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Physico-chemical, nutritional, and flavor properties of soybean extracts processed by rapid-hydration hydrothermal cooking

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Physico-chemical, nutritional, and flavor properties of soybean extracts processed by rapid-hydration hydrothermal cooking

by

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DEDICATION

This work is dedicated with love to my wife, Il-Sun, and my daughter, Ah-Young, who share every moment of our lives in Ames, especially when we are tired, homesick and have no mental capacity.

Most of all, it is dedicated to the one and only; the way, the truth and the life.

INTRODUCTION

Soybeans (Glycine max (L.)) are an important food source due to their high oil and protein contents. However, soybeans also contain antinutritive factors that may cause difficulties for digestion by humans and other monogastric animals; lipoxygenase enzymes which catalyze the production of the undesirable beany flavor; and undigestible carbohydrates which cause flatulence. All soy products should be heat treated prior to use in food in order to maximize their nutritive value. One of the simplest procedures for converting soybeans to a nutritious food is to extract the beans with water and heat near 100°C for 60 min to produce a beverage known as soymilk (Tou-chiang, China; Kong Kook or Doo Yoo, Korea; Tonyu, Japan).

Soymilk has been of considerable interest as a substitute for bovine milk and to feed infants who are allergic to animal milks. It was estimated in 1984 that about 130,000 metric tons of soybeans were processed worldwide into soymilk. Soymilk consumption has experienced rapid growth in Japan (increased from 4,000 metric tons in 1978 to 131,750 metric tons in 1983) and other Far Eastern countries. Because of increasing concern for cholesterol and saturated fats in animal products, interest by Western consumers in soymilk is escalating, especially for its secondary products of tofu, Tofutti®, frozen desserts and bases for extending sodium caseinate in imitation cheese. Recent U.S. consumer interest in foreign foods is also contributing to this trend. The potential for American industries to add value and export finished foods,

such as soymilk, seems great.

Soymilk is a traditional soybean food which has been used for thousands of years in East Asia. Traditional Oriental soymilk is processed by grinding soaked soybeans with cold water. No attempt is made to remove off-flavors which have been described as beany, painty, bitter and rancid. The acceptability of soymilk in Western cultures is limited by this characteristic off-flavor.

Bland flavored soymilk has been produced by a variety of techniques developed since 1900 and especially since 1966. Early inactivation of the soybean enzyme lipoxygenase using moist heat will produce soymilk having an improved, relatively bland, appealing flavor. Acid grinding, dry heating-extrusion cooking, alkaline soaking and pre-blanching techniques have also been developed to improve the flavor of soymilk. However, further improvement in the flavor and the development of low-cost processes with high product yields could significantly increase the consumption of soymilk and other soymilk-derived foods.

Recently, a promising method for production of soymilk using the continuous steam infusion cooking process known as rapid-hydration hydrothermal cooking (RHHTC) was developed, which increased yields of solids and protein, and produced soymilk with remarkable dispersion stability. High-temperature short-time cooking and high shear of steam infusion were believed to be critical to improved functional properties and the yield of a stable dispersion. It is anticipated that RHHTC processing will result in improved flavor when coupled with improved slurrying methods.

The overall objective of this study was to explore approaches to improve the flavor properties of RHHTC soymilk while still achieving the improved yield, dispersed soybean solids and protein, and nutritional quality inherent to the RHHTC process. Specific objectives include: (1) to evaluate the effects of moisture, particle size and storage time on lipid and flavor deterioration during short-term storage of full-fat soy flours; and (2) to evaluate the effects of slurrying and cooking conditions on physico-chemical, nutritional and flavor properties of soymilk produced by RHHTC.

PART I. FACTORS AFFECTING LIPID DETERIORATION IN FULL-FAT SOY FLOUR

ABSTRACT

Corsoy 79 soybeans were ground into 8-(coarse) and 24-mesh (fine) full-fat soy flours. From the particle size analysis, the 8-mesh full-fat soy flours were found to have larger values for geometric mean diameter and geometric standard deviation. However, the distribution moduli of coarse and fine soy flours were similar and indicated soybeans were nearly "brittle".

Development of hydrolytic and oxidative rancidities of coarsely and finely ground full-fat soy flours were followed from grinding to 24 hrs later. No increases in peroxide value and conjugated dienes in the oil and hexanal content in the headspace of the flour were observed when the moisture content was 10.7% or less. At 14.9% moisture and above, lipid oxidation increased with increased moisture content and storage time. Free fatty acid contents increased slightly at all moisture contents. However, hydrolysis did not exceed 0.06% over the moisture range of 4 to 18%, which is of little practical significance. Fine grinding increased oxidative and hydrolytic rancidities, especially at 14.9% moisture and above. These findings indicate that raw soybeans can be ground to full-fat soy flours and stored up to 24 hrs without undergoing significant lipid and flavor deterioration if the moisture content is 11% or less.

INTRODUCTION

Utilization of Soy Flour

In many parts of the world, soybeans are important components of human food and animal feed. They are used as whole beans, grits, flours, isolates and concentrates. Soy flours are used for functional properties, as well as nutritional. Full-fat soy flour is often used for fortifying bread to increase protein content, improve the balance of essential amino acids and contribute other nutrients (Dubios and Hoover, 1981). Other advantages of using soy flours in making products are increased mixing tolerance, easier machining, improved moisture retention, reduced fat absorption and improved crust color. Though up to 28% soy flour can be incorporated with the help of sodium stearoyl-2-lactylate (Tsen and Hoover, 1973), the U.S. baking trade often uses only 3 to 5% (basis wheat flour) of soy flours to extend shelf life and to replace part of the milk and egg in their dough formulae (Langsdorf, 1981). Soy fortification of corn, rice and manioc flours is economically practiced in countries where protein deficiency is a problem as in many developing countries (Torun, 1981).

In addition to enrichment of bakery products, full-fat soy flours can be used in baby foods, low calorie foods, meats, beverages, soups and sauces. In such products, full-fat soy flours partially replace more expensive and scarce ingredients, such as egg, milk and meat (Pringle, 1974).

Commercially available soy flours are the products obtained by

grinding and screening sound, clean, dehulled soybeans either before or after the removal of the oil. They are the least refined form of soy protein products and may have varying fat content, particle size and degree of heat treatment. Types, methods of preparation, protein contents and applications for commercial soy flours are given in Table 1.

Lo (1971) used full-fat soy flours to prepare a soy beverage. The soy flour was suspended in water to provide a beverage having substantially the same consistency as bovine milk. The resultant beverage was rich in protein, fat and vitamins and yet extremely economical.

Another procedure to utilize full-fat soy flours to prepare a soy beverage was developed by Mustakas et al. (1971). This process produced a spray-dried beverage powder from extruded full-fat soy flour that was easily reconstituted by adding water. A smooth, low-viscosity formulation was produced that approached the composition and beverage characteristics of bovine milk.

Recently, Johnson et al. (1981) developed a new method for processing aqueous extracts from soybeans, which used steam infusion cooking. This process has become known as rapid-hydration hydrothermal cooking (RHHTC). This process is unique in that it involved a simple method to produce a soybean beverage from full-fat soy flours with very high yield. They reported that coupling rapid slurrying with steam infusion cooking minimized off-flavor development and that the resultant soymilk was bland.

Stability of Soy Flour

Raw full-fat soy flours are enzyme active during/after grinding until they are further processed, usually with heat. Crushing or macerating raw soybean tissue triggers lipolysis through release of lipolytic enzymes and mixing them with substrate. Lipase catalyzes the hydrolysis of free fatty acids from triglycerides, a phenomenon called hydrolytic rancidity. Lipolysis can even occur at very low relative humidity (10%), such as in oat flakes (Acker and Beutler, 1965). In dry substrates, the water for hydrolysis is provided by equilibration with humidity in air. Among the released free fatty acids, the polyunsaturates are then oxidized to fatty acid hydroperoxides by lipoxygenase. Lipid hydroperoxides dismutate into a cascade of oxygenated fatty acids and chain-cleavage products. Lipoxygenase is an enzyme that oxidizes polyunsaturated fatty acids. In order to act as a substrate for lipoxygenase, a fatty chain containing a cis,cis-1,4-pentadiene moiety is a prerequisite. Soybeans possess three isoenzymes of lipoxygenase designated L-1, L-2 and L-3 with pH optima 8.3, 6.5 and 6.5, respectively (Christopher et al., 1970). Linoleic acid and structurally related fatty acids are substrates for both L-1 and L-2. L-2 is able to oxidize methyl linoleate and mono- and trilinolein (Koch et al., 1958). Vernooy-Gerritsen et al. (1984) found that lipoxygenase-1 and -2 are localized in cotyledon storage tissues. Lipoxygenase is not associated with either protein bodies in storage parenchyma cells, lipid bodies, mitochondria and other cellular organelles. In the dry soybean, oxygen is apparently limiting and enzyme-substrate contact is limited by

substrate immobility and, thus, lipoxygenase is apparently inactive. Upon hydration, oxygen can diffuse into the tissue, enzyme and substrate may gain mobility, and oxidation occurs.

Water is a major factor affecting the rate of lipid oxidation in foods. Karel (1980) studied the importance of water expressed as water activity and the dependence of the oxidation rate on moisture content in potato chips. Water plays several different roles in enzyme-substrate reactions: (1) acting directly on the structure of enzyme protein and substrate; (2) disrupting hydrogen bonds and, consequently, alternating of protein structure; (3) acting as a solvating medium facilitating the diffusion of reagents; and (4) acting as a reactant as in the case of hydrolysis (Drapron, 1985).

Studies of Acker and Beutler (1965) and Brockmann and Acker (1977) indicate both lipase and lipoxygenase are active at very low relative humidities (10 and 15%, respectively). With increased water activity there is an increase in the speed of enzymatic reactions. If the storage temperature is lowered sufficiently for the substrate, triglycerides, to solidify lipid oxidation comes abruptly to a standstill. These enzymes are responsible for off-flavor problems which hinder consumer acceptability of soybean products and the marketability of soy foods. Furthermore, Wolf (1975) indicated that the significance of lipoxygenase action is less certain when soybeans are processed under low moisture conditions as in the commercial extraction of oil. The potency of flavor compounds due to the decomposition of hydroperoxides generated by lipoxygenase suggests that very little oxidation may be needed to give

rise to objectionable levels of flavor constituents. Mustakas et al. (1969) reported that inactivation of lipoxygenase was a key step in the preparation of good-flavored full-fat soy flours.

In addition to flavor considerations, lipid hydroperoxides can lead to loss of nutritive value, such as the destruction of some vitamins. Also, lipid hydroperoxides and their secondary scission products may affect color and taste of soy protein products (Eriksson, 1982).

Measurement of Lipid Oxidation

Techniques for measuring lipid oxidation include organoleptic evaluation, chemical methods and physical methods. The method of choice depends upon a number of factors including the nature and history of the oxidized sample, type of information required, time available and test conditions. There is a need for a more thorough assessment of available methods so that unreliable, cumbersome methods may be discarded and modifications made to the remaining methods to maximize the information obtained (Gray, 1978). Table 2 shows widely-used methods for determining lipid oxidation.

Recently, gas liquid chromatography has been shown to be effective for quantitative determination of volatile compounds in the headspace vapors of food samples. The concentration of hexanal and other fatty acid oxidation products in the headspace of soy flours and crude oils can be readily determined and, thus, used to evaluate the quality of soybeans (Snyder et al., 1985; Frankel et al., 1987). The advantages of this technique are: sample handling is minimized; time required for sample

preparation is low; the vapor phase above a food must contain the chemical responsible for the olfactory stimuli causing the perceived odor; and the volumes of vapor samples can be increased while chromatographic resolution remains.

Hexanal is believed to be one of the major lipid oxidation products contributing to off-flavors of soy proteins. Wilkens and Lin (1970) observed some 80 volatile compounds contributing to the flavor of soymilk, but hexanal predominated accounting for 25% of the total peak area. Hexanal and other volatile carbonyls collectively impart a grassy, beany flavor to soymilk. Other authors have used hexanal as an indicator of rancidity in soy foods and soybean oil (Snyder et al., 1985; Warner et al., 1988). Hexanal content was shown to closely follow the development of off-flavor (Bengtsson et al., 1967), and to be a simple and rapid yet effective analytical tool for measuring oxidative deterioration (Fritsch and Gale, 1977).

Research Objectives

The objective of this study was to determine how quickly full-fat soy flours must be processed. Moisture content and particle size were expected to affect the extent of hydrolytic and oxidative rancidity of full-fat soy flours during short-term storage.

MATERIALS AND METHODS

Selection of Soybeans

Seed-grade Corsoy 79 soybeans grown at Ames, IA during 1984 were used in this study. Proximate analysis is shown in Table 3. Protein, moisture, crude oil and ash content were determined by AACC standard procedures 46-11, 44-15A, 30-25 and 08-01, respectively (AACC, 1976). Fatty acid composition of the oil was determined by using methods of Graef et al. (1985).

Adjustment of Moisture Content

Two 4-kg samples of soybeans were adjusted to the desired level of moisture by mixing calculated amounts of distilled water and soybeans in air-tight bags, and allowing several days for the absorbed moisture to equilibrate within the beans at 5 - 6°C. Also, 8-kg samples of soybeans were dried to various moisture contents in an open-top drum drier equipped with a blower motor and a temperature controller. The drying temperature was less than 41°C (White et al., 1976). Samples were ground and moisture content was determined by the vacuum oven method (AACC, 1976).

Grinding and Storage of Soy Flour

Soybeans were ground through 8- and 24-mesh screens using a Fitz-mill (Model D, Fitzpatrick Co., Elmhurst, IL). The flour (300 g) was immediately placed in 1-quart Mason jars, sealed and stored at 25°C for

5 sec and 6, 12 and 24 hrs until analyzed. The Mason jars were about two-thirds full and oxygen should not be limiting.

Particle Size Analysis

The U.S. standard sieve series were used for sizing. All sieves (12 sieves) were first weighed empty. Particle size distributions were determined by using methods of the ASAE (1985); 50 g of sample was used instead of the recommended 100 g. At the end of the sieving period the sieves were weighed and differences between them and their empty weights gave the weights of the fractions retained on the sieves. Duplicate analyses were performed. The size of particles for each sample was reported in terms of geometric mean diameter (dgw) and geometric standard deviation (sgw) by weight. From a log-log plot of "percent finer than" against screen size for each sample, product size modulus (k) and distribution modulus (a) were calculated (Hansen and Henderson, 1972).

Crude Oil Extraction

Samples of full-fat soy flours (300 g each) were transferred to 1-L brown-colored separatory funnels with a wide-opened top. The separatory funnel was loosely plugged with glass wool at the exit. Chloroform-methanol (2:1 v/v) was used to extract free and bound fat. Duplicate samples were extracted. The extraction procedure is shown in Figure 1. Crude oils were stored in glass vials flushed with N_2 gas at 5°C and lipid analysis was conducted as quickly as possible after extraction.

Measurement of Lipid Oxidation

The free fatty acid (FFA) contents of crude oils were determined in duplicate using AOCS (1964) method Ca5a-40. The sample size was 28.2 g of crude oil.

Peroxide value (PV) and conjugated dienoic acid (CD) were also determined in duplicate by AOCS official methods Cd8-53 and Ti 1a-64, respectively (AOCS, 1964). In the latter method, 1 g oil was dissolved in 1 L isooctane and the absorbance was measured at 233 nm with a Gilford Spectrophotometer 250 (Gilford Instrument Laboratories, Oberlin, OH). To calculate percent conjugated dienoic acid, a k_o value of 0.07 was chosen.

Static Headspace Capillary Gas Chromatography of Soy Flours

An internal standard was prepared by adding 25 μl of 4-heptanone (Aldrich Chemical Company, Inc., Milwaukee, WS) to a 1-L volumetric flask filled about three-fourths with deionized distilled water. After mixing, the flask was made up to volume with deionized distilled water.

Headspace volatiles, especially hexanal concentration, were determined in soy flour samples. A 100-ml bottle was filled with 50 ml of boiling distilled water and placed into a boiling water bath. A 6-g sample of soy flour was suspended and 1 ml of internal standard was added. The bottle was sealed with a septum secured by an aluminum cap. After 30 min passed in a boiling water bath, the bottle was placed in a 5°C refrigerator until analyzed. Before injection, the bottles were warmed to 37°C and equilibrated for 3 hrs using a shaking water bath (Model 127, Fisher Scientific, Springfield, NJ). A 1-ml sample of the

headspace was introduced into a Varian Model 3700 gas chromatograph (Varian Associates, Inc., Walnut Creek, CA) equipped with a Durabond DB-5 fused silica capillary column, 30 m x 0.32 mm, 1 micron film thickness (J&W Scientific, Rancho Cordova, CA) in a split mode (20:1). The column temperature was programmed at the rate of 5°C/min from 40°C (zero hold time) to 125°C (5 min hold time). A hydrogen flow rate of 30 ml/min, nitrogen flow rate of 30 ml/min (column: 1.5 ml/min; makeup: 28.5 ml/min) and air flow rate of 300 ml/min were used. A 10-ml gas tight syringe (Hamilton Company, Reno, NV) was used and cleaned at a syringe cleaner (Hamilton Company, Reno, NV) after each run. The results were expressed as the peak area ratio of hexanal to 4-heptanone. Volatile analyses were performed in duplicate on each sample.

Statistical Analysis

Data were analyzed by using a Statistical Analysis System (SAS, 1984) program package. The General Linear Models (GLM) procedure was used to determine the main and interaction effects. Significant differences among treatment means were determined by Duncan's multiple range test or the least significant difference (LSD) procedure. Probability levels of $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Characterization of Soybeans

Seed-grade Corsoy 79 soybeans were 99.9% pure with 95.0% germination potential (Table 3). Protein and crude oil contents were 41.7% and 20.0%, respectively. The composition of fatty acids was shown to be typical of soybeans (Erickson, 1983).

Particle Size Analysis

Figure 2 shows a log-probability plot of cumulative percent by weight against particle size for full-fat soy flours ground through 8- and 24-mesh screens. The plots were relatively straight lines indicating that the distributions were evenly spread throughout the sieves. It was of interest to note that the distribution of full-fat soy flours ground through the 8-mesh screen consisted of coarser particles with a wider range of sizes than soybeans ground through the 24-mesh screen.

Two important distribution parameters, the geometric mean diameter (dgw) and the geometric standard deviation (sgw), were obtained for each flour from Figure 2 and listed in Table 4. The dgw of flours ground through the 8-mesh screen (609.8 micron) was larger than that of flours ground through the 24-mesh screen (293.7 micron). The sgw is a measure of the dispersion of particles sizes relative to the dgw. The sgw of flours ground through the 8-mesh screen was larger than that of flours ground through the 24-mesh screen (2.05 verses 1.44). Therefore, the soy flours ground through the 8-mesh screen had a wider distribution of

particle sizes and were less uniform in size.

Another important distribution parameter, distribution modulus, was obtained from the log-log plot of cumulative weight versus particle size. The distribution modulus should be constant for a product ground through different screen sizes. Therefore, the distribution modulus has the same value whether it refers to breakage of a single particle or to the entire product. Hansen and Stewart (1965) stated that the distribution modulus of 1.00 indicates complete "brittleness". Table 4 showed the distribution moduli had 1.24 for flours ground through the 8-mesh screen and 1.22 for flours ground through the 24-mesh screen. Therefore, under the conditions of this experiment soybeans approached complete "brittleness".

Determination of Lipid Oxidation

Corsoy 79 soybeans whose original moisture content was 10.7% were treated to adjust their moisture content by tempering or drying and then ground into the flours with two different particle size distributions. These flours had 4.0, 6.8, 7.6, 10.7, 14.9 and 17.5% moisture.

Free fatty acid contents were determined in soy flour stored up to 24 hrs at different moisture levels (Figures 3 and 4). In general, lipase activity increased with increased moisture content and storage time, but at all moisture contents hydrolytic activity was low and of little practical significance. The hydrolysis of triglycerides in full-fat soy flours did not exceed 0.06% free fatty acid over the moisture range of 4.0 to 17.5% during the 24-hr storage period. This amount of

hydrolysis is not organoleptically significant. Fritsch (1981) indicated that at least 2% free fatty acid level in soybean oil is necessary for an adverse effect upon the odor or the flavor of foods.

Figure 4 illustrates the effect of particle size on free fatty acid contents of crude oils in the 8- and 24-mesh full-fat soy flours. Grinding to small particle sizes slightly increased hydrolytic rancidity. Fine grinding increased the rate of hydrolysis more at higher moisture contents than at lower moisture contents. Fine grinding destroys the natural compartmentalization of cells. Oil is protected in spherosomes from lypolytic enzymes in the cytoplasm. Fine grinding more extremely breaks these structures down and provides more mixing of enzymes with substrates.

Enzyme-catalyzed Oxidation

The primary product of lipid oxidation, hydroperoxides, was determined in the 8- and 24-mesh full-fat soy flours stored for 24 hrs at different moisture levels (Figures 5 and 6). Peroxide values increased with storage time especially at 14.9% moisture and higher. However, oxidative rancidity, as indicated by peroxide values, was not practically significant over the 24-hr storage time at moisture contents of 10.7% or less. Figure 6 shows that fine grinding increased oxidative rancidity. This effect was more statistically significant at the 5% level over the 24-hr storage time at the moisture content of 17.5%. The results (Figures 5 and 6) indicate full-fat soy flours with 10.7% moisture content or less can be stored for at least 24 hrs without incurring

significant increases in hydroperoxides.

Additional evidence of oxidative changes during storage of full-fat soy flours with different moisture contents was obtained by measuring diene conjugation in extracted oil. Compared to the peroxide value, the conjugated diene hydroperoxide method was faster, was simpler, required no chemical reagents, did not depend upon a chemical reaction for color development and was conducted on smaller samples. As shown in Figure 7, conjugated dienes increased initially with increased storage time and moisture. The production of conjugated dienes was pronounced at 14.9% moisture or higher. Conjugated diene contents changed little in soybeans with 10.7% moisture and less.

More conjugated dienes were observed in the 24-mesh flour than the 8-mesh flour. At high moisture content (17.5%), fine grinding greatly accelerated conjugated diene formation. However, at 14.9% moisture and less, the increased production of conjugated dienes due to fine grinding was small (Figure 8).

Headspace Analysis of Volatiles

Figure 9 shows the headspace gas chromatographs (GC) obtained from aqueous dispersion of 8-mesh full-fat soy flours after 12-hr of storage time at 10.7% moisture. This figure was a typical chromatogram. Generally, eleven GC peaks were detected. The hexanal peak, which was of interest, was identified on the basis of relative retention time to a hexanal standard. Heptanone was used as an internal standard, which eluted after the hexanal peak. Heptanone was found to be a reliable

internal standard and no interactions with the volatiles from the homogenates were observed. The peak ratio of hexanal to heptanone was highly reproducible.

Hexanal contents in the headspace of soy flour slurries increased at higher flour moisture contents and with smaller particle size. A drastic increase in hexanal content was noted at 14.9 to 17.5% moisture and this increase was significant during 6 hrs of storage. However, hexanal content did not significantly increase during storage of soy flours at moisture contents of 10.7% and below. Therefore, raw full-fat soy flours can be stored at 10.7% moisture or less without significant lipid deterioration (Figures 10 and 11).

Soybeans possess high lipoxygenase activity and the enzyme is distributed throughout the cotyledons. In the dry bean, oxygen is apparently limiting and enzyme-substrate contact is limited by substrate immobility or compartmentalization and, thus, lipoxygenase is apparently inactive. However, upon hydration, oxygen can diffuse into the tissue, enzyme and substrate may gain mobility and oxidation occurs. When cell structures are disrupted during grinding, conditions can be ideal for oxidation. Finer particles provide more surface area to accelerate the reaction. However, based on the results of this study a critical moisture content of 11% must be exceeded for this mechanism to proceed.

Relationship Between Peroxide Value and Conjugated Diene Content in Extracted Oil and Headspace Volatiles

Peroxide value and conjugated diene measurements were compared to gas chromatographic analysis of headspace volatiles from the aqueous dispersion of full-fat soy flours (Figure 12). Linear relationships between them were observed. Their regression coefficients were 0.75 and 0.81 for conjugated dienes and peroxide value, respectively.

Recently, gas chromatographic analysis of headspace volatile compounds has been widely used to evaluate the quality of soybeans (Frankel et al., 1987) and soybean oils (Snyder et al., 1985; Warner et al., 1988). They, also concluded that headspace gas chromatographic analysis of volatiles provides a sensitive method to evaluate oxidative deterioration of beans and crude oils.

CONCLUSIONS

Corsoy 79 soybeans were ground through 8- and 24-mesh screens. The geometric mean diameter for 8-mesh full-fat soy flours was larger than that for 24-mesh full-fat soy flours (609.8 vs 293.7 microns). Distribution moduli for 8- and 24-mesh soy flours were 1.24 and 1.22, respectively, indicating soybeans were almost completely "brittle".

Free fatty acid contents increased slightly at all moisture contents. However, hydrolysis did not exceed 0.06% over the moisture range of 4 to 18%. Therefore, hydrolytic rancidity was not a practical problem during short-term storage.

Peroxide value, conjugated dienes and hexanal content did not increase when moisture content was 10.7% or less. Fine grinding increased oxidative, especially at 14.9% moisture content and above. Raw soybeans can be ground to full-fat soy flours and stored for up to 24 hrs without undergoing significant lipid and flavor deterioration if the moisture content is equivalent to or less than 11%.

Gas chromatographic analysis of headspace volatiles was found to be simple, sensitive and a valuable tool for evaluating oxidative deterioration in soy flours.

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Table 1. Type, preparation method and application of soy flours

Type	Preparation method	Application
Full-fat flours (40% protein[a])	Dehulled cotyledons are milled to specific size.	Produced primarily in Europe for the baking industry.
Enzyme-active flours (52-54% protein)	Produced from defatted flakes with minimum heat. High NSI[b].	Increasing mixing tolerance and bleaching in bread; preparation of dispersible concentrates and isolates.
Defatted flours (52-54% protein)	Finely ground to pass through a No. 100 U.S. standard screen size. Controlled moist heat-treatment used to provide "white" (NSI 85-90), "cooked" (NSI 20-60), and "toasted" (NSI below 20) grades.	Varied uses requiring a wide range of protein solubilities.
Lecithinated/ Refatted flours	Lecithin or vegetable oil is recombined with defatted flakes (0.5-30% fat).	Improved water dispersibility and emulsification capacity in baking applications.

[a]Protein conversion factor was 6.25 g protein/g N.

[b]NSI denotes nitrogen solubility index.

Table 2. Methods for determining lipid oxidation

Method
Chemical methods
Peroxide value
Thiobarbituric acid
Kreis test
Oxirane determination
Total volatile carbonyl compounds
Physical methods
Conjugated diene
Fluorescense
Infrared spectroscopy
Polarography
Gas chromatography
Refractometry
Color
Dielectric constant
Oxygen uptake
High pressure liquid chromatography
Organoleptic method
Sniff test and Schaal oven test
Tasting

Table 3. Characterization of experimental soybeans

Variety	Corsoy 79
Growing location	Ames, Iowa
Crop year	1984
Pure seed (%)	99.9
Inert material (%)	0.10
Weed seed (%)	0.00
Crop seed (%)	0.00
Secondary noxious weeds	None Found
Germination potential (%)	95.0
Seeds per pound	3,558.0
Proximate analysis[a]	
Moisture (%)	10.7
Protein (%)[b]	41.7
Crude oil (%)	20.0
Ash (%)	4.9
Fatty acid composition	
Palmitic acid (%)	10.7
Stearic acid (%)	4.2
Oleic acid (%)	27.5
Linoleic acid (%)	51.7
Linolenic acid (%)	5.9

[a]Dry basis except moisture.

[b]Protein conversion factor was 6.25 g protein/g N.

Table 4. Particle size distribution parameters of full-fat soy flours

Screen mesh size	Geometric mean diameter dgw (micron)	Geometric standard deviation sgw (micron)	Distribution modulus a	Size modulus k (micron)
8	609.8	2.05	1.24	1,300
24	293.7	1.44	1.22	535

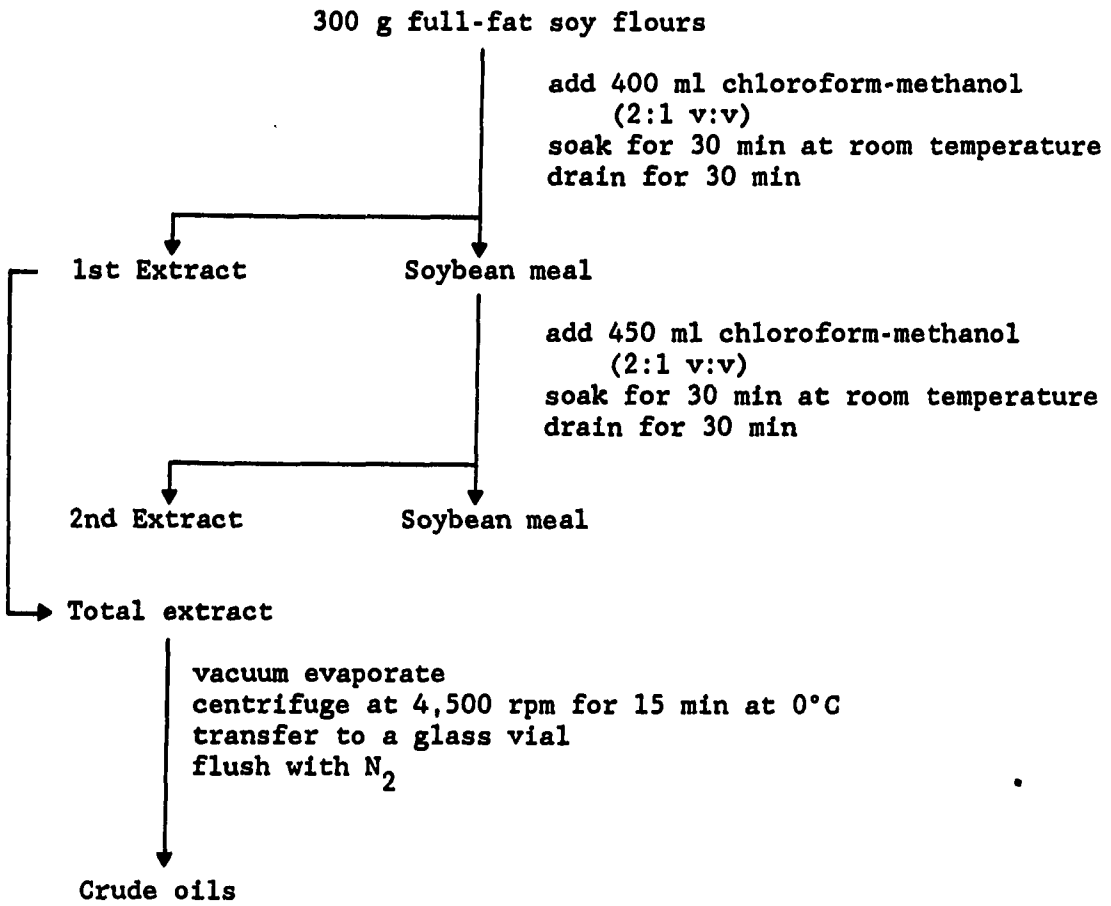

Figure 1. Extraction of crude oil from full-fat soy flours

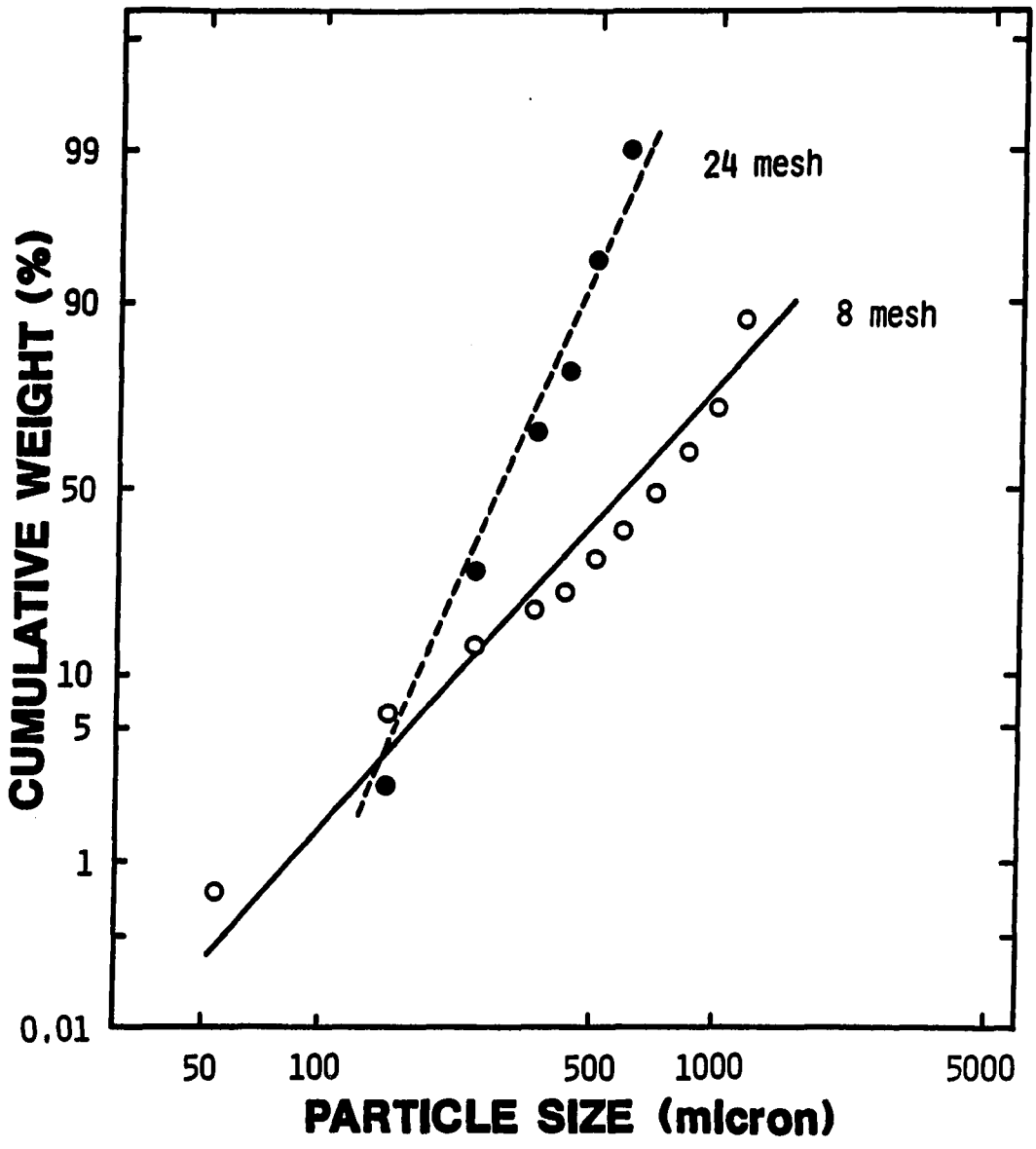

Figure 2. Particle size distribution of full-fat soy flours ground through 8- and 24-mesh screens

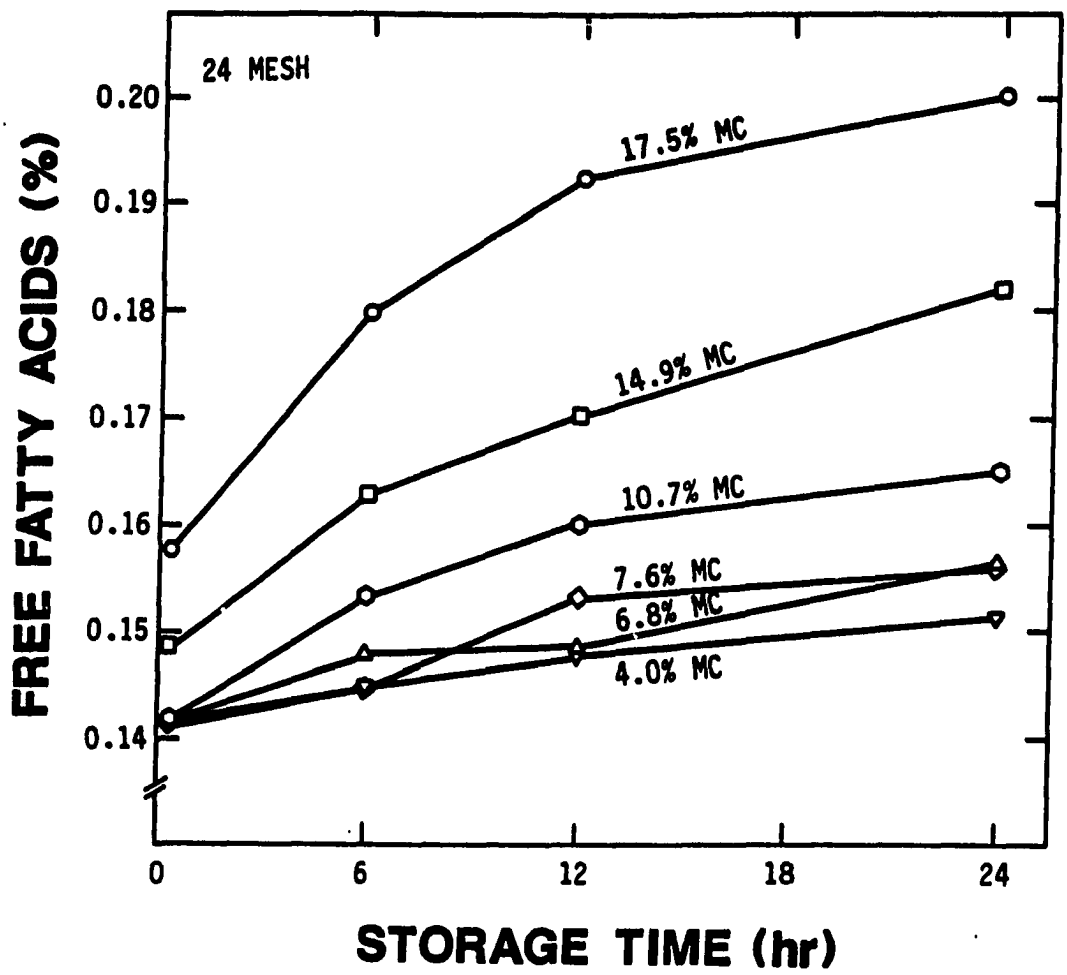

Figure 3. Effect of moisture content (MC) on hydrolytic rancidity of oil in full-fat soy flours during short-term storage (LSD was 0.02)

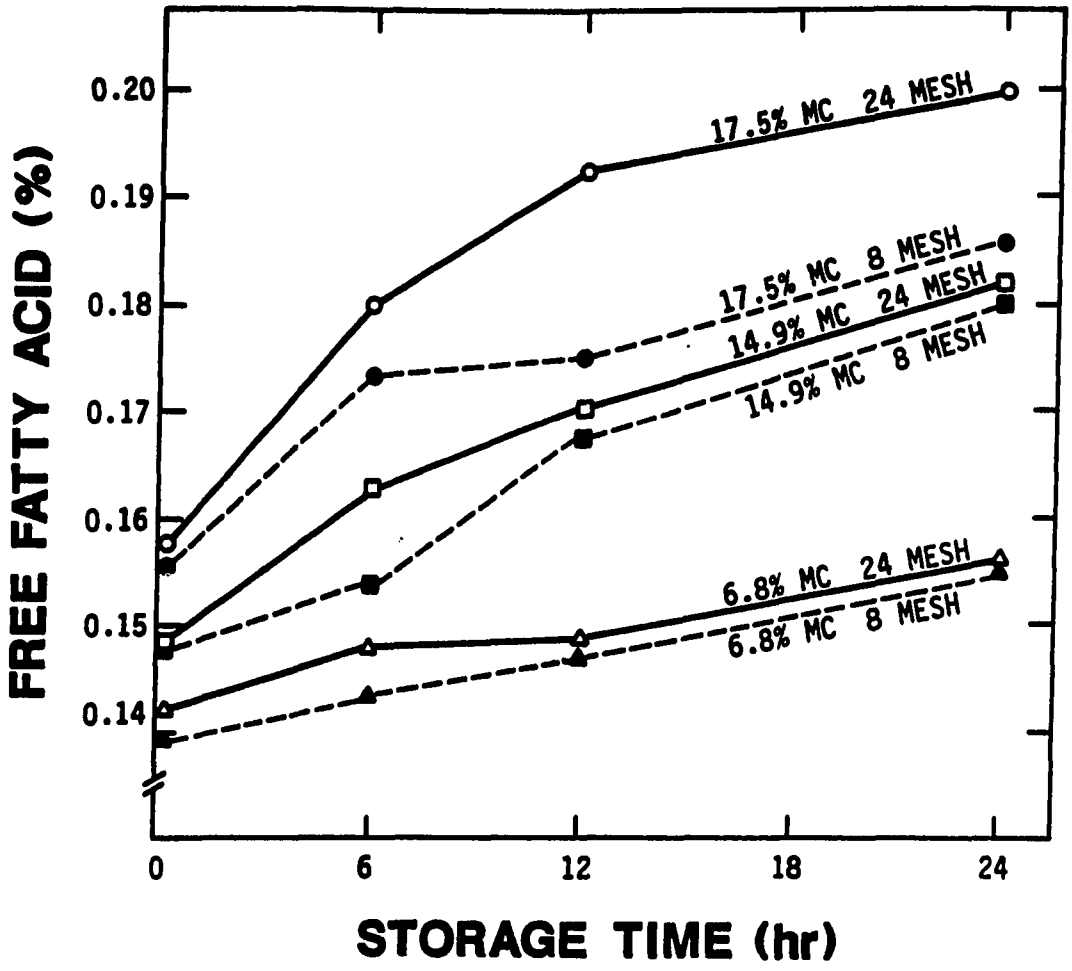

Figure 4. Effect of particle size on hydrolytic rancidity of oil in full-fat soy flours at different moisture contents (MC) during short-term storage (LSD was 0.02)

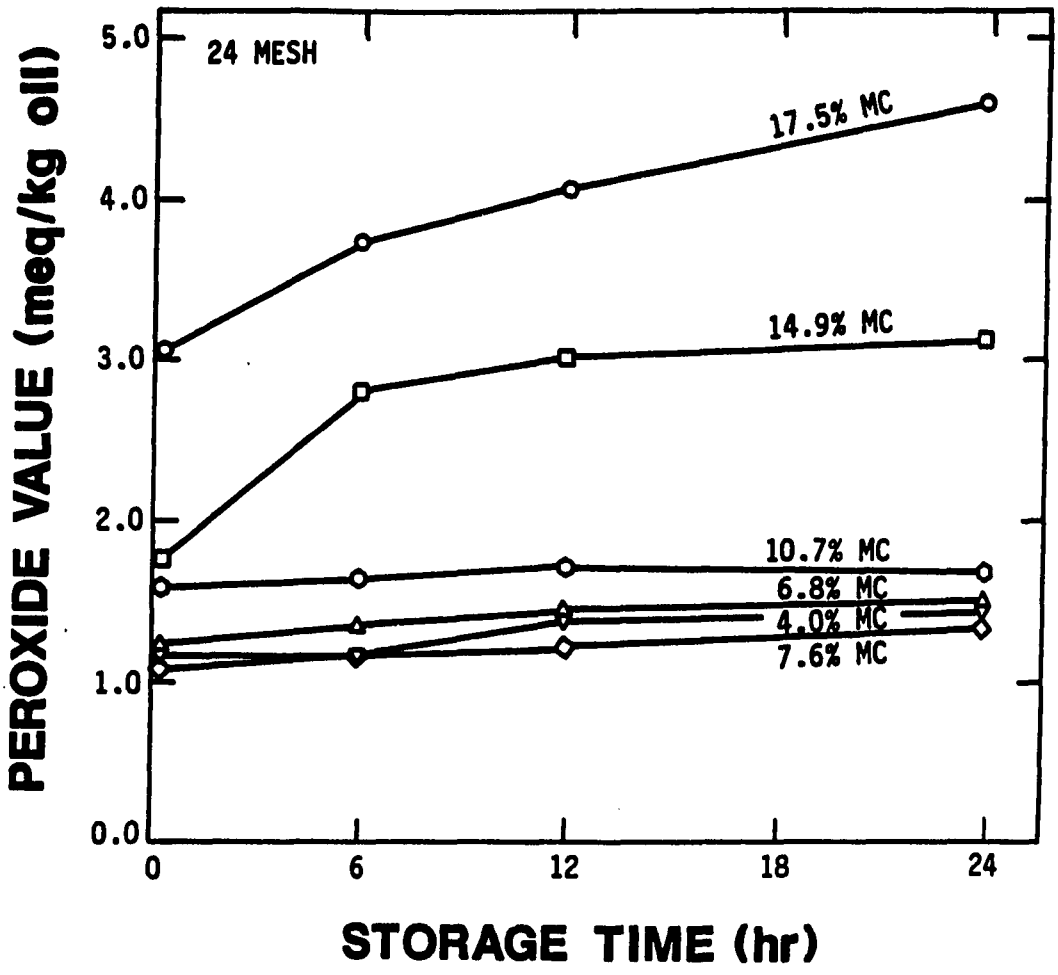

Figure 5. Effect of moisture content (MC) on peroxide value of oil in full-fat soy flours during short-term storage (LSD was 0.34)

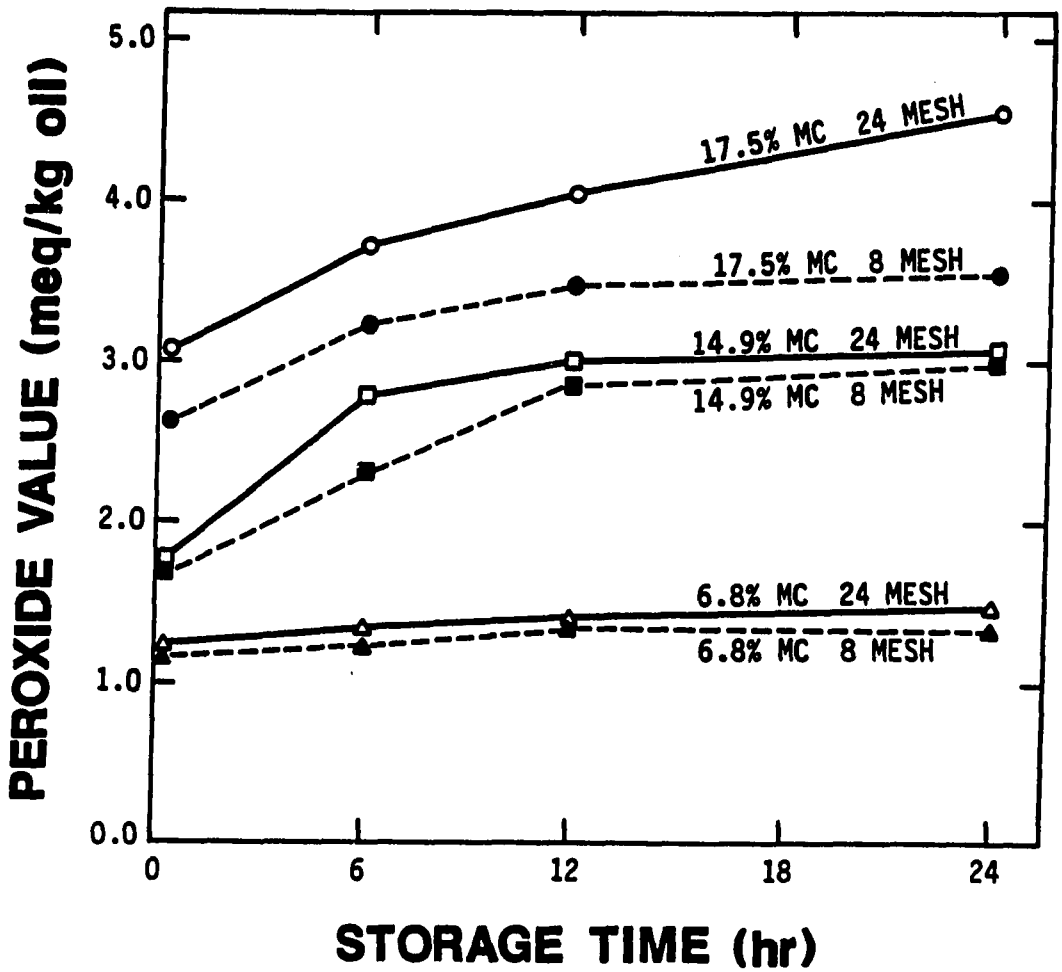

Figure 6. Effect of particle size on peroxide value of oil in full-fat soy flours at different moisture contents (MC) during short-term storage (LSD was 0.43)

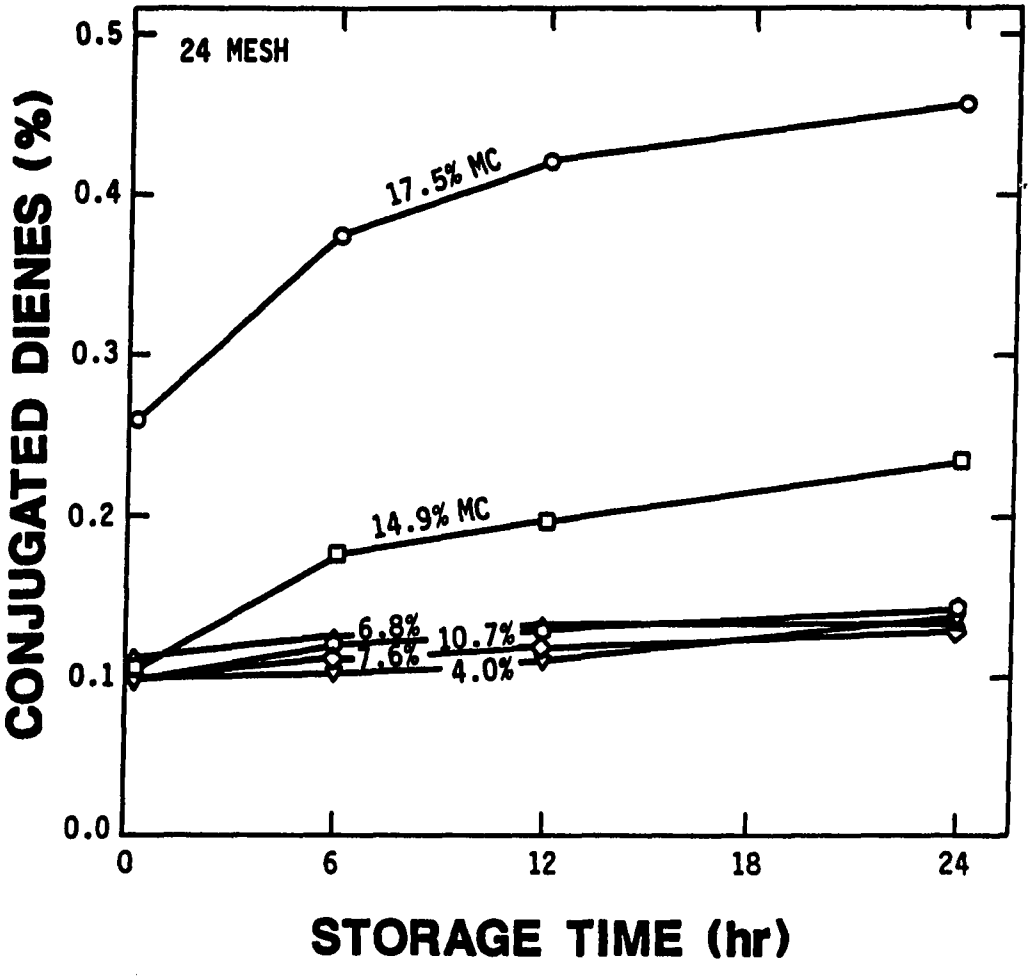

Figure 7. Effect of moisture content (MC) on conjugated dienes of oil in full-fat soy flours during short-term storage (LSD was 0.02)

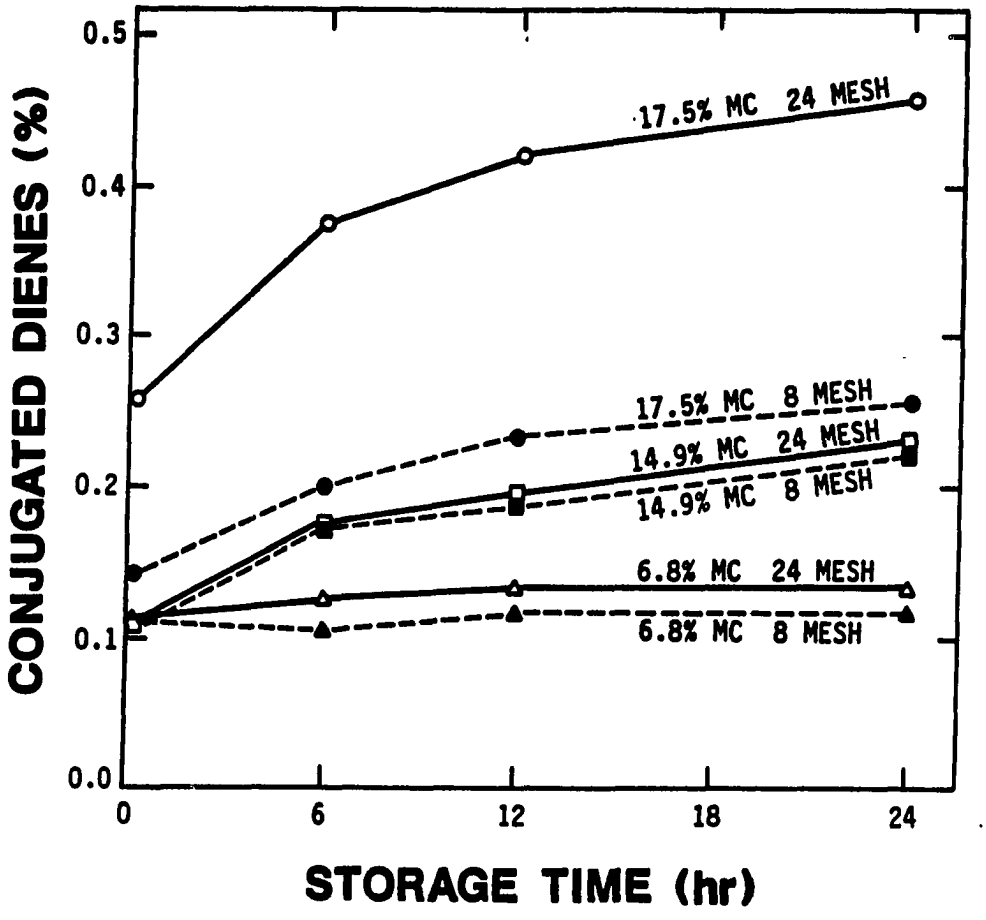

Figure 8. Effect of particle size on conjugated dienes of oil in full-fat soy flours at different moisture contents (MC) (LSD was 0.02)

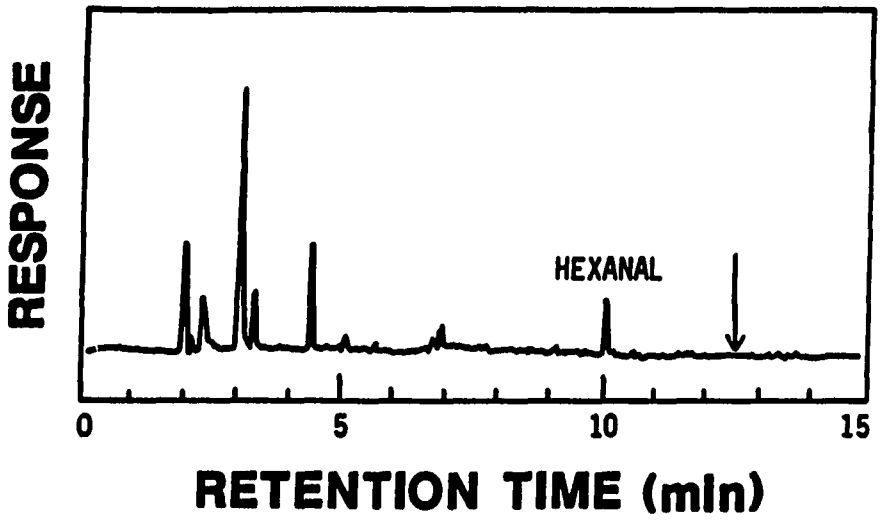

Figure 9. GLC volatiles after 12-hr storage from 8-mesh full-fat soy flours at 10.7% moisture content (arrow indicates retention time for 4-heptanone internal standard)

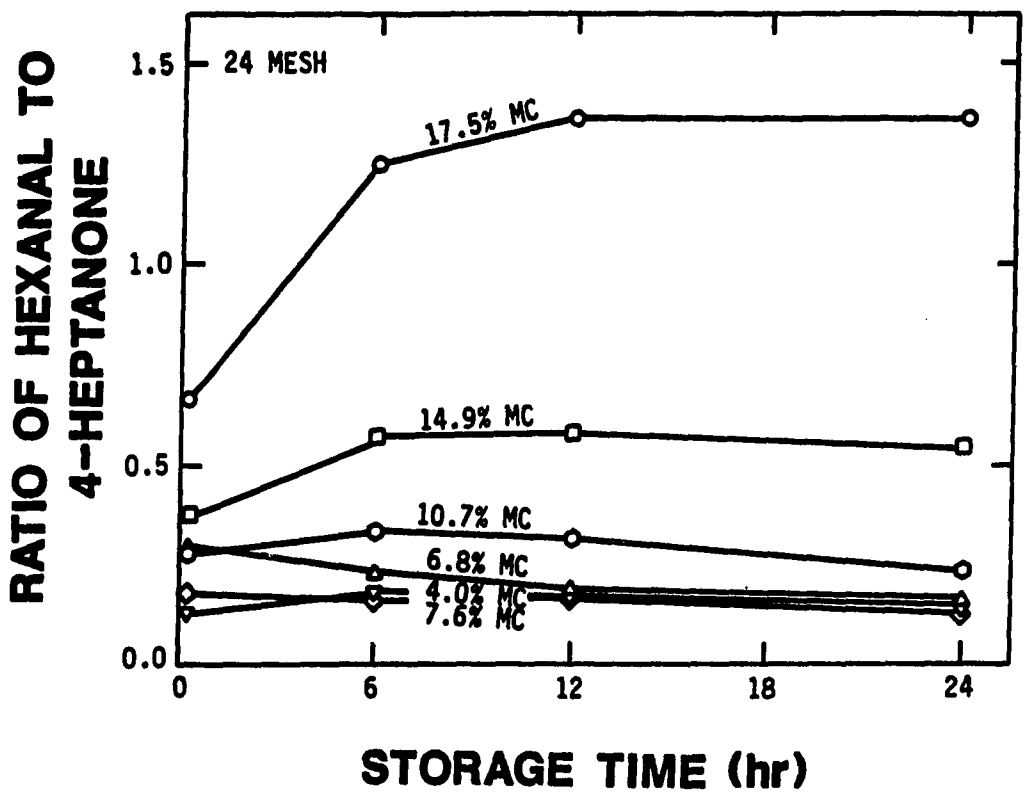

Figure 10. Effect of moisture content (MC) on hexanal content of full-fat soy flours during short-term storage (LSD was 0.19)

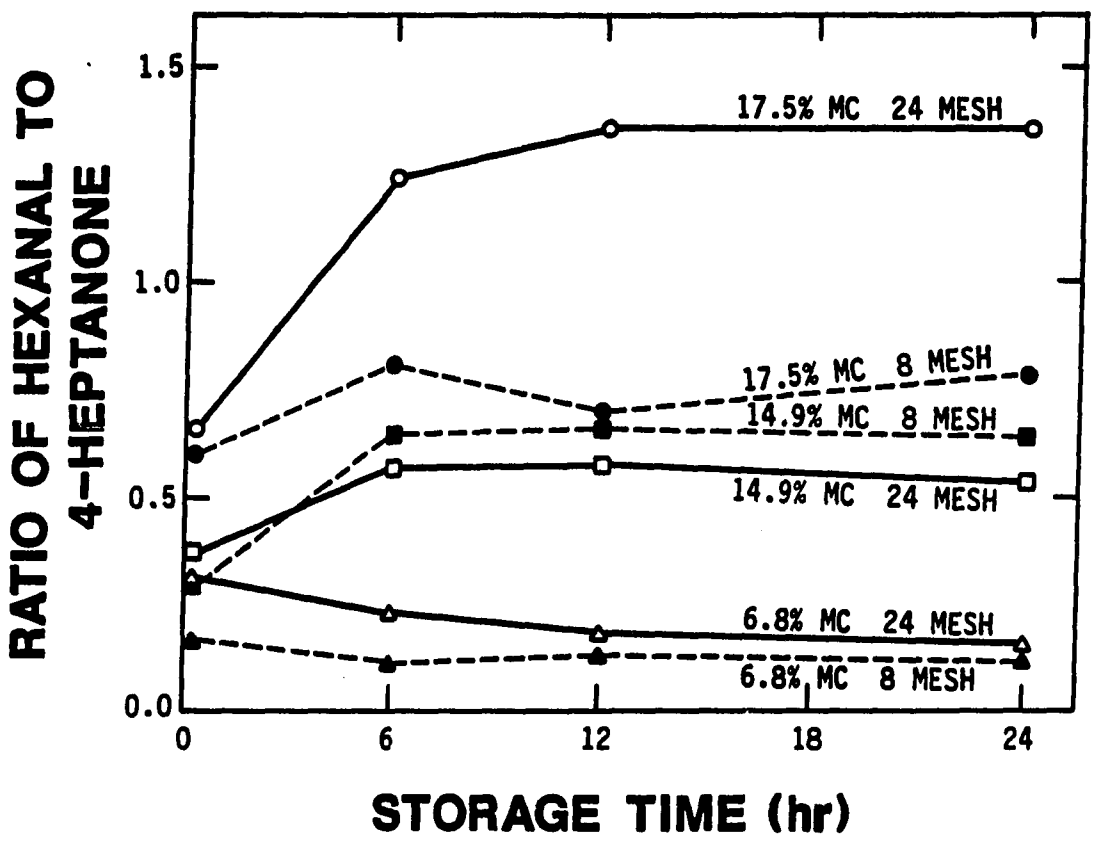

Figure 11. Effect of particle size on hexanal content of full-fat soy flours at different moisture contents (MC) during short-term storage (LSD was 0.21)

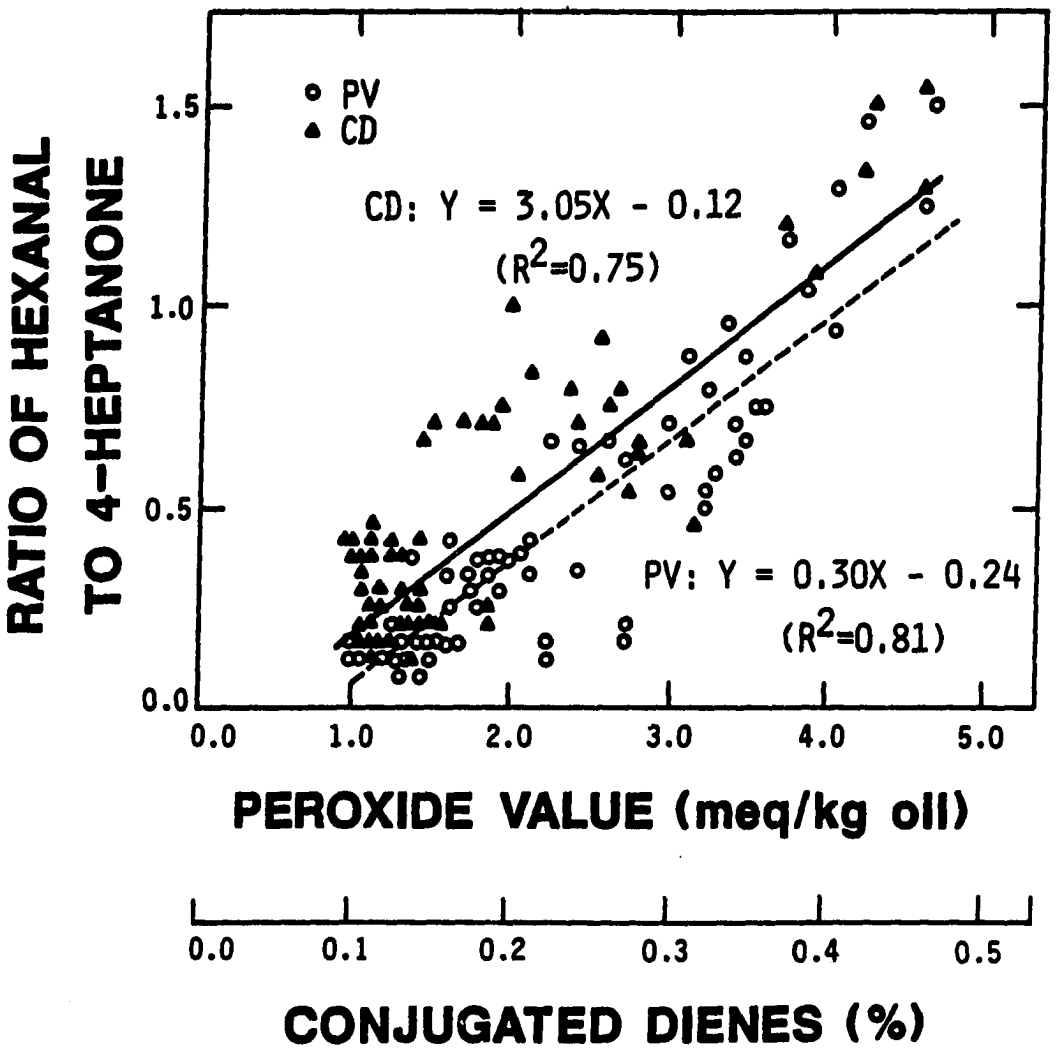

Figure 12. Relationship between hexanal content in full-fat soy flours to peroxide value and conjugated dienes in the oil

PART II. EFFECT OF PROCESSING CONDITIONS ON PHYSICO-CHEMICAL PROPERTIES OF SOYMILK PROCESSED BY RAPID-HYDRATION HYDROTHERMAL COOKING (RHHTC)

ABSTRACT

A pilot-plant scale rapid-hydration hydrothermal cooking (RHHTC) system was assembled in which the distance between the point where raw soy flour contacts water to the hydroheater was minimized. Minimizing water contact time prior to cooking reduced the development of off-flavor during slurrying due to lipoxygenase activity.

Soy flour was slurried continuously at 25 and 80°C with water contact times of 11, 240 and 1,200 sec prior to cooking at 154°C. Soymilk was processed at five different cooking times by changing the holding tube length. About 82% of the soybean solids was recovered as a stable dispersion compared to 40% for traditional soymilk. Maximum yields of soymilk fraction, total solids and protein were obtained with 20 - 26 sec cooking at 154°C. Slightly less yield was obtained by hot water slurrying than with cold water slurrying. The flow of soymilk processed by RHHTC exhibited mildly thixotropic behavior. Soymilk made by hot water slurrying had lower viscosity. High viscosity soymilks did not always have high yields. Both yield and viscosity increased with cooking time at 154°C to maximum values and then decreased with longer cooking time. The centrifuged cooked slurry showed no signs of instability during a 10-day storage period.

RHHTC processing employing either cold or hot water slurrying produced a stable soymilk with higher yields of soymilk fraction, solids and protein, and much more viscous dispersions than soymilk processed by the traditional method.

INTRODUCTION

Preparation of Soymilk

Soymilk (Tou-chiang, China; Kong Kook or Doo Yoo, Korea; Tonyu, Japan) is the aqueous dispersion achieved by grinding soaked soybeans in water, heating and filtering (Snyder and Kwon, 1987). Soymilk has been of considerable interest as a substitute for bovine or human milk, particularly in the feeding of infants who are allergic to animal milk. Also, soymilk is useful when bovine milk is either too expensive or unavailable. Soymilk can be converted into a dried powder by either spray or roller drying and used similarly to dry milk solids from bovine milk.

Shurtleff and Aoyagi (1984) classify soymilk in three different ways: those with added flavor or nutrients; those produced by filtration; and those utilizing methods for controlling off-flavors. The latter method classifies soymilk into traditional soymilk and low beany flavor soymilk.

Traditional soymilk is processed from whole soybeans. Beans are soaked in cold water overnight and finely ground with a stone mill in the presence of a small stream of water. The ground mass (slurry) is heated to boiling for 15-30 min with constant stirring. The heating improves nutritive quality and flavor, and pasteurizes soymilk. The cooked ground mass is strained through cloth and soymilk, a highly stable oil in water emulsion, passes through the cloth. To insure a high recovery of protein and solids, a water to dry bean ratio of 10:1 is usually used. From

100 g of soybeans, approximately 1 L of soymilk is obtained (Snyder and Kwon, 1987). No attempt is made to remove or control beany flavors in traditional soymilk. The acceptance of soymilk produced by the traditional method has been limited in Western populations because of the characteristic flavor and odor (Hand et al., 1964). The flavor and odor have been described as beany, painty, bitter and rancid (Nelson et al., 1976). Wilkens et al. (1967) suggested that undesirable flavors and odors are caused by lipoxygenase. The ultimate result of enzyme activity is the formation of numerous volatile carbonyl compounds, which are decomposition products from lipid oxidation and are responsible for the beany flavors (Gardner, 1980). Once the off-flavor is produced it is not possible to eliminate or mask it (Nelson et al., 1971).

There has been considerable research on soymilk processing to improve its quality. Numerous modifications to the traditional process reportedly improve the flavor and odor, and make it acceptable to Western cultures. These modifications include alkaline soaking (Badenhop and Hackler, 1970), hot water grinding (Wilkens et al., 1967), acidic grinding (Kon et al., 1970) and pre-blanching (Nelson et al., 1976). However, in more recent years, several entirely different processes have been developed, such as extrusion cooking, wet-milling (Mustakas et al., 1971) and enzyme application (Eriksen, 1983; Mital and Steinkraus, 1976).

Wilkens et al. (1967) found that bland soymilk was produced by grinding soybeans with water at temperatures between 80 and 100°C, and maintaining the temperature above 80°C for 10 min to completely inactivate lipoxygenase. Later, Wilkens and Hackler (1969) found that

the soymilk produced by this method lacked the chalky mouth-feel that is characteristic of many full-fat soy products or defatted soy bases when reconstituted. However, Badenhop and Wilkens (1969) found that when high-temperature grinding is preceeded by soaking, a significant quantity of 1-octen-3-ol is formed. It can arise from linoleic acid by the catalytic action of lipoxygenase in soybean.

Kon et al. (1970) ground soybeans in acidified water (pH 2.0) to suppress off-flavor development and produce a bland soymilk by neutralizing after heating. Protein extraction at pH 2.0 was approximately 80 - 85% which was nearly maximum. Unfortunately, irreversible conformation changes to the 11S protein fraction occurred in the pH range of two to three (Wolf and Briggs, 1958).

A soy beverage was developed using extruded full-fat soy flours which can be reconstituted with water and combined with additives to approach the composition and beverage characteristics of bovine milk (Mustakas et al., 1971). The resulting soy beverage had a mild nutty flavor with good flavor stability.

Badenhop and Hackler (1970) evaluated the effects of soaking soybeans in sodium hydroxide solutions on flavor, acceptability and nutritional value of soymilk using the hot water grinding technique to prepare soymilk. Taste panel members preferred the flavor of soymilk at pH 7.37 (soybeans soaked in 0.05 N NaOH) to higher or lower pH.

Nelson et al. (1975, 1976) patented a process known as the Illinois Process which utilized a bean blanching step before grinding to reduce lipoxygenase activity and the homogenization of the soybean slurry

without filtration to disperse solubles and increase solids yields. About 95% of the protein and 89% of the solids in whole beans were recovered in the beverage. Fine grinding and homogenization reportedly ensured the high yield of protein and solids from blanched soybeans. However, Johnson and Snyder (1978) showed that blanching intact soybeans as in the Illinois Process fixed protein bodies and resulted in much lower yields of soymilk and protein when subjected to low-speed centrifugation. Moreover, the Illinois Process is complex, requires much sophisticated equipment, produces large amounts of waste water and is energy intensive.

An improved method for production of soymilk using a continuous steam infusion cooking known as rapid-hydration hydrothermal cooking (RHHTC) was recently developed (Johnson et al., 1981). RHHTC processing involves dry grinding the unsoaked whole soybeans to flour, forming a slurry of soy flour in water, injecting steam under pressure to inactivate trypsin inhibitor (TI) and lipoxygenase without fixing protein bodies or substantially denaturing the soybean protein, cooling the slurry and centrifuging to separate the hulls from the slurry. Yields of RHHTC soymilk of over 87% of the solids and 90% of the protein have been achieved when cooked at 154°C for 30 sec. A recent report indicates all but the hull can be recovered as a stable soymilk (Hung, 1984). Over 90% of TI was inactivated under these processing conditions (Johnson et al., 1980). Based on studies conducted at much lower temperature (Hackler et al., 1963), maximum nutritional quality should result at these conditions. Maximum protein efficiency ratio (PER) occurs when about 90%

of the native TI activity in soybeans is inactivated. Also, Hackler et al. (1963) has shown that the insoluble solids filtered out of the soymilk had a higher PER than the soymilk itself. Therefore, any increase in yield should improve the PER of soymilk. Improved nutritional quality in RHHTC was reflected in a 10.6% improvement in protein efficiency ratio (PER) over traditional soymilk (Hung, 1984). However, most previous work on soymilk processed by RHHTC was done with a slurrying time prior to cooking for about 20 min which allowed excessive development of lipoxygenase-catalyzed off-flavors. Nor did they optimize hot water slurrying which may reduce off-flavors. There are two opportunities for lipoxygenase to develop off-flavors in RHHTC processing: during grinding and storage of full-fat soy flours and during slurrying just prior to steam injection. Previous work in this study has shown that if the beans are not ground at moisture contents exceeding 11% nor stored more than 24 hrs, little flavor deterioration occurred. Reducing water contact time and/or hot water slurrying are approaches to controlling flavor development in the second area. However, reducing water contact time is likely to reduce hydration of protein and other soybean solids and alter the optimum cooking conditions for yield and nutritional properties. Hot water slurrying is likely to fix protein bodies and reduce yield.

Yields of Aqueous Soybean Extracts

Because functional properties are directly related to the physico-chemical properties of the proteins, a detailed knowledge of the

structural and physical characteristics of soy proteins is essential for understanding and manipulating their properties in foods (Kinsella et al., 1985). It is generally felt that the proteins are the principal functional components in soy flours through the carbohydrates may play a role in water binding, swelling and controlling viscosity. The predominant structural proteins are the two major globulin species β-conglycinin (7S) and glycinin (11S). Though normally occurring in the approximate ratio of 1:1, these proportions have been shown to vary significantly in some varieties (Gayler and Sykes, 1981).

Protein denaturation is any modification of the native structure of protein usually altering the tertiary and/or quaternary structure. Generally, it involves disrupting noncovalent forces responsible for the organization of the native structure, although in some cases it may also include reduction of disulfide bonds. Unlike most proteins, soy proteins are quite heat stable, especially glycinin (Hermansson, 1978). However, upon heating, soy proteins undergo stepwise dissociation of subunits followed by unfolding of the polypeptides that subsequently associate and aggregate to form precipitates or progels. Short exposure to heat above 100°C results in an increase in protein solubility due to dissociation and degradation of the polypeptide (Wolf, 1978). Prolonged heating causes aggregation and precipitation of protein. Degree of denaturation is affected by pH, temperature, salt concentration, chaotropic agents and agitation.

The degree of solubility of a protein in a given aqueous solution is the net result of both electrostatic and hydrophobic interactions between

protein molecules. Conditions under which electrostatic repulsions between the molecules is greater than the hydrophobic interaction between nonpolar surfaces on the protein favor increased solubility. Conversely, conditions under which hydrophobic interactions are greater than electrostatic repulsions result in intermolecular aggregation and decreased solubility. Physico-chemical changes during commercial preparations, which result in irreversible changes in the oligomeric state of soy protein, alter the delicate balance of the above two forces and, thereby, affect solubility (Kinsella et al., 1985).

The solubilities of commercial soy proteins are generally given as nitrogen solubility indices (NSI) or protein dispersibility indices (PDI). These indices are also known as the fast stir and slow stir methods, respectively; the fast stir giving higher values (AOCS, 1969). Which one is more useful remains controversial. Nevertheless, NSI and PDI are widely used in the soy protein industry as practical estimates of the extent of denaturation and in product specifications. Neither of these official methods relate well to soymilk extracts (Johnson, 1978). Consequently, Johnson and Snyder (1978) have used the term "yield of solids" to denote that fraction of soybean solids remaining dispersed in the aqueous extract after filtering or centrifuging. Yield of solids (%) was expressed as the following equation:

$$\% \text{ yield of solids} = \% \text{ Soymilk} \times \left[\frac{\% \text{ solids in soymilk}}{\% \text{ solids in soy slurry}} \right]$$

From this equation, soymilk contains only the stable water-soluble dispersion of the cooked slurry, not the water insoluble residue which

contributes a throaty mouth-feel. However, various methods have been applied to remove the water-insoluble residue from the stable dispersion. Plate filters and centrifuges have been used in pilot plant studies while cheesecloth and batch centrifuges have been used in laboratory situations. Various pressures on the filter chamber and centrifugal forces in the centrifuge have been used. Furthermore, data may be reported on the basis of total solids of dehulled soybeans or undehulled soybeans. Because of this, it has been difficult to standardize the procedure, generalize the findings and compare results from one situation to another.

Approximately 60% of the total solids is recovered in the milk processed by the traditional method. The protein yield in the aqueous extract is more favorable than the solids yields. Around 70% of the total protein is extracted (Smith and Circle, 1972).

Use of toasted soybeans (Wilkens et al., 1967) or toasted full-fat soy flours eliminates the beany flavor, but greatly reduces protein extraction and yield of soymilk. Also, the recovery of solids in hot water grinding increased with increasing water temperature to 60°C to where 67% of the solids from dehulled soybeans were recovered. Recovery decreased over the range of 60 - 100°C. At 100°C, the normal extraction temperature, the solids recovery was only 60%.

Nelson et al. (1976) used blanching of soaked beans prior to wet grinding to control lipoxygenase activity, which drastically reduced the recovery of solids because protein bodies became fixed and settle out due to their large size. The Illinois Process reportedly overcame this

problem of dispersing fixed protein bodies by using homogenization at high pressure and temperature. They reported yields of 89% of the solids and 95% of the protein, but no filtering or centrifugation was conducted. Subsequent low-speed centrifugation decreased the solids yield to 49% (Johnson and Snyder, 1978). The Illinois group believes that tenderization of the soybeans in combination with homogenization of the slurry results in the formation of hydrophilic protein-lipid complexes which are responsible for the dispersibility.

Johnson and Snyder (1978) investigated the effect of heating soaked beans before grinding (Illinois Process) and during wet grinding (hot water grinding process). Their results showed that heating before grinding resulted in solids yields as low as 26% due to heat fixation of protein bodies that are removed by the low-speed centrifuge (642 x g). Wet grinding in hot water increased the yield to around 43%. Homogenization at high pressure (8,000 psi) and high temperature (75°C) increased the yields in both processes to 49% and 59%, respectively. They concluded that homogenization was helpful in redispersing heat-fixed protein bodies.

Eriksen (1983) applied commercially available enzymes to soymilk production to improve yields of solids and protein. Soymilk was produced from toasted full-fat soy flours by preparing a slurry of flours in water at a water to flour ratio of 10 to 1. The slurry was heated to 50°C and the pH of slurry was adjusted to the pH optimum of the selected enzyme. After the prescribed period of hydrolysis, the slurry was boiled for 15 min to terminate the reaction and then centrifuged at 1,500 x g for

5 min. The best results were obtained with neutral proteinases which increased protein and solids yields from 33 and 42% to 73 and 66%, respectively.

Flow Behavior of Soymilk

The tendency of a fluid to flow easily or difficultly has been the subject of practical and intellectual importance to mankind for centuries (Bourne, 1982). Numerous papers have been published on the flow behavior of soy products to define their role in baked goods, meat products and soy beverages (Lee and Rha, 1979; Hermansson, 1975; Shen, 1976). Flow properties of protein dispersions have practical significance in pumping, mixing, heating, cooling, spray drying and other operations.

Tung (1978) stated that flow properties of protein dispersions are governed by composition, as well as, molecular shape, size and charge. These are influenced by processing conditions, such as temperature, concentration, pH, ionic strength and previous processing history.

Lee and Rha (1979) stated that protein demonstrates its complex and dynamic nature in solution. Upon absorbing solvent the protein swells, unfolds and becomes flexible. Such interactions of protein with the solvent determines the degree of solubility and flow behavior. A higher degree of swelling is likely to lead to increased flexibiity of molecules. Swelling and unfolding will increase the effective volume or hydrodynamic volume, decrease the distance between protein molecules, and, thus, will increase viscosity.

Tanford (1961) has shown that globular proteins with a wide range of

molecular sizes exhibited intrinsic viscosities between 3.3 and 4.0 cm^3/g, whereas, fibrous proteins of equivalent molecular weight exhibited much higher viscosities. Such large differences in viscosity arise from the molecular shape and orientation.

Hermansson (1975) explained that in most cases viscosities of soy protein solutions increase exponentially with increasing protein concentration. At concentrations above 8%, the viscosity of soy isolate became high due to protein-protein interaction leading to the formation of a protein network (i.e., gel). She also mentioned the effect of commercial processing condition upon the modification of the flow properties of soy proteins. For example, heat treatment and oxidation increased viscosity. Factors influencing the state of the sulfhydryl groups also seem to be important (Circle et al., 1964).

Ionic strength has a marked effect on viscosity of protein dispersions. Circle et al. (1964) demonstrated that adding NaCl to soy protein dispersions decreased apparent viscosity. This phenomenon may be the result of decreased hydration of the protein via neutralization of charges by counterions and by increased stabilization of soy protein in the presence of NaCl.

The effect of particle size of aggregates upon flow properties was also studied (Lee and Rha, 1979). Under food processing conditions, such as separation, extraction, drying, formulation, fabrication and sterilization, proteins are often denatured and aggregated. When aggregated protein is placed in a solvent, the size of the dispersed units can range from unimolecular to large agglomerates. The viscosity

of a dispersion containing particles of large size is higher than that of a dispersion containing small particles. However, particle size is not the only factor responsible for the high viscosity. Particle shape and interaction between the particles also play a role.

Little quantitative data is available on the effect of processing on viscosity of soymilk. Viscometric characteristics of soymilk are needed to understand processing and formulation variables as well as to design a production plant for soymilk manufacture (Forster and Ferrier, 1979).

Lo et al. (1968) found that the apparent viscosity of soymilk processed by hot water grinding has a logarithmic relationship with solids content. They also indicated that soymilk is a non-Newtonian fluid since its viscosity depends upon the force of shear and concentrated soymilk (20% solids content) is thixotropic in which the apparent viscosity decreases with increased time of shearing.

Viscosities of soymilks prepared from beans soaked in water, sodium carbonate and sodium hydroxide, and cooked by the traditional method were studied by Khaleque et al. (1970). They found that soymilk prepared by sodium carbonate soaking had higher viscosity than those prepared by water or sodium hydroxide soaking. The recovery of protein in soymilk was slightly higher for sodium bicarbonate soaking than for water soaking but sodium hydroxide soaking significantly reduced viscosity.

Forster and Ferrier (1979) showed that soymilk produced by the Illinois Process displayed pseudoplastic flow behavior and mild thixotropic behavior. At higher solids levels the apparent viscosity and degree of pseudoplasticity of soymilk increased. Inclusion of soybean

hulls in soymilk caused higher apparent viscosity than observed in soymilk made from dehulled cotyledons. Soymilk prepared from cotyledons blanched in 0.25% $NaHCO_3$ had higher apparent viscosity and was more pseudoplastic than soymilk prepared from cotyledons blanched in an acid solution or in tap water.

Hung (1984) measured the apparent viscosity of soymilk prepared from whole or dehulled soybean flours processed by RHHTC. Viscosity gradually increased to a maximum at 157°C and then decreased with increasing cooking temperature. However, soymilk prepared from whole bean flour had much higher apparent viscosity than corresponding samples of soymilk prepared from dehulled soybean flours.

Suspension Stability of Soymilk

Few studies have been made on suspension stability of soymilk. However, one of the most severe problems encountered with previous soybean beverages has been instability of the suspension system leading to visual separation into two or more layers (Wei et al., 1976).

Mustakas (1974) developed a lipid-protein concentrate beverage which had excellent suspension properties. These properties were attributed to reduction of the lipid-complex curd into fine particles by using a colloid mill and homogenizer. This method utilized an isoelectric wash to remove carbohydrate followed by centrifugation at alkaline pH to remove insolubles.

Soymilk made by the Illinois Process was reported to have good suspension stability (Priepke et al., 1980; Nelson et al., 1976). They

found that this characteristic was due to the formation of lipid-protein complexes in which both free and bound lipid played major roles. Protein concentration, particle size distribution and viscosity of the Illinois soybean beverage were not related to suspension stability.

Recently, Hung (1984) evaluated the stability of RHHTC soymilk. The suspension stability was expressed as the solids content ratio of top to bottom layers after 10 days of quiescent storage at 5°C. He found little detectable separation in RHHTC soymilk. This result was attributed the extreme shear force during steam infusion cooking.

Research Objectives

The objective of this study was to determine the effects of slurrying and cooking conditions on physico-chemical properties, such as the yields of total solids, protein and soymilk fraction, and viscosity and suspension stability of RHHTC soymilk. Changes in slurrying conditions to improve flavor are likely to alter optimum RHHTC cooking conditions due to differences in hydration of protein and fixation of protein bodies.

MATERIALS AND METHODS

Description of RHHTC System

A pilot-plant scale steam infusion cooking system was assembled according to Figures 1 and 2. Full-fat soy flours were fed by a screw feeder (The VersiFeeder, Vibrascrew, Inc., Totowa, NJ) to a vibroreactor (Model JM14/E/3, Cherry-Burrell Corporation, Cedar Rapids, IA) where the flours were continuously slurried with water. The vibroreactor further reduced particle size of the soy flours and dispersed it in water. Water was fed continuously into the vibroreactor and controlled by a variable-area flowmeter and valve (Cole-Parmer Instrument Co, Chicago, IL). The slurry was pumped with a variable speed Moyno pump (2MI type SSQ, Robin and Myers, Inc., Springfield, OH) to a hydroheater valve (Size 300 type B, Hydrothermal Corp., Milwaukee, WS) where it was infused with steam. The slurry enveloped the steam flowing into the combining tube. Extreme turbulence and shear were produced when the two combined. This transferred heat instantaneously and prevented temperature stratification. The hydroheater valve was integrated with a variable speed Moyno pump and back pressure control valve. After passing through the combining tube, the cooked slurry flowed through a 2.54 cm (1 inch)-diameter stainless-steel holding tube (2.66 cm/1.05 inch ID) insulating with 2.54 cm (1 inch) of fiber glass. Temperature sensing and data logging components (OM 302 series Thermocouple Digital Data Logger, Omega Engineering, Inc., Stanford, CN) were used. The system was fitted with four copper-constantan thermocouples (Omega Engineering, Inc.,

Stanford, CN) to automatically record temperature. Pressure gauges were positioned immediately after the hydroheater valve and before the flash chamber.

The length of the holding tube was adjusted from 2.51 m (8.3 ft) to 10.7 m (35.3 ft) to obtain different cooking times. The cooking temperature was controlled by adjusting the back pressure valve within the system by means of a control gate valve at the flashing end of the holding tube. Cooking temperature was variable from 110 to 160°C with 5.62 kg/cm^2 (80 psi) gauge steam pressure in the holding tube. A constant temperature of 154°C was used to cook most samples and required 4.43 to 4.50 kg/cm^2 (63 to 64 psi) gauge steam pressure in the holding tube. The steam was flashed in a 25.3 cm (10 inch) flash chamber.

The distance from the point of initial contact of soy flours with water to the point of steam injection was reduced to achieve as little as 11 sec of water contact time. Longer water contact times were achieved by adding a surge tank at the top of the Moyno pump. The cooked slurry discharged from the flash chamber into a stainless-steel container in an ice bath with continuous stirring. The cooked slurry was cooled to 5 - 10°C and was stored at 5°C overnight before removing sludge.

Full-fat Soy Flour Preparation

Seed-grade Corsoy 79 soybeans were purchased from Department of Agronomy, Iowa State University (Table 1). The beans were ground in a Fitz-mill (Model D, Fitzpatrick Co., Elmhurst, IL) through a 32-mesh opening screen (in preliminary trials, much higher yields of soymilk

fraction, protein and solids were observed when processing soy flours ground through a 32-mesh screen rather than through a 24-mesh screen; due to the small effect of particle size on lipolysis in soy flour found in the previous section, this finer grinding was not expected to adversely affect flavor of RHHTC soymilk). Grinding was always done immediately before cooking. Soy flours were slurried in tap water in proportions of 17 parts water to 3 parts soy flours to give 15% solids content in the raw slurry.

Processing of Control Soymilk (Traditional)

One part of soybeans (900 g) was soaked overnight (about 18 hrs) with 3 parts of tap water (2,700 ml) at room temperature. The soaked beans were drained and rinsed with tap water two times, and then 8 parts (7,200 ml) of water were added. Water and beans were ground in a vibroreactor (Model JM14/E/3, Cherry-Burrell Corporation, Cedar Rapids, IA) in two passes. The raw slurry was allowed to stand for 20 min with occasional stirring. The slurry was cooked for 60 min in a steam-jacketed kettle (Lee Metal Product Co., Inc., Philipsburg, PA) at 97 to 100°C. The sample was continuously agitated during cooking.

Composition and Yield of Fractions

Samples of the cooked slurry were adjusted to 10% solids content with distilled water after the solids content of the cooked slurry was determined by using AOAC procedure 16.032 (AOAC, 1980). The slurry was centrifuged (Beckman Instruments, Inc., Palo Alto, CA) at 1,050 x g

centrifugal force and 5°C for 5 min. Soymilk was carefully decanted and the residual material weighed. The yields of fractions and solids were calculated by the procedures of Johnson and Snyder (1978). Three determinations comprised the mean. Duplicate 5-g samples of both soymilk and residue were oven-dried according to the AOAC procedure 16.032 (AOAC, 1980) to determine the solids balance. Nitrogen determinations were run in duplicate by the macro-Kjeldahl method using a Tecator Kjeltec System (Digestion System 6 and 1002 Distilling Unit, Tecator, Hogansas, Sweden). Protein was estimated by using a factor of 6.25 g protein/g N.

Suspension Stability

Suspension stability of 500 ml soymilk was determined by measuring solids distribution after 10 days of undisturbed storage at 5°C in a 500 ml cylinder (4.8 cm inside diameter and 35 cm height). A 15-ml aliquot of soymilk was withdrawn using a pipet from the center of the upper one-third (designated as the upper layer) and another 15-ml sample was withdrawn from the center of the lower one-third (designated as the lower layer) of cylinder. Each 15-ml sample was thoroughly mixed and analyzed for the total solids as described in AOAC procedure 16.032 (AOAC, 1980). The ratio of the solids content of the upper layer to lower layer was calculated. Duplicate analyses were performed on each sample.

Apparent Viscosity

A Brookfield LVT Synchro-Lectric Viscometer (Brookfield Engineering Laboratories, Stoughton, MA) was used to determine viscosity of soymilk. A 200 ml sample was placed in a 200-ml beaker and equilibrated at 25°C for 30 - 40 min in a constant temperature water bath. Determinations were made at 60 rpm using a No. 2 spindle at 1 min intervals for 10 min. Duplicate analyses were performed for each sample.

Statistical Analysis

Data were analyzed by using a Statistical Analysis System (SAS, 1984) program package. The General Linear Models (GLM) procedure was used to determine the main and interaction effects. Significant differences among treatment means were determined by Duncan's multiple range test or the least significant difference (LSD) procedure. Probability levels of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Yields of Soymilk, Solids and Protein

Control soymilk was made from whole soybeans soaked for 18 hrs, ground in cold water and cooked at 97 - 100°C for 60 min. Hackler et al. (1965) found that these conditions produced maximum nutritional quality for soymilk protein and destroyed about 90% of the native TI activity. Under these conditions, the yield of soymilk fraction was 48.6%, the yield of soybean solids was 39.7% and the yield of protein in the soymilk fraction was 47.5% (Table 2). Hand et al. (1964) reported that 65% of the soybean solids and 83% of the protein were the maximum possible yields before heat treatment. Johnson and Snyder (1978) found that the maximum yield of soymilk fraction was 68.7%, solids yield was 62% and protein yield was 75% when soybeans were extracted by wet grinding, homogenized at 562.4 kg/cm^2 (8,000 psi) and 75°C, and centrifuged at 642 x g. The lower recoveries observed in this study were attributed to the use of centrifugation to separate undispersed solids instead of plate filtering or cheesecloth straining.

Higher yields of dispersed protein and solids were accomplished by RHHTC processing than traditional processing (Table 2). It is believed that the optimum heat treatment at high temperature and the extreme shear generated in steam infusion partially denatured the native structure of soy proteins. Subunits dissociated by disrupting noncovalent forces responsible for native quaternary structures of soy proteins by unfolding the polypeptide tertiary structure. Exposed hydrophobic areas of the

peptide sequester and emulsify fine droplets of fat.

The effects of cold and hot water slurrying in RHHTC processing on the yields of soymilk fraction, solids and protein for three different water contact times were studied (Table 2 and Figure 3). Regardless of slurrying procedure, yields of soymilk fraction, solids and protein increased as cooking times increased up to 20 - 26 sec, but decreased at cooking times longer than 26 sec. The increase in solids yield with increasing cooking time was presumably due to increased protein solubility and emulsification. When the cooking time was increased beyond 26 sec, the solids yield decreased due to protein aggregation from excessive denaturation. The patterns were consistent; only the length of the trend was dependent upon cooking time and water contact time. Maximum yields of soymilk fraction in cold slurrying occurred at cooking times of 26, 26 and 20 sec for 11, 240 and 1,200 sec of water contact times, respectively. Maximum yields of soymilk fraction were 89.6, 90.7 and 89.1% for 11, 240 and 1,200 sec of water contact times, respectively. The yield of solids in soymilk was 84.2% for 11 sec, 85.0% for 240 sec and 82.8% for 1,200 sec of water contact. Johnson (1978) obtained 86.0% solids recovery in soymilk cooked for 34 sec and 154°C with more than 20 min of hydration time. Reducing water contact time in cold water slurrying did not significantly affect yields. This was surprising in the light of findings (Johnson and Snyder, 1978) that protein bodies can become fixed if not solubilized before heat treating. Apparently, even 11 sec of water contact time was sufficient to prevent fixation of protein bodies.

The yields of solids in RHHTC soymilk prepared with hot water slurrying were less than those of soymilks prepared with cold water slurrying. The effect of water contact time on the yield of solids in soymilk at 80°C slurrying was considerably larger than in cold water slurrying prior to cooking. The longer the contact time with hot water prior to steam injection the poorer the yield. Yields of solids were the lowest in samples processed with 1,200 sec of water contact time. This was attributed to protein bodies becoming fixed during hot water slurrying. Maximum yields of solids were observed at cooking times of 20 sec for 11 and 240 sec of water contact times while soymilk with 1,200 sec of water contact time had maximum yield at 26 sec of cooking time. The yields at each cooking time were significantly different at the 5% level. The highest yield of solids (80.6%) occurred at 11 sec of water contact time. Therefore, rapid slurrying was necessary to improve the yield of solids when employing hot water.

Viscometric Characteristics of Soymilk

Viscosity is a functional property of beverages and reflects physico-chemical characteristics of the protein. Viscosity decreased during the 10 min shearing period at 25°C (Figure 4) indicating that RHHTC soymilk was mildly thixotropic. Viscosity measured again after 24 hrs at the same shear rate was the same as the original viscosity. Flow behavior of RHHTC soymilk was similar to that of traditional soymilk reported by Lo et al. (1968) and Forster and Ferrier (1979). Thixotropic behavior may contribute to the stability of soymilk enhancing its shelf

life and improving mouthfeel (Forster and Ferrier, 1979).

Apparent viscosities of soymilk increased to a maximum and then decreased at longer cooking times (Figure 5). This trend was similar to trends observed in this study for solids yield of soymilk (Figure 3). Protein swells on hydration, unfolds on heating and becomes flexible with a concomitant increase in hydrodynamic volume and a decrease in the distance between protein molecules. Thus, viscosity increases. Prolonged cooking caused aggregation of protein; thereby, reducing viscosity.

The viscosity of RHHTC soymilk processed with hot water slurrying was less viscous than those observed in soymilks processed by cold water slurrying (Figure 5). This observation was partially attributed to differences in solids and protein contents. Overall mean solids content in soymilk processed with cold and hot water slurrying were 9.16 and 8.64%, respectively. The difference between the two values was significantly different at the 5% level. Overall mean protein contents were 3.75% for cold water slurrying and 3.45% for hot water slurrying. These two values were also significantly different at the 5% level. Hermansson (1975) and Lo et al. (1968) found that the apparent viscosities of soy protein dispersions rose exponentially with increasing protein concentrations. It is also probable that hot water slurrying caused some protein bodies to become fixed. These were removed with the insoluble residue during centrifuging.

Viscosities of soymilk processed by RHHTC (41.7 - 231.3 cp) were much higher than that of the control soymilk (14.6 cp). Hung (1984)

found that the apparent viscosity of RHHTC soymilk had a high positive correlation with fiber content and that crude fiber contents were higher in RHHTC soymilk than traditional soymilk. Urbanski et al. (1982) showed that the small fiber fraction contributed more to viscosity than the much larger protein fraction. Because low viscosity is desired in soymilk that is used as a bovine milk substitute, the soymilk made from the traditional process may be preferred to that made by RHHTC processing. However, RHHTC soymilk may be better utilized to make dairy analogs other than milks, such as milkshakes, yogurts and other high viscosity dairy analogs which are becoming increasingly popular. The high viscosity of RHHTC soymilk may be advantageous in these applications.

Relation of Solids Yields to Viscosity

Since the response of solids yield and viscosity of RHHTC soymilk to processing conditions were similar (Figures 3 and 5), the relationships between these two responses were determined at both water slurrying temperatures (Figure 6). Yield was not highly correlated to viscosity. High viscosity does not necessarily result in high yield of RHHTC soymilk and high yields at lower viscosity are possible by selecting proper conditions.

Suspension Stability

Suspension stability was evaluated by measuring the solids distribution in soymilk after a 10-day period of quiescent storage at 5°C. All soymilks showed good suspension stabilities (Table 3) with

solids ratios of upper to lower layers being nearly 1.0. No separation or sedimentation were observed during the 10-day period. Traditional soymilk also exhibited good dispersibility with solids ratios of the upper to lower layer approaching 1.0. Optimum RHHTC conditions probably result in optimum denaturation. Unfolded proteins interacted with oil droplets acting as emulsifiers at their interface with water. Less than optimum unfolding reduces protein-lipid interaction. Overheating causes protein to aggregates, thus preventing protein-lipid interaction. Hung (1984) found that the percentage of bound lipid was high (93%) in soymilk processed by RHHTC indicating the oil in RHHTC soymilk was bound by hydrophillic complexing with protein. Nelson et al. (1975) reported that an acceptable Illinois soymilk had greater than 50% bound lipids, and it was preferable to have 85% or more of the soybean oil bound.

Comparison of RHHTC Soymilk with Traditional Soymilk

When physico-chemical properties of soymilks processed by RHHTC and the traditional method were compared (Table 4), it was found that solids, protein and soymilk fractions in RHHTC soymilk when employing either cold or hot water slurrying were higher than those of traditional soymilk. Higher yields of dispersed protein and solids were accomplished by RHHTC processing than traditional processing. Suspension stabilities were equally stable during the 10-day storage period. However, RHHTC soymilks were more viscous.

CONCLUSIONS

The recovery of soybean solids in RHHTC soymilk increased with cooking time to a maximum and then decreased with longer cooking times. Yields of soymilk fraction and protein followed similar behavior. Maximum yield of RHHTC soymilk slurried at 25°C was obtained at 26 sec of cooking for 11 sec of cold water contact time. Under these conditions, the yields of solids was 84.2%. In hot water slurrying the maximum yield of solids in soymilk was 80.6% where 11 sec of water contact time and 20 sec of cooking at 154°C were used. Yields of RHHTC soymilk were much higher than that recovered from traditional soymilk (39.7%).

Rheological properties of RHHTC soymilk were mildly thixotropic which may improve the stable dispersion, enhance the shelf life and improve mouthfeel. The apparent viscosity followed a similar pattern with respect to cooking time as solids yield. However, the viscosity of RHHTC soymilk was not highly correlated to solids yield. RHHTC soymilks with higher solids yield did not necessarily have higher viscosity. Viscosities of soymilks with hot water slurrying were lower than those found in cold water slurrying. All RHHTC conditions produced soymilk with much higher viscosity than that observed in traditional soymilk.

All RHHTC soymilks exhibited good suspension stabilities; equivalent to traditional soymilk. RHHTC processing employing either cold or hot water slurrying produced a stable soymilk with higher yields of soymilk fraction, solids and protein, higher contents of solids and protein, and higher viscosity than traditional soymilk.

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Table 1. Characterization of experimental soybeans

Variety	Corsoy 79
Growing location	Ames, Iowa
Crop year	1985
Pure seed (%)	99.9
Inert weed (%)	0.1
Crop weed (%)	0.0
Weed seed (%)	0.0
Secondary noxious weeds	None Found
Germination potential (%)	95.0
Seeds per pound	2,925.0
Approximate analysis[a]	
Moisture (%)	11.1
Protein (%)[b]	41.2
Crude oil (%)	19.1
Ash (%)	5.0
Fatty acid composition	
Palmitic acid (%)	11.2
Stearic acid (%)	3.2
Oleic acid (%)	23.1
Linoleic acid (%)	54.3
Linolenic acid (%)	8.2

[a]Dry basis except moisture.

[b]Protein conversion factor was 6.25 g protein/g N.

Table 2. Effect of slurrying and cooking conditions on fraction, solids and protein yields in RHHTC and traditional soymilks

Process	Cooking time (sec)	Slurrying conditions: Water temp (°C)	Slurrying conditions: Water contact time (sec)	Soymilk[a]: Fraction yield (%)	Soymilk[a]: Solids yield (%)	Soymilk[a]: Protein yield (%)
RHHTC[b]	13	25	11	86.7	81.3	82.4
			240	81.8	73.9	74.6
			1,200	84.4	78.1	79.1
	20		11	88.3	81.0	84.3
			240	89.4	83.4	86.9
			1,200	89.1	82.8	87.1
	26		11	89.6	84.2	88.7
			240	90.7	85.0	89.6
			1,200	87.8	80.3	83.4
	35		11	87.0	79.4	81.8
			240	87.9	80.7	82.1
			1,200	83.6	75.9	77.1
	47		11	85.9	76.8	76.3
			240	86.1	78.1	76.6
			1,200	85.2	75.5	73.0
	13	80	11	84.5	77.4	77.8
			240	75.2	64.7	64.9
			1,200	69.6	55.6	53.6
	20		11	87.0	80.6	80.9
			240	86.4	79.7	79.2
			1,200	72.4	59.3	51.7
	26		11	86.3	78.4	81.2
			240	84.5	74.8	77.5
			1,200	84.4	73.2	74.0
	35		11	83.5	74.0	75.5
			240	79.0	66.3	66.3
			1,200	75.9	61.8	56.9
	47		11	79.3	68.0	63.9
			240	75.5	59.5	52.7
			1,200	81.2	51.7	51.7
Control[c]	3,600	25	66,000	48.6	39.7	47.5

[a]LSD for fraction yield was 1.37, LSD for solids yield was 2.14 and LSD for protein yield was 2.54.

[b]Cooking temperature in RHHTC was 154°C.

[c]Cooking temperature in traditional processing was 97 - 100°C.

Table 3. Suspension stability of RHHTC soymilk

Processing conditions		Solids ratio[a]	
Cooking time[b] (sec)	Water contact time (sec)	25°C[c]	80°C[c]
13	11	1.000	0.999
	240	0.999	1.001
	1,200	1.002	0.999
20	11	1.000	0.999
	240	1.000	0.999
	1,200	0.999	1.001
26	11	1.002	1.000
	240	1.000	0.999
	1,200	1.002	1.001
35	11	1.000	1.003
	240	1.000	1.000
	1,200	0.996	0.999
47	11	1.001	1.001
	240	0.999	1.002
	1,200	1.001	1.000

[a]LSD for 25°C slurrying was 0.003 and LSD for 80°C slurrying was 0.004.

[b]Cooking temperature was 154°C.

[c]Slurrying temperature.

Table 4. Comparison of physico-chemical properties of optimally processed RHHTC soymilk with those of traditional soymilk

Properties	RHHTC		Traditional[3]
	25°C[1]	80°C[2]	
Solid content in soymilk (%)	9.22[a4]	9.27[a]	8.17[b]
Protein in soymilk (%)	3.82[a]	3.86[a]	3.44[b]
Fraction as soymilk (%)	88.3[a]	87.0[b]	48.6[c]
Yield of total solid (%)	84.0[a]	80.6[b]	39.7[c]
Yield of protein (%)	84.3[a]	80.9[b]	47.6[c]
Viscosity (cp)	168[a]	133[b]	14.6[c]
Suspension stability (ratio[5])	1.001[a]	0.999[a]	1.000[a]

[1]Soymilk processed by RHHTC with cold water slurrying at 154°C for 26 sec cooking time with 11 sec water contact time.

[2]Soymilk processed by RHHTC with hot water slurrying at 154°C for 20 sec cooking time with 11 sec water contact time.

[3]Soymilk processed by the traditional method at 97-100°C for 60 min.

[4]Letters not in common denote significant differences at the 5% level.

[5]Ratio of solids contents of upper to lower layers.

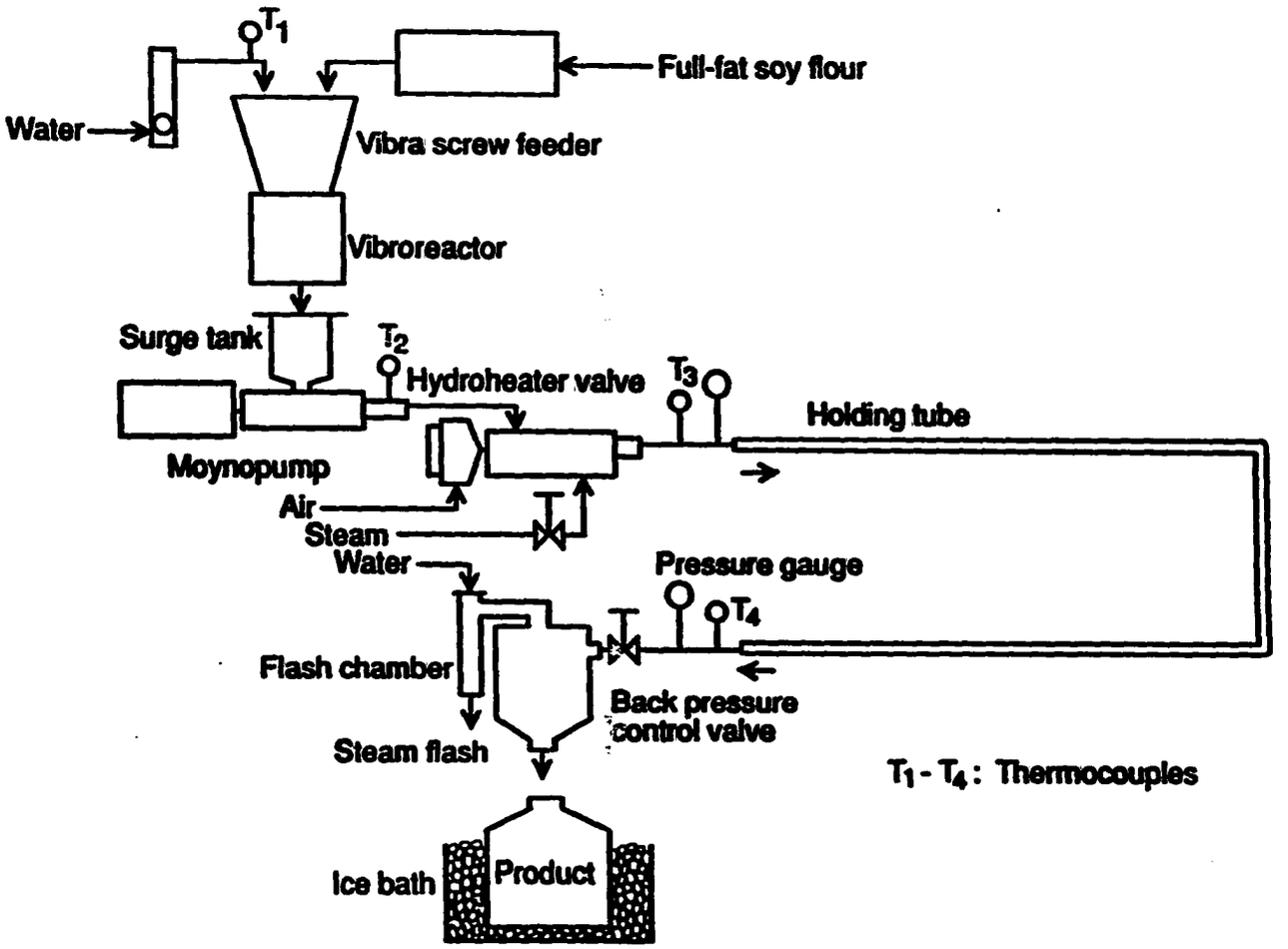

Figure 1. Schematic diagram of rapid-hydration hydrothermal cooking (RHHTC) system

Figure 2. The pilot-plant scale rapid-hydration hydrothermal cooking (RHHTC) system

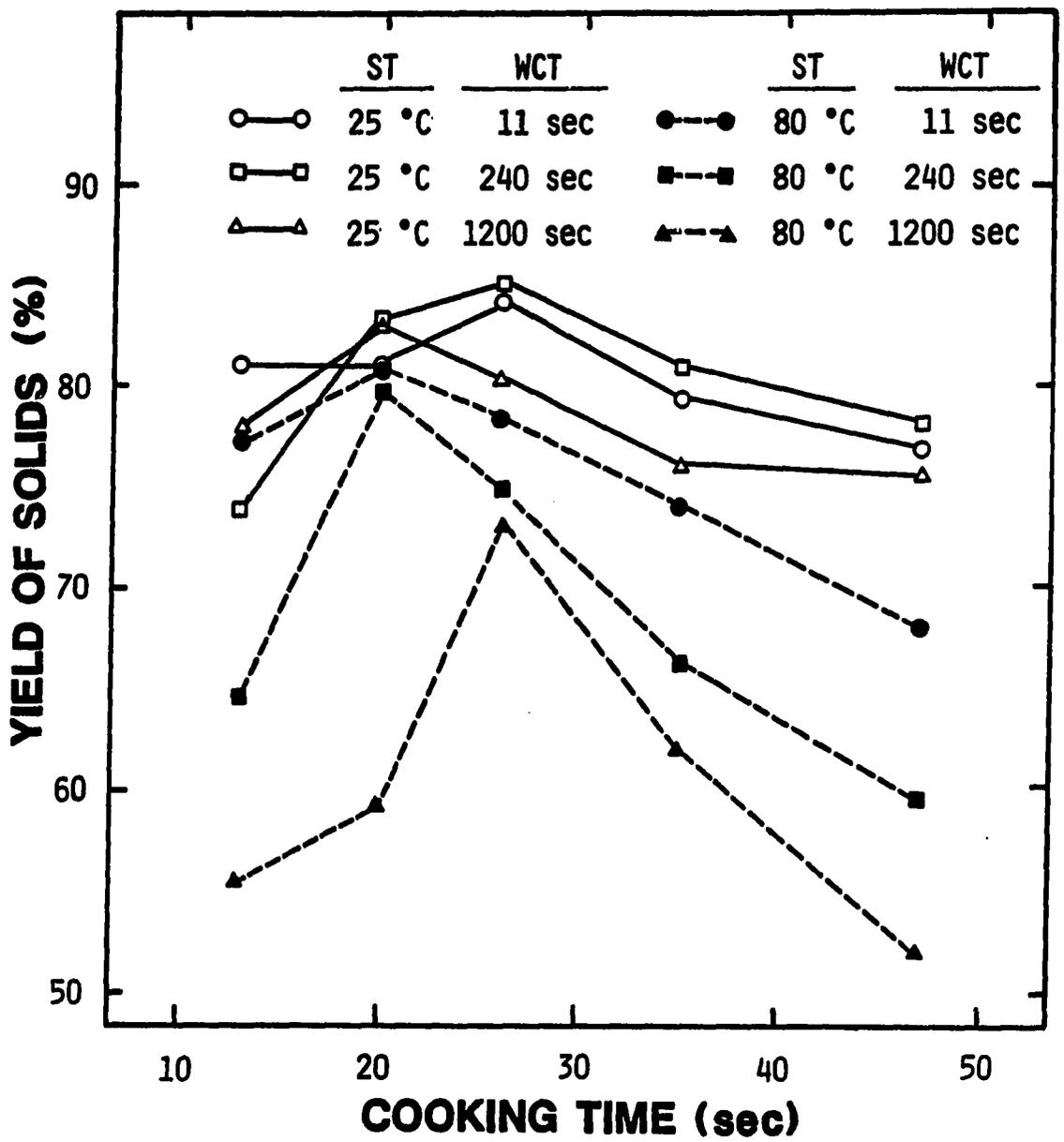

Figure 3. Effect of soy flour slurrying conditions on yield response of RHHTC soymilk processed at 154°C for different times (ST denotes slurrying temperature and WCT denotes water contact time; LSD was 2.15)

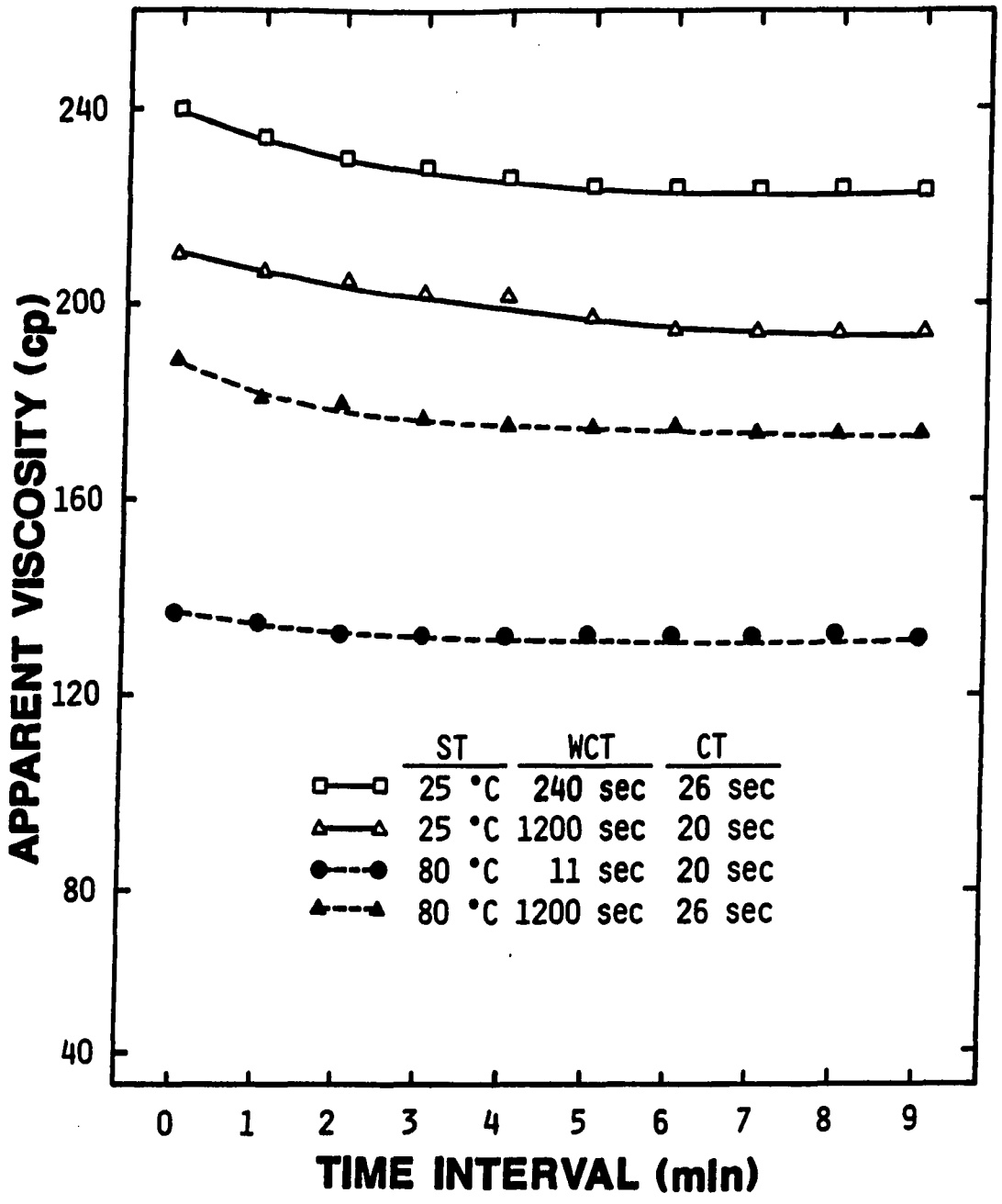

Figure 4. Effect of slurrying conditions on the apparent viscosity of RHHTC soymilk (ST denotes slurrying temperature, WCT denotes water contact time and CT denotes cooking time)

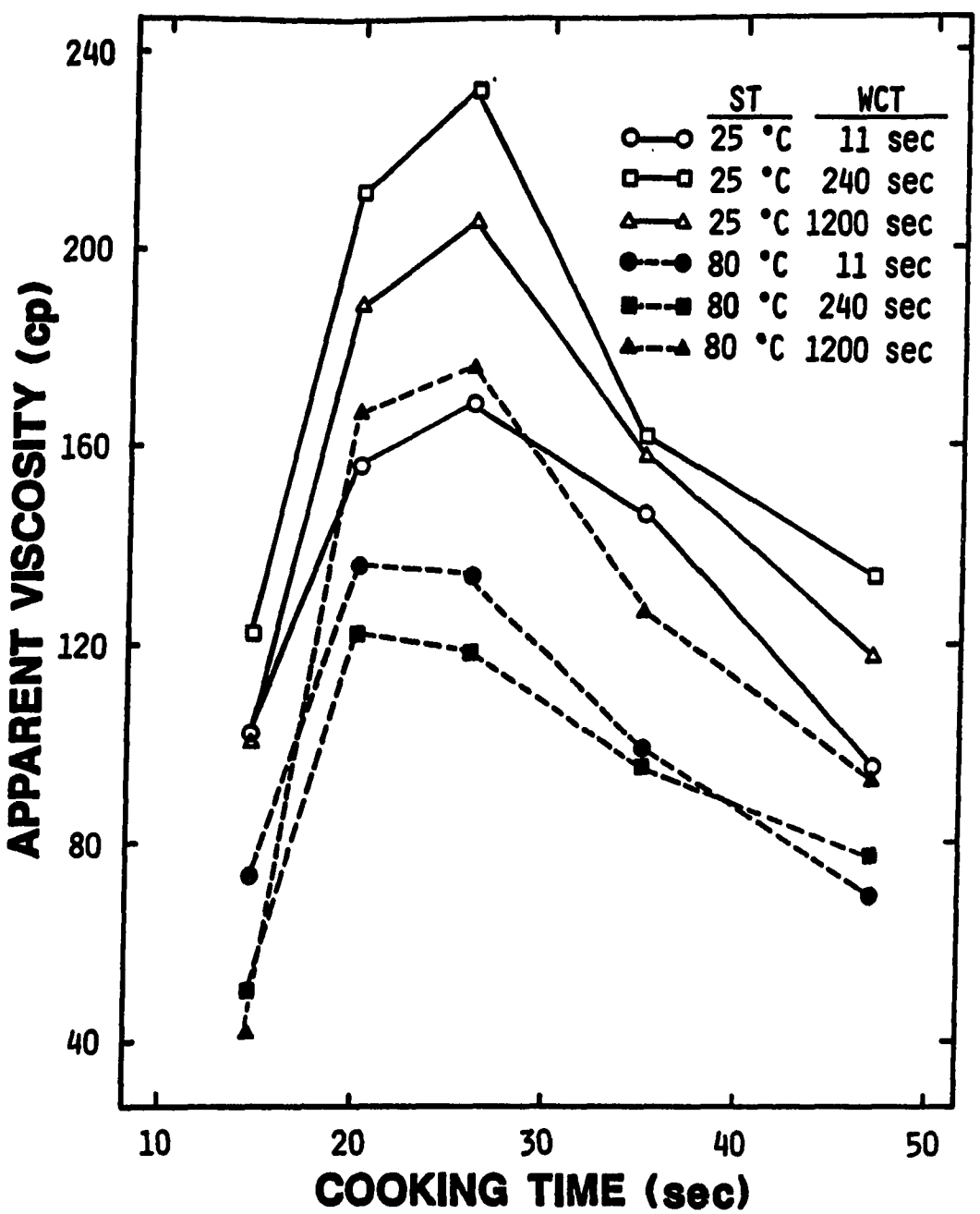

Figure 5. Effect of soy flour slurrying conditions on the apparent viscosity of RHHTC soymilk processed at 154°C for different times (ST denotes slurrying temperature and WCT denotes water contact time; LSD was 32.2)

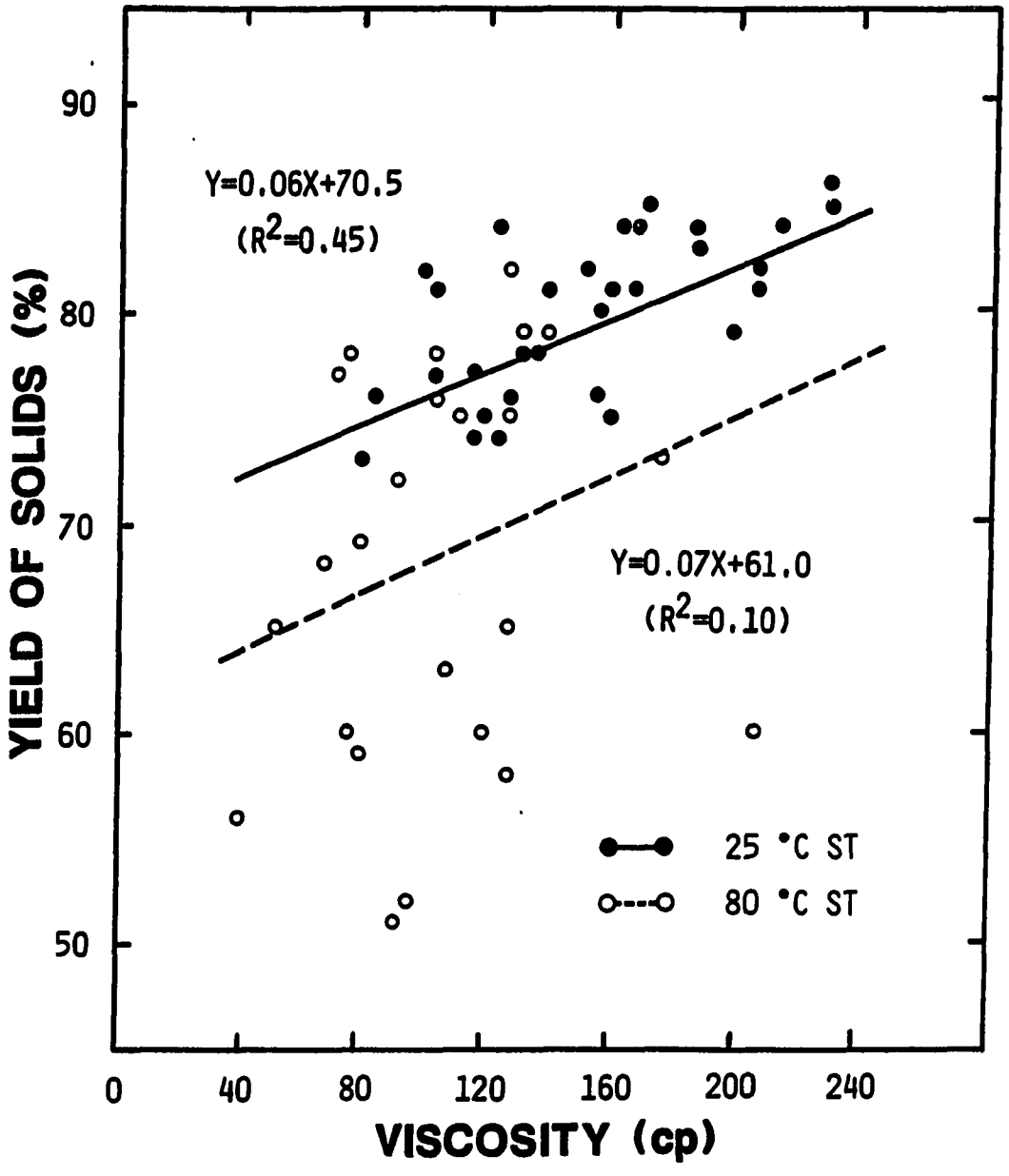

Figure 6. Relationship between yield of solids and the apparent viscosity of RHHTC soymilk processed at 154°C using cold and hot water slurrying

PART III. EFFECT OF PROCESSING CONDITIONS ON NUTRITIONAL PROPERTIES OF SOYMILK PROCESSED BY RAPID-HYDRATION HYDROTHERMAL COOKING (RHHTC)

ABSTRACT

The effect of soy flour slurrying and cooking conditions upon the nutritional properties of RHHTC soymilk were studied. Optimum RHHTC processing conditions for maximum nutritional value were identified as 26 sec of cooking at 154°C with 11 sec of water contact time for cold water slurrying and 18 sec of cooking time with 11 sec of water contact time for hot water slurrying. Losses of available lysine increased and colors darkened with increasing cooking time in RHHTC. Longer water contact time gave lower values for available lysine and darker colored soymilks. RHHTC soymilks processed with hot water slurrying (processed at equivalent residual TI values) had higher values for available lysine and were lighter than those with cold water slurrying. Apparently, oxidation products produced during slurrying contributed to browning of soymilk and losses of available lysine. Maximum in vitro digestibility occurred in soymilk processed under the same conditions which maximized solids yield and viscosity.

RHHTC soymilks processed under optimum conditions gave better nutritional qualities than observed in traditional soymilk. RHHTC soymilk had higher available lysine, less chemical browning and more digestibility and equivalent residual TI. Nutritional properties were better when employing hot water slurrying than cold water slurrying.

INTRODUCTION

Heat treatment is one of the most important methods for extending the storage life of perishable foodstuffs. However, heat treatment oftentimes adversely affects other food qualities. Color, texture and flavor are often damaged during heat treatment, whereas heat-labile antinutritional substances that may be present naturally are inactivated, thereby improving nutritive quality. It is desirable to have control over these changes to derive maximum advantage from desirable physico-chemical changes and to reduce detrimental chemical reactions to minimum during heat treatment. Therefore, it is important to evaluate the adequacy of heat treatments which influence the quality of the final product.

In order for soybeans to provide maximum nutrition, deleterious substances must be removed from raw soybeans. Through the application of heat it is possible to inactivate harmful materials, such as trypsin inhibitors (Van Buren et al., 1964). However, heat treatment may affect the protein quality and quantity in soyfoods.

Trypsin Inhibitors

It was not long after soybeans were introduced into the U.S., primarily as a source of oil, that Osborne and Mendel (1917) found that soybeans had to be heated to support of the growth of rats. It has been generally assumed that beneficial effects of heat treatment could be ascribed to the destruction of trypsin inhibitors (TI). The inactivation

of TI parallels the improvement in nutritive value to rats (Rackis, 1972). In addition to the inactivation of TI, heat treatment also increases the susceptibility of protein to proteolytic enzymes present in the intestinal tract. Liener et al. (1949) demonstrated that TI alone did not account for all of the growth inhibition observed with raw soybeans. Further evidence of the role of TI in the growth of rats comes from the experiment in which it was shown that if TI activity of a crude extract of soybean was removed by affinity chromatography on Sepharose-bound trypsin the resulting unheated extract was still capable of growth inhibition and pancreatic hypertrophy (Kakade et al., 1973). They estimated that approximately 40% of the growth inhibition, as well as, 40% of the pancreatic hypertrophic effect of a soybean extract was accounted for by TI. In their in vitro digestion studies, heat treatment of soybean protein isolate increased the digestiblity of protein above the digestibility of a similar preparation from which the inhibitor had been removed. This observation suggested that native, undenatured soy protein is resistant to enzyme attack unless denatured by heat. Fukushima (1968) has reported that most soybean proteins are globulins which are folded to sequester hydrophobic regions in the interior and show little susceptibility to proteinases unless the internal structure is disrupted by denaturation. Therefore, TI and the refractory nature of soybean protein act to inhibit the growth of rats.

Two types of trypsin inhibitors have been isolated from soybeans. The Kunitz inhibitor has a molecular weight of 20,000-25,000 with 181 amino acid residues, two disulfide bonds and a specificity for trypsin.

The Bowman-Birk inhibitor has a molecular weight of only 6,000-10,000 with 71 amino acid residues, 7 disulfide bonds and capability of inhibiting trypsin and chymotrypsin at different binding sites (Liener and Kakade, 1980). The Kunitz inhibitor has very little α - helical structure and is largely in the form of a random coil in its native state. Thus, it is reasonably susceptible to heat treatment, whereas the Bowman-Birk inhibitor exhibits remarkable stability toward heat, acid and alkali, a property attributed to the stabilizing effect of the large number of disulfide bonds for its small size.

The extent to which TI activity is destroyed by heat is a function of temperature, duration of heating, particle size, pH and moisture content. Controlling these variables is very important to produce a product having maximum nutritive value (McKinney and Cowan, 1956).

Klose et al. (1948) found that maximum nutritive value was obtained after steaming whole, dry soybeans for 20 - 30 min at atmospheric pressure or for 15 - 20 min at 1.05 kg/cm^2 (15 psi) steam pressure at 120°C. Up to 90 and 92% of the original TI activity was destroyed at atmospheric pressure. Complete destruction of TI by atmospheric cooking results in degradation of essential amino acids and, thereby, adversely affects protein quality. Atmospheric cooking inactivates most TI in whole soybeans in 15 - 20 min if the initial moisture content is 20%. If the beans are soaked in water overnight to 60% moisture, 5 min in boiling water is sufficient to inactivate most of TI (Albrecht et al., 1966).

Most studies have shown that when properly heat processed, soymilk has a nutritive value almost equivalent to that of bovine milk. The TI

activity present in soymilk can be effectively eliminated by heating the milk for 30 - 75 min at 93°C or 5 - 10 min at 121°C (Hackler et al., 1965). In these studies, maximum protein efficiency ratios were obtained when about 90% of TI had been destroyed (60 min at 97 - 100°C).

Available Lysine

Heat treatment of soybeans may cause some of the nutrients to be destroyed or made unavailable. Since the value of soybeans as a food is due in a large part to their high contents of protein and lysine (an essential amino acid), it is important to be able to measure the extent to which the nutritional quality of the protein may have been harmed during thermal processing.

It is well known that the bioavailability of lysine in foods depends upon the amount of lysine possessing free ϵ-amino groups. This lysine is usually regarded as available lysine. The ϵ-amino groups of lysine can become blocked by reacting with carbonyl groups from reducing sugars or from secondary fat carbonyls of fat oxidation and polyphenols (Hurrell and Carpenter, 1981).

A variety of different methods have been developed to determine available lysine (Carpenter, 1960; Booth, 1971; Kakade and Liener, 1969; Walker, 1979; Rabasseda et al., 1988). Even though there are drawbacks, the most widely used method was developed by Carpenter (1960) and modified by Booth (1971). This method uses 1-fluoro-2,4-dinitrobenzene (FDNB) for derivatization; the N-ϵ-dinitrophenyl-lysine (ϵ-DNP-lysine) is measured spectrophotometrically after acid hydrolysis

and extraction.

The estimation of available lysine has been applied to plant protein (Jokinen et al., 1976; Ringe and Love, 1988), animal protein (Carpenter, 1960), fish meal (Rabasseda et al., 1988) and soymilk (Van Buren et al, 1964; Hand et al., 1963). Wolf et al. (1979) found that the FDNB method was a good indicator of changes in lysine availability in severely heat-treated soybean samples. Moreover, Van Buren et al. (1964) found that determination of available lysine was a reliable index for evaluating adequacy of heat treatment and protein quality damage in processed soymilk. The extent of lysine damage is a good index of heat damage to nutritional quality although lysine is present in high amounts in soy products and may not be the growth limiting amino acid.

Color

Color is another major criterion used as an index to evaluate nutritionally important quality changes. The color of fresh fruit indicates ripeness and the time the fruit is most likely to taste best. Off-colors in cheese and meats are associated with poor flavor quality. One expects each food product to have a certain color. Deviation from the expected color can result in rejection of a product even when this color does not adversely influence the flavor or the nutritional value (Blouin et al., 1981).

Soymilk can be used as a beverage or as an extract for making tofu. Color is an important factor in both products. In soymilk, the dispersed protein can participate in Maillard reactions with other components of

soybeans. Since protein is involved in this reaction, the color of soymilk indicates protein damage. The first step in Maillard reaction involves the condensation reaction between the carbonyl group of the reducing sugar and the free amino group of protein. The condensation product is rapidly converted via Schiff's base and Amadori rearrangement to biologically unavailable deoxyketosyl compounds. Condensation products are the major form of blocked amino acids after Maillard reaction. Mild heat treatment causes little change of this form so that color and digestibility of the product changes little. After the formation of the deoxyketosyl derivative, the reaction leads to the formation of brown pigments or melanoidins. These reactions are responsible for numerous flavors and odors, possible toxicity, as well as, further reduction in nutritive value due to destruction or alteration of amino acids and reduced protein digestibility. Furthermore, cross-linking between the protein chains and these breakdown products reduces the availability of all amino acids.

Apart from lysine, cystine and methionine are sensitive to heat treatment and to the presence of sugar (Miller et al., 1965). Tryptophan will degrade by reacting with reducing sugars and splitting of the indole ring due to heat or oxidation (Dworschak and Hegedus, 1974). Methionine can be damaged after relatively short periods of heating due to oxidation to methionine sulphoxide or to methionine sulphone since the thioether group of methionine does not combine with reducing sugars (Hurrell and Carpenter, 1981).

McNaughton (1981) showed that color was a good indicator of TI

content and may be used to predict adequate heat treatment of soybean meals for broiler chicks. Hand et al. (1963) reported that overheating of soy products resulted in loss of available lysine and increased the degree of browning. Van Buren et al. (1964) indicated that measuring the extent of browning was a good indicator of heat damage during processing. The protein efficiency ratio (PER) could be predicted using the Hunter L value (measuring the darkness of color). They found that the regression equation PER = 0.027H - 0.04 (H = Hunter L value) predicted PER. This relation applied when 90% or more of TI was denatured. The Hunter color difference measurement was a simple, rapid and highly reproducible test. This color measuring test was useful in evaluating relative heat damage to the nutritional quality of soymilk.

In Vitro Digestibility

Protein quality and quantity in foods have become important factors to food manufacturers with the advent of nutritional labeling regulations for foods in 1973. The official assay for nutritional quality of protein is the rat-based protein efficiency ratio (PER) (AOAC, 1984). Major drawbacks of the PER assay are the length of time required to conduct the test (4 weeks), the expense involved and the assumption that growth requirements of weanling rats are the same for all humans. Net protein ratio (NPR), a 10 - 14 day bioassay, and the 2 - week PER are alternatives to the PER assay although neither method sufficiently reduces time and expense for routine testing (Harris et al., 1988).

Several enzymatic methods have been developed to measure in vitro

digestion. A pepsin-pancreatin enzyme system gave reasonably accurate estimation of protein digestibility (Akeson and Stahmann, 1964). Buchanan (1969) described a procedure that utilized papain to measure the in vitro protein digestibility of wheat leaf protein concentrate. His results agreed with in vivo results from the rat bioassay. Saunders et al. (1973) compared digestibilities of alfalfa concentrate by in vivo and in vitro methods. They found that a papain-trypsin enzyme method correlated well with in vivo digestibility ($r = 0.91$). Maga et al. (1973) indicated that the initial rate of hydrolysis by trypsin was a good indicator of protein digestibility.

Hsu et al. (1977) developed a simplified in vitro procedure which utilized a mixture of enzymes (porcine trypsin, bovine chymotrypsin, porcine intestinal peptidase) to estimate protein digestibility. Protein digestibility was expressed as pH of the assay mixture after 10 min of incubation with the enzyme solution. The linear correlation between in vitro and in vivo digestibilities was 0.90 for 23 foods or food ingredients.

Satterlee et al. (1979) developed the computed PER (C-PER) assay for protein quality. This C-PER assay utilizes essential amino acid profile and in vitro protein digestibility data to estimate PER. They incorporated a mixed enzyme system to obtain protein digestibility. One of the advantages of the C-PER assay was that a predicted PER can be obtained in 72 hr or less at much lower cost than can the rat PER assay. This assay was recommended as an alternative method for routine quality control screening of food and food ingredients. In 1984, the C-PER and

the discriminant computed PER (DC-PER) computer prediction models of Satterlee et al. (1982) were adopted as official first actions by the Association of Official Analytical Chemists.

In vitro digestibility has been applied to soymilk in only a few instances. Hung (1984) measured digestibilities of the dried RHHTC soymilk and compared them to that of traditional soymilk using the procedure of Hsu et al. (1977). His results indicated that in vitro digestibility of RHHTC soymilk was better than that of traditional soymilk.

Research Objectives

The objective of this study was to determine the effects of slurrying and cooking conditions on nutritional properties and their indicators, such as residual trypsin inhibitor activity, available lysine, in vitro digestibility and color of RHHTC soymilk. Changes in slurrying conditions to improve flavor are likely to alter optimum RHHTC cooking conditions due to differences in hydration of protein and lipid oxidation.

MATERIALS AND METHODS

Characterization of Soybeans

Seed-grade Corsoy 79 soybeans, which were grown at Ames, IA during 1985, were used in this study. These soybeans are characterized in Table 1 of Part II.

Soy Flour Slurry Preparation

Corsoy 79 soybeans were ground in a Fitz-mill (Model D, Fitzpatrick Co., Elmhurst, IL) through a 32-mesh screen. Soy flours were slurried in tap water (25 and 80°C) at 17 part of water to 3 part of soy flours. Soy flours were metered at a rate of 120 to 130 g/min into the vibroreactor. Either cold or hot water was continuously supplied from a steam-jacketed tank through a pump into the vibroreactor.

Soymilk Processing

A control sample was prepared by cooking in the traditional manner in a steam-jacketed kettle. Whole soybeans were soaked overnight with 3 parts water to 1 part soybeans at room temperature. The soaked beans were drained and rinsed with tap water two times. Eight parts of water added and the soybeans were ground in two passes through the vibroreactor. The raw slurry was allowed to stand for 20 min with occasional stirring and then cooked for 60 min at 97 - 100°C. Cooking time was measured when the temperature reached 97°C. The slurry was continuously agitated during cooking. After cooking, the sample was

cooled in an ice bath and stored at 5°C overnight. The cooked slurry was adjusted to 10% solids and centrifuged at 2,500 rpm in a centrifuge (Model J2-21, Beckman Instruments, Inc., Palo Alto, CA) generating 1,050 x g for 5 min at 5°C. The soymilk was carefully decanted.

A RHHTC system as described in Part II was assembled. The holding tube was varied in length to achieve different cooking times at 154°C. Three water contact times of 11, 240 and 1,200 sec were used in both cold and hot water slurrying with RHHTC processing. In hot water slurrying, the vibroreactor, Moyno pump, surge tank and piping were preheated to near 90°C by using silicon heating tapes and recycling hot water through the system. Temperatures during slurrying were intensely monitored and the slurry always exceeded 80°C.

Determination of Trypsin Inhibitor

Trypsin inhibitor (TI) activity was assayed by the procedure of Swartz et al. (1977) as detailed in Johnson et al. (1980). A 4.5 g sample of soymilk was diluted to 100 ml with double distilled water. The diluted sample was centrifuged at 30,000 x g for 30 min at 5°C with a refrigerated centrifuge. The supernatant was used for assaying TI activity without further dilution. In addition, 10% raw soy flour in distilled water was slurried and continuously mixed for 3 hrs which maximized the extraction of TI in raw soy products (Kakade et al., 1974). A 4.0 g sample of raw slurry was prepared in the same manner as soymilk samples. Raw samples were further diluted 1:2 for assaying TI activity.

Trypsin (bovine pancrease Type III, Sigma Chemical Co., St. Louis,

MO), 0.0010 g, was placed into a 100-ml volumetric flask and diluted to 100 ml with 0.001 N HCl. Fresh standard solution was made with each run. Benzoyl-DL-arginine-p-nitroanalide hydrochloride (BAPA) was used as substrate for trypsin. BAPA substrate solution was made by dissolving 0.003 g BAPA (Sigma Chemical Co., St. Louis, MO) in 1 ml of dimethyl sulfoxide and diluting to 100 ml with 0.05 M tris buffer (pH 8.2) with 0.02 M $CaCl_2$.

Aliquots of soymilk (0.30 - 0.65 ml) were placed into test tubes and volumes were brought to 0.65 ml with distilled water. A 0.35 ml aliquot of trypsin solution was added and the preparation mixed on a Vortex mixer. The mixture was incubated at 37°C in a water bath for 5 min and 3.0 ml of substrate solution was added. The reaction mixture was mixed on a Vortex mixer and the rate of hydrolysis was measured spectrophotometically. Absorbance at 410 nm was measured for 5 min with a Gilford Spectrophotometer 250 (Gilford Instrument Laboratories Inc., Oberlin, OH) and recorded by a Sargent-Welch Recorder Model SRG (Sargent-Welch Scientific Company, Skokie, IL). For each run, a reference assay was performed on distilled water. The initial rates of hydrolysis were determined and converted percent inhibition as shown in Table 1. One trypsin inhibitor unit (TIU) was defined as the amount of trypsin inhibitor which caused 50% of inhibition of trypsin activity in each assay. TI units in each soymilk sample was calculated and compared to that of raw soymilk to obtain a value for residual TI concentration.

Determination of Available Lysine

Available lysine was determined by the 1-fluoro-2,4-dinitrobenzene (FDNB) method of Carpenter (1960) as modified by Booth (1971). Samples with approximately 12 mg lysine were weighed into 100 ml round-bottom flasks. Ten milliters of 8% (w/v) $NaHCO_3$ were added to each flask and stirred for 10 min at room temperature. Fifteen milliters of FDNB solution containing 0.4 ml FDNB (Sigma Chemical Co., St. Louis, MO) in 15 ml 95% ethyl alcohol were added and stirred for 2 hrs at room temperature. Thereafter, the procedure described by Booth (1971) was followed. A recovery test was conducted to calculate a correction factor for 100% recovery. A standard solution (7.5 mg lysine) containing known amounts of dinitro-L-lysine HCl (Sigma Chemical Co., St Louis, MO) was used as a recovery marker. Triplicate analyses were performed. Correction factors were 1.095 and 1.101 for soymilks processed with hot and cold water slurrying, respectively. Available lysine values were reported as g Lys/16 g N.

Color Measurement

Color of soymilk was measured using a Hunter Labscan Spectro Colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). A Hunter color standard plate, No. LS-12414, was used to standardize with the illumination source being CIE 1965 10° standard observer, D_{65}. The X, Y and Z values of the standard plate were 78.46, 83.14 and 85.14, respectively. A Falcon 1007 Petri Dish (60 x 15 mm type) (Becton, Dickman and Co., Oxnard, CA) was used to hold the sample. Quadruplicate

analyses were performed on each sample.

In Vitro Protein Digestibility

In vitro protein digestibility of soymilk protein was determined by the multienzyme procedure of Hsu et al. (1977). The enzymes used were bovine pancreatic trypsin (Type III), bovine pancreatic -chymotrypsin (Type II) and porcine intestinal peptidase. The multienzyme solution (10 ml) was added to 50 ml soymilk (6.25 mg/ml protein) at pH 8 and the drop in pH was recorded for 10 min at 37°C. The in vitro digestibility of the sample was calculated by using the following equation:

$$\% \text{ digestibility} = 210.464 - 18.103X$$

where X was the pH value after 10-min digestion (Hsu et al., 1977). Duplicate analyses were performed.

Statistical Analysis

Data were analyzed by using a Statistical Analysis System (SAS, 1984) program package. The General Linear Models (GLM) procedure was run to determine main and interaction effects. Significant differences among treatment means were determined by Duncan's multiple range test or the least significant difference (LSD) procedure. Probability levels of $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Residual Trypsin Inhibitor Activity of Soymilk

Soymilk produced by wet grinding is traditionally heated for 60 min at 93°C to achieve soymilk with maximum nutritional quality (Hackler et al., 1965). In this study, traditional soymilk had 12.2% of residual trypsin activity after cooking for 60 min at 97 - 100°C. The effect of slurrying and cooking conditions on residual TI activity is shown in Figure 1. After 13 sec of cooking at 154°C, 18.0 - 19.0% residual TI remained when slurrying at 25°C and 12.8 - 14.9% at 80°C. Apparently, considerable amount of TI was inactivated during hot water slurrying. The original content of TI in raw soymilk was 188.0 trypsin inhibitor units (TIU). The times for 88% inactivation of TI at 25°C slurrying temperature were 26, 21 and 19 sec for 11, 240 and 1,200 sec of water contact times, respectively and 18, 14 and 17 sec for 11, 240 and 1,200 sec of water contact time at 80°C of slurrying temperature, respectively.

The effect of water contact time on residual TI activities was evident in both cold and hot water slurrying where the residual TI activities were the same as traditional soymilk. Increasing water contact time increased the rate of TI inactivation.

Optimum cooking conditions for RHHTC processing were identified as 26 sec of cooking time with 11 sec of water contact time for cold water slurrying and 18 sec of cooking time with 11 sec of water contact time for hot water slurrying. Residual TI activities in RHHTC soymilk processed in one of these two ways were equivalent to traditional

soymilk.

Available Lysine

The effects of water contact time and slurrying temperature on available lysine were studied. Loss of available lysine was very rapid during the first 20 sec of cooking and then the rate decreased at longer cooking time (Figure 2). Little change occurred after 35 sec of cooking at 154°C regardless of slurrying condition.

Water contact time during slurrying of soy flour significantly affected lysine availability in RHHTC processing. The results showed that lysine availability generally decreased with increased water contact time. Generally, shorter contact time favored retention of available lysine.

Available lysine content of the control sample (traditional soymilk processed at 97 - 100°C for 60 min) was 5.60 g Lys/16 g N. RHHTC soymilk processing with either cold or hot slurrying retained higher values of available lysine at equivalent TI residuals (indicated by arrows in Figure 2). Only in a few instances where hot slurrying was used with long cooking times did available lysine contents of RHHTC approach those of traditional soymilk. Available lysine of raw soy flour was 6.51 g Lys/16 g N. Losses in available lysine ranged from 1.54 to 10.9% for cold slurrying and from 15.8 to 18.1% for hot slurrying over the range in cooking times from 13 to 47 sec. Traditional soymilk lost 14.0% available lysine during cooking. In cold water slurrying, the losses ranged from 1.54 to 9.09%, 4.60 to 10.9%, and 2.70 to 7.55% for 11, 240

and 1,200 sec of water contact times, respectively. Difference of processing method, slurrying temperature and duration of water contact time prior to cooking as well as cooking time influenced protein quality of the resultant soymilk.

Effect of Processing Conditions on Browning

Browning is one of the more important reactions impairing the quality of soymilk during processing. Amino acids, such as lysine, cysteine, methionine, arginine, tryptophan, histidine and serine, may be partially destroyed as a result of heating (Del Valle, 1981) and, thus, lower protein quality. In soymilk processing reducing sugars may react with amino acids, especially lysine. The effect of RHHTC processing on browning of soymilk was studied.

RHHTC soymilk became darker with increasing cooking time (Figure 3). The rate of browning was faster during the first 20 sec of cooking. Water contact time of soy flours also influenced browning. Less water contact time gave lighter color in soymilk. At 20 sec of cooking, Hunter L values decreased from 76.1 at 11 sec of water contact time to 74.5 at 240 sec of water contact time to 73.8 at 1,200 sec of water contact time when slurrying at 25°C.

The L value of traditional soymilk was 73.9. RHHTC soymilks with 88% reduction in TI (indicated by arrows in Figure 3) had higher L values. Moreover, traditional soymilk was darker than those processed by RHHTC at 35, 26 and 20 sec of cooking with 11, 240 and 1,200 sec of water contact times at 80°C slurrying (values were significantly different at

the 5% level).

RHHTC soymilks processed with hot water slurrying were lighter than those with cold water slurrying when achieving 88% of inactivation of TI. In cold water slurrying (25°C), lipoxygenase was active and produced hydroperoxides which in turn underwent decomposition to aldehyde, ketones, alcohols, ester acids, etc. These decomposition products, especially aldehydes, are reactive and amino acids, such as histidine, cysteine/cystine, methionine, tyrosine and lysine, are susceptible to attack by those products. Nonenzymatic browning results from reaction between the aldehydes and amino groups of proteins to form a Schiff's base. Aldol polycondensation leads to formation of pigments. Therefore, the more lipid oxidation, the greater the browning. Cold water slurrying and longer water contact times are more favorable for lipid oxidation.

The effects of processing on the Hunter color values a and b were also studied (Table 2). Even though the range of Hunter a values (redness-greenness) was relatively broad, especially at 80°C slurrying temperature (-1.5 to +0.6), soymilk samples generally became more red as cooking time increased. This tendency was more pronounced with increasing slurrying temperature and water contact time. The Hunter color b values (yellowness-blueness) of soymilk samples generally decreased as cooking time and water contact time increased; however, this change was not as pronounced as Hunter color L values.

The relationship between residual TI activities and Hunter color L values in RHHTC soymilks was observed in the present work (Figure 4). The relationship was reasonably well correlated but was different for

each slurrying temperature. Correlation coefficients were 0.75 and 0.73 for hot and cold water slurrying, respectively. Therefore, color irrespective of cooking temperature is not a suitable indicator of adequate heat treatment.

Positively correlated relationships were observed between L values and available lysine (Figure 5). The relationship was more highly correlated in hot water slurrying versus cold water slurrying (0.71 vs 0.60) and the response was more rapid in hot water slurrying than in cold water slurrying (0.06 vs 0.13).

In Vitro Digestibility

The multienzyme digestion technique was used to evaluate the susceptibility of soymilk protein to enzymatic attack. In this test proteolytic digestion is accompanied by increased acidity. Typical pH drops during 10 min of incubation time are shown in Figure 6. The fastest drop in pH of the assay mixture occurred during the first 1 min (56.5 - 72.9% hydrolysis) after which it changed very little during the 10-min assay. In one experiment, the incubation time was increased to 1 hr, but the change in pH was essentially the same as that obtained at 10 min. Differences in pH drop were due to differences in level of residual trypsin inhibitor activity, as well as, differences in susceptibility of the protein to enzymatic attack. These pH drop values can be converted into estimates of digestibility (Hsu et al., 1977). In vitro digestibility of traditional soymilk was 83.3%.

The effect of slurrying and cooking conditions on in vitro

digestibilities among RHHTC soymilk was studied (Figure 7). All soymilks digested similarly with the exception of soymilks processed with hot water slurrying for 11 sec of water contact time. Digestibility increased until it reached a maximum and decreased with prolonged cooking. Highest digestibilities were obtained at the same cooking times which gave the highest yields of solids and viscosity. At these cooking times, soy proteins were probably dissociated into subunits and partially denatured so that the polypeptides were highly susceptible to proteolysis. Longer cooking times produced lower molecular weight browning reaction products which prevented proteolysis (Oste et al., 1986). Longer water contact times gave higher digestibilities in cold water slurrying, whereas, the reverse situation was observed in hot water slurrying. Thus, the duration of water contact time was significant in protein hydrolysis. Higher digestibilities occurred when employing rapid slurrying (11 sec) with hot water. RHHTC soymilks processed by either cold or hot water slurrying were more digestible except in a few instances (47 sec cooking with 11, 240 and 1,200 sec of water contact times at 80°C slurrying).

Comparison of RHHTC Soymilk with Traditional Soymilk

Nutritional properties of soymilks processed by RHHTC employing either cold or hot water slurrying were compared to those of traditional soymilk (Table 3). Optimum RHHTC processing conditions, which resulted in 12% residual TI, produced soymilk with more available lysine, lighter color, less chemical browning and more digestible protein than the

traditional method. Residual TI activities were not significantly different among the three soymilks, but RHHTC soymilks trended towards lower values.

CONCLUSIONS

Eighty eight percent reduction in TI occurred at 19 - 26 sec cooking times for 25°C slurrying and at 14 - 18 sec for 80°C slurrying. Slurrying of soy flour at 80°C prior to cooking reduced the cooking time required to inactivate TI. TI inactivation was highly correlated to Hunter color L value, but the relationships were different depending on whether hot or cold water was used in slurrying.

Losses of available lysine occurred with increasing cooking time in RHHTC soymilks. The loss of lysine was more rapid during the first 20 sec of cooking. Soymilk produced by RHHTC with cold or hot water slurrying of soy flour had higher available lysine contents than that of traditional soymilk when processed to equivalent levels of residual TI. Lysine availability was lower using 240 sec of water contact time in soymilk processed with either cold or hot water slurrying.

The extent of browning increased with increasing cooking time. Rapid browning occurred during the first 20 sec of cooking time. Water contact time was a major factor in browning; longer water contact time led to darker color in soymilk. RHHTC soymilks processed with hot water slurrying had lighter color than those with cold water slurrying.

The relationship between available lysine content and Hunter color L value exhibited a positive correlation. The correlation was better and its slope was steeper in hot water slurrying than cold water slurrying.

In vitro digestibilities ranged from 82.3 to 84.6%, while traditional soymilk had 83.3% digestibility. Maximum digestion occurred

at the same point where the solids yield and viscosity were also at their maximum values.

RHHTC processing produced better nutritional quality as evidenced by more available lysine, less chemical browning and higher protein digestibility when processed to equivalent residual TI activities.

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Table 1. Calculation of residual trypsin inhibitor activity

$$\text{Inhibition} = (1.00 - R_s/R_c) \times 100$$

$$\text{TIU concentration} = \frac{\text{\% inhibition}}{\text{50\% inhibition/TIU}} \times \frac{1}{V} \times D_1 \times D_2$$

$$\text{Residual trypsin inhibitor activity (\%)} = (TIU_c/TIU_r) \times 100$$

R_s = rate of hydrolysis with soy extract (absorbance units/min)

R_c = rate of hydrolysis without soy extract (absorbance units/min)

V = sample volume of soymilk extract in assay mixture (0.30 to 0.65 ml)

D_1 = initial dilution before centrifugation at 30,000 x g (100 ml/4.5 g)

D_2 = dilution after sample preparation (1 to 3)

TIU_c = trypsin inhibitor units of cooked soymilk

TIU_r = trypsin inhibitor units of raw soymilk

Table 2. Effect of processing conditions on Hunter color values of RHHTC soymilk processed at 154°C for different times

Cooking time (sec)	Water contact time (sec)	Slurrying temperature (°C)[a] 25 L	25 a	25 b	80 L	80 a	80 b
13	11	79.4	0.08	10.3	80.8	-1.61	13.3
	240	78.4	-0.36	10.8	79.6	-1.41	13.4
	1,200	77.7	-0.44	10.8	78.2	-1.63	11.6
20	11	76.1	0.41	10.1	76.5	-1.04	12.0
	240	74.5	-0.03	10.8	75.3	-0.98	11.6
	1,200	73.8	-0.43	10.4	76.2	-1.48	11.4
26	11	74.0	0.48	11.4	75.6	-0.20	11.9
	240	73.9	0.12	11.2	74.5	-0.15	11.7
	1,200	73.4	-0.46	11.1	72.4	-0.68	11.4
35	11	73.3	0.49	11.5	75.5	0.32	12.6
	240	72.1	0.25	11.4	73.2	0.35	12.5
	1,200	71.7	-0.29	11.4	72.1	-0.24	11.7
47	11	72.2	0.53	12.0	71.7	0.63	12.4
	240	71.8	0.33	11.9	72.0	0.43	12.6
	1,200	71.1	0.40	11.9	71.5	0.11	13.2

[a]LSD was 1.06 for L value, 0.08 for a value and 0.24 for b value.

Table 3. Comparison of nutritional properties of optimally processed RHHTC soymilk with those of traditional soymilk

Properties	RHHTC		Traditional[3]
	25°C[1]	80°C[2]	
Available lysine (g Lys/16 g N)	6.12[a4]	6.03[a]	5.60[b]
Hunter color L	74.0[a]	76.5[b]	73.9[a]
Hunter color a	0.48[a]	-1.04[b]	0.17[c]
Hunter color b	11.4[a]	12.0[b]	13.8[c]
In vitro digestibility (%)	84.0[a]	84.5[a]	83.3[b]
Residual TI activity (%)	11.8[a]	11.0[a]	12.2[a]

[1]Soymilk processed by RHHTC with cold water slurrying at 154°C for 26 sec cooking time with 11 sec water contact time.

[2]Soymilk processed by RHHTC with hot water slurrying at 154°C for 20 sec cooking time with 11 sec water contact time.

[3]Soymilk processed by the traditional method at 97 - 100°C for 60 min.

[4]Letters not in common denote significant difference at the 5% level.

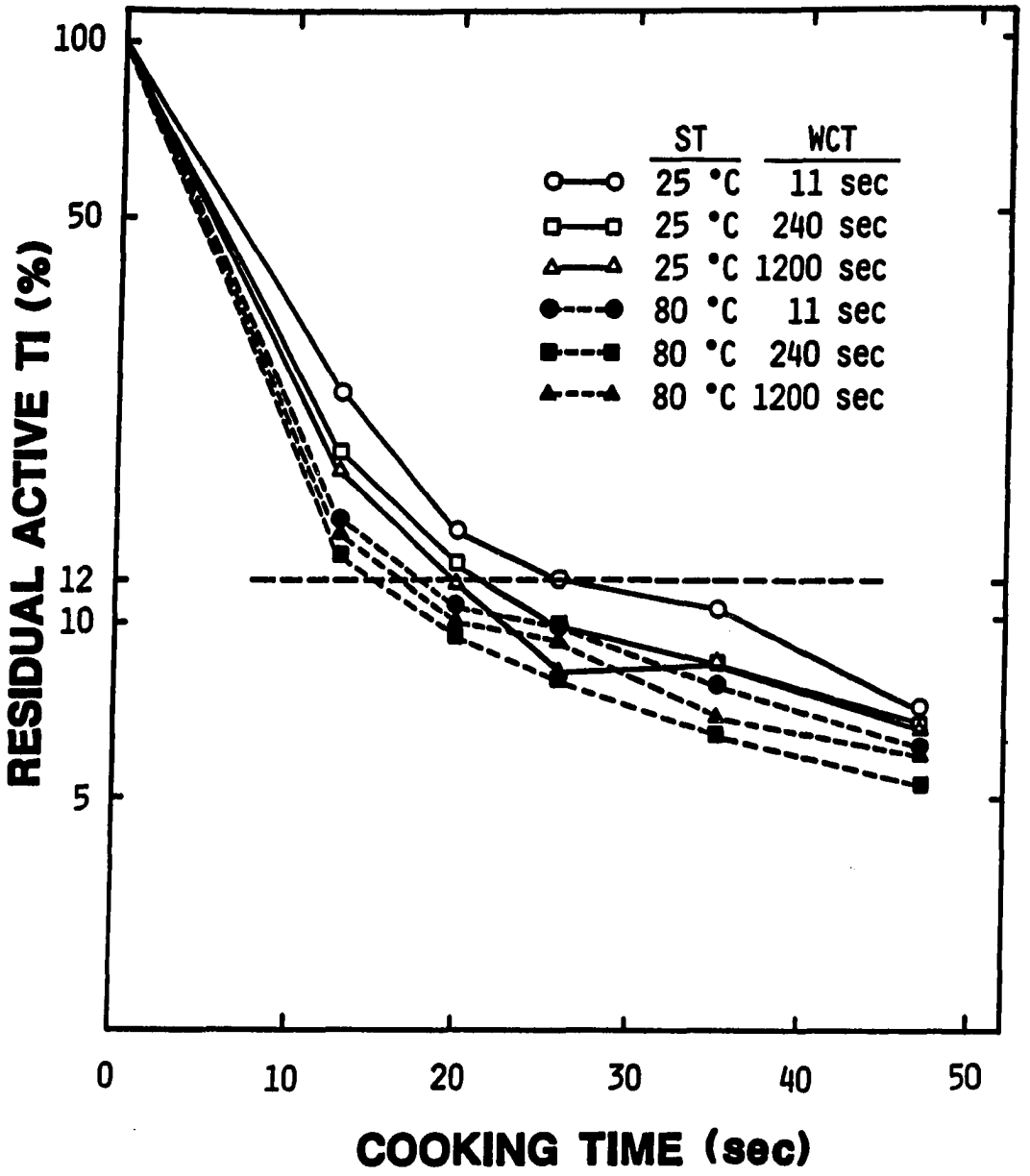

Figure 1. Effect of RHHTC on residual trypsin inhibitor of soymilk processed at 154°C for different times (ST denotes slurrying temperature, WCT denotes water contact time and horizontal dotted line indicates 12% residual TI activity; LSD was 1.58)

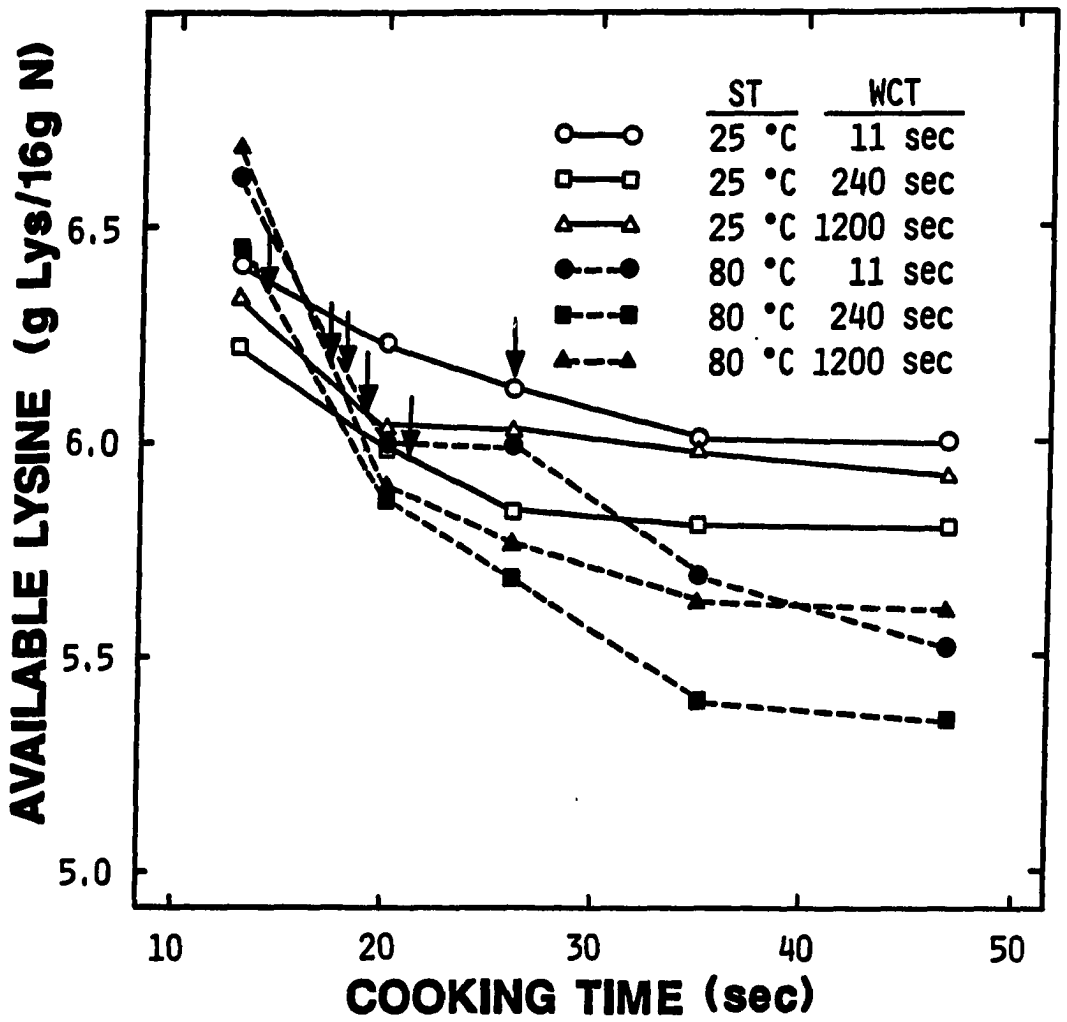

Figure 2. Effect of RHHTC on available lysine content of soymilk processed at 154°C (ST denotes slurrying temperature, WCT denotes water contact time and arrows indicate 12% residual TI activity; LSD was 0.30)

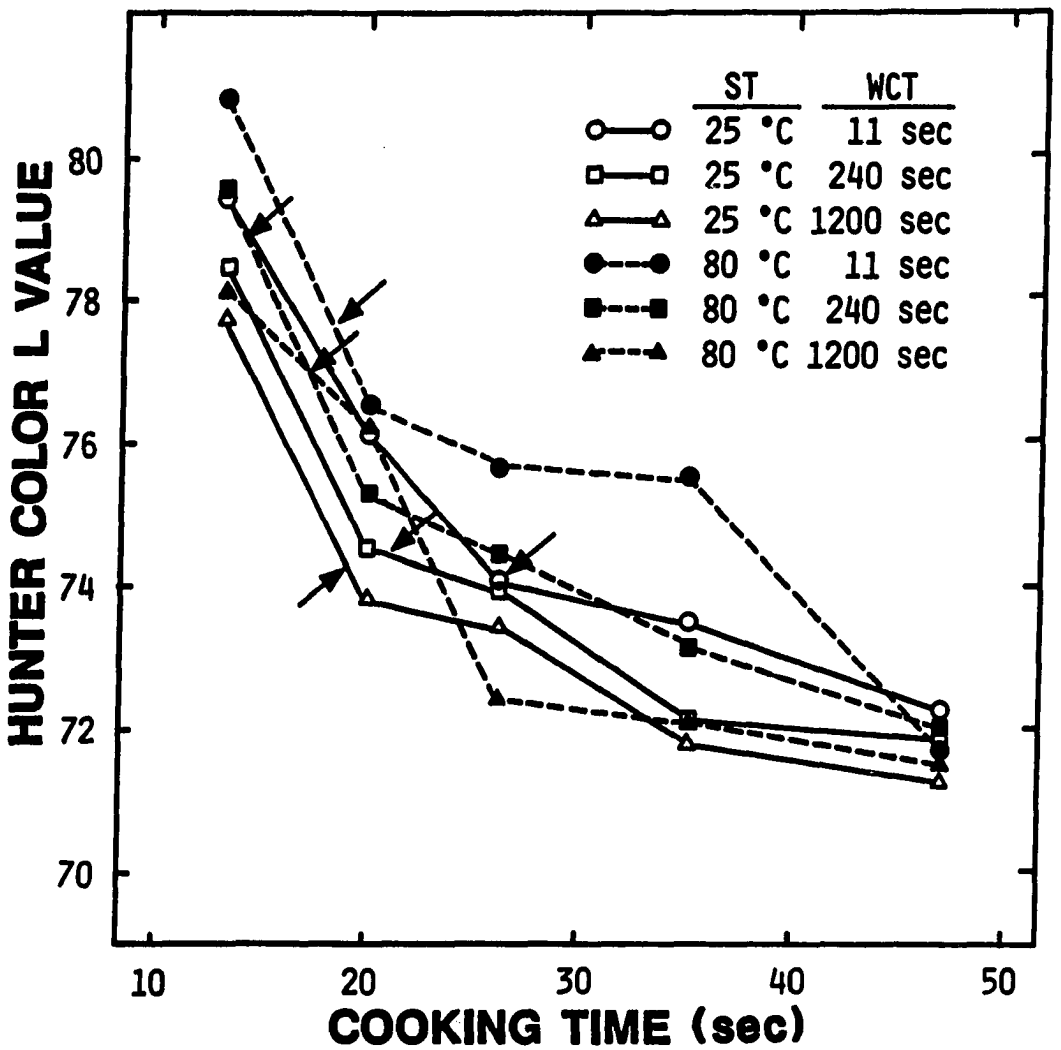

Figure 3. Effect of RHHTC on Hunter L value of soymilk processed at 154°C (ST denotes slurrying temperature, WCT denotes water contact time and arrows indicate 12% residual TI activity; LSD was 1.06)

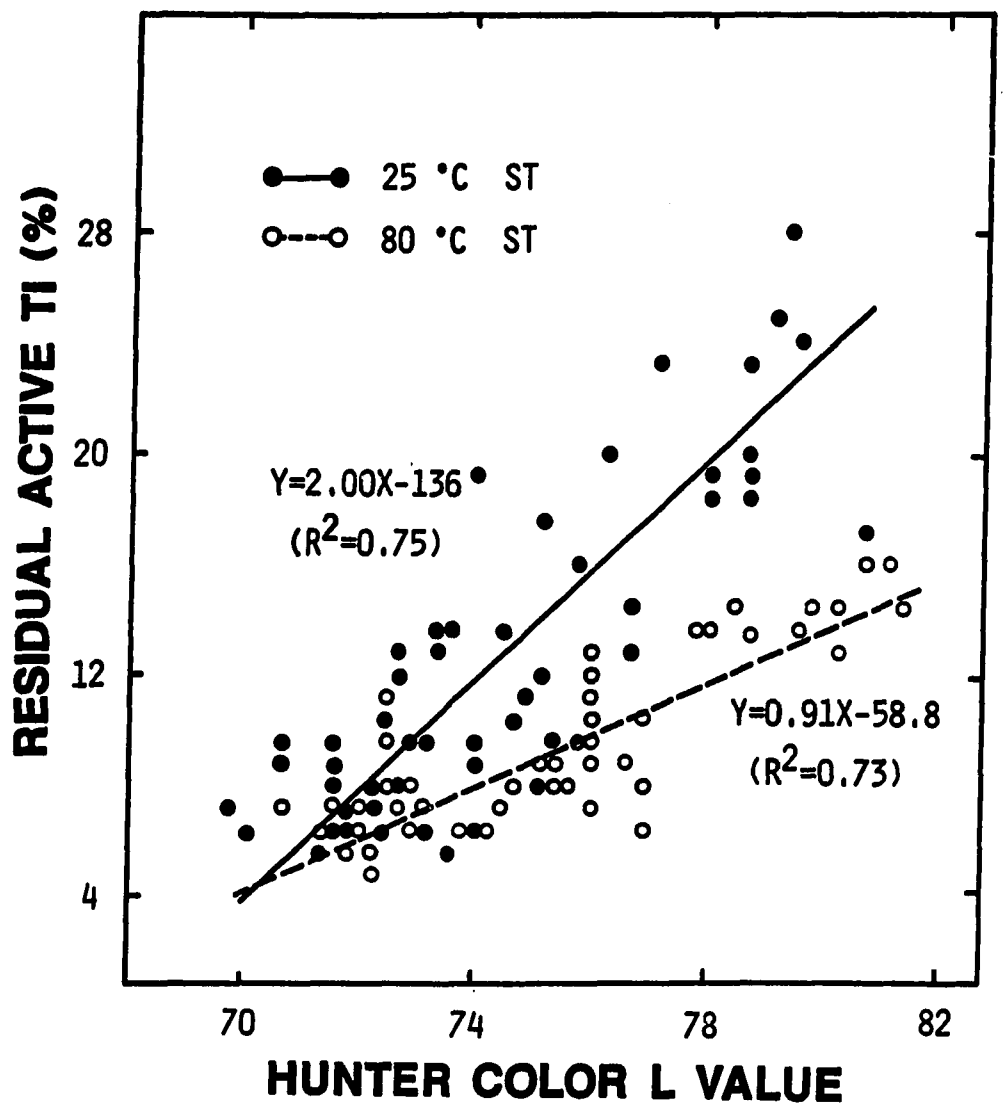

Figure 4. Relationship between residual trypsin inhibitor level and Hunter L value in RHHTC soymilk processed at 154°C for different times (ST denotes slurrying temperature)

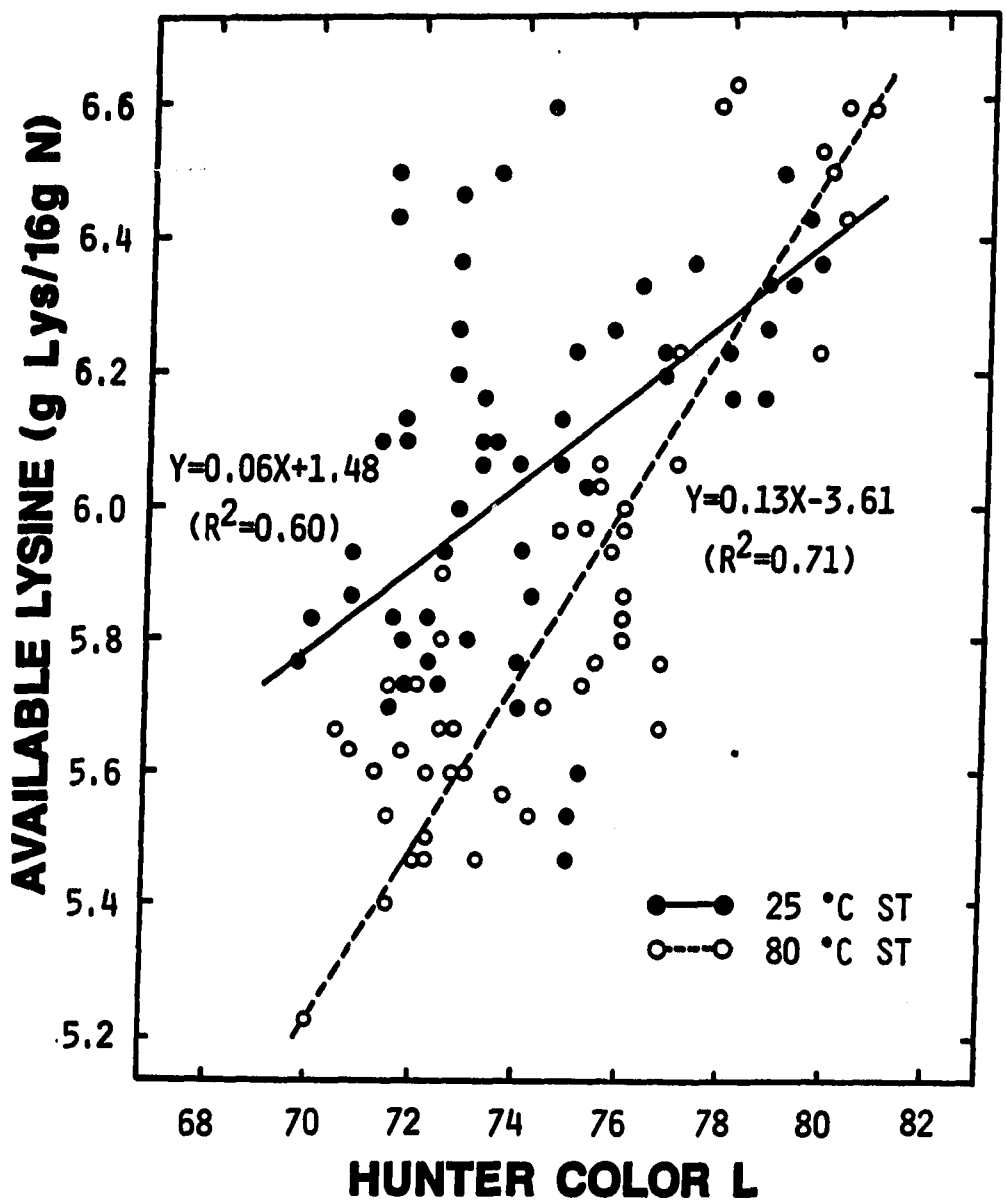

Figure 5. Relationship between available lysine content and Hunter L value in RHHTC soymilk processed at 154°C (ST denotes slurrying temperature)

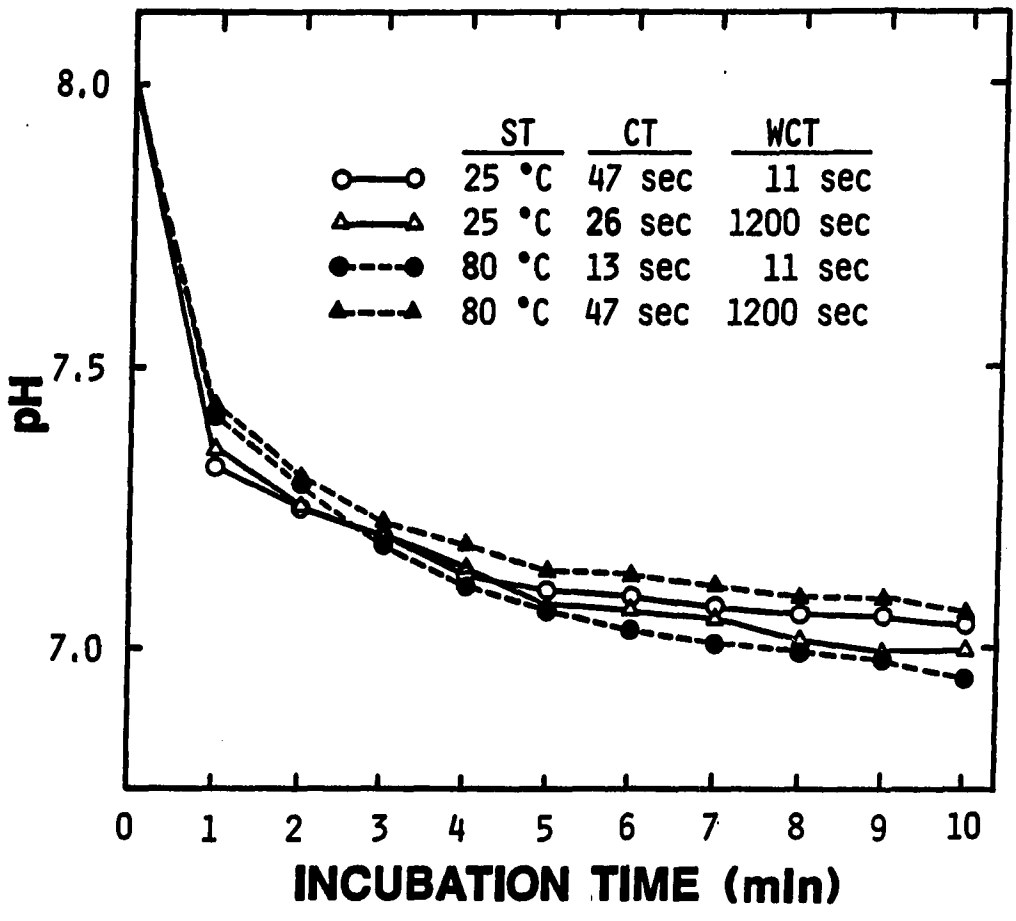

Figure 6. Hydrolysis of RHHTC soymilk by multienzymes (ST denotes slurrying temperature, CT denotes cooking time and WCT denotes water contact time)

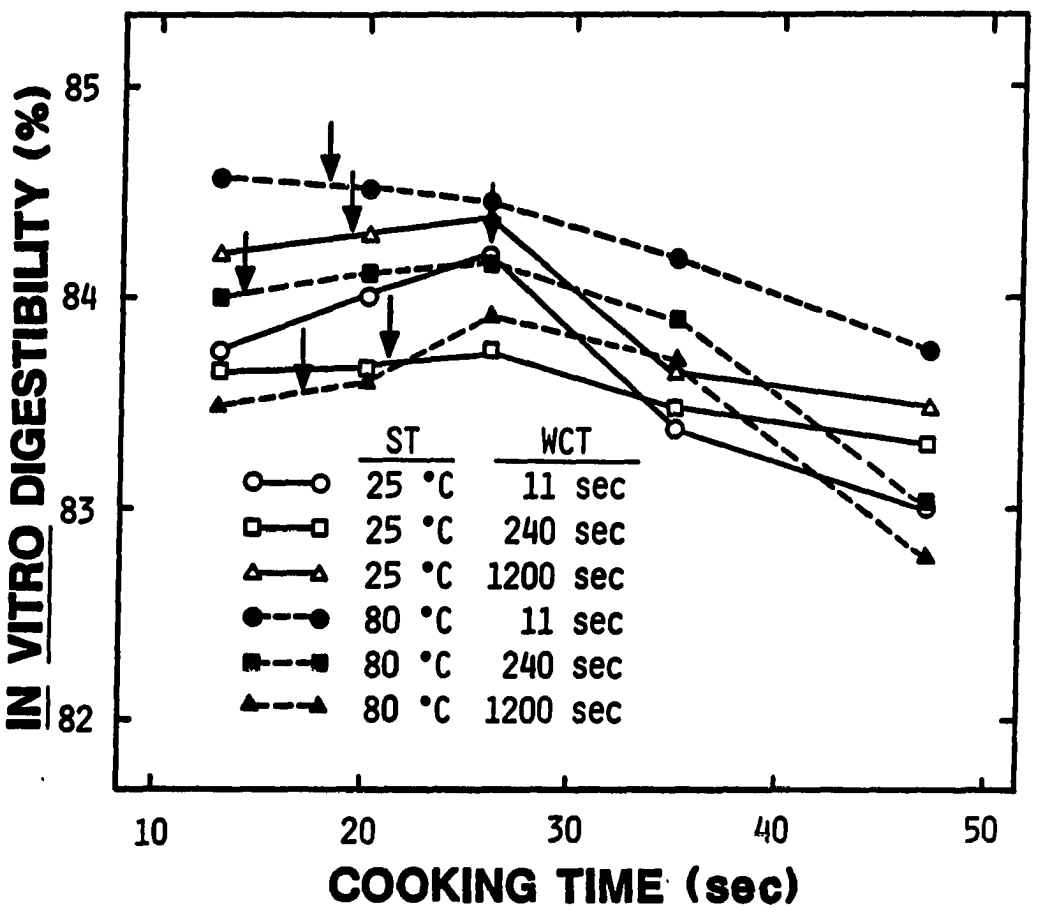

Figure 7. Effect of RHHTC on in vitro digestibility of soymilk processed at 154°C (ST denotes slurrying temperature, WCT denotes water contact time and arrows indicate 12% residual TI activity; LSD was 1.06)

PART IV. EFFECT OF PROCESSING CONDITIONS ON FLAVOR CHARACTERISTICS OF SOYMILK PROCESSED BY RAPID-HYDRATION HYDROTHERMAL COOKING (RHHTC)

ABSTRACT

Off-flavor development in RHHTC soymilk was studied and compared with that of traditional soymilk. RHHTC soymilk was prepared by employing either cold (25°C) or hot (80°C) water slurrying of soy flours prior to cooking, five cooking times (13, 20, 26, 35 and 47 sec) at 154°C and three water contact times (11, 240 and 1,200 sec). Longer cooking times reduced TBA-reactive compounds and hexanal contents when using cold water slurrying. This was attributed to degrade to non-TBA reactive compounds, volatilize to gas phase in cooker, or become bound to protein. RHHTC processing employing either cold or hot water slurrying was superior in producing soymilk with low levels of oxidation. TBA values and hexanal contents in RHHTC soymilk were much less than those of traditional soymilk. However, it was not feasible to eliminate off-flavor development due to lipoxygenase by mere rapid slurrying prior to steam injection. Hot water slurrying produced soymilk with much lower TBA values and hexanal contents than rapid slurrying in cold water.

INTRODUCTION

Polyunsaturated fatty acids and lipolytic enzymes in soybeans are presumed to be responsible for development of off-flavors at various stages of food processing. Beany and raw flavor has been one of the major limiting factors to increased utilization of soy products in human foods (Hammonds and Call, 1972; Rackis et al., 1979). The undesirable flavors have been characterized as green, beany, painty, grassy and bitter (Wolf, 1975; Rackis et al., 1979). The compounds causing these volatile and nonvolatile off-flavors include carbonyls, alcohols, furans, hydroxy fatty acids, oxidized lecithin and some phenolic compounds (Wolf, 1975; Rackis et al., 1979; Sessa, 1979). These off-flavors are largely derived from the oxidation of lipids. Lipoxygenase-catalyzed oxidation involved causing a wide range of oxidation products including ethyl vinyl ketones, n-hexanal, 3-cis-hexenal and n-pentyl furan, etc. Lipoxygenase enzymes attack fatty acids with cis, cis-1,4-pentadiene structures. Unsaturation at ω-6 (e.g., 12 position in C_{18} acids) has been reported to be essential for lipoxygenase activity of soybeans. Attempts to control these problems can be alleviated by physical or chemical and biochemical treatments. A number of methods to prevent, remove or mask off-flavors in soy protein have been developed (Table 1). These methods are not very compatible with the preparation of highly functional and nutritional soy proteins because they require conditions that extremely denature and alter functional properties.

Wilkens et al. (1967) found that the off-flavors of soymilk were not

present in dry soybeans but were formed during processing. Grinding the beans in boiling water prevented the formation of strong beany flavors by inactivating lipoxygenase. Mustakas et al. (1969) showed that peroxide values and rancid odors developed rapidly upon soaking of soybeans. Johnson (1978) showed that lipoxygenase activity was extremely rapid in slurrying soy flour in water. Therefore, he recommended rapid hydration techniques to minimize off-flavor development in soybean flour slurries for RHHTC processing. Nelson et al. (1971) concluded that lipoxygenase-catalyzed oxidation takes place instantaneously whenever soybeans are ground in water at temperatures below 80°C. In the Illinois Process blanching of soaked beans prior to grinding was found to prevent formation of oxidized flavors and resulted in an acceptable flavored product (Nelson et al., 1976). Mustakas et al. (1969) showed that lipid hydroperoxides formed during tempering of raw soybeans (8% H_2O), cracking soybeans (10% H_2O) and subsequent steam tempering to 12% moisture prior to flaking.

Potentially reactive lipid hydroperoxides and their secondary degradation products are capable of damaging protein and amino acids (Pattee et al., 1982). Secondary products of hydroperoxide decomposition (particularly aldehydes) readily react with and damage amino acids. Malon(di)aldehyde, which can cross-link proteins via Schiff's base formation, appears to be important among the aldehydes.

One molecule of malonaldehyde condenses with two molecules of thiobarbituric acid (TBA) to form a chromagen which can be quantitated by measuring the absorbance at 532 nm. This is the basis for the TBA test.

The TBA test is one of the more commonly used chemical methods for the detection of lipid oxidation (Gray, 1978). The TBA test may be performed in two ways, either directly on a food product followed by extraction of the colored pigment (King, 1962; Vyncke, 1975) or on steam distillate of the food (Tarladgis et al., 1960).

One major area of research into off-flavor problems has centered on the isolation and identification of reaction products from lipoxygenase activity. A considerable number of studies on the volatile compounds from raw soybean, soybean flour and soybean oil have been conducted; however, only a few studies have dealt with soymilk. Mattick and Hand (1969) isolated and identified ethyl vinyl ketone in soymilk by using headspace techniques and gas chromatography/mass spectrometry. They also indicated that ethyl vinyl ketone was only part of the total green beany flavors but its flavor intensity was stronger than other volatiles. Wilkins and Lin (1970) observed over 50 volatile compounds in beany-flavored soymilk by employing gas chromatographic and mass spectral techniques. The major components were hexanal, hexanol, hexenal, 1-pentin-3-ol, 1-octen-3-ol, ethyl vinyl ketone and 2-pentyl furan. Hexanal comprised about 25% of the volatiles in the sample. Hexanal is believed to be one of the major compounds contributing to the disagreeable aroma of soymilk.

Research Objectives

The objective of this study was to identify RHHTC processing conditions which minimize lipid oxidation. The effects of slurrying

conditions (temperature and time) and cooking time at 154°C on the development of off-flavors and off-aromas in soymilk were determined.

MATERIALS AND METHODS

Characterization of Soybeans

Seed-grade Corsoy 79 soybeans grown at Ames, IA during 1985 were used in this study. Characteristics of the soybean have already been discussed (Part II).

Soy Flour Slurry Preparation

Corsoy 79 soybeans were ground in a Fitz-mill (Model D, Fitzpatrick Co., Elmhurst, IL) through a 32-mesh screen. The soy flours were slurried in tap water (25 and 80°C) at 17 parts water to 3 parts soy flours. The soy flours were continuously fed to a vibroreactor (Model JM 14/E/3, Cherry-Burrell Corporation, Cedar Rapids, IA) at a rate of 120 to 130 g/min. Either cold or hot water was supplied from a steam-jacketed tank through a pump into the vibroreactor. A variable-area flowmeter (Cole-Parmer Instrument Co., Chicago, IL) was used to control the flow rate between 680 to 740 ml/min. Complete mixing of soy flour with water took only 2 sec in the vibroreactor.

Soymilk Processing

A steam injection cooker was used to process soy flour slurries by rapid-hydration hydrothermal cooking (RHHTC) at 154°C. A holding tube 10.76 m (35.3 ft) in length gave average cooking times of 47 sec. Shorter cooking times were achieved by shortening the holding tube length to 2.53 m (8.3 ft), 3.75 m (12.3 ft), 5.58 m (18.3 ft) and 7.71 m

(25.3 ft) which gave the cooking times of 13, 20, 26 and 35 sec, respectively. Water contact times were 11, 240 and 1,200 sec for both cold and hot water slurrying with RHHTC processing. Without a surge tank between the Moyno pump and the vibroreactor the slurry time was less than 11 sec. This was the shortest water contact time deemed practical. Longer holding times were achieved by use of a surge tank to hold the slurry. In hot water slurrying, the vibroreactor, Moyno pump, surge tank and pipes were preheated to more than 90°C by using heating tapes (Fisher Scientific Co., Itasca, IL) and recycling water through the system. The temperatures during slurrying was intensely monitored and always exceeded 80°C.

A control sample was prepared by cooking in the conventional manner in a steam-jacketed kettle (Lee Metal Product Co, Inc., Philipsburg, PA). Whole soybeans were soaked overnight at room temperature with 3 parts water to 1 part soybeans. The soaked beans were drained and rinsed with tap water two times, and then 8 parts of water was added, and the mass was ground in the vibroreactor in two passes. The raw slurry was allowed to stand for 20 min with occasional stirring and then cooked for 60 min at 97 - 100°C. Cooking time was measured when the temperature reached at 97°C. The slurry was continuously agitated during cooking. After completion of the cooking, the sample was cooled in an ice bath and was stored overnight at 5°C. The cooked slurry was adjusted to 10% solids and centrifuged at 2,500 rpm in a refrigerated centrifuge generating 1,050 x g for 5 min at 5°C. The soymilk was carefully decanted from the undispersed residue.

Detection of Lipid Oxidation

The procedure of King (1962) for measuring TBA (thiobarbituric acid) values in bovine milk was modified and adapted to measure TBA value in soymilk as described by Johnson (1978). A 34-ml sample of soymilk was pipetted into a 125 ml Erlenmeyer flask fitted with a stopper and warmed to 25°C. A 10-ml aliquot of trichloroacetic acid (1 g TCA per ml) was added and followed by 4.0 ml 95% ethanol. The sample was vigorously shaken for 15 sec. After 60 min, the sample was filtered through No. 42 Whatman filter paper. To each of four test tubes, 1.0, 2.0, 4.0 and 4.0 ml of filtrate were transferred and diluted with diluting solution to bring the sample volume to 8.0 ml. The diluting solution was composed of 5 parts TCA solution, 10 parts 95% ethanol and 85 parts distilled water. A 2-ml aliquot of TBA reagent, 1.4 g 2-thiobarbituric acid (Sigma Chemical Company, St Louis, MO) in 100 ml of 95% ethanol were added to the first three test tubes. A 2-ml aliquot of 95% ethanol was added to the latter test tube serving as a blank. All test tubes were stoppered and placed in 60°C water bath for 60 min. After cooling, the absorbance was read against the blank at 532 nm using a Gilford Spectrometer 250 (Gilford Instrument Laboratories Inc., Oberlin, OH). TBA values were determined using a standard curve of malonaldehyde tetraethylacetal (Sigma Chemical Company, St. Louis, MO) (Figure 1). TBA values were reported in μg malonaldehyde/ml soymilk.

Analysis of Headspace Volatiles

Fifty milliliters of soymilk were placed in a 100-ml bottle. The internal standard, 0.5 ml of 4-heptanone, was added and the sample was mixed. The internal standard was prepared daily by adding 50 µl of 4-heptanone (Aldrich Chemical Company, Inc., Milwaukee, WS) to a 1-L volumetric flask filled about three-fourths with deionized distilled water. After mixing, the flask was made up to volume with deionized distilled water. The sample bottle was sealed with a septum secured by an aluminum cap. The bottles warmed to 37°C in an air oven for 3 hrs. After the 3 hrs a 10-ml gastight syringe (Hamilton Company, Reno, NV) was used to inject 1 cc headspace into a Varian Model 3500 gas chromatograph (Varian Associates, Inc, Walnut Creek, CA). The volatiles were eluted onto a Durabond DB-5 fused silica capillary column (30 m x 0.32 mm, 1 micron film thickness, J & W Scientific, Rancho Cordova, CA) in the split mode (20:1). A column flow rate of 1.5 ml/min, a hydrogen (makeup gas) flow rate of 28.5 ml/min, a detector air flow of 300 ml/min and a detector nitrogen flow rate of 30 ml/min were used. Column temperature was programmed between 40°C (hold 0 min) and 125°C (hold 5 min) at 5°C/min. The syringe was flushed into a Hamilton syringe cleaner (Hamilton Co., Reno, NV) and prewarmed to 37°C. A cotton glove was used to maintain the temperature of the sample bottle and the syringe when injecting. Volatile analyses were performed in duplicate.

Statistical Analysis

Data were analyzed by using a Statistical Analysis System (SAS, 1984) program package. The General Linear Models (GLM) procedure was run to determine the main and interaction effects. Significant differences among treatment means were determined by Duncan's multiple range test or the least significant difference (LSD) procedure. Probability levels of $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

TBA Values

TBA values were used to quantify the extent of lipoxygenase-catalyzed oxidation in soymilk treatments. The effects of water contact time and temperature upon TBA value were determined (Figure 2). TBA values decreased with decreasing water contact time. Nelson et al. (1971) hypothesized that the beany flavor is not present in the original bean but when cell tissue is disrupted in the presence of moisture highly objectionable flavors develop. Moreover, Johnson (1978) studied the effect of hydration times upon lipoxygenase-catalyzed oxidation of 20% raw soy flour slurries. His results showed that TBA value almost doubled after 1 min of slurrying and increased six times after 15 min of slurrying.

Figure 2 in this study indicates that rapid slurrying (11 sec) with cold water was not adequate in reducing lipoxygenase activity. TBA values were 1.32, 1.43 and 1.68 μg malonaldehyde/ml soymilk for 11, 240 and 1,200 sec of water contact times, respectively. The TBA value at 11 sec of water contact time was 80% of that at 1,200 sec of water contact time (13 sec of cooking). However, TBA values of RHHTC soymilks processed with 11 sec of water contact time were generally lower than that of traditional soymilk (1.84 μg malonaldehyde/ml soymilk). TBA values also decreased with increasing cooking time as shown in Figure 2. RHHTC soymilks which have 88% reduction in TI are indicated by arrows. Presumably, TBA reactive-compounds produced during slurrying degrade to

non-TBA reactive compounds, volatilize to gas phase in cooker or become bound to protein. One of most important TBA-reactive compounds is malonaldehyde which arises from decomposition of hydroperoxides produced during the oxidation of polyunsaturated fatty acids (Dahle et al., 1962). Malonaldehyde may react with amino groups of protein, particularly the ϵ-amino group of lysine, through formation of Schiff's base adducts. Nonenzymatic browning results from this reaction and leads to formation of melanoidin pigments. Heating, pH and hydration conditions of are important factors in this reaction (Adrian, 1974).

Indeed, hot water slurrying was very effective in reducing lipoxygenase activity as shown in Figure 2. TBA values were much lower compared with those in cold water slurrying (25°C), especially at shorter cooking times. Water contact time was not as important in hot water slurrying as in cold water slurrying. TBA values in soymilks with different contact times in hot water were not significantly different at each cooking time except at 13 sec of cooking (Table 2).

Headspace Volatiles

The equilibra of the volatiles between the gas and the liquid phase were studied. As shown in Figure 3, total volatiles increased up to 4 hr of incubation. Peak areas for 4-heptanone were relatively constant; however, those for hexanal gradually decreased. Area ratios of hexanal to 4-heptanone did not change during the first 3 hrs of incubation. Thereafter, the ratio decreased rapidly. This rapid decrease was due to the decrease in hexanal content. Hexanal may be binding to soymilk

protein during incubation. A 3-hr period was judged to be optimum for headspace equilibration in soymilk.

Figure 4 shows a typical gas chromatograph (GC) profile of headspace volatiles obtained from soymilk processed by RHHTC processing. Eight peaks were detected but no attempt was made to identify any except hexanal. Identification was based on the relative retention time of a hexanal standard to the internal standard.

In cold water slurrying (25°C), the hexanal content decreased with increasing of cooking time (Figure 5). Hexanal contents behaved similarly to TBA-reactive compounds. Longer water contact times led to higher hexanal contents.

Hexanal contents decreased as cooking time increased. However, in hot water slurrying (80°C), water contact and cooking times were not important. Water contact times had no significant effect on hexanal content (Table 4). Modest levels of hexanal were present in soymilk processed by hot water slurrying with RHHTC processing. This suggests that the presence of some preformed lipid oxidation products in raw soybeans. Rackis et al. (1972) have shown that the TBA value of raw soybeans ranged between 1.10 and 1.40 μg malonaldehyde/g soybeans during maturation. Further studies are needed to determine whether this level of hexanal adversely affects flavor.

Relationship Between TBA Value and Headspace Hexanal in RHHTC Soymilk

There was a positive correlation between TBA value and headspace hexanal content in soymilk processed by cold-water slurrying with RHHTC

(Figure 6). There was a fairly good linear relationship in which the regression coefficient was 0.77.

Comparison of RHHTC Soymilk with Traditional Soymilk

The TBA value and hexanal content of traditional soymilk were compared with those of optimally cooked RHHTC soymilk (Table 4). RHHTC processing employing either cold or hot water slurrying was superior in producing soymilk with low levels of oxidation. TBA and hexanal contents in RHHTC soymilk were much less than those of traditional soymilk. RHHTC soymilk with hot water slurrying had the lowest level of oxidative degradation.

CONCLUSIONS

TBA values of RHHTC soymilk slurried at 25°C decreased with increased the cooking time. Longer water contact times gave higher TBA values. It does not appear to be feasible to eliminate off-flavor development by mere rapid slurrying. However, hot water slurrying minimized the development of TBA reactive compounds. Rapid slurrying in even hot water is desirable.

The hexanal content in soymilk processed at 154°C exhibited similar patterns to those of TBA values. Hexanal contents were much higher in cold water slurrying than in hot water slurrying.

Longer cooking times reduced TBA-reactive compounds and hexanal contents when using cold water slurrying. This was attributed to degrade to non-TBA reactive compounds, volatilize to gas phase in cooker, or become bound to protein.

RHHTC soymilk employing cold or hot water slurrying resulted in soymilk with lower TBA values and hexanal content than traditional soymilk. To minimize the off-flavor development in RHHTC soymilk, rapid slurrying with hot water (> 80°C) was recommended.

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Table 1. Methods of off-flavor control and removal

Method	Reference
Addition of antioxidants	Buck (1981)
Alkaline soaking	Badenhop and Hackler (1970)
Blanching	Nelson et al. (1976)
Dry heat or moist heat	Mustakas et al. (1969)
Enzyme application	Arai et al. (1970)
Grinding with aqueous ethanol with heat	Borhan and Snyder (1979)
Grinding with hydrogen peroxide	Paulsen (1963)
Grinding in hot water	Wilkens et al. (1967)
Grinding with solvent azeotrope	Eldridge et al. (1971)
Grinding at acidic pH	Kon et al. (1970)
Inhibition by acetylenic compounds	Blain and Shearer (1965)
Masking	McDaniel and Chan (1988)

Table 2. Effect of cooking time on TBA value (μg malonaldehyde/ml) of RHHTC soymilk processed with hot water slurrying of soy flour[1]

Cooking time[2] (sec)	Water contact time (sec)		
	11	240	1,200
13	0.20[a]	0.26[a]	0.27[a]
20	0.25[b]	0.25[a]	0.33[b]
26	0.28[b]	0.32[a]	0.37[b]
35	0.26[b]	0.29[ab]	0.35[b]
47	0.274[b]	0.324[b]	0.349[b]

[1]Means underlined in the same horizontal row do not differ significantly at the 5% level. Values in the same vertical column for cooking time bearing different letters differ significantly at the 5% level.

[2]At 154°C.

Table 3. Effect of cooking time on the headspace hexanal content of RHHTC soymilk processed with hot water slurrying of soy flour[1]

Cooking time[2] (sec)	Water contact time (sec)		
	11	240	1,200
13	0.02^{ab}	0.05^{a}	0.03^{a}
20	0.05^{bc}	0.06^{a}	0.05^{a}
26	0.04^{bc}	0.04^{a}	0.07^{a}
35	0.06^{c}	0.07^{a}	0.06^{a}
47	0.00^{a}	0.03^{a}	0.03^{a}

[1]Means underlined in the same horizontal row do not differ significantly at the 5% level. Values in the same vertical column for cooking time bearing different letters differ significantly at 5% level. Hexanal content is expressed as the area peak ratio of hexanal to 4-heptanone.

[2]At 154°C.

Table 4. Comparison of flavor properties of optimally processed RHHTC soymilk with those of traditional soymilk[1]

Properties	RHHTC		Traditional[4]
	25°C[2]	80°C[3]	
TBA value (μg malonaldehyde/ml)	1.04[a]	0.25[b]	1.84[c]
Hexanal content[5]	0.45[a]	0.05[b]	0.85[c]

[1]Letters not in common denote significant differences at the 5% level.

[2]Soymilk processed by RHHTC with cold water (25°C) slurrying at 154°C for 26 sec cooking time with 11 sec of water contact time.

[3]Soymilk processed by RHHTC with cold water (80°C) slurrying at 154°C for 20 sec cooking time with 11 sec of water contact time.

[4]Soymilk processed by the traditional method at 97 - 100°C for 60 min.

[5]Hexanal content is the peak area ratio of hexanal to the 4-heptanone standard in headspace volatiles above soymilk.

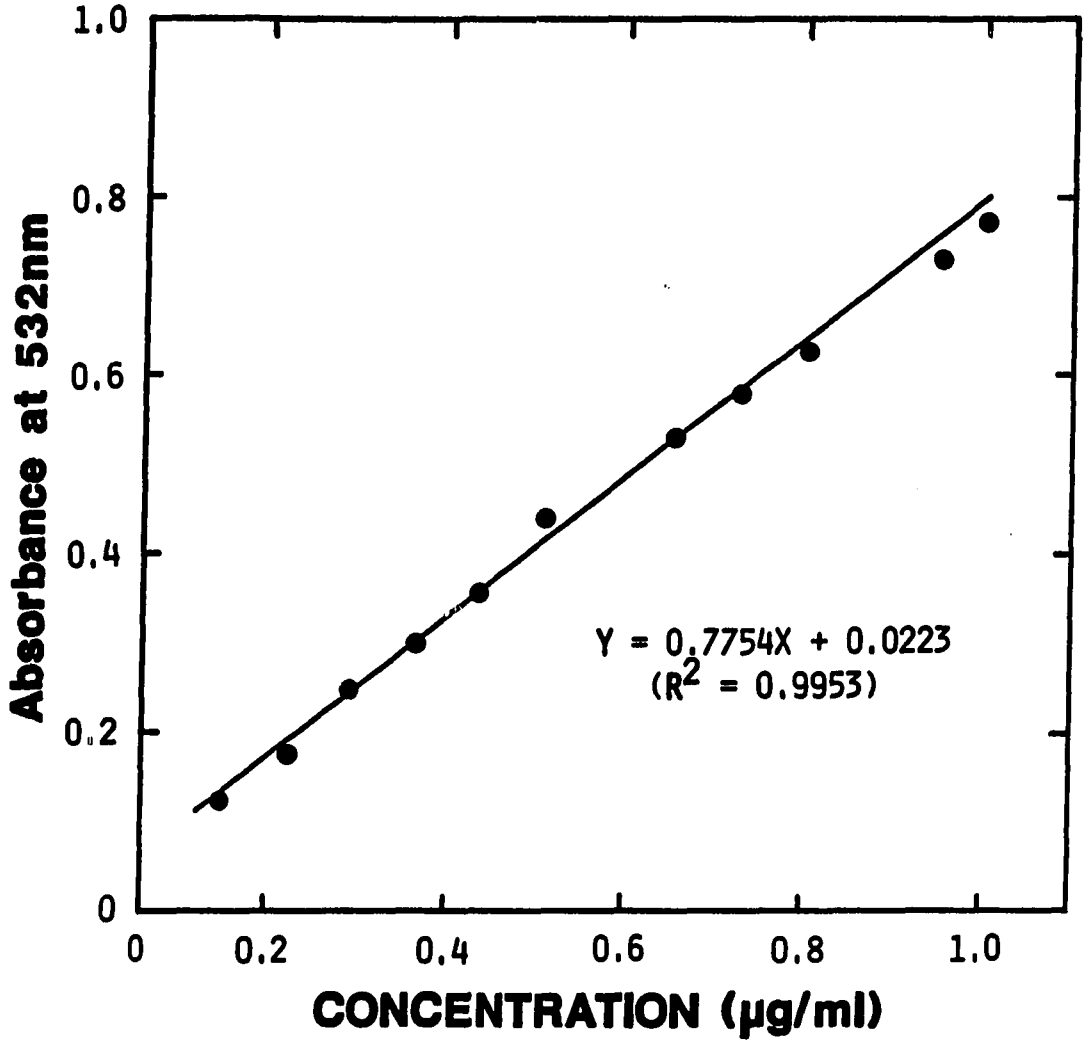

Figure 1. Standard curve for measuring malonaldehyde concentration

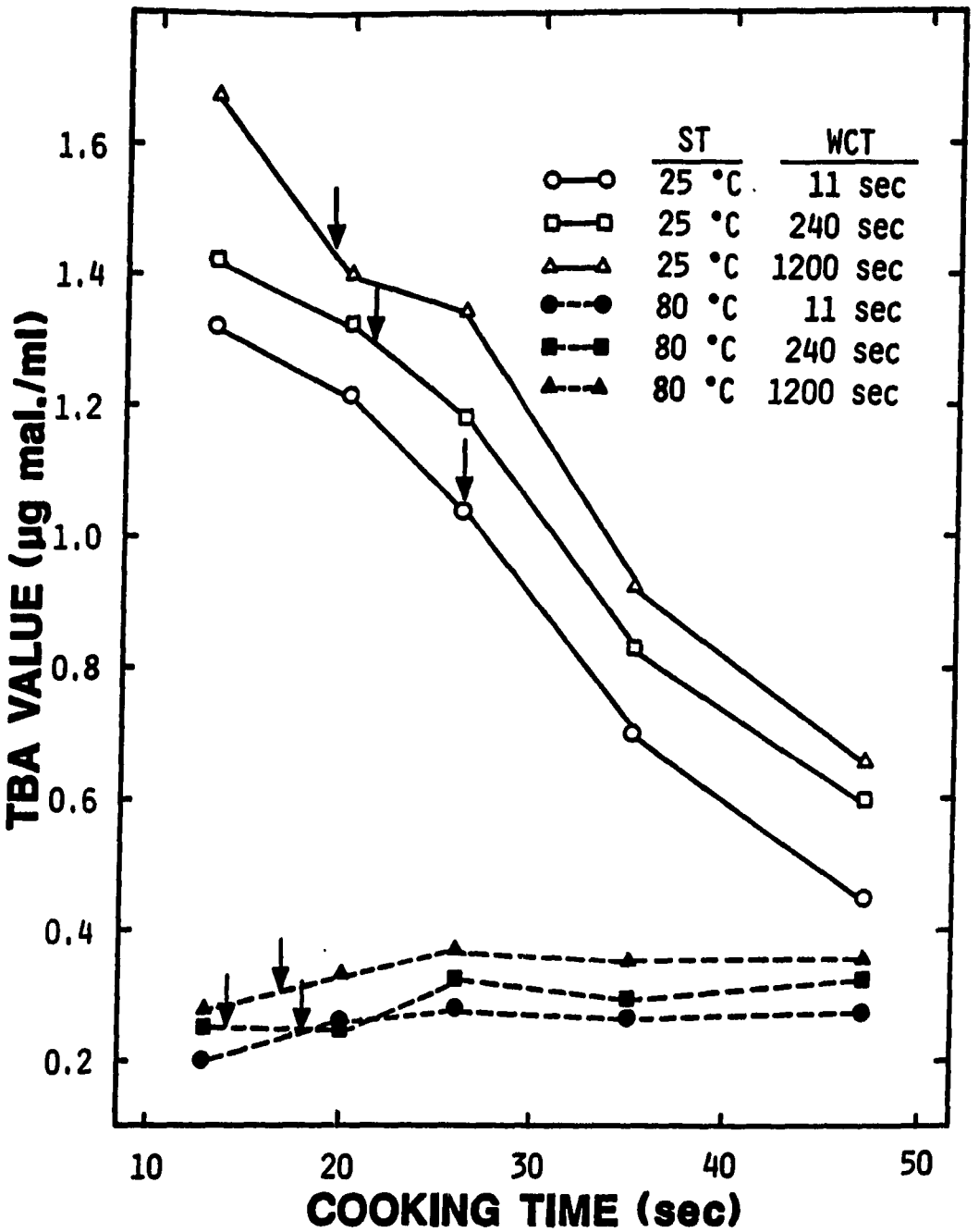

Figure 2. Effect of soy flour slurrying conditions on TBA value of RHHTC soymilk processed at 154°C for different times (ST denotes slurrying temperature, WCT denotes water contact time and arrows indicate 12% residual TI activity; LSD was 0.07)

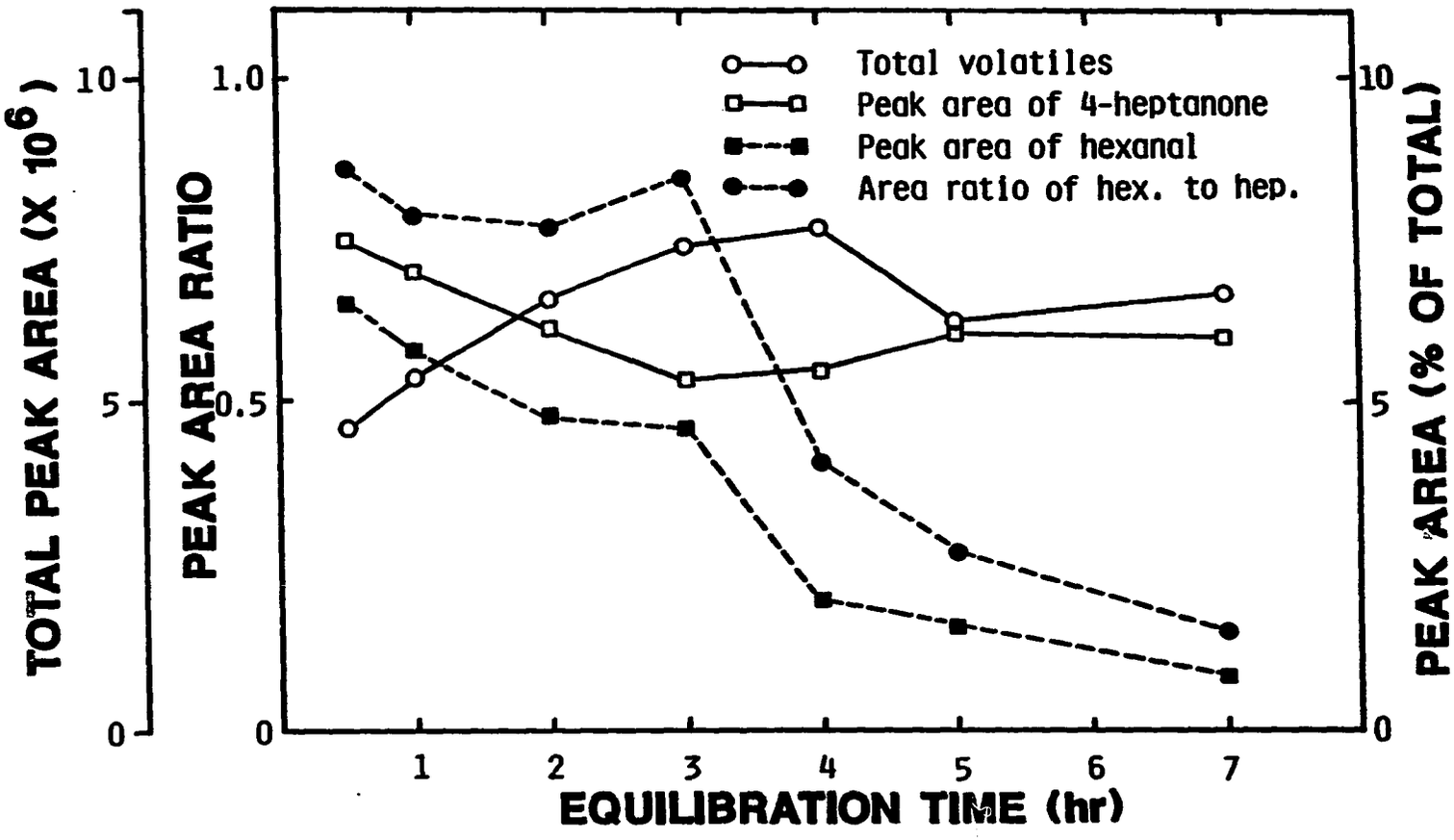

Figure 3. Effect of incubation time on concentration of total volatiles, hexanal and 4-heptanone in headspace of RHHTC soymilk (LSD was 1.59 x 10^5 for total volatiles, 3.25 for peak area for 4-heptanone, 2.56 for peak area of hexanal and 0.32 for area ratio of hexanal to 4-heptanone)

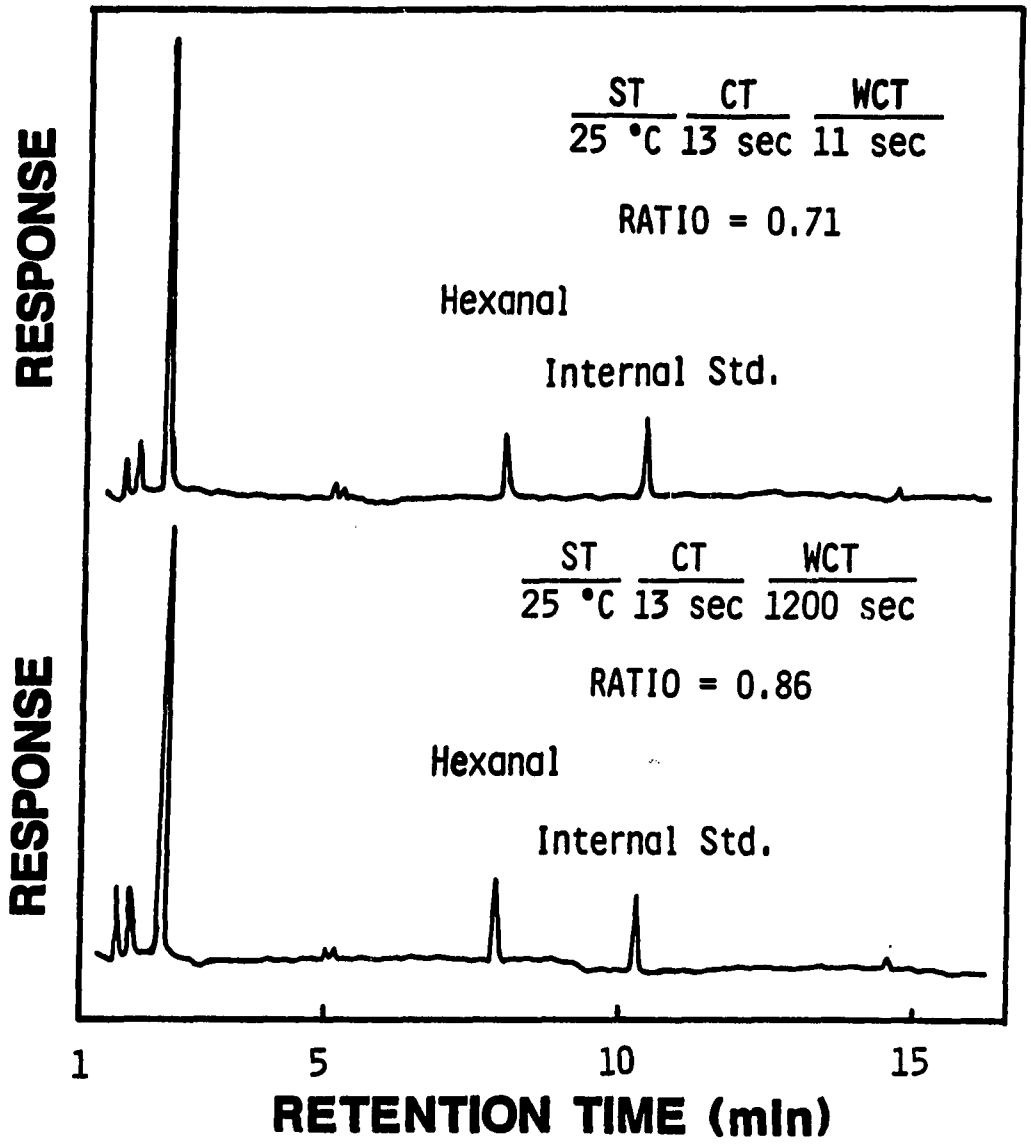

Figure 4. Gas chromatogram profile of headspace volatiles of RHHTC soymilk (ST denotes slurrying temperature, CT denotes cooking time and WCT denotes water contact time)

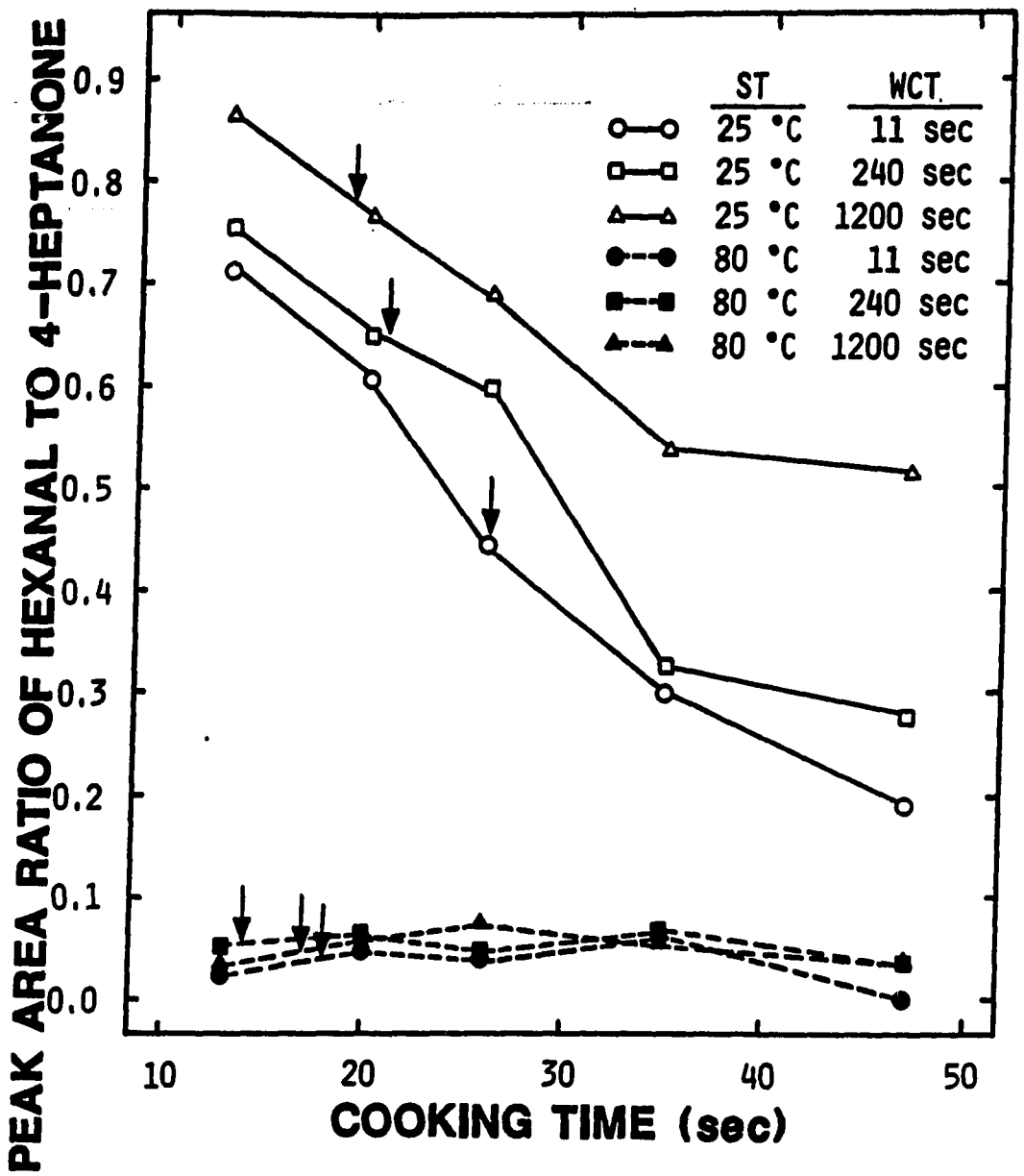

Figure 5. Effect of soy flour slurrying conditions on headspace hexanal content of RHHTC soymilk processed at 154°C for different times (ST denotes slurrying temperature, WCT denotes water contact time and arrows indicate 12% residual TI activity; LSD was 0.14)

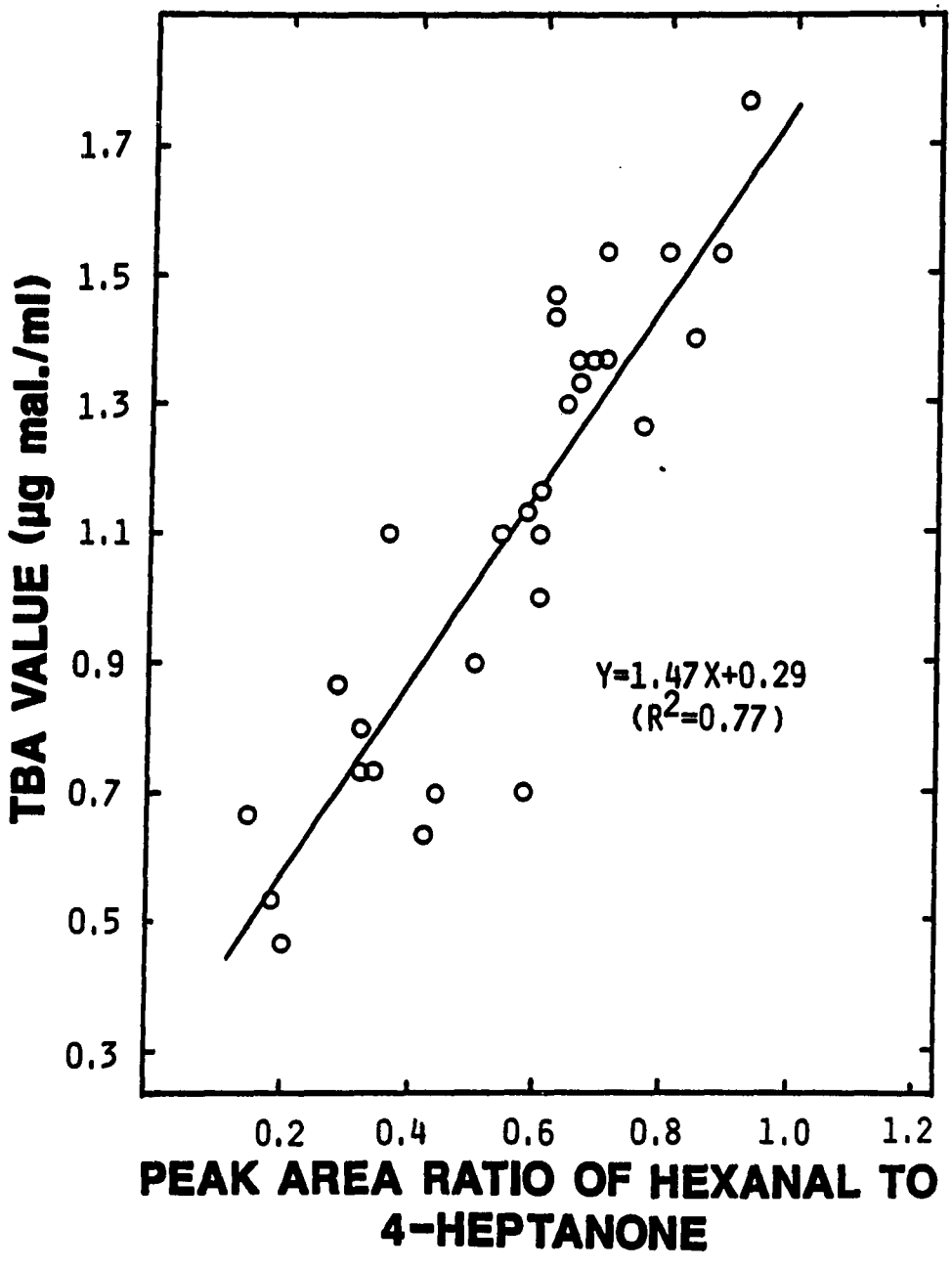

Figure 6. Relationship between TBA value and headspace hexanal content of RHHTC soymilk processed with cold water slurrying of soy flour

GENERAL SUMMARY

The continuous steam infusion process known as rapid-hydration hydrothermal cooking (RHHTC) system was explored as a means of processing aqueous extracts of soybeans. Effects of processing conditions on flavor, nutritional value and yields of soybean solids and protein were studied in an attempt to improve the quality of RHHTC soymilk.

The effects of particle size (8- and 24-mesh) and moisture content on the stability of full-fat soy flours stored for 24 hr were studied. No increase in peroxide value, conjugated dienes and hexanal contents were observed when moisture contents were 10.8% or less. At 14.9% moisture and above, lipid oxidation increased proportionally with increased moisture content and storage time. Free fatty acid contents increased slightly (0.06% maximum) at all moisture contents over the 24-hr storage period. Finer grinding (8 vs 24 mesh) only slightly increased oxidative and hydrolytic rancidities with greater effects observed at 14.9% moisture content and above. Hexanal content in soy flour volatiles was highly correlated to peroxide value and conjugated dienes in extracted oil having correlation coefficients of 0.81 and 0.74, respectively. These findings indicate that raw soybeans can be ground to full-fat soy flours and stored up to 24 hrs without undergoing significant lipid and flavor deterioration if the moisture content is equivalent to or less than 11%.

The solids recoveries in RHHTC soymilk showed a pattern of gradual increase to a maximum followed by a steady decline. Protein yields also

exhibited a similar pattern. Maximum yields of solids when slurrying of 25°C were obtained at 26, 26 and 20 sec of cooking time for 11, 240 and 1,200 sec of water contact time. At these conditions, the yields were 84.2, 85.0 and 82.8%, respectively. In hot water slurrying, maximum yields of solids were 80.6% for 11 sec of water contact time, 79.7% for 240 sec and 73.2% for 1,200 sec which occurred at 20, 20 and 26 sec of cooking, respectively. The recoveries of soymilk fraction, total solids and protein were higher in RHHTC processing, especially in cold water slurrying, than in the traditional method. High yield was attributed to partial denaturation (the dissociation and the unfolding of the polypeptides). This facilitates greater interaction between hydrophobic areas in protein with fat.

Rheological properties of RHHTC soymilk were mildly thixotropic which may improve the stable dispersion, enhance the shelf life and improve mouthfeel. The pattern of apparent viscosity with respect to cooking time was similar to that of yields of solids. However, low correlation was observed between them, indicating high solids RHHTC soymilk did not always have high viscosity. Viscosities of soymilks prepared with hot water slurrying were lower than those prepared with cold water slurrying. However, RHHTC soymilks were much more viscous than traditional soymilk. RHHTC soymilk showed excellent suspension stability.

Losses of available lysine increased with increasing cooking time in RHHTC. This loss was most rapid during the first 20 sec of cooking. However, these losses in available lysine in RHHTC soymilk were lower

than that observed traditional soymilk indicating that the high-temperature short-time treatment of RHHTC causes less heat damaged to protein and results in more nutritious soybean extracts.

Browning of soymilk increased with increased cooking time. Water contact time was also an important factor in browning; longer water contact times gave darker colored soymilks. Apparently, oxidation products produced during slurrying contribute to browning of soymilk.

In vitro digestibilities of RHHTC soymilks ranged from 82.3 to 84.7% compared to 83.3% for traditional soymilk. Maximum digestion occurred in soymilk processed under the same conditions which maximized solids yield and viscosity.

A cooking time of 60 min at 100°C has been reported to produce maximum nutritional quality for soymilk protein and destroy about 90% of the native TI activity. This level of inactivation in this study occurred at 19 - 26 sec and 14 - 18 sec for 25 and 80°C slurrying, respectively.

The effect of processing conditions on lipid oxidation in RHHTC soymilk was also studied. At 25°C slurrying TBA values of soymilk decreased with increasing cooking time. Apparently, TBA-reactive compounds became bound to protein or were destroyed. The longer the water contact time, the higher the TBA value. It was not feasible to sufficiently reduce lipoxygenase-catalyzed oxidation by mere rapid slurrying in cold water. However, when slurrying at 80°C, TBA values only slightly increased with increasing water contact time. Cooking time at 154°C had no effect on TBA values of soymilk when slurried at 80°C.

Rapid slurrying in hot water temperature was the preferred means of minimizing TBA values.

Hexanal contents in RHHTC soymilk exhibited identical patterns to those of TBA values. Differences in water contact time influenced hexanal content in soymilk. Rapid slurrying with hot water (> 80°C) was preferred to minimize hexanal development.

Optimum RHHTC processing conditions were found to be 18 sec of cooking time with 11 sec of water contact time and hot water slurrying. When RHHTC soymilk processed under these optimum conditions was compared to traditional soymilk the yield, nutritional properties and flavor qualities were significantly better. However, RHHTC soymilk was more viscous than traditional soymilk. Hot water (> 80°C) slurrying is preferred in RHHTC processing and it is not feasible to eliminate off-flavor development by mere rapid slurrying with cold water prior to steam infusion.

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