1983

Effect of heat denaturation of whey proteins on the rheological properties of cornstarch-milk systems

Li-Hsiang Ling
Iowa State University

Ling, Li-Hsiang

EFFECT OF HEAT DENATURATION OF WHEY PROTEINS ON THE RHEOLOGICAL PROPERTIES OF CORNSTARCH-MILK SYSTEMS

Iowa State University PH.D. 1983

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __√__.

1. Glossy photographs or pages __✓__
2. Colored illustrations, paper or print _____
3. Photographs with dark background __✓__
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages _____
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

Effect of heat denaturation of whey proteins on the rheological properties of cornstarch-milk systems

by

Li-Hsiang Ling

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Food and Nutrition
Major: Food Science

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1983

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Both milk and starch are important constituents of many foods and have been the subjects of many investigations. However, study of the interaction of the two has been extremely limited (24,35,60,73).

Small amounts of other substances are always associated with starch as it occurs in nature and more are present in foods. The kind and amount of these compounds appear to influence starch behavior, which in turn affects the textural properties of foods. Model systems containing only one or two substances besides starch and water have been studied (2,59,60,62,65). Addition of milk to starch yields a considerably more complex system in which, besides starch and protein, the presence of lactose, numerous salts and fat (even in skimmilk) must be considered.

Study of stability of starch pastes and white sauces to freeze-thaw treatment by Osman and Cummisford (61) indicated constituents of milk in sauces exert appreciable effect on stability, which was evident by the fact that less liquid separated in the sauces than in the starch pastes. Studies of effects of preheating of milk on starch-milk pastes by Hwang (35) and Stalder (73) showed that the differences in Amylograph viscosity patterns during heating as well as gel strengths were related to the temperature at which

preheating had been carried out and the resulting degree of denaturation of whey proteins. Grant (24) later showed that addition of a large amount of sugar did not eliminate the effects of preheating the milk.

Although this effect of previous heat treatment of milk on viscosity and gel strength of starch-thickened foods has been generally unrecognized, it is important to the food industry and appeared to need further investigation. The present study was designed to learn more about the mechanism of this apparent interaction between starch and milk, including such factors as swelling of the starch granules, extent of denaturation of whey proteins and the possible importance of individual whey protein.

LITERATURE REVIEW

Starch

Starch is used to perform different functions in foods. The thickening and gel forming properties are by far the most important. The consistency of sauces, the firmness of pie fillings, the baking of bread, and the fabrication of snack foods all rely on proper gelatinization of starch to produce desirable viscosity and texture. The rheological properties of starch are influenced by the source of the starch and sometimes its modification and by other constituents present in the food system.

Swelling and viscosity

Raw starch is insoluble in water. When starch is mixed with cold water, water can penetrate into amorphous regions of the starch granules, but swelling of the granule is too small to be readily observed. Proton magnetic resonance supported previous observations that absorption of water by the granules before initial gelatinization occurs is reversible (38). It was shown that when the temperature of a starch suspension was increased but remained below the gelatinization range, water mobility was reversibly decreased.

With rising temperature to supply sufficient energy to break intermolecular hydrogen bonds in the amorphous

regions, granules begin to swell. The swelling of granules causes a loss of the radial orientation of the crystalline regions and results in a loss of birefringence. This phenomenon, gelatinization, starts at the hilum of the granules and spreads rapidly to the periphery (45). It has been found that undamaged wheat starch granules immersed in water at 50°C for 72 hrs gelatinized at a higher temperature and the gelatinization occurred more suddenly than in control samples. This study indicates a modification of the internal structure of the granules (23).

Further heating of starch granules causes more swelling and allows additional water to enter into the granules. The adsorption of water during and following gelatinization becomes irreversible. As a result of the granules swelling, there is an increase in starch solubility, paste viscosity and paste clarity. In a concentrated starch paste, the swollen granules gradually become susceptible to shear disintegration with a reduction in viscosity. Granules which are capable of swelling to a high degree are more susceptible to breakdown on heating than are granules that undergo more limited swelling.

The major factor contributing to the viscosity of a starch paste is the presence of highly swollen starch granules (45). Water entering the granules causes the starch to swell greatly, leaving less available water

in solution. Therefore, the probability of granules coming into contact with each other increases and results in an increase in viscosity. However, soluble starch components leached from the swollen granules may associate with the granules and form a matrix held together by hydrogen bonding. It is believed that the apparent viscosity of a starch paste is caused by both the individual swollen granules and the interaction between soluble starch components and the swollen granules.

Three methods have been applied most often to study starch gelatinization temperature: loss of birefringence, increase in optical transmission and rise in viscosity. Others, such as susceptibility to hydrolysis by α-amylase, have recently been used (22). Measurement of the loss of birefringence is the most sensitive, accurate and reproducible technique. Because individual granules of each starch species differ not only in size and shape but also in the degree of association in amorphous regions, the granules gelatinize over a temperature range rather than at a single temperature. Microscopy employing a heating-stage and polarized light provides a simple and rapid means for detecting the loss of birefringence (68). The gelatinization temperature determined by this method is independent of concentration and heating rate but is a function of granule structure. It supplies information only on the beginning of swelling

of granules. The further swelling and amount of rupturing of granules are more interesting to the industry and can be obtained with a Brabender Amylograph. In this instrument, the starch suspension is heated at a constant rate of 1.5°C per min in a container that is revolving at a constant speed. A sensing device, consisting of a disk with several rods extending into the revolving starch suspension, provides consistent shearing as well as measuring the torque and transmitting it to a recording device. The Amylograph viscosity is dependent on concentration, heating rate and shearing conditions.

Gel formation and retrogradation

Upon cooling, the colloidally dispersed hot paste normally increases in viscosity. If it is allowed to stand without stirring, both amylose and amylopectin molecules have a tendency for intermolecular bond formation within the swollen granules and in the aqueous solution between the granules. This causes formation of crystalline micelles and results in a three dimensional gel network except at extremely low concentration, in which the starch precipitates. When only amylopectin molecules are present, the outer branches prevent the degree of association required for gel formation except when a 30% or greater concentration is present.

Retrogradation can be regarded as a progression in the

firming of a starch gel. When gels are held for periods of time, crystallization of the starch molecules continues to increase. This process makes some of the liquid separate from the lattice, resulting in syneresis and a toughening of the gel as well as the appearance of a skin on the surface of the cooled paste. It occurs most rapidly when the gel is frozen and thawed. Under this condition, even waxy starch retrogrades. Birefringence caused by crystalline micelles has been observed after retrogradation of gels (69).

Ott and Hester (63) studied the amount of soluble amylose needed for gel formation. They specified four types of gel structures with regard to amylose and degree of swelling of starch granules. In the first gel system (amylose gel), water was trapped with amylose (1.4%) to form a three dimensional network. In the second gel system (unmodified waxy corn starch), highly swollen and fragmented starch granules were associated with water. One percent of amylose was required to produce a gel with strength equivalent to that of the amylose gel. In the third type of gel (slightly cross-linked waxy corn starch), highly swollen but intact granules which bound a large proportion of water existed. Only 0.5% amylose was needed to give a gel of equivalent strength. The fourth gel (highly cross-linked waxy corn starch) contained intact starch granules which were less highly swollen. Such granules could provide some

structure but 0.7% amylose was needed in this gel.

Several factors are involved in determining characteristics of starch gels, such as the type and size of starch granules, the paste concentration, cooking time and temperature, agitation during cooking, storage time and temperature after heating, type and amount of added ingredients.

Effect of other substances on starch behavior

Rheological properties of starch are influenced by other substances present in the system. These substances may be impurities in the starch or other constituents of the system.

Salts A progressive increase in effects of ions on gelatinization temperature was found for the lyotropic anion series (SCN^-, I^-, NO_3^-, Br^-, Cl^-, F^-, SO_4^{2-}). In general, for concentrations, below 1 M, SO_4^{2-}, F^-, and Cl^- ions increased gelatinization temperature, while Br^-, NO_3^-, I^-, and SCN^- ions decreased this temperature (49). The effect of salts on the overall pasting properties is more complicated. The literature on this subject is conflicting. The reason is because a different cooking temperature might completely reverse the relative values for a certain salt at two concentrations or two salts at the same concentration. Osman (59) found that 0.5 N Na_2SO_4 delayed an increase in viscosity shown by the Amylograph until 85°C and it introduced a second increase in viscosity during a holding period at 95°C. However, at a concentration of 0.001 N Na_2SO_4, the

Amylograph curve started to increase below 80°C and it began to decrease at 95°C. The Amylograph curves from these two pastes crossed each other. In spite of these variations, certain values for temperature and viscosity during gelatinization fall in an order of lyotropic series. It has been reported that increasing temperature at maximum viscosity and decreasing maximum viscosity were in the order of lyotropic series at 0.43 M salts concentration (52). In proteinaceous foods, the effect of salts on proteins appeared to mask the effect that salts had on starch (33).

Tipton, as cited by Stalder (73), reported that NaCl, KCl, and Na_3PO_4 at a concentration of 0.1 N altered the Amylograph pattern only slightly from that obtained with water. She also found that higher concentrations of NaCl and other salts, usually at concentrations higher than those found in milk, were required to produce any differences in the Amylograph curves. However, Hwang (35) found that a protein-free milk system prepared by dialyzing 300 ml of 5% lactose solution against approximately 610 liters of pasteurized skimmilk produced an Amylograph pattern that differed greatly from that produced by the milk or by a 5% lactose solution. This dialyzed solution contained all the soluble milk salts naturally present in skimmilk as well as the lactose.

A more interesting effect of salt on the gelatinization of starch was studied by Ganz (19). He showed that when

0.43 M NaCl was added to wheat starch paste at 70°C, the peak viscosity was lower than the control containing no NaCl. When the NaCl was added at 60°C, the peak viscosity was enhanced and more time was required to reach it. He suggested that the salt inhibited the opening of the crystalline regions in the granules and altered the course of fragmentation at high temperatures.

Lipids Osman and Dix (62) reported that when triglycerides (soybean oil hydrogenated to different degrees) were added to an aqueous slurry of cornstarch (not defatted), the maximum viscosity remained unchanged but occurred at a lower temperature than in the control. The characteristic step in the cooling curve was removed. Degree of unsaturation had no influence on the results. When polar lipids, e.g., monoglycerides but not lecithin, were added to the above mixture, the temperature at maximum viscosity was raised and the step reintroduced into the cooling curve. They suggested that an amylose-containing starch and polar lipids are needed to produce this effect. This theory was supported by later work of Gray and Schoch (25), who reported that polar lipids restricted the swelling and solubilization of ordinary starch by forming a complex with the amylose. Polar lipids extracted from wheat starch (53) reduced the maximum viscosity attained by starch paste in the Amylograph, but nonpolar lipids had the opposite effect.

Carbohydrates The presence of pentosans in wheat flour increased dough development time. Jelaca and Hlynka (39) reported that pentosans isolated from flour had a high affinity for water. In a Farinograph, water-soluble pentosans absorbed 4.8 g H_2O per g pentosans in a gluten-water system in which starch had been washed from the flour, 6.9 g in a starch-water system, and 6.5 g in reconstituted gluten-starch dough. This study showed that water-binding capacity of pentosans depended not only on the amount of pentosans present but also on the nature of the flour components.

The effect of saccharides on the rheological properties of starch is derived from the ability of saccharides to bind water. Sucrose at 50% concentration of the weight of water in a slurry competes with starch for the available water and therefore inhibits the swelling of the granule, increase the temperature at which birefringence disappears, lowers maximum viscosity, and reduces the thinning of the paste after completion of granule swelling (65). This competition reduces the gel strength and, therefore, leads to a decrease in syneresis. But at low concentration of sugars (5%), the delay in swelling is not great and the maximum viscosity, in some instances, slightly increases. Disaccharides exhibit a greater effect than monosaccharides on retarding the swelling of granules when present at the same concentration by weight (2). The competition effect of sugars with starch

for water is true for starch-water system but a more complicated situation arises when flour is studied. Hester et al. (29) indicated that wheat flour heated in the presence of sucrose (40%) had higher maximum and final viscosities and gels seemed to be firmer than those made with starch alone. It is apparent that sucrose has an influence on constituents of flour other than starch, which alters the paste and gel properties. The effects of 5% lactose, the approximate concentration found in milk, on the viscosity of 5% cornstarch was studied by Hwang (35). She found a slight increase in the maximum viscosity but the temperature at which the maximum viscosity occurred did not change.

Milk Protein has a significant effect on starch behavior because it interacts with it. Interaction between starch and milk protein was investigated by Hwang (35). She found that the Amylograph curves of 5% cornstarch in skimmilk of two different sources differed. Skimmilk from University of Illinois herds, pasteurized in the Dairy Technology Laboratory at approximately 71°C for 15 seconds, produced an Amylograph pattern with a first increase of viscosity at 70°C, after which a second increase in viscosity at 95°C was produced. Skimmilk from commercial sources did not show this second peak viscosity. Stalder (73) continued this work and found that the second peak viscosity was due to milk protein that was not denatured before

the starch-milk suspension was heated. Further evidence of of the importance of the milk protein was shown by the pronounced increase of viscosity in a paste made with a milk formed from the serum of unheated milk and the precipitate of heated milk, which contained both undenatured and denatured whey protein. It was thus apparent that the differences in the milk from the two sources were related to the heat treatment they had received and that the commercial product, in order to prolong its shelf-life, had been pasteurized at a higher temperature than required to destroy pathogenic organisms.

The two-step gelatinization pattern of the Amylograph curve produced by the milk from University of Illinois herds could be eliminated by addition of 4 μg/ml NaOH to the skim-milk, which raised the pH less than 0.1 unit. The Amylogram was similar to that of commercial milk. Similar addition of HCl to commercial milk caused it to produce an Amylograph pattern characteristic of University milk (35). This sensitivity of starch-milk system to acid and base was also confirmed by Stalder (73). She found the addition of 0.004 mg/ml of HCl to the heated milk recovered the second peak viscosity. Tipton, as cited by Stalder (73), added 0.002 milliequivalent/ml $CaCl_2$ to commercial milk to produce the step characteristic of University milk. The increase in viscosity was also reversed by addition of NaOH. The

effect of these small changes in concentrations of acid and base on the Amylograph curve of starch-milk pastes is not yet explained. Upon examination of the Amylograph response, it seems that rheological properties of starch-milk systems are dependent on the state of the milk protein. The effects of acid and base on the state of protein are reflected by changes that occur in the properties of the starch-protein systems.

Cummisford, as cited by Stalder (73), used Ostwald viscometers to measure the viscosity of tapioca amylopectin which had been heated at 95°C in milk or its fractions. The result showed that the high viscosity attained with skimmilk was not obtained by the addition of casein and whey protein to the protein-free milk system. However, the viscosity of the fabricated milk-amylopectin mixture was considerably higher than that produced by heating amylopectin in the protein-free milk system itself. Certainly, starch interacts with some proteins of milk, but more work is necessary to clarify the mechanism of this reaction.

Mahdi and Bradley (48) isolated a residue that remained after ice cream prepared with 65-fluidity starch substituted for half the sucrose was washed. They found the isolated residue gave positive anthrone and biuret tests and speculated a carbonyl-amine reaction had occurred between

the amylose fraction of the starch and casein during pasteurization of the ice cream mixture. However, they presented no evidence of a chemical reaction. A physical entanglement of the protein with retrograded amylose cannot be ruled out the possible structure of the precipitate.

Moore and Carter (57) obtained a precipitate by heating together a maltodextrin (10 D.E.) and β-lactoglobulin at 85°C. Gel filtration of solutions of the dextrin, the protein, and a mixture of the two before and after heating gave further evidence of some type of interaction. Attempts to determine whether this interaction was or was not covalent were not conclusive. They proposed a mechanism involving non-covalent entrapment of carbohydrate in protein aggregates.

Examination of electron micrographs of modified tapioca starch-milk gels showed that the casein micelles in the interstitial spaces between starch granules were intact and resembled those in fluid milk. The micelles were not aggregated nor had the micelles interacted with the starch granules (34).

The amylose-iodine reaction was used to demonstrate the formation of amylose-wheat protein complex (8,9). The reduction in amylose blue value resulting from the presence of wheat protein was suggested as evidence of interaction. However, Jones and Wilson (42) found some iodine was taken

up by the protein itself through substitution in the tyrosine ring and thus made the use of the reaction as a quantitative measurement of protein-starch complex formation unreliable.

Erlander and Erlander (16) hypothesized a chelate type hydrogen bond formation for interaction between protein and carbohydrate. They suggested that an amide group in protein can hydrogen bond to the C-2 and C-3 hydroxyl groups of any glucose unit in the starch chain.

Takeuchi (74) measured electrolytic conductance of a mixture composed of α-casein and an acidified paste of potato starch, which is unlike cereal starches in having some covalently bonded phosphate monoester groups distributed along the α-(1→4)-glucan branches of the amylopectin fraction and possibly on the amylose molecules. When the protein was titrated with the oppositely charged starch, an inflection was shown on the plot of electrolytic conductivity vs. volume of potato starch solution. This behavior was regarded to be the evidence of interaction of the protein and the starch.

It is generally believed that the major driving force for the interaction between acidic polysaccharides and protein molecules is electrostatic or ionic in character (46). For example, Ganz (20) showed that soluble complexes formed when the pH of several proteins and carboxymethyl

cellulose mixtures were decreased to the isoelectric points of the proteins. Further decrease in pH resulted in precipitation of the complexes and a decrease in the overall net charge. Imeson et al. (36) found more stable complexes were obtained when denatured protein, rather than native protein, interacted with anionic polysaccharide. It was suggested that conformation changes occurred more readily in denatured protein and resulted in greater protein interaction with the polysaccharide. This suggestion is not applicable to the interaction between unmodified cereal starches and protein because these starches carry no electric charge.

The similarity of the interaction of protein with carbohydrate to the antibody-antigen system has been suggested by several workers (21,27). Lectins serve as carbohydrate-specific antibodies in the interaction with saccharides. They interact with sugar moieties of glycoprotein and glycolipid and thus are used as probes to label cell surface carbohydrates (47). So and Goldstein (72) found that concanavalin A reacted specifically to form a precipitate with dextran. Various parameters optimal for this interaction, including pH range, buffer system, and solubility of precipitate were investigated by them. They proposed that the interaction takes place when

binding sites on the carbohydrate and protein are complementary to each other.

Milk

Milk is the secretion of the mammary gland. It is commonly described simply as a mixture of fat, protein, lactose, and ash, which represents a total solids content of 11-14%. Water is the other component. Among the factors which influence the composition of milk, breed characteristics, seasonal effects, and time in the lactation cycle are significant. Variations in composition are minimized by use of composite milk samples.

The milk proteins are a heterogeneous mixture consisting of two fractions, casein and whey, based on solubility at a specific pH. Whey proteins are frequently designated as those nitrogen compounds remaining in the milk serum after precipitation of the casein at pH 4.6. According to this classification, the whey proteins consist of about 20% of the proteins in milk. Major proteins in whey are beta-lactoglobulins A and B (β-Lg A, β-Lg B), alpha-lactalbumin (α-La), bovine serum albumin (BSA), and heterogeneous mixtures of immunoglobulin (Ig). In contrast to the casein, whey proteins are not associated in micelles but are molecularly dispersed in a globular structure and are relatively heat labile.

Heat treatments like pasteurization at high temperature, sterilization, and dehydration can cause denaturation of milk proteins. Protein denaturation is not an all-or-nothing phenomenon and intermediate steps exist during the transition of a protein from the native state to the irreversibly denatured form. There has been controversy about the meaning of the term, protein denaturation. In general, denaturation is defined as an unfolding of the compact three dimensional protein molecule into a less organized structure. Under appropriate condition, a protein may refold again into its original state (7). Usually, however, these unfolding molecules associate into aggregates irreversibly.

Most milk for food use in developed countries is given a heat treatment, either pasteurization or sterilization. According to the Code of Federal Regulations on dairy products (5), the term pasteurization means that such products shall be heated to at least 62°C (145°F) and held at such temperature for 30 min, or to 71°C (161°F) for 15 sec (high-temperature-short-time pasteurization) in properly operated equipment. The purpose of pasteurization is to guarantee that milk is free from Mycobacterium tuberculosis and Coxiella burnetti, which are the most heat-resistant pathogenic organisms in the milk, and to inactivate enzymes. In order to prolong the keeping quality of milk, the increase

of temperature to 138°C (280°F) for 2 sec (ultrapasteurization) has been suggested. Heat treatment at 82°C for 30 min is still common practice within the dairy industry for cultured dairy products (1). A pasteurization temperature of 88°C and above for 1 sec has been designated as ultra-high-temperature (UHT) and now is used in the high-heat-short-time (HHST) process.

Chemical reactions may occur during pasteurization. β-Lg induces the pH to increase to around pH 6.8 because of a loss of CO_2 and volatile sulfur compounds. This increase is partially compensated by release of phosphates from casein. Interaction of these liberated phosphates with calcium salts leads to formation of tricalcium phosphate and development of a corresponding acidity. Polymerization between β-Lg and κ-casein induced by -S-S- formation and/or interaction may occur at pH 6.7. The complex formation is sensitized by calcium, whose activity is affected by phosphate and citrate salts. Casein micelles are relatively heat stable. The resistance to the denaturing process of casein has led several investigators to regard it as an inherently denatured protein. McMeekin (51) characterized casein as a denatured molecule because of lack of α-helix or β-pleated sheet structure.

Effect of heat treatment

The precise changes which occur in milk during heat treatment at high temperatures are complex and not well understood. This review emphasizes only changes of milk proteins heat-treated under 95°C, which is the highest temperature used for heat treatment of milk in this study. Harland et al. (26) determined the denaturation of whey protein at different temperatures by measuring the turbidity developed in the heated non-casein filtrate when HCl was added. They confirmed the importance of time-temperature relationship in determining the extent of denaturation of whey proteins. There was a ten-fold decrease in the time required for a given percentage denaturation of the whey proteins for each 7.5°C increase in the temperature. Quantitative electrophoretic analysis of whey protein denaturation was conducted by Larson and Rolleri (44). The denaturation curves obtained for individual whey proteins indicated α-La as being most heat resistant, and Ig being the least heat resistant. β-Lg and BSA showed an intermediate sensitivity. A more precise electrophoretic method was later developed (30), incorporating β-Lg A as an internal standard for polyacrylamide gel electrophoresis. Use of the internal standard improved precision 2- or 3-fold because it could confirm that each gel behaved in the

same way during the experimental process. By use of polyacrylamide gel electrophoresis, a kinetic study of skimmilk heated at 74°C for different times (31) indicated the denaturation of α-La to be a first order, or probably pseudo-first order reaction. Both β-Lgs A and B followed second order kinetics, while that of BSA could not be described as a simple first or second order reaction. These facts suggested that an irreversible step involving -S-S interchange for both α-La and β-Lg occurred.

Effects of heat on the denaturation of β-Lg in the absence of other proteins was summarized by McKenzie (50). The heat denaturation of β-Lg buffered near pH 7 involves primary and secondary stages. The primary reaction results in the formation of a series of molecular aggregates through the formation of intermolecular -S-S- bonds. The secondary reaction takes place after the first occurs and produces much heavier components at the expense of the aggregates formed in primary reaction, but -S-S- bonds are not involved in their formation. The heavy components formed in the secondary reaction undergo aggregation, and precipitates develop in the solution.

In the milk system, the effect of heat on the functionality of the -SH in β-Lg is more complicated. Sawyer (66), in a comprehensive review of the interaction of κ-casein and β-Lg, pointed out that there was no unequivocal

evidence that the interaction between these two fractions involved -S-S- bridge formation. However, many recent investigations have supported the existence of such interaction (6,10,17). Smits and Brouwershaven (71) concluded that intermolecular -S-S- bonds between β-Lg and κ-casein played a primary role in their interaction, but that hydrophobic bonds were also involved. These non-covalent bonds played an important part in the ultimate effect of the interaction. They also found that the extent of interaction was independent of the presence of α-La. This was due to only -S-S- bonds being present in α-La, whereas free -SH in the β-Lg was believed to be involved with the interaction.

Effect of environmental conditions on heat stability of milk

Several factors have effects on the thermal denaturation of whey proteins in food systems.

pH Rose (64) reported a heat stability/pH curve that showed most individual milk samples had a characteristic maximum stability at pH 6.7 and a minimum stability at pH 6.9. However, milk samples collected near the end of the lactation period increased in stability progressively with increasing pH and showed no maximum and minimum. The former were called type A and the latter, type B. The type A and type B milks were made interchangeable by controlling the ratio of concentration of κ-casein and

β-Lg.

Changes in thermal behavior of whey protein between pH 6 and pH 7 were studied by differential scanning calorimetry, which measures the difference in heat flow between a sample and a reference as a function of temperature (13). When pH was increased from 6 to 7, the peak size of heat absorption (denaturation) decreased. This stabilization mechanism is probably caused by the additional formation of a -S-S- bond in partly folded β-Lg molecules because the amount of active -SH increased sharply near pH 6.8 (75). This observation is in agreement with Dunnill and Green (15). They measured the rate of reaction between chloromercuribenzoate and -SH of β-Lg at several pH values in the range from 2.8 to 8.5. At low pH, the reaction was slow and remained so up to pH 6.8, at which point a rapid increase began. From these studies, it seems that the small change around pH 6.8 appears to be crucial for the thermal behavior of β-Lg. McKenzie (50) reported a change in the -SH activity and the release of one proton per monomer of β-Lg from a buried carboxyl group at pH 6.8.

Sugars Sugars exert a protective action on β-Lg against thermal denaturation. Differential scanning calorimetry (37) indicated 4% glucose or lactose had a stabilizing effect on β-Lg. This stabilization is in agreement with the report of Hillier et al. (32), who dialyzed

cheese whey against concentrated skimmilk to increase lactose and salts concentration and then determined the concentration of undenatured whey protein. Lactose also protected α-La against heat denaturation.

Salts The susceptibility of whey proteins to denaturation is dependent on the pH of solution. However, the extent of whey protein aggregation is controlled by the presence of inorganic salts. The calcium/phosphate ratio in the whey is of great importance to the heat stability of milk. Reduction of the soluble phosphate content by dialysis caused a progressive shift of the heat stability/pH curve to more alkaline values and the milk protein had a higher heat stability (18). Morrissey (58) concluded that β-Lg, soluble phosphate, and calcium were all required for a typical heat stability/pH curve of type A milk and that the minimum in the curve was due to heat-induced precipitation of calcium phosphate on a casein β-Lg complex. He further showed that colloidal calcium phosphate, which is an integral factor in the casein micelle, was not an essential factor that determined heat stability in milk. Colloidal calcium phosphate-free milk enriched in soluble calcium by dialysis against calcium-enriched milk showed the characteristics of a type A milk. Increases in calcium concentration up to 0.4 mg/ml

(32), compared to the original calcium content (0.34 mg/ml) in whey, tended to slow the denaturation of α-La and β-Lg. Further increases in calcium up to 0.9 mg/ml produced little effect.

EXPERIMENTAL PROCEDURES

Materials

Cornstarch used in this study was unmodified commercial powdered cornstarch, Buffalo brand, from the Corn Products Company.

Bovine milk samples were obtained from Iowa State University herds. In order to eliminate the variation among individual cows, pooled milk samples were obtained from the bulk tank of the barn. After being stored in a refrigerator overnight, sample was then skimmed in an electric separator (model 100AE, De Laval Separator Co.).

Heat Treatment of Skimmilk

The skimmilk was divided into five 2-liter portions. Four of them were individually heated at 74°C, 78°C, 82°C, and 95°C. Each sample was contained in a 4-liter Erlenmeyer flask equipped with a condenser to prevent water loss by evaporation. Each was heated on a hot plate with magnetic stirrer to the desired temperature, which was determined by an electronic Tele-Thermometer (model 42-SC, yellow Springs Instrument Co.). It was then held at the specified temperature for 20 min in a constant temperature water bath. After being heated, the flask was quickly cooled in an ice-water bath to 4°C and stored in a refrigerator for future use.

Amylograms and Gel Strength of Starch-Milk Pastes

Thirty g (dry substance) of cornstarch was placed in a 250 ml beaker. A small portion of the milk was added to the beaker to disperse the starch. The dispersion was transferred to a 500 ml volumetric flask. Another portion of the milk was used to rinse the beaker and then added to the flask. Additional milk was added to give a total volume of 500 ml. The mixture was shaken vigorously and quickly transferred to the Brabender Amylograph cup. It was heated from 30°C to 95°C at 1.5°C/min and held for 15 min at 95°C. The viscosity was continuously recorded during the heating period.

After the 15 min holding period, the surface layer on the paste from Amylograph was discarded and a portion of the paste was poured into each of two 250 ml beakers. They were then covered, and copper disks measuring 19 mm in diameter and 2 mm in thickness attached to rods, 90 mm long and 1.5 mm thick, were immersed in the hot paste. The disks were immersed 3 cm below the surface of the pastes and the rods were supported by slotted covers. A thin layer of mineral oil was poured on the surface of the hot paste samples, which were then placed in a refrigerator at 4°C for 18 hr to form gels. After that period, the samples

were removed from the refrigerator and were allowed to stand at room temperature for 1 hr. The gel strength was measured by a method essentially the same as that described by Bechtel (3). The rod was hooked to one arm of a balance and a small stream of water was added to an aluminum can on the other arm until the disk was pulled from the gel. The weight of water was used as a measure of gel strength.

Gelatinization Temperature Range

The gelatinization of starch in the milk samples was measured following the standard method as described by Watson (77). A drop containing a dilute dispersion of starch in the medium, e.g., water or milk, was observed under a Leitz microscope equipped with a polarizing filter, analyzer and a hot stage at magnification of 125X during heating. The gelatinization temperature is defined as being the temperature range between the point at which the first 2% of granules in a given field start to lose birefringence and the point at which 98% of the granules have lost their birefringence.

Swelling Power

Swelling power was obtained by the method of Schoch (67) with modification in time of heating and speed of

centrifugation. Preliminary experiments showed that the heating time had to be extended to allow swelling of the granules in milk to reach a constant value; an increase in speed of centrifugation was necessary because of the greater swelling of the granules in milk at higher temperatures. The swelling power was determined by placing 4.5 g (dry substance) sample of cornstarch into 250 ml centrifuge bottle. A weighed magnetic stirring bar and 200 g of milk were added. The bottle was shaken thoroughly and placed in a constant temperature water bath. Stirring of 4 samples heated simultaneously was induced by a group of magnets attached to the stirring shaft of a variable-speed stirrer placed in the water bath between the bottles. A slow constant speed, just fast enough to keep the starch slurry suspended, was used. After 1 hour, the sample was removed and centrifuged in a superspeed centrifuge at 850 times gravity for 30 min. The swelling power was determined by dividing the weight of the swollen starch granules by the weight of the dry starch used.

Because there is about 10% soluble material from milk in the supernatant, no correction factor was used in calculating the swelling power to account for soluble starch that is lost into the supernatant.

Micrography

Photomicrographs of swollen starch granules were made according to the method described by Miller et al. (54). A drop containing starch that had been suspended in raw milk or preheated milk was observed under a Leitz microscope equipped with phase contrast objectives and photographed at a magnification of 400X after the suspension had been heated at 95°C for 1 hr, the condition used to measure swelling power. A 6% paste prepared in the Amylograph after heating to 95°C and holding there for 7 min was also sampled and diluted to reduce crowding of the granules and produce a satisfactory image under microscope examination. The 7 min holding time was chosen because this represented the time when those samples that swelled after reaching to 95°C attained their maximum viscosity. All micrographs were made at room temperature without applying a cover glass, which was reported to produce physical pressure and alter the integrity of the granules.

Determination of Heat Denaturation of Whey Proteins

Polyacrylamide gel electrophoresis was used to observe changes in the milk proteins in samples which had been

heated as described previously. The procedure used followed the Hillier method (30) with modifications in stain solution and in preparing the sample for loading on the gel.

Polyacrylamide gel electrophoresis was performed in pH 8.3 tris-(hydroxymethyl)-methylamine (TRIS) buffer. The reagents used were as follows:

Stock buffer: 4.6 g TRIS[1], 0.38 ml conc. HCl[2], distilled water to 100 ml, adjusted pH to 8.9 by addition of conc. NaOH[2] or HCl[2] solutions.

Running buffer: 2.4 g TRIS, 11.6 g glycine[1], distilled water to 4 liter, adjusted pH to 8.3.

Tracking dye: 2 g sucrose[2], 14 mg bromophenol blue[2], running buffer to 20 ml.

Stain and fixative solution: 0.4 g Coomassie Brilliant Blue R-250[1], 200 ml methanol[2], 20 ml acetic acid[2], distilled water to 400 ml.

Destain solution: 2 liter methanol[2], 300 ml acetic acid[2], distilled water to 4 liter.

Nine percent polyacrylamide gels were prepared by dissolving 9 g acrylamide[1] and 0.4 g methylene-bis-acrylamide[1] in stock buffer to a total volume of 100 ml. Twenty ml of this gel solution were mixed with 20 µl

---

[1]Bio-Rad Laboratories.

[2]Fisher Scientific Co.

N,N,N',N',-tetramethylethylenediamine[1] and 0.002 g ammonium persulfate[1] to initiate gelation. The mixture was then immediately poured into tubes (0.6 cm x 12.5 cm) to a height of 9 cm, and each gel was overlayed with a few drops of water so that a flat surface resulted after polymerization. The water was subsequently removed from the top of the polymerized gel, and the running buffer was used to rinse the gel several times and to overlay the gel. The gel was thus ready for loading samples.

Acid whey was prepared by acidifying milk samples to pH 4.6 with diluted acetic acid and filtering the precipitated casein and denatured whey. In an early experiment, the whey filtrate was dialyzed against deionized distilled water containing pH 7.0 phosphate buffer. However, preliminary experiments showed that dialysis of the filtrate was not necessary. A similar finding was also reported by Hillier (30) and dialysis therefore was subsequently omitted.

The whey protein in the filtrate could be separated by gel electrophoresis into several bands. Studies indicated that each whey protein fraction had a different capacity for binding Coomassie Brilliant Blue stain. A

---

[1]Bio-Rad Laboratories.

dye binding capacity curve for each whey protein was therefore needed. Use of β-Lg A as an internal standard allowed the gels to be monitored and this insured that each behaved in the same way, giving an obvious improvement in precision. In this study, only 4 whey proteins, β-Lg A, β-Lg B, α-La, and BSA, were analyzed quantitatively. A standard mixture consisting of a known amount of each protein studied was applied to one of the gels in a run and the proteins on the other gels were estimated by direct comparison with this standard. Each gel was also loaded with a known amount of β-Lg A as an internal standard.

A standard solution of each whey protein was prepared by dissolving the individual protein, 0.65 mg β-Lg A, 0.65 mg β-Lg B, 0.40 mg α-La, or 0.60 mg BSA, in 5 ml 0.1 M phosphate buffer at pH 7. All standard proteins were obtained from the Sigma Chemical Company. The absorbance was measured at 280 nm using a Gilford spectrophotometer. The concentration was calculated using extinction coefficients of 0.95 for β-Lg A and B, 2.09 for α-La, and 0.66 for BSA (40). The standard mixture was then prepared by combining the volumes of the 4 pure protein solutions required to produce a mixture that contained 1.9 μg β-Lg A, 1.7 μg β-Lg B, 1.5 μg α-La, and 0.4 μg BSA. This mixture was then freeze-dried for

future use to simulate the freeze-drying step in the preparation of the unknown samples.

The following procedure was used to determine the dye binding capacity curve. A series of samples was prepared with known amounts of the standard solution. Each aliquot was freeze-dried, then dissolved in 100 µl of tracking dye and incubated in a water bath at 37°C for 15 min. Before loading the samples, 1.9 µg β-Lg A in the tracking dye was applied with a micropipette underneath the buffer solution in the tubes, which were then placed in the electrophoresis cell (model 155, Bio-Rad Laboratories) and run for 10 min. The voltage was maintained at 250 V. The current was then turned off. The samples were loaded on the gel beneath the buffer solution and the current re-started. An average of 2 hr was required for the tracking dye to migrate out of the tubes. After electrophoresis, the gels were removed from the tubes by insertion of a syringe needle between the gel and glass tube and rotation of the tube while water was forced gently out of the syringe. The gels were stained for 2 hr and then destained until good resolution of bands was obtained, usually about 4 days. The gels were preserved in 7.5% acetic acid overnight and then scanned at 590 nm using a Gilford spectrophotometer (model 240) with a gel scanner (model

2520). The scan rate was 4 cm/min and the gel was suspended in a 10 cm cuvette containing 7.5% acetic acid. The optical density was recorded as a series of peaks on a chart recorder (model 242, Gilford Instrument Co.). The area under each peak represented a measure of the amount of protein in the gel and was determined by a planimeter.

To prepare the skimmilk samples for gel electrophoresis, 3 ml of each sample were pipetted into a beaker. The casein with denatured whey protein was precipitated by adding diluted acetic acid to the milk until pH was lowered to 4.6. The amount of acetic acid was recorded as a dilution factor. The precipitate was removed by filtration through Whatman No. 1 filter paper. Volumes of filtrate containing an average of 5-10 μg were freeze-dried. The freeze-dried samples were loaded onto gels and subjected to gel electrophoresis as described previously. The concentration of each protein that remained undenatured was determined from the area of the peak according to the following formula:

$$P_x = \frac{A_s}{A_{std}} \times \frac{A_{issm}}{A_{iss}} \times P_s$$

$$P_w = P_x \times \frac{\text{dilution factor}}{\text{volume of filtrate}}$$

P_x = μg of individual protein in a sample.

P_s = µg of the corresponding protein in the standard protein mixture.

P_w = concentration of individual protein in a sample (µg/µl).

A_s = peak area of a sample.

A_{std} = peak area of standard mixture.

A_{issm} = internal standard peak area of standard mixture.

A_{iss} = internal standard peak area of sample.

Additional Heat Treatment of Whey Proteins

The further changes that whey proteins undergo when preheated milk is used in the Amylograph were determined by heating 500 ml samples of milk in the Amylograph in the absence of starch. When the desired temperature (or time-temperature) treatment had been attained, the Amylograph cup was removed immediately and approximately 10 ml of the heated milk was poured into a vial and placed in an ice-water bath. The amount of whey protein that remained undenatured was measured by polyacrylamide gel electrophoresis as before.

Determination of Effect of Added Whey Proteins on Paste Viscosity and Gel Strength

To samples of milk that had been preheated at 82°C, β-Lg (combined A and B, Sigma Co.) and α-La were added in the amount necessary to restore the minimum levels of these proteins found in raw milk, milk preheated at 74°C,

and that preheated at 78°C. Samples were stirred gently overnight to ensure complete dispersion of the added protein before being heated with 6% cornstarch in an Amylograph. The resulting pastes were used to prepare samples for tests of gel strength. Results were compared with those obtained when raw milk was used.

Model Reaction for the Starch Interaction with Milk Protein

To study the interaction between β-Lg and starch, free from possible complications caused by other components in natural milk, a buffer solution of β-Lg was substituted for milk in a study with the Amylograph. The Jenness-Koops buffer, with a composition similar to milk ultrafiltrate, was chosen (41). If the buffer solution without added protein produced the same Amylograph with starch that water did and if added β-Lg appeared to be denatured at approximately the same rate in the buffer as in milk, it was assumed that any differences between the Amylograms obtained with water and with the buffered β-Lg solution were probably the result of interaction of the starch and β-Lg.

Preparation of buffer solution

The reagents (Fisher Scientific Co.) used were as follows:

Solution I: 6.32 g KH_2PO_4, 7.16 g Na_3citrate, 2.40 g KCl, 4.80 g K_3citrate.H_2O, 0.72 g K_2SO_4, dissolved in distilled water and diluted to 80 ml.

Solution II: 3.59 g $CaCO_3$, 4.43 g $MgCO_3.Mg(OH)_2.3\ H_2O$, dissolved in 60 ml distilled water and 10 ml conc. HCl in an evaporating dish covered with a watch glass. After evaporating to dryness, the solid was dissolved in distilled water and diluted to 80 ml. This procedure avoided uncertainties in weighing of the hygroscopic $CaCl_2$ and $MgCl_2$.

Buffer solution was prepared by adding solution I and solution II to 3740 ml of distilled water. The amount of 1.2 g of K_2CO_3 was added to the solution, which was then adjusted to pH 6.63 with 1.0 N KOH and diluted to 4 liter with distilled water.

Determination of heat stability of β-Lg in buffer solution

Three concentrations of β-Lg in buffer, 2.0 mg/ml, 2.3 mg/ml, and 2.6 mg/ml, were prepared in 5 ml volumetric flasks. The flasks were placed in the Amylograph cup and surrounded by water. The cup was then placed on the instrument and heated in the usual fashion from 30°C to 88°C. After heating, the flasks were cooled in an ice-water bath. Denaturation of β-Lg was determined by gel electrophoresis as described earlier.

Amylogram of starch-β-Lg paste

An Amylogram was prepared by dispersing 6.6% corn-starch in a buffered β-Lg solution that had been shown in

the above experiment to have the same concentration of undenatured β-Lg when the mixture reached 88°C as is present at that temperature in an Amylogram made with milk which has been preheated at 74°C. The concentration used was 2.3 mg/ml.

RESULTS AND DISCUSSION

Effect of Heat Treatment of Skimmilk on the Amylograms of Starch-Milk Pastes

Samples of skimmilk for use in these experiments were individually preheated at 4 different temperatures within the range in which denaturation of whey proteins is known to occur.

The Amylograms of 6% cornstarch pastes made with a skimmilk which had received different heat treatments are shown in Figure 1. All milk samples had almost identical effects on viscosity until the temperature reached 88°C, where viscosity of the paste made with milk preheated at 95°C became constant. On the contrary, viscosities of pastes made with raw milk or that preheated at 74°C began to increase sharply at this point, and then decrease during a holding time at 95°C. Paste made with milk preheated at 78°C gave intermediate result showing an increase above 88°C which leveled off at a lower viscosity than that reached by samples made with raw milk or that had been preheated at 74°C. Paste made with milk preheated at 82°C showed a much lower but continuous viscosity increase after the temperature reached 88°C. These results agree with previous observations by

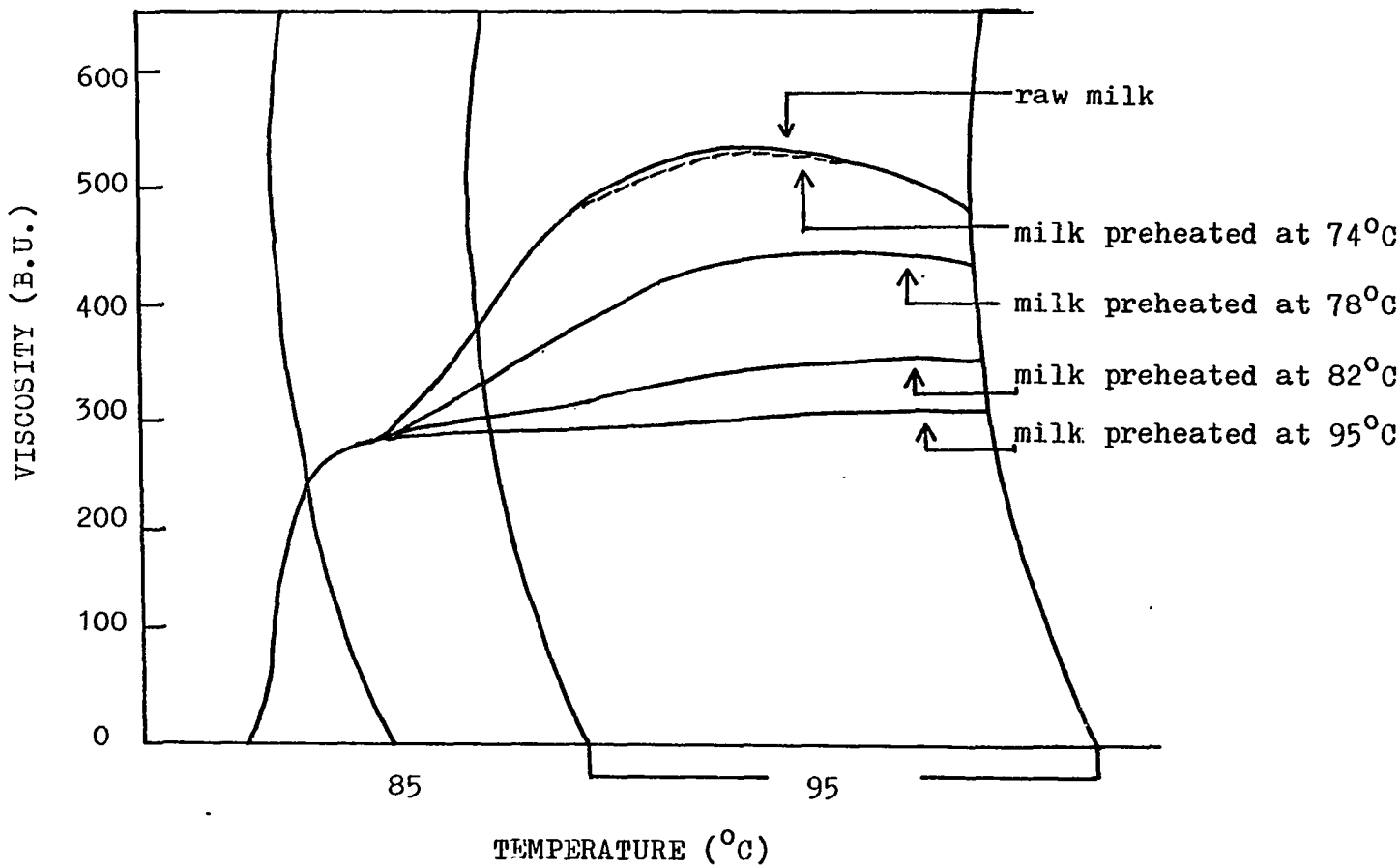


Figure 1. Effect of preheat treatment of milk on Amylogram of cornstarch-milk paste

Stalder (73). She found that the mildest heat treatment of the milk which caused a lowering of the subsequent Amylograph curve with starch was between 75°C and 77°C.

If skimmilk is assumed to contain 10% solids, the Amylograph of 6.6% cornstarch paste would be comparable to that of 6% starch-milk paste based on the same amount of available water. The starch-water paste began to increase in viscosity at a higher temperature than the starch-milk pastes. The increase was continuous until a maximum was reached, after which a slow decrease occurred (Figure 2).

It should be mentioned that the change of viscosity in pastes caused by the preheat treatment of milk may alter final quality of some starch-containing foods. According to the results of these experiments, milk pasteurized at the temperature required for the high-temperature-short-time methods (71°C) might not affect viscosity produced during subsequent heating with starch, compared to raw milk. But lower viscosity in pastes might be expected if milk samples were pasteurized at higher temperatures.

This information about effects of preheating of milk on the viscosity of starch-thickened milk products is also valuable in controlling the texture of gravies and sauces, which contain both starch and milk. Different preheat treatments of milk can change the final texture of products which in turn affects their mouth feel.

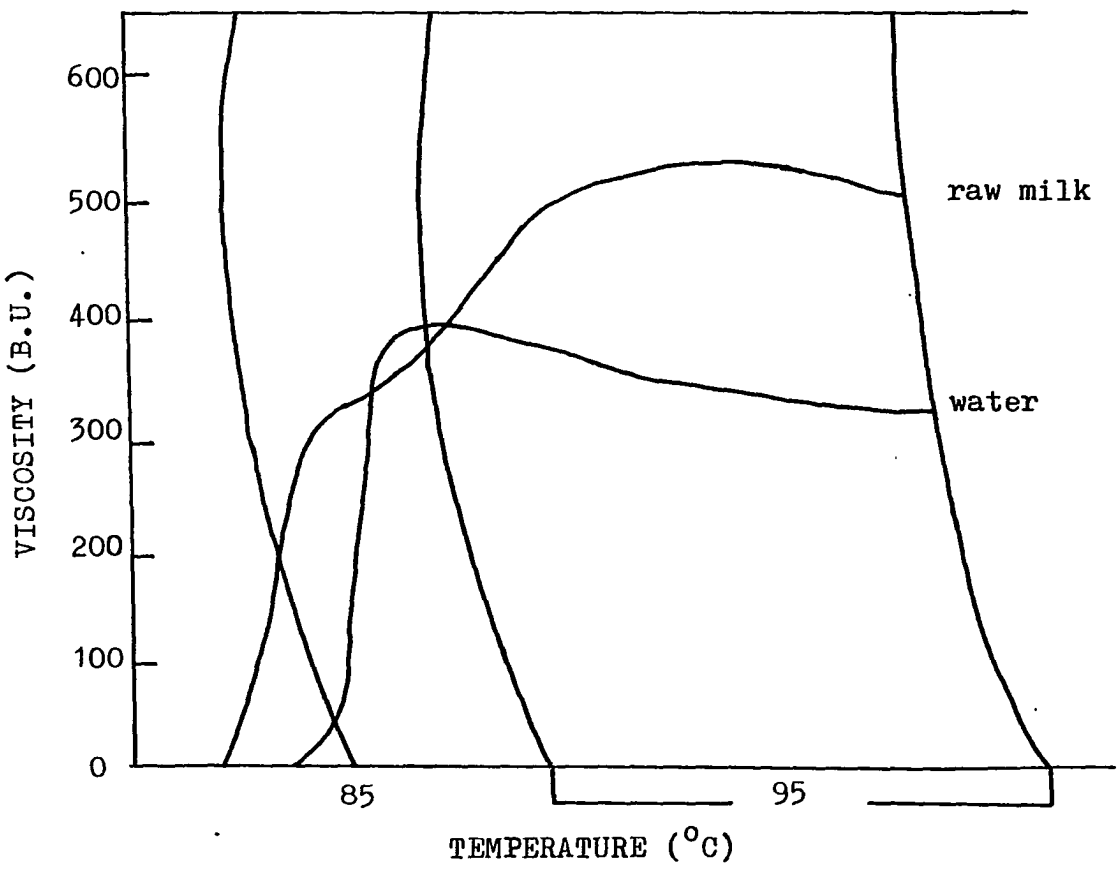


Figure 2. Amylograms of starch pastes made with raw milk and water based on approximately the same amount of available water

Effect of Heat Treatment of Milk on the Gel Strength

Gels formed from the above pastes by refrigeration at 4°C for 18 hr were also affected by the preheating of the milk, but there was no direct relationship between viscosity, final or otherwise, and gel strength (Table 1). Milk samples A and B produced paste with different viscosities and gel strengths when used raw or after each of the heat treatments but the trend of effect of preheat treatments on these properties was the same. With milk preheated at 78°C, the gel strength was slightly lower than that formed with the milk preheated at 74°C, but the gel strength continued to decrease as the preheat temperature was increase to 82°C and to 95°C. The reason for milks that had received increasingly greater heat treatment producing a continuous decrease in final viscosity, but giving gels that had maximum strength after heat treatment at 74°C, is not obvious.

Wood (78) indicated that test methods that measure the force required to rupture a gel will not necessarily rank a series of gels in the same order as tests that measure the gel only by a small deformation without rupture. The gel strength measured by the imbedded-disk method as shown in Table 1 would be impossible to compare with the results measured by Bloom gelometer. Mitchell (56) reported that the force required to rupture a gel correlated better with the gel strength assessed in the

Table 1. Comparison of breaking strength of gels with Amylograph viscosity of pastes after 15 min holding at 95°C

Sample[a]	Maximum viscosity[b] (B.U.)	Final viscosity[b] (B.U.)	Gel strength[c] (g)
raw A	533±3	490±15	130±3
74 A	530±0	490±5	173±6
78 A	430±0	415±0	167±1
82 A	338±3	330±0	126±1
95 A	288±3	285±5	115±2
raw B	488±3	460±12	124±2
74 B	485±5	455±0	157±4
78 B	373±3	364±1	135±3
82 B	305±5	298±3	104±1
95 B	270±0	265±0	96±1

[a]The number denotes the temperature (°C) of preheating of milk. The letter indicates two separated milk samples.

[b]Average of two paste samples ± standard error.

[c]Average of four gel samples ± standard error.

mouth than measurement of the elastic modulus at small deformation of the gel.

The molecular weight dependence of the rupture strength of low ester pectin gels has been studied (43). Instron tests indicated that increasing the molecular weight of pectin increased the firmness and strength of the corresponding gels. But the information about the internal structure of the gel cannot be obtained by this method. Mitchell (55) investigated rheological test methods for gels. He concluded that it was desirable to employ fundamental test methods rather than empirical test methods to elucidate gel structure. To prove the effect of heat treatment of milk on the gel strength would require more measurements of a number of parameters such as creep compliance, stress relaxation, and rigidity modulus.

The rate at which the gel is deformed during the measurement is important. It has been reported that the initial slope of the force-displacement curve obtained for a compression test of alginate gels depended on the rate of compression (70). In the present study, a constant and reproducible increase in force used to raise the imbedded disk out of the gel on one arm of a balance was obtained by adding a small stream of water to an aluminum

can, about 50 ml/min from a separatory funnel on the other arm.

Effect of Heat Treatment of Milk on Swelling Power and Gelatinization Temperature of Starch Granules

Increase in viscosity of starch pastes during heating is generally attributed to swelling of the starch granules.
granules. A study of the gelatinization temperature and swelling power of the starch granules was investigated to see if these two properties would explain any differences in viscosity of starch-milk pastes resulting from different treated and untreated milk samples.

The gelatinization temperature ranges of cornstarch in water and in samples of milk which had received no heat treatment and preheat temperature at 78°C and 95°C were obtained. These heat treatments gave the highest, intermediate, and lowest Amylograph viscosities, yet showed no difference in gelatinization temperature, determined with a heating-stage microscope (Table 2). Starch granules gelatinized at lower temperature in water than in any of the milk samples.

The swelling power of starch in water and in samples of milk given the 3 heat treatments was also studied. The swelling power of starch in water was higher than that in milk when measured at temperatures of 70°C and below,

Table 2. Gelatinization temperature range of cornstarch in water and in milk which has received different preheat treatments

Suspension medium[a]	Gelatinization temperature range[b] (°C)
water A	62.3-68.2
raw A	68.0-74.4
78 A	67.4-74.2
95 A	68.1-74.3
water B	62.2-68.4
raw B	68.1-74.3
78 B	68.2-74.2
95 B	68.1-74.4

[a]The number denotes the temperature of preheating of milk.

[b]Each number is an average of five determinations.

but lower at 80°C and above (Figure 3). Heat treatment of the milk did not affect the swelling of the starch.

That differences in the Amylograph viscosity caused by milk samples preheated at the 3 temperatures were not related to effects on granular swelling, as evidenced by gelatinization temperature and swelling power, is also confirmed by photographs of swollen granules. Figure 4 shows starch granules heated at 95°C for 1 hr during measurement of swelling power (a,b,c) and in the Amylograph (d,e,f) at the point at which the largest difference in the Amylograph viscosity among samples was obtained (after being held at 95°C for 7 min). Comparison of swollen granules taken during swelling power determination showed no appreciable difference among milk samples. The same observation was also found among samples taken from the Amylograph. Because of possible drying effects resulting from the photography being performed without use of cover glass, as described by Miller et al. (54), the appearance of samples from the two methods of preparation may not be comparable.

It should be noted that the concentration of starch and heating conditions for determination of the swelling power and gelatinization temperature were different from those measuring Amylograph viscosity. Measurement of swelling power and gelatinization temperature take place in a large

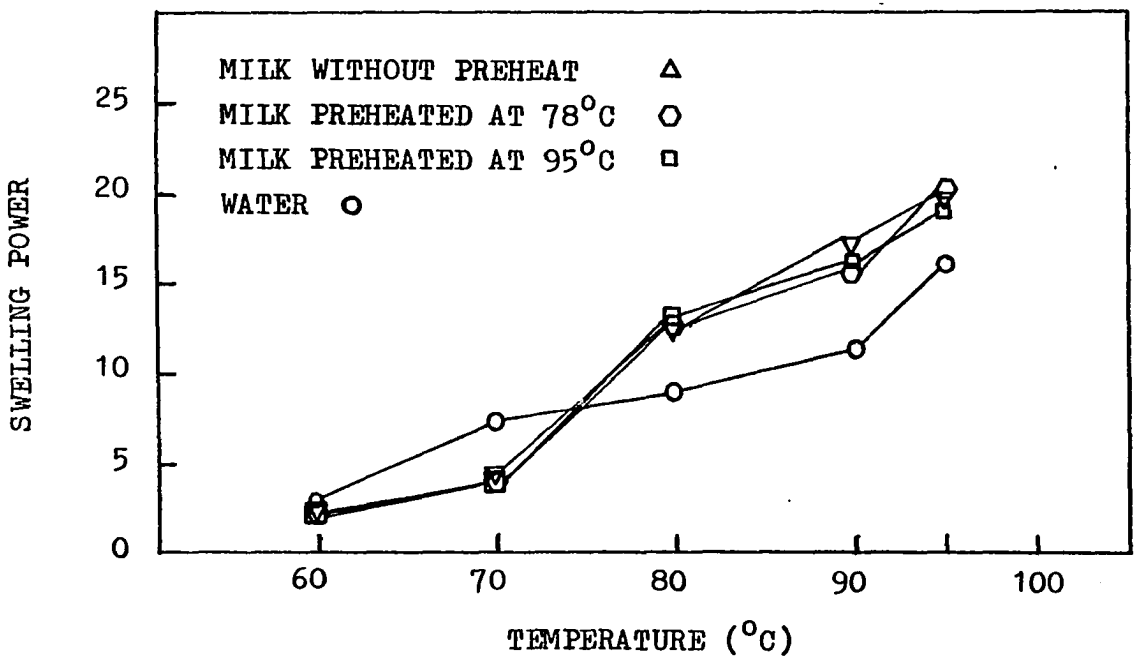
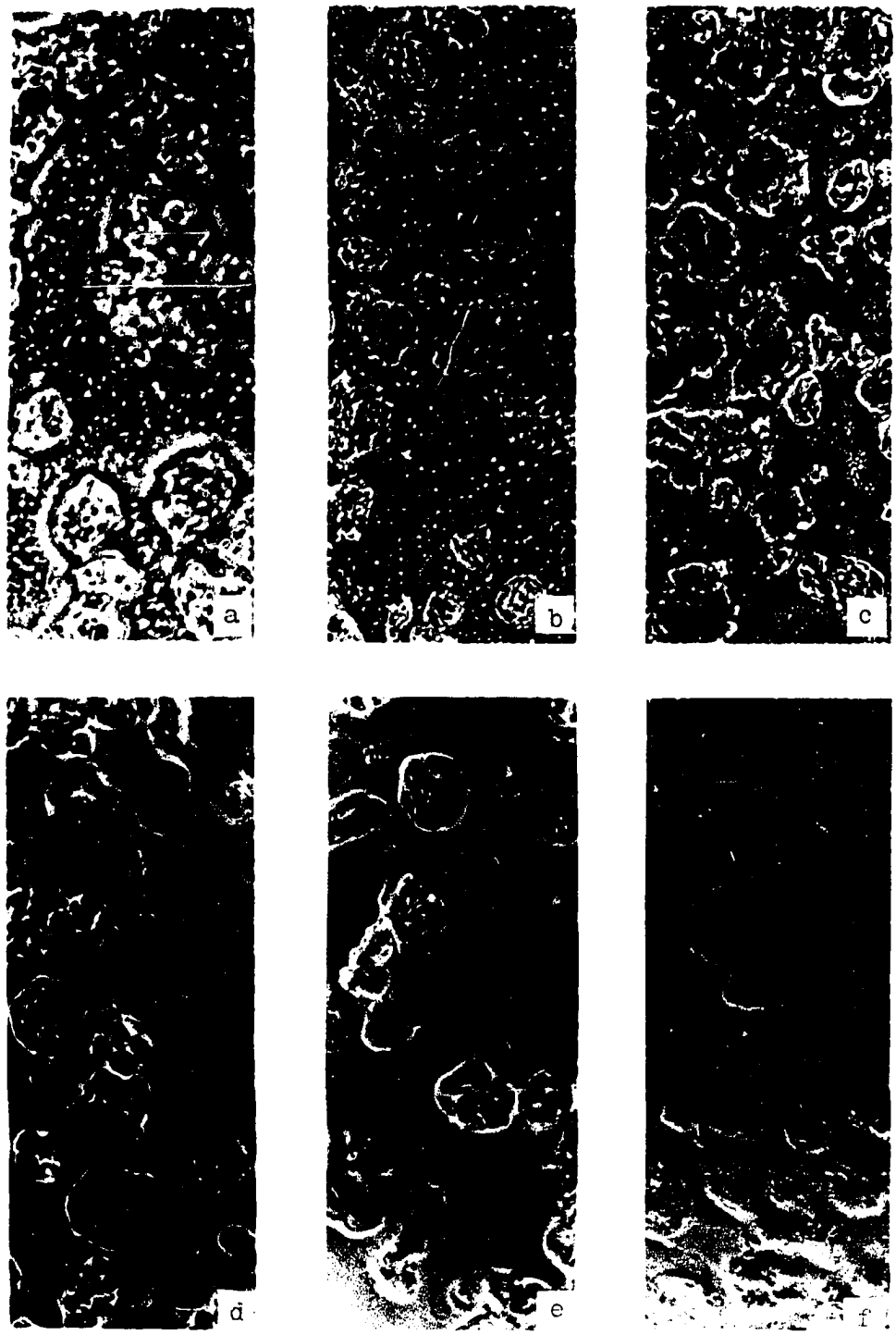


Figure 3. Swelling power of cornstarch in different media

Figure 4. Light micrograph view (400X) of granule structure in the milk taken from centrifuge bottles after heating at 95°C for 1 hr: (a) raw milk (b) milk preheated at 78°C (c) milk preheated at 95°C. Granule structure in diluted aliquot of the paste made from Amylograph at 95°C for 7 min: (d) raw milk (e) milk preheated at 78°C (f) milk preheated at 95°C


a
b
c
d
e
f

excess of water and changes in the granules are therefore a function of only temperature. However, in the Amylograph, there was less excess water; the changes of Amylograph viscosity were a function of time, temperature, and amount of available water. The amount of water available to starch is a major factor in controlling its thermal gelatinization.

Effect of Heat Treatment on Whey Proteins in Milk

To determine whether or not the decrease in viscosity caused by heating the milk was related to the denaturation of milk proteins, and, if so, which proteins, polyacrylamide gel electrophoresis was used to observe changes in the milk proteins quantitatively. Due to the lack of α-helix or β-pleated sheet structure of casein protein in milk, it is regarded as undenaturable. Only whey protein, which is heat sensitive, was examined.

There are two major sources of error in the staining system of polyacrylamide gel electrophoresis. First, dye does not stain all proteins to the same extent. Second, the overall degree of staining in any one experiment is influenced by several variables such as time, temperature, gel homogeneity, and the destaining process. Such a large number of variables makes it

difficult to repeat an experiment on separate occasions and to reproduce the same overall degree of staining. In order to overcome the two problems, a binding capacity curve of each whey protein to dye and a standard solution with an internal standard for each run are necessary.

The binding capacity curve, as expected, indicated that each whey protein had a different capacity for taking up stain (Figure 5). The protein, BSA, gave the highest binding capacity for Coomassie Brilliant Blue dye, α-La and β-Lg B showed the least, and β-Lg A gave intermediate results.

A standard solution prepared by combining the individual purified proteins in the approximate ratio found in whey was separated at the same time that unknown whey proteins were separated. Each gel was also loaded with a known amount of β-Lg A to serve as an internal standard. Further, the gel with the standard solution and internal standard was stained and destained with the unknown samples so that the standard solution received the same treatment as the test samples. Since each gel had the same amount of internal standard, the ratio of the internal standard peak area in the sample to that in the standard solution gels provided the factor for correction caused by gel variation.

The improvement in precision resulted from using an

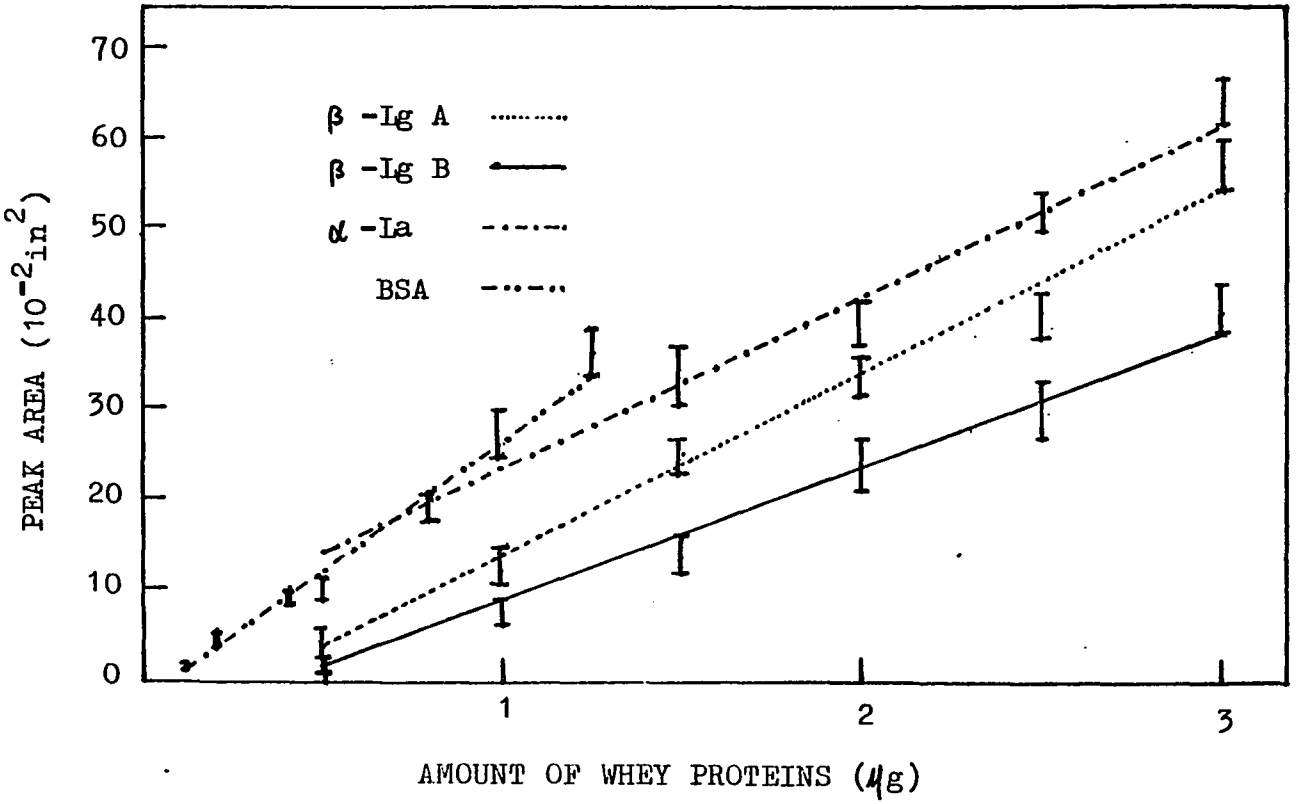


Figure 5. Relationship between the amount of protein and dye binding of each whey protein

internal standard can be observed in Table 3. These were data from the dye-binding capacity curves of solution of the pure proteins. It can be seen that use of the internal standard led to an obvious improvement in precision. The two β-Lg peaks appeared visually separated on gels but not completely resolved in scans of the gels (Figure 6). This fact may introduce some error in estimating whey protein concentrations. The application of samples to gels and the accuracy of densitometric traces are other factors related to the accuracy of determination of whey protein concentration by disk polyacrylamide gel electrophoresis in this study.

Table 4 shows the result of an experiment where three separate milk samples were analyzed for denaturation of whey proteins. In this study, only the concentration of β-Lg A, β-Lg B, α-La, and BSA were estimated by direct comparison with the corresponding standard. The results indicated the concentration of each whey protein decreased with increasing heat treatment. Each protein possessed a different resistance to heat. After the milk was heated at 82°C, 7-30% of the α-La remained undenatured, but only 3-13% of the β-Lg A, 0.5-3% of the β-Lg B, and none of the BSA.

The amount of total protein and the percentage of each individual whey protein differ among milk samples (40). Milk samples A and B in the present study did not differ greatly from one another, but sample C differed considerably from them both in whey proteins composition and in response

Table 3. Improvement in precision of measuring whey proteins using an internal standard

Whey protein	Amount of protein (μg)	Average peak area without internal standard[a] ($10^{-2}in^2$)	Average peak area corrected by use of internal standard[a] ($10^{-2}in^2$)
β-Lg A	0.5	4.2±1.8	4.5±0.7
"	1.0	11.0±2.7	12.2±1.3
"	1.5	22.5±2.6	25.2±2.1
"	2.0	34.3±11.8	31.8±3.8
"	2.5	45.0±2.8	40.6±1.4
"	3.0	62.0±3.6	57.0±3.9
β-Lg B	0.5	2.3±1.7	3.0±1.0
"	1.0	6.5±0.9	8.8±0.7
"	1.5	7.0±1.0	14.0±1.2
"	2.0	18.3±5.7	23.8±3.4
"	2.5	13.8±1.7	30.1±2.4
"	3.0	18.5±6.0	41.3±3.4
α-La	0.5	12.6±1.2	10.4±0.7
"	1.0	26.2±3.7	28.0±1.3
"	1.5	38.2±5.3	34.3±2.6
"	2.0	40.0±4.0	39.3±1.3
"	2.5	43.3±9.1	46.8±1.3

[a]Average of three replicates ± standard deviation.

Table 3. (continued)

Whey protein	Amount of protein (μg)	Average peak area without internal standard[a] ($10^{-2}in^2$)	Average peak area corrected by use of internal standard[a] ($10^{-2}in^2$)
α-La	3.0	59.2±3.7	64.0±2.1
BSA	0.1	1.0±0.3	1.8±0.3
"	0.2	3.3±1.1	4.9±0.4
"	0.3	3.8±1.6	7.0±0.6
"	0.4	5.9±1.7	9.2±0.8
"	0.8	14.0±0.7	19.0±0.6
"	1.2	19.3±1.1	38.1±0.3

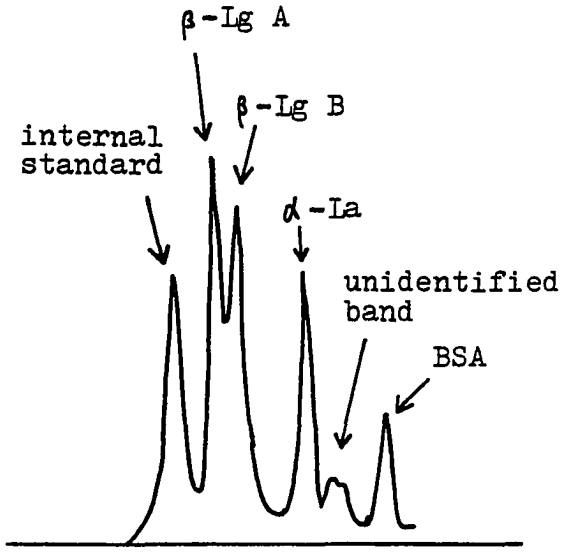


Figure 6. Densitometric trace of stained gel containing separated whey proteins in raw milk

Table 4. Effect of heating milk at various temperatures on whey protein denaturation

Sample[a]	Concentration of undenatured whey proteins (μg/ml)[b]			
	β-Lg A	β-Lg B	α-La	BSA
raw A	1890±30	2000±140	1200±40	151±0
74 A	1000±70	1000±70	620±60	60±7
78 A	500±40	330±7	320±20	18±1
82 A	48±3	12±1	89±30	N.D.[c]
95 A	N.D.[c]	N.D.[c]	16±1	N.D.[c]
raw B	1900±200	1863±0	1300±100	160±10
74 B	1360±6	962±1	913±6	111±7
78 B	459±2	270±4	278±6	7±1
82 B	96±4	10±2	160±7	N.D.[c]
95 B	13±0	13±1	15±0	N.D.[c]
raw C	1490±40	1250±10	1300±70	230±20
74 C	1320±30	1040±40	1100±60	191±4
78 C	800±100	550±20	800±60	64±3
82 C	200±20	38±5	390±30	N.D.[c]
95 C	22±1	N.D.[c]	31±5	N.D.[c]

[a]The number indicates the temperature of preheating of milk.

[b]Average of three replicates ± standard error.

[c]Not detectable.

to heat treatment. This sample was used with wheat starch instead of cornstarch and showed a similar reduction in maximum viscosity of Amylograms with increasing heat treatments at 78°C or above. In this case, preheating at 74°C caused an increase in the maximum viscosity, an effect that had been observed with cornstarch and milk on an occasion before analysis for protein denaturation was began. The cause of this anomaly is unknown. As expected, wheat starch produced lower viscosity than the same concentration of cornstarch. Maximum viscosities were 190, 225, 180, 130, and 115 B.U., respectively, for 6% pastes prepared with raw milk and with milk that had been preheated at 74°C, 78°C, 82°C, and 95°C.

A band of unknown protein was found between α-La and BSA. It was present even when the freeze-dried sample for protein analysis was prepared from milk immediately after it was removed from the holding tank while milking was still in progress. Storage of the milk did not change its amount appreciably. Heating this milk at 74°C also gave no effect but heating at 78°C doubled its concentration. No obvious difference in the amount of unknown protein was detected between samples prepared at 78°C or 82°C. Amount of the four whey proteins being studied were so small in samples that had been preheated at 95°C that the band of the unknown protein overshadowed them and made their quantitative assessment difficult or impossible. In an attempt to

compensate for this effect, the sample size was decreased. However, this decrease in sample size caused the 4 whey protein bands to become too weak to be measured accurately.

As expected, when milk samples with or without previous heat treatment were heated in the Amylograph in the absence of starch, the concentration of undenatured whey proteins continued to decrease (Table 5). α-La was still the most resistant to heat treatment. β-Lg A appeared more thermostable than β-Lg B when the temperature of heating was below 95°C. However, during holding 7 min at 95°C, β-Lg B became more stable than β-Lg A. Similar results were also reported by Hillier and Lyster (31). Because the presence of the unknown protein band masked other protein bands, none of the whey proteins in 95°C preheated milk and no BSA in any of the samples could be detected.

Figure 7 shows that the extent of heat denaturation of whey proteins affects the maximum viscosity of pastes. The more whey protein denatured, the lower viscosity obtained, with one exception. With 74°C preheated milk, although an appreciable portion of the whey proteins was denatured, the viscosity was almost the same as that obtained with raw milk. All the data in Figure 7 originated from those of sample B milk in Table 1 and Table 4. A comparison of Figure 1 with Table 4 and Table 5 indicated that, although large losses of native protein occurred when milk was

Table 5. Effect of additional heat treatment in the Amylograph on whey protein denaturation of milk samples

Sample[a]	Concentration of undenatured whey proteins (μg/ml)[b]		
	β-Lg A	β-Lg B	α-La
raw 88	700±100	280±40	1200±200
raw 95	41±5	36±7	990±50
raw 95(7)	14±1	14±3	380±90
74 88	600±100	300±90	1000±300
74 95	22±0[c]	20±1	500±100[c]
74 95(7)	12±0[c]	14±1	270±20[c]
78 88	310±70	130±30	600±100
78 95	20±1[c]	21±1	370±30[c]
78 95(7)	9±0[c]	14±0	140±20[c]
82 88	59±2	32±3	140±20
82 95	10±0[c]	15±1[c]	115±0[c]
82 95(7)	6±0[c]	13±1[c]	62±3[c]

[a]First column indicates temperature (°C) of preheating; second column, the temperature to which sample was heated in the Amylograph; number in parentheses, the number of minutes sample was held at highest temperature in the Amylograph.

[b]Average of three replicates ± standard error except where noted.

[c]Average of duplicates ± standard error.

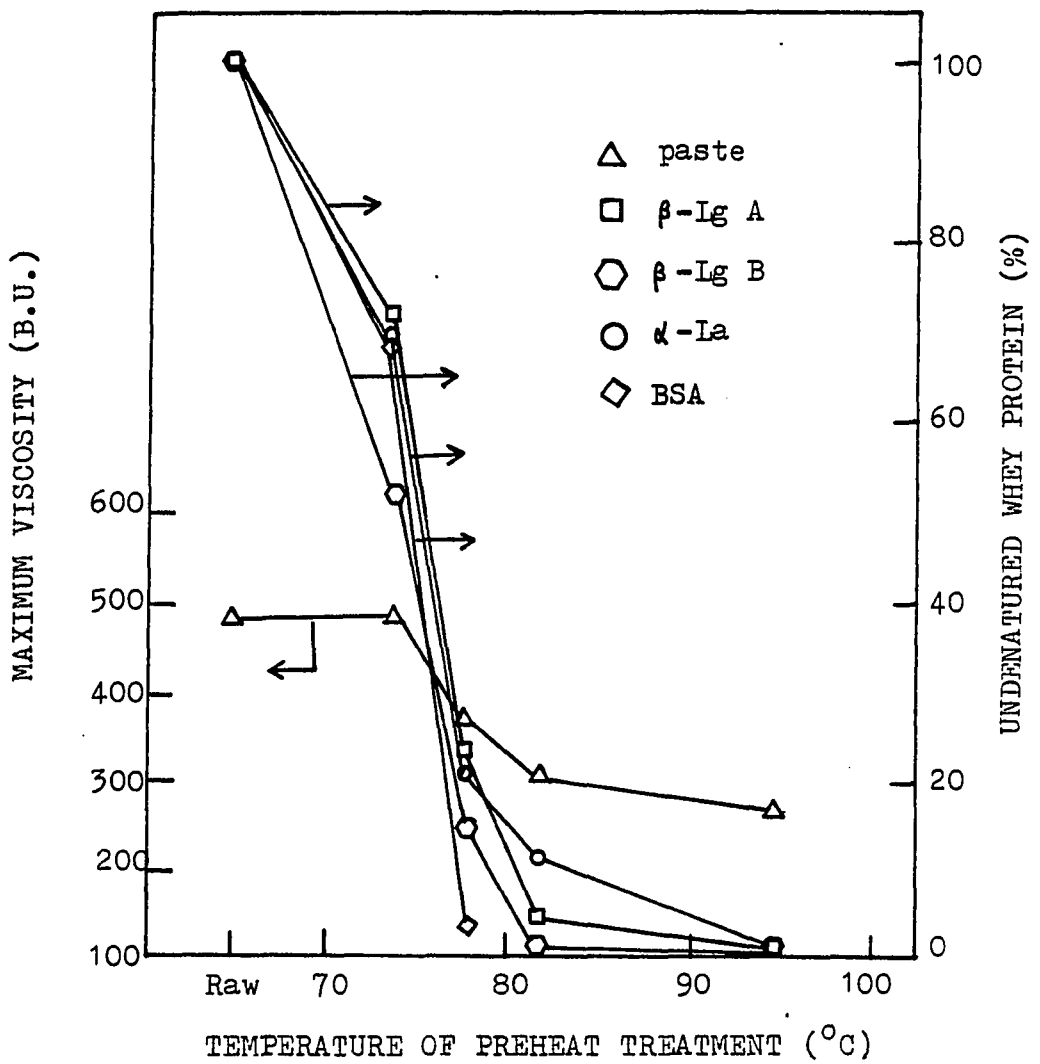


Figure 7. Comparison of Amylograph maximum viscosity with heat denaturation of whey proteins in cornstarch-milk paste

preheated at 74°C, the conditions which were necessary to produce an Amylograph viscosity change in the 6% cornstarch paste were not reached. The greater reduction in undenatured protein produced by treatment at 78°C, 82°C, and 95°C appeared to greatly affect the Amylograph curves. Treatment at 95°C eliminated any viscosity increase in the paste above 88°C, presumably because proteins were nearly completely denatured. The increase of viscosity of the pastes is apparently primarily dependent on the amount of unaltered or partially folded whey proteins and there still remains sufficient such whey proteins after 74°C preheating to yield a viscosity comparable to that of the raw milk. At higher heat treatments, insufficient undenatured whey proteins remain to develop as much viscosity as with milk preheated at 74°C. These results suggest that an important factor in the development of viscosity in a cornstarch-milk system may be denaturation of one or more of the whey proteins during rather than before the swelling of the starch occurs.

Effect of Individual Whey Proteins on Viscosity and Gel Strength

Milk preheated at 82°C with only about 0.060-0.230 mg/ml undenatured β-Lg (Table 4) gave an Amylograph viscosity that rose gradually at temperatures above 88°C (Figure 8). When 0.6 mg/ml β-Lg (a commercial preparation containing both A and B) was added to bring the concentration of undenatured

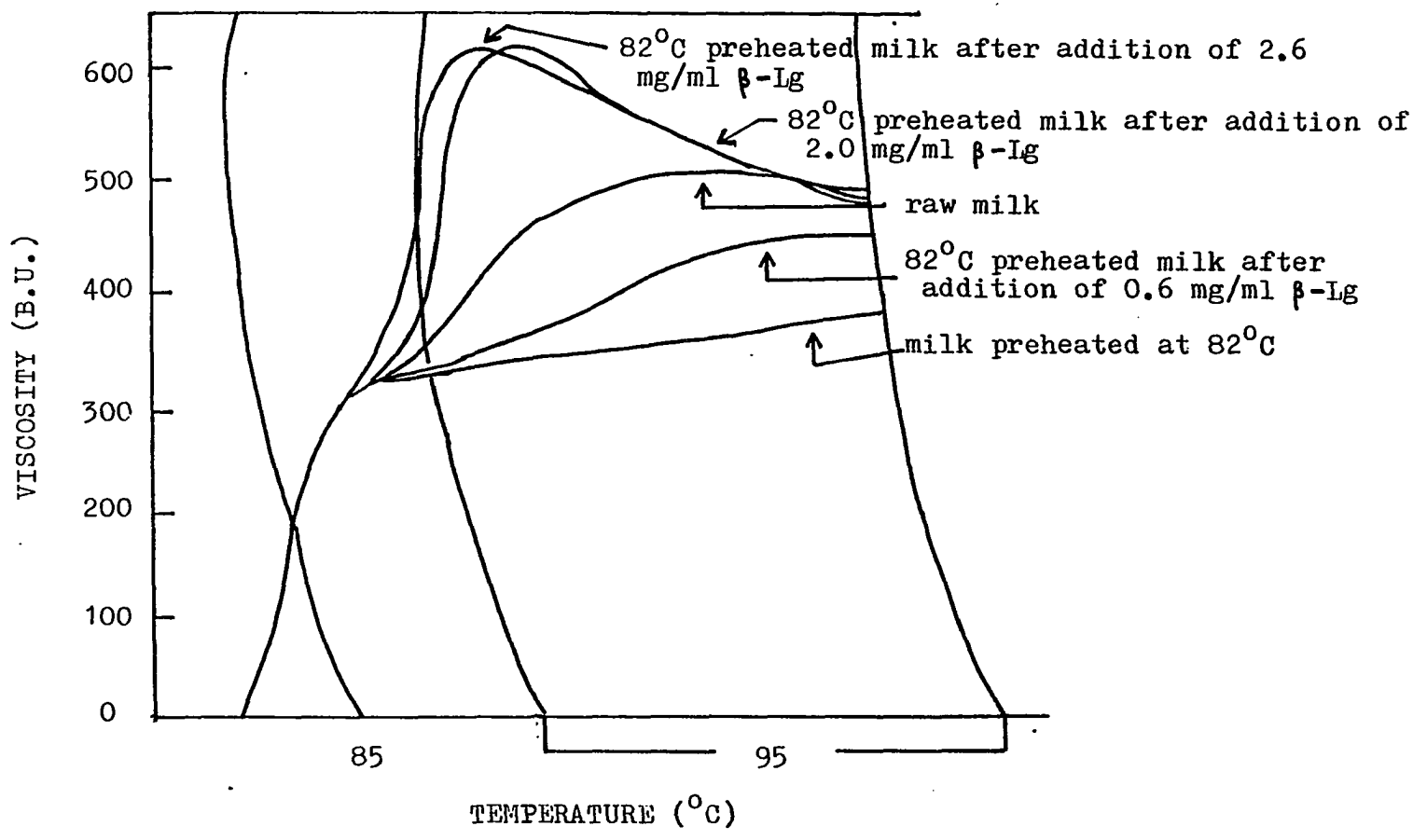


Figure 8. Effect of added undenatured β-Lg on Amylograms of cornstarch-82°C preheated milk pastes

β-Lg to that present in milk preheated at 78°C, the resulting Amylogram was similar to that previously found with milk that had been preheated at 78°C (Fig. 1). Addition of 2.0 mg/ml β-Lg, bringing the level of undenatured β-Lg to that in milk preheated at 74°C (Table 4), caused a very pronounced increase in viscosity with a miximum even higher than that shown by the control sample of raw milk, and the maximum occurred sooner. Just as raw milk, with about 2.6-3.9 mg/ml undenatured β-Lg, produced no higher maximum viscosity than milk that had been preheated at 74°C, so addition of 2.6 mg/ml of β-Lg, to raise the concentration to that of raw milk, caused no further increase in viscosity, although the maximum viscosity occurred sooner.

Unlike β-Lg, α-La failed to produce any effect on the Amylogram given by milk that had been preheated at 82°C (Figure 9). When 0.5 mg/ml α-La was added to restore the concentration to that found in raw milk (Table 4), no effect was produced unless undenatured β-Lg concentration was also restored to its original level. With both addition of α-La and β-Lg, the Amylogram was almost identical to that obtained when only β-Lg alone was added (Fig. 9). Although larger amounts of α-La than β-Lg are present in the sample after preheat treatment at 82°C (Table 4) and also after heating in the Amylograph (Table 5), only the β-Lg appears to make a contribution to the viscosity of the starch-milk pastes.

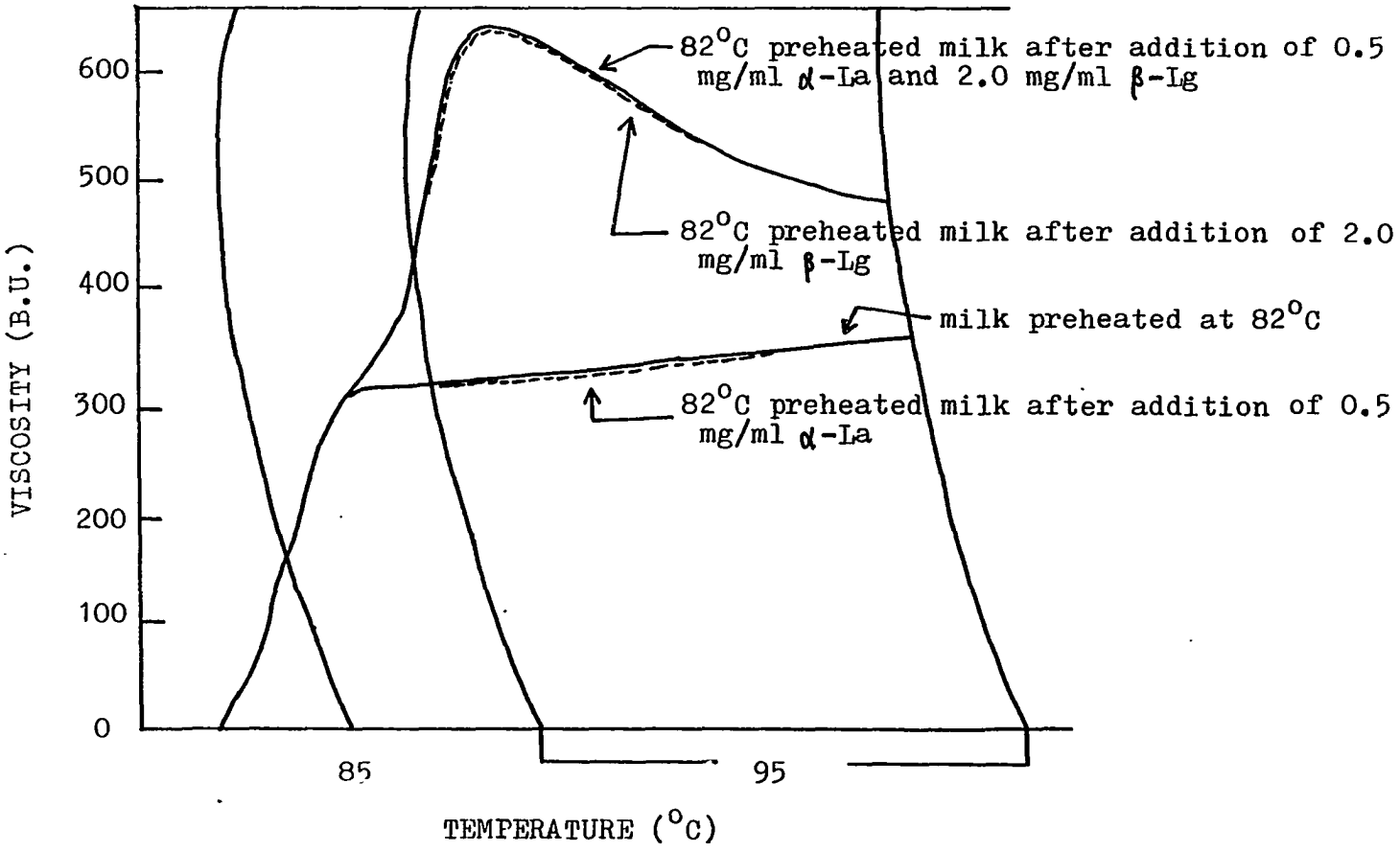


Figure 9. Effect of added undenatured α-La and added undenatured α-La with β-Lg on viscosity of cornstarch-milk paste

Possibly the differences in the responses in paste viscosity to added β-Lg and α-La are related to the activity of the -SH group during heat treatment of the milk. β-Lg is the principal source of the -SH group. When milk is heated, some -SH groups in β-Lg associate with κ-casein to form intermolecular -S-S- bonds (6,17), which may serve as one means of the formation of filaments that produce the increased viscosity by entanglement with the highly swollen starch granules and their filaments.

Strengths of gels formed from starch-milk pastes prepared in the Amylograph using raw milk, milk that had been preheated at 82°C, and the preheated milk after addition of β-Lg are shown in Table 6. Just as addition of increasing amounts of β-Lg caused increased viscosity, so it also resulted in stronger gels.

Interaction of Starch and β-Lactoglobulin Solution

In an attempt to find a dispersion medium for whey proteins that would have little effect on the behavior of starch but would allow the effects of individual whey proteins unaffected by other milk constituents to be tested, the Jenness-Koops buffer solution was investigated. The Amylograms of 6.6% cornstarch pastes prepared with water and with this buffer were nearly identical (Figure 10). The Amylogram made with the buffer solution after the

Table 6. Amylograph viscosity and gel strength of pastes prepared after addition of β-Lg to milk preheated at 82°C

Sample	Maximum viscosity (B.U.)	Final viscosity (B.U.)	Gel strength[a] (g)
raw	570	490	161±3
preheated without β-Lg	360	360	143±1
preheated with 0.6 mg/ml β-Lg	430	430	173±1
preheated with 2.0 mg/ml β-Lg	620	450	223±4
preheated with 2.6 mg/ml β-Lg	620	460	243±7

[a]Average of two samples ± standard deviation.

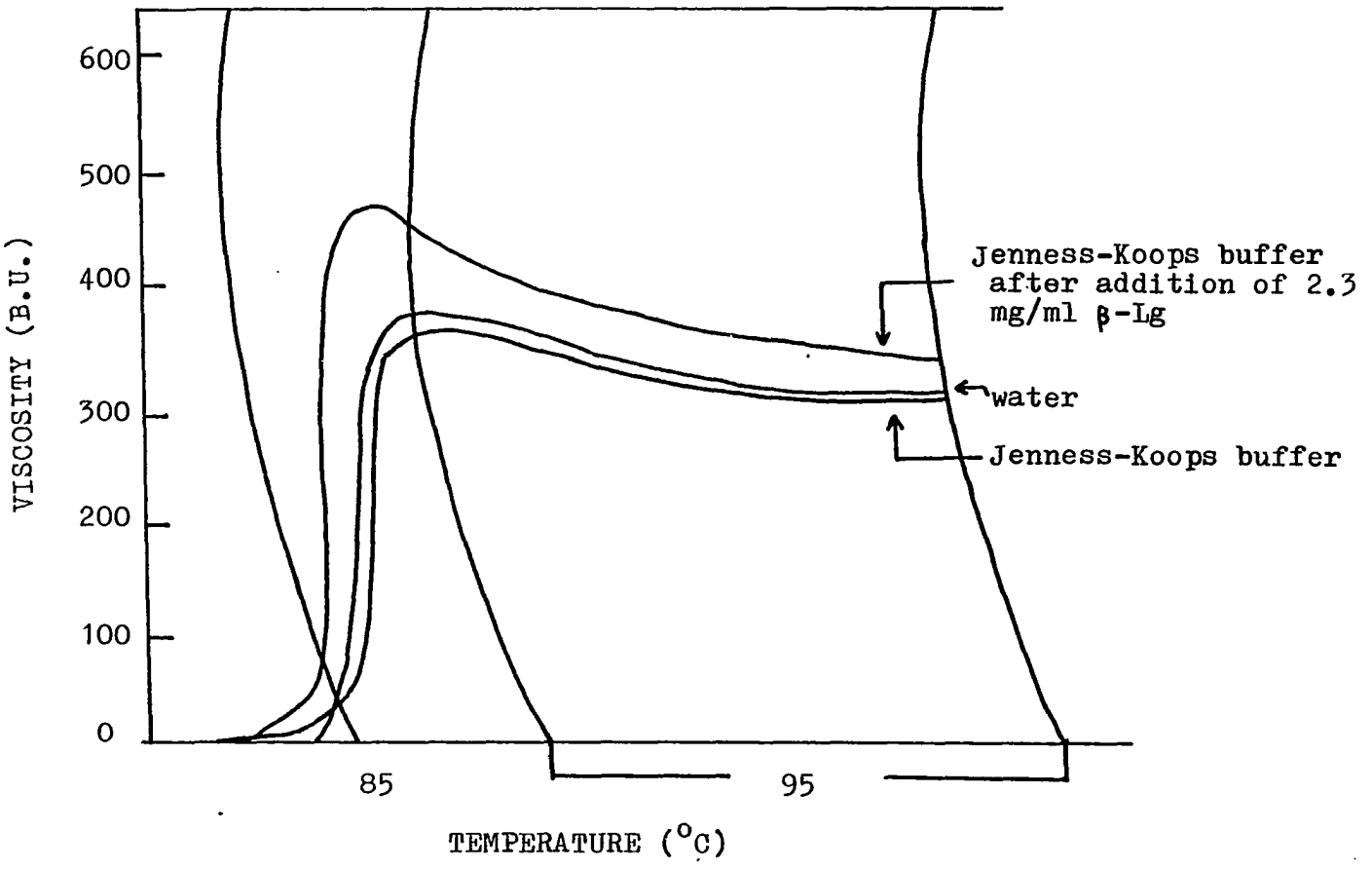


Figure 10. Comparison of Amylograms of cornstarch in different media

addition of 2.3 mg/ml β-Lg is also shown in Figure 10. This concentration of undenatured β-Lg is approximately equivalent to that of milk that had been preheated at 74°C and then heated in the Amylograph to 88°C. An appreciably higher maximum viscosity was obtained, indicating the importance of β-Lg in developing viscosity in starch-milk pastes.

From the above discussion, there is little doubt that the extent of heat denaturation of whey protein affects the Amylogram of the paste. Microscopic examination and measurement of swelling power indicated differences in Amylograms were not caused by effects on granular swelling. This finding appears to be contradictory to the theory that has been proposed (4,76) for the effect of gluten on the swelling of starch in bread, which assumes that water freed during denaturation of the gluten proteins causes increased swelling of the starch granules. However, the much greater ratio of water to starch (14:1) in this study with milk compared to that (0.6:1) in a typical yeast bread dough, as well as the much smaller ratio of β-Lg to starch in the milk systems, a very different situation in which the increase in water caused by denaturation of the β-Lg could not be expected to have any measurable effect.

The results reported in the present study indicated the effect of starch-protein interaction. When starch

was heated in milk samples in the Amylograph to about 80°C, starch granules took up water and swelled sufficiently to cause an initial rise in the Amylograph viscosity. When the temperature reached 88°C, the starch granules continued to swell and most of the whey proteins were denatured (Table 5). It appears possible that whey protein molecules may intertwine with the swollen starch granules and soluble starch components which are leached from the swollen granules as well as fragments of ruptured granules as denaturation progresses to form a loose matrix held together by hydrogen bonds. Such entanglement of denatured protein molecules with swollen starch granules and with soluble starch may produce the increase in viscosity observed in the pastes made with raw milk and that preheated at 74°C, as shown in Figure 1. For milk samples which were preheated at temperature higher than 74°C, considerable amounts of denatured whey protein molecules may associate themselves to form precipitates (50) and thus lose their ability to intertwine with swollen granules before the granules have swollen enough to make such interaction possible. Figure 1 indicated that pastes made with milk samples preheated at 82°C and 95°C fail to give a similar increase in viscosity. Further evidence for the starch-protein interaction is shown in Figure 8. When β-Lg was added to the milk preheated at 82°C to replace that which had been denatured, the milk recovered its

original ability to produce high viscosity with starch.

Protein-protein reactions in milk during heating also need to be considered because they may serve as a means of strengthening the starch-protein meshwork described. The common type of reaction is -S-S- bond formation between β-Lg and κ-casein (6,10,17). Although such -S-S- bond formation may occur, the appreciably higher maximum viscosity produced by addition of β-Lg to a buffer solution (Fig. 10) indicates that this single protein alone undergoes changes during its denaturation that causes an interaction with the swollen starch granules.

The problem of directly determining the extent of heat denaturation of whey proteins in starch-milk pastes is difficult. There is no available method to measure the exact extent of heat denaturation of whey proteins in the starch-protein complex quantitatively. It needs to be noted that when milk is mixed with starch and heated, the amount of available water decreases with increasing temperature because starch granules take up most of the water during swelling. The heat denaturation of whey proteins in such conditions may be different from that in the absence of starch. It has been found that 1% dextrin had a protective effect against denaturation of β-Lg by heat, shown by a reduced tendency of β- Lg to aggregate after heating (57). Starch may have a similar effect.

Apart from theoretical interpretations, the characteristic curves developed with different preheating of skimmilk can be useful for assessing uniformity of product quality and in helping to predict behavior of ingredients in product systems. One needs to be aware of the existence of interactions between starch and protein and the effects of previous treatment of the protein on such interactions. Recently, there has been an increased use of cheese whey in fabricating processed foods. Whey protein has excellent nutritive quality, even superior to casein, and can be used to supplement cereals (11,12). Hernandez and coworkers (28) investigated dehydrated cheese whey as a possible supplement for cereals. They concluded that the addition of cheese whey significantly increased the nutritional value of cereals, especially corn and wheat flours. When whey is substituted for skimmilk, the previous heat treatment that may have denatured part of the whey proteins becomes an important consideration.

SUMMARY AND CONCLUSIONS

The present study was undertaken to obtain information which may explain the previously reported effect on the two-step gelatinization pattern of the Amylograph curves of starch-milk pastes caused by preheating the milk at different temperatures. Because the major change in milk caused by heat treatment is denaturation of whey proteins, the amount of each of the whey proteins that remained undenatured after different heat treatments was determined by polyacrylamide gel electrophoresis. The possible relation of this denaturation of whey proteins to changes of rheological properties in starch-milk systems was examined.

Determination of the swelling power of cornstarch in milk samples that had received various heat treatments, as well as microscopic examination, showed that swelling of the starch granules was not affected. Therefore, the swelling of the granules was not the cause of the effects on viscosity and gel strength of starch-milk systems.

The extent of denaturation of whey proteins was clearly related to the differences in the Amylograph curves. To obtain an approximation of the denaturation of the milk protein during the viscosity tests in the

Amylograph, samples of raw milk and samples that had received each of the preheat treatments studied were heated in the Amylograph in the absence of starch. Samples were removed when the temperature in the Amylograph reached 88°C (the temperature at which the second viscosity increase began when 6% starch was present) and after the temperature had been held for 7 min at 95°C (the time-temperature condition at which the second rise in viscosity reached its maximum). In raw milk heated to 88°C, only 40% of the original β-Lg A, 18% of the β-Lg B, 94% of the α-La, and none of the BSA remained undenatured. In a sample of the same milk held at 95°C for 7 min, only about 1% of either β-Lg A or B and 30% of α-La remained undenatured.

Milk that had been preheated at 74°C produced an Amylogram with 6% starch almost identical to that formed with raw milk. Likewise, when it was heated alone in the Amylograph to 88°C, it contained close to the same amount of undenatured β-Lg A, β-Lg B, and α-La as raw milk heated to the same temperature.

Milk samples that had been preheated at 78°C and 82°C produced much reduced second steps in the Amylograph curves with starch and also showed appreciably greater protein denaturation when heated in the Amylograph to 88°C. Milk that had been preheated at 95°C had very little

undenatured protein even before any additional heating and produced an Amylograph curve completely devoid of a second step.

The importance of β-Lg in the production of the second increase in viscosity was further indicated by its effect when added to milk which had been preheated at 82°C. Such preheated milk had its total content of undenatured β-Lg (combined A and B) reduced by 96% and produced no second step in the Amylograph. When β-Lg was added to bring the total undenatured β-Lg level to that found in raw milk (2.6 mg/ml) or in milk that preheated at 74°C (2.0 mg/ml), a large second increase in viscosity was reintroduced. Addition of only enough β-Lg to bring the level to that remaining undenatured in milk preheated at 78°C (0.6 mg/ml) produced a curve very similar to that obtained with milk preheated at 78°C, although with a lower second increase in viscosity than that caused by addition of 2.0 mg/ml β-Lg.

When β-Lg was added to a solution of inorganic salts (Jenness-Koops buffer) in the amount present in milk that had been preheated at 74°C, the viscosity developed in the Amylograph was increased. However, the viscosity increase was appreciably less than it had been when β-Lg was added to preheated milk at 82°C in the amount needed to bring the concentration to the same level.

Although the amount of α-La remaining undenatured at 88°C was larger than that of β-Lg and most of β-Lg was denatured during the heat treatment that produced the second viscosity increase in the starch paste, addition of 0.5 mg/ml α-La to restore the level to that of milk preheated at 74°C failed to cause any viscosity increase.

Gels formed by refrigeration of the starch-milk pastes heated in the Amylograph were also affected by the preheating of the milk. Those made from milk preheated at higher temperatures gave lower strengths. But for reasons not apparent from this study, gels prepared from raw milk showed intermediate strength.

From the results reported in the present study, it seems that when starch is heated in the Amylograph to 88°C with milk that has previously had only mild heat treatment or none, β-Lg molecules, as they become denatured, may intertwine with swollen granules and soluble starch components to form a loose matrix held together by hydrogen bonds. Such entanglement causes the second increase in viscosity. If milk has received severe heat treatment, considerable amounts of β-Lg molecules have associated themselves to form colloidal particles and thus are no longer available to intertwine with the swollen granules.

Several further investigations are suggested by this study. Examination of the starch-milk pastes by such means as scanning electron microscopy might give further insight into the mechanism by which the higher viscosity is produced. Although the presence of the unknown band appearing on all the polyacrylamide gels made for determination of undenatured whey proteins does not appear to be related to the viscosity changes in the starch pastes, identification of this band would be of interest. Extension of this study to determine the effects on starch-protein systems of heat treatments used in dehydration of milk and eggs would be valuable. For the last study, it is recognized that normal variation in both milk and eggs would need to be considered and that, ideally, the same pooled samples would be used for controls and for the processed samples to eliminate variation.

REFERENCES

1. Babel, F. J. 1976. Technology of dairy products manufactured with selected microorganisms. Pages 213-271 in W. J. Harper and C. W. Hall, eds. Dairy technology and engineering. The AVI Publishing Co., Inc., Westport, Conn.

2. Bean, M. L., and E. M. Osman. 1959. Behavior of starch during food preparation. II. Effects of different sugars on the viscosity and gel strength of starch pastes. Food Res. 24:665-671.

3. Bechtel, W. G. 1950. Measurements of properties of corn starch gels. J. Colloid Sci. 5:260-270.

4. Bechtel, W. G. 1959. Gelatinization of starch during baking. Baker's Dig. 33(12):74.

5. Code of Federal Regulations. 1982. Title 21. Milk and creams §131.3. General provisions. The Office of the Federal Register. National Archives and Records Service, General Services Administration, Washington, D. C.

6. Creamer, L. K., G. P. Berry, and A. R. Matheson. 1978. The effect of pH on protein aggregation in heated skim-milk. N. Z. J. Dairy Sci. Technol. 13:9-15.

7. Creighton, T. E. 1978. Experimental studies of protein folding and unfolding. Prog. Biophys. Mol. Biol. 33: 231-279.

8. Dahle, L. K. 1971. Wheat protein-starch interaction. I. Some starch-binding effects of wheat-flour proteins. Cereal Chem. 48:706-714.

9. Dahle, L. K., E. P. Montgomery, and V. W. Brusco. 1975. Wheat protein-starch interaction. II. Comparative abilities of wheat and soy proteins to bind starch. Cereal Chem. 52:212-225.

10. Davies, F. L., P. A. Shankar, B. E. Brooker, and D. G. Hobbs. 1978. A heat-induced change in the ultrastructure of milk and its effect on gel formation in yoghurt. J. Dairy Res. 45:53-58.

11. Delaney, R. A. M. 1976. Composition, properties, and uses of whey protein concentrates. J. Soc. Dairy Technol. 29:91-100.

12. de Rham, O., P. V. de Rovaart, E. Bujard, F. Mottu, and J. Hidalgo. 1977. Fortification of soy protein with cheese whey protein and the effect of alkaline pH. Cereal Chem. 54:238-245.

13. de Wit, J. N., and G. Klarenbeek. 1981. A differential scanning calorimetric study of the thermal behavior of bovine β-lactoglobulin at temperature up to 160°C. J. Dairy Res. 48:293-302.

14. Dickerson, R. W., Jr., W. H. Stroup, H. E. Thompson, Jr., and R. B. Read, Jr. 1973. Recent developments in pasteurization of grade "A" milk and milk products. J. Environ. Health 35:560-564.

15. Dunnill, P., and D. W. Green. 1965. Sulphydryl groups and the N⇔R conformational change in β-lactoglobulin. J. Mol. Biol. 15:147-151.

16. Erlander, S. R., and L. G. Erlander. 1969. Explanation of ionic sequences in various phenomena. X. Protein-carbohydrate interactions and the mechanism for the staling of bread. Staerke 21:305-315.

17. Euber, J. R., and J. R. Brunner. 1982. Interaction of κ-casein with immobilized β-lactoglobulin. J. Dairy Sci. 65:2384-2387.

18. Fox, P. F., and M. C. T. Hoynes. 1975. Heat stability of milk: influence of colloidal calcium phosphate and β-lactoglobulin. J. Dairy Res. 42:427-435.

19. Ganz, A. J. 1965. Effect of sodium chloride on the pasting of wheat starch granules. Cereal Chem. 42:429-431.

20. Ganz, A. J. 1974. How cellulose gum reacts with proteins. Food Eng. 46:67-69.

21. Goldstein, I. J., C. E. Hollerman, and J. M. Merrick. 1965. Protein-carbohydrate interaction. I. The interaction of polysaccharides with concanavalin A. Biochim. Biophys. Acta 97:68-76.

22. Goshima, G., K. Ohashi, H. Tsuge, and Y. Ohno. 1978. Methods for estimating the characteristic temperature of starch gelatinization. Res. Bull. Fac. Agric. Gifu Univ. 41:115-121.

23. Gough, B. M., and J. N. Pybus. 1971. Effect on the gelatinization temperature of wheat starch granules of prolonged treatment with water at 50°C. Staerke 23:210-212.

24. Grant, V. M. 1968. The effects of heat treatment of milk on selected food systems. M.S. Thesis. Univ. of Iowa, Iowa City, Iowa.

25. Gray, V. M., and T. J. Schoch. 1962. Effects of surfactants and fatty adjuncts on the swelling and solubilization of granular starches. Staerke 14:239-246.

26. Harland, H. A., S. T. Coulter, and R. Jenness. 1952. The effect of the various steps in the manufacture on the extent of serum protein denaturation in nonfat dry milk solids. J. Dairy Sci. 35:363-368.

27. Hehre, E. J. 1960. Contribution of classical immunology to the development of knowledge of dextran structure. Bull. Soc. Chim. Biol. 42:1581-1585.

28. Hernandez, M., A. Vega, and A. Sotelo. 1981. Nutritional evaluation of cereal-cheese whey mixtures. J. Food Sci. 47:81-83.

29. Hester, E. E., A. M. Briant, and C. J. Personius. 1956. The effect of sucrose on the properties of some starches and flours. Cereal Chem. 33:91-101.

30. Hillier, R. M. 1976. The quantitative measurement of whey proteins using polyacrylamide gel electrophoresis. J. Dairy Res. 43:259-265.

31. Hillier, R. M., and R. L. J. Lyster. 1979. Whey protein denaturation in heated milk and cheese whey. J. Dairy Res. 46:95-102.

32. Hillier, R. M., R. L. J. Lyster, and G. C. Cheeseman. 1979. Thermal denaturation of α-lactalbumin and β-lactoglobulin in cheese whey: effect of total solids concentration and pH. J. Dairy Res. 46:103-111.

33. Hodge, J. E., and E. M. Osman. 1976. Carbohydrates. Pages 41-138 in O. R. Fennema, ed. Principles of food science. Part I. Marcel Dekker, Inc., New York.

34. Hood, L. F., A. S. Seifreid, and R. Meyer. 1974. Microstructure of modified tapioca starch-milk gels. J. Food Sci. 39:117-120.

35. Hwang, Q. S. 1963. A comparison of some characteristics of milk samples with their action on starch-milk pastes. M.S. Thesis. Univ. of Illinois, Urbana, Illinois.

36. Imeson, A. P., D. A. Ledward, and J. R. Mitchell. 1977. On the nature of the interaction between some anionic polysaccharides and proteins. J. Sci. Food Agric. 28: 661-668.

37. Itoh, T., Y. Wada, and T. Nakanishi. 1976. Differential thermal analysis of milk proteins. Agric. Biol. Chem. 40:1083-1086.

38. Jaska, E. 1971. Starch gelatinization as detected by proton magnetic resonance. Cereal Chem. 48:437-444.

39. Jelaca, S. L., and I. Hlynka. 1971. Water-binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. Cereal Chem. 48:211-222.

40. Jenness, R. 1970. Protein composition of milk. Page 17 in H. A. McKenzie, ed. Milk protein: chemistry and molecular biology. Vol. I. Academic Press, New York.

41. Jenness, R., and J. Koops. 1962. Preparation and properties of a salt solution which simulates milk ultrafiltrate. Neth. Milk Dairy J. 16:153-164.

42. Jones, M. G., and K. Wilson. 1976. Milk protein-amylose interaction in solution. Staerke 28:338-341.

43. Kim, W. J., V. N. M. Rao, and C. J. B. Smit. 1978. Effect of chemical composition on compressive mechanical properties of low ester pectin gels. J. Food Sci. 43: 572-575.

44. Larson, B. L., and G. D. Rolleri. 1955. Heat denaturation of the specific serum proteins in milk. J. Dairy Sci. 38:351-360.

45. Leach, H. W. 1965. Gelatinization of starch. Pages 289-307 in R. L. Whistler and E. F. Paschall, eds. Starch: chemistry and technology. Vol. I. Academic Press, New York.

46. Ledward, D. A. 1978. Protein-polysaccharide interactions. Pages 205-217 in J. M. V. Blanshard and J. R. Mitchell, eds. Polysaccharides in food. Butterworths, London.

47. Lotan, R. 1979. Qualitative and quantitative aspects of labeling cell surface carbohydrates using lectins as probes. Scanning Electron Microsc. 3:549-564.

48. Mahdi, S. R., and R. L. Bradley, Jr. 1969. Fat destabilization in frozen desserts containing low dextrose equivalent corn sweeteners. J. Dairy Sci. 52:1738-1741.

49. Mangels, C. E., and C. H. Bailey. 1933. Relation of concentration to action of gelatinizing agents on starch. J. Am. Chem. Soc. 55:1981-1988.

50. McKenzie, H. A. 1971. β-Lactoglobulins. Pages 257-330 in H. A. McKenzie, ed. Milk protein: chemistry and molecular biology. Vol. II. Academic Press, New York.

51. McMeekin, T. L. 1952. Milk proteins. J. Milk Food Technol. 15:57-61.

52. Medcalf, D. G., and K. A. Gilles. 1966. Effect of a lyotropic ion series on the pasting characteristics of wheat and corn starches. Staerke 18:101-105.

53. Medcalf, D. G., V. L. Youngs, and K. A. Gilles. 1968. Wheat starches. II. Effect of polar and nonpolar lipid fractions on pasting characteristics. Cereal Chem. 45:88-95.

54. Miller, B. S., R. I. Derby, and H. B. Trimbo. 1973. A pictorial explanation for the increase in viscosity of a heated wheat starch-water suspension. Cereal Chem. 50:271-280.

55. Mitchell, J. R. 1976. Rheology of gels. J. Texture Stud. 7:313-339.

56. Mitchell, J. R. 1980. The rheology of gels. J. Texture Stud. 11:315-337.

57. Moore, W. E., and J. L. Carter. 1974. Protein-carbohydrate interactions at elevated temperatures. J. Texture Stud. 5:77-88.

58. Morrissey, P. A. 1969. The heat stability of milk as affected by variations in pH and milk salts. J. Dairy Res. 36:343-351.

59. Osman, E. M. 1967. Starch in the food industry. Pages 163-215 in R. L. Whistler and E. F. Paschall, eds. Starch: chemistry and technology. Vol. II. Academic Press, New York.

60. Osman, E. M. 1975. Interaction of starch with other components of food systems. Food Technol. 29(4):30-35.

61. Osman, E. M., and P. D. Cummisford. 1959. Some factors affecting the stability of frozen white sauces. Food Res. 24:595-604.

62. Osman, E. M., and M. R. Dix. 1960. Effects of fats and nonionic surface-active agents on starch pastes. Cereal Chem. 37:464-475.

63. Ott, M., and E. E. Hester. 1965. Gel formation as related to concentration of amylose and degree of starch swelling. Cereal Chem. 42:476-484.

64. Rose, D. 1961. Variations in the heat stability and composition of milk from individual cows during the lactation cycle. J. Dairy Sci. 44:430-441.

65. Savage, H. L., and E. M. Osman. 1978. Effects of certain sugars and sugar alcohols on the swelling of corn starch granules. Cereal Chem. 55:447-454.

66. Sawyer, W. H. 1969. Complex between β-lactoglobulin and κ-casein. J. Dairy Sci. 52:1347-1355.

67. Schoch, T. J. 1964. Swelling power and solubility of granular starches. Page 106-108 in R. L. Whistler, ed. Methods in carbohydrate chemistry. Vol. IV. Starch. Academic Press, New York.

68. Schoch, T. J., and E. C. Maywald. 1956. Microscopic examination of modified starches. Anal. Chem. 28:382-387.

69. Selby, A. E. 1967. A comparison of changes occurring in starch pastes during retrogradation. Ph.D. Dissertation. Univ. of Illinois, Urbana, Illinois.

70. Smidsrod, O., A. Haug, and B. Lian. 1972. Properties of poly-(1,4 hexuronates) in the gel state. I. Evaluation of a method for the determination of stiffness. Acta Chem. Scand. 26:71-78.

71. Smits, P., and J. H. V. Brouwershaven. 1980. Heat-induced association of β-lactoglobulin and casein micelles. J. Dairy Res. 47:313-325.

72. So, L. L., and I. J. Goldstein. 1967. Protein-carbohydrate interaction. IV. Application of the quantitative precipitin method to polysaccharide-concanavalin A interaction. J. Biol. Chem. 242:1617-1622.

73. Stalder, M. J. 1964. The amylograph viscosity of starch-milk pastes: effects of source and preheating of milk. M.S. Thesis. Univ. of Illinois, Urbana, Illinois.

74. Takeuchi, I. 1969. Interaction between protein and starch. Cereal Chem. 46:570-579.

75. Tanford, C. 1968. Protein denaturation. Adv. Protein Chem. 23:121-282.

76. Watson, C. A., and J. A. Johnson. 1965. Studies on the gelatinization of starch. I. Competition for water by protein and cellulose derivatives. J. Food Sci. 30: 450-456.

77. Watson, S. A. 1964. Determination of starch gelatinization temperature. Pages 240-242 in R. L. Whistler, ed. Methods in carbohydrate chemistry. Vol. IV. Starch. Academic Press, New York.

78. Wood, F. W. 1979. Psychophysical studies on liquid foods and gels. Pages 21-31 in P. Sherman, ed. Food texture and rheology. Academic Press, London.

APPENDIX: PHYSICAL PROPERTIES OF STARCH FROM CAVENDISH BANANA FRUIT[1]

Introduction

Conversion of agricultural residues to usable products has occupied mankind for millennia. Generally the probability of success is highest when four conditions are met: 1) The residue is centrally located; 2) Its supply is uninterrupted; 3) It is composed of materials that are easily extracted or altered; 4) The potential product is of at least moderately high value.

Bananas culled from those grown for export, however, fulfill all four conditions: 1) They are packed at central locations; 2) Production is year-round; 3) The chief components are starch and sugars, which are readily extracted or fermented; 4) Most products may be consumed by humans.

There are a number of products that may be made from ripe or green fruit, but none has attained even close to 1%

---

[1]Ling, L. H., E. M. Osman, J. B. Fernandes, and P. J. Reilly. 1982. Staerke 34:184-188. The banana starch was isolated by Dr. Fernandes and the size distribution was determined by K. Ramanarayanan and K. A. Berglund. The published paper, written by Dr. Reilly, has been reorganized with a small amount of additional description of some of the experimental methods, designated by numbers at the beginning of the paragraph.

of the tonnage of the fresh fruit itself. Those requiring only limited processing include puree, powder, and flour (3,20); products of fermentation processes potentially are alcohol (9) and vinegar (1,9). Enzymatic processing leads to glucose and fructose syrups (18).

It appeared to us that starch as a product from reject bananas would be preferable to any of the preceding. It can be made essentially pure and will not degrade under proper storage. Its potential acceptance in foods is made easier by its absence of flavor. If its physical properties differ sufficiently from starches of other plants, it could command a premium price as a food ingredient.

The potential tonnage of this product is not large compared to corn starch, but it is certainly substantial. Between 6 and 7 million tons of Cavendish bananas are exported annually (2); it is generally accepted that a further 10% to 20% beyond this weight are culled in processing because of overripening, over- or undersizing, blemishes, or cuts. Assuming the average implies that about one million tons are rejected. Since green bananas with skins are approximately 15% starch (14), roughly 150,000 tons of starch are potentially available. To this could be added perhaps a third as much in discarded stalks and a substantial amount in the pseudostems left in the fields.

It should be pointed out that not all of the culled

fruit is wasted. Appreciable amounts go to domestic markets and to animal feed in the exporting countries, and to this extent the potential tonnage is decreased.

Rather little is known about the physicochemical properties of banana starch. Two articles deal with pesudostem starch (13,16); several more cover the starch from banana fruit (5,6,11,12,17). There appears to be no information on the properties of starch from banana stalks. These papers contain some data that relate to the suitability of banana starch for food uses; these consist of measurement of swelling and solubility in hot water, amylograms, and amylase digestibilities.

This paper is an extension of the prior work to include more data related to the potential use of banana fruit starch as a food. It deals with the physical properties of banana starch granules, pastes, and gels, in many cases in comparison with starches from other sources, and presents scanning electron micrographs of swelling granules and new data on paste length, gel strength, light transmittance and reflectivity by the gel, and granule size distribution.

Materials

Green Cavendish bananas (Musa cavendishii) were obtained in 40 lb boxes 5 to 7 days after they had been

harvested in Central America. They had not been subjected to artificial ripening. Starch was isolated by a modification of a previously published method (5,7). Skins were removed, and banana chunks were blended with 1% sodium hydroxide, 3% sodium bisulfite, and 0.03% sodium azide, the percentages based on the wet weight of banana pulp, dissolved in water. During 48 h of standing at room temperature, the mixture separated into three layers, the upper two containing little starch. They were decanted away, and the bottom layer was washed repeatedly with water. It was then passed, as an aqueous slurry, through three screens with successively smaller clearances of 149 μm, 125 μm, and 105 μm to remove fibrous material. A thin brown layer on the filtrate after decanting was removed by scraping, and the rest was dried at room temperature for several days, at which time its moisture, measured by the method of Smith (15), was 15.5%.

Other starches were obtained from the following companies: corn, waxy corn, waxy corn diphosphate, and acetylated waxy corn diphosphate from American Maize-Products Company, Hammond, Indiana, potato from A. E. Staley Manufacturing Co., Decatur, Illinois, and tapioca from National Starch and Chemical Corp., Bridgewater, New Jersey. Moistures were 10.7% for corn, 10.7% for waxy corn, 10.8% for acetylated waxy corn diphosphate, 14.3% for potato, and

12.6% for tapioca.

Starch Properties

Size distribution

This measurement was conducted with a Coulter Counter Model TAII with a 280 μm orifice. Starch was dispersed in a 5% sodium chloride solution previously filtered through an Amicon PM10 membrane. A plot of fractional number density vs. characteristic length is shown in Figure 1.

Amylose content

Amylose contents were measured following the method of French et al. (4). Four samples of banana starch averaged 19.5%, while four of corn starch (American Maize-Products) had an average of 22.1%. A second corn starch, this one from National Starch and Chemical, had an amylose content of 28.2%, closer to the generally accepted level.

In adapting the method for use with whole granular starch instead of isolated amylose, starch was defatted by extraction with 95% ethanol in a Goldfish apparatus[1]. Approximately 0.1 mg of starch (dry basis) was dissolved in 5 ml of 1 N KOH, then diluted with about 85 ml of water and neutralized with 5 ml of 1 N HCl. Five ml of

---

[1]Additional description beyond the original published paper.

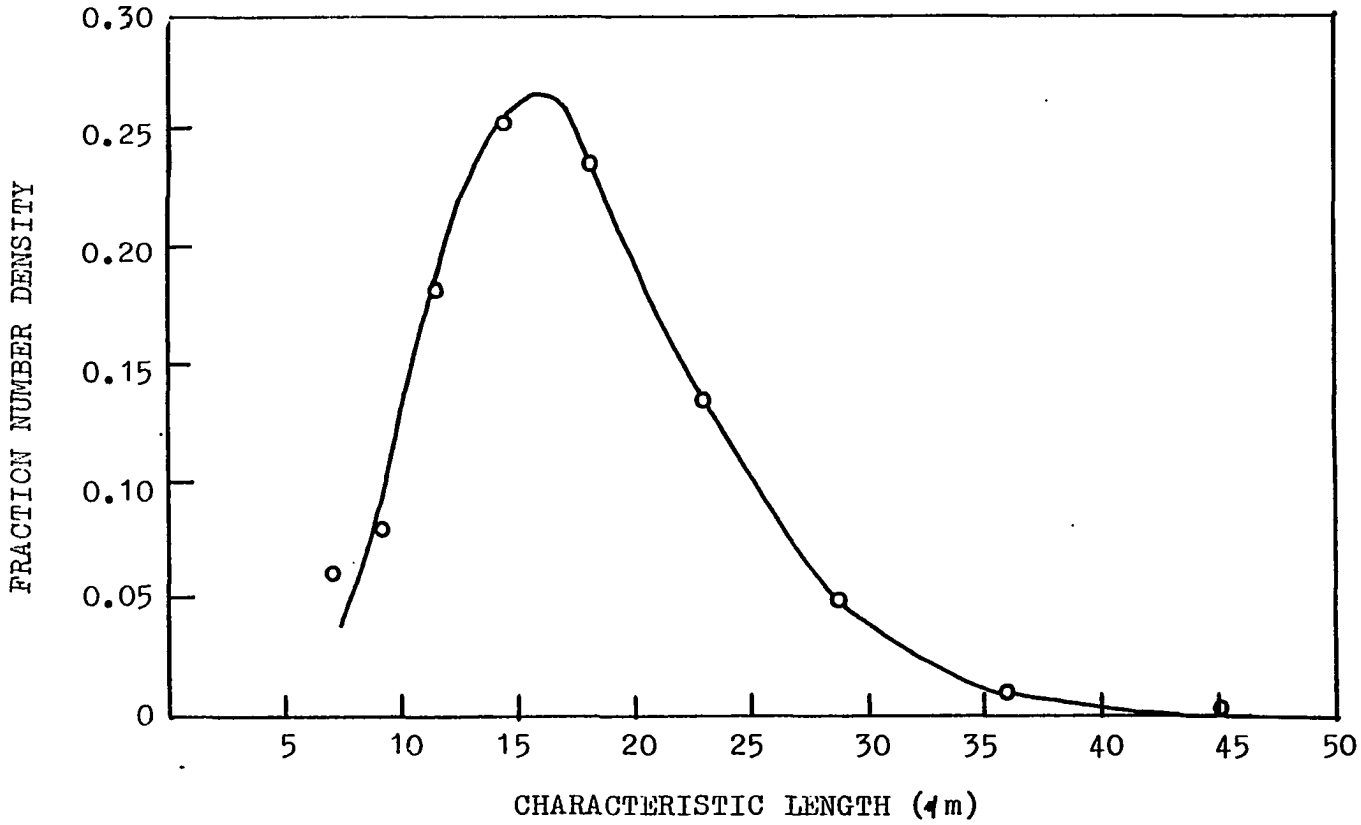


Figure 1. Size distribution of banana starch granules at room temperature in 5% sodium chloride

1 M KI solution was added to bring the total volume to 100 ml. The solution was titrated with standard iodine solution (0.01 M I_2, 0.05 M KI and 0.05 M KCl). After initial additions of 0.1, 0.1, 0.3 and 0.5 ml of iodine solution to dissolved starch, increments of 1 ml were added up to a total of 10 ml. The solution was stirred and the potential was read after each addition on a pH/mv meter, model 701A (Orion Research, Inc.) with bright platinum and calomel electrodes. A blank was prepared in the same way except that the starch sample was omitted.

The blank titration was used to prepare a calibration curve of iodine concentration vs. potential[1]. When plotted on semi-log graph paper, the blank data gave a straight line and was used to determine the free iodine in the sample titrations. The iodine binding capacity was obtained by plotting bound iodine against free iodine and extrapolating to zero iodine concentration. By assuming the iodine binding capacity is 200 mg of I_2 per g of dry amylose, the amylose content in the sample was calculated.

Gelatinization temperature range

These values were obtained by the method of Watson (19). Five samples of banana starch had an average

---

[1]Additional description beyond the original published paper.

gelatinization temperature range of 70.1°C to 74.6°C, while the average range of five samples of corn starch was 64.6°C to 70.5°C.

These were measured under a Leitz microscope equipped with a polarizing filter and a heating-stage as mentioned in the text of this thesis[1].

Scanning electron micrography

A 1% suspension of banana or corn starch granules in 100 ml of water contained in a mechanically shaken 250 ml centrifuge bottle was heated at a rate of 0.5 - 1.0°C/min. Samples were removed at specified times, placed on cover glasses, and frozen in liquid nitrogen. They were immediately freeze-dried without thawing, given a 60% gold - 40% palladium coating, and photographed at 200x and 1100x magnification with a JEOL 35 scanning electron microscope.

Banana starch granules, which are very variable in both shape and size, exhibit surface cracking at 65°C, and progressively greater swelling, deformation, and erosion between 70°C and 90°C (Figure 2). Corn starch granules, which are more regular in shape and size, swell greatly at 75°C and above, but are more resistant to deformation than are those of banana starch (Figure 3 and 4).

---

[1]Additional description beyond the original published paper.

Figure 2. Scanning electron micrographs of banana starch granules at (a) room temperature (200X), and heated to (b) 65°C (200X) (c) 75°C (200X) (d) 80°C (200X)

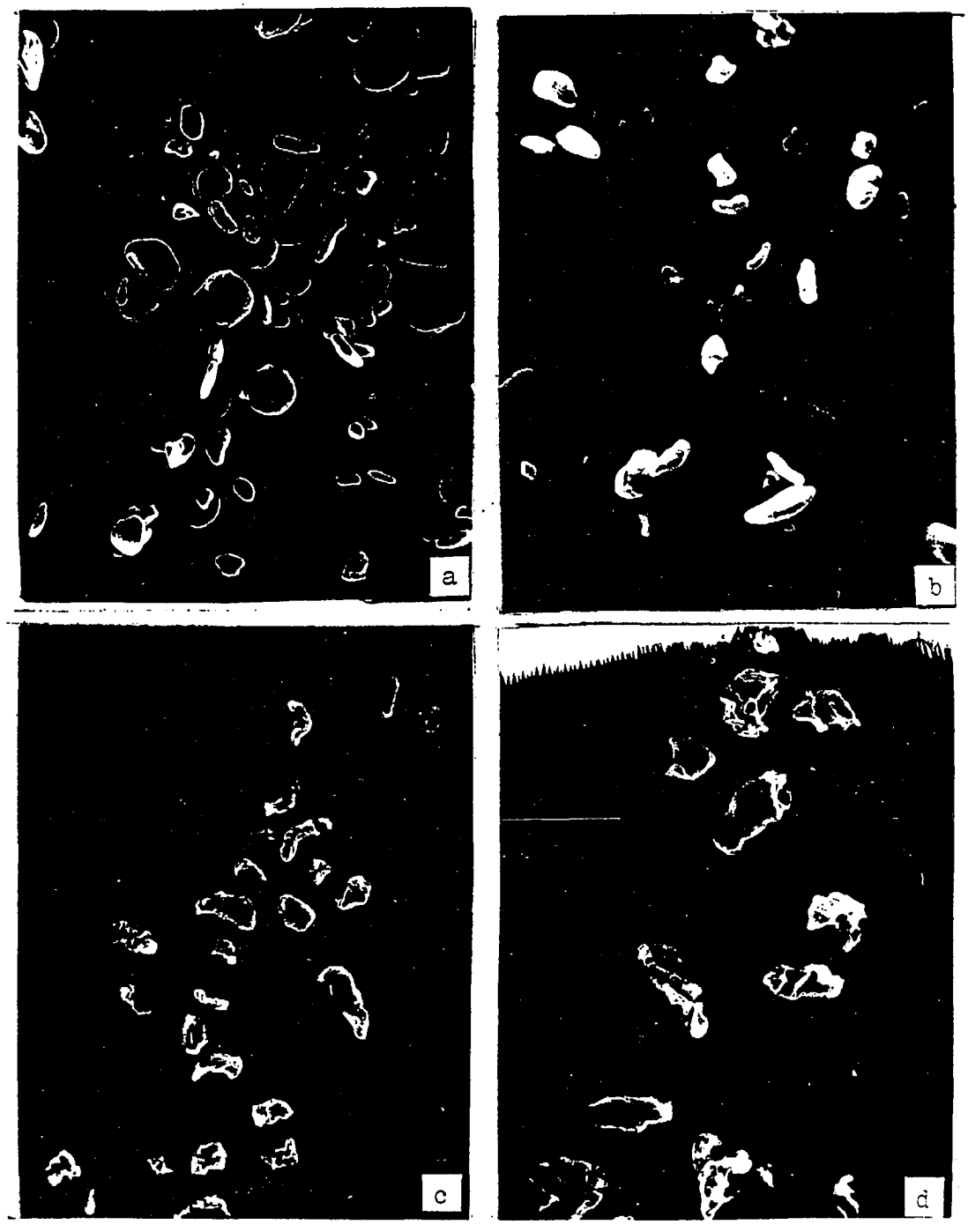

a
b
c
d

Figure 2. (continued)
(e) 90°C (200X)
(f) room temperature (1100X)
(g) 65°C (1100X)
(h) 70°C (1100X)

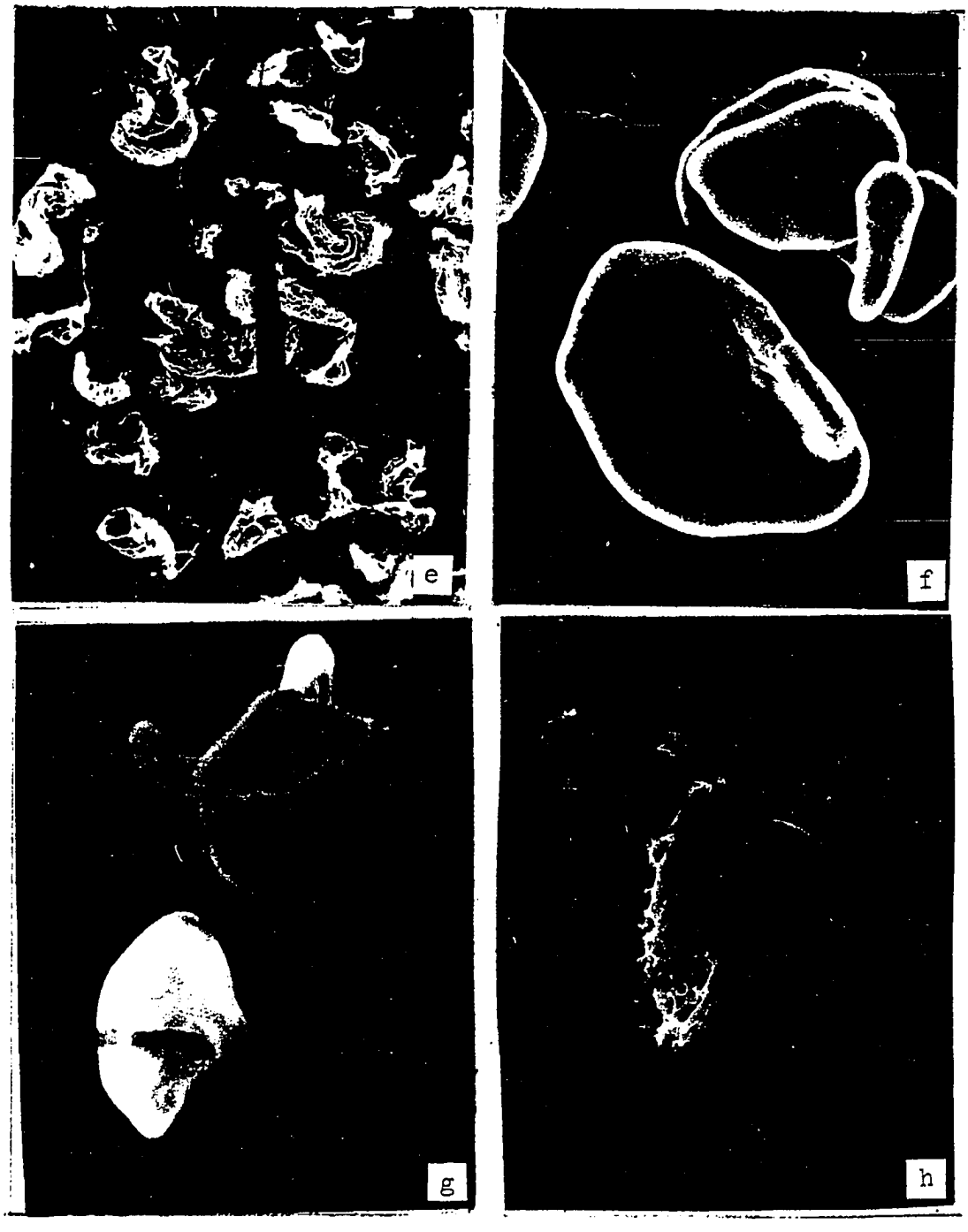

e
f
g
h

Figure 2. (continued)
(i) 75°C (1100X)
(j) 80°C (1100X)
(k) 90°C (1100X)


i


j

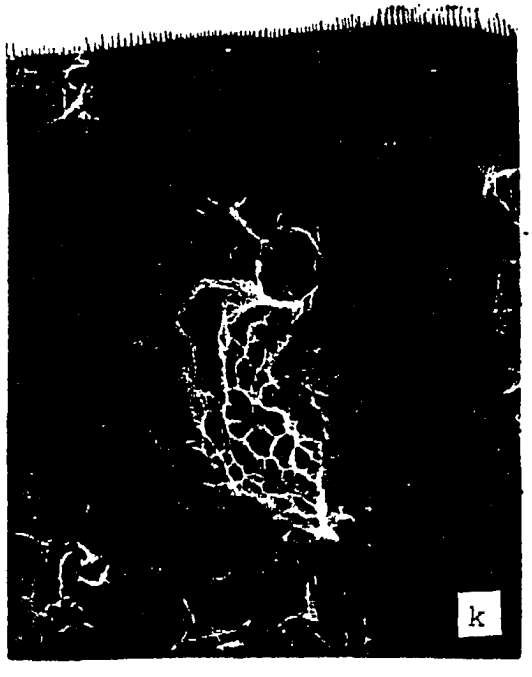

k

Figure 3. Scanning electron micrographs at 200X magnification of corn starch granules at (a) room temperature, and heated to (b) 75°C (c) 80°C (d) 90°C

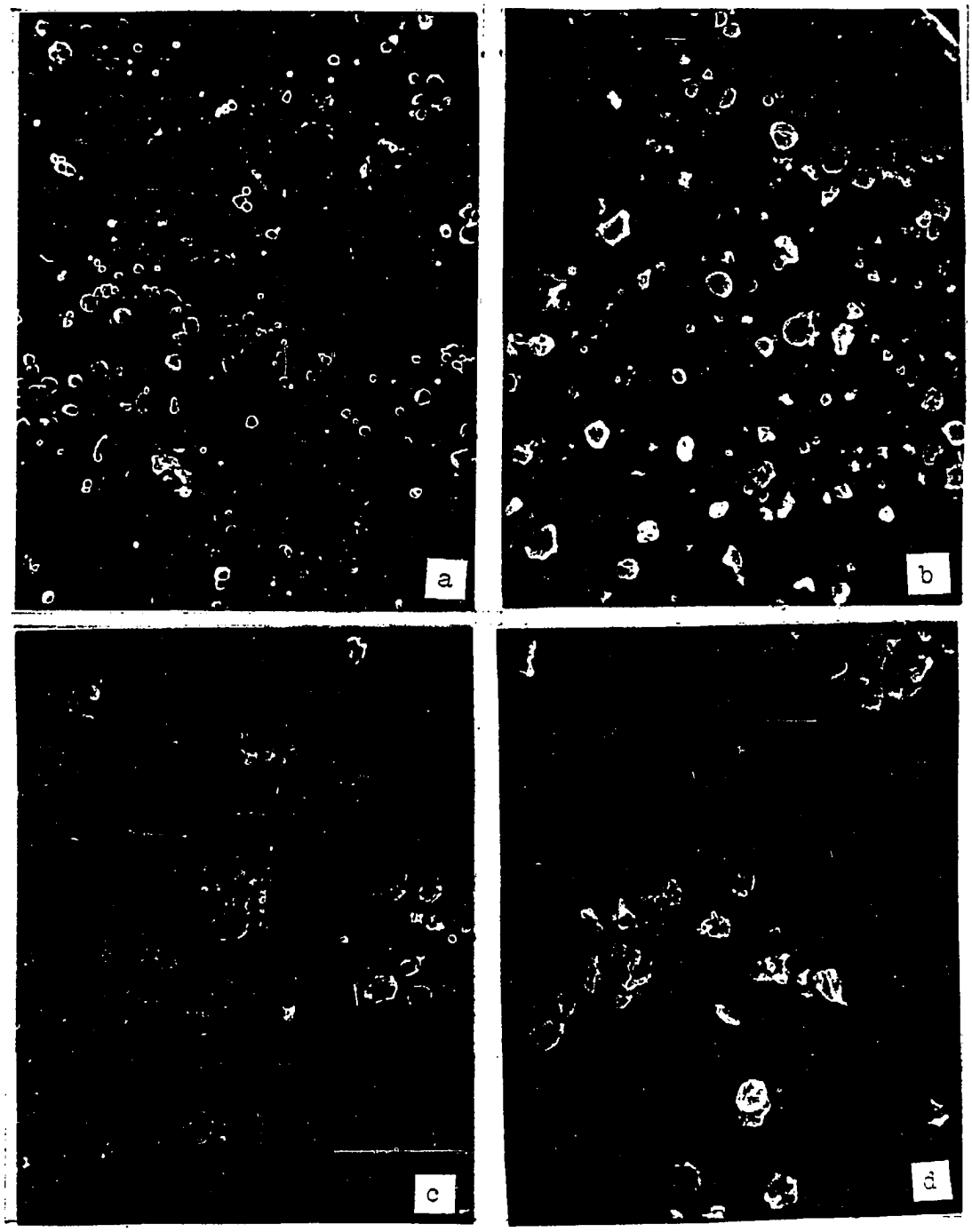
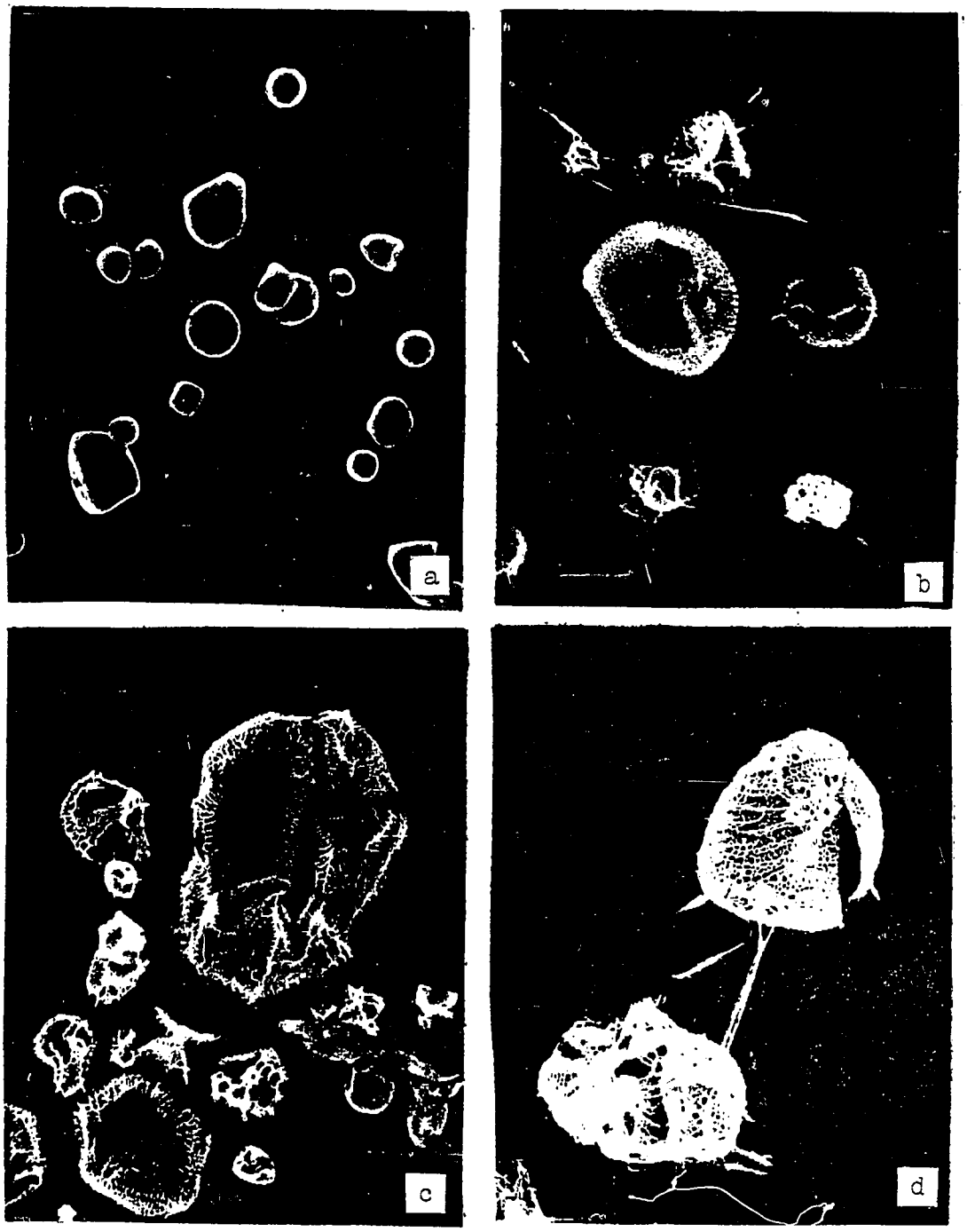

a
b
c
d

Figure 4. Scanning electron micrographs at 1100X magnification of corn starch granules at (a) room temperature, and heated to (b) 75°C (c) 80°C (d) 90°C


a
b
c
d

The sampling was accomplished by dipping the cover glass into the starch suspension in the centrifuge bottle[1]. The cover glass was then introduced quickly into liquid nitrogen and held in a vertical position until boiling ceased. The specimen was then placed on the frozen plate of a freeze-drier (VirTis Company, Model 10-MR-TR) and dried under a vacuum of 60 millitorr of mercury to prevent artifacts caused by ice crystals. When the frozen plate reached room temperature (about 10 hrs), the specimen was mounted on a copper disk.

Amylograms

A Brabender type VA-VE Visco-amylograph with 75 rpm stirring was employed to obtain amylograms of 6% (dry basis) banana, corn, and tapioca starch suspensions. They were heated from 30°C to 95°C at 1.5°C/min, held for 1 hr at 95°C, and then cooled at 1.5°C/min to 30°C.

Banana and tapioca starches both had maximum viscosities much higher than that of corn starch; however, tapioca starch reached its peak at a much lower temperature, and its viscosity decreased appreciably faster than the other two (Figure 5). Viscosities of both banana and corn starch pastes increased sharply with decreasing temperature in a

---

[1]Additional description beyond the original published paper.

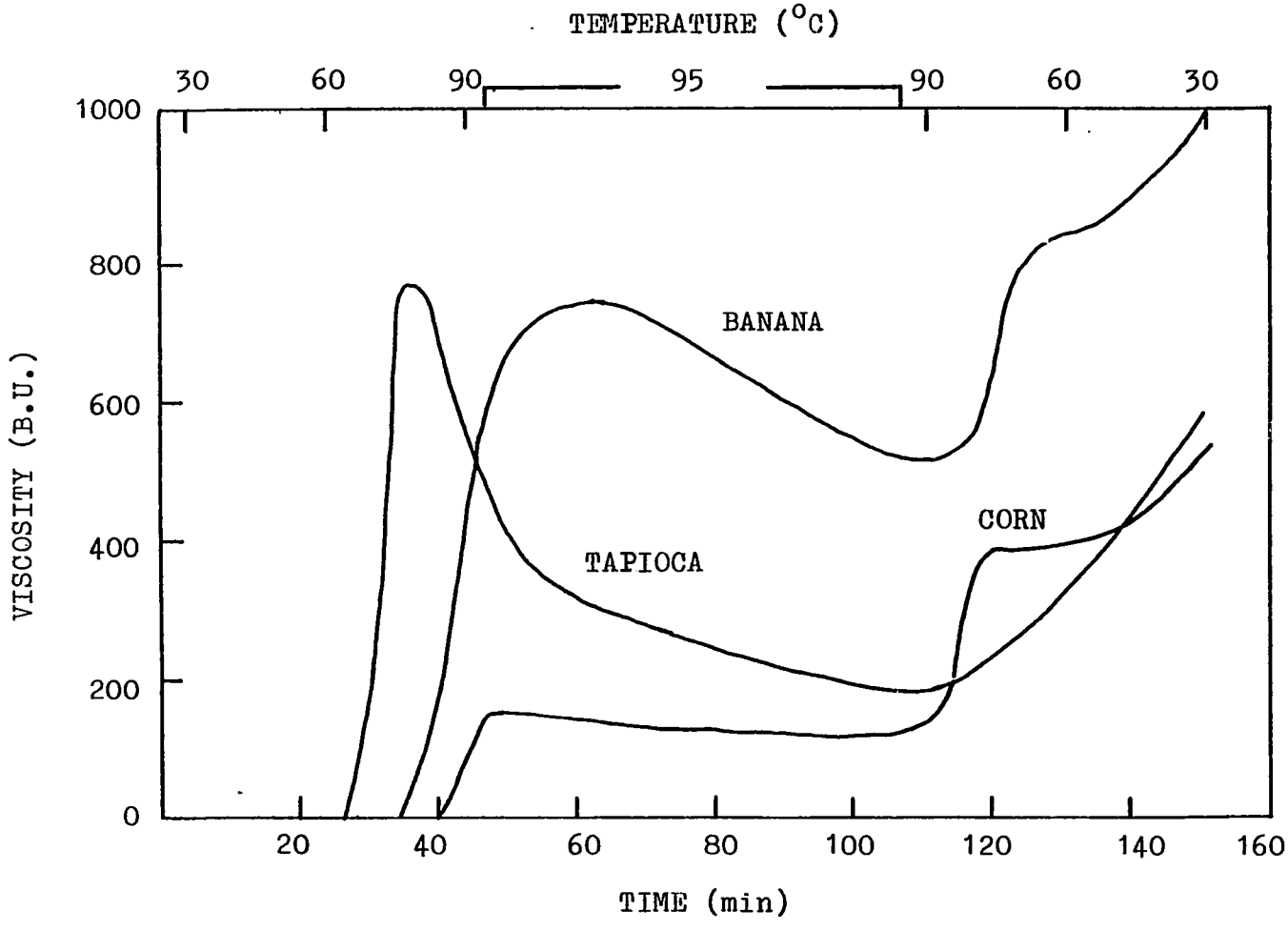


Figure 5. Brabender Amylograms of 6% starch pastes

stepwise fashion, while the viscosity of tapioca starch increased more gradually.

Paste length

Banana, corn, and tapioca starch suspensions were treated by Brabender Visco-amylograph as described above, and held at 95°C for 15 min. After dipping a glass rod into the pastes, it was apparent that banana starch paste was somewhat longer than the corn starch paste but appreciably shorter than that of tapioca.

In the absence of any generally accepted reliable test for this property, such casual inspection of the manner in which the hot paste falls from a stirring rod dipped into it forms the customary method of estimating stringiness or length of paste[1].

Gel strength

Gel strength was measured by a modification of the Saare-Martens method, cited by Osman and Mootse (10). Banana and corn starch suspensions were heated in the Brabender Visco-amylograph as described in the measurement of paste length, and a portion of each paste sample was poured into a 250 ml beaker. In the middle of the beaker,

---

[1]Additional description beyond the original published paper.

30mm deep in the paste, was suspended a 19 mm diameter, 2 mm thick copper disc, attached to a 90 mm long, 1.5 mm thick hooked rod. After allowing the paste, covered by a thin layer of mineral oil, to stand for 18 hrs at 4°C followed by 0.5 - 1 hr at room temperature, the rod was connected to one arm of a balance, and an increasing weight was added to a beaker on the other arm by means of a fine stream of water until the disc broke loose from the gel. With three samples of banana starch, an average of 189 g were required for this, while an average of 241 g were required for four samples of corn starch.

Light transmittance and reflectance by the gel

Transmittances and reflectances of four samples each of a number of starch gels were measured by the method of Kite et al. (8). Starch samples (5% dry basis, in water) were heated in a Brabender Visco-amylograph as in the measurement of paste length, degassed under vacuum, and allowed to stand for 48 hrs at room temperature in 11 mm i.d. test tubes (Bausch and Lomb cat. no. 33-17-80). Transmittance was measured at 550 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. For reflectance determination, starch pastes were held under a microscope cover glass in a black plastic bottle cap for 48 hrs at room temperature and measured with the reflectance attachment of a Spectronic 20, using a magnesium carbonate block

as the white standard.

Corn starch transmitted the least light and reflected the most, followed by banana starch (Table 1). Waxy corn diphosphate starch and acetylated waxy corn diphosphate starch also transmitted little light, while waxy corn, potato, and tapioca starches were relatively transparent. The five other starches reflected much less light than corn and banana starches.

Discussion

Because banana starch had been so little studied, and because the methods of starch separation and the geographical source of the bananas in this work differed from those of previous workers, several properties measured by others were measured again. Moisture was very similar to that found by Rasper (10) in granules from the fruit of the very closely related plantain (Musa paradisiaca) and by Fujimoto et al. (5,6) in granules from banana (M. Cavendishii) fruit, but was higher than the moisture in starch granules from banana pseudostems (13,16). Characteristic lengths of granules measured by electronic means varied roughly six-fold; therefore, the ratio of volumes between the largest and smallest particles was the cube of this, assuming the shapes of large and small granules were the same. This wide distribution was confirmed by the

Table 1. Transmittances and reflectances of 5% gels of seven starches

Starch	Transmittance (%)	Reflectance (%)
banana	2.3	20.5
corn	1.6	26.3
waxy corn	51.9	11.3
waxy corn diphosphate[a]	4.0	12.2
acetylated waxy corn diphosphate	5.5	12.0
potato	50.3	13.1
tapioca	57.1	11.0

[a]Two samples.

photographic evidence from both this study (Figure 2a) and others (5,6). The average size was smaller than that reported by Patil and Magar (11) for granules from banana and plantain fruit but similar to that of Fujimoto et al. (5) for granules from the former. Amylose content agreed with that found previously for starch from banana fruit (11) and pseudostems (13).

Our amylograms of corn starch are similar to those conducted under the same conditions by Rasper (12) and Fujimoto et al. (6), but we measured somewhat higher viscosities on banana starch paste than did Rasper on plantain starch or Fujimoto et al. on banana starch of the same concentration, and higher viscosities on tapioca starch than did the former, when his data were adjusted to 6% starch concentration. However, the shapes of our curves for all three starches are similar to those previously reported, except that the viscosity of banana starch in our hands first rose at a higher temperature and then showed greater setback on cooling than reported by Fujimoto et al. Rasper, Fujimoto et al., and we all found that banana starch paste was appreciably more viscous than an equal concentration of corn starch paste, but that both were very resistant to breakdown of viscosity on heating at 95°C. Based on a logarithmic relationship between maximum viscosity of many starch pastes and their concentration found by Rasper (12),

the same maximum viscosity found with 6% corn starch should be attained by banana starch of roughly 4% concentration.

Scanning electron micrography of banana and corn starch granules demonstrated that both underwent great swelling, erosion, and deformation at elevated temperatures in water, but that in corn starch these changes occurred at higher temperatures than in banana starch. Occurrence of the initial viscosity rise of corn starch in a Brabender amylograph also occurs at a higher temperature than that of banana starch, although its gelatinization measured by loss of birefringence occurs at a lower temperature. This can doubtless be attributed to the larger size and lower resistance to swelling of banana starch granules.

Banana and corn starch are similar in three other properties that are important in food applications: paste length, gel strength, and gel optical properties. Both starches give short pastes that upon cooling yield gels of high strength; corn starch paste is somewhat shorter and its gel is somewhat stronger. The latter finding is similar to that of Rasper (12), who measured gel consistency of plantain and corn starches by a totally different method and found the latter to be higher. Corn starch gel transmits somewhat less light and reflects somewhat more than banana starch gel, but they are more similar to each other in optical properties than to any other starch tested.

Fujimoto et al. (6), using granule size, moisture, bulk fluidity, swelling capacity, solubilities in water, sodium hydroxide, and dimethyl sulfoxide, amylogram characteristics, α-amylase and glucoamylase digestion, and x-ray diffractograms, placed banana starch generally between sweet potato and potato starch, with corn starch closest to sweet potato starch and most removed from potato starch in properties. If, however, one considers just those properties important in food applications - swelling and degradation at high temperatures, susceptibility to amylase attack, amylogram characteristics, paste length, gel strength, and gel optical properties - one could make the case that corn and banana starches are quite similar, perhaps an unexpected conclusion for two starches of such different origins (though both are from monocotyledonous plants). Banana starch is somewhat less resistant than corn starch to swelling and degradation and appreciably more resistant to α-amylase and glucoamylase attack (6); however, their amylograms have similar shapes and they are very similar in paste length, gel strength, and gel optical properties.

The foregoing would indicate that the two starches in general would compete for the same markets, which because of advantages in transportation costs would be more likely to fall to banana starch near its origin than in temperate

countries.

References

1. Adams, M. R. 1978. Small-scale vinegar production from banana. Trop. Sci. 20:11-19.

2. Anonymous. 1980. 1978 world trade annual. Vol. I. Walker, New York.

3. Crowther, P. C. 1979. The processing of banana products for food use. Rept. Trop. Prod. Inst. G122. London.

4. French, D., A. O. Pulley, and W. J. Whelan. 1963. Starch fractionation by hydrophobic complex formation. Staerke 15:349-354.

5. Fujimoto, S., K. Saishoji, T. Nagahama, and M. Kanie. 1977. Isolation of starch from green banana. Dempun Kagaku 24:31-35.

6. Fujimoto, S., K. Shimoozono, T. Nagahama, and M. Kanie. 1977. Properties of starch from green banana. Dempun Kagaku 24:36-41.

7. Kanie, M., T. Nagahama, S. Fujimoto, and K. Saishoji. 1976. Production of banana starch. Japan. Kokai 76 79:742-747.

8. Kite, F. E., E. C. Maywald, and T. J. Schoch. 1963. Functional properties of starches of foods. Staerke 151:131-138.

9. Maldonado, O., C. Rolz, and S. S. de Cabrera. 1975. Wine and vinegar production from tropical fruits. J. Food Sci. 40:262-265.

10. Osman, E. M., and G. Mootse. 1958. Behavior of starch during food preparation. I. Some properties of starch-water systems. Food Res. 23:554-564.

11. Patil, D. L., and N. G. Magar. 1974. Physicochemical properties of banana fruit starch and starch components. J. Ind. Chem. Soc. 51:1004-1007.

12. Rasper, V. 1969. Investigations on starches from major starch crops grown in Ghana. I. Hot paste viscosity and gel-forming power. J. Sci. Food Agr. 20:165-171.

13. Shantha, H. S., and G. S. Siddappa. 1970. Physico-chemical nature of banana pseudostem starch. J. Food Sci. 35:72-74.

14. Simmonds, N. W. 1959. Bananas. Longmans, Green, London.

15. Smith, R. J. 1964. Determination of moisture. Pages 36-41 in R. L. Whistler, ed. Methods in carbohydrate chemistry. Vol. IV. Starch. Academic Press, New York.

16. Subrahmanyan, V. G. Lal, D. S. Bhatia, N. L. Jain, G. S. Bains, K. V. Srinath, B. Anandaswamy, B. H. Krishna, and S. K. Lakshminarayana. 1957. Studies on banana pseudostem starch: production, yield, physico-chemical properties and uses. J. Sci. Food Agric. 8:253-261.

17. Sugimoto, Y. S. F., T. Takaya, and H. Fuwa. 1980. In vivo digestion of banana starch granules. Staerke 32:290-294.

18. Van Wyk, P. J., E. A. Heinen, and L. G. J. Ackermann. 1978. Preparation of glucose and high fructose syrups from bananas. Lebensm. Wiss. Technol. 11: 29-30.

19. Watson, S. A. 1964. Determination of starch gelatinization temperature. Pages 240-242 in R. L. Whistler, ed. Methods in carbohydrate chemistry. Vol. IV. Starch. Academic Press, New York.

20. Wilson, R. J. 1975. The international market for banana products for food use. Rept. Trop. Prod. Inst. G103. London.

ACKNOWLEDGMENTS

The author gratefully acknowledges the patience, encouragement, criticism, and facilities provided by Dr. Elizabeth M. Osman during the entire course of this research and the preparation of this manuscript. Special appreciation for her gentle personality and unfailing support when the author was in hospital.

The author also wishes to thank Dr. Peter J. Reilly for his encouragement in every aspect of this work, especially in the publication of the paper of banana starch.

Appreciations are also due to Dr. Mark H. Love for his inspiring discussions both on laboratory works and on life prospectives.

Gratitudes are due to Dr. Allen A. Kraft and Dr. Robert E. Serfass for serving on the graduate committee and providing helpful comments.

Special appreciation is due to Dr. Jacqueline Dupont for providing gel electrophoresis equipment.

Personal gratitude is extended to all graduate students in this department, especially Carolyn Shimazu for her assistance in time of need. Acknowledgement is also made to Christian brothers and sisters in Chinese Bible Study, to Tony Chou, Mae Chen for their warm encouragement and assistance of many sorts.

The author dedicates this to his parents for their love to see him through it all.

Praise the LORD.