 13. Apart from Delica, gel firmness after 1 d storage at 4°C was higher for starch from fruit grown in 2000 season than other two seasons, and this trend was not as evident after gels were stored for 7 d. Gels were stickier from starches of fruit grown in 1999 than the two other seasons, and this trend was also less evident after 7 d storage of gels. After 7 d storage, some starch gels showed stickiness of double or triple magnitude higher for starch extracted from fruit grown in 1999 season compared to 2000 season. There have been no previous reports in the literature of variation in starch gel properties over different growing seasons for starches obtained from any botanical source.

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Table 1

Climatic data for Ames, Iowa, during the main squash cultivation period, from May 20 to September 30 between 1998 and 2001. MnT = Average daily mean temperature (°C), MxT = Average daily maximum Temperature (°C), MmT = average minimum daily temperature (°C), Rnfl = rainfall (mm) and SolR = average daily solar radiation (langleys).

	May 20-31	June 1-15	June 16-30	July 1-15	July 16-31	Aug 1-15	Aug 16-31	Sept 1-15	Sept 16-30
1998 MnT	19.6	16.6	23.4	24.1	23.3	22.2	24.6	21.3	20.1
1998MxT	24.5	21.7	29.1	29.4	29.1	26.7	29.1	28.5	27.3
1998MmT	14.5	11.4	17.6	18.9	17.5	17.5	19.7	12.9	13.0
1998Rnfl	82.9	162.3	123.7	39.9	17.0	18.4	56.2	4.8	19.3
1998SolR	419	393	547	556	563	448	416	476	365
1999MnT	18.4	21.5	20.7	23.6	27.3	21.4	21.9	18.1	15.5
1999MxT	24.8	26.1	25.7	29.4	32.2	26.9	27.4	24.4	23.3
1999MmT	12.2	16.5	15.4	17.3	22.1	15.5	16.2	11.3	7.6
1999Rnfl	37.8	131.6	37.3	56.1	86.1	48.5	95.0	38.6	14.7
1999SolR	569	435	489	583	548	485	424	436	373
2000MnT	18.9	21.1	20.0	24.3	20.7	23.7	22.4	22.5	16.0
2000MxT	25.5	26.9	26.2	29.1	26.6	29.7	27.7	30.3	23.7
2000MmT	11.9	15.3	14.1	19.2	14.5	17.3	17.0	14.7	8.1
2000Rnfl	36.3	36.3	46.0	30.7	35.8	18.0	13.0	6.9	16.5
2000SolR	417	513	565	462	533	527	357	451	328
2001MnT	12.5	19.6	22.5	23.6	25.8	24.3	21.8	18.2	13.9
2001MxT	15.5	25.3	29.2	30.0	30.7	29.9	27.2	22.6	20.1
2001MmT	9.3	13.5	15.5	17.1	20.8	18.3	16.1	13.6	7.7
2001Rnfl	78.1	38.1	4.3	11.2	31.5	26.2	17.3	99.3	34.5
2001SolR	329	477	622	548	460	515	470	362	333

Table 2

Water and starch content of squash fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Water content (%)			
Cultivar	1998	1999	2000	2001
Cha Cha			71.8 ± 2.1	80.3 ± 0.8
Delica	78.8 ± 0.7	77.2 ± 0.6	80.1 ± 1.4	80.3 ± 0.7
Hyvita		81.4 ± 0.4	84.4 ± 0.8	
Kurijiman	73.9 ± 0.2	74.1 ± 0.1	78.9 ± 1.9	
Lakota	91.2 ± 0.2	87.5 ± 0.3	89.5 ± 0.8	
Rouge Vif D'Etampes			94.3 ± 0.3	94.7 ± 0.2
Sweet Mama	84.1 ± 1.7	78.6 ± 0.4	79.4 ± 0.3	81.3 ± 0.6
Warren Scarlet	92.1 ± 1.3	88.5 ± 0.9	88.9 ± 1.1	
Whangaparoa Crown	93.0 ± 0.2	89.9 ± 0.7	91.0 ± 0.5	

	Starch content (% dry weight)*		
Cultivar	1999	2000	2001
Cha Cha		61.2 ± 8.4	81.9 ± 1.7
Delica	69.9 ± 6.3	56.7 ± 3.8	74.0 ± 3.9
Hyvita	82.3 ± 3.1	54.4 ± 4.1	
Kurijiman	76.1 ± 0.9	55.2 ± 1.2	
Lakota	31.0 ± 4.1	17.6 ± 3.5	
Rouge Vif D'Etampes		14.5 ± 0.1	50.2 ± 0.4
Sweet Mama	61.8 ± 0.7	52.3 ± 4.7	83.9 ± 0.1
Warren Scarlet	34.7 ± 6.3	17.4 ± 11.2	
Whangaparoa Crown	21.2 ± 5.8	14.2 ± 2.6	

* Data for 1998 season starch content is omitted due to loss of samples.

Table 3

Seasonal variation in iodine affinity of amylopectin fraction, and the apparent and absolute amylose contents of squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Iodine Affinity			
Cultivar	1998	1999	2000	2001
Cha Cha			3.88 ± 0.04	3.11 ± 0.18
Delica	1.93 ± 0.13	4.74 ± 0.19	3.89 ± 0.09	2.55 ± 0.16
Hyvita		3.53 ± 0.19	3.07 ± 0.41	
Kurijiman	2.35 ± 0.19	4.22 ± 0.02	2.92 ± 0.29	
Lakota	1.65 ± 0.11	3.54 ± 0.02	3.84 ± 0.15	
Rouge Vif D'Etampes			4.76 ± 0.18	2.29 ± 0.04
Sweet Mama	2.33 ± 0.10	4.57 ± 0.13	3.51 ± 0.05	2.43 ± 0.02
Warren Scarlet	1.81 ± 0.07	4.42 ± 0.13	4.05 ± 0.05	
Whangaparoa Crown	1.96 ± 0.15	4.42 ± 0.01	3.78 ± 0.12	

	Apparent Amylose (%)			
Cultivar	1998	1999	2000	2001
Cha Cha			37.7 ± 0.9	41.0 ± 0.2
Delica	27.8 ± 0.8	43.0 ± 0.0	36.5 ± 1.7	40.5 ± 0.1
Hyvita		36.9 ± 0.4	33.7 ± 1.0	
Kurijiman	28.3 ± 0.9	41.1 ± 0.8	34.6 ± 0.5	
Lakota	22.2 ± 1.1	38.4 ± 1.0	32.4 ± 0.6	
Rouge Vif D'Etampes			34.7 ± 1.4	30.4 ± 1.8
Sweet Mama	24.9 ± 1.4	38.3 ± 1.8	38.7 ± 1.2	38.5 ± 0.4
Warren Scarlet	31.3 ± 3.0	39.6 ± 0.1	37.0 ± 0.5	
Whangaparoa Crown	27.9 ± 0.8		36.0 ± 0.3	

	Absolute Amylose (%)			
Cultivar	1998	1999	2000	2001
Cha Cha			18.2 ± 0.9	25.3 ± 0.7
Delica	18.2 ± 0.7	19.2 ± 1.0	17.0 ± 1.7	27.7 ± 0.7
Hyvita		19.1 ± 0.5	18.3 ± 1.3	
Kurijiman	16.5 ± 0.7	19.9 ± 0.8	20.0 ± 1.5	
Lakota	14.0 ± 0.7	20.7 ± 1.1	13.1 ± 5.4	
Rouge Vif D'Etampes			10.8 ± 0.8	18.9 ± 1.6
Sweet Mama	13.2 ± 1.1	15.3 ± 2.4	21.1 ± 1.3	26.3 ± 0.5
Warren Scarlet	17.9 ± 3.3	17.4 ± 0.1	16.7 ± 3.3	
Whangaparoa Crown	18.0 ± 1.1		17.0 ± 0.7	

Table 4

Seasonal variation in weight-average molecular weight (M_w), polydispersity and gyration radius of squash amylopectin from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	M_w (x 10^8)			
Cultivar	1998	1999	2000	2001
Cha Cha			3.46 ± 0.26	4.82 ± 0.16
Delica	3.19 ± 0.27	4.02 ± 0.22	2.83 ± 0.26	4.22 ± 0.02
Hyvita		4.49 ± 0.76	4.00 ± 0.33	
Kurijiman	2.68 ± 0.48	4.61 ± 0.27	3.05 ± 1.80	
Lakota	3.68 ± 0.25	6.21 ± 1.84	5.52 ± 1.78	
Rouge Vif D'Etampes			3.48 ± 0.19	2.32 ± 0.01
Sweet Mama	3.19 ± 0.14	3.82 ± 0.10	4.15 ± 0.39	3.71 ± 0.02
Warren Scarlet	3.87 ± 0.89	4.17 ± 0.22	3.73 ± 0.38	
Whangaparoa Crown	3.38 ± 0.27		3.88 ± 0.27	

	Polydispersity			
Cultivar	1998	1999	2000	2001
Cha Cha			1.38 ± 0.07	1.48 ± 0.07
Delica	1.44 ± 0.15	1.83 ± 0.14	1.95 ± 0.20	2.10 ± 0.13
Hyvita		2.91 ± 0.09	1.51 ± 0.10	
Kurijiman	1.81 ± 0.40	1.90 ± 0.01	1.82 ± 0.24	
Lakota	1.21 ± 0.09	2.40 ± 0.80	1.82 ± 0.30	
Rouge Vif D'Etampes			1.51 ± 0.15	2.33 ± 0.00
Sweet Mama	1.30 ± 0.06	2.69 ± 0.16	1.43 ± 0.14	1.46 ± 0.04
Warren Scarlet	1.35 ± 0.08	2.99 ± 0.02	1.54 ± 0.10	
Whangaparoa Crown	1.29 ± 0.08		1.41 ± 0.09	

	Gyration Radius (nm)			
Cultivar	1998	1999	2000	2001
Cha Cha			309 ± 5	350 ± 2
Delica	311 ± 7	346 ± 10	297 ± 5	357 ± 1
Hyvita		380 ± 21	318 ± 6	
Kurijiman	294 ± 13	366 ± 6	299 ± 27	
Lakota	324 ± 6	410 ± 44	349 ± 28	
Rouge Vif D'Etampes			304 ± 3	308 ± 0
Sweet Mama	311 ± 3	354 ± 5	329 ± 11	320 ± 6
Warren Scarlet	337 ± 17	363 ± 5	317 ± 9	
Whangaparoa Crown	304 ± 10		313 ± 5	

Table 5

Seasonal variation in short- and intermediate-length amylopectin branch chains of squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	DP 6-12			
Cultivar	1998	1999	2000	2001
Cha Cha			14.1 ± 0.3	14.5 ± 0.2
Delica	14.9 ± 0.2	13.7 ± 0.5	15.3 ± 0.2	14.8 ± 0.3
Hyvita		16.2 ± 0.1	16.1 ± 0.3	
Kurijiman	15.6 ± 0.2	14.0 ± 0.3	15.4 ± 0.3	
Lakota	12.6 ± 0.4	18.2 ± 0.4	15.6 ± 0.4	
Rouge Vif D'Etampes			14.9 ± 0.1	15.5 ± 0.8
Sweet Mama	15.2 ± 0.1	14.4 ± 0.2	14.4 ± 0.2	14.4 ± 0.2
Warren Scarlet	15.4 ± 0.3	14.6 ± 0.2	16.7 ± 0.7	
Whangaparoa Crown	15.9 ± 0.2		15.9 ± 0.3	

	DP 13-24			
Cultivar	1998	1999	2000	2001
Cha Cha			38.4 ± 0.3	37.9 ± 0.2
Delica	40.6 ± 0.1	37.7 ± 0.2	39.5 ± 0.3	39.0 ± 0.3
Hyvita		39.8 ± 0.3	39.9 ± 0.7	
Kurijiman	41.0 ± 0.2	38.3 ± 0.2	40.5 ± 0.2	
Lakota	44.7 ± 0.3	38.6 ± 0.4	39.5 ± 0.9	
Rouge Vif D'Etampes			40.1 ± 0.8	37.4 ± 0.7
Sweet Mama	40.1 ± 0.1	38.2 ± 0.1	39.5 ± 0.1	38.6 ± 0.2
Warren Scarlet	40.4 ± 0.2	38.7 ± 0.3	41.0 ± 0.9	
Whangaparoa Crown	41.2 ± 0.1		38.2 ± 0.2	

	DP 25-36			
Cultivar	1998	1999	2000	2001
Cha Cha			17.1 ± 0.2	18.2 ± 0.3
Delica	14.7 ± 0.1	17.0 ± 0.3	16.0 ± 0.3	17.8 ± 0.2
Hyvita		17.4 ± 0.2	17.4 ± 0.5	
Kurijiman	14.2 ± 0.2	15.2 ± 0.4	16.6 ± 0.8	
Lakota	16.5 ± 0.1	17.0 ± 0.3	16.9 ± 0.2	
Rouge Vif D'Etampes			17.5 ± 0.5	18.7 ± 0.3
Sweet Mama	15.0 ± 0.1	15.0 ± 0.1	16.5 ± 0.3	19.1 ± 0.2
Warren Scarlet	16.0 ± 0.2	16.1 ± 0.2	16.7 ± 0.7	
Whangaparoa Crown	14.6 ± 0.1		16.4 ± 0.7	

Table 6

Seasonal variation in long amylopectin branch chain-lengths and average amylopectin branch chain-length of squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	DP ≥ 37			
Cultivar	1998	1999	2000	2001
Cha Cha			30.2 ± 0.5	28.8 ± 0.4
Delica	29.3 ± 0.2	31.1 ± 0.5	28.9 ± 0.6	27.7 ± 0.5
Hyvita		25.5 ± 0.3	26.3 ± 0.7	
Kurijiman	28.4 ± 0.3	31.8 ± 0.4	27.1 ± 1.1	
Lakota	25.4 ± 0.6	29.5 ± 0.2	27.4 ± 0.6	
Rouge Vif D'Etampes			28.0 ± 0.7	26.4 ± 0.3
Sweet Mama	28.8 ± 0.2	32.0 ± 0.3	29.3 ± 0.2	27.1 ± 0.3
Warren Scarlet	27.1 ± 0.3	30.2 ± 0.5	25.6 ± 1.3	
Whangaparoa Crown	27.3 ± 0.2		29.1 ± 0.7	

	Average Chain-length (DP)			
Cultivar	1998	1999	2000	2001
Cha Cha			28.8 ± 0.3	28.5 ± 0.1
Delica	28.1 ± 0.1	29.4 ± 0.2	28.0 ± 0.3	27.7 ± 0.2
Hyvita		26.9 ± 0.1	26.9 ± 0.3	
Kurijiman	27.4 ± 0.3	29.3 ± 0.1	27.3 ± 0.5	
Lakota	27.0 ± 0.2	28.7 ± 0.3	27.3 ± 0.1	
Rouge Vif D'Etampes			27.1 ± 0.6	26.4 ± 0.3
Sweet Mama	27.9 ± 0.1	29.5 ± 0.1	28.3 ± 0.1	27.8 ± 0.2
Warren Scarlet	26.9 ± 0.4	28.8 ± 0.4	25.7 ± 0.2	
Whangaparoa Crown	27.2 ± 0.2		28.3 ± 0.3	

Table 7

Seasonal variation in gelatinisation temperatures (°C) of native squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Onset Gelatinisation Temperature (T_o)			
Cultivar	1998	1999	2000	2001
Cha Cha			64.4 ± 0.4	62.4 ± 0.0
Delica	63.4 ± 0.4	61.8 ± 0.3	63.8 ± 0.2	63.3 ± 0.2
Hyvita		63.8 ± 0.4	61.3 ± 0.1	
Kurijiman	63.5 ± 0.2	61.9 ± 0.2	65.0 ± 0.2	
Lakota	62.9 ± 0.3	63.7 ± 0.8	62.9 ± 0.1	
Rouge Vif D'Etampes			64.4 ± 0.3	61.3 ± 0.1
Sweet Mama	63.5 ± 0.3	59.5 ± 0.1	64.4 ± 0.2	63.3 ± 0.0
Warren Scarlet	61.7 ± 0.4	61.9 ± 0.4	61.2 ± 0.4	
Whangaparoa Crown	60.6 ± 0.7		61.8 ± 0.2	

	Peak Gelatinisation Temperature (T_p)			
Cultivar	1998	1999	2000	2001
Cha Cha			67.1 ± 0.4	66.9 ± 0.1
Delica	66.4 ± 0.3	65.7 ± 0.1	67.0 ± 0.2	66.7 ± 0.4
Hyvita		66.5 ± 0.3	64.2 ± 0.2	
Kurijiman	66.5 ± 0.1	65.1 ± 0.2	67.7 ± 0.1	
Lakota	66.4 ± 0.1	67.0 ± 0.0	67.6 ± 0.1	
Rouge Vif D'Etampes			67.9 ± 0.2	65.3 ± 0.1
Sweet Mama	66.2 ± 0.3	64.3 ± 0.3	67.5 ± 0.2	66.5 ± 0.0
Warren Scarlet	65.7 ± 0.3	65.3 ± 0.4	65.8 ± 0.3	
Whangaparoa Crown	64.0 ± 0.7		64.9 ± 0.2	

	Conclusion Gelatinisation Temperature (T_c)			
Cultivar	1998	1999	2000	2001
Cha Cha			70.4 ± 0.4	70.4 ± 0.1
Delica	69.7 ± 0.2	69.2 ± 0.1	70.0 ± 0.3	70.4 ± 0.3
Hyvita		70.0 ± 0.3	67.8 ± 0.1	
Kurijiman	69.8 ± 0.2	68.8 ± 0.3	70.7 ± 0.1	
Lakota	70.4 ± 0.1	71.2 ± 0.5	71.8 ± 0.2	
Rouge Vif D'Etampes			72.1 ± 0.3	70.1 ± 0.1
Sweet Mama	69.8 ± 0.4	69.1 ± 0.2	70.8 ± 0.2	69.7 ± 0.0
Warren Scarlet	70.4 ± 0.4	69.7 ± 0.5	70.2 ± 0.4	
Whangaparoa Crown	67.7 ± 0.7		68.4 ± 0.3	

Table 8

Seasonal variation in range of gelatinisation temperature and enthalpy change of gelatinisation of native squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Range of Gelatinisation Temperature (°C)			
Cultivar	1998	1999	2000	2001
Cha Cha			6.0 ± 0.1	8.0 ± 0.1
Delica	6.3 ± 0.3	7.4 ± 0.4	6.3 ± 0.1	7.1 ± 0.1
Hyvita		6.2 ± 0.1	6.5 ± 0.1	
Kurijiman	6.2 ± 0.2	6.9 ± 0.1	5.7 ± 0.1	
Lakota	7.5 ± 0.2	7.4 ± 1.3	8.9 ± 0.2	
Rouge Vif D'Etampes			7.7 ± 0.1	8.8 ± 0.1
Sweet Mama	6.3 ± 0.2	9.6 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Warren Scarlet	8.6 ± 0.3	7.7 ± 0.2	8.9 ± 0.2	
Whangaparoa Crown	7.1 ± 0.2		6.7 ± 0.4	

	Enthalpy Change of Gelatinisation (J/g)			
Cultivar	1998	1999	2000	2001
Cha Cha			17.8 ± 0.2	16.6 ± 0.3
Delica	17.3 ± 0.4	16.7 ± 0.4	16.5 ± 0.2	17.0 ± 0.4
Hyvita		18.2 ± 0.3	15.8 ± 0.2	
Kurijiman	16.9 ± 0.4	16.7 ± 0.2	17.2 ± 0.2	
Lakota	16.8 ± 0.2	17.4 ± 0.4	17.6 ± 0.3	
Rouge Vif D'Etampes			16.3 ± 0.2	17.5 ± 0.6
Sweet Mama	17.4 ± 0.4	15.1 ± 1.3	17.7 ± 0.3	15.5 ± 0.8
Warren Scarlet	16.4 ± 0.2	17.3 ± 0.2	14.8 ± 0.3	
Whangaparoa Crown	16.3 ± 0.8		16.3 ± 0.2	

Table 9

Seasonal variation in thermal transition temperatures (°C) of retrograded squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Onset Thermal Transition Temperature			
Cultivar	1998	1999	2000	2001
Cha Cha			37.0 ± 0.5	35.6 ± 0.7
Delica	36.3 ± 0.4	37.5 ± 0.0	35.7 ± 0.5	36.6 ± 0.1
Hyvita		35.1 ± 0.2	36.4 ± 0.5	
Kurijiman	36.6 ± 0.4	37.1 ± 0.3	37.5 ± 0.3	
Lakota	35.2 ± 0.6	38.3 ± 0.3	36.8 ± 0.4	
Rouge Vif D'Etampes			37.4 ± 0.7	34.3 ± 0.6
Sweet Mama	35.1 ± 0.6	35.7 ± 0.6	36.2 ± 0.8	33.8 ± 1.4
Warren Scarlet	36.0 ± 0.4	35.4 ± 0.5	36.4 ± 0.9	
Whangaparoa Crown	35.6 ± 0.5		35.6 ± 1.0	

	Peak Thermal Transition Temperature			
Cultivar	1998	1999	2000	2001
Cha Cha			56.3 ± 0.5	53.7 ± 0.1
Delica	54.0 ± 0.9	53.7 ± 0.3	53.9 ± 0.4	53.8 ± 0.2
Hyvita		53.5 ± 0.8	54.1 ± 0.2	
Kurijiman	54.5 ± 0.6	53.9 ± 3.9	54.5 ± 0.4	
Lakota	53.3 ± 0.6	54.9 ± 2.1	53.5 ± 0.6	
Rouge Vif D'Etampes			57.3 ± 0.4	53.3 ± 0.3
Sweet Mama	52.4 ± 0.9	55.9 ± 1.8	54.4 ± 0.6	53.7 ± 0.1
Warren Scarlet	52.6 ± 0.5	53.2 ± 0.6	56.6 ± 0.5	
Whangaparoa Crown	53.3 ± 0.6		54.1 ± 0.6	

	Conclusion Thermal Transition Temperature			
Cultivar	1998	1999	2000	2001
Cha Cha			65.9 ± 0.3	66.2 ± 0.2
Delica	64.5 ± 0.9	66.3 ± 0.1	65.4 ± 0.3	66.7 ± 0.3
Hyvita		63.9 ± 0.1	65.3 ± 0.3	
Kurijiman	64.8 ± 0.2	65.6 ± 0.3	65.4 ± 0.4	
Lakota	65.4 ± 0.3	67.1 ± 0.9	65.1 ± 0.5	
Rouge Vif D'Etampes			68.5 ± 0.4	68.2 ± 0.3
Sweet Mama	65.0 ± 0.3	66.3 ± 0.0	65.9 ± 0.3	66.8 ± 0.2
Warren Scarlet	65.5 ± 0.3	64.0 ± 0.1	66.1 ± 0.4	
Whangaparoa Crown	65.0 ± 0.2		65.1 ± 0.2	

Table 10

Seasonal variation in enthalpy change of thermal transition of retrograded starch and percent retrogradation of squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Enthalpy Change of Thermal Transition (J/g)			
Cultivar	1998	1999	2000	2001
Cha Cha			8.8 ± 0.4	8.6 ± 1.1
Delica	8.1 ± 0.4	7.7 ± 0.1	9.2 ± 0.4	8.9 ± 0.1
Hyvita		7.0 ± 0.3	8.1 ± 0.3	
Kurijiman	7.8 ± 0.3	6.0 ± 0.1	7.5 ± 0.2	
Lakota	8.9 ± 0.4	10.6 ± 2.6	8.3 ± 0.3	
Rouge Vif D'Etampes			9.8 ± 0.6	13.8 ± 1.0
Sweet Mama	9.5 ± 0.3	7.4 ± 0.4	9.0 ± 0.4	9.9 ± 0.9
Warren Scarlet	8.8 ± 0.4	7.0 ± 0.4	8.1 ± 0.4	
Whangaparoa Crown	8.2 ± 0.6		8.3 ± 0.4	

	Percent Retrogradation			
Cultivar	1998	1999	2000	2001
Cha Cha			49.2 ± 2.0	51.8 ± 6.3
Delica	46.6 ± 1.6	46.0 ± 1.5	56.2 ± 2.6	52.6 ± 1.2
Hyvita		39.2 ± 1.7	51.2 ± 2.1	
Kurijiman	45.8 ± 1.4	36.4 ± 0.1	43.5 ± 1.1	
Lakota	53.3 ± 1.7	61.3 ± 16.5	47.2 ± 1.5	
Rouge Vif D'Etampes			59.9 ± 3.9	79.4 ± 7.7
Sweet Mama	54.7 ± 1.3	49.8 ± 7.1	51.2 ± 2.6	65.8 ± 3.2
Warren Scarlet	53.4 ± 3.1	40.6 ± 2.3	55.6 ± 3.7	
Whangaparoa Crown	49.8 ± 1.5		51.1 ± 2.5	

Table 11

Seasonal variation in pasting properties of peak viscosity, breakdown and final viscosity (Rapid Viscoanalyser units (RVU)) of squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Peak Viscosity (RVU)			
Cultivar	1998	1999	2000	2001
Cha Cha			224 ± 5	264 ± 1
Delica	175 ± 11	221 ± 9	179 ± 14	279 ± 3
Hyvita		183 ± 1	192 ± 9	
Kurijiman	179 ± 15	207 ± 3	187 ± 8	
Lakota	233 ± 8	203 ± 0	184 ± 12	
Sweet Mama	206 ± 12	180 ± 1	207 ± 12	197 ± 3
Warren Scarlet	177 ± 7	193 ± 8	184 ± 25	
Whangaparoa Crown	174 ± 10		221 ± 11	

	Breakdown (RVU)			
Cultivar	1998	1999	2000	2001
Cha Cha			64 ± 5	66 ± 4
Delica	52 ± 11	81 ± 4	75 ± 5	111 ± 3
Hyvita		79 ± 9	66 ± 5	
Kurijiman	63 ± 13	78 ± 4	71 ± 8	
Lakota	89 ± 6	83 ± 1	70 ± 3	
Sweet Mama	67 ± 12	47 ± 1	54 ± 13	42 ± 2
Warren Scarlet	53 ± 8	63 ± 9	36 ± 12	
Whangaparoa Crown	65 ± 9		82 ± 12	

	Final Viscosity (RVU)			
Cultivar	1998	1999	2000	2001
Cha Cha			268 ± 6	318 ± 5
Delica	207 ± 24	268 ± 6	163 ± 20	259 ± 0
Hyvita		190 ± 7	218 ± 10	
Kurijiman	195 ± 36	228 ± 8	191 ± 9	
Lakota	244 ± 7	219 ± 1	191 ± 29	
Sweet Mama	232 ± 7	215 ± 1	257 ± 13	240 ± 3
Warren Scarlet	218 ± 10	225 ± 4	236 ± 40	
Whangaparoa Crown	193 ± 9		247 ± 4	

Table 12

Seasonal variation in pasting properties of setback (Rapid Viscoanalyser units (RVU)) and pasting temperature of squash starches from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Setback (RVU)			
Cultivar	1998	1999	2000	2001
Cha Cha			108 ± 5	121 ± 2
Delica	84 ± 10	129 ± 1	60 ± 9	92 ± 1
Hyvita		86 ± 1	92 ± 3	
Kurijiman	79 ± 15	99 ± 1	75 ± 4	
Lakota	100 ± 5	99 ± 0	77 ± 16	
Sweet Mama	93 ± 3	82 ± 0	104 ± 8	86 ± 2
Warren Scarlet	93 ± 6	95 ± 5	88 ± 27	
Whangaparoa Crown	85 ± 8		108 ± 1	

	Pasting Temperature (°C)			
Cultivar	1998	1999	2000	2001
Cha Cha			67.7 ± 0.6	67.5 ± 0.2
Delica	68.8 ± 1.1	64.9 ± 0.1	66.1 ± 0.7	66.2 ± 0.2
Hyvita		67.1 ± 0.2	66.0 ± 0.7	
Kurijiman	67.8 ± 0.6	64.9 ± 0.2	66.9 ± 0.7	
Lakota	67.2 ± 0.1	67.2 ± 0.0	68.6 ± 0.5	
Sweet Mama	68.4 ± 0.7	70.3 ± 0.3	68.0 ± 0.8	67.1 ± 0.2
Warren Scarlet	68.0 ± 0.9	66.0 ± 0.3	73.8 ± 1.5	
Whangaparoa Crown	66.5 ± 0.9		65.9 ± 0.4	

Table 13
Seasonal variation in firmness and stickiness of squash starch gels after 1 or 7 d storage, in which starch was extracted from fruit at harvest grown between 1998 and 2001. Values after ± represent the standard error of the mean.

	Gel Firmness (g) 1 d		
Cultivar	1999	2000	2001
Cha Cha		20.0 ± 1.1	16.8 ± 0.2
Delica	24.2 ± 0.3	16.1 ± 0.5	16.8 ± 0.6
Hyvita	13.4 ± 0.3	18.4 ± 1.1	
Kurijiman	16.4 ± 0.2	18.3 ± 0.7	
Lakota	13.8 ± 0.3	21.7 ± 1.2	
Sweet Mama	18.2 ± 0.9	26.7 ± 2.4	12.6 ± 0.0
Warren Scarlet	15.1 ± 0.2	16.2 ± 1.0	

	Gel Firmness (g) 7 d		
Cultivar	1999	2000	2001
Cha Cha		27.5 ± 2.1	21.3 ± 0.2
Delica	38.9 ± 1.3	20.0 ± 1.6	31.5 ± 0.9
Hyvita	14.7 ± 0.2	25.6 ± 2.1	
Kurijiman	25.7 ± 0.3	23.2 ± 0.6	
Lakota	17.1 ± 0.7	27.5 ± 1.4	
Sweet Mama	23.3 ± 0.4	35.4 ± 0.2	15.5 ± 0.2
Warren Scarlet	18.8 ± 0.4	25.9 ± 0.7	

	Gel Stickiness (g/sec) 1 d		
Cultivar	1999	2000	2001
Cha Cha		-9.7 ± 1.5	-11.4 ± 0.2
Delica	-14.1 ± 4.1	-11.5 ± 2.0	-3.0 ± 0.3
Hyvita	-9.2 ± 2.9	-9.2 ± 1.7	
Kurijiman	-13.7 ± 0.2	-12.4 ± 1.0	
Lakota	-16.0 ± 2.0	-13.3 ± 1.6	
Sweet Mama	-20.8 ± 0.9	-8.8 ± 1.4	-9.4 ± 0.0
Warren Scarlet	-16.5 ± 2.0	-12.7 ± 1.6	

	Gel Stickiness (g/sec) 7 d		
Cultivar	1999	2000	2001
Cha Cha		-15.4 ± 1.0	-18.1 ± 1.4
Delica	-12.1 ± 8.9	-15.7 ± 2.2	-13.2 ± 1.0
Hyvita	-17.2 ± 2.8	-11.3 ± 0.8	
Kurijiman	-16.8 ± 7.4	-18.3 ± 1.1	
Lakota	-33.0 ± 5.3	-18.6 ± 1.2	
Sweet Mama	-39.7 ± 2.6	-11.6 ± 1.3	-15.2 ± 1.6
Warren Scarlet	-20.6 ± 4.2	-13.4 ± 1.2	

CHAPTER 8. SEASONAL VARIATION IN WINTER SQUASH (*Cucurbita maxima* D.) FRUIT. II. VARIATION IN TEXTURE OF RAW AND COOKED FRUIT.

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Abstract Seven winter squash cultivars were grown in Ames, Iowa for 2 or 3 years and textural attributes of hardness, fracturability and springiness of squash fruit were measured using an Instron Universal Testing Machine. Squash fruit were steamed for 0, 2, 5, 10, 15 or 20 min and texture of fruit flesh, with skin excluded, was measured. Hardness, fracturability and springiness showed high seasonal variation for all steaming times. Hardness and fracturability showed greatest seasonal variation for squash fruit steamed 10 min. Springiness of squash fruit exhibited greater seasonal variation when fruit was raw or at short cooking durations. After studying climatic conditions, variation in all three textural attributes among the seasons could be due to differences in rainfall over the entire growing seasons or average daily solar radiation and average daily maximum temperatures at periods during fruit development. Results suggest researchers studying texture of squash should consider taking measurements over several seasons to account for variation.

Keywords winter squash; buttercup squash, *Cucurbita maxima*; texture; seasonal variation;

INTRODUCTION

Texture of buttercup squash is an important attribute influencing consumer preferences in markets such as Japan (Harvey & Grant 1991, Nagao et al. 1991). In order to meet consumer demands, it is important to gain an understanding of the determinants of winter squash texture. Seasonal variation between growing years is one factor that could be influencing the textural attributes of winter squash.

Studies reporting seasonal variation effects on textural attributes are not very common. To our knowledge there have been no previous reports on seasonal variation in the textural attributes of any squash or pumpkin. For fruit crops, similar firmness of apples between two years has been reported (Cliff et al. 1998b), but another study by same author reports crispness of apples to differ between two years (Cliff et al. 1998a). Two cultivars of pear were also reported to have different flesh firmness between two years (Chen et al. 1993). Similar firmness during the first 120 d after fruit set was observed for kiwifruit but the final 40 d after fruit set, prior to harvest, resulted in differences between two seasons (González Rodriguez et al. 1993). Seasonal differences in firmness, and differences in frequency of sensory panelist's textural descriptors (such as soft, mushy, stringy, smooth and mealy) were reported for kiwifruit (Stec et al. 1989). Firmness of strawberries measured using sensory panelists and Instron Universal Testing Machine differed among three growing seasons (Sims et al. 1997).

Seasonal differences in textural attributes have also been reported for vegetable crops. Seasonal differences in texture of seed, cotyledon and testa of green peas among three seasons that spanned six years apart, was reported (Edelenbos et al. 2001). Leaf lettuce grown in spring has been rated by sensory panelists to have softer texture than lettuce grown

in winter (Cuppett et al. 1999), and both green and white asparagus have been reported to have greater fibrousness grown in spring compared to summer (Brovelli et al. 1998). Flouriness of cooked potatoes has been reported to differ among three seasons (O'Beirne & Cassidy 1990), while crispness and firmness of carrots has been reported to increase when carrot plants experience higher temperatures and solar radiation (Rosenfeld et al. 1998).

MATERIALS AND METHODS

Climatic data

Daily mean, maximum and minimum temperatures for Ames between the primary squash cultivation period of May 20 to September 30, from 1998 to 2000 was obtained from Wunderground.com historical weather database and is shown in Chapter 7.

Plant material

In total, seven winter squash (*Cucurbita maxima* D.) cultivars were grown for three seasons, except for one cultivar, between 1998 and 2000 in Iowa. Squash cultivars studied were three buttercups (Delica, Kurijiman and Sweet Mama), one cross between a buttercup, Green Delicious, and a non-buttercup, Table Queen (Hyvita), one Hubbard-type (Warren Scarlet, also known as Red Warren), one Crown-type (Whangaparoa Crown) and one Native American Indian squash (Lakota). Cultivation, field site location, field experimental planting layout design, harvesting, storage and source of seeds has been described previously (Chapter

7). The 1998 and 2000 season squash were cultivated on the same field and the 1999 season squash were cultivated at an adjacent field. All squash fruit were stored for 5 weeks at 12°C prior to textural analysis.

Texture measurements

Four fruit, from each replicate for all seven squash cultivars, were randomly selected as described previously (Chapter 2). Squash fruit were steamed 0 to 20 minutes as described previously (Chapter 4). Texture measurements of raw and cooked fruit flesh, excluding the skin, were carried out as described previously (Chapter 4) using the Instron Universal Testing Machine (Instron Corp., Canton, MA). Measurements of hardness, fracturability and springiness were made by using Series 12 software (Instron Corp., Canton, MA) based on calculations described by Szczesniak (1963) and Bourne (1968).

RESULTS AND DISCUSSION

Ames seasonal climatic variance

Climatic conditions in Ames, Iowa during the squash growing seasons from 1998 to 2000 have been mentioned previously (Chapter 7).

Hardness of fruit at short duration steaming times

Hardness of raw squash fruit, and fruit steamed for 2 or 5 min is shown in Table 1 for all squash cultivars during each of the three seasons. Raw fruit of Hyvita did not vary in hardness between 1999 and 2000 seasons, but all other squash cultivars had at least two seasons in which taking into consideration their standard error of the mean, were considerably different in hardness. Delica raw fruit exhibited the greatest variation among seasons as hardness was not similar in any of the three seasons. Seasonal variation for the hardness of squash cultivar raw fruit did not show any consistent trend between seasons

making it difficult to attribute any of the variation in texture to the climatic variation over the three seasons.

Squash fruit steamed 2 min has similar trends to raw fruit. Hyvita was once again the only cultivar that had no variation in hardness between seasons and Delica was the only cultivar to have different hardness for all three seasons. Hardness of squash fruit after 5 min resulted in substantial differences in which cultivars varied. Hyvita, like when raw or steamed 2 min, had no variation between growing seasons, but Delica also had no significant differences in hardness among the three seasons for fruit steamed 5 min. Whangaparoa Crown showed no variation between 1998 and 2000 season for hardness of raw fruit and fruit steamed 2 min, but had substantial differences after 5 min steaming.

Hardness of fruit at longer steaming durations

Seasonal variation in hardness of squash fruit steamed 10, 15 or 20 min for each cultivar is shown in Table 2. Large differences between cultivars were observed for the 1998 and 2000 season for hardness of fruit steamed 10 min, but differences were much less for the 1999 season. Additionally, some cultivars had large differences in hardness among the three seasons. Relative to other cultivars, the buttercup cultivars Delica and Kurijiman, and the close genetic relative, Hyvita, had low hardness after fruit were steamed for 10 min for all three seasons but differences among seasons were still observed. Lakota, Sweet Mama, Red Warren and Whangaparoa Crown all had greater than two-fold magnitude difference among growing seasons for fruit steamed 10 min.

Cultivar differences in hardness of squash fruit steamed 15 min was much less than for fruit steamed 10 min, but seasonal variation was still observed for most cultivars. Hyvita was the only squash cultivar having no seasonal variation in hardness of fruit steamed 15

min. Whangaparoa Crown had the greatest seasonal variation in hardness of fruit steamed 15 min, with squash grown in 1998 at least eight times harder than fruit from the 1999 season. Lakota and Warren Scarlet also showed at least two-fold magnitude difference in hardness among seasons when fruit were steamed 15 min. Overall variation of squash cultivar hardness among the three season was reduced after fruit were steamed 20 min with all hardness values ranging between 8 and 50 N and standard error of the mean for each cultivar was generally low. Despite this, only Lakota did not have any seasonal variation in hardness of fruit steamed 20 min. Measurements of squash hardness over the three seasons have demonstrated that variation of raw and cooked fruit is substantially large enough that future studies investigating the texture of winter squash should consider analysis over more than one growing season to obtain values of textural attributes that more accurately reflect the typical fruit texture.

Fracturability of fruit at short duration steaming times

Seasonal variation in fracturability of raw fruit, and fruit steamed for 2 or 5 min, for all squash cultivars is shown in Table 3. Unlike for hardness of raw fruit, fracturability of uncooked fruit showed a clear consistent seasonal effect for all squash cultivars. Force required to fracture raw fruit grown in the 2000 season was higher for all squash cultivars than for the other two seasons. The most obvious seasonal difference between the 2000 season and the combination of 1998 and 1999 season is rainfall. Rainfall during the growing season, from May 20 to September 30, was 525 mm in 1998, 547 mm in 1999, but only 234 mm in 2000 (Chapter 7). Surprisingly, despite the considerably lower rainfall during the entire growing season, solar radiation from July1 to September 30, when fruit development is occurring, was lower for 2000 season. Mean daily solar radiation between July 1 and

September 30 in 1998 was 471 langleys, in 1999 was 475 langleys and in 2000 was 443 langleys (Chapter 7). Higher solar radiation has been reported to result in increased chemical composition of carrots (Rosenfeld et al. 1998). We have already established previously that starch, the principal storage carbohydrate of winter squash, is highly correlated to the hardness of squash fruit (Chapter 5). There were no clear differences in temperature throughout the growing season for the three season, but temperatures between August 1 to 15 were considerably hotter in 2000 than for the other two seasons. Softer leaf lettuce has been reported when grown in winter compared to spring (Cuppett et al. 1999). Further research would be needed to determine if rainfall, solar radiation or temperatures during fruit development are critical in determining the fracturability of raw fruit.

Fracturability of fruit steamed 2 min did not show the same clear seasonal effect of raw fruit, but most cultivars had higher fracturability in the 2000 season. Large differences in force required to fracture fruit steamed 2 min was observed for Delica, Kurijiman, Warren Scarlet and Whangaparoa Crown. Seasonal variation in fracturability of fruit steamed 5 min differed from fruit steamed 2 min. Fracturability of Delica was not different among the three seasons for fruit steamed 5 min, but all other cultivars had seasonal variation. Apart from Warren Scarlet, all other squash cultivars had lower fracturability of fruit steamed 5 min and grown in 1999 season compared with the 1998 season. During the last one and half months of fruit development, squash grown in 1998 experienced considerably higher maximum daily temperatures (mean of 28.3°C) than squash grown in 1999 season (mean of 25.0°C, Chapter 7), which may contribute to changes in fruit composition that influence the fracturability of fruit during cooking. Increased crispness of carrots grown in growth chambers with elevated temperatures has been previously reported (Rosenfeld et al. 1998). Lower temperatures have

been reported to result in greater fibrousness of asparagus (Brovelli et al. 1998). Fibrousness is a textural attribute of winter squash described by sensory panelists (Corrigan et al. 2001, Cumarasamy et al. 2002) but it is difficult to determine if fibres would influence hardness or fracturability.

Fracturability of fruit at longer steaming durations

Squash cultivar seasonal variation in fracturability of fruit steamed for 10, 15 or 20 min is shown in Table 4. Similar to hardness measurements, dramatic seasonal variation in fracturability of fruit steamed 10 min was observed. The two buttercup cultivars, Delica and Kurijiman, plus the close genetic relative, Hyvita, all had no seasonal variation in fracturability of fruit steamed 10 min. However, the remaining buttercup cultivar, Sweet Mama, had large seasonal variation with fracturability of fruit steamed for 10 min thirteen times higher in 1998 season than in the other two seasons. Lakota and Whangaparoa Crown also showed large seasonal variation, with both cultivars requiring at least eight times greater force to fracture fruit steamed 10 min and grown in 1998 season compared with 1999 season.

Once squash fruit was steamed for 15 min, seasonal variation in fracturability was greatly reduced compared with fruit steamed for 10 min, but seasonal variation was still observed. Similar to fruit steamed 10 min, no seasonal variation was observed for Delica, Hyvita and Kurijiman fruit steamed for 15 min. Lakota, Sweet Mama and Whangaparoa Crown had greater than two-fold magnitude difference in force required to fracture fruit steamed 15 min grown in 1998 compared with 1999 season, and fruit steamed 20 min showed similar trend for all three cultivars. Delica and Hyvita fruit steamed for 20 min had no seasonal variation in fracturability. Although differences in fracturability of Kurijiman and Warren Scarlet steamed for 20 min were small, the differences were significant when

taking into consideration their standard error of the mean. Measurements of fracturability indicate future studies interested in investigating fracturability of fruit should consider reporting this textural attribute from fruit grown more than one season to obtain values that reflect typical squash fracturability.

Springiness of fruit at short steaming duration times

Seasonal variation in springiness of raw fruit, and fruit steamed 2 or 5 min for the squash cultivars is shown in Table 5. Similar to observations of fracturability, raw fruit showed a seasonal effect in springiness with all cultivars springier in the 1999 and 2000 seasons compared with 1998 season although Warren Scarlet was not significantly different. Delica, Hyvita, Kurijiman, Warren Scarlet and Whangaparoa Crown all had very similar springiness of raw fruit for the 1999 and 2000 seasons. Since rainfall and solar radiation were similar for most of the fruit development duration in 1998 and 1999, and both seasons were considerably different from 2000 season, these environmental factors are unlikely to be contributing to the lower springiness for squash grown in 1998. Squash grown in 1998 did experience warmer average daily maximum temperatures during the last one and a half months of fruit development (28.3°C) than 1999 (25.0°C) and 2000 (27.2°C) seasons (Chapter 7). In particular average daily maximum temperature between September 15 to 30, the later stages of fruit development, was 4°C higher in 1998 than the other two seasons (Chapter 7).

Springiness of fruit steamed 2 min had no variation for all squash cultivars between 1999 and 2000 seasons, but all cultivars except Warren Scarlet had lower springiness for squash grown in 1998. When squash fruit was steamed for 5 min, Hyvita and Whangaparoa Crown had variation in springiness between 1999 and 2000 season, but all other cultivars had

no variation between these two seasons. Springiness of fruit from all cultivars steamed 5 min was lower in 1998 season than the other two seasons.

Springiness of fruit at longer steaming durations

Variation in springiness of squash fruit among the three seasons that was steamed for 10, 15 or 20 min for all squash cultivars is shown in Table 6. Compared with the shorter steaming durations, changes in seasonal variation for springiness of fruit started to emerge after fruit were steamed 10 min. Kurijiman, which was less springy for fruit steamed 0 to 5 min and grown in 1998 compared with other seasons, showed no seasonal variation in fruit springiness among all three seasons for fruit steamed 10 min. All cultivars except Kurijiman had seasonal variation in springiness of fruit steamed 10 min with Lakota and Whangaparoa Crown fruit substantially springier grown in 1999 and 2000 compared with 1998 season.

Lower springiness for the 1998 season observed for squash fruit steamed 10 min or less was a trend that was not found for fruit steamed 15 min. Kurijiman, Warren Scarlet and Whangaparoa Crown all had no difference in springiness of fruit steamed 15 min for the 1998 season compared with 1999 season. Kurijiman, Sweet Mama and Warren Scarlet also had no variation in springiness of fruit steamed 15 min for the 1998 season compared with 2000 season. Hyvita, Sweet Mama and Whangaparoa Crown had differences in springiness of fruit steamed 15 min between 1999 and 2000 seasons but the trend was not consistent for all three cultivars. For fruit steamed 20 min, springiness of Hyvita, Lakota, Warren Scarlet and Whangaparoa Crown was very similar between 1999 and 2000 season. Sweet Mama was also similar for these two seasons, largely because of a large range in its standard error of the mean. Apart from Warren Scarlet which had no variation among the three seasons, squash fruit steamed 20 min was less springy for squash cultivar fruit grown in the 1998

season, compared with 1999 season, and this may be due to differences in temperature during fruit development mentioned for springiness of fruit at the shorter cooking times.

Our results show that hardness, fracturability and springiness of winter squash fruit can vary greatly between growing seasons and this variation in textural attributes is likely to be observed throughout the entire cooking process. Therefore, future research on the texture of winter squash should seriously consider evaluating texture over at least two seasons to account for seasonal variation and obtain textural measurements that more accurately reflect the characteristics of individual squash cultivars.

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Table 1 Seasonal variation in hardness of winter squash fruit raw and steamed for 2 or 5 min. Values after ± represent the standard error of the mean.

	Hardness (N) raw		
Cultivar	1998	1999	2000
Delica	536 ± 52	825 ± 9	943 ± 45
Hyvita		487 ± 26	454 ± 25
Kurijiman	773 ± 93	955 ± 175	1012 ± 52
Lakota	876 ± 15	699 ± 42	769 ± 35
Sweet Mama	855 ± 162	616 ± 72	730 ± 38
Warren Scarlet	355 ± 92	477 ± 95	625 ± 47
Whangaparoa Crown	553 ± 39	404 ± 59	554 ± 30

	Hardness (N) steamed 2 min		
Cultivar	1998	1999	2000
Delica	398 ± 16	790 ± 64	850 ± 55
Hyvita		388 ± 36	417 ± 25
Kurijiman	583 ± 37	764 ± 101	873 ± 50
Lakota	980 ± 71	713 ± 36	763 ± 30
Sweet Mama	823 ± 51	476 ± 51	640 ± 26
Warren Scarlet	385 ± 59	357 ± 62	633 ± 55
Whangaparoa Crown	577 ± 50	398 ± 55	544 ± 63

	Hardness (N) steamed 5 min		
Cultivar	1998	1999	2000
Delica	331 ± 46	364 ± 27	418 ± 64
Hyvita		236 ± 15	245 ± 36
Kurijiman	354 ± 39	511 ± 55	404 ± 42
Lakota	807 ± 23	462 ± 89	594 ± 35
Sweet Mama	674 ± 144	383 ± 82	397 ± 46
Warren Scarlet	335 ± 49	298 ± 93	494 ± 63
Whangaparoa Crown	269 ± 10	305 ± 62	448 ± 42

Table 2 Seasonal variation in hardness of winter squash fruit steamed for 10, 15 or 20 min. Values after ± represent the standard error of the mean.

	Hardness (N) steamed 10 min		
Cultivar	1998	1999	2000
Delica	23 ± 3	46 ± 9	57 ± 3
Hyvita		32 ± 2	45 ± 9
Kurijiman	39 ± 9	46 ± 10	66 ± 10
Lakota	415 ± 138	107 ± 37	266 ± 26
Sweet Mama	427 ± 77	46 ± 37	43 ± 6
Warren Scarlet	49 ± 19	94 ± 43	196 ± 58
Whangaparoa Crown	229 ± 50	77 ± 11	423 ± 77

	Hardness (N) steamed 15 min		
Cultivar	1998	1999	2000
Delica	13 ± 1	26 ± 3	30 ± 2
Hyvita		17 ± 1	19 ± 2
Kurijiman	17 ± 3	23 ± 5	31 ± 2
Lakota	82 ± 8	30 ± 3	73 ± 9
Sweet Mama	38 ± 4	16 ± 2	22 ± 2
Warren Scarlet	15 ± 4	14 ± 1	43 ± 11
Whangaparoa Crown	192 ± 72	14 ± 1	94 ± 31

	Hardness (N) steamed 20 min		
Cultivar	1998	1999	2000
Delica	10 ± 1	18 ± 1	23 ± 2
Hyvita		12 ± 0	14 ± 1
Kurijiman	16 ± 2	15 ± 2	23 ± 2
Lakota	23 ± 2	22 ± 3	25 ± 2
Sweet Mama	21 ± 4	11 ± 2	15 ± 1
Warren Scarlet	12 ± 2	8 ± 1	19 ± 2
Whangaparoa Crown	49 ± 22	11 ± 1	23 ± 3

Table 3 Seasonal variation in fracturability of winter squash fruit raw and steamed for 2 or 5 min. Values after ± represent the standard error of the mean.

	Fracturability (N) raw		
Cultivar	1998	1999	2000
Delica	425 ± 29	735 ± 24	829 ± 62
Hyvita		447 ± 18	512 ± 25
Kurijiman	687 ± 69	826 ± 177	898 ± 55
Lakota	522 ± 19	576 ± 45	806 ± 46
Sweet Mama	650 ± 107	540 ± 92	852 ± 71
Warren Scarlet	223 ± 63	325 ± 70	693 ± 104
Whangaparoa Crown	397 ± 34	339 ± 50	660 ± 60

	Fracturability (N) steamed 2 min		
Cultivar	1998	1999	2000
Delica	285 ± 20	646 ± 50	635 ± 85
Hyvita		388 ± 36	424 ± 41
Kurijiman	401 ± 54	728 ± 94	710 ± 74
Lakota	585 ± 12	450 ± 61	821 ± 54
Sweet Mama	654 ± 97	476 ± 51	657 ± 41
Warren Scarlet	208 ± 50	250 ± 19	509 ± 90
Whangaparoa Crown	373 ± 30	344 ± 41	605 ± 55

	Fracturability (N) steamed 5 min		
Cultivar	1998	1999	2000
Delica	234 ± 22	212 ± 60	194 ± 65
Hyvita		63 ± 24	132 ± 47
Kurijiman	310 ± 56	250 ± 95	187 ± 55
Lakota	702 ± 2	294 ± 87	641 ± 44
Sweet Mama	503 ± 96	305 ± 59	204 ± 66
Warren Scarlet	204 ± 58	247 ± 67	400 ± 108
Whangaparoa Crown	269 ± 10	175 ± 18	407 ± 56

Table 4 Seasonal variation in fracturability of winter squash fruit steamed for 10, 15 or 20 min. Values after ± represent the standard error of the mean.

	Fracturability (N) steamed 10 min		
Cultivar	1998	1999	2000
Delica	18 ± 2	16 ± 1	15 ± 1
Hyvita		15 ± 1	15 ± 1
Kurijiman	22 ± 7	19 ± 4	18 ± 2
Lakota	227 ± 62	18 ± 2	279 ± 76
Sweet Mama	282 ± 52	14 ± 2	17 ± 1
Warren Scarlet	118 ± 87	49 ± 29	87 ± 49
Whangaparoa Crown	229 ± 50	17 ± 2	131 ± 76

	Fracturability (N) steamed 15 min		
Cultivar	1998	1999	2000
Delica	8 ± 0	8 ± 2	6 ± 1
Hyvita		7 ± 1	6 ± 1
Kurijiman	6 ± 1	5 ± 2	7 ± 1
Lakota	58 ± 13	14 ± 2	42 ± 19
Sweet Mama	29 ± 5	4 ± 2	7 ± 1
Warren Scarlet	10 ± 4	5 ± 1	14 ± 5
Whangaparoa Crown	92 ± 38	7 ± 1	17 ± 4

	Fracturability (N) steamed 20 min		
Cultivar	1998	1999	2000
Delica	4 ± 0	3 ± 0	3 ± 0
Hyvita		2 ± 1	4 ± 1
Kurijiman	5 ± 1	3 ± 0	3 ± 1
Lakota	17 ± 1	9 ± 2	11 ± 1
Sweet Mama	13 ± 2	3 ± 1	3 ± 1
Warren Scarlet	5 ± 1	2 ± 0	6 ± 1
Whangaparoa Crown	37 ± 16	3 ± 1	10 ± 1

Table 5 Seasonal variation in springiness of winter squash fruit raw and steamed for 2 or 5 min. Values after ± represent the standard error of the mean.

	Springiness (mm) raw		
Cultivar	1998	1999	2000
Delica	11.34 ± 0.18	12.20 ± 0.10	12.19 ± 0.05
Hyvita		12.43 ± 0.09	12.41 ± 0.04
Kurijiman	11.29 ± 0.10	11.70 ± 0.38	11.71 ± 0.15
Lakota	11.35 ± 0.13	11.92 ± 0.16	12.32 ± 0.05
Sweet Mama	11.42 ± 0.18	12.38 ± 0.05	12.06 ± 0.14
Warren Scarlet	11.78 ± 0.25	11.91 ± 0.23	11.98 ± 0.15
Whangaparoa Crown	11.55 ± 0.05	12.63 ± 0.07	12.60 ± 0.04

	Springiness (mm) steamed 2 min		
Cultivar	1998	1999	2000
Delica	11.75 ± 0.06	12.25 ± 0.11	12.31 ± 0.02
Hyvita		12.58 ± 0.01	12.49 ± 0.08
Kurijiman	11.61 ± 0.06	11.92 ± 0.22	11.98 ± 0.06
Lakota	10.43 ± 0.54	12.39 ± 0.04	12.25 ± 0.14
Sweet Mama	11.43 ± 0.23	12.49 ± 0.09	12.33 ± 0.10
Warren Scarlet	11.99 ± 0.21	12.06 ± 0.26	12.07 ± 0.19
Whangaparoa Crown	11.55 ± 0.09	12.62 ± 0.08	12.56 ± 0.05

	Springiness (mm) steamed 5 min		
Cultivar	1998	1999	2000
Delica	11.87 ± 0.15	12.67 ± 0.05	12.60 ± 0.09
Hyvita		12.64 ± 0.03	12.95 ± 0.05
Kurijiman	11.87 ± 0.06	12.50 ± 0.14	12.32 ± 0.10
Lakota	11.53 ± 0.07	12.71 ± 0.14	12.70 ± 0.03
Sweet Mama	11.65 ± 0.28	12.78 ± 0.12	12.53 ± 0.12
Warren Scarlet	11.84 ± 0.17	12.38 ± 0.13	12.39 ± 0.17
Whangaparoa Crown	11.79 ± 0.09	13.05 ± 0.07	12.91 ± 0.06

Table 6 Seasonal variation in springiness of winter squash fruit steamed for 10, 15 or 20 min. Values after ± represent the standard error of the mean.

	Springiness (mm) steamed 10 min		
Cultivar	1998	1999	2000
Delica	12.22 ± 0.10	12.65 ± 0.03	12.48 ± 0.03
Hyvita		12.62 ± 0.03	12.87 ± 0.03
Kurijiman	12.17 ± 0.18	12.31 ± 0.18	12.12 ± 0.18
Lakota	12.38 ± 0.01	13.42 ± 0.17	13.19 ± 0.06
Sweet Mama	12.32 ± 0.13	12.97 ± 0.14	12.48 ± 0.03
Warren Scarlet	12.57 ± 0.10	12.94 ± 0.05	12.87 ± 0.08
Whangaparoa Crown	12.32 ± 0.07	13.02 ± 0.12	13.27 ± 0.05

	Springiness (mm) steamed 15 min		
Cultivar	1998	1999	2000
Delica	11.83 ± 0.00	12.49 ± 0.11	12.40 ± 0.14
Hyvita		12.37 ± 0.07	12.62 ± 0.05
Kurijiman	12.25 ± 0.15	12.11 ± 0.32	12.05 ± 0.15
Lakota	12.16 ± 0.08	12.83 ± 0.10	12.99 ± 0.12
Sweet Mama	11.97 ± 0.08	12.57 ± 0.07	12.21 ± 0.02
Warren Scarlet	12.60 ± 0.08	12.70 ± 0.14	12.48 ± 0.19
Whangaparoa Crown	12.50 ± 0.04	12.43 ± 0.39	13.14 ± 0.05

	Springiness (mm) steamed 20 min		
Cultivar	1998	1999	2000
Delica	12.17 ± 0.00	12.75 ± 0.14	12.53 ± 0.23
Hyvita		12.67 ± 0.12	12.77 ± 0.05
Kurijiman	12.42 ± 0.07	12.73 ± 0.09	12.36 ± 0.17
Lakota	12.00 ± 0.24	12.77 ± 0.21	12.78 ± 0.21
Sweet Mama	12.32 ± 0.16	12.68 ± 0.34	12.43 ± 0.24
Warren Scarlet	12.51 ± 0.10	12.46 ± 0.22	12.46 ± 0.24
Whangaparoa Crown	12.58 ± 0.05	13.13 ± 0.05	13.13 ± 0.03

CHAPTER 9. VARIATION IN AGRONOMIC TRAITS, STARCH STRUCTURAL PROPERTIES, STARCH FUNCTIONAL PROPERTIES AND TEXTURAL ATTRIBUTES OF *Cucurbita maxima* D. cv. Zapallo Macre WINTER SQUASH FRUIT.

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ABSTRACT

Zapallo Macre squash were found to vary dramatically in fruit size, shape and colour. At least eight phenotypes were identified and five were selected for detailed study of their starch structural and functional properties, as well as their textural attributes raw and steamed up to 20 min. The five phenotypes varied in fruit weight, water content, starch content, apparent and absolute amylose content, amylopectin molecular weight, amylopectin polydispersity, amylopectin gyration radius and amylopectin branch chain-length distribution. The five phenotypes also varied in starch gelatinisation temperatures, starch pasting properties and gel properties. Hardness, fracturability and springiness of fruit varied among the phenotypes at all cooking times. Commercialisation of the starches in Zapallo Macre fruit may be limited due to the high variability in its properties. The large difference in starch accumulation between fruit on same plant could make Zapallo Macre a useful model crop to study starch biosynthesis.

Key words: Zapallo Macre, winter squash, pumpkin, *Cucurbita maxima*, starch structure, starch function, texture.

INTRODUCTION

The genus *Cucurbita* is a great untapped resource of squash and pumpkins which may produce fruit with unusual compounds of commercial benefit. Currently, less than half of one percent of all squash and pumpkins have had any research conducted (Nayar and Moré 1998). However, crops that have received no plant breeding have a propensity for large variation in phenotype and composition. The many squash cultivars that are loosely referred to as Zapallo have been reported to vary in fruit shape (Lizana and Monardes 1978).

In this study we investigate in detail the starch structural properties, starch functional properties, and textural attributes of raw and cooked squash fruit of *Cucurbita maxima* D. cv. Zapallo Macre phenotypes to evaluate the economic importance of this accession line.

EXPERIMENTAL

Plant material

Cucurbita maxima D. cv. Zapallo Macre seeds were planted along with eleven other winter squash cultivars in summer, 2000, at an Iowa State University farm site 1.7 miles south of Ames, Iowa (geographical location 41° 58' 57.5" N, 93° 38' 22.9"), in a randomized complete block (8.23 m x 3.05 m blocks) with 3 replicates (36 plants/replicate). Normal crop husbandry was followed as required. Zapallo Macre, according to USDA Plant Introduction database, originates from the Bolivian/Peruvian border. Seeds were obtained from the USDA, ARS Plant Genetic Resources Unit, Cornell University, Geneva, NY with the accession number being PI 298818. Squash fruit maturity was adjudged when stalks became woody (Hawthorne 1990), and this stage had been previously shown to have the highest starch content (Irving *et al* 1997).

Starch isolation and quantification, and water content

Starch was isolated from squash fruit using method reported by Badenhuizen (1964) with slight modification (Kasemsuwan *et al* 1995) and further modification as described previously (Chapter 2). Due to the drastic variation in fruit weight, shape and colour, fruit were separated into eight categories (types) and two fruit for each of five different types were selected for each of three replicates, peeled and deseeded, and used for starch isolation. Specific information about the five types selected for starch isolation is mentioned in the results section and in Fig 1. Squash fruit was ground through a meat grinder ("The Butcher Shop", item#402, Krups North America Inc., Peoria, IL), due to its hardness, and immediately blended in 0.3% (w/v) sodium metabisulfite and then filtered through 106 μm mesh. Filtrate was washed with 10% toluene in 0.1 M NaCl. Toluene/salt washed starch was washed three times with distilled water, twice with ethanol, and then recovered by filtration using Whatman No. 4 filter paper. Purified starch cake was dried in a convection oven at 35°C for 48 h. Starch yields varied among individual fruit, therefore results presented in this study are for the fruit in which sufficient starch was present to conduct analysis. Water content of squash fruit, with skins and seeds removed, was determined by freeze-drying. Total starch content of freeze-dried squash fruit powders, measured in duplicate, was determined using total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), based on AOAC method 996.11, AACC method 76.13 and ICC standard method No. 168, in which fruit powders are hydrolyzed with α-amylase and amyloglucosidase, and subsequent glucose content determined using glucose oxidase-

peroxidase. Internal standards of corn starch were added to samples to check quantitation and recovery of starch.

Molecular weight distribution and gyration radius of amylopectin by high-performance size-exclusion chromatography (HPSEC)

Weight-average molecular weight and *z*-average gyration radius of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). Starch samples, duplicate measurements of each replicate for all fruit types, were prepared as described by Yoo and Jane (2002a). The HPSEC system consisted of a HP 1050 series isocratic pump (Hewlett Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., Tokyo, Japan) were used. Operating conditions and data analysis are described by Yoo and Jane (2002b), except flow rate was 0.4 mL/min and sample concentration was 0.8 mg/mL.

Absolute amylose contents by potentiometric autotitration Absolute amylose content of starch was determined following the procedure of Lu et al. (1996). Analysis was based on iodine affinities of defatted whole starch and amylopectin fraction using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). Starch samples were

defatted using a 90% dimethyl sulfoxide (DMSO) solution, followed by alcohol precipitation. Determination of amylose content was duplicated for each squash fruit type for each replicate.

Amylopectin branch chain-length distribution by high-performance anion-exchange chromatography (HPAEC)

Amylopectin was fractionated by complexing amylose with *n*-butanol as described by Schoch (1942). Amylopectin (2 mg/mL) was defatted in boiling 90% DMSO for 1 h, followed by stirring for 24 h and then debranched using isoamylase (EC 3.2.1.68 from *Pseudomonas amyloderamosa*) (EN102, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) as described by Jane and Chen (1992). Branch chain-length distribution of amylopectin was determined using an HPAEC system (Dionex-300, Sunnyvale, CA) equipped with an amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, A-7255, Sigma Chemical Co., St Louis, MO) post-column, on-line reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Wong and Jane 1997). PA-100 anion exchange analytical column (250 x 4 mm, Dionex, Sunnyvale, CA) and a guard column were used for separating debranched amylopectin samples. Gradient profile of eluents and operating conditions were described previously (McPherson and Jane 1999). Branch chain-length distribution of amylopectin was also analyzed to determine extra-long branch-chains by using a HPSEC equipped with a RI detector. Operating conditions have been described earlier (McPherson and Jane 1999), except flow rate was 0.4 mL/min, analytical column used for analysis was Shodex OH pak SB-803HQ (Showa Denko K.K., Tokyo, Japan) and sample concentration

was 0.8 mg/mL. HPAEC-ENZ-PAD and HPSEC analysis were duplicated for each replicate of all fruit types.

Starch thermal properties by differential scanning calorimetry (DSC)

Thermal properties of starch were determined using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) (Jane *et al* 1999). Approximately 2 mg of starch was weighed in an aluminum pan, mixed with 6 mg of deionized water and sealed. Sample was allowed to equilibrate for 2 h and scanned at a rate of 10°C/min over a temperature range of 10-100°C. An empty pan was used as the reference. Rate of starch retrogradation was determined using the same gelatinized samples, stored at 4°C for 7 d, and analyzed using DSC as described previously (White *et al* 1989). All thermal properties were carried out in triplicate for each replicate of each fruit type.

Starch pasting properties by Rapid Visco-Analyser (RVA) and gel properties. Starch pasting properties were analyzed using a Rapid Visco-Analyser (RVA-4, Newport Scientific, Sydney, Australia) (Jane *et al* 1999). Starch suspension (8%, w/w), in duplicate for each replicate of each fruit type was prepared by weighing starch (2.24 g, dry starch basis (dsb)) into a RVA canister and making up the total weight to 28 g with distilled water. Starch suspension was equilibrated at 30°C for 1 min, heated at a rate of 6.0°C/min to 95°C, maintained at that temperature for 5.5 min, and then cooled to 50°C at a rate of 6.0°C/min. Constant paddle rotating speed (160 rpm) was used throughout entire analysis. Immediately after the completion of the RVA sample run, the spindle was removed, and the canister was wrapped in several layers of Saran® wrap, to minimize dehydration, and placed in a refrigerator at 4°C. After 1 or 7 d, canisters were removed from the refrigerator, equilibrated to room temperature, and gel firmness and stickiness were measured using a Stable Micro

Systems TAXT2*i* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with Texture Expert for Windows software (v 1.22). Each gel was measured five times by using a 4 mm diameter cylindrical stainless steel punch probe (TA54). Pretest speed was 2.0 mm/s, and gels were compressed at a test speed of 0.9 mm/s and a penetration test distance of 7.5 mm. Peak force of the curve was reported as the firmness of gels and stickiness of gels was defined as the negative load portion of the curve as described previously (Takahashi and Seib 1988).

Steaming of squash fruit and texture analysis. Two fruit, from each of the three replicates for all five Zapallo Macre fruit types, were randomly selected as described previously (Chapter 2). All fruit skins were marked into quarters, and one quarter was randomly selected for texture analysis. From this quarter, six 3-cm wide at the equator, longitudinal segments were used for texture analysis. Depending on fruit size, allocation of segments for texture may be greater than one quarter of the fruit's circumference. Therefore, approximately two-thirds to three-quarters was randomly allocated for isolation of starch and the remainder for textural analysis, and the groundspot (part of squash fruit with skin discoloration due to contact with ground) was not excluded from the textural analysis. Squash fruit longitudinal segments, with skins remaining, were randomly selected to be steamed in a 10-cup size rice steamer (Zojirushi America Corporation, Commerce, CA, model NHS18) for 0, 2, 5, 10, 15 or 20 min. The plane of the squash fruit skin was perpendicular to the water surface, so that the pieces of fruit did not impede heat flow. After each segment was steamed for its specified time, a 20 mm diameter apple corer (Oxo brand, BASF Corp., Mount Olive, NJ) with a recessed cutting edge, preventing further compression, was used to immediately remove a fruit cylinder, cut from the direction of seed cavity to

skin. The fruit cylinder was then placed in a metal cylinder with cut grooves 12 mm apart, allowing a sliced fruit cylinder to have flat surfaces with height of 12 mm and width of 20 mm, and skin excluded. Sliced fruit cylinders were placed on the base plate of the Instron Universal Testing Machine (Instron Corp., Canton, MA) with the side closest to skin against the base plate. The texture profile analysis was started exactly 40 seconds after removal of squash fruit from the steamer. Texture profile analysis of squash cylinders involved a two-cycle compression, with 75% compression of their original height, using an Instron Universal Testing Machine. Compression, using the 57 mm compression anvil (Instron part 2830-009), was at a crosshead speed of 30 mm/min. Measurements of hardness, fracturability and springiness were made by using Series 12 software (Instron Corp., Canton, MA) based on calculations described by Szczesniak (1963) and Bourne (1968). Hardness was defined as the maximum force of the first compression cycle that was not associated with the first fracture, unless the sample experienced first fracture at end of the first compression. Fracturability is the force at which material fractures in the first peak (compression force decreases). Springiness is the height that food recovers during the time that elapses between end of the first compression peak and start of the second compression peak.

RESULTS

Agronomic trait differences

Zapallo Macre seeds received from the Plant Introduction Station at USDA, Geneva, NY, all appeared to be identical in colour and size and although not measured, we suspect there was little variation in seed weight. Zapallo Macre accession line was reported by the USDA to have 100% germination and our observations of over 75 seeds confirm this finding. All Zapallo Macre squash plants growing in field appeared to have similar leaf morphology,

vining tendencies and flower morphology. Therefore prior to fruit development, no indication was evident that the fruit would vary drastically in phenotype. At harvest, we observed at least eight distinctive types of fruit based on startling differences in fruit size, shape and colour, in which some of this diversity is illustrated in Fig 1. Five of these eight types were selected for studies of their starch structure, starch functional properties and fruit textural attributes because there was greater occurrence of these Zapallo Macre fruit types. Descriptions of the five types are: Type I = very large, oval, grey to white skin with unusual fluorescent-lime green/yellow internal flesh; Type II = large, round to oval, variegated green skin fruit with bright yellow internal flesh; Type III = small to medium size, round, ribbed, grey-skin fruit with bright orange internal flesh; Type IV = small, oval, variegated orange/green-skin fruit with bright orange/yellow internal flesh; Type V = large, oblong, pink skin fruit with yellow/orange internal flesh. Illustration of the fruit interior for all five Zapallo Macre types selected is shown in Fig 2a-c. Average fruit weight for the five Zapallo Macre types is shown in Fig 1. Type I was significantly heavier than all other four types. Although we could not be absolutely certain, from the best that we could ascertain following individual vines, the lightest fruit (Type IV) were found on the same plants that produced the heaviest fruit (Type I). Type IV fruit, based on standard error of the mean, were considerably lighter than Type I and V.

Water and starch content

Water and starch content (dry weight basis) of Zapallo Macre squash fruit is shown in Fig 1. Type IV fruit had the highest starch content and lowest water content of the five Zapallo Macre fruit types and based on standard error of the means, appeared to be different from the four other types. Type II had lower water content than Type I. All five Zapallo Macre fruit

types starch content was different from each other based on their standard error of the mean. Dramatic differences in starch content were observed, with Type IV squash fruit having over five times greater starch content than Type V. When considering all eight types found, even greater differences in starch content were observed because two of the three fruit types not selected for further study did not accumulate any starch. These fruit that lacked any starch accumulation were found on the same plants that had fruit with greater than 40% of their dry matter as starch.

Amylose content

The iodine affinities and amylose contents of Zapallo Macre fruit are shown in Table 2. Type IV had higher iodine affinity of the whole starch and the associated apparent amylose content than Type II, III and V. Absolute amylose content was similar but Type IV was lower than Type I and V.

Amylopectin molecular size, polydispersity and gyration radius

Amylopectin molecular size, polydispersity and gyration radius for the Zapallo Macre squash fruit type starches are shown in Table 3. Type I and II starch had amylopectin with lower weight-average molecular weight than Type III, IV and V. Amylopectin polydispersity was higher for Type II than all four other types. Type IV starch exhibited extremely low amylopectin polydispersity and was lower than the four other types. Differences were also observed in amylopectin gyration radius among the starches from the five Zapallo Macre fruit types, with Type III amylopectin molecules wider than all four other types. Type I amylopectin molecules were narrower than all four other types.

Amylopectin branch chain-length distribution

Amylopectin branch chain-length distribution for starches from the five Zapallo Macre fruit types is shown in Table 4. Type V fruit starch had lower average amylopectin branch chain-length than Type II and IV. Type II fruit starch, which had the longest average amylopectin branch chain-length, had lower proportion of very short chains (DP 6-9) than Type I and V. Short amylopectin branch chain-lengths (DP 6-12) were in higher proportion for Type I and V starches than for the other Zapallo Macre fruit-type starches. Type V fruit starch had considerably higher proportion of intermediate amylopectin branch chain-lengths (DP 13-24) and considerably lower proportion of long amylopectin branch chain-lengths (DP 25-36) than all four other fruit-type starches. Type V fruit starch had lower proportion of very long amylopectin branch chain-lengths (DP $\geq$ 37) than Type II and IV.

Starch thermal properties

Gelatinisation temperatures and enthalpy change of gelatinisation of the different Zapallo Macre type fruit starches are shown in Table 5. Onset gelatinisation temperature (T_o) of Type I fruit starch was higher than the other four type starches, and T_o of Type V was lower than Type III and IV. Type I and II Zapallo Macre fruit starches had higher peak gelatinisation temperature than the other three types. Type II had higher conclusion gelatinisation temperature (T_c) than the four other types and Type I also had higher T_c than Type III, IV and V. The range of gelatinisation temperature (T_c-T_o) (ROG) showed large variation among the Zapallo Macre fruit-type starches. Type I fruit starch ROG was considerably lower than the four other types. Type II ROG was considerably higher than the four other types and range was almost twice as broad as Type I. Type V ROG was higher than Type I and III. Enthalpy change of gelatinisation (ΔH) for the Zapallo Macre type fruit

starches also showed considerable differences. Type IV ΔH was lower than all other types except Type V. Starch from Type I had higher ΔH than the other four type starches.

Thermal transition temperatures, enthalpy change of thermal transition and percent retrogradation for the Zapallo Macre fruit-type retrograded starches are shown in Table 6. No differences were observed in onset temperature of the thermal transition. However, differences were observed in peak temperature (T_{pR}) of the thermal transition with Type I fruit starch being lower than all four other types. Type I and IV fruit starches had lower conclusion temperature of the thermal transition than Type V. Enthalpy change of the thermal tranisiton of retrograded starch was higher for Type III than Type II and IV. Percent retrogradation of Type III and Type V starch was greater than Type II.

Starch pasting and gel properties

Pasting properties of the Zapallo Macre type fruit starches are shown in Table 7. Pasting properties of Type V were not investigated due to insufficient starch yield to conduct analysis. Type I fruit starch had lower peak viscosity than Type II, III and IV. Type II starch also had higher peak viscosity than Type III. Breakdown of Type III paste was substantially lower than that of Type I, II and IV starch paste. Final viscosity and setback of Type III and IV starch pastes were higher than Type I and II. Pasting temperature of Type IV starch was considerably lower than the other three starches and Type I pasting temperature was also higher than Type II.

Properties of gel firmness and stickiness for the Zapallo Macre type fruit starches are shown in Table 8. After gelatinised starch had been stored for 1 d at 4°C, Type I starch gels were less firm than starch from Type II, III and IV fruit. Type III starch gels were also firmer than Type II starch gels. After starch gels were stored 7 d, Type I starch gels were

still considerably softer than other three fruit-type starch gels. Type III gels were firmer than other three fruit-type starch gels. Type I and IV fruit starch gel stickiness was lower than Type II and III after 1 d storage at 4°C. No differences in gel stickiness among the fruit-type starches was observed for starch gels stored 7 d at 4°C.

Fruit hardness

Hardness of the five Zapallo Macre fruit types steamed 0 to 20 min is shown in Table 9. Large variation in hardness was observed among fruit of the same type category for raw fruit and fruit steamed 10 min or less. Despite the large variation, Type I fruit had lower hardness than Type II, IV and V. Type V fruit hardness was also lower than Type II and IV. For fruit steamed 2 min, Type I hardness was substantially lower than the other four types that all had very similar hardness. An almost identical trend was observed for fruit steamed 5 min except Type V was harder than Type I but not similar in hardness to the other three types. Type III fruit steamed 10 min was harder than Type I and V. After 15 and 20 min steaming, hardness for all types was in a similar range but Type IV was still harder than Type II.

Fruit fracturability

Fracturability of the five Zapallo Macre fruit types steamed 0 to 20 min is shown in Table 10. Similar to the trend for fruit hardness, fracturability of fruit within the same fruit type was very variable for raw fruit and fruit steamed up to 10 min. Type I fruit required less force to fracture raw fruit and fruit steamed 2 or 5 min compared with Type II, III and IV. Fracturability of raw fruit from Type V was lower than Type II and IV. Type I fruit steamed for 10 min had higher fracturability than Type II, IV and V fruit. Variability of fracturability for fruit steamed 15 or 20 min was reduced with Type I and IV fruit requiring more force to

fracture than other fruit steamed 15 min and no major differences in fruit fracturability observed for fruit steamed 20 min.

Fruit springiness

Springiness of raw and cooked Zapallo Macre squash fruit is shown in Table 11. For raw fruit and fruit steamed 2 min, Type II fruit had significantly lower springiness than the other four types based on their standard error of the mean. Type II fruit was also less springy than Type I, IV and V after 5 min steaming. After 10, 15 or 20 min steaming, Type I fruit was springier than the four other fruit-types. Type IV fruit steamed 20 min was less springy than the four other fruit-types.

DISCUSSION AND CONCLUSIONS

Very few studies have involved the *Cucurbita maxima* cultivar Zapallo, and of the nine studies we found, seven focused on diseases of Zapallo. One study compared six Zapallo cultivars but the findings are unknown as the study was reported in Spanish (Montesinos-Vassalla and Morales-Deza 1972). Another study, also of unknown conclusions because it was written in Spanish, did clearly find variability in shape of Zapallo fruit based on the title of the publication (Lizana and Monardes 1978). Our study confirms the dramatic variability in fruit size, shape and colour for Zapallo cultivars (Fig 1.).

The great variability in fruit phenotypes was also reflected in their starch content. Fruit with over 60% of their dry matter as starch were found attached to the same plants that produced fruit that accumulated little or no starch. Type I fruit is to our knowledge the first squash fruit to weigh over 10 kg and have over 40% of its dry matter as starch (Table 1). Large squash fruit which accumulate a high percentage of starch could be important from a commercial viewpoint as this may enable a rapid harvest that obtains a high yield of starch.

Many studies have been investigating the mechanisms and regulation of starch biosynthesis in storage organs (Smith *et al* 1995, Kavakli *et al* 2000, Asano *et al* 2002), but many questions remain unanswered. The fact that Zapallo Macre fruit varied so dramatically in amount of starch accumulated makes this squash cultivar a potentially useful model system for advancing the knowledge of starch biosynthesis in storage organs.

The variation in starch structural and functional properties of Zapallo Macre fruit is not typically observed for a cultivar of any crop and highlights the effects of plant breeding in creating crops with more consistent starch structure. With the current wide variation in starch functional properties among the Zapallo fruit-types, the potential of the starch for industrial purposes is probably limited until plant breeding first produces a Zapallo Macre line with greater consistency in starch properties.

Texture of Zapallo Macre fruit varied substantially between the different fruit types, but also varied considerably among fruit of the same type, especially hardness. There was no clear trend of high-starch Zapallo Macre fruit types becoming softer or requiring less force to fracture at the early steaming times. The large differences in springiness of squash fruit types suggests there was variability in the degree of pectin breakdown and cell wall rupturing during the cooking process that can result in increased cell sliding and viscoelastic properties to the squash fruit (Batisse *et al* 1981, Redgwell *et al* 1997).

Studies of Zapallo Macre fruit found at least eight different fruit phenotypes. Five of the phenotypes that were investigated in detail revealed great differences in starch content, starch structure, starch functional properties, and textural attributes throughout the entire cooking process. Considerations of commercialising Zapallo Macre squash fruit would have to consider the inherent variability when making a feasibility assessment. Additionally the

large variation in starch accumulation among fruit on the same plant could make Zapallo Macre an interesting model system for studying the mechanisms and regulation of starch biosynthesis in storage organs.

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TABLE 1

Mean fruit weight, water content of fruit and starch content (% dry weight) of Zapallo Macre squash fruit.[#] Values after ± represent the standard error of the mean.

Zapallo Macre type	Fruit weight (kg)	Water content (%)	Starch content (%)
Type I	15.1 ± 0.3	91.2 ± 1.1	42.6 ± 0.4
Type II	3.7 ± 0.6	84.7 ± 1.7	16.1 ± 0.1
Type III	4.4 ± 1.9	87.6 ± 5.5	19.0 ± 2.2
Type IV	2.7 ± 0.4	78.9 ± 2.8	62.8 ± 3.0
Type V	4.3 ± 0.5	90.5 ± 4.2	12.1 ± 0.9

[#] Fruit weight, water and starch contents were averaged from three replicates.

TABLE 2

Iodine affinities, apparent amylose and absolute amylose contents for Zapallo Macre squash fruit defatted starches at harvest. Values after ± represent the standard error of the mean.

Zapallo Macre type	Iodine Affinity		Apparent amylose	Absolute amylose
	whole starch	amylopectin fraction	content (%)*	content (%)#
Type I	6.92 ± 0.13	3.89 ± 0.13	34.8 ± 0.7	19.6 ± 0.7
Type II	6.46 ± 0.32	3.60 ± 0.16	32.5 ± 1.6	18.1 ± 0.8
Type III	6.75 ± 0.12	3.86 ± 0.03	33.9 ± 0.6	19.4 ± 0.2
Type IV	7.15 ± 0.18	3.63 ± 0.09	35.9 ± 0.9	18.3 ± 0.5
Type V	6.39 ± 0.00	3.92 ± 0.04	32.1 ± 0.0	19.7 ± 0.2

Apparent amylose contents were averaged from two analyses for each of three replicates.; Values were calculated from dividing iodine affinity by a factor of 0.199.

Absolute amylose contents were averaged from two analyses for each of three replicates.; Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.

TABLE 3

Average amylopectin molecular weight, polydispersity and gyration radius of Zapallo Macre squash fruit starches extracted from fruit at harvest*. Values after ± represent the standard error of the mean.

Zapallo Macre type#	M_w x 10^8 (g/mol)♣	Polydispersity (M_w)	R_z (nm)♦
Type I	3.03 ± 0.25	1.41 ± 0.01	300 ± 1
Type II	3.01 ± 0.19	1.82 ± 0.17	310 ± 4
Type III	3.92 ± 0.26	1.40 ± 0.01	321 ± 3
Type IV	3.68 ± 0.02	1.23 ± 0.03	310 ± 2
Type V	3.59 ± 0.18	1.35 ± 0.01	313 ± 1

* Data were obtained from two injections of all three replicates.

Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; Freshly prepared starch aqueous solution (100 μL; 0.8 mg/mL) was injected to HPSEC system.

♣ *weight*-average molecular weight.

♦ *z*-average radius of gyration.

TABLE 4

Branch chain-length distributions of squash fruit amylopectins purified from starch extracted from Zapallo Macre fruit at harvest[*♦]. Values after ± represent the standard error of the mean.

Zapallo Macre type	Average CL	Percent distribution				
		DP 6-9	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37
Type I	27.2 ± 0.3	6.3 ± 0.6	16.5 ± 0.9	39.5 ± 0.5	17.2 ± 0.5	26.3 ± 1.0
Type II	29.6 ± 1.6	4.2 ± 0.7	12.1 ± 2.6	33.4 ± 6.3	21.2 ± 5.1	32.9 ± 3.8
Type III	28.4 ± 0.9	4.5 ± 0.1	13.7 ± 0.3	39.1 ± 0.6	17.7 ± 1.2	29.3 ± 2.1
Type IV	29.0 ± 0.1	4.5 ± 0.2	13.5 ± 0.1	38.4 ± 0.6	17.7 ± 0.0	30.0 ± 0.4
Type V	25.8 ± 1.7	5.7 ± 0.4	16.5 ± 1.4	44.0 ± 3.8	15.7 ± 0.6	23.3 ± 4.6

[*] Grouping of degree of polymerization (DP) numbers followed that of Hanashiro *et al* (1996).
[♦] Values comprise of two injections for all three replicates.

TABLE 5

Thermal properties of native Zapallo Macre squash starches isolated from fruit at harvest. Values after ± represent the standard error of the mean.

Zapallo Macre type*	T_o (°C)#	T_p (°C)	T_c (°C)	Range (°C)♣	ΔH (J/g)
Type I	64.3 ± 0.4	67.4 ± 0.7	70.2 ± 0.5	5.9 ± 0.3	17.3 ± 0.8
Type II	60.3 ± 0.6	66.9 ± 0.3	71.6 ± 0.5	11.3 ± 1.9	15.5 ± 0.6
Type III	61.3 ± 0.8	65.3 ± 0.9	68.4 ± 0.9	7.1 ± 0.1	15.3 ± 0.2
Type IV	61.3 ± 0.4	65.3 ± 0.5	68.8 ± 0.6	7.5 ± 0.3	12.5 ± 0.6
Type V	60.0 ± 0.4	64.2 ± 0.7	68.1 ± 0.7	8.1 ± 0.4	13.4 ± 0.3

* Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.

Values were calculated from three analyses for each of three replicates.

♣ Range of gelatinization is equal to T_c-T_o.

TABLE 6

Thermal properties of starch isolated from Zapallo Macre squash fruit at harvest and retrograded.
Values after ± represent the standard error of the mean.

Zapallo Macre type*	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	% retrogradation
Type I	36.9 ± 1.7	52.8 ± 0.9	65.5 ± 0.2	8.7 ± 0.6	50.7 ± 4.0
Type II	37.6 ± 1.0	57.1 ± 0.7	66.0 ± 0.8	7.0 ± 0.3	45.3 ± 1.8
Type III	37.7 ± 1.0	55.4 ± 1.0	65.0 ± 1.6	8.9 ± 0.7	58.0 ± 4.9
Type IV	36.5 ± 0.8	56.8 ± 0.1	65.2 ± 0.1	6.4 ± 0.6	51.0 ± 2.7
Type V	37.8 ± 1.2	57.3 ± 0.9	66.5 ± 0.3	7.7 ± 1.1	57.0 ± 7.6

Same starch samples after gelatinization (see Table 5) were left for 7 days at 4°C and rescan using DSC.

TABLE 7

Pasting properties of Zapallo Macre squash fruit starches, extracted at harvest, measured by Rapid Visco-Analyzer. Values after ± represent the standard error of the mean.

Zapallo Macre type*	Peak Viscosity#	Breakdown#	Final Viscosity#	Setback#	Pasting Temperature (°C)
Type I	195 ± 1	72 ± 1	212 ± 2	89 ± 0	68.1 ± 0.0
Type II	228 ± 17	83 ± 12	234 ± 5	89 ± 1	67.2 ± 0.6
Type III	204 ± 5	55 ± 5	259 ± 3	109 ± 0	67.9 ± 0.2
Type IV	219 ± 2	71 ± 4	251 ± 10	104 ± 4	65.5 ± 0.0
Type V	na	na	na	na	na

* 8% (w/w) starch suspension measured in duplicate for all three replicates.

Viscosity measured in Rapid Visco-Analyzer units (RVU), 1 RVU = 12 centipoise.

na = not analyzed due to insufficient starch yield.

TABLE 8

Gel firmness (g) and gel stickiness (g/sec) from starches extracted from Zapallo Macre fruit at harvest, heated under RVA temperature profile and stored at 4°C for 1 or 7 d[#]. Values after ± represent the standard error of the mean.

	Gel Firmness		Gel Stickiness	
Zapallo Macre type	1 Day	7 Days	1 Day	7 Days
Type I	14.7 ± 0.6	18.5 ± 0.4	-7.1 ± 1.0	-17.8 ± 0.9
Type II	17.6 ± 0.3	21.2 ± 0.8	-12.3 ± 0.9	-18.5 ± 2.4
Type III	18.9 ± 0.3	27.9 ± 0.7	-11.9 ± 1.3	-16.4 ± 1.9
Type IV	18.0 ± 0.4	23.5 ± 0.5	-8.4 ± 0.8	-15.1 ± 2.0
Type V	na	na	na	na

[#] Values were obtained from five measurements for each of three replicates.
na = not analyzed due to insufficient starch yield.

TABLE 9

Hardness (N) of Zapallo Macre squash fruit at harvest and steamed for 0 to 20 minutes[#].
Values after ± represent the standard error of the mean.

	Steaming Time (min)					
Zapallo Macre type	0	2	5	10	15	20
Type I	345 ± 31	226 ± 17	218 ± 12	49 ± 8	22 ± 2	17 ± 1
Type II	682 ± 81	646 ± 10	404 ± 77	120 ± 90	18 ± 0	12 ± 1
Type III	525 ± 153	622 ± 143	457 ± 139	110 ± 1	23 ± 9	15 ± 1
Type IV	661 ± 73	664 ± 236	457 ± 26	84 ± 44	33 ± 3	19 ± 3
Type V	537 ± 6	620 ± 73	276 ± 30	53 ± 26	22 ± 7	19 ± 6

[#] Hardness measurements are from four fruit from each of three replicates.

TABLE 10

Fracturability (N) of Zapallo Macre squash fruit at harvest and steamed for 0 to 20 minutes[#]. Values after ± represent the standard error of the mean.

	Steaming Time (min)					
Zapallo Macre type	0	2	5	10	15	20
Type I	333 ± 24	190 ± 13	117 ± 9	47 ± 3	10 ± 1	1 ± 0
Type II	647 ± 3	501 ± 82	241 ± 16	13 ± 2	4 ± 2	2 ± 0
Type III	464 ± 131	535 ± 168	355 ± 186	64 ± 45	5 ± 3	3 ± 1
Type IV	720 ± 87	549 ± 187	348 ± 73	16 ± 1	12 ± 0	4 ± 2
Type V	369 ± 141	384 ± 101	131 ± 110	11 ± 3	9 ± 7	6 ± 4

[#] Fracturability measurements are from four fruit from each of three replicates.

TABLE 11

Springiness (mm) of Zapallo Macre squash fruit at harvest and steamed for 0 to 20 minutes[#].
Values after ± represent the standard error of the mean.

	Steaming Time (min)					
Zapallo Macre type	0	2	5	10	15	20
Type I	12.91 ± 0.02	12.95 ± 0.02	13.00 ± 0.03	13.53 ± 0.09	13.40 ± 0.05	13.43 ± 0.07
Type II	11.65 ± 0.15	12.08 ± 0.02	12.40 ± 0.10	12.92 ± 0.13	12.72 ± 0.13	13.12 ± 0.04
Type III	12.58 ± 0.01	12.29 ± 0.05	12.66 ± 0.24	12.92 ± 0.32	13.02 ± 0.23	13.10 ± 0.00
Type IV	12.39 ± 0.11	12.32 ± 0.08	12.63 ± 0.07	12.86 ± 0.19	12.79 ± 0.11	12.58 ± 0.22
Type V	12.53 ± 0.02	12.97 ± 0.12	12.97 ± 0.12	13.16 ± 0.16	12.88 ± 0.43	13.05 ± 0.25

[#] Springiness measurements are from four fruit from each of three replicates.

Fig 1. Illustration of some, but not all, of the diversity in size, shape and color of Zapallo Macre winter squash fruit. This photo shows the five fruit types selected. The large white-skin squash at centre of picture is Type I. The dark green large fruit to the left of centre is Type II. The grey skin squash to the left of centre and in front of the dark green squash is Type III. The small orange and green variegated squash located at front centre of picture is Type IV. The large oblong pink skin squash at front right is Type V.

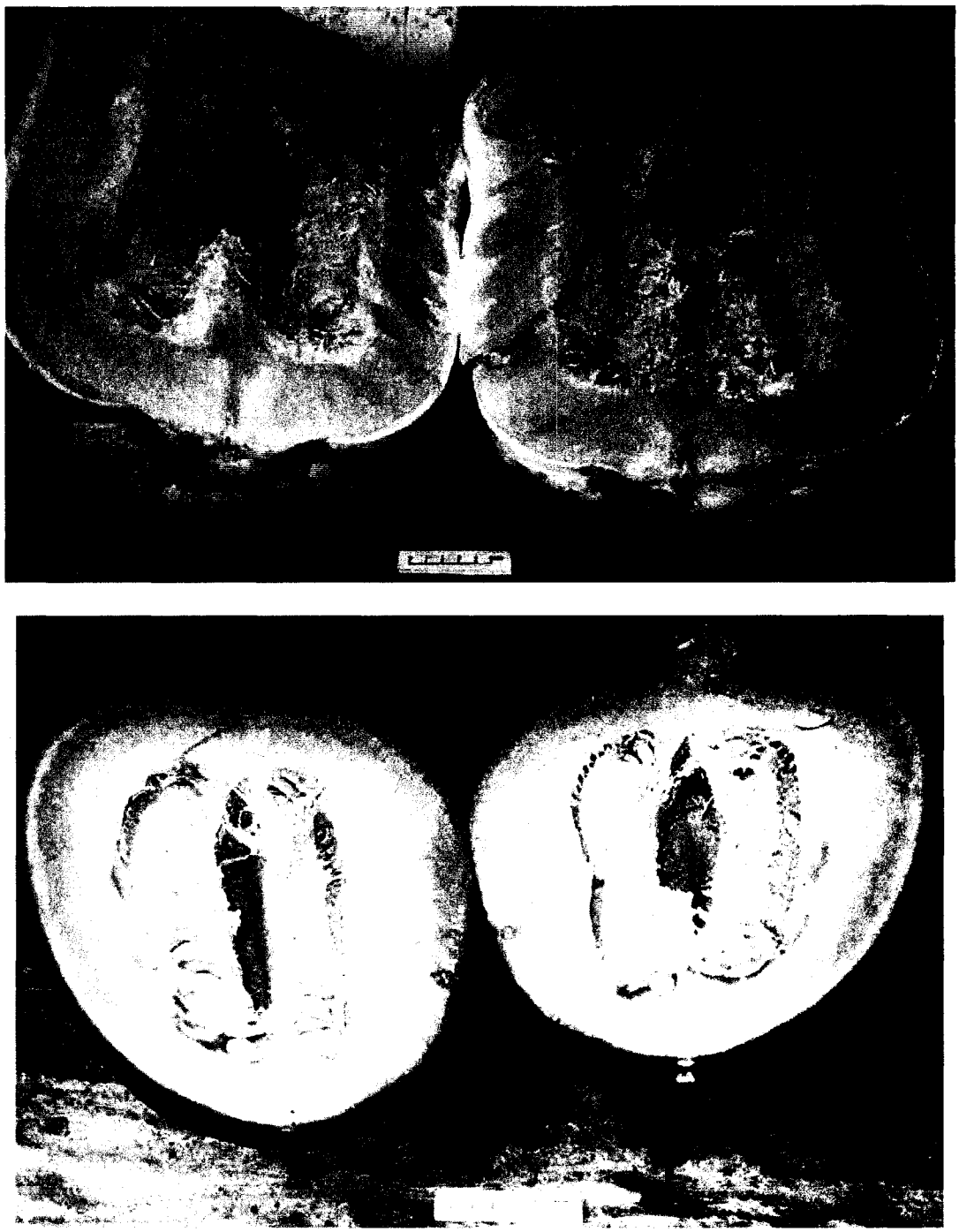

Fig 2a. The fruit interior of Zapallo Macre winter squash fruit type I (top) and type II (bottom). Extremities on scale = 5 cm.

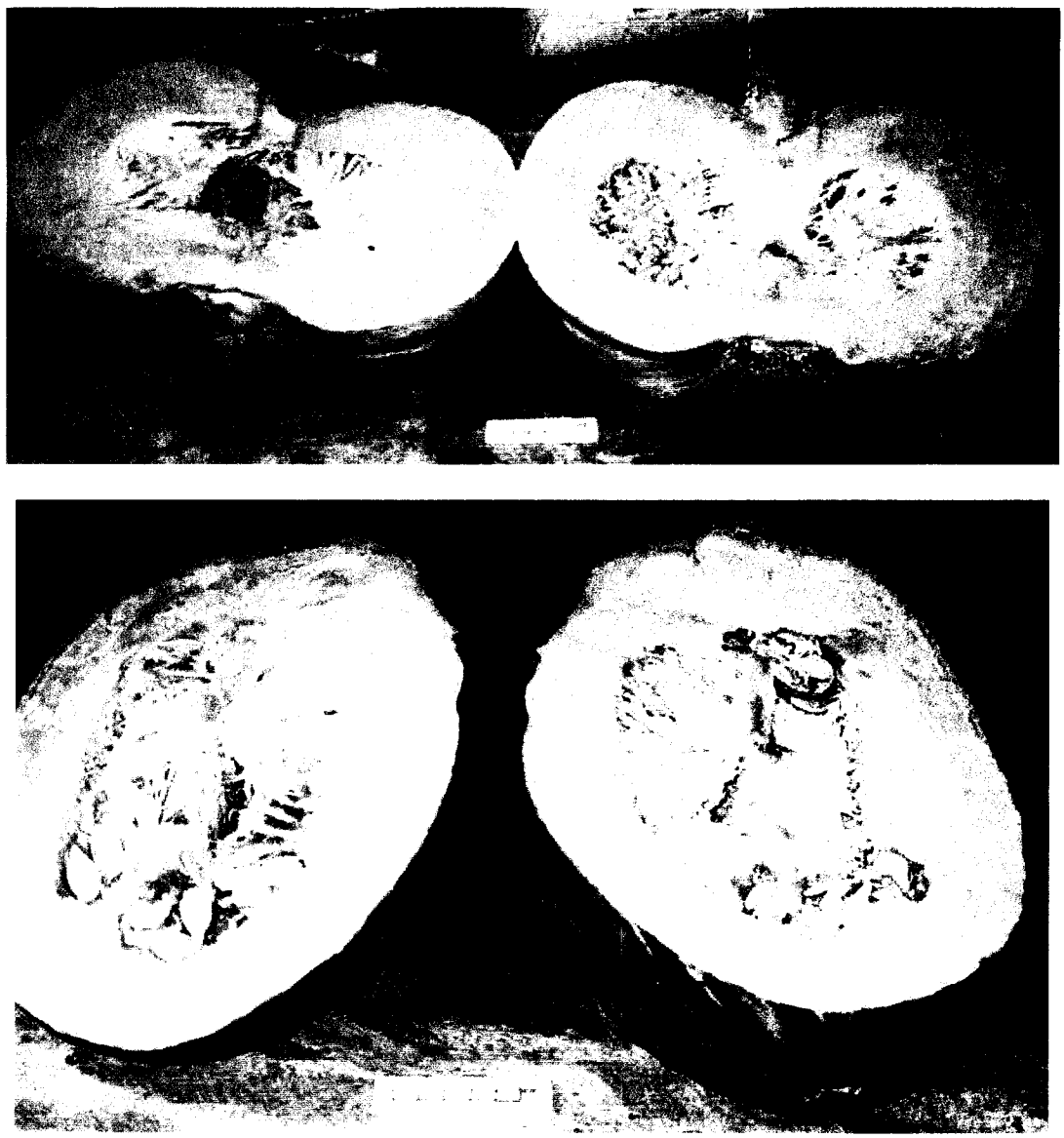

Fig 2b. The fruit interior of Zapallo Macre winter squash fruit type III (top) and type IV (bottom). Extremities on scale = 5 cm.

Fig 2c. The fruit interior of Zapallo Macre winter squash fruit type V. Extremities on scale = 5 cm.

CHAPTER 10. STRUCTURAL AND FUNCTIONAL PROPERTIES OF APPLE (*Malus domestica* Borkh) FRUIT STARCH.

A paper to be submitted to Carbohydrate Polymers

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Abstract

Structural and functional properties of six apple cultivars (Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala) fruit starch were investigated. Apple starches exhibit C-type X-ray diffraction patterns, and granules ranged from 2-12 μm. Apple fruit had 44-53% of dry matter as starch. Apparent amylose content was high (40-48%) but iodine affinity of amylopectin was high, resulting in absolute amylose content of 26-29%. Amylopectin weight-average molecular weight ranged from 4.6 to 11.1 x 10^8. Proportion of long-chain amylopectins was high. Onset gelatinization temperature ranged from 64-66°C and ΔH was high (16-18 J/g). Retrogradation percentage, after 7 d at 4°C ranged from 42-47%. Most distinctive characteristic of apple starch was that three cultivars had extremely low breakdown (< 4 RVU) and very high setback (> 100 RVU). Peak and final viscosities ranged from 99-148 and 144-224 RVU, respectively. Pasting temperature was about 70°C. Significant differences were observed in gel firmness and stickiness among cultivars.

Keywords: Apple starch, Starch structure, Starch function, Amylose, Amylopectin, *Malus*,

1. Introduction

Starch is the main carbohydrate of plant storage organs. Starch has been extensively characterized in cereals, root and tuber crops, but little research has been done characterizing fruit starches. Apples, like many other fruit crops, accumulate starch at early stages of maturation and progressively degrade starch to increase sweetness as maturity nears (Warrington, Fulton, Halligan, & de Silva 1999). Hail damage is a frequent cause of crop loss in the apple industry, resulting in premium apples being downgraded for juicing and other processing (Dodds, Penrose, Bower, & Nicol 1994). Hail damage often occurs when fruit are immature and substantial amount of starch is present. Therefore, if apple starches possess some unique characteristics, fruit may be more valuable for their starch in niche industries rather than for juicing.

Very little is known about the characteristics of apple starch, and in fact only one publication exists investigating starch characteristics of one apple cultivar (Potter, Hassid, & Joslyn 1949). Techniques and instrumentation to analyze starches have greatly advanced since then. Unfortunately starch content was not reported. Amylose was reported to consist 24% of total starch based on pentasol precipitation method, and 26.5% based on potentiometric iodine titration method. β-amylase hydrolysis of amylose yielded 90% maltose, and hydrolysis ceased for amylopectin when 64% was degraded to maltose. Acetylation of amylopectin was used to determine its molecular weight to be 1.2×10^6. End-group determination by periodate oxidation showed average of 24 glucose residues per end-group of amylopectin. Amylose chain-length was determined to be 530 glucose residues.

In this study we investigate starch structural and functional properties of six apple cultivars to determine if apple starches possess some distinctive characteristics. We also correlate structural and functional starch properties to explain the starch properties.

2. Materials and methods

2.1 Plant Material

Immature fruit from trees of six apple (*Malus domestica* Borkh) cultivars were harvested on August 8 and 9, 2002, at the Iowa State University Horticultural Farm, 2 miles east of Gilbert, Iowa. Cultivars studied were Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala and illustrations of these cultivars can be found in Appendix C. Three replicates, each consisting of 20 apples collected randomly, which included fruit from center of tree and towards end of branches, as well as varying tree canopy heights, were collected for each cultivar.

2.2 Starch Isolation and Quantification, and Water Content of Apple Fruit

Starch was isolated from apple fruit using method reported by Badenhuizen (1964) with slight modification by Kasemsuwan, Jane, Schnable, Stinar, & Robertson (1995) and further modification by Stevenson & Jane (Chapter 2). On the same day as being harvested, apple fruit were sliced into quarters and blended in 0.3% (w/v) sodium metabisulfite using Osterizer blender (Oster® Designer® Slope Blender 14 speed, grind mode used, Sunbeam Products Inc., Boca Raton, FL). Apple starch puree was then filtered through 106 μm mesh and the filtrate was spun at 7,000 rpm for 40 min to deposit starch. To remove protein and lipids such as chlorophyll pigments, starch pellet was washed by mechanical stirring for 1 h with 10% toluene in 0.1 M sodium chloride and left standing for at least 4 h to allow starch to settle out. The washing procedure was repeated several times until the supernatant remained clear. Toluene waste was repeatedly left standing for 24 h to allow physically entrapped

starch to deposit, until no more starch was obtained. Starch obtained from toluene waste was combined with starch that initially deposited from toluene washes. Toluene/salt washed starch was washed three times with distilled water, twice with ethanol, and then recovered by filtration using Whatman No. 4 filter paper. Purified starch cake was dried in a convection oven at 35°C for 48 h. Water content of apple fruit was determined by freeze-drying finely diced fruit. Total starch content of freeze-dried apple fruit powders, measured in duplicate, was determined using total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland), based on AOAC method 996.11, AACC method 76.13 and ICC standard method No. 168, in which fruit powders are hydrolyzed with α-amylase and amyloglucosidase, and subsequent glucose content determined using glucose oxidase-peroxidase. Internal standards of corn starch were added to samples to check quantitation and recovery of starch.

2.3 Starch Granule Morphology by Scanning Electron Microscopy

Starch granules, spread on silver tape and mounted on a brass disk, were coated with gold/palladium (60/40) for each cultivar. Sample images were observed at 1500x magnification under a scanning electron microscope (JOEL model 1850, Tokyo, Japan) following the method of Jane, Kasemsuwan, Leas, Zobel, & Robyt (1994).

2.4 Starch Crystalline Structure by X-ray Diffractometry

Crystallinity of starch granules was studied using X-ray diffractometry. X-ray diffraction patterns were obtained with copper, Kα radiation using a Siemens D-500 diffractometer (Siemens, Madison, WI). Analysis was conducted following procedure of Song & Jane (2000). Degree of crystallinity was calculated based on method of Hayakawa,

Tanaka, Nakamura, Endo, & Hoshino (1997). The following equation was used to determine percent crystallinity:

$$\text{Crystallinity (\%)} = A_c/(A_c + A_a) \times 100$$

where A_c = crystalline area on the X-ray diffractogram and A_a = amorphous area on the X-ray diffractogram.

2.5 Molecular Weight Distribution and Gyration Radius of Amylopectin by High-Performance Size-Exclusion Chromatography (HPSEC)

Weight-average molecular weight and z-average gyration radius of amylopectin were determined using high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors (HPSEC-MALLS-RI). Starch samples, duplicate measurements of each replicate for all cultivars, were prepared as described by Yoo & Jane (2002a). The HPSEC system consisted of a HP 1050 series isocratic pump (Hewlett Packard, Valley Forge, PA), a multi-angle laser-light scattering detector (Dawn DSP-F, Wyatt Tech. Co., Santa Barbara, CA) and a HP 1047A refractive index detector (Hewlett Packard, Valley Forge, PA). To separate amylopectin from amylose, Shodex OH pak KB-G guard column and KB-806 and KB-804 analytical columns (Showa Denko K.K., Tokyo, Japan) were used. Operating conditions and data analysis are described by Yoo & Jane (2002b), except flow rate used was 0.3 mL/min and sample concentration was 0.8 mg/mL.

2.6 Apparent and Absolute Amylose Contents by Potentiometric Autotitration

Apparent and absolute amylose contents of starch were determined following the procedure of Lu, Jane, Keeling, & Singletary (1996). Analysis was based on iodine affinities of defatted whole starch and amylopectin fraction using a potentiometric autotitrator (702 SM Titrino, Brinkmann Instrument, Westbury, NY). Starch samples were defatted using a 90% dimethyl sulfoxide (DMSO) solution, followed by alcohol precipitation. Determination of amylose content was duplicated for each replicate of each apple cultivar.

2.7 Amylopectin Branch Chain-Length Distribution by High-Performance Anion-Exchange Chromatography (HPAEC)

Amylopectin was fractionated by selective precipitation of amylose with n-butanol as described by Schoch (1942). Amylopectin (2 mg/mL) was defatted in boiling 90% DMSO for 1 h, followed by stirring for 24 h and then debranched using isoamylase (EC 3.2.1.68 from *Pseudomonas amyloderamosa*) (EN102, Hayashibara Biochemical Laboratories Inc., Okayama, Japan) as described by Jane & Chen (1992). Branch chain-length distribution of amylopectin was determined using an HPAEC system (Dionex-300, Sunnyvale, CA) equipped with an amyloglucosidase (EC 3.2.1.3, from *Rhizopus* mold, A-7255, Sigma Chemical Co., St Louis, MO) post-column, on-line reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) (Wong & Jane 1997). PA-100 anion exchange analytical column (250 x 4 mm, Dionex, Sunnyvale, CA) and a guard column were used for separating debranched amylopectin samples. Gradient profile of eluents and operating conditions were described previously (McPherson & Jane 1999). HPAEC-ENZ-PAD and HPSEC analysis was duplicated for each replicate of each cultivar.

2.8 Thermal Properties by Differential Scanning Calorimetry (DSC)

Thermal properties of starch were determined using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) (Jane et al. 1999). Approximately 2 mg of starch was weighed in an aluminum pan, mixed with 6 mg of deionized water and sealed. The sample was allowed to equilibrate for 2 h and scanned at a rate of 10°C/min over a temperature range of 10-100°C. An empty pan was used as the reference. Rate of starch retrogradation was determined using the same gelatinized samples, stored at 4°C for 7 d, and analyzed using DSC as described previously (White, Abbas & Johnson, 1989). All thermal properties were carried out in triplicate for each replicate of each cultivar.

2.9 Pasting Properties by Rapid Visco-Analyser (RVA) and Gel Properties

Starch pasting properties were analyzed using a rapid visco-analyser (RVA-4, Newport Scientific, Sydney, Australia) (Jane et al. 1999). Starch suspension (8%, w/w), in duplicate for each replicate of each cultivar, was prepared by weighing starch (2.24 g, dry starch basis (dsb)) into a RVA canister and making up the total weight to 28 g with distilled water. Starch suspension was equilibrated at 30°C for 1 min, heated at a rate of 6.0°C/min to 95°C, maintained at that temperature for 5.5 min, and then cooled to 50°C at a rate of 6.0°C/min. Constant paddle rotating speed (160 rpm) was used throughout entire analysis. Immediately after completion of RVA sample run, spindle was removed, and canister was wrapped in several layers of Saran® wrap, to minimize dehydration, and placed at 4°C.

After 1 or 7 d, canisters were removed from 4°C, equilibrated to room temperature, and gel properties were measured using a Stable Micro Systems TAXT2*i* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with Texture Expert for Windows software (v 1.22). Each gel was measured five times using a 4 mm diameter cylindrical stainless steel punch probe (TA54). Pretest speed was 2.0 mm/s, and gels were compressed at a test speed of 0.9 mm/s and a penetration test distance of 7.5 mm. Peak force was reported as the firmness of gels and stickiness of gels was defined as the negative load portion of the curve as described previously (Takahashi & Seib, 1988).

2.10 Statistical analysis

All statistical significance tests were calculated using SAS (1999) and applying Tukey difference test (Ramsey & Schafer 1996) at the 5% level of significance. Correlations between apple starch structural and functional properties were conducted using SAS (1999) and the PROC CORR function specifying use of the Pearson correlation coefficient. A 5% level of significance was used to discriminate correlations of importance. Means of the apple cultivars were correlated, with n =6 for all correlations.

3. Results and discussion

3.1 Starch content

Apple cultivar starch contents, shown in Table 1, were significantly different (P = 0.04). Granny Smith apples had significantly greater starch content than Royal Gala, and this appeared to be irrespective of fruit maturation stage since average fruit weight and

pigmentation skin color were similar. Average fruit weight, as well as red anthocyanin pigments present (from visual observations and see Appendix C), indicate that Jerseymac fruit maturity may have been more advanced than other cultivars, possibly explaining its relatively low starch content. Starch content of immature apple fruit is difficult to compare with other literature data because most report starch content at harvest, and most researchers measure starch index during fruit development, especially for apples. Apple starch content of 28 mg/g fresh weight has been previously reported for Royal Gala fruit during development (Brookfield, Murphy, Harker, & MacRae 1997), but no dry matter contents were provided to make valid comparisons with our data, although their starch content measurements appear low relative to our results. Bowen & Watkins (1997) studied Fuji apples during maturation and reported 4% starch content on dry weight basis, which is somewhat below the values we report, although this study reported highest starch content at first sampling time, in which some starch may have degraded. Studies of developing pear fruit also report highest starch levels at 3-4% dry weight (Singh & Dhillon 1982). Besides from some winter squash fruit, which have higher starch contents than apple fruit (Hurst, Corrigan, Hannan, & Lill 1995, Chapter 2), the only other report of substantial starch content in fruit is 20% of dry matter in tomato fruit 20 d post anthesis (Brampton, Asquith, Parke, Barraclough, & Hughes 1994).

3.2 Starch granule morphology

Scanning electron micrographs show all six apple cultivars have similar starch granule morphology and size distribution, with starch granule diameters typically ranging

between 2 to 12 μm (Fig. 1). Starch granule shapes included spherical, polyhedral and dome shapes. Many dome-shaped granules exhibited the typical characteristics of a compound starch. The starch granule size range for apple fruit is similar to that of squash fruit (Chapter 1, Chapter 2). Unlike squash fruit, apple starch has greater proportion of starch granules in the 6-9 μm diameter range, suggesting one initiation phase of starch biosynthesis, which has also been reported for tomato fruit (Brampton et al. 1994). Predominance of starch granule diameters between 6-8 μm has been reported for kiwifruit starch (Sugimoto, Yamamoto, Abe, & Fuwa 1988). Apple starch is also in similar granule size distribution range to starch from pineapple stem (Jane et al. 1994). A mixture of polyhedral and dome-shaped starch granules has also been observed from pineapple stem, babassu coconut, acorn (Jane et al. 1994) and kiwifruit (Sugimoto et al. 1988). In complete contrast to apple fruit starch granule morphology and distribution, avocado and banana starch have been shown to have considerably longer elongated granules (Fuwa, Sugimoto, Takaya, & Nikuni 1979, Jane et al. 1994).

3.3 Starch Crystalline structure

Apple starches all exhibited C-type X-ray diffraction patterns (Fig. 2), with a minor peak at $2\theta = 17.2°$ and a small peak at $2\theta = 5.5°$, characteristic of all B-type starches, a single peak at $2\theta = 22\text{-}24°$ and another peak at $2\theta = 14.6°$, characteristic of all A-type starches. The A-type X-ray diffraction pattern exhibits greater intensity, indicating that although apple starches are C-type, a greater proportion of the crystalline packing is A-type. Percentage crystallinity of Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold, and Royal Gala

apple starches, calculated based on X-ray diffractograms, was 43.7, 47.3, 41.3, 45.7, 40.6 and 46.4, respectively. Starch crystallinity percentages of apple fruit starches are similar to squash fruit starches (Chapter 1, Chapter 2), but differences between cultivars are considerably smaller for apple starches. Starch crystallinity percentage was correlated to starch content (r = -0.79, P = 0.05) and the pasting parameter, trough (r = 0.91, P = 0.01). The C-type X-ray diffraction pattern of apple fruit starch is similar to that of banana fruit starch (Jane et al. 1999) but different from the B-type X-ray diffraction pattern found for starch of squash fruit (Sugimoto et al. 1998, Chapter 1) and kiwifruit (Sugimoto et al. 1988).

3.4 Iodine affinity and amylose content

Iodine affinities for defatted whole starches and the corresponding apparent amylose contents were significantly different among the apple cultivars (P = 0.05), with apparent amylose content of Jerseymac significantly higher than Gala (Table 2). Absolute amylose content, calculated by subtracting iodine affinity of amylopectin fraction from that of the defatted whole starch, was not significantly different for the apple cultivars. Iodine affinities of apple whole starch and amylopectin fraction were larger than that of most native starches. Iodine affinity of defatted whole apple starch was higher than that of potato (7.20) and mungbean starches (7.58) (Jane et al. 1999). Iodine affinity of apple amylopectin fraction was higher than that of all A-type starches, but was comparable with that of B-type starches, and was slightly lower than that of the B-type squash fruit starch (Chapter 2), suggesting high proportion of long-branch chains present in the amylopectin. The apparent amylose content

of apple fruit starch was two to three times higher than that reported for kiwifruit starch (Sugimoto et al. 1988).

3.5 Amylopectin molecular weight and size

Weight-average amylopectin molecular weight (M_w), polydispersity and gyration radius (R_Z) of apple starches is shown in Table 3. Apple starch M_w ranged from 4.63×10^8 to 1.11×10^9 g/mol for the six cultivars, with no significant differences. Apple starch M_w was larger than most starches reported (Yoo & Jane 2002b). Apple cultivars, Granny Smith, Jonagold and Royal Gala, had fruit starch with very low amylopectin molecular weight polydispersity (M_w/M_n) relative to most native starches (Chapter 1), a characteristic similar to squash fruit starches and mungbean starch (Chapter 1, Chapter 2). Amylopectin polydispersity of Granny Smith, Jonagold and Royal Gala starch was significantly lower than Gala. Differences in amylopectin polydispersity between Gala and Royal Gala are surprising since their genetic similarity (Kruczynska, Rutkowski, & Czynczyk 2001, Hansen & Zanon 1982). All previously analyzed B- and C-type starches have polydispersities above two, except for potato (1.79) and squash (< 1.4) (Chapter 1), therefore it is surprising for some apple cultivars to have amylopectin with high uniformity. There is no evidence that the low polydispersity of apple and squash fruit starches provide a structure that allows rapid degradation by amylases to increase sweetness because other fruits with rapid starch degradation, such as banana, do not have low polydispersities (Chapter 1).

Gyration radius of amylopectin was not significantly different among the apple cultivars, suggesting similar spatial arrangement of amylopectin chains within molecules.

Gyration radius of amylopectin from apple cultivars ranged from 406 to 435 nm, which is more than 40% wider than reported for any other C-type starch amylopectin, and is wider than all B-type starches except green leaf canna, and wider than all A-type starches except normal rice, waxy rice, sweet rice and Chinese taro (Yoo & Jane 2002b). Despite the wider dimension of apple amylopectin, density of these molecules was comparable or higher than lotus root and green banana C-type starches (Yoo & Jane 2002b).

3.6 Amylopectin branch chain-length distribution

Amylopectin branch chain-length distributions for the starches of apple cultivars are shown in Table 4. There was no significant difference among apple cultivars in amylopectin branch chain-length distribution. The most notable characteristic of all apple cultivar amylopectins was the very high proportion (29.7-32.4%) of long amylopectin chains (DP $\geq$ 37) (Table 4, Figure 3a and 3b), which far exceeded the proportion reported previously for any other C-type starch (green banana highest with 24% DP $\geq$ 37) and also larger proportion than that of all B-type starches reported (26.1-29.5%) (Jane et al. 1999). Average chain-length of apple amylopectin was also longer than that of other C-type starches but was comparable to that of B-type starches, because B-type had less (1.8-3.5%) short amylopectin chains (DP 6-9) (Jane et al. 1999). The peak DP(II) for apple amylopectin distribution (DP 45.3-47) are shorter than that of the B- or C-type starch (DP 48-53), but are comparable with that of the A-type starches (DP 41-51) (Jane et al. 1999).

3.7 Thermal properties

Thermal properties of native apple starches are shown in Table 5. Onset gelatinization temperature (T_o) was significantly higher for Gala and Granny Smith than Golden Delicious, Jerseymac and Jonagold ($P = 0.0001$). Royal Gala had significantly higher T_o than Jonagold. Apple starch T_o tended to be 1-4°C higher than squash fruit starch (Chapter 1, Chapter 2) and kiwifruit starch (Sugimoto et al. 1988). Peak gelatinization temperature (T_p) was not significantly different for the apple cultivars, with extremities separated by just 0.9°C. Conclusion gelatinization temperature (T_c) was significantly higher for Gala, Jerseymac, Jonagold and Royal Gala than Granny Smith ($P = 0.007$).

Retrograded apple starches (Table 6) had lower T_{oR} than all other starches (Jane et al. 1999), except squash fruit starches (Chapter 1, Chapter 2). Additionally, T_{oR} of apple starches is surprising since A-type retrograded starches typically have lower T_{oR} (Jane et al. 1999). T_{pR}, T_{cR}, ΔH_R and percentage retrogradation of retrograded apple starches were similar to other C-type retrograded starches (Jane et al. 1999). Percent retrogradation of apple fruit starches was lower than most squash fruit starches (Chapter 1, Chapter 2). There were no significant differences in starch retrogradation properties among apple cultivars.

3.8 Pasting properties

Pasting properties of apple starches is shown in Table 7. The pasting property of breakdown was significantly different among the apple cultivars ($P < 0.0001$), with three cultivars exhibiting very low breakdown, a distinctive feature only previously reported for green leaf canna starch (Jane et al. 1999). Peak viscosities for Gala, Granny Smith and Royal

Gala were significantly higher than that of Golden Delicious, Jerseymac and Jonagold ($P < 0.0001$). The same three apple cultivars with higher peak viscosity, all had significantly higher breakdown ($P < 0.0001$). Golden Delicious, Jerseymac and Jonagold had extremely low breakdown (Fig. 4). Despite little difference in trough among apple cultivars, final viscosity was higher for the three apple cultivars with extremely low breakdown, with Golden Delicious and Jerseymac significantly higher than Gala and Granny Smith ($P = 0.0008$). Golden Delicious, Jerseymac and Jonagold, which had low peak viscosity and breakdown, but high final viscosity, all had setback over 100 RVU, which is only been previously reported for cattail millet, mungbean, green leaf canna (Jane et al. 1999) and squash starches (Chapter 2). Setback of these three apple cultivars was significantly higher than other three cultivars ($P < 0.0001$). Gala, Granny Smith and Royal Gala, which had higher peak viscosity, tended to have a lower peak time, and all three were significantly lower than Jerseymac ($P = 0.002$). No large differences in pasting temperature were observed but Granny Smith was significantly lower than Jonagold ($P = 0.01$).

3.9 Gel properties

Firmness and stickiness of apple gels stored for 1 or 7 d at 4°C are shown in Table 8. Jerseymac gels, after 1 d storage, were significantly firmer than Gala, Golden Delicious, Jonagold and Royal Gala ($P < 0.0001$). Firmness of Gala gels, stored for 1 d, was significantly lower than all other cultivar gels. After 7 d at 4°C, Jerseymac still had the firmest gel, and was significantly firmer than Gala, Golden Delicious and Royal Gala ($P = 0.0002$). Firmness of gels was found to only be correlated to apparent amylose content, long

amylopectin chain-lengths and T_p. Since no correlation was established between gel firmness and absolute amylose content, and T_p can be influenced by amylopectin chain-length, long-chain amylopectins are the likely contributor to firmer gels.

No significant differences among apple cultivars were observed for stickiness of gels stored 1 d, but significant differences were observed after 7 d storage ($P = 0.001$). Gels from Golden Delicious and Jonagold starch were stickier than gels from Gala and Granny Smith.

3.10 Correlations to Amylose Contents

Correlation coefficients among selected apple starch structural and functional properties are shown in Table 9. Correlation coefficients are mentioned in text when not included in Table 9. Amylose content of apples was correlated with thermal, pasting and gel parameters. Apparent amylose content of apple cultivars was correlated to onset gelatinization temperature (T_o), peak gelatinization temperature (T_p), enthalpy change of retrograded starch (ΔH_R), peak viscosity, breakdown, final viscosity, setback, peak time ($r = 0.86$, $P = 0.03$), second DP peak of amylopectin chain-length distribution ($r = -0.79$, $P = 0.05$), average amylopectin chain-length ($r = 0.92$, $P = 0.008$), proportion of amylopectin chains that were either DP 13-24 ($r = -0.92$, $P = 0.008$) or DP $\geq$ 37 and firmness of gels after 1 d ($r = 0.83$, $P = 0.04$) and 7 d storage at 4°C. Higher apparent amylose content has been reported previously to result in lower T_o (Inouchi, Takei, Asaoka, Kawamura, Sakamoto, & Fuwa 1993, Visser, Suurs, Steeneken, & Jacobsen 1997, Demenke, Hucl, Abdel-Aal, Båga, & Chibbar 1999) and lower peak viscosity (Wang, White, & Pollak 1993, Jane et al. 1999, Kuno, Kainuma, & Takahashi 2000). Iodine affinity of amylopectin fraction was correlated

to T_o (r = -0.89, P = 0.01), ΔH_R (r = 0.87, P = 0.02), peak viscosity (r = -0.86, P = 0.03), breakdown (r = -0.89, P = 0.01), final viscosity (r = 0.82, P = 0.05), setback (r = 0.88, P = 0.02), peak time (r = 0.87, P = 0.02), average amylopectin chain-length (r = 0.79, P = 0.05), proportion of amylopectin chains of either DP 13-24 (r = -0.91, P = 0.01) or DP $\geq$ 37 (r = 0.79, P = 0.05), and gel stickiness after 1 d (r = -0.79, P = 0.05) or 7 d (r = -0.80, P = 0.05) storage at 4°C. Despite all the correlations involving iodine affinity of amylopectin fraction, absolute amylose content was only correlated to pasting temperature, proportion of amylopectin chains of either DP 3-6 (r = 0.84, P = 0.04) or DP 25-36 (r = -0.79, P = 0.05) and gel stickiness after 7 d storage. Correlations between apparent amylose and long-chain amylopectins indicate that the latter was contributing to high iodine affinities observed for apple starches.

3.11 Correlations to Amylopectin Molecular Weight and Gyration Radii

M_w of apple cultivars was correlated to R_z (r = 0.94, P = 0.006), T_c (r = -0.81, P = 0.05) and ΔH (r = -0.79, P = 0.05). Polydispersity of apple cultivars was correlated to retrogradation percentage (r = -0.84, P = 0.04). Apart from M_w, R_z was not correlated to any other starch property. Amylopectin density was correlated to proportion of amylopectin chain-lengths of DP 6-9 (r = -0.79, P = 0.05).

3.12 Correlations to Amylopectin Branch Chain-length Distribution

Amylopectin chain-length distribution was correlated to other starch structural and functional properties. The second DP peak of amylopectin branch chain distribution was correlated to previously mentioned apparent amylose content, as well as T_o of native (r = 0.79, P = 0.05) and T_{oR} of retrograded starch (r = 0.83, P = 0.04), and the pasting properties of peak viscosity (r = 0.91, P = 0.01), breakdown (r = 0.82, P = 0.05), setback (r = -0.81, P = 0.05) and peak time (r = -0.84, P = 0.04). Average amylopectin chain-length was correlated to previously mentioned iodine affinity of amylopectin fraction and apparent amylose content, as well as T_p (r = -0.95, P = 0.004), and gel firmness after 1 (r = 0.90, P = 0.01) or 7 d (r = 0.94, P = 0.005). T_o was specifically correlated to proportion of amylopectin chains with lengths of DP 13-24 (r = 0.82, P = 0.05), and T_p was correlated to DP 6-12, DP 25-36 (r = 0.79, P = 0.05) and DP $\geq$ 37. Proportion of amylopectin chains with length DP 13-24 was correlated to pasting properties of peak viscosity (r = 0.91, P = 0.01), breakdown (r = 0.81, P = 0.05), setback (r = -0.79, P = 0.05), peak time (r = -0.80, P = 0.05) and gel firmness after 7 d storage (r = -0.79, P = 0.05). Peak viscosity and gel firmness after 7d storage were also correlated to proportion of amylopectin chains of length DP $\geq$ 37.

3.13 Correlations to Starch Thermal Properties

T_o was correlated to many starch properties including ΔH, ΔH_R, peak viscosity, breakdown, final viscosity, setback, peak time (r = -0.98, P = 0.002), pasting temperature, stickiness of gels stored 1 d (r = 0.92, P = 0.008), stickiness of gels stored 7 d and also previously mentioned correlations with apparent amylose content, iodine affinity of amylopectin fraction, second DP peak of amylopectin chain-length distribution, and

proportion of amylopectin chains of length DP 13-24. Proportion of amylopectin chain-lengths of DP 13-24 was correlated to ΔH_R (r = -0.84, P = 0.04).

3.14 Correlations to Starch Pasting Properties

Correlation analysis can provide some clues to explain the low breakdown of Golden Delicious, Jerseymac and Jonagold. Breakdown was correlated to pasting parameters of peak viscosity, final viscosity, setback, peak time (r = -0.99, P = 0.0001) and pasting temperature. Breakdown was also correlated to gel stickiness after 1 d (r = 0.91, P = 0.01) and 7 d. As previously mentioned, breakdown was negatively correlated with apparent amylose content, iodine affinity of amylopectin fraction, ΔH, ROG, and ΔH_R, and positively correlated to T_o, T_{oR} and T_{pR}, second peak DP of amylopectin distribution and proportion of amylopectin chains of length DP 13-24. Correlations tend to suggest that apple starches with low breakdown have greater amount of amylopectin with long chains. No amylose-lipid complex was observed for all apple starches, therefore the ability of amylose to help hold granule together during swelling was likely to be similar for all cultivars.

3.15 Correlations to Starch Gel Properties

Gel stickiness was correlated to most pasting parameters. Stickiness of gels stored for 1 d was correlated to peak viscosity (r = 0.79, P = 0.05), breakdown (r = 0.91, P = 0.01), final viscosity (r = -0.90, P = 0.01), setback (r = -0.88, P = 0.02), peak time (r = -0.88, P = 0.02) and pasting temperature (r = -0.90, P = 0.01). Stickiness of gels stored for 7 d was correlated to peak viscosity, breakdown, setback, peak time (r = -0.79, P = 0.05) and pasting

temperature. Apple gel stickiness has been previously mentioned to be correlated positively to absolute amylose content, T_o, T_{oR}, T_{pR} and T_{cR}, and negatively correlated to iodine affinity of amylopectin fraction, ΔH, ROG, and ΔH_R. Amylose content had previously been reported to be correlated to gel stickiness (Yamin, Lee, Pollak, & White 1999). Correlations suggest apple starch with higher amylose content and shorter amylopectin chains have stickier gels.

Firmness of gels stored for 1 or 7 d at 4°C was correlated to apparent amylose content, Tp and apple fruit weight ($r = 0.84$, $P = 0.03$ for both). Proportion of very long amylopectin branch chain-lengths (DP $\geq$ 37) was correlated to firmness of gels stored for 7 d.

4. Conclusion

Six apple cultivars had starch granules ranging 2-12 μm, and starches exhibited C-type X-ray diffraction patterns. Absolute amylose content ranged from 26-29% and amylopectin molecular weight was high. Average amylopectin branch chain-length was very long and this may have contributed to the distinctive pasting properties of very low breakdown and very high setback. Onset gelatinization temperature was 64-66°C and ΔH was high (16-18 J/g). Apple starches exhibited high gel firmness characteristics.

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Table 1

Average fruit weight (g), water content (%) and starch content (% dry weight) of apple fruit*.

Cultivar	Average Fruit Weight♦	Water Content	Starch Content#
Gala	103	89.4	45.8^{ab}
Golden Delicious	100	90.4	47.5^{ab}
Granny Smith	114	88.3	53.2^{a}
Jerseymac	159	89.9	44.3^{ab}
Jonagold	123	88.5	51.0^{ab}
Royal Gala	109	88.9	44.0^{b}
		$P = 0.18$	$P = 0.04$♣

Starch contents were averaged from two duplicates of each of three replicates.

♦ Average of 60 fruit (20 fruit per replicate).

*Values with different letters denote cultivar differences at the 5% level of significance for each comparison between cultivars in the respective column.

♣ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 2

Iodine affinities, apparent amylose and absolute amylose contents for defatted apple fruit starches[♦].

Cultivar	Iodine Affinity		Apparent amylose	Absolute amylose
	whole starch	amylopectin fraction	content (%)[*]	content (%)[#]
Gala	7.92[b]	2.12	39.8[b]	29.1
Golden Delicious	9.16[ab]	3.59	45.4[ab]	28.0
Granny Smith	8.62[ab]	2.80	43.3[ab]	29.3
Jerseymac	9.57[a]	3.77	48.1[a]	29.1
Jonagold	9.02[ab]	3.84	46.1[ab]	26.0
Royal Gala	8.42[ab]	3.13	42.4[ab]	26.6
	$P = 0.05$[♠]	$P = 0.09$	$P = 0.05$	$P = 0.95$

Apparent amylose contents were averaged from two analyses for each of three replicates.; Values were calculated from dividing iodine affinity by a factor of 0.199.

[#] Absolute amylose contents were averaged from two analyses for each of three replicates.; Values were calculated by subtracting iodine affinity for the amylopectin fraction from the iodine affinity for the whole starch, divided by a factor of 0.199.

[♦] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♠] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 3

Average amylopectin molecular weight, polydispersity, gyration radius and density of apple fruit starches.[*♥]

Cultivar[#]	M_w x 10^8 (g/mol)[♣]	Polydispersity (M_w)	R_z (nm)[♦]	ρ (g/mol/nm^3)[♠]
Gala	4.63	3.16[a]	406	6.9
Golden Delicious	7.20	2.14[ab]	422	9.6
Granny Smith	11.10	1.47[b]	435	13.5
Jerseymac	6.41	1.80[ab]	419	8.7
Jonagold	6.47	1.54[b]	413	9.2
Royal Gala	7.79	1.51[b]	428	9.9
	$P = 0.79$[☼]	$P = 0.02$	$P = 0.94$	$P = 0.83$

[*] Data were obtained from two injections of all three replicates.

[#] Starch samples were dissolved in 90% DMSO solution and precipitated with 5 vol. ethanol; Freshly prepared starch aqueous solution (100 µL; 0.8 mg/mL) was injected to HPSEC system.

[♣] *weight*-average molecular weight.

[♦] *z*-average radius of gyration.

[♠] Density is equal to M_w/R_z^3.

[♥] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[☼] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 4

Branch chain-length distributions of apple fruit amylopectins[*#].

Cultivar	Peak DP		Average	Percent distribution				
	I	II	CL	DP 6-9	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37
Gala	13.0	46.3	27.9	4.6	15.9	39.0	15.0	29.7
Golden Delicious	13.0	45.7	29.1	4.3	14.7	38.0	14.4	31.8
Granny Smith	13.0	46.3	29.2	4.2	14.9	38.2	14.0	31.9
Jerseymac	13.0	45.3	29.6	4.9	15.3	37.5	14.1	32.1
Jonagold	13.0	45.3	29.1	4.5	14.9	37.2	15.2	32.4
Royal Gala	12.7	47.0	28.4	4.4	15.6	38.5	15.7	29.9
			$P = 0.39$[♣]	$P = 0.57$	$P = 0.61$	$P = 0.76$	$P = 0.69$	$P = 0.53$

[*] Grouping of degree of polymerization (DP) numbers followed that of Hanashiro, Abe, & Hizukuri (1996).

[#] Values were calculated from two injections for each of three replicates.

[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 5

Thermal properties of native apple fruit starches.

Cultivar*	T_o (°C)#♦	T_p (°C)	T_c (°C)	Range (°C)♣	ΔH (J/g)
Gala	66.1^{a}	70.9	77.1^{a}	11.0^{b}	17.1
Golden Delicious	64.7^{bc}	70.0	76.2^{ab}	11.4^{ab}	17.7
Granny Smith	66.5^{a}	70.1	75.1^{b}	8.5^{c}	15.8
Jerseymac	64.2^{bc}	70.0	76.9^{a}	12.8^{a}	17.3
Jonagold	64.1^{c}	70.3	77.2^{a}	13.1^{a}	17.4
Royal Gala	65.5^{ab}	70.7	77.3^{a}	11.8^{ab}	16.5
	$P = 0.0001$♠	$P = 0.14$	$P = 0.007$	$P < 0.0001$	$P = 0.68$

* Starch samples (~2.0 mg, dsb) and deionized water (~6.0 mg) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.

Values were calculated from three analyses for each of three replicates.

♣ Range of gelatinization is equal to T_c-T_o.

♦ Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♠ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 6

Thermal properties of starch, retrograded for 7 days at 4°C, isolated from apple fruit[#].

Cultivar[*]	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	% retrogradation
Gala	36.9	53.3	64.3	7.1	41.6
Golden Delicious	36.1	52.4	63.3	7.4	42.3
Granny Smith	37.5	53.4	64.5	7.1	45.2
Jerseymac	36.5	53.1	64.4	7.8	46.1
Jonagold	36.5	52.8	63.5	7.9	45.5
Royal Gala	37.6	53.1	64.7	7.5	47.3
	$P = 0.34$[♣]	$P = 0.20$	$P = 0.21$	$P = 0.88$	$P = 0.95$

[*] Same starch samples after gelatinization (see Table 5) were left for 7 days at 4°C and rescan using DSC.
[#] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.
[♣] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 7

Pasting properties of apple fruit starches measured by Rapid Visco-Analyser.

Cultivar*	Peak Viscosity#♣	Trough#	Breakdown#	Final Viscosity#	Setback#	Peak Time (min)	Pasting Temperature (°C)
Gala	148^{a}	102	46^{a}	162^{bc}	59^{b}	9.5^{bc}	70.3^{ab}
Golden Delicious	113^{b}	109	4^{b}	216^{a}	107^{a}	11.6^{ab}	70.7^{ab}
Granny Smith	141^{a}	90	51^{a}	144^{c}	54^{b}	9.0^{c}	69.9^{b}
Jerseymac	113^{b}	111	2^{b}	224^{a}	113^{a}	12.2^{a}	70.6^{ab}
Jonagold	99^{b}	97	3^{b}	199^{ab}	102^{a}	11.8^{ab}	71.3^{a}
Royal Gala	148^{a}	111	37^{a}	180^{abc}	69^{b}	9.8^{bc}	70.6^{ab}
	$P < 0.0001$♦	$P = 0.10$	$P < 0.0001$	$P = 0.0008$	$P < 0.0001$	$P = 0.002$	$P = 0.01$

* 8% (w/w) starch suspension measured in duplicate for all three replicates.

Viscosity measured in Rapid Visco-Analyser units (RVU), 1 RVU = 12 centipoise.

♣ Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

♦ *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 8

Gel firmness (g) and stickiness (g sec) from starches extracted from apple fruit, heated under RVA temperature profile and stored at 4°C for 1 or 7 d[#♣].

Cultivar	Firmness		Stickiness	
	1 day	7 days	1 day	7 days
Gala	21.2[c]	23.8[c]	-15.8	-13.3[a]
Golden Delicious	28.7[b]	34.2[bc]	-18.1	-18.5[b]
Granny Smith	34.0[ab]	38.6[ab]	-14.2	-12.3[a]
Jerseymac	40.2[a]	47.2[a]	-17.4	-15.3[ab]
Jonagold	31.5[b]	37.1[ab]	-18.1	-18.9[b]
Royal Gala	29.4[b]	33.0[bc]	-16.7	-16.0[ab]
	$P < 0.0001$[♦]	$P = 0.0002$	$P = 0.35$	$P = 0.001$

[#] Values were obtained from five measurements for each of three replicates.

[♣] Values with different letters denote differences at the 5% level of significance for each comparison between cultivars in the respective column.

[♦] *P* represents the probability of F-statistic exceeding expected for each comparison between cultivars in the respective column.

Table 9

Correlation coefficients (r x 100) among apple fruit starch of selected structural and functional properties.

	St	Ap_A	Ab_A	T_o	T_p	ROG	ΔH	ΔH_R	DP_{6-12}	$DP_{\geq 37}$	PV	BK	FV	SB	PT	GF
St	100															
Ap_A	5	100														
Ab_A	0	-14	100													
T_o	22	-83*	47	100												
T_p	-37	-83*	-18	44	100											
ROG	-51	51	-57	-88**	2	100										
ΔH	37	42	-25	-77*	-14	76*	100									
ΔH_R	-18	78*	-59	-92**	-33	87*	52	100								
DP_{6-12}	-65	-62	16	36	85*	10	-13	-25	100							
$DP_{\geq 37}$	56	85*	-4	-56	-90**	14	21	51	-86*	100						
PV	-21	-85*	35	89**	65	-62	-68	-78*	66	-83*	100					
BK	16	-86*	36	97**	58	-77*	-81*	-83*	49	-64	93**	100				
FV	-44	79*	-26	-93**	-49	79*	82*	76*	-30	44	-76*	69	100			
SB	-22	87*	-27	-96**	-61	76*	80*	80*	-47	63	-89**	-99***	97**	100		
PT	-12	50	-80*	-86*	-7	88**	71	85*	-24	32	-76*	-78*	68	71	100	
GF	10	89**	8	-52	-80*	22	-1	60	-49	76*	-55	-53	49	57	13	100
GS	8	-53	76*	82*	27	-72	-73	-71	47	-38	76*	82*	-74	-76*	-91**	-13

St = starch content, Ap_A = apparent amylose content, Ab_A = absolute amylose content, T_o = onset gelatinization temperature, T_p = peak gelatinization temperature, ROG = range of gelatinization temperature, ΔH = enthalpy change of gelatinization, ΔH_R = enthalpy change of retrograded thermal transition, DP_{6-12} = proportion of amylopectin branch chain-lengths DP 6-12, $DP_{\geq 37}$ = proportion of amylopectin branch chain-lengths DP $\geq$ 37, PV = peak viscosity, BK = breakdown, FV = final viscosity, SB = setback, PT = pasting temperature, GF = gel firmness after 7 d storage at 4°C and GS = gel stickiness after 7 d storage at 4°C. * = 0.05, ** = 0.01 and *** = 0.001 level of significance.

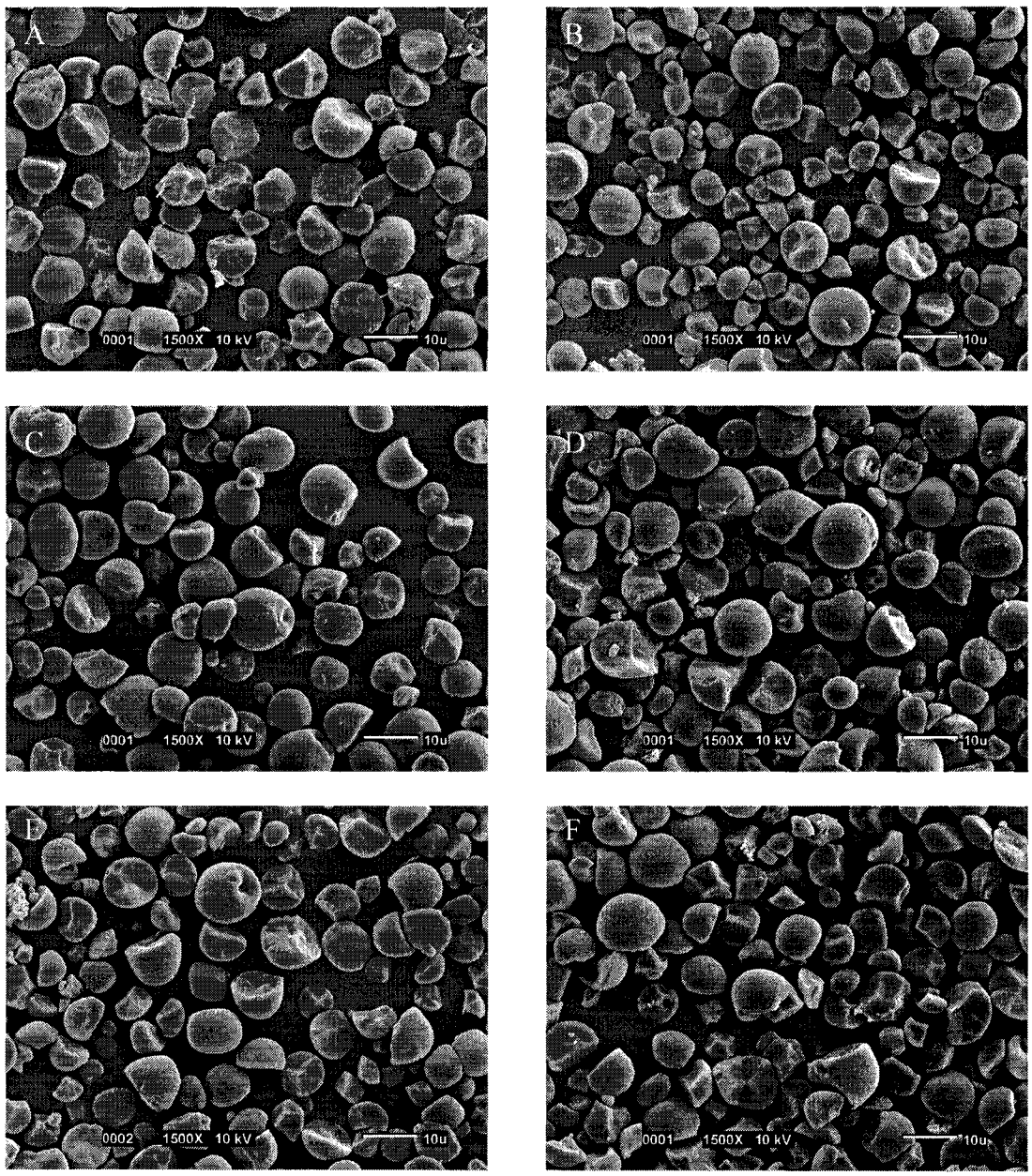


Figure 1. Scanning electron micrographs of Gala (A), Golden Delicious (B), Granny Smith (C), Jerseymac (D), Jonagold (E) and Royal Gala apple fruit starches (scale bar = 10 μm).

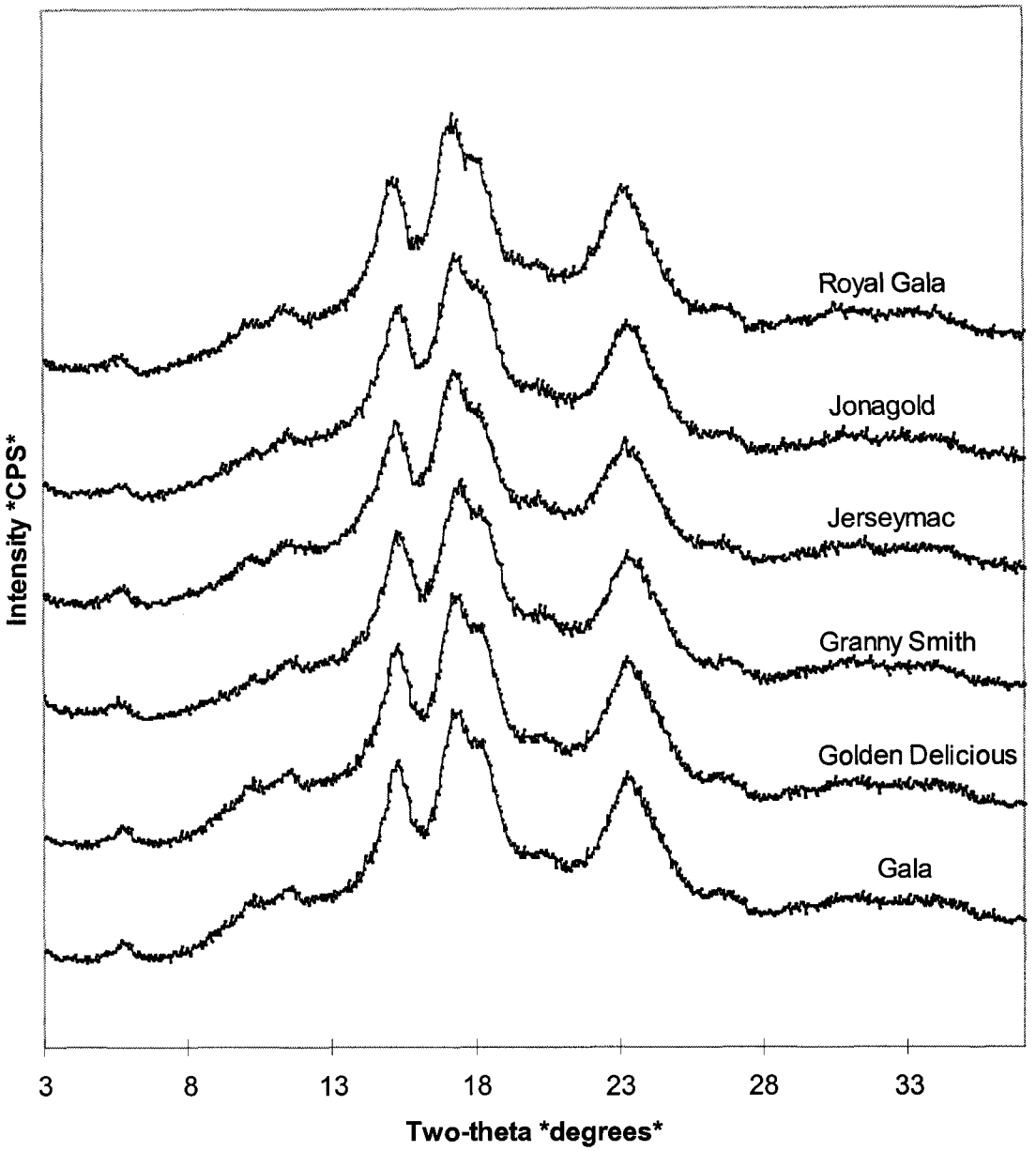


Fig. 2. X-ray diffraction patterns of Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala apple fruit starches.

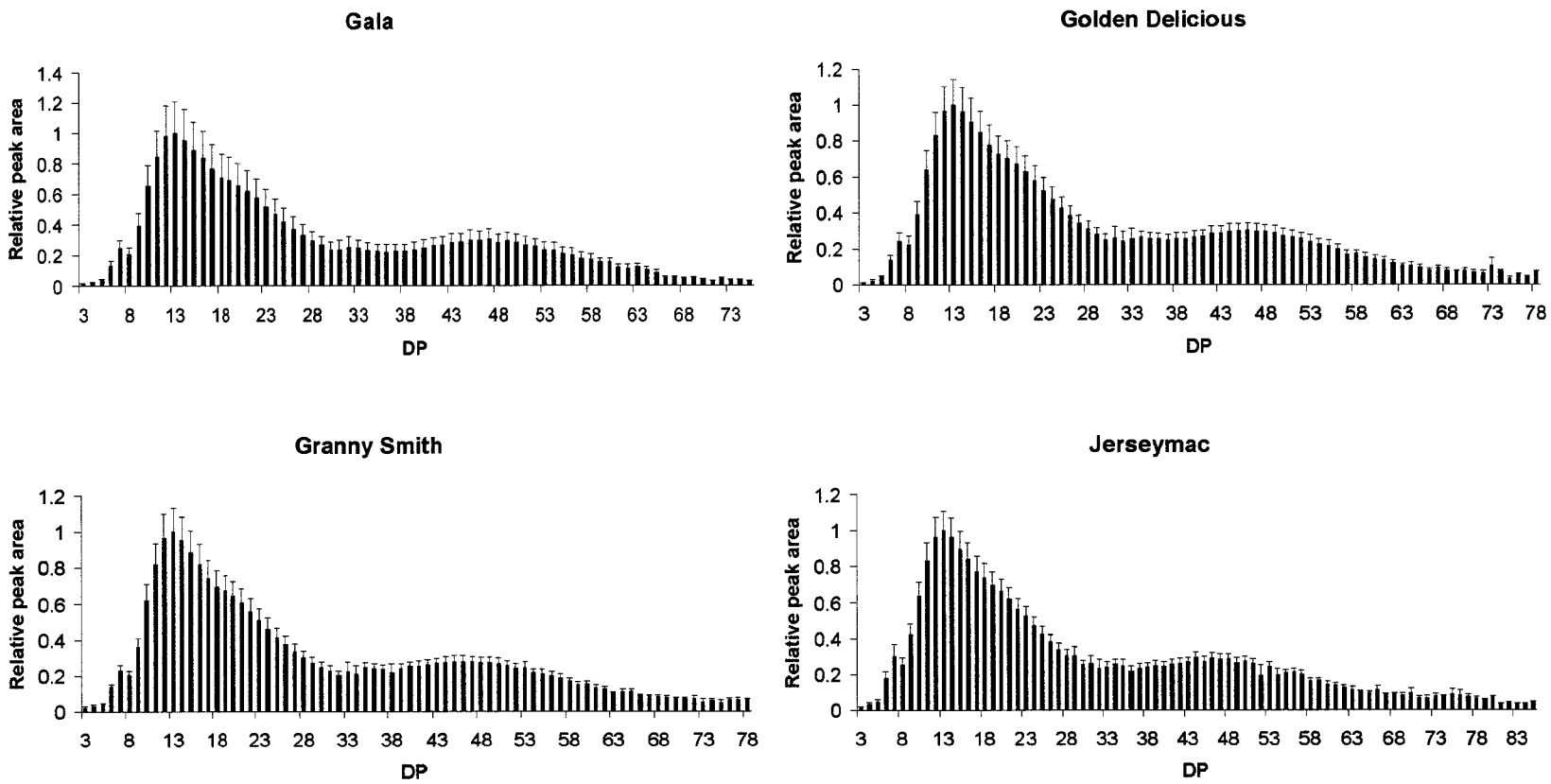


Figure 3a. Relative peak area distributions of Gala, Golden Delicious, Granny Smith and Jerseymac apple fruit amylopectin branch chain-lengths analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of three replicates. DP = Degree of polymerization.

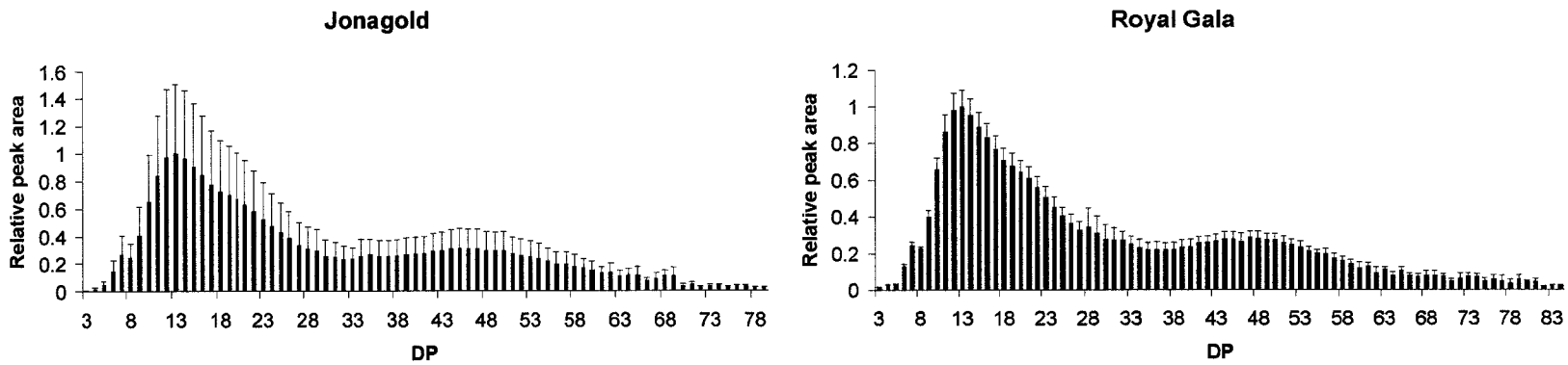


Figure 3b. Relative peak area distributions of Jonagold and Royal Gala apple fruit amylopectin branch chain-lengths analyzed by using a HPAEC-ENZ-PAD. Error bars represent standard error of the mean for each individual DP from two analyses of three replicates. DP = Degree of polymerization.

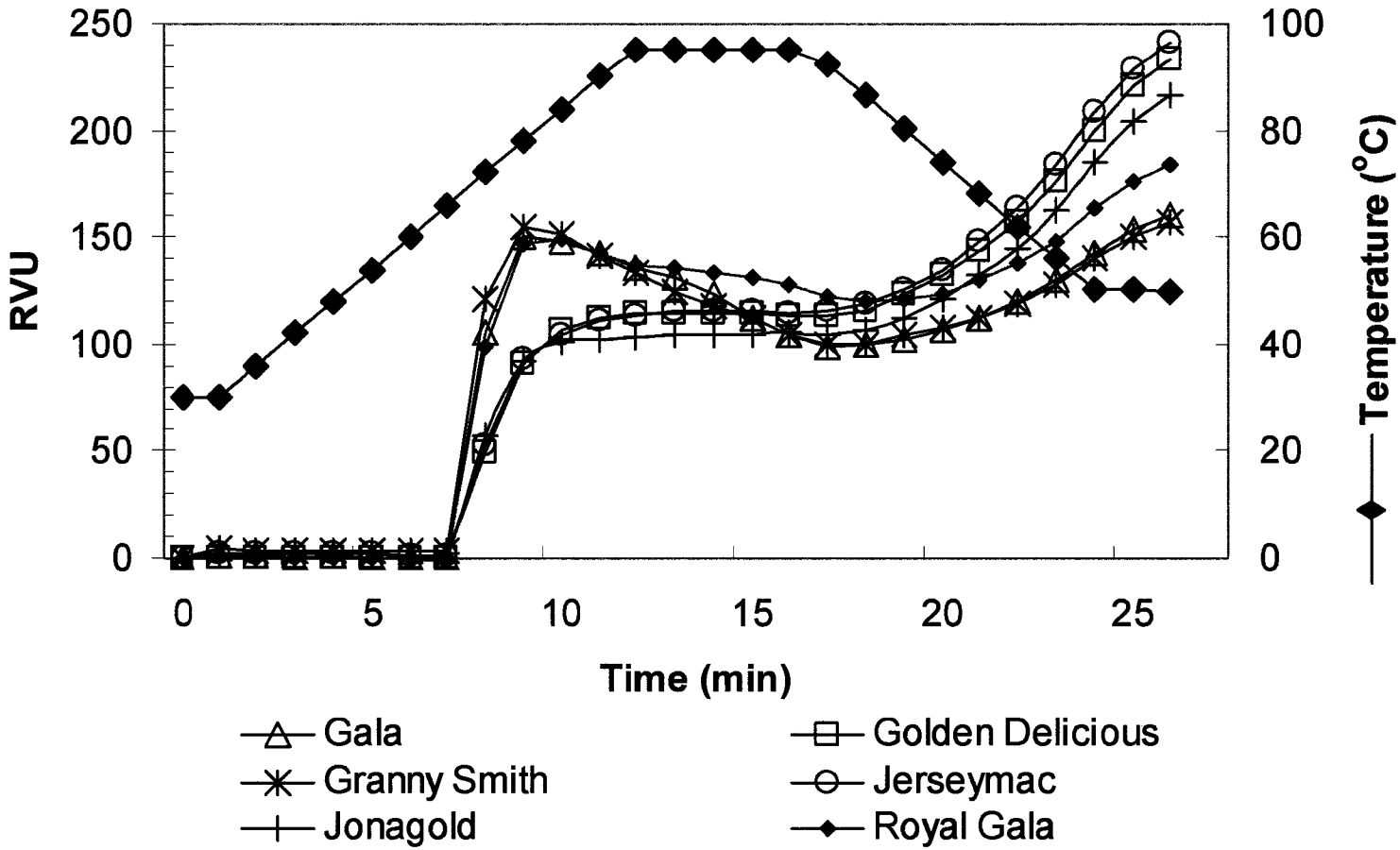


Fig. 4. Rapid Visco-Analyser pasting profiles of Gala, Golden Delicious, Granny Smith, Jerseymac, Jonagold and Royal Gala apple fruit starches (8.0% dsb, w/w).

GENERAL CONCLUSIONS

Starch structural and functional properties of 13 winter squash (*Cucurbita maxima* D.) cultivars were investigated at harvest and after 5 or 10 weeks storage at 12°C. Texture profile analysis was carried out on winter squash fruit steamed at 6 different cooking times from 0 to 20 minutes, and for all three storage times. Correlations among squash starch structural and functional properties, and fruit textural attributes were calculated to investigate the role of starch structure in texture of winter squash and starch functional properties.

Winter squash cultivars could be separated into three groups based on their starch content at harvest: (1) cultivars that accumulate < 1% of dry matter as starch; (2) cultivars that have 11-18% of dry matter as starch; (3) cultivars accumulating high levels of starch with over 50% of dry matter as starch. Squash starches have continuous granule size distribution, ranging from 1.5 to 14 μm in diameter, and exhibit B-type X-ray diffraction patterns. Average starch granule diameter tended to increase after storage. Squash amylopectin fraction had high iodine affinity resulting in large differences between apparent and absolute amylose contents. Absolute amylose content ranged from 11 to 21% at harvest and tended to decrease after 10 weeks storage. Squash starch weight-average amylopectin molecular weight ranged from 2.03 to 5.52 x 10^8 g/mol at harvest and increased after storage. A distinctive feature of squash amylopectins was their very low polydispersity, frequently observed below 1.3 for many cultivars. Polydispersity decreased after storage suggesting there was selective degradation of amylopectin molecules. Amylopectin gyration radius ranged from 294 to 349 nm at harvest and was largely unchanged after storage. Average amylopectin branch chain-length of starch extracted from fruit at all storage times ranged from 25.2 to 29.7. Squash amylopectins had high proportion of long branch chain-lengths

(DP $\geq$ 37) ranging from 25.4 to 30.2 at harvest, and increased after storage for most squash cultivars that had sufficient starch remaining to perform analysis.

Onset and conclusion gelatinization temperature of squash starches from fruit at harvest ranged from 60.6 to 65.0°C and 67.7 to 72.1°C, respectively, and decreased after storage. A distinctive thermal property of squash starches was several cultivars, especially the high-starch content buttercup squash, had very low range of gelatinization temperature (5.7 to 6.5°C for several cultivars). Enthalpy change of gelatinization of squash starches is high ranging from 14.2 to 17.8 J/g. Retrograded squash starches, for 7 days at 4°C, had low onset of thermal transition temperature (typically < 38°C) and high enthalpy change of thermal transition, ranging from 6.5 to 9.8 J/g, and retrogradation percentage ranged from 44 to 60%.

Squash starches had high peak viscosity, ranging from 174 to 233 RVU, high final viscosity (163 to 268 RVU) and high setback (60 to 108 RVU), for starch from fruit at harvest. Breakdown was moderate to high (36 to 89 RVU) and pasting temperature ranged from 65.6 to 73.8°C for starch from fruit at harvest. Pasting properties were not greatly affected by storage time for most squash cultivars. Squash starches exhibit firm and sticky gel properties. In general, gel firmness increased, while gel stickiness decreased for increasing storage duration of fruit that starch was extracted from.

A wide range in hardness of raw squash fruit at harvest was observe with Halloween-type squash fruit hardness (304 and 312 N) considerably lower than buttercup squash hardness (853 to 1083 N). The four buttercups, which had high-starch content, had the four hardest fruit and were considerably harder than the close genetic relative, Hyvita (512 N). Hardness decreased substantially for high-starch squash cultivars between 5 and 10 minutes

steaming, but decreases were much less for low-starch cultivars. There was no change in hardness of fruit after storage at all cooking times and fracturability of raw fruit increased after 10 weeks storage. High-starch buttercup squash cultivars tended to have lower springiness of raw fruit at harvest compared to low-starch squash cultivars. Springiness increased during cooking relative to raw, and was higher for low-starch cultivars, especially Halloween-type squash. Springiness was not greatly influenced by storage time.

Correlations among squash fruit texture and starch structural or functional properties depended on fruit storage time and cooking time. Starch content was positively correlated to hardness and fracturability, and negatively correlated to springiness. Apparent amylose content correlated negatively to hardness and fracturability of squash fruit, but absolute amylose content correlated positively to hardness and fracturability, suggesting long branch chains of amylopectin play a role in texture of squash fruit. Furthermore, hardness and fracturability of squash fruit was consistently correlated to short (DP $\leq$ 12) and long (DP $\geq$ 37) amylopectin branch chain-lengths and negatively correlated to intermediate amylopectin branch chain-lengths (DP 13-36), regardless of storage time. Amylopectin molecular weight and polydispersity correlated positively to hardness of squash fruit, whereas gelatinization temperatures were negatively correlated to hardness. Paste viscosity was negatively correlated to hardness and fracturability of squash fruit, while breakdown was positively correlated to springiness.

Many correlations among squash structural and functional properties were also observed. Apparent amylose was correlated positively to peak viscosity, final viscosity and setback, whereas absolute amylose was positively correlated to peak viscosity and breakdown, at harvest, but negatively correlated to breakdown and positively correlated to

final viscosity for starch extracted from fruit after 10 weeks storage. Higher molecular weight amylopectin with lower polydispersity resulted in lower gelatinization temperatures. High enthalpy change of gelatinization was observed for squash starches with low polydispersity. Squash starches with high enthalpy change of gelatinization had higher peak viscosity. Apparent amylose was positively correlated to gel firmness and negatively correlated to gel stickiness. Wider amylopectin molecules, determined by gyration radius, produce stickier, less firm gels. Starch pastes with low breakdown produced firmer gels. Amylopectins with shorter average branch chain-lengths, particularly less DP $\geq$ 37, produced stickier gels. Squash with high enthalpy of thermal transition of retrograded starch, and paste with high breakdown and low final viscosity result in softer gels. Amylopectin polydispersity was negatively correlated to pasting properties of final viscosity and setback, as well as gel firmness. Average amylopectin branch chain-length was correlated positively to peak viscosity and negatively to pasting temperature. Proportion of long amylopectin branch chain-lengths (DP $\geq$ 37) was positively correlated to peak viscosity.

Low-frequency ultrasound (100 KHz) was transmitted through raw and cooked squash fruit flesh that was stored 7.5 weeks, as a nondestructive method of measuring squash fruit texture. Ultrasonic velocity (UV) transmitted through raw squash fruit was comparable or slower than through air, ranging from 190 to 362 m s^{-1}. UV increased after 10 min steaming with the five high-starch squash cultivars having the fastest UV. Despite squash fruit becoming at least 20 times softer after 20 min steaming, UV increased rapidly, ranging from 1,950 to 2,800 m s^{-1}. Light micrographs show the five high-starch cultivars steamed 10 min have cells engorged 50 to 100% with gelatinized starch, which the seven low-starch cultivars lack, suggesting swollen gelatinized starch mass contributes to higher UV. Fruit

cell wall rupturing depended on cultivar and cooking time. Light micrographs indicate starch and cell walls contribute to squash fruit texture, but an additional factor, possibly turgor pressure, contributes to texture. UV seemed related to behavior of starch within cells and cell wall structure.

Winter squash fruit starches exhibited seasonal variation. Starches extracted from fruit grown 2, 3 or 4 years had variation in the starch structural properties of starch content, amylose content, average amylopectin molecular weight, amylopectin polydispersity, amylopectin gyration radius and amylopectin branch chain-length distribution. Starch functional properties of gelatinization temperatures, enthalpy change of gelatinization, thermal transition temperatures and enthalpy change of thermal transition of retrograded starch, starch paste peak viscosity, breakdown, final viscosity, setback and pasting temperature all varied among the four seasons. Only variation in starch content, amylose content, amylopectin molecular weight and amylopectin branch chain-length distribution showed consistent trends for all cultivars between seasons and this variation could be attributed to rainfall over entire season, and average daily temperatures and solar radiation during squash fruit development.

Variation in textural attributes of winter squash fruit across 2-3 seasons (years) was investigated. Hardness, fracturability and springiness showed high seasonal variation for fruit steamed at various times between 0 and 20 min. Greatest variation between seasons in fruit hardness and fracturability was observed for fruit steamed 10 min. Springiness exhibited greater seasonal variation when fruit was raw or at short duration steaming times. Rainfall over entire season or average daily maximum temperature and solar radiation during fruit development may be contributing to variation in textural attributes observed. Results

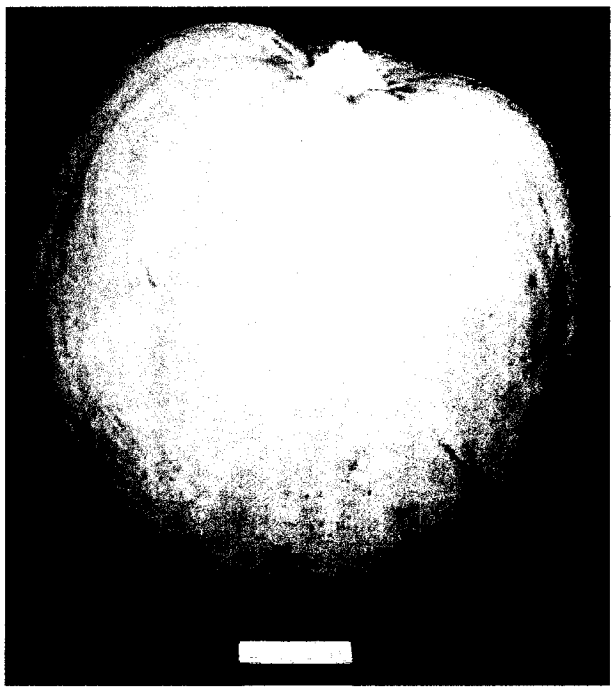
suggest that future studies on the texture of winter squash should consider textural measurements over more than one season to account for seasonal variation.

During cultivation of winter squash, surprising observations were made for the cultivar Zapallo Macre which originates from the Peruvian/Bolivian border. Zapallo Macre fruit vary drastically in fruit size, shape and color. Zapallo Macre fruit also had large differences in starch content with fruit accumulating greater than 60% of their dry matter as starch harvested from the same plants that produced other squash fruit that lacked any starch. Zapallo Macre fruit variation could be divided up into about eight phenotypes, in which starch properties and textural attributes of five phenotypes were studied. The five phenotypes varied in starch content, amylose content, amylopectin molecular size, amylopectin branch chain-length distribution, gelatinization temperatures, enthalpy change of gelatinization, thermal transition temperatures of retrograded starch, all starch pasting parameters, gel firmness and gel stickiness. The five phenotypes also varied in hardness, fracturability and springiness of raw and cooked fruit. Possible commercial utilization of starches from Zapallo Macre could be limited due to its variation. However, the large variation in starch accumulation of Zapallo Macre fruit could make a potential model system for studying the mechanism and regulation of starch biosynthesis in storage organs.

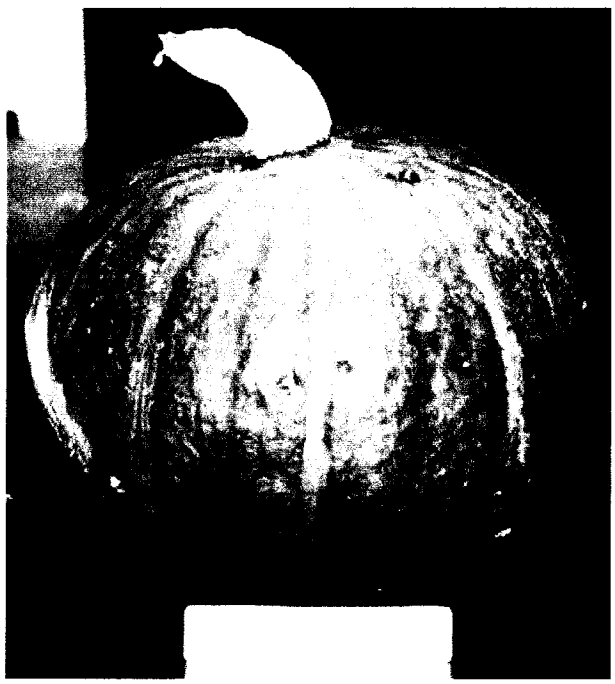
Structural and functional properties of six apple cultivar starches were also characterized. Starch comprised 44 to 53% of the dry matter of immature apple fruit. Apple starch granules varied in diameter from 2 to 12 μm and granules were compound. Apple starches exhibited C-type X-ray diffraction patterns, but A-type crystalline packing was predominant. Apple starch had high apparent amylose content (40 to 48%), but iodine affinity of amylopectin fraction was also high, resulting in absolute amylose content of 26 to

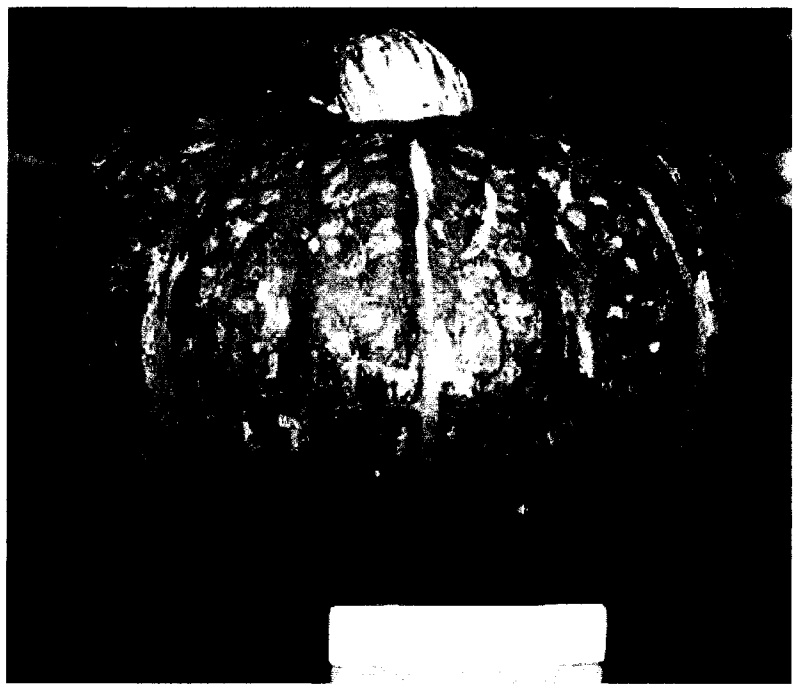
29%. Weight-average amylopectin molecular weight was large (4.6 to 11.1 $x10^8$ g/mol). Proportion of long amylopectin branch chains (DP $\geq$ 37) was higher than any other starch and ranged from 29.7 to 32.4. Onset gelatinization temperature ranged from 64 to 66°C and enthalpy change of gelatinization was high (16-18 J/g). A distinctive characteristic of apple starches was three cultivars had extremely low breakdown (< 4 RVU) and very high setback (> 100 RVU). Peak and final viscosities ranged from 99 to 148 RVU and 144 to 224 RVU, respectively, and pasting temperature was about 70°C. Apple starches produced both firm and sticky gels.

APPENDIX A – SQUASH FRUIT AND OTHER PICTURES OF CULTIVATION.

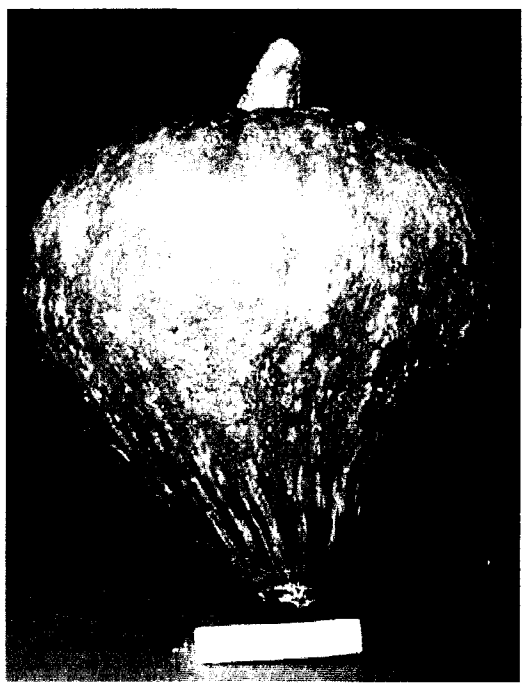


Big Max (above) and Cha Cha (below).

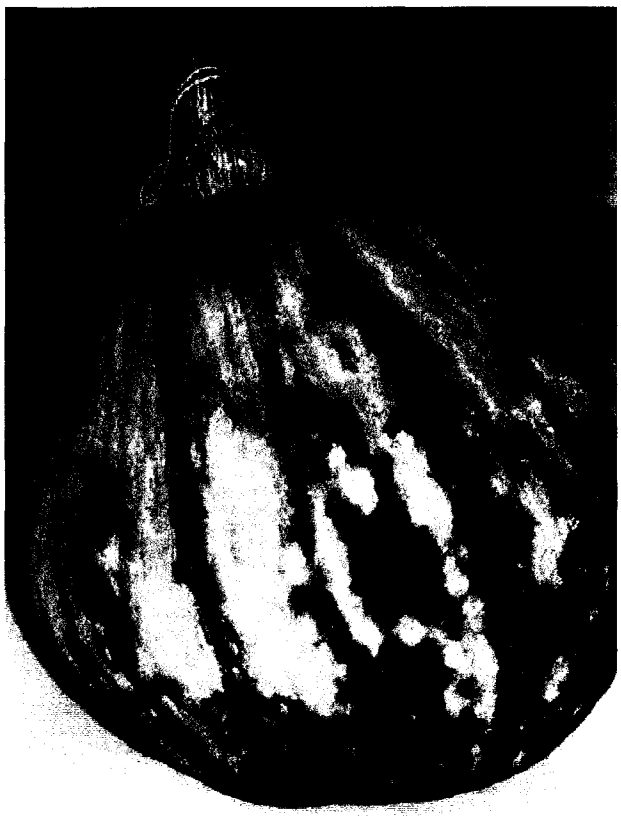




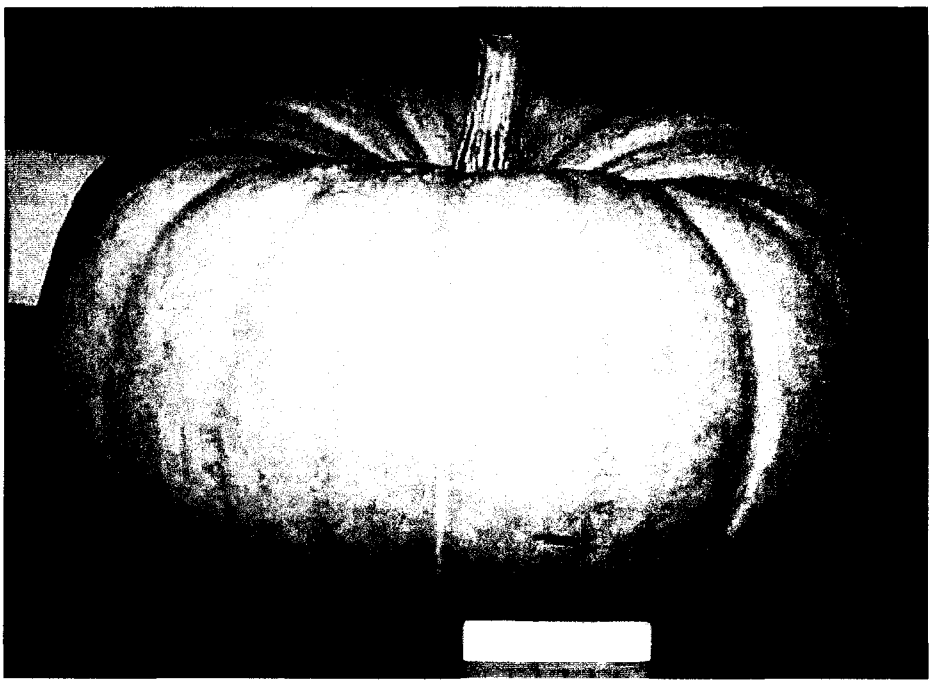
Delica (above) and Hyvita (below).



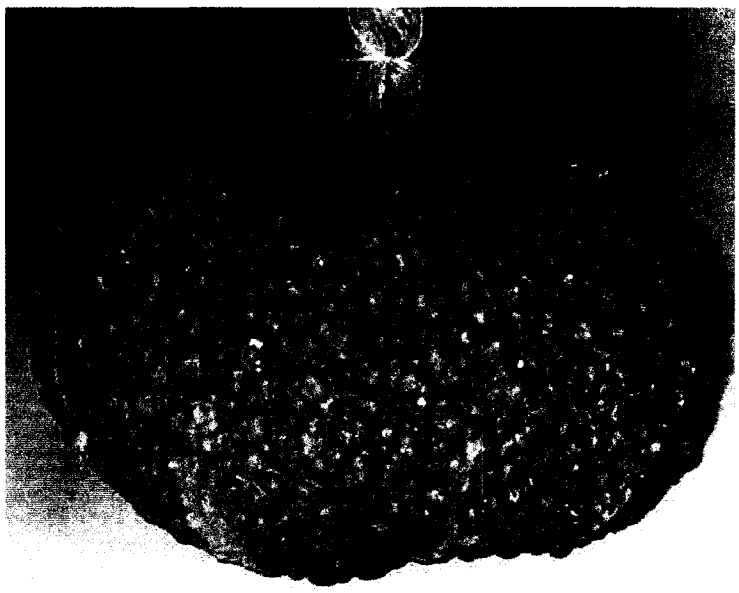
Kurijiman (above) and Lakota (below).

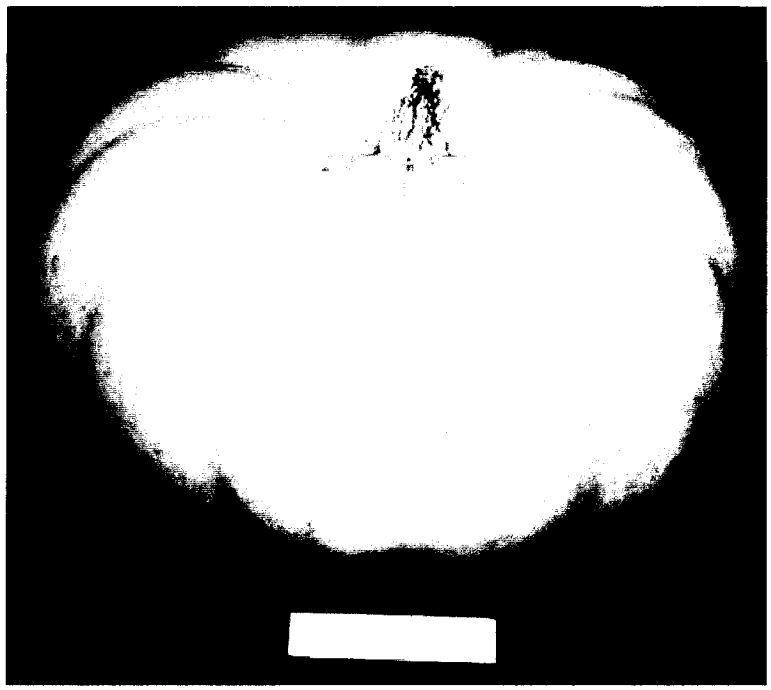


Prizewinner (above) and Rouge Vif D'Etampes (below).

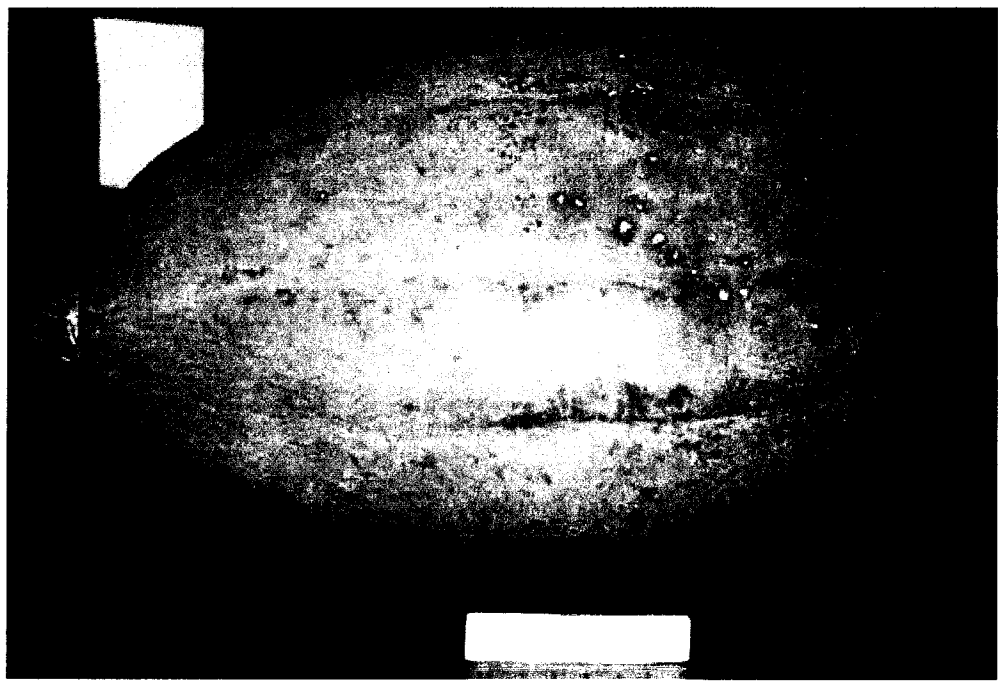


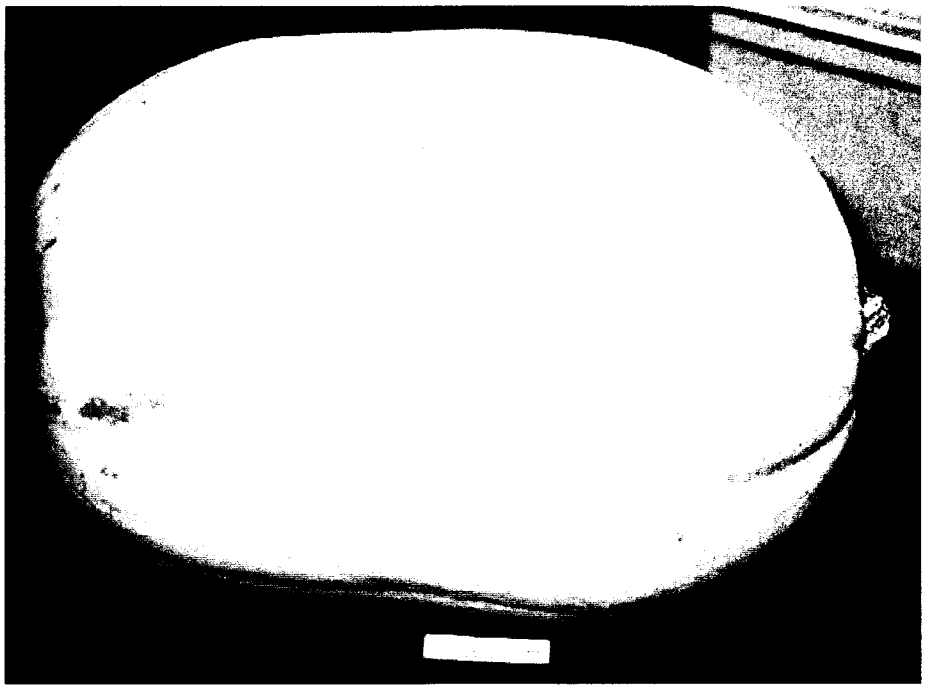
Sweet Mama (above) and Warren Scarlet (below).



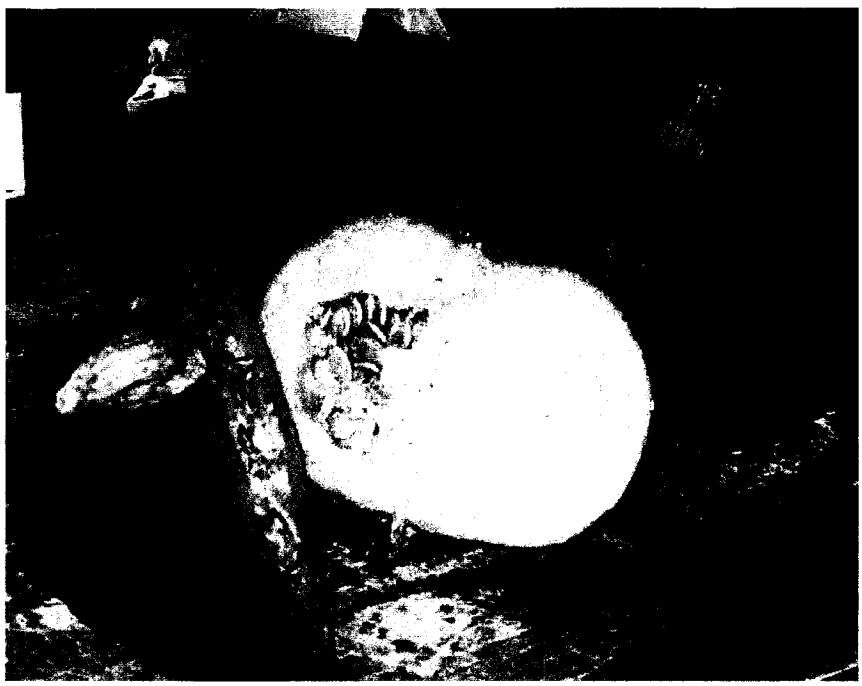


Whangaparoa Crown (above) and Yogorou (below).

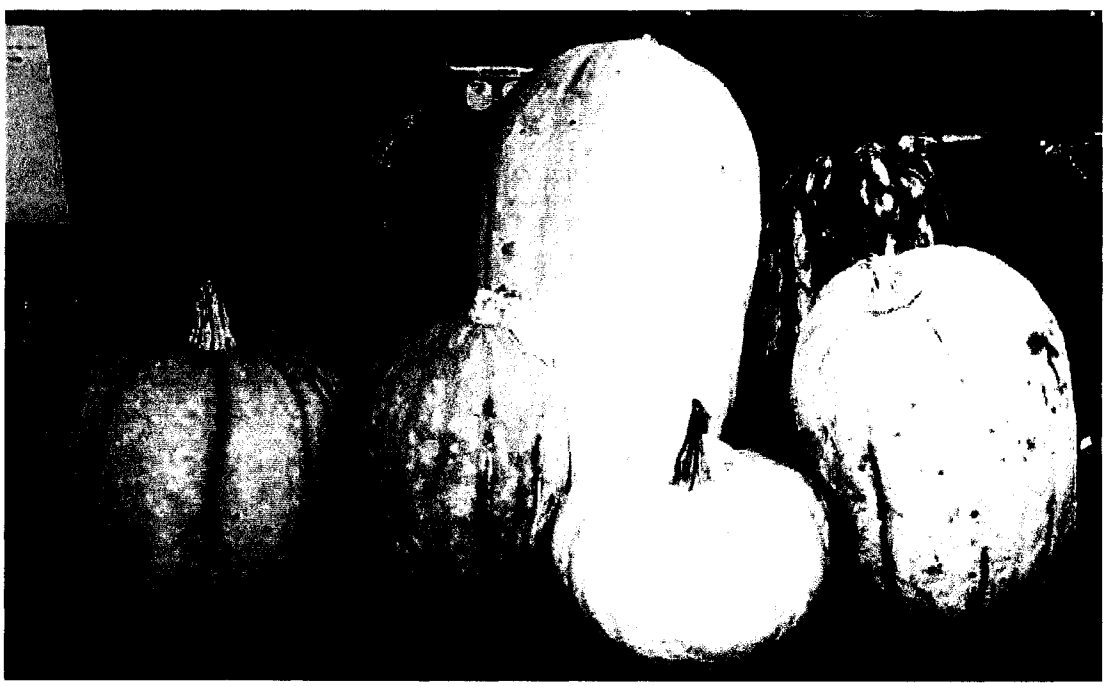




Zapallo Macre (above) and interior of buttercup squash (below).

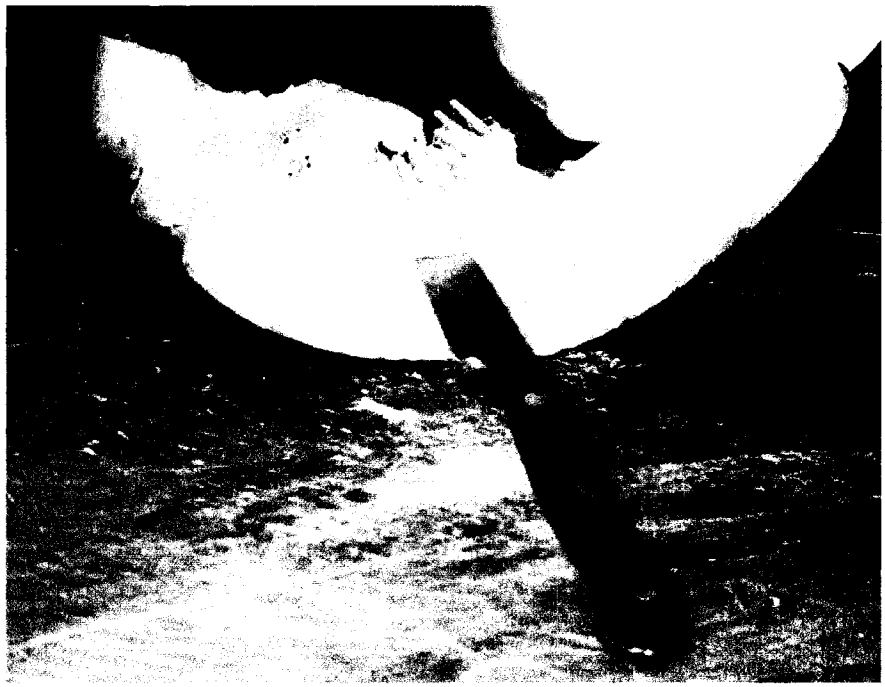


Some cultivars showed incredible diversity. Shown here is the diversity for Lakota (above) and Zapallo Macre (below).



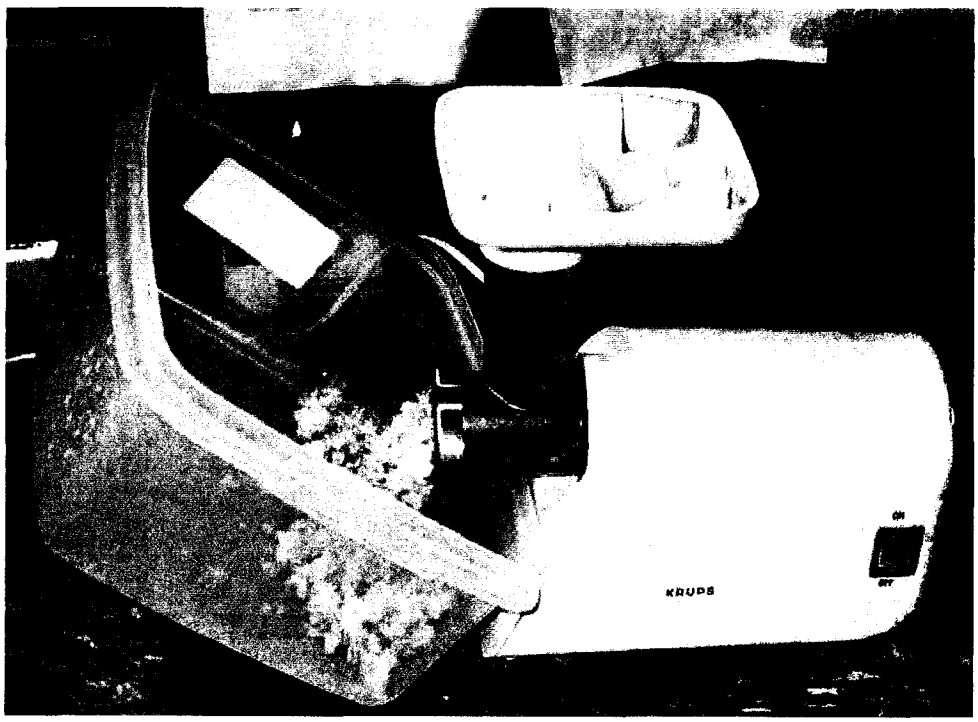
Above: Squash seeds were germinated in greenhouse and seedlings transplanted.
Below: Field site in August, 2000.

Above: Squash fruit stored at 12°C.
Below: Squash fruit sliced longitudinally for starch extraction.



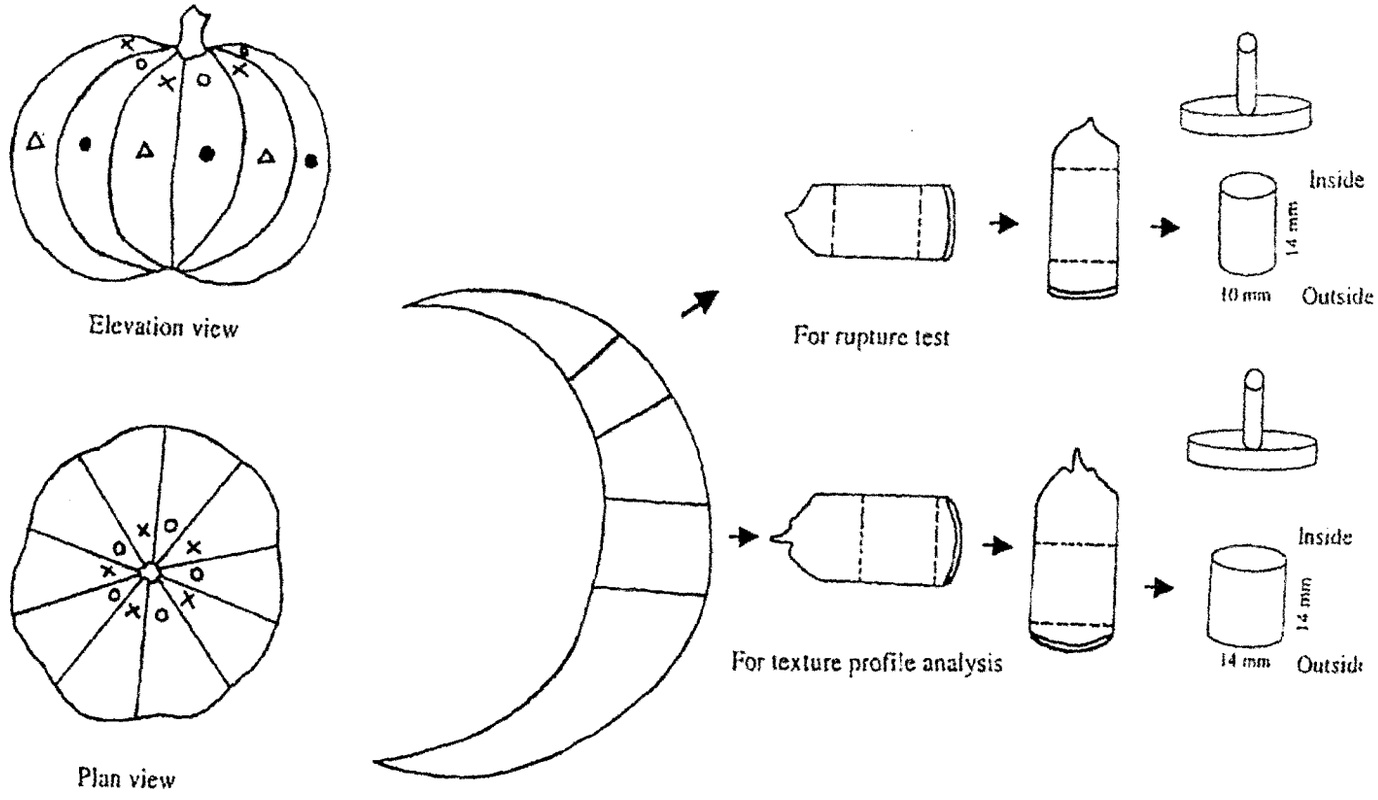
Above: Deseeding of fruit for starch extraction.
Below: Removal of skins for starch extraction.

Above: Squash fruit cut into cubes so it could fit into meat grinder.
Below: Squash fruit ground in meat grinder prior to blender because of its hardness.



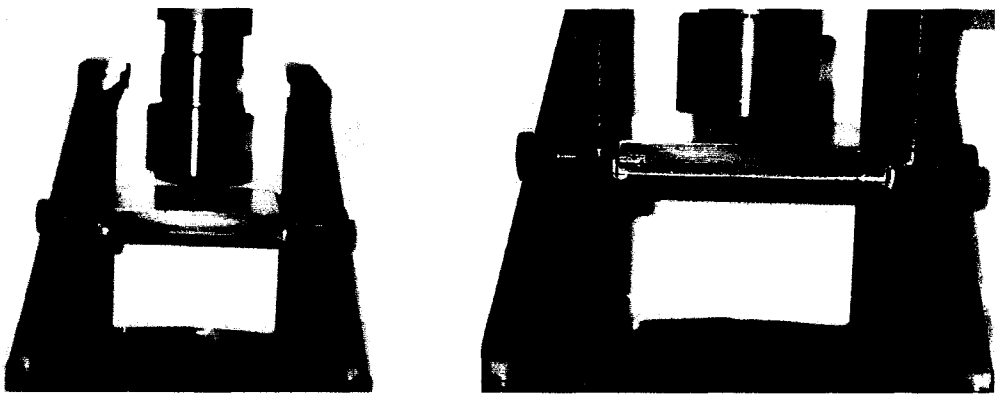
Whangaparoa Crown and Delica sold as whole fruit (above) and as segments (below) at a store in New Zealand. Photos courtesy of Jina's Fruit and Vegetable Market, Upper Hutt, New Zealand.

APPENDIX B – INSTRON UNIVERSAL TESTING MACHINE MEASUREMENTS OF TEXTURE

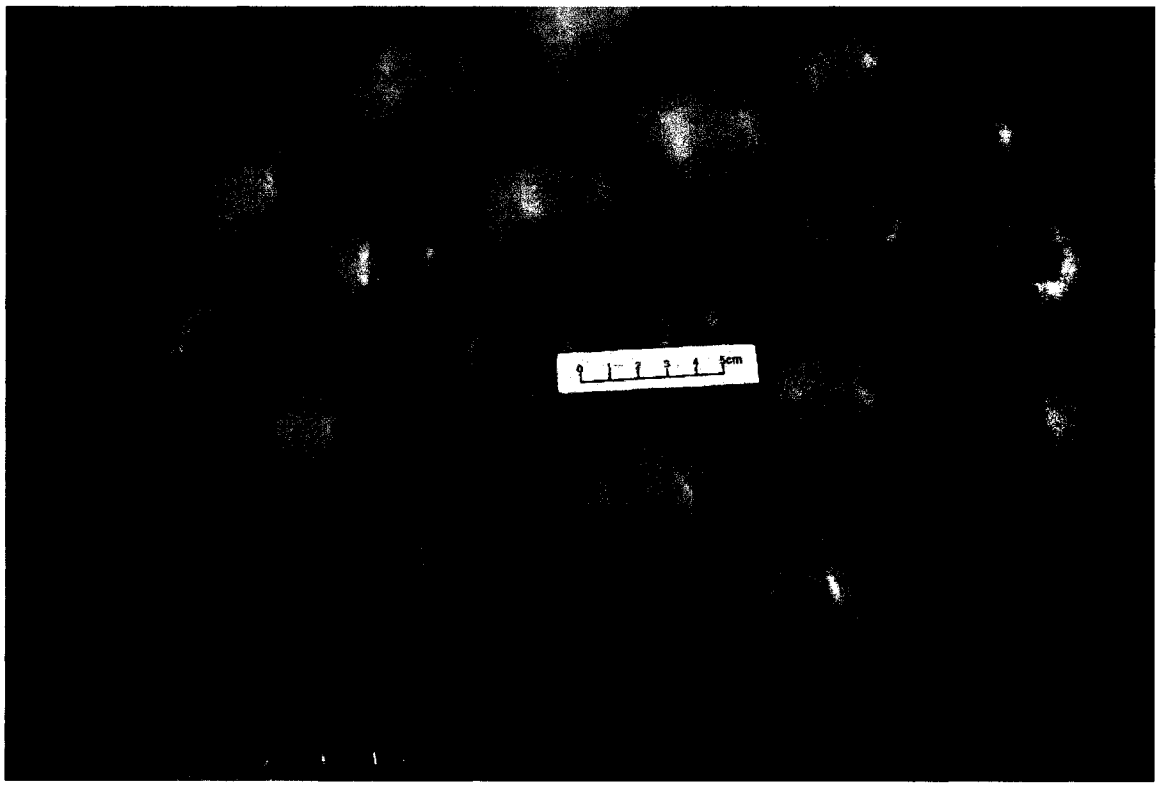


For squash texture profile analysis, samples of raw and steamed longitudinal fruit slices had a cored fruit cylinder removed from equatorial region and trimmed to have a height of 12 mm and diameter of 20 mm, similar to this above diagram obtained from Ratnayake (2001) Physical properties of squash (*Cucurbita maxima*) cell walls. Ph.D. thesis, Auckland University, Auckland, New Zealand. pp. 54.

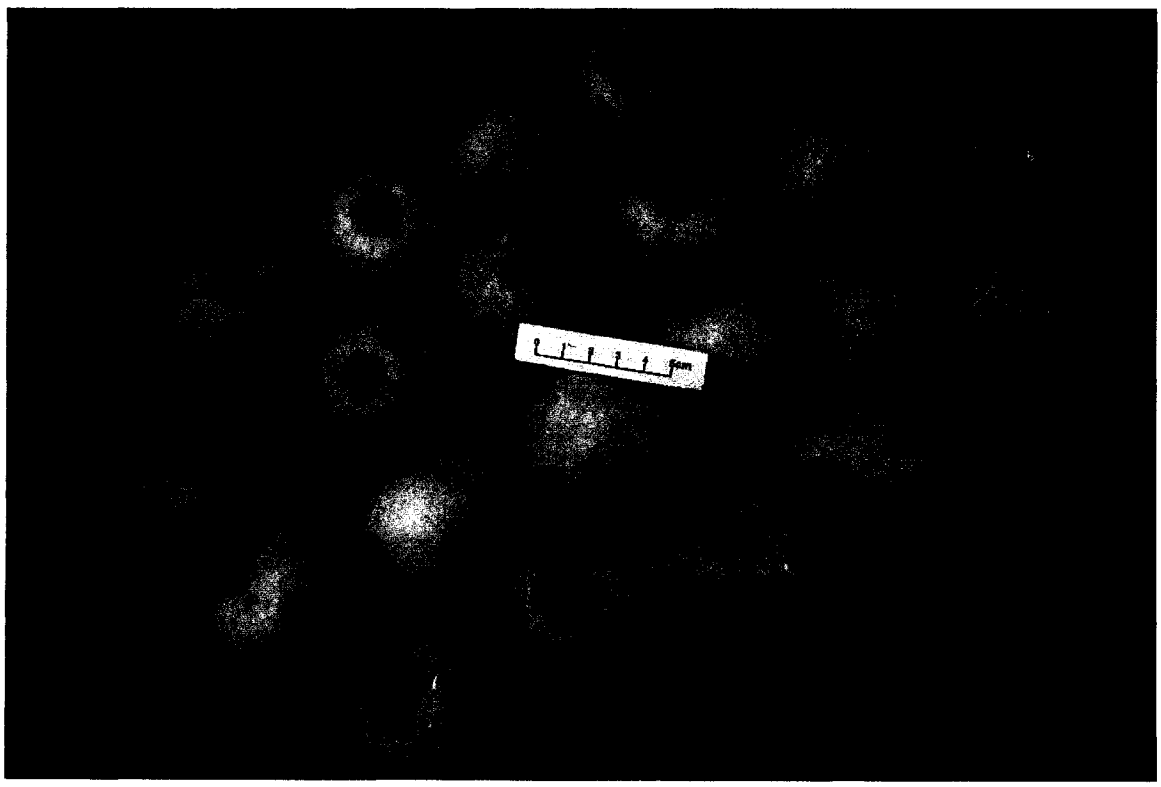
Above: Squash fruit steamed
Below: Fruit cylinders used for Instron Universal Testing Machine textural profile analysis.

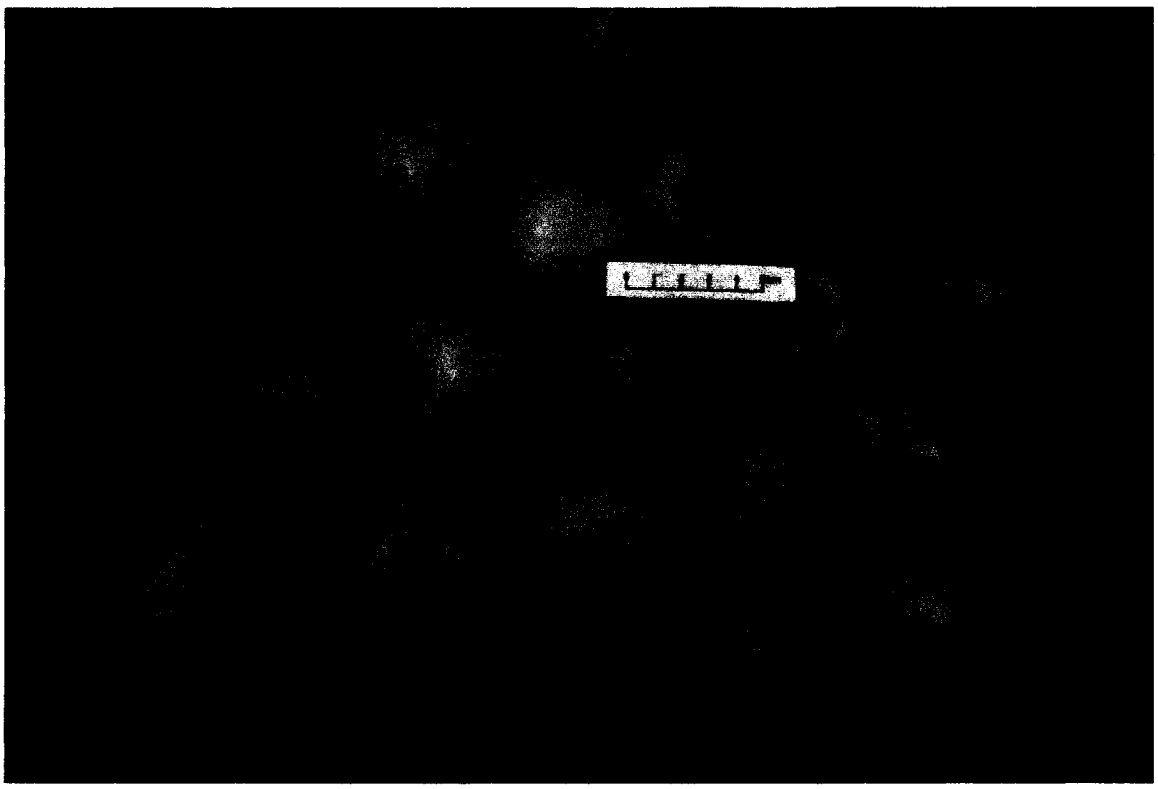


APPENDIX C – APPLE CULTIVARS SELECTED FOR STARCH STUDY

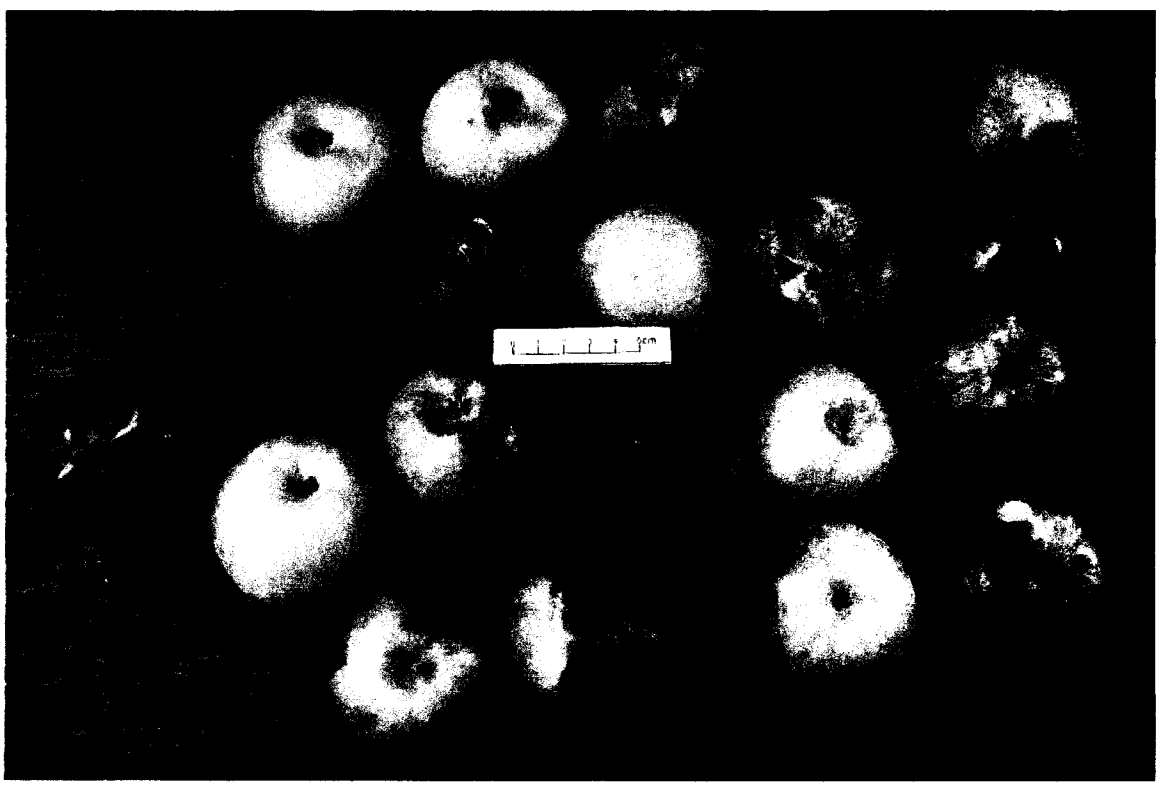


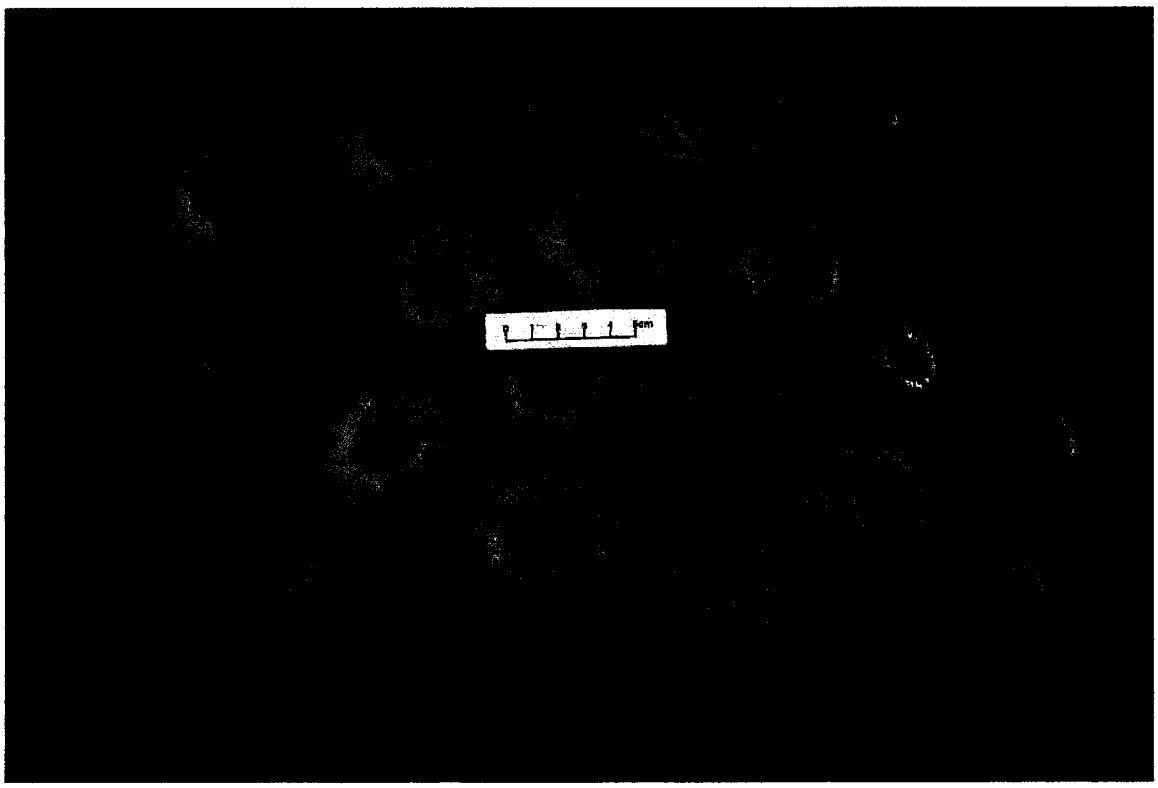
One apple replicate selected for Granny Smith (above) and Gala (below)



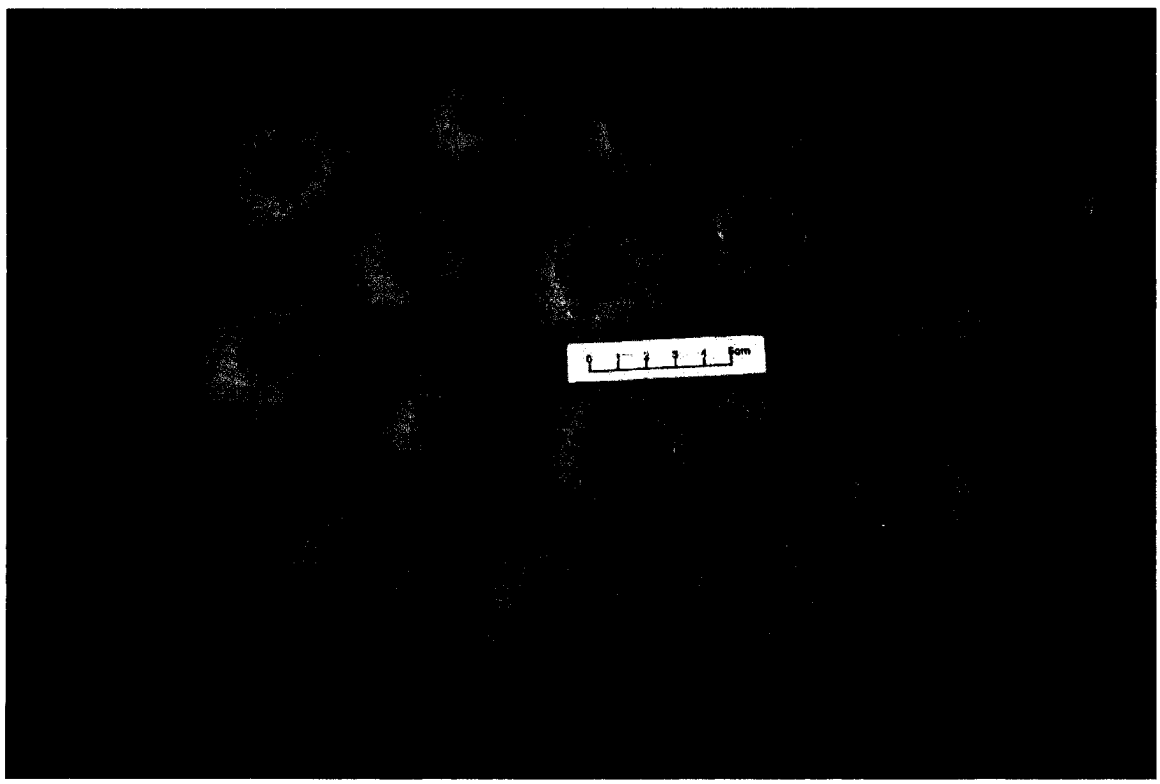


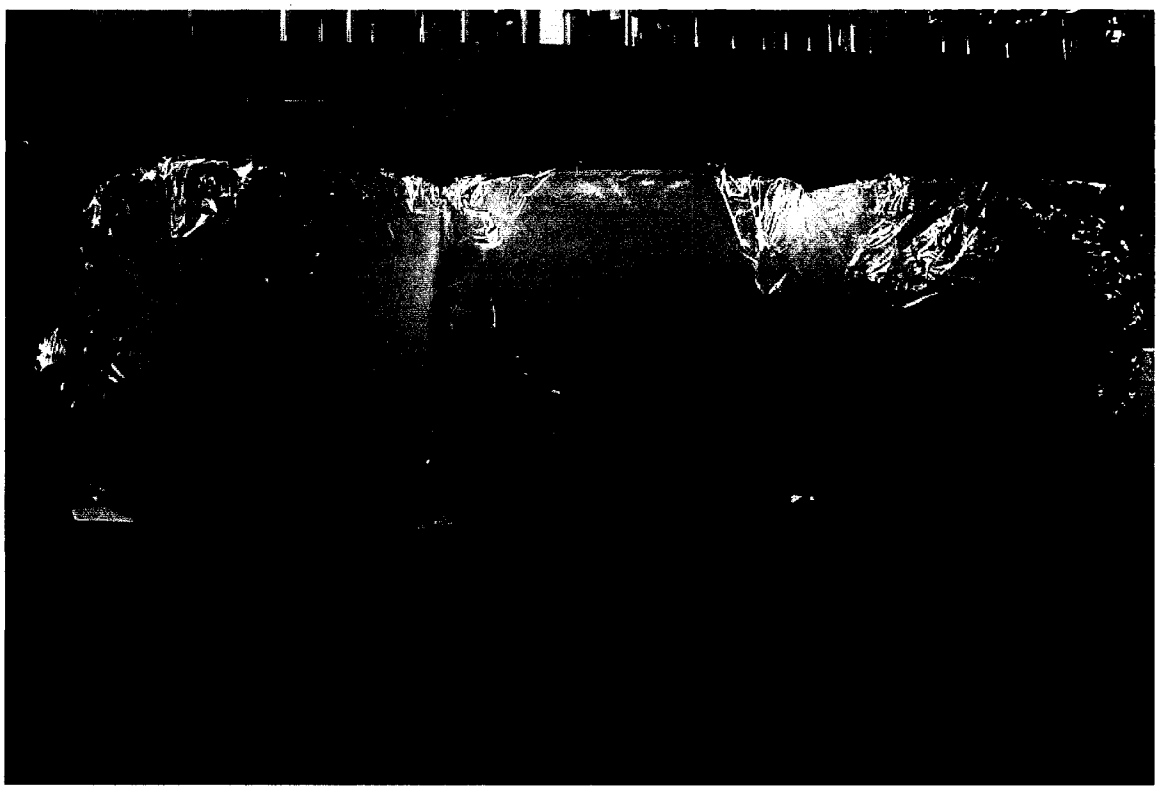
One apple replicate selected for Royal Gala (above) and Jerseymac (below)





One apple replicate selected for Jonagold (above) and Golden Delicious (below)



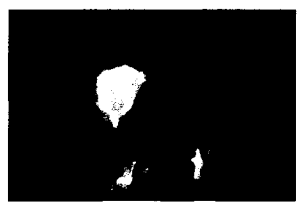


Above picture highlights the importance of not using a meat grinder initially to grind apple fruit prior to blending apple fruit in sodium metabisulfite. The three samples are Granny Smith apple extracts. The extract on left had apple fruit ground in meat grinder and then immediately blended in sodium metabisulfite. The center and right extract both had apple fruit sliced and immediately blended in sodium metabisulfite. All three replicates had almost identical apple fruit weight that was extracted. The meat grinder resulted in less sodium metabisulfite needed to ground fruit but as can be seen from the starch depositing at bottom of each container, apples ground in meat grinder first resulted in a considerable loss of starch suggesting that apples have very active amylases, and therefore studies involving apple starch must extract fruit by immediately placing sliced fruit in sodium metabisulfite.

APPENDIX D – WHY ARE JACK-O-LANTERN PUMPKINS USED FOR HALLOWEEN CARVING?

The Halloween pumpkins of Prizewinner and Big Max accumulated very little starch and Rouge Vif D'Etampes degraded its starch within 5 weeks of storage. Therefore I propose that Jack-o-Lantern type pumpkins are used for Halloween because they either lack starch or have degraded it by October 31, and this lack of starch decreases their hardness making them easy to carve.

Carvings by David Stevenson



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