A comparison of processing methods on the proximate composition and physical properties of soymilk

by

Kenneth Wayne Johnson

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major: Food Technology

Iowa State University
Ames, Iowa

1976

TABLE OF CONTENTS

INTRODUCTION

Soybeans and their products have been a traditional food staple and an important source of protein and other nutrients for oriental cultures for centuries, while the use of soybeans as food in the United States has found only limited acceptance.

The major use of soybeans in the U.S. is for the production of edible soybean oil. Approximately 57% of the 2.8 billion bushel crop of soybeans for 1973 and 1974 was used for oil production, with the meal from the extracted beans used chiefly as an animal feed. The major use of the remaining portion of the crop was for export and yearly carry-over. Only about 2% of the crop was used for producing edible plant protein for human consumption (Pomeranz, 1976).

One of the simplest methods for converting soybeans to food is to grind the soybeans with water, thereby producing a product known as soymilk. While this approach has been used successfully for centuries by the Chinese, western cultures have been reluctant to accept this type of product, principally due to the various bitter and oxidized off-flavors associated with the soymilk.

Two basic approaches can be used to correct the flavor problem: removal of the volatile components that are formed during processing and are responsible for the off-flavors, or modification of the process to prevent the formation of volatile components during processing. The latter method is most often used.

Many of the processes that have been developed use heat at some stage in the processing of soymilk both to improve the flavor and eliminate antinutritional factors. However, heating can also cause changes in the soymilk such as protein denaturation and decreased yields of soymilk.

While many processes have been developed for producing soymilk, little good comparative information on the composition of soymilk as affected by these processes exists. This is due to the differing techniques and equipment used by various laboratories in the preparation of soymilk.

The purpose of this study is to compare the composition of soymilk made by different processes which vary the soaking conditions, the amount of heating, the sequence of heating and grinding, and the degree of homogenization.

LITERATURE REVIEW

Composition

The worldwide demand for protein coupled with increasing costs of animal sources of protein have created a renewed interest in the utilization of soybeans as a food source.

The unique composition of the soybean is responsible for the tremendous amount of interest and work in converting the raw soybean into edible food products. A typical analysis would give the following composition of the soybean on a dry basis:

Protein	40%
Fat	20%
Carbohydrate	35%
Ash	5%

Soybean protein

Most of the protein in soybeans is found in subcellular inclusions in the soybean cotyledon called protein bodies or aleurone grains, believed to be a reserve storage form of protein for the seed during germination and growth (Dieckert and Dieckert, 1976). An extensive study of the protein bodies by Tombs (1967) has shown them to be from 2-20 μm in diameter. They are believed to be covered by a membrane consisting of phospholipids 7.5 nm in thickness. Protein bodies isolated by Tombs from defatted soyflour using sucrose density gradient centrifugation contained approximately 83% protein. The protein bodies are believed to contain about 60-70% of the total protein in the seed.

Polyacrylamide gel electrophoresis of the protein in the protein bodies isolated by Tombs suggested only the 11S protein component was present, however disc immunoelectrophoresis studies by Catsimpoolas et al. (1968) showed at least 6 different components present. Ultracentrifuge patterns of the protein in the protein bodies support the idea that several fractions are present in the protein bodies (Wolf, 1970).

Soybean lipid

The lipid in the soybeans is present in the soybean cotyledons in storage packets known as spherosomes, about 0.2-0.5 μ in diameter. The majority of the fatty acids are unsaturated, approximately 80% according to Smith and Circle (1972), with linoleic and oleic comprising about 63% and 29%, respectively, of the unsaturated fatty acids of the soybean with the remainder as linolenic acid.

Extraction of crushed soybeans also removes phospholipids along with the lipid. These phospholipids, lecithin and cephalin, are good emulsifying agents and are especially abundant in the soybean.

Carbohydrate and ash

The soybean contains a high proportion of carbohydrate, including the soluble sugars sucrose, raffinose, and stachyose with minor amounts of verbascose as well as several insoluble carbohydrates of the pectic group. The soybean hull is high in cellulosic type material.

The ash content of soybeans is high in potassium and phosphorous with lesser amounts of sodium, calcium, sulfur, and magnesium as well as several other trace minerals.

A comprehensive review of the soybean carbohydrate and ash content is available in Smith and Circle (1972).

Soymilk Utilization

One product from soybeans that has received a great deal of attention is soymilk. Soymilk, the aqueous extract of soybeans, was introduced to the Chinese over 2000 years ago by Whai Nain Tze (Piper and Morse, 1923).

Soymilk has been marketed in several developing countries recently. In Brazil, Coca Cola has developed a chocolate flavored soy beverage, "Saci." An oilseed protein drink, "Puma," was developed by Monsanto for marketing in British Guiana (Wilding, 1970). Other examples of soymilk are: Vitasoy in Hong Kong, Vitamilk in Thailand, Philsoy in the Philippines, Beanvit and Vitabean in Singapore and Malaysia and Super-D and Vegimil in South Korea (H. E. Snyder, personal communication, Iowa State University, Ames, Iowa, 1975).

The use of soymilk in the U.S. and European countries has generally been limited to proprietary types of infant foods for children who are allergic to cow's milk. In 1973, it was estimated that as many as 10% of infants in the U.S. were on soy-based formulas (Weisburg, 1974).

The advantages in using soymilk are its relatively cheap price and its high nutritional quality. Mustakas et al. (1971) estimated that with soybeans at $2.70/bu., their soymilk base could be produced at a "cost to make" of 10 cents per gallon.

Soy Flavor

Flavor is one of the major deterrents to greater acceptability and use of soy protein in foods (Cowan et al., 1973). The off-flavors associated with soy products can be divided into two basic groups: green, beany flavors and bitter off-flavors (Gossens, 1974).

The green, beany flavors are due mainly to the breakdown of fatty acids, especially linoleic and linolenic, by the enzyme lipoxygenase. Lipoxygenase catalyzes the oxidation of lipids containing cis, cis-1,4-pentadiene system to cis-transdiene hydroperoxides which decompose into a number of volatile compounds, some of which are related to the green, beany off-flavors (Rackis, 1972). Gini and Koch (1961) have shown that the decomposition of hydroperoxides is catalyzed by a soybean peroxidase system. Four isoenzymes of lipoxygenase have been shown to be present in soybeans using polyacrylamide gel electrophoresis (Guss et al., 1967). The isoenzymes differ according to their specificity for substrates at varying pH's (Christopher et al., 1970, 1972), their activation by calcium (Restrepo et al., 1973), and the types of carbonyl compounds formed (Grosch and Laskawy, 1975).

Wilkens et al. (1967) found that the off-flavors formed by lipoxygenase were not present in the dry soybean, but were formed almost instantly during processing or when soybeans were damaged.

Fujimaki (1969), using paper, thin layer, column and gas chromatography, as well as ultraviolet, infrared and mass spectrometry, identified a large number of organic components related to off-flavors in the soybean and processed products. The alcohol n-hexanol was found to be a major contributor to the green, beany flavor in both quantity and quality, with n-hexanal a lesser contributor.

Mattick and Hand (1969) isolated over 80 compounds from unheated soybeans ground with water and identified 40, mostly aldehydes, ketones and alcohols. Many of these were the same as those found by Fujimaki. Most of the compounds isolated were believed to be the result of lipoxygenase activity. Ethyl vinyl ketone was reported as having a typical beany flavor with a threshold of taste at 5 ppm in soymilk.

Anderson (1976) reports that removal of the acid-sensitive protein fraction improves flavor of soy isolates. The acid-sensitive fraction is believed to bind to the nonprotein material responsible for the green, beany flavors.

Snyder (1973) has found that a blanch time of 2 min at 100 C for soaked soybeans was sufficient to prevent development of green, beany tastes in soybeans upon grinding. This heat treatment was not sufficient to destroy growth inhibitors as shown in growth studies using young chicks with raw soybeans as a control.

The bitter off-flavors are formed mostly through the oxidative and enzymatic breakdown of proteins and amino acids. Using proteolytic enzymes to hydrolyze soy proteins, Fujimaki (1969) found most of the bitterness to be caused by diffusible bitter peptides and amino acids, particularly isoleucine, leucine, phenylalanine and valine. Leucine at the C-terminal was characteristic of almost all bitter peptides studied. Bitterness could be removed from proteolyzates by the addition of α-chymotrypsin, resulting in the formation of plasteins (Fujimaki et al., 1970). Plasteins are high molecular weight protein-like substances formed from protein hydrolyzates by the action of certain proteolytic enzymes such as α-chymotrypsin by reverse hydrolysis of the peptides.

Sessa et al. (1974, 1976) found that residual lipids in defatted soy flakes may contribute to the bitter taste upon autoxidation of unsaturated fatty acid constituents on the soybean phosphatidylcholine.

The formation of 1-octen-3-ol from lipids through autoxidation or enzymatically during the soaking of soybeans was found by Badenhop and Wilkens (1969). This compound is reported to be formed early during the germination process and possesses a mushroom, musty or earthy odor.

Arai et al. (1966) isolated 7 phenolic acids by ethanol extraction of hexane defatted soy flakes. These substances have sour, bitter, astringent and phenol-like tastes; however, Rackis et al. (1967) states that phenolic acids present may be in too low a concentration to contribute to the flavor of defatted soyflour.

The formation of 4 vinylphenol and 4 vinylguaicol from p-coumaric acid and ferulic acid, respectively, during heating of defatted soymeal have been reported as being responsible for the cooked off-flavors of soymeals (Greuell, 1974).

Flatulence

Another problem associated with the consumption of soy products is flatulence. It is generally believed to be caused by consumption of indigestible oligosaccharides, mainly stachyose and raffinose, that are decomposed anaerobically by microorganisms in the lower intestine with the production of gas (Rackis et al., 1967).

Hand (1967) reported a loss of the oligosaccharides from the soybeans during soaking that could not be accounted for in the soak water. He suggested that this could possibly be due to enzymatic activity within the bean, degrading the oligosaccharides to simple sugars.

Wagner et al. (1975) have patented a process in which a lowering of the pH of the soak water to 5.0-5.5 is used to activate enzymes within the soybean in order to optimize the degradation of the oligosaccharides during soaking, thereby removing the flatulence-causing factors from the soybean prior to processing.

Ku (1972) investigated the effect of water ratio and pH upon extraction of oligosaccharides into the soak water from soybeans. Increasing the ratio of soak water to beans increased the loss of oligosaccharides from the beans by diffusion into the soak water. Increasing the pH of the soak water with $NaHCO_3$ also increased losses of oligosaccharides but caused increased protein losses. Lo et al. (1968a) found that increasing the soak time increased the loss of solids from the beans into the soak water, from 5% after 24 h to 10% at 72 h. Analysis of the soak water showed it to be composed of 23.6% crude protein, about one-half of this was nonprotein nitrogen, and 75% carbohydrate.

Sugimoto and Van Buren (1970) have shown that oligosaccharides can be degraded in soymilk using an enzyme preparation from <u>Aspergillus saitoi</u> that contained both α-galactosidase and invertase activity.

Smiley et al. (1976) were able to degrade both the raffinose and stachyose in soymilk to glucose using a crude protein extract from *Aspergillus awamori* with both α-galactosidase and invertase activity. The use of a hollow fiber reactor prevented contamination of the soymilk with the crude extract and allowed for conservation of the enzyme preparation.

DeMan et al. (1975) analyzed samples of soymilk made from 55 varieties of soybeans grown in southern Ontario. They found five samples with no sucrose and one with no raffinose. Proper breeding and selection could lead to a lowering of the flatulence-causing factors in the soybean.

Nutritional Aspects of Soybeans as Food

The nutritional quality of soymilk and soy products is of importance since these products may make up a substantial portion of an individual's food intake, especially in the case of infants using soymilk as a substitute for cow's milk.

The quality of soymilk is very close to cow's milk in its nutritional value (Rice, 1970). According to Rackis (1974), the first limiting amino acid of the soybean is methionine. Fortification of soy isolates with 1% methionine can increase the protein efficiency ratio (PER) of the isolate from around 1.8-1.9 to that of standard milk casein, 2.5 (Decock, 1974).

The high lysine content makes soy protein a useful complement to the cereal proteins which are generally deficient or limiting in lysine. A mixture of soy protein concentrate and wheat flour is superior to either protein source alone (Wilding et al., 1968).

The soybean also has a high content of polyunsaturated fatty acids. This is of particular importance to those individuals who are interested in lowering their serum cholesterol.

Raw full fat and defatted soy flours have been shown to inhibit growth, depress metabolizable energy and fat absorption, reduce protein digestibility and cause other physiological problems. Many of these problems are due to the animal's inability to utilize the essential nutrients, rather than an actual toxic effect of the raw bean (Rackis, 1974). Good reviews of the antinutritional factors and their action are available (Wolf, 1967; Rackis, 1972, 1974; Pomeranz, 1976).

Osborne and Mendel (1917) first established the superiority of heat processed soybeans over raw soybeans in weanling rat feeding studies. Arnold et al. (1971) has shown that a critical quantity of heat is necessary to ensure maximum nutritional quality in whole soybeans.

Heat treatment has been shown by a number of workers to result in destruction of the heat-labile growth inhibitors, trypsin inhibitor and hemagglutinin.

According to Liener (1958), the nutritional value of soybeans is inversely proportional to the trypsin inhibitor content. At least 7-10 trypsin inhibitors are known to exist

(Rackis, 1974). Two of these, the Bowman-Burke (Odani and Ikenaka, 1973) and the Kunitz (Koide and Ikenaka, 1973), trypsin inhibitors have been completely sequenced and their active site of interaction with trypsin determined.

The adequacy of heat treatment in preparing soymilk powders was investigated by Van Buren et al. (1964). Neither the soluble nitrogen nor urease activity could substitute as an indicator for adequate heating for destruction of trypsin inhibitors in the soymilk powders.

Albrecht et al. (1966) have shown that trypsin inhibitor and urease activity are destroyed at about the same rate. When heating soaked whole soybeans, approximately 60% moisture in the beans, a 5 minute blanch was sufficient to destroy the trypsin inhibitor and urease activity.

A 0.4M sodium carbonate soak increased the rate of destruction of trypsin inhibitor upon heating due to the increased pH (Wallace et al., 1971). In vitro protein digestibility of the soymilk with trypsin was increased 19% over a similar sample using a water soak before heating. No significant difference was noted using pepsin to digest the proteins.

Baker and Mustakas (1973) found trypsin inhibitor to be more heat resistant than either lipoxygenase or urease with or without acidic or basic additives in the cooking water of dehulled soybeans. Both NaOH and HCl at 1% in the cooking water increased the inactivation of lipoxygenase and urease. Acid retarded the destruction of trypsin inhibitor at temperatures above 180 F.

The heat treatment of soybeans using a fluctuating temperature of 125 ± 25 C was 30% more effective in inactivating purified soybean trypsin inhibitor in 6 min than heat treatment at a constant 125 C for 6 min (Wu et al., 1975).

Liener (1953), using isolated soybean hemagglutinin, reported that it can account for as much as 50% of the growth inhibition in feeding raw soybeans to rats. However, there was a significantly lower intake of food in those animals on feed containing hemagglutinin. When the food intake of the control group was restricted to the same level as those receiving hemagglutinin, no growth inhibition was observed.

No significant difference was noted between feeding rats a raw soyprotein diet in which hemagglutinin was removed by passage through a column of sepharose bound concavalin A and feeding a similar diet with hemagglutinin present (Turner and Liener, 1975).

Birk and Gertler (1961) found that gastric digestion readily inactivated hemagglutinin activity; therefore, it could account for no more than a small portion of growth inhibition noted in feeding studies with chickens and rats.

Hemagglutinin activity is readily destroyed by moist heat. Any heat treatment sufficient to inactivate the trypsin inhibitors would be adequate for the destruction of hemagglutinin activity.

Processing Soymilk

The traditional oriental method for preparing soymilk is by soaking beans overnight, grinding in excess tap water, filtering to remove the insoluble residue and then heating the resultant soymilk to improve the nutritional quality and flavor. This product has found only limited acceptance outside the orient, due mainly to the poor flavor of the soymilk.

Many processes have been developed over the years to increase the nutritional value of the soymilk, to increase yields and to make the resultant soymilk more palatable.

Hackler et al. (1963) have found that the residue remaining after making soymilk had the highest PER of several fractions tested and was the most acceptable as a diet for growing rats. Simply increasing yields by incorporation of the insoluble residue could increase the nutritional value of the soymilk.

Good reviews of the patent literature on soymilk processing are available (Tsui, 1947; Noyes, 1969; Hansen, 1974). Some of the treatments used in these processes include: heating, soaking, washing, pressure cooking and removal of portions of the soybean, either physically or chemically, along with the use of acids, bases, neutralizing agents, salts, and inert gases, as well as the use of a number of other additives.

The effect of various alkalies and salts upon palatability and the nutritional value of soymilk have been studied extensively. As mentioned previously, an alkaline soak will increase the loss of oligosaccharides into the soak water from the beans and render trypsin inhibitor and lipoxygenase more sensitive to heat destruction.

Badenhop and Hackler (1970) found that the PER decreased as the pH was increased from 6.55 to 9.18 with NaOH in soymilk produced by grinding soaked soybeans with boiling water. Niacin increased to a maximum at pH 8.04, then decreased. American taste panelists noticed increasingly soapy tastes in the soymilk with increasing pH. The decrease in PER when using an alkaline soak and blanch is believed to be the result of the destruction of the essential amino acid cystine. Methionine supplementation of soymilk can be used to correct for the loss of cystine from alkaline soaking (Badenhop and Hackler, 1973) and to increase the nutritional value of soy-based foods (Brookwalter et al., 1975).

Lysinoalanine is one product formed in the soymilk during alkaline soaking above pH 8, causing a decrease in the availability of the two essential amino acids lysine and cystine (Bohak, 1964). Lysinoalanine has been shown to be toxic in some studies. A review of the toxicity of lysinoalanine is available (Pomeranz, 1976).

Steinkraus et al. (1968) found that the variety of soybeans used can affect the influence of alkalies upon taste of the soymilk. Only 50% of a Filipino taste panel preferred soymilk made from Hsieh-Hseih variety soybeans soaked in 0.1% NaOH prior to grinding compared to water soaking. All preferred the Taichung variety presoaked in 0.1% NaOH over a simple water soak prior to grinding.

Khaleque et al. (1970) found that an alkaline soak of soybeans in 0.5% sodium carbonate was more effective than several other alkalies including sodium bicarbonate and sodium hydroxide in reducing the green, beany flavor in the resultant soymilk. Using sodium carbonate, they produced a soymilk with better mouth feel that was easier to process and had improved yields of protein and fat in the soymilk. Khaleque et al. (1970) also question whether lipoxygenase is actually responsible for the green, beany taste by comparing flavor evaluations of soymilk produced by grinding with boiling water and by the conventional oriental procedure. However, the procedure as outlined for making the milk by the hot grind process, 250 ml boiling water plus 100 g, dry weight, of soaked beans, would not result in a sufficiently high grinding temperature to inactivate the lipoxygenase (Wilkens et al., 1967).

Bourne et al. (1976) studied the effect of various sodium salts and alkalies upon the flavor acceptability of soymilk made by grinding soaked beans with boiling water. The level of sodium ion was found to be most significant in flavor acceptability. Sodium citrate was superior to other sodium salts and alkalies and they propose a possible synergistic effect of the citrate ion with the sodium ion upon flavors in the soymilk.

A soymilk has been developed by Nelson et al. (1976) in which a 0.5% $NaHCO_3$ soak and blanch solution is used to reduce the off-flavors and softened the beans sufficiently to yield good mouth feel and stability. High pressure homogenization was used to achieve colloidal stability. With proper pasteurization, this beverage is reported to remain stable for about two months at 34 F with no separation. Elimination of the filtering step resulted in increased yields by utilizing the whole soybean, 95% of the protein and 89% of the solids are recovered in the soymilk with the remainder lost in the water used to soak, rinse and blanch the beans. Particle size for the soymilk, determined using a coulter counter, is reported to be in the range of 3.4 to 7.3 microns for 80% of the particles with none smaller than 2.7 microns.

The use of a high temperature grinding method for preparing soymilk has been developed by workers at Cornell University. This procedure is said to inactivate lipoxygenase and bring protein into solution before it is denatured (Steinkraus et al., 1968). Wilkens et al. (1967) found that lipoxygenase could be inactivated, based upon gas chromatographic analysis of the soymilk volatiles, by grinding either soaked or unsoaked soybeans at temperatures above 80 C. Use of an antioxidant, nordihydroguairetic acid at a concentration of 4 mg/ml, allowed the use of grinding temperatures as low as 60 C while still reducing the formation of volatiles in the milk. The use of filtering rather than centrifugation for separation of the insoluble residue from the milk created difficulties in assessing yields due to the formation of gels at higher temperatures which plugged the filters.

Lo et al. (1968b) found that soaked beans yielded a higher percent of solids in the soymilk than either nonsoaked beans or preground flour at various extraction temperatures using the hot grind method. Extraction temperatures above 85 C, however, resulted in decreased yields due to formation of gels that plugged the filters.

A soymilk prepared by the hot grind method developed at Cornell, with added sucrose and vanilla flavoring, was found to be highly acceptable to Filipino school children (Steinkraus et al., 1968).

A process in which extruded full fat soyflour is used as a base for blending soymilk has been developed by Mustakas et al. (1971). Off-flavors were eliminated by extrusion cooking and addition of cream flavorings. With proper formulation, this soymilk is reported to be similar to cow's milk in nutritional value. A major advantage of this product is its redispersibility in water after spray drying of the soymilk. This provides better storage and keeping qualities along with lower transportation costs.

Mital and Steinkraus (1976) compared soymilk made using defatted soybean flour blended with refined soybean oil to soymilk made using the Cornell hot grind method and also to cow's milk. The hot grind processed soymilk was found to be significantly inferior in flavor acceptability, while soymilk from defatted flour was only slightly inferior to cow's milk.

MATERIALS AND METHODS

Soybeans

Soybeans of the Amsoy 71 variety were used for all experiments. The soybeans were sorted by hand to remove broken or discolored beans as well as any debris and were stored at 4 C prior to use to minimize changes in moisture content. Proximate analysis of the soybeans showed: protein 41.50%, crude lipid 20.42%, carbohydrate 32.34%, and ash 5.75% on a dry basis with soybeans at 10.69% moisture.

Procedure for Preparing Soymilk

Soybeans were soaked overnight, approximately 18 h, at 21 C in three times their weight of either tap water or a 0.5% $NaHCO_3$ solution. After soaking, the soybeans were drained and rinsed twice with fresh tap water. Blanching of the soybeans, when applicable, was done in 0.5% $NaHCO_3$ solution for 30 min. After blanching, the soybeans were drained, rinsed twice in fresh tap water, then cooled to room temperature before grinding.

Soybeans were ground with tap water at a 1:7 ratio (original dry weight of beans:tap water), except when the effect of the soybeans to water ratio during grinding was studied. Grinding was done by one of two methods: for small batches, less than one liter total volume, the soybeans were ground in a Waring blender for three minutes; larger batches of soymilk were prepared by grinding in a Cherry Burrell Vibroreactor Model JV. The soy slurry was passed through the Vibroreactor a total of eight times to insure completeness of grinding.

Homogenization of the soy slurry, when used, was done with either a Gaulin model 15M8TA laboratory scale homogenizer at various pressures, or a Manton Gaulin 200-500CGD homogenizer at 2000 pounds per square inch (psi) pressure. Temperature of the soy slurry before homogenization was either 21-25 C or 70-75 C.

The soy slurry after grinding, or after homogenization when used, was adjusted to pH 6.95-7.05 with either 1N HCl or NaOH using a Corning model 12 pH meter, then centrifuged at 642 x G for 10 min in a Sorvall RC-2B centrifuge using the GS-3 head.

The supernatant, or soymilk, was freeze-dried in a VirTis cabinet model freeze-drier to less than 5% moisture. The freeze-dried soymilk was stored in tightly stoppered bottles at 4 C for later testing.

A detailed outline of each of the procedures used for the preparation of soymilk is presented in Table 1. The procedures differ from each other basically in the amount of heat applied and in the order of processing.

Table 1. Outline of processes used for the preparation of soymilk

Process A	Process B	Process C
Soak soybeans in 0.5% $NaHCO_3$ solution for 18 h	Soak soybeans in tap water for 18 h	Soak soybeans in tap water for 18 h
Drain	Drain	Drain
Rinse twice with tap water	Rinse twice with tap water	Rinse twice with tap water
Blanch 30 min at 100 C in 0.5% $NaHCO_3$ solution	Preheat soybeans 15-20 sec by dipping in hot water 95-100 C	
Grind at 20-25 C	Grind with boiling water	Grind at 20-25 C
Homogenize	Homogenize	Homogenize
Adjust pH to 6.95-7.05	Adjust pH to 6.95-7.05	Adjust pH to 6.95-7.05
Centrifuge at 642 x G	Centrifuge at 642 x G	Centrifuge at 642 x G

Process A

A procedure similar to that described by Nelson et al. (1976) was used. The unique feature of this process is that the soybeans were blanched for 30 min before grinding.

Process B

This process was essentially as described by Steinkraus et al. (1968). It differed from the other processes in using a hot grinding step. To insure the grinding temperature of the soybeans remained above 80 C, the soaked soybeans were preheated for 15-20 sec by immersion in 95-100 C tap water, then ground immediately in a preheated grinder with boiling tap water. The grinder was preheated with boiling water. Constant monitoring of the temperature of the soy slurry during grinding was done. Only samples in which the temperature remained above 80 C during the grinding were used for subsequent investigations.

Process C

Soybeans were soaked and ground in tap water with no heating prior to grinding. This process is similar to the traditional oriental method for preparing soymilk.

Analysis of the Soymilk

The constituents of the solids in the soymilk as determined in the following section have been corrected for the percent moisture in the freeze-dried samples, thereby eliminating any variation in the constituents of the solids due to differing levels of moisture in the samples. All results are averages of duplicate determinations for each constituent in the sample.

Total solids

The total solids were determined gravimetrically by drying approximately 5 g samples of soymilk or soy slurry, or 2 g samples of freeze-dried soymilk, in a hot-air oven at 80-85 C, then cooling in a $CaCl_2$ desiccator. Approximately 6 h drying time was generally sufficient to reach a constant weight in the samples.

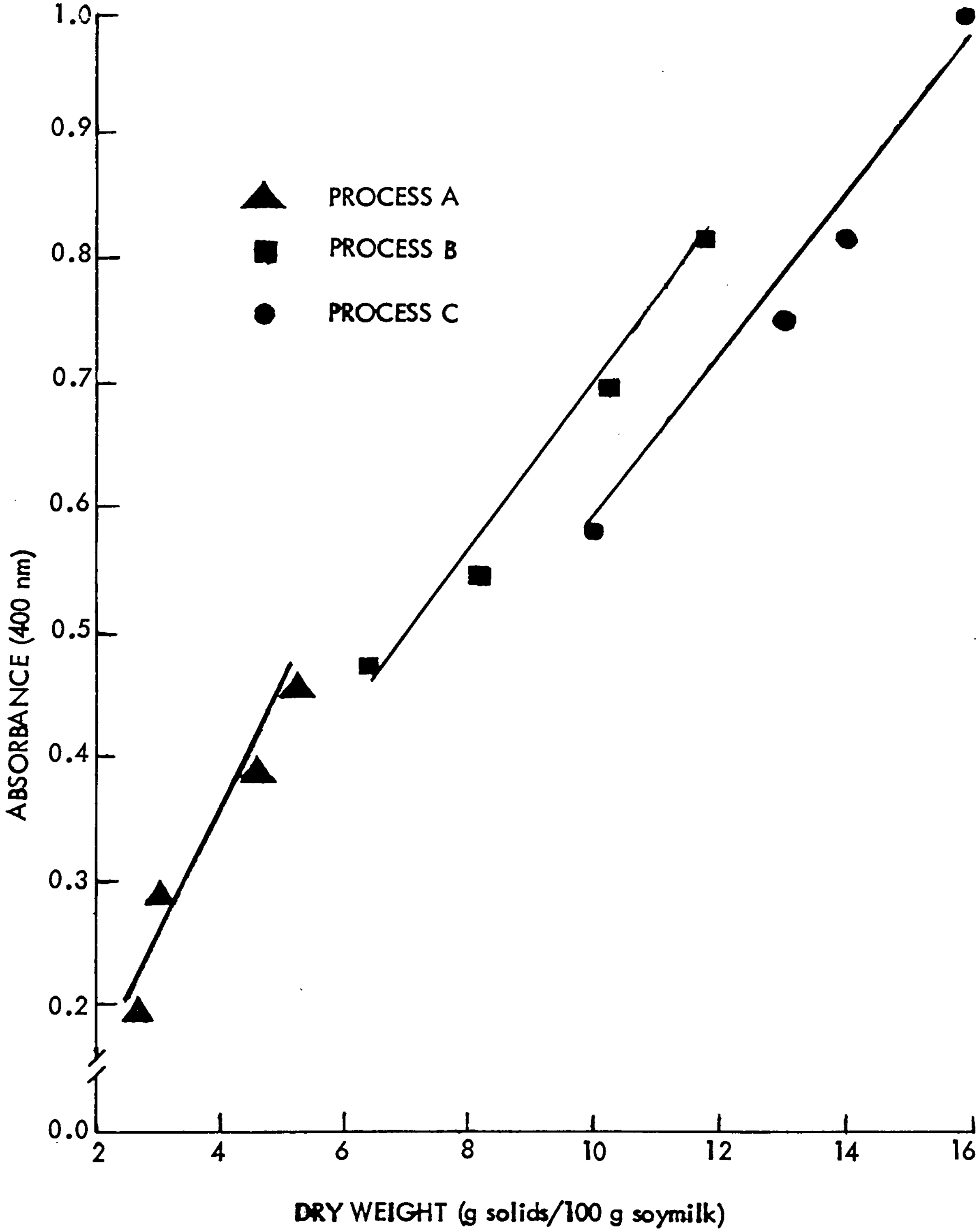
Optical measurement of solids

The absorbance of a 1:250 dilution of soymilk in distilled deionized water was determined using a Bausch and Lomb Spectronic 20 spectrophotometer at 400 nm. The absorbance of soymilk at 400 nm is a good indicator of the percent solids in the soymilk as shown in Fig. 1. However, for each procedure used, a new set of standards must be made since the procedure for preparing the soymilk does change the slope of the line and placement of the line on the graph.

Protein determinations

A modification of the micro-Kjeldahl method 38.012 (A.O.A.C., 1970) was used to determine total nitrogen in all samples. The total nitrogen was converted to protein using a

Figure 1. Effect of processing method upon the absorbance of a 1:250 dilution of soymilk


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.0
ABSORBANCE (400 nm)
PROCESS A
PROCESS B
PROCESS C
2
4
6
8
10
12
14
16
DRY WEIGHT (g solids/100 g soymilk)

conversion factor of 6.25.

Freeze-dried samples of soymilk, 0.05 g, were digested with 2 ml of concentrated sulfuric acid on a Lab Con Co digestion apparatus. Cupric selenite, 0.2 g, was used as a catalyst during digestion along with 0.3 g of K_2SO_4 to raise the boiling point of the acid. The samples were digested until light blue to clear in color, approximately 30-45 min, then additional acid was added to rinse the neck of the digestion flask. The digestion was allowed to continue for another 15-30 min until no further fuming of the sample was noted. A doubling of the time used for digestion of the sample resulted in less than a 1% increase in the percent protein of the solids of soymilk containing approximately 40% protein.

After cooling the digested samples to room temperature, 5 ml of distilled water was added, then the mixture was quantitatively transferred to a Lab Con Co distillation apparatus. Excess sodium hydroxide solution, 40%, was added to release the ammonia, which was then trapped in 4.0% boric acid solution. The ammonia in the solution was titrated with standard HCl prepared according to A.A.C.C. (1969) method 70-20. Tashiro's indicator, 0.25 g methylene blue and 0.375 g methyl red in 300 ml of 95% ethyl alcohol, was used during the titration to reach a clear to greyish color for the end point of the titration.

The macro-Kjeldahl method 2.044 of A.O.A.C. (1970) was used to determine the percent protein in some samples to compare the efficiency of the micro-Kjeldahl method previously described.

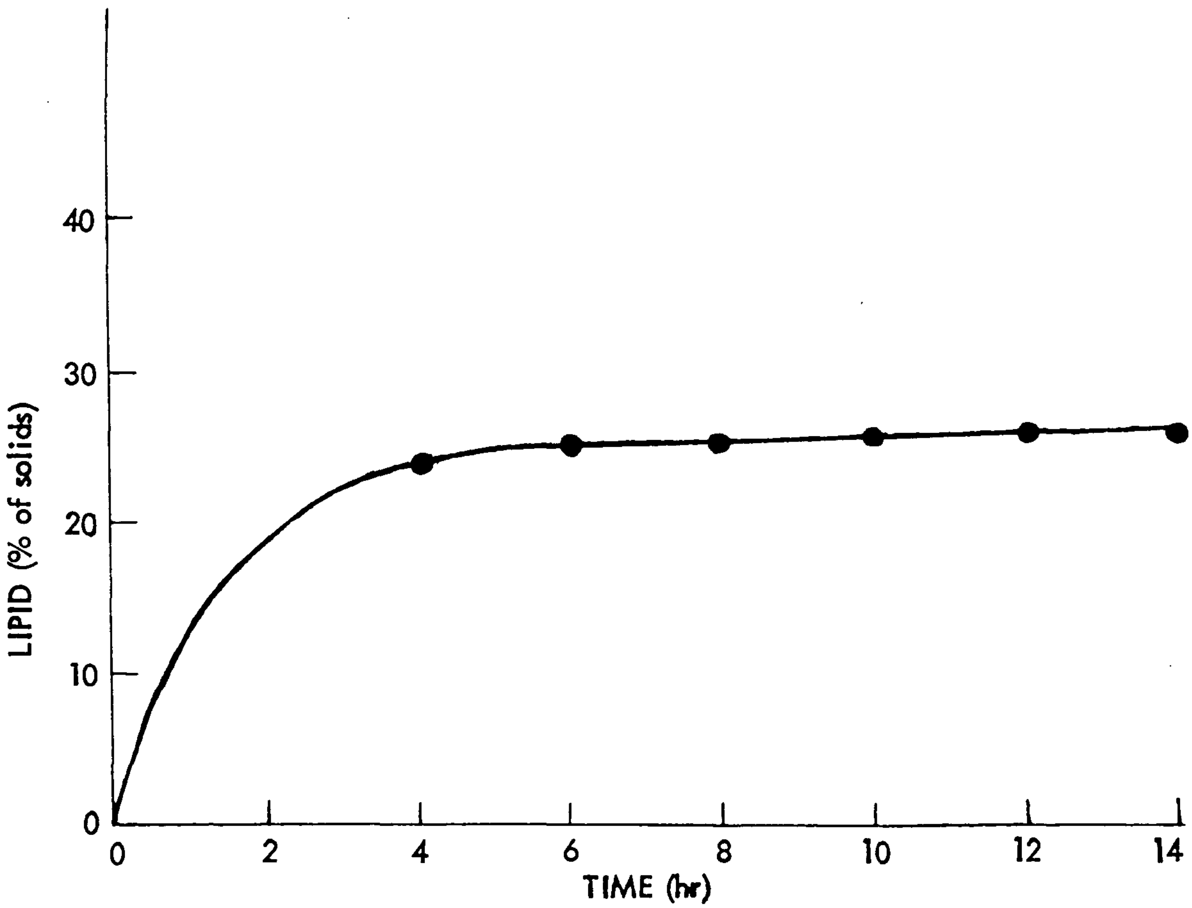
Three samples of freeze-dried soymilk prepared by process A containing three different levels of protein, approximately 30, 40 and 45% of the solids, were analyzed by both the micro and macro-Kjeldahl methods for protein. Very little difference was found in comparing the averages of duplicate determinations of protein between the two processes. Therefore the micro-Kjeldahl method was used for all further protein determinations.

Crude lipid

Crude lipid was determined in all samples by hexane extraction using a Goldfisch extraction apparatus utilizing the procedure of A.A.C.C. (1969) method 30-20. Samples of freeze-dried soymilk weighing between 0.8 and 1.2 g were extracted 16-18 h with hexane in tared extraction thimbles. The percent lipid was determined gravimetrically as weight loss due to extraction. As shown in Fig. 2, the fat extraction with hexane required a minimum of 8-10 h for completeness of extraction.

Some samples of freeze-dried soymilk were extracted with a 2:1 chloroform:methanol solution (v/v) instead of hexane in a process similar to the hexane extraction of crude lipids.

Figure 2. Extraction of crude lipid from oven dried soymilk samples with hexane as solvent using a Goldfisch extraction apparatus



40
30
20
10
0
LIPID (% of solids)
0
2
4
6
8
10
12
14
TIME (hr)

A comparison of the percent crude lipid extracted by each of the solvent systems from freeze-dried soymilk made according to process A with solids increased by homogenization is shown in Table 2. The chloroform:methanol solution extracted approximately 10% more material than the hexane extracted from the solids. This is due to the ability of chloroform:methanol to strip emulsifiers from the oil, thus releasing more oil for extraction, and to the extraction of phospholipids by the chloroform:methanol solution.

Ash determination

The method of A.O.A.C. (1970) 15.016 was used, with some modification, for the determination of ash. Approximately 2 g samples of freeze-dried soymilk were charred using a Bunsen burner, being careful not to allow the samples to burn with an open flame, then ignited in a muffle furnace at 525 C for 12-16 h to get carbon-free ash. Samples were cooled in a $CaCl_2$ desiccator and the percent ash was determined gravimetrically.

Carbohydrate

Carbohydrate was determined by difference, therefore it includes all material not included as protein, lipid, ash or moisture.

Table 2. Comparison of the percent lipid extracted from freeze-dried soymilk using either hexane or chloroform:methanol with a Goldfisch extraction apparatus

Sample	Solvent system	
	Hexane	Chloroform:methanol (2:1)
1	33.69	44.70
2	31.04	42.77
3	32.42	40.89
4	29.39	39.55

Percent soymilk

Weighed samples of soy slurry were centrifuged at 642 x G for 10 min. The percent soymilk is used to indicate the ratio by weight of soymilk recovered from the soy slurry and is defined for the purpose of this paper as follows:

$$\% \text{ soymilk} = \frac{\text{g of soymilk after centrifuging}}{\text{g of soy slurry centrifuged}} \times 100.$$

Recovery and yield

The definition used for recovery is as defined by Al-Kishtaini (1971), and is similar to that used to define percent soymilk.

$$\% \text{ recovery} = \frac{\text{g of constituent in soymilk}}{\text{g of constituent in soy slurry}} \times 100.$$

Lo et al. (1968b) and Al-Kishtaini (1971) define yield as the volume of soymilk times the percent solids content of the soymilk. This definition works well when all samples are held constant with respect to the weight of soybeans used in preparing the soymilk and the volume of water, but does not take into account the variation when either of these factors is changed. The definition of yield will be as follows:

$$\text{yield} = \frac{\text{g solids in soymilk}}{\text{g solids in soy slurry}} \times \frac{\text{g soymilk}}{\text{g soy slurry}} \times 100.$$

Based upon the previous definitions of percent soymilk and percent recovery, yield would be defined as follows:

$$\text{yield} = \frac{\text{\% recovery of solids x \% soymilk}}{100}.$$

Experimental Procedures

Effect of temperature of the soak solution and addition of salts upon hydration of soybeans

Samples of soybeans, 20 g, were loosely wrapped in bags made of a single layer of cheesecloth. The samples were soaked in excess tap water at either 4, 21 or 100 C or in 0.5% $NaHCO_3$ solution at 21 C. Water uptake by the soybeans was determined gravimetrically after removal of the soybeans from the bag and lightly blotting to remove any excess water.

Temperature-time relationship for elimination of the green, beany taste

Whole soaked soybeans, 18 h soak, were added to water at various temperatures from 60 to 100 C. Soybeans were removed periodically and tasted by the author for determination of the point in heating where no green, beany tastes developed upon maceration. After this point had been reached in each of the samples, the remainder of the soybeans were removed from the heat, then refrigerated overnight in tap water and retasted with and without the hulls.

It was felt unnecessary to undertake further organoleptic studies, including taste panel work, because of the sharp difference in taste of soybeans with and without the green, beany taste.

Sonication

Unsoaked soybeans, 100 g, were simultaneously hydrated and blanched by immersion in boiling water for 10 min. The soybeans were then cooled and ground with tap water at approximately a 1:7 ratio (original dry weight of soybeans to water). The soy slurry was sonicated with a Blackstone sonicator adjusted to maximum frequency output. Samples were removed at various times following the start of sonication and centrifuged at 1086 x G for 10 min. The absorbance of a 1:250 dilution was determined on the resultant soymilk to follow changes in the solids content of the soymilk.

Effect of soybean:water ratio during grinding

Soymilk was made according to procedures A, B, and C with differing soybean:water ratios used during grinding in the Waring blender. The total weight of the water plus soaked beans was held constant at approximately 700 g, with approximate ratios of soybeans to water of 1:5, 1:6, 1:8, and 1:10 used in each procedure.

Effect of homogenization upon solids and protein content of soymilk

Soymilk was made by soaking soybeans in tap water (1:3 soybeans to water) for 18 h, blanching 5 min at 100 C in the soak water, then grinding in the Cherry Burrell Vibroreactor in a 1:7 ratio (original dry soybean weight to tap water) after cooling. The soy slurry was passed through the large capacity Manton Gaulin homogenizer up to 7 times at 2000 psi. Samples were removed at various intervals and centrifuged at 642 x G for 10 min. The percent solids in the soymilk and percent protein of the soymilk was determined for each sample.

<u>Effect of homogenization pressure and temperature</u>

Soymilk was prepared by process A, B, and C. Grinding was done in the Cherry Burrell Vibroreactor. After grinding the soy slurry was divided in half. One-half was homogenized at room temperature, cold homogenization, while the other half was heated to 70-75 C and homogenized while hot, hot homogenization. Homogenization pressures used were 0, 2000, 4000, 6000, and 8000 psi with the laboratory scale Manton Gaulin homogenizer. The procedure was performed in duplicate for each of the processes and at each temperature of homogenization and results are the average of the duplicate results from each sample at each pressure and process.

RESULTS AND DISCUSSION

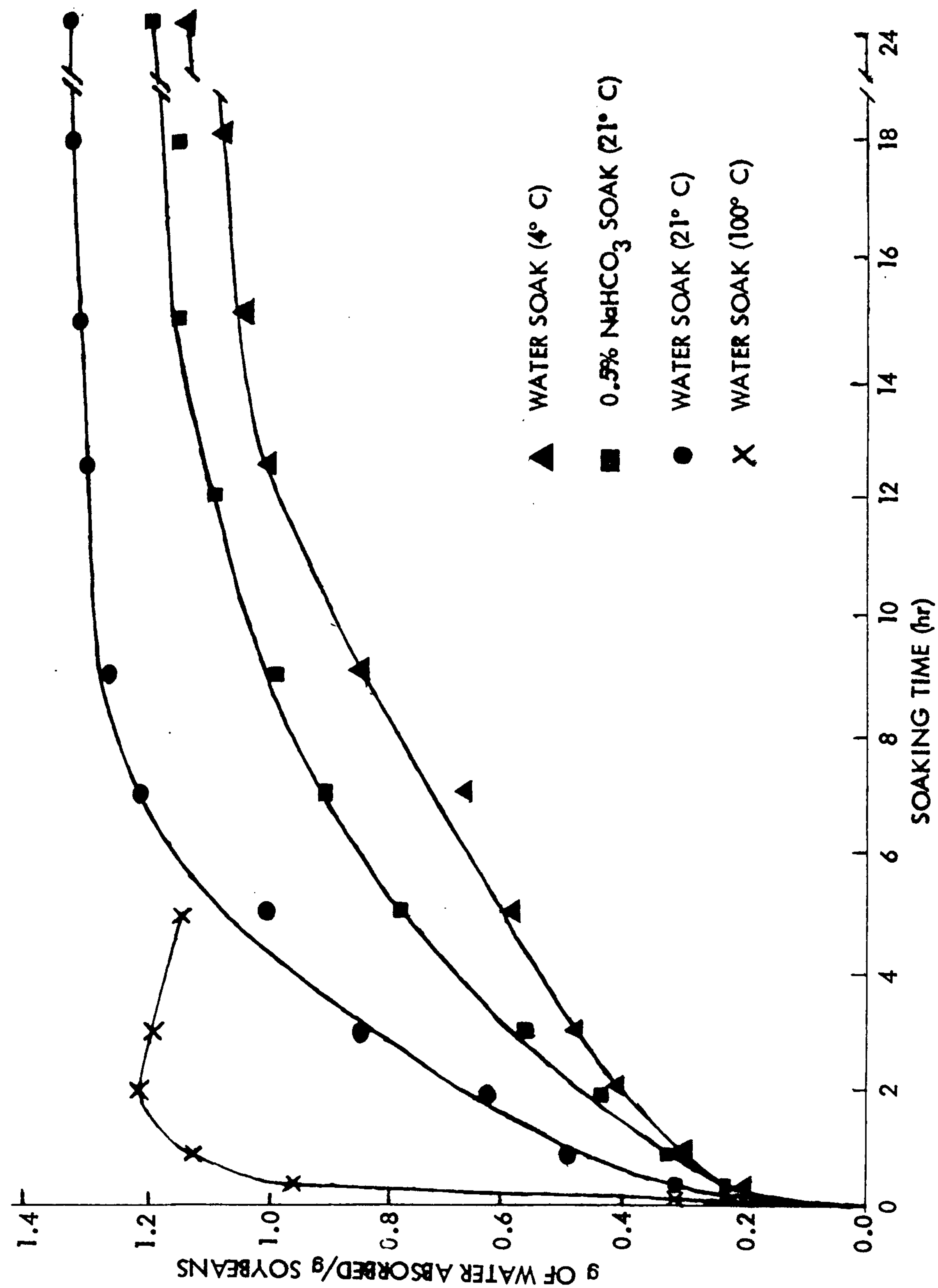
The purpose of the following experiments was to determine the effect of various processing conditions upon soymilk. Heating of the soy preparation at some stage in its processing is widely used to prevent formation of off-flavors, remove off-flavors from the soymilk and to improve the nutritional value of the soymilk. Three basic processes were studied with differences in when the heat was applied. Also, a variety of methods were investigated to improve the solids recovery and the nutritional quality of the soymilk as determined by the protein content.

Effect of Soaking Temperature and of Salt Addition upon Hydration of Soybeans

The rate of water uptake by soybeans is affected by both the solution used for soaking the soybeans and the temperature of the soak solution (Fig. 3). Soybeans soaked at 21 C in tap water absorbed water at a faster rate, 1.3 times the original soybeans weight in 10 h, than soybeans soaked in sodium bicarbonate solution, only 1.05 times the original soybean weight in 10 h.

The apparent water uptake that was measured is the result of two different processes occurring simultaneously: the increase in weight of the soybean due to water diffusing inward and the loss of soluble carbohydrates, peptides and amino acids into the soak water. The concentration gradient between the

Figure 3. Effect of soaking temperature and of $NaHCO_3$ upon the hydration of soybeans


WATER SOAK (4° C)
0.5% NaHCO$_3$ SOAK (21° C)
WATER SOAK (21° C)
WATER SOAK (100° C)
SOAKING TIME (hr)
g OF WATER ABSORBED/g SOYBEANS
0 2 4 6 8 10 12 14 16 18 24
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

solubles inside the soybean and the soak solution is responsible for the flow of water into the soybean. Decreasing the difference in concentration by addition of sodium bicarbonate to the soak water lowers the chemical potential to drive moisture into the beans. The cotyledon cell membranes and the hull are the major physical barriers that hinder bean solubles from migrating into the soak water. Ku (1972) found that a 0.5% $NaHCO_3$ soak retarded the hydration rate during soaking and increased the loss of solubles into the soak water during blanching of the beans, apparently by making the cotyledon cell membrane more permeable.

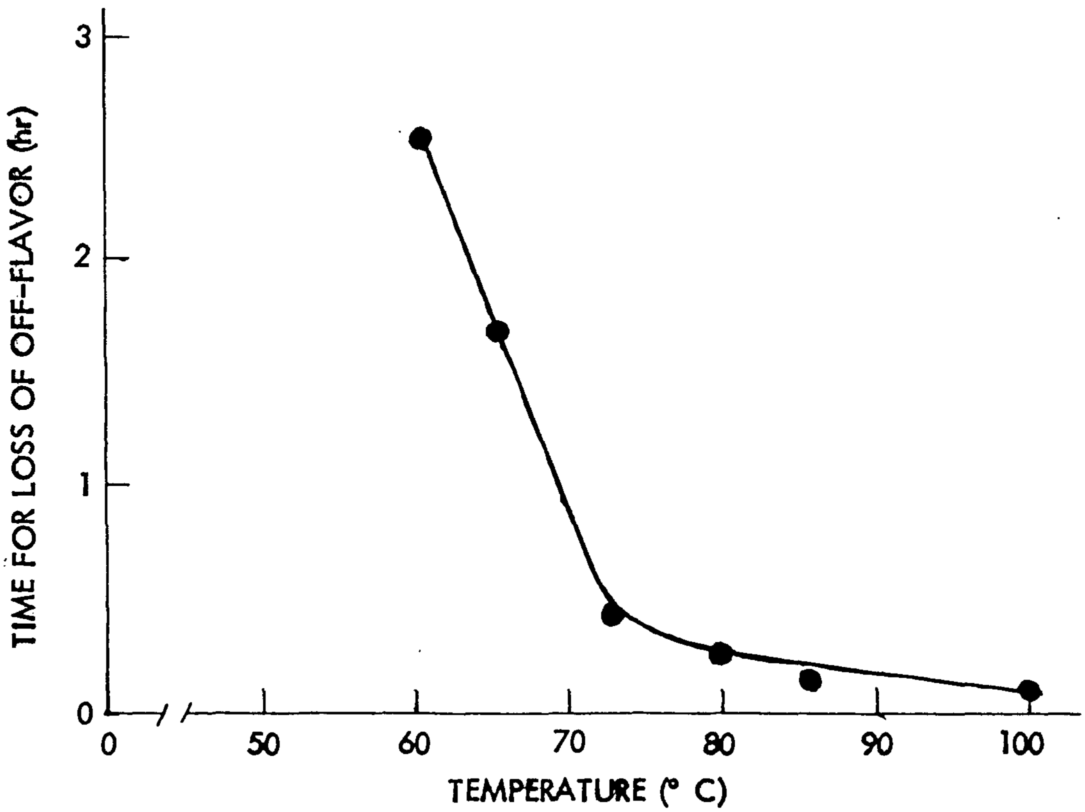
Hamad and Powers (1965) found that both the seed coat and the temperature of the soak water affected the rate of hydration of Great Northern beans. Soybeans soaked at 4 C in tap water had the lowest rate of water absorption (Fig. 3), while simultaneous hydration and blanching by soaking at 100 C resulted in the most rapid water uptake by the soybeans. After two hours soaking in boiling water, a decrease in total weight of the soybeans is observed. This is probably due to the destruction of cellular components by the prolonged heating and leakage of soluble components into the soak water. The soak water became very discolored with the longer heating times.

Temperature-Time Relationship for Elimination of Green, Beany Flavors

The length of time necessary to prevent the formation of the green, beany flavor in soaked soybeans is dependent upon the time and temperature at which the beans are heated (Fig. 4). Higher temperatures result in shorter times of heating necessary for elimination of the green, beany flavor. A minimum time of two minutes was required at 100 C. This is the same time as determined by Snyder (1973) in similar heating experiments and correlates well with the theory that the green, beany flavors are caused by enzymatic action rather than by preformed components within the soybeans.

Samples of soybeans treated for a sufficient period of time to prevent development of the green, beany flavor upon maceration were retasted after overnight refrigeration. In all samples except the 100 C heated sample, a bitter taste was present that could be removed by dehulling and rinsing the soybean cotyledons with fresh water. Heating the soybeans for twice the time necessary to prevent formation of the green, beany flavor at all temperatures except 100 C was ineffective in preventing the development of bitter tastes after overnight refrigeration of the samples. These bitter tastes may be due to either bitter peptides from protein degradation or to autoxidation of the soybean phosphatidylcholine. However, since they were easily removable by simply rinsing, this would

Figure 4. Temperature-time relationship to prevent the development of green, beany taste upon maceration



TIME FOR LOSS OF OFF-FLAVOR (hr)
3
2
1
0
0
50
60
70
80
90
100
TEMPERATURE (° C)

indicate the smaller peptides are responsible for the bitter tastes.

Sonication

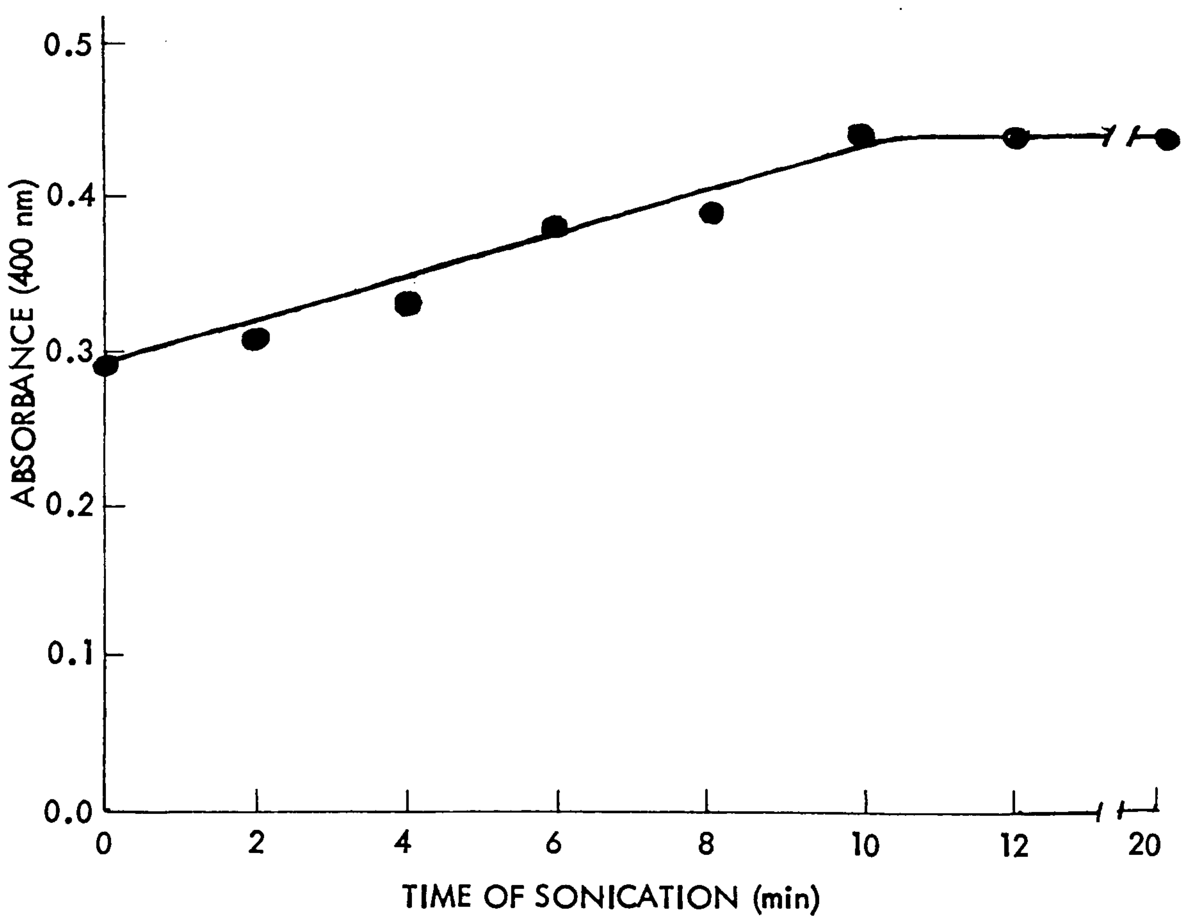
Sonication of the soy slurry resulted in a slight increase in the total solids of the soymilk as shown by the absorbance of the soymilk (Fig. 5). Ten minutes of sonication were necessary to achieve maximum absorbancy. These results are similar to those found by Wang (1975) when sonicating autoclaved defatted soyflakes in a 1:10 ratio with distilled water. Wang found that the soluble protein increased to a maximum after 6 min of sonication with 48% of the protein solubilized as compared to 32% soluble protein in nonsonicated flakes. A comparison of the absorbancy to solids relationship in Fig. 1, using process A which is most closely related, shows this to be about a one percent increase in the solids content of the soymilk.

Effect of Soybean:Water Ratio During Grinding

Percent soymilk, solids recovery and yield

The percent soymilk recovered increased in all three processes as the difference in the soybean:water ratio became greater (Table 3). Since the water would constitute a larger proportion of the system as the dilution factor became greater, it is expected that the percent soymilk would increase with increasing dilution of the system since more water would be

Figure 5. Effect of sonication of soy slurry upon absorbancy of a 1:250 dilution of soymilk. The soy slurry was prepared by boiling soybeans 10 min prior to grinding at a 1:7 soybeans:water ratio



0.5
0.4
0.3
0.2
0.1
0.0
ABSORBANCE (400 nm)
0
2
4
6
8
10
12
20
TIME OF SONICATION (min)

Table 3. Effect of processing conditions and the ratio of soybeans to water during grinding upon the % solids in soymilk, the composition of solids, % soymilk, % solids recovered and yield

Process	Ratio soybean:water	% solids in soymilk	Constituents of solids (%)	
			Protein	Fat
A	1:5	5.27	33.05	32.43
	1:6	4.58	31.59	33.78
	1:8	3.33	30.00	35.93
	1:10	2.79	28.43	42.08
B	1:5	13.48	43.28	25.14
	1:6	11.45	43.40	23.08
	1:8	9.28	44.42	21.01
	1:10	8.15	42.53	20.53
C	1:5	11.81	45.30	24.13
	1:6	10.22	45.40	23.51
	1:8	8.18	44.52	23.19
	1:10	6.40	46.23	26.01

Table 3. Continued

Process	Constituents of solids (%)		% soymilk	% solids recovered	Yield
	Ash	CHO			
A	5.23	29.29	44.5	43.1	19.2
	5.54	29.09	47.3	41.0	19.3
	5.72	28.35	58.7	38.7	22.7
	5.24	24.25	64.8	40.6	26.3
B	5.28	26.30	33.7	83.9	28.2
	5.78	27.74	40.7	81.6	33.2
	5.50	29.07	53.1	71.4	37.9
	5.75	31.19	57.1	74.4	51.0
C	5.24	25.33	47.5	86.6	41.1
	5.35	25.74	45.3	89.6	40.6
	5.38	24.91	58.7	86.8	51.0
	5.59	22.17	71.8	81.1	58.4

recovered.

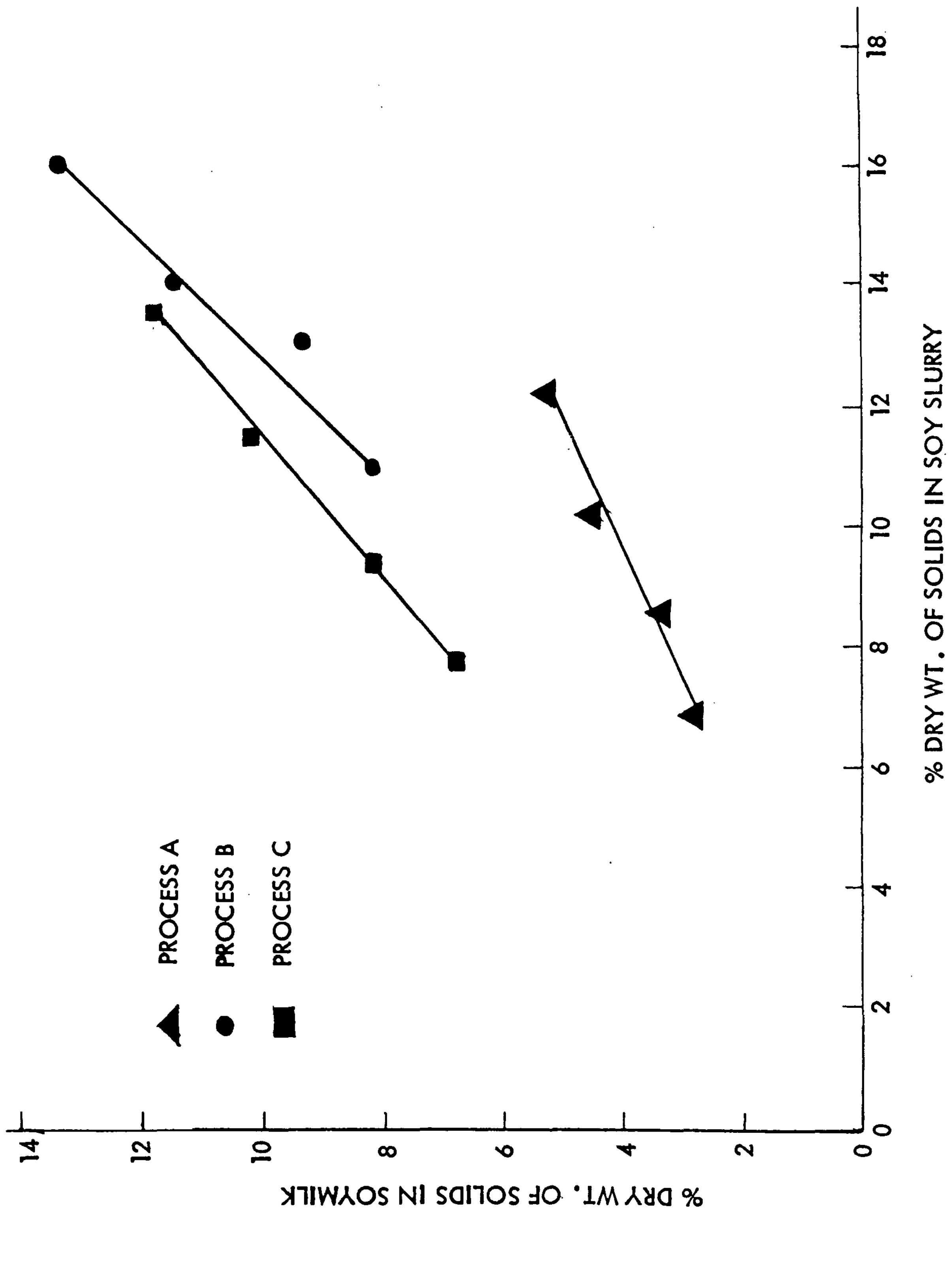
The recovery of solids remained nearly constant with changing dilutions within each processing system as shown in Fig. 6. Heating had a marked effect upon the recovery of solids as shown by the placement and slope of the lines in Fig. 6. The heat used in process A, a 30 min blanch prior to grinding, decreased significantly the overall recovery of solids in the system at each dilution to about 41% of the solids recovered. The heat applied in process B, hot grinding the soybeans, also decreased the recovery of the solids but to a lesser extent than that found in process A. About 78% of the solids were recovered in process B, compared to 87% for process C where no heating was used.

The yield increased with increasing dilution of each processing system (Table 3). The increase in yield in each process was due to the increased recovery of soymilk from the soy slurry with the ratio of solids recovered remaining approximately the same.

Protein

The protein, as a percent of solids, remained nearly constant in process B and C at the different ratios of soybeans to water, while the protein in process A decreased as the ratio of water to soybeans increased.

Figure 6. Relationship of the dry weight of soymilk to the solids present in the soy slurry with changing ratios of soybeans to water during grinding


PROCESS A
PROCESS B
PROCESS C
% DRY WT. OF SOLIDS IN SOY SLURRY
% DRY WT. OF SOLIDS IN SOYMILK
0
2
4
6
8
10
12
14
16
18

The decrease in protein as a percent of solids in process A with increasing dilution may have been due to several factors. The length of time of heating of each sample was held constant at 30 min prior to grinding; however, the quantity of heat applied may have varied with more heat applied to the soybeans used to make the more dilute batches of soymilk. Since the total volume during grinding was held constant during the grinding, there were less total beans in the batches at the higher dilutions. With less beans during the heating, more heat could have been applied, i.e., a fast boil vs. a slow boil, with less problems of foaming and boil over during heating. This could have resulted in increased protein denaturation and lower protein solubility at the higher dilutions.

Crude lipid

The percent lipid of the solids increased from 32% to 42% of the solids in process A, remained nearly constant in process C at 25%, and decreased slightly, from 25% to 21%, in process B with the increasing water to soybean ratios (Table 3).

The increase in the extractable lipids in process A is probably due to the decreased emulsification properties of the protein, which is reflected in the decrease in protein of the solids as the ratio of water to soybeans is increased in process A. In process B where less heat was applied and the protein content remained nearly constant, the lipid decreased, indicating better emulsification of lipid in the more dilute soy slurry.

More efficient grinding may have resulted in more crude lipid released in the more dilute systems as the cells containing the spherosomes were disrupted or through stripping of the natural emulsifiers from the oil in solution.

Ash

The percent ash of the solids remained nearly constant in all three processes. It was about 5.5% at the various soybean to water ratios used (Table 3).

Carbohydrate

The carbohydrate content varied with both the process and the dilution (Table 3). However, these values, since they are obtained by difference, may not reflect completely the effect of either the process or the dilution on the carbohydrate content.

Effect of Homogenization upon Solids and Protein Content of Soymilk

A soy slurry was made by a modification of process A in which a tap water soak and blanch with only 5 min boiling were used prior to grinding in the Cherry Burrell Vibroreactor. Homogenization of the soy slurry was done by repeatedly passing the slurry through the homogenizer at 2000 psi. The solids content of the resultant soymilk was increased most rapidly during the first few passes through the homogenizer with a slower increase in the solids during later passes (Fig. 7). Protein increased over half again as much as percent of solids with one pass through the homogenizer over nonhomogenized soymilk (Fig. 7) and leveled off at 41.5% of the solids after 5 passes through the homogenizer.

Microscopic observation of the soy slurry from soybeans that are heated prior to grinding reveal numerous particles similar to those described by Tombs (1967) and called protein bodies. These particles are generally absent in preparations of soy slurry from soybeans that receive no heat treatment prior to grinding or in soybeans simultaneously blanched and ground as in process B.

Photomicrographs of the soy slurry prepared both by heating before grinding and with no heat prior to grinding are shown in Fig. 8a and 8b. These pictures are representative of the appearance of the soy slurry when examined microscopically.

The protein bodies, as can be seen in the photomicrographs, are present only in the heated soy slurry while unheated soy slurry has a grainy appearance with much smaller particles present. Large pieces of cellular debris appear in both photomicrographs. The protein bodies appear to be "fixed" in some manner by the heat treatment prior to grinding and are much more resistant to disruption by grinding than the protein

Figure 7. Effect of repeated homogenization at 2000 psi upon the solids and protein content of soymilk prepared with 5 min heating prior to grinding

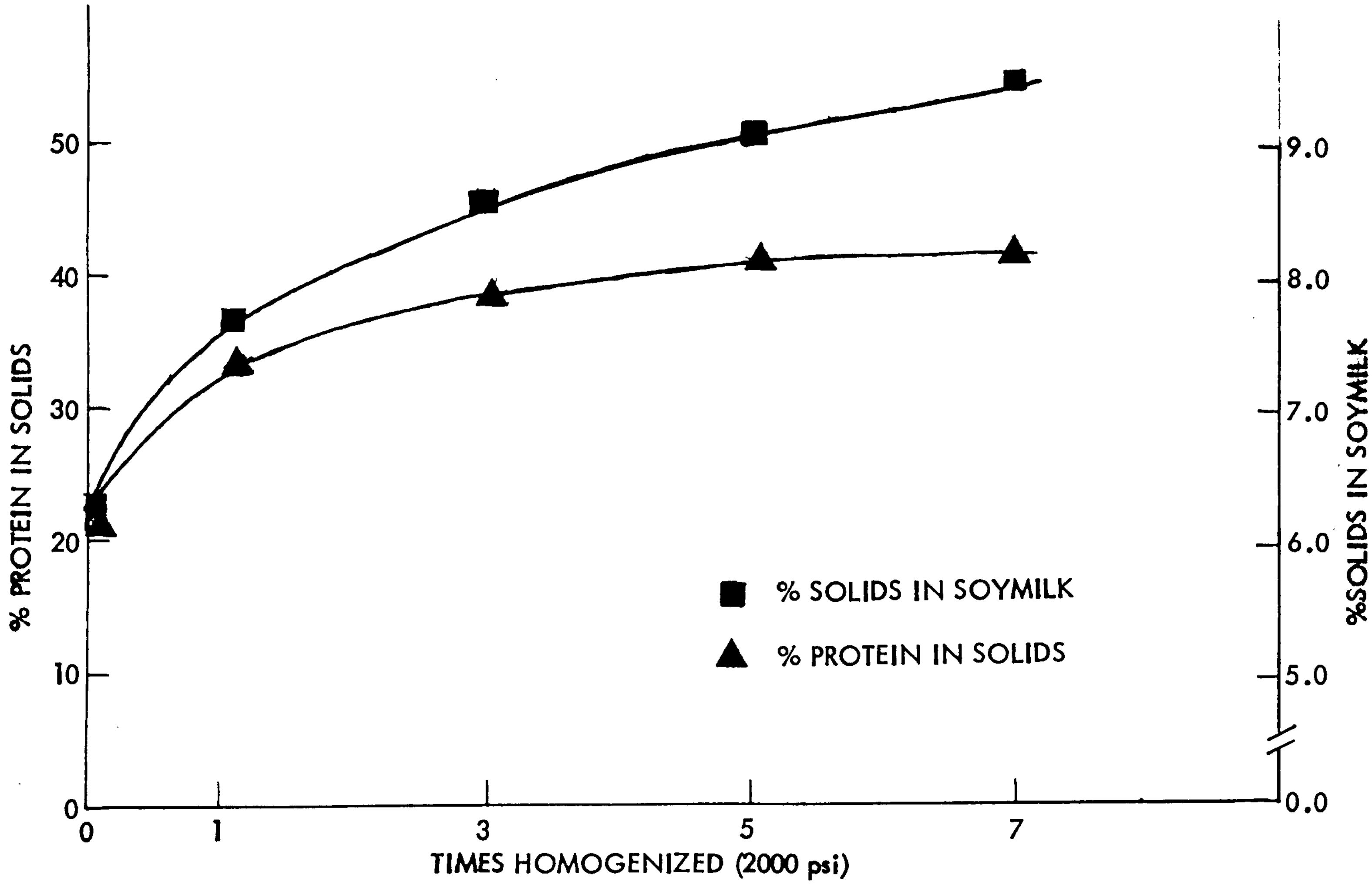
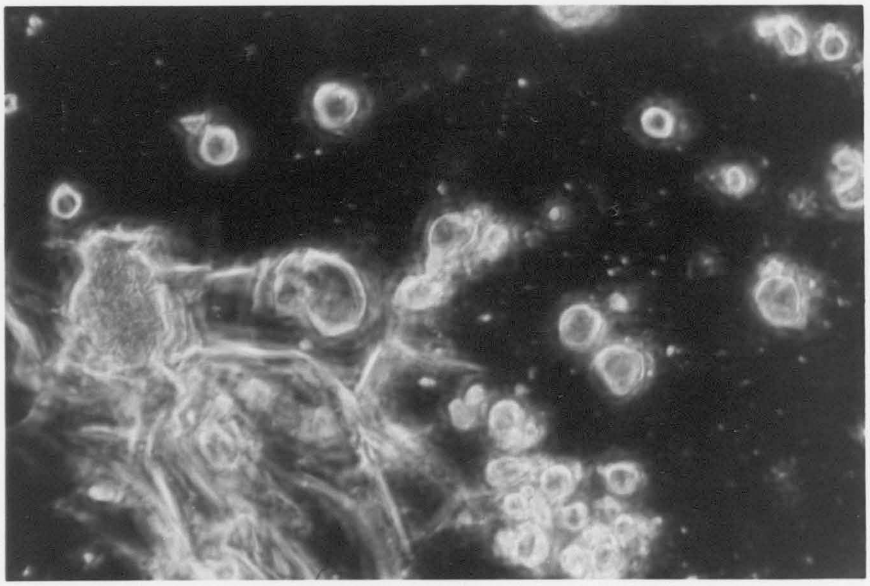
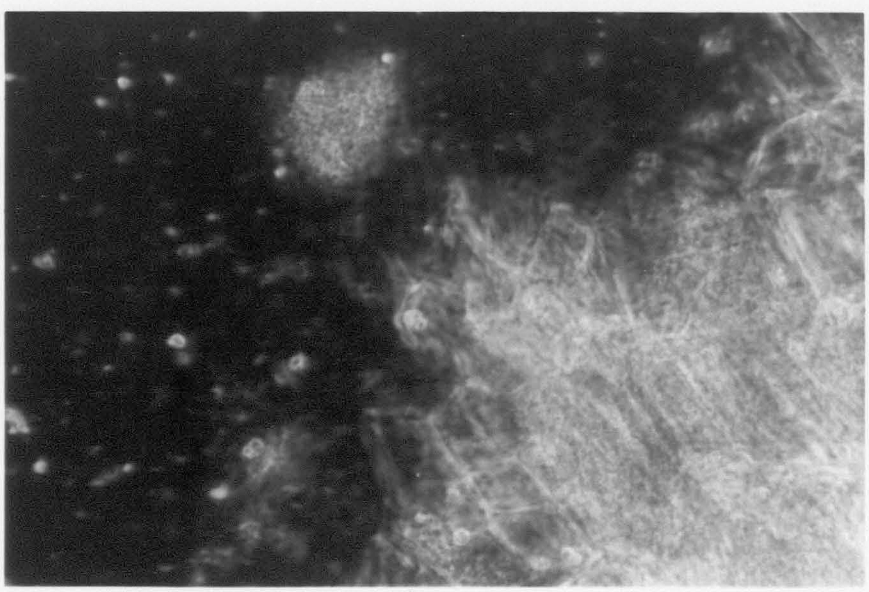

% PROTEIN IN SOLIDS
50
40
30
20
10
0
%SOLIDS IN SOYMILK
9.0
8.0
7.0
6.0
5.0
0.0
% SOLIDS IN SOYMILK
% PROTEIN IN SOLIDS
0
1
3
5
7
TIMES HOMOGENIZED (2000 psi)

Figure 8a. Photomicrograph of soy slurry prepared from soybeans boiled 5 min prior to grinding. The numerous inclusions about 10 μm in diameter are believed to be protein bodies (magnification 430x)

Figure 8b. Photomicrograph of soy slurry from unheated soybeans. No protein bodies are present in this preparation. Small grainy appearance may be due to spherosomes (magnification 430x)





bodies in unheated preparations.

Low speed centrifugation or filtering the soy slurry is effective for removal of the protein bodies from the soymilk prepared as in process A, heating prior to grinding.

While no quantitative methods were used to determine the number of protein bodies present in the soy slurry before and after homogenization, it is believed that the protein bodies were disrupted by the pressures used and that disruption of the protein bodies released protein in a form that remained in the soymilk rather than being removed by centrifuging.

These protein bodies appear much more sensitive to homogenization than the other constituents. The increase in protein was nearly equal to the increase in solids of the soymilk upon homogenizing and leveled off only when the percent protein of the solids was approximately equal to that found in the soybean.

The Effect of Homogenization Pressure and Soy Slurry Temperature on Yield and Solids Content of Soymilk

The objective of this experiment was to determine how homogenization pressure and the temperature of the soy slurry during homogenization effected the yield and solids content of the soymilk made by processes A, B, and C.

Percent soymilk, solids recovery and yield

The percent soymilk recovered was increased in all three processes by increasing homogenization pressures (Tables 4, 5, and 6). The amount of heat and when applied was an important factor in the percent soymilk recovered; decreases in the percent soymilk recovered from the soy slurry were observed with the 30 min heating time prior to grinding used in process A. Homogenization of the soy slurry hot, compared to the cold homogenization, slightly improved the recovery of soymilk as shown in process A and B (Tables 4 and 5), while decreasing the recovery in process C (Table 6), which had received no heating prior to or during grinding.

The increase in recovery of soymilk in all three processes was evident in the precipitate after centrifuging. The centrifuge speed was chosen as the lowest possible to pack the precipitate sufficiently to allow removal of the supernatant soymilk, while still high enough to give as little separation as possible upon refrigeration of the soymilk. The precipitate seemed to pack much more tightly at the higher homogenization pressures, probably due to the particle size being reduced by homogenization but not reduced sufficiently to prevent removal by centrifugation. Also, there was more moisture removed from the precipitate at higher homogenization pressures, which is indicated by the increase in soymilk recovered.

Table 4. Effect of hot and cold homogenization upon some constituents of solids, the percent soymilk, and yield in soymilk prepared by process A

Homogenization temperature	Homogenization pressure	Constituents of solids (%)		% soymilk	Yield
		CHO	Ash		
20-25 C	0 psi	28.24	6.13	51.4	23.5
	2000 psi	22.37	5.38	52.1	31.8
	4000 psi	20.12	5.38	54.7	38.9
	6000 psi	17.64	4.97	53.2	40.4
	8000 psi	18.65	4.85	57.4	45.6
70-75 C	0 psi	28.20	5.38	49.9	23.2
	2000 psi	20.73	4.89	52.6	37.6
	4000 psi	19.15	4.84	54.3	41.3
	6000 psi	19.23	4.70	56.1	44.9
	8000 psi	17.54	4.54	59.2	48.8

Table 5. Effect of hot and cold homogenization upon some constituents of solids, the percent soymilk, and yield in soymilk prepared by process B

Homogenization temperature	Homogenization pressure	Constituents of solids (%)		% soymilk	Yield
		CHO	Ash		
20-25 C	0 psi	26.47	5.74	48.4	43.4
	2000 psi	22.95	5.73	56.6	48.6
	4000 psi	21.44	5.82	62.6	55.1
	6000 psi	22.29	5.53	65.5	58.4
	8000 psi	22.35	5.44	68.1	61.5
70-75 C	0 psi	26.17	5.18	48.9	41.8
	2000 psi	22.97	5.04	57.9	49.5
	4000 psi	21.35	5.15	62.6	54.5
	6000 psi	23.64	5.04	64.4	56.9
	8000 psi	24.44	5.23	67.1	58.9

Table 6. Effect of hot and cold homogenization upon some constituents of solids, the percent soymilk, and yield in soymilk prepared by process C

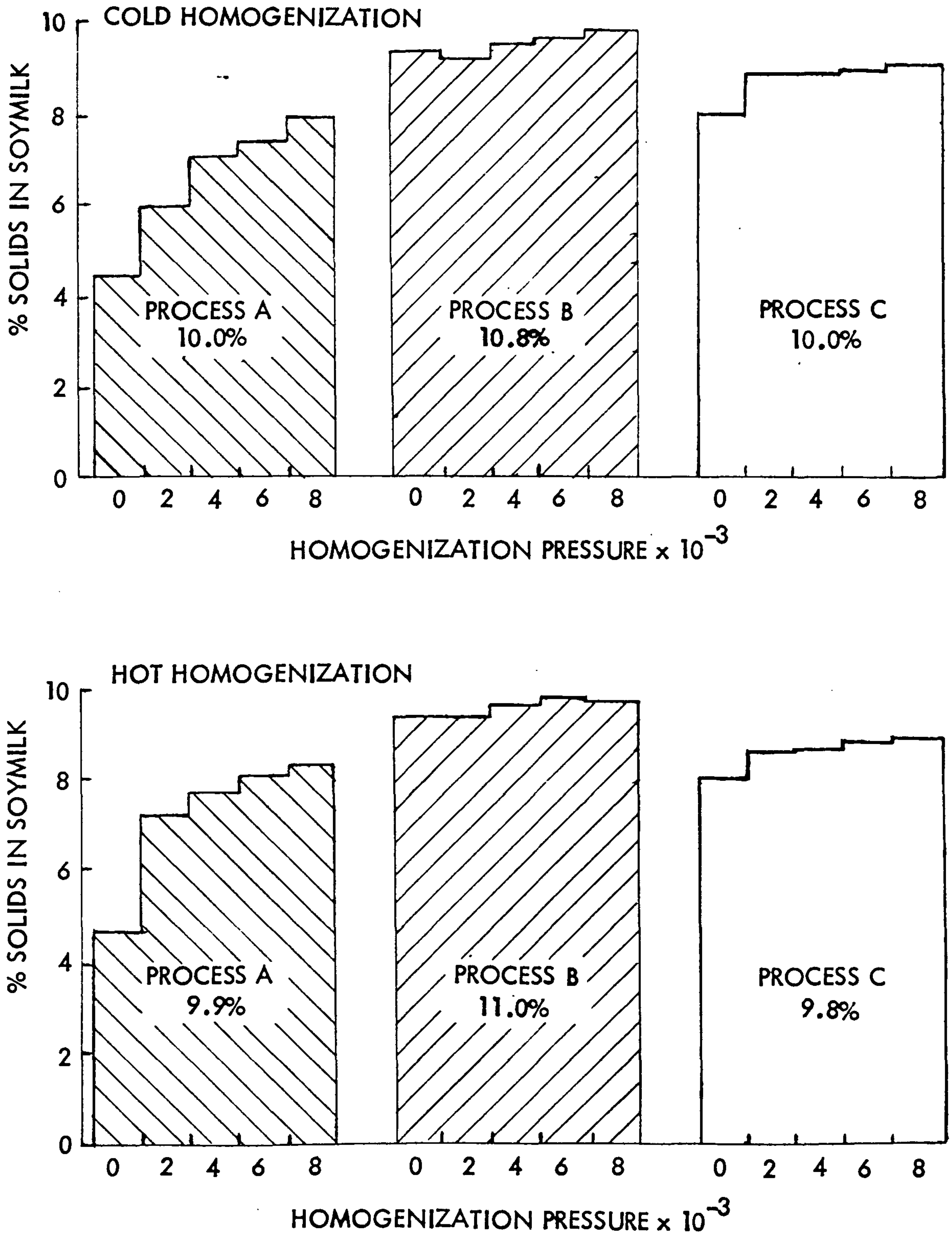
Homogenization temperature	Homogenization pressure	Constituents of solids (%)		% soymilk	Yield
		CHO	Ash		
20-25 C	0 psi	25.40	5.61	57.5	50.2
	2000 psi	30.30	5.29	68.4	60.9
	4000 psi	34.58	5.39	71.1	63.0
	6000 psi	37.54	5.27	72.4	64.6
	8000 psi	39.10	5.43	73.5	66.7
70-75 C	0 psi	24.83	5.67	59.6	49.3
	2000 psi	23.22	5.23	61.6	54.5
	4000 psi	24.83	5.23	66.5	59.6
	6000 psi	25.46	5.36	67.9	61.0
	8000 psi	27.47	5.44	68.7	62.0

The percent solids of the soymilk was nearly doubled, from 4.5% to 7.95% at 0 and 8000 psi, respectively, in process A by increased homogenization pressures (Fig. 9). Hot homogenization of the soy slurry from process A increased the solids content more than the cold homogenization at each pressure used with the largest increase in percent solids at 2000 psi (Fig. 9).

The percent solids in the soymilk only increased slightly with increasing homogenization pressures in processes B and C (Fig. 9). Hot homogenization was only slightly more effective than cold homogenization in increasing solids at all homogenization pressures in process B (Fig. 9), while decreasing the solids content of soymilk made by process C.

The yield of soymilk was increased in all three processes by homogenization (Tables 4, 5, and 6). In process A, the increase in yield was due to increases in both the solids recovery and the percent soymilk recovered with the increasing homogenization pressures. The increase in yield in processes B and C was due mainly to increases in the percent soymilk recovered with little change in the percent solids of the soymilk. Hot homogenization resulted in increased yields over cold homogenization in process A, the yield in process B remained about the same at the two homogenization temperatures and the yield decreased in process C with hot homogenization.

Figure 9. Effect of homogenization pressure and temperature upon soymilk solids using three different processes for preparing soymilk. The percent solids of the soy slurry is included with each process


COLD HOMOGENIZATION
% SOLIDS IN SOYMILK
10
8
6
4
2
0
PROCESS A
10.0%
PROCESS B
10.8%
PROCESS C
10.0%
0 2 4 6 8
0 2 4 6 8
0 2 4 6 8
HOMOGENIZATION PRESSURE x 10^-3
HOT HOMOGENIZATION
% SOLIDS IN SOYMILK
10
8
6
4
2
0
PROCESS A
9.9%
PROCESS B
11.0%
PROCESS C
9.8%
0 2 4 6 8
0 2 4 6 8
0 2 4 6 8
HOMOGENIZATION PRESSURE x 10^-3

Constituents of Soymilk

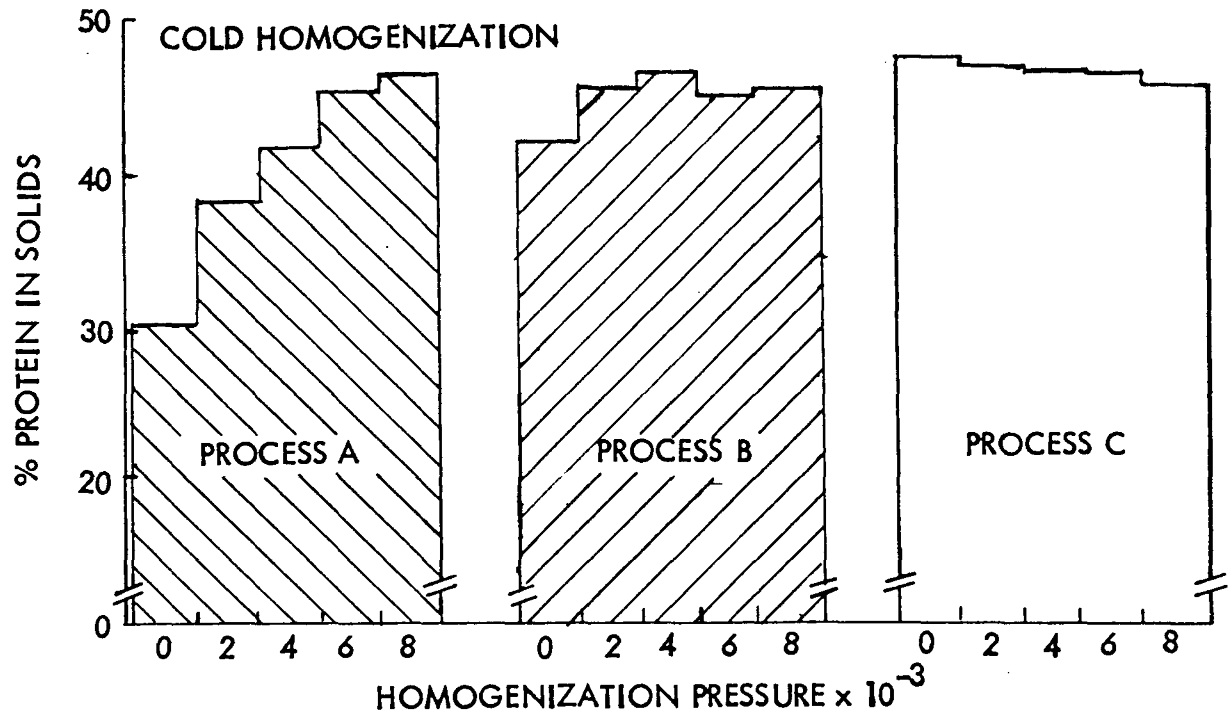
Protein

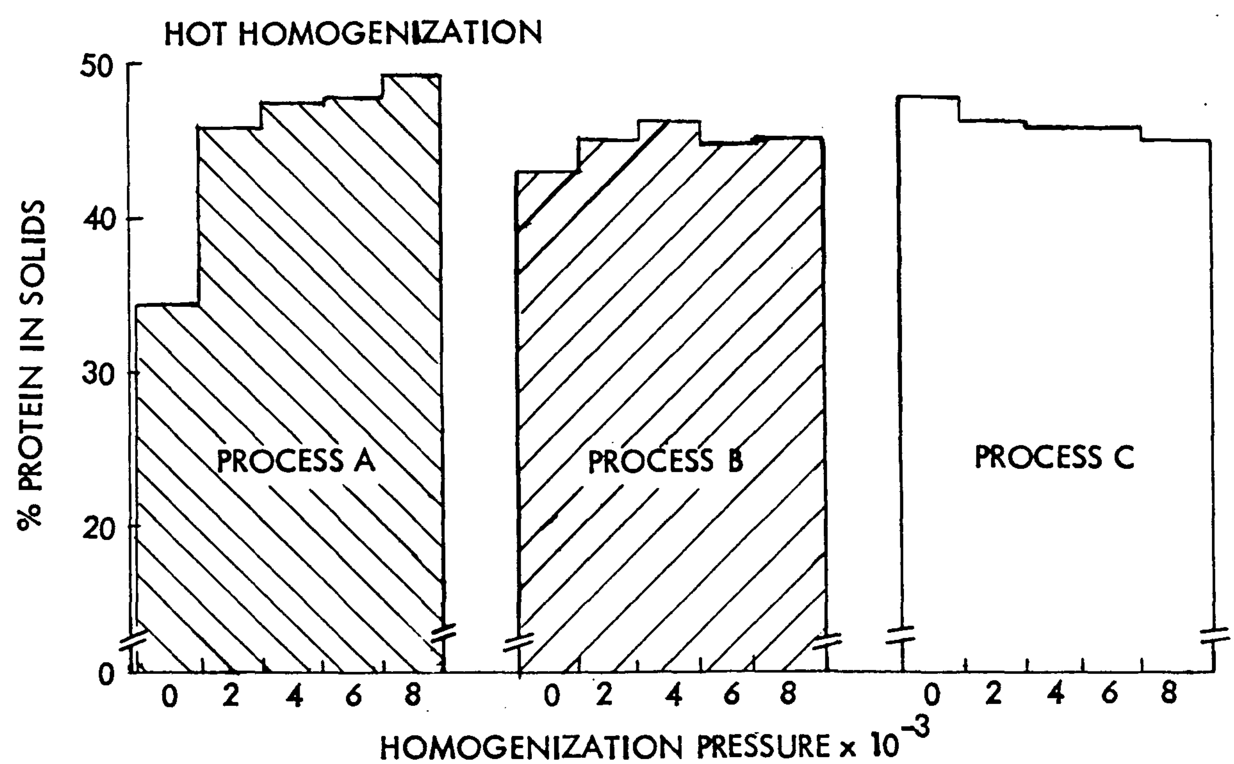
The protein content both quantitatively and qualitatively is an important consideration in the use of soymilk as a replacement for cow's milk and as an aid to improve nutrition in underdeveloped countries. Hackler et al. (1963) found the residue in comparison to soymilk yielded the higher PER and the more acceptable diet.

Homogenization was most effective in increasing the protein content of soymilk in process A, increasing the protein from around 30% of the solids to nearly 47% of the solids at 0 and 8000 psi, respectively (Fig. 10). This is an increase from 1.4 up to 3.7 g of protein per 100 g of soymilk, nearly a threefold increase in the protein content of the soymilk.

Hot homogenization of the soy slurry from process A resulted in better recovery of the protein at all pressures used compared to the cold homogenization. The protein recovery by hot homogenization over cold homogenization was increased by 0.26 g and 0.45 g per 100 g of soymilk at 0 and 8000 psi, respectively. The solids content increased by a similar amount, indicating that almost all of the increase due to high temperature homogenization is from the increases in protein of the soymilk. Here again the protein bodies are probably the major factor in determining the protein content of the soymilk. Apparently the hot homogenization increases their

Figure 10. Effect of homogenization pressure and temperature upon the percent protein of the solids using three different processes for preparing soymilk


COLD HOMOGENIZATION
% PROTEIN IN SOLIDS
50
40
30
20
0
PROCESS A
PROCESS B
PROCESS C
0 2 4 6 8
0 2 4 6 8
0 2 4 6 8
HOMOGENIZATION PRESSURE x 10-3


HOT HOMOGENIZATION
% PROTEIN IN SOLIDS
50
40
30
20
0
PROCESS A
PROCESS B
PROCESS C
0 2 4 6 8
0 2 4 6 8
0 2 4 6 8
HOMOGENIZATION PRESSURE x 10-3

susceptibility to breakage and aids in the dispersion of these bodies into the soymilk.

The protein as a percent of solids increased from 42% at 0 psi to a maximum of 46% at 4000 psi and then decreased to slightly below 45% at 8000 psi in soymilk prepared by process B (Fig. 10). Homogenization of the hot soy slurry showed little difference in protein recovery, with only a slight increase at both 0 and 2000 psi (Fig. 10).

Protein as a percent of solids decreased with higher homogenization pressures in soymilk prepared by process C (Fig. 10). The heating of the soy slurry prior to homogenization had little effect upon the percent protein of the solids, as shown in Fig. 10.

Homogenization is an effective method to increase the protein content of soymilk in processes requiring a heating period before grinding the soybeans. This is probably due to the effect of homogenization upon the protein bodies. Homogenizing the soy slurry while hot improved the protein recovery and allows for lower pressures during homogenization to be used.

Nelson et al. (1976) studied the effect of the temperature of homogenization upon soymilk made in a manner similar to process A, except no separation step of the soymilk from the precipitate was used. They noted less graininess in soymilks homogenized at higher temperatures and found that higher pressures were necessary when lower homogenization temperatures were used to produce a soymilk with low graininess and resistance to separation upon refrigeration.

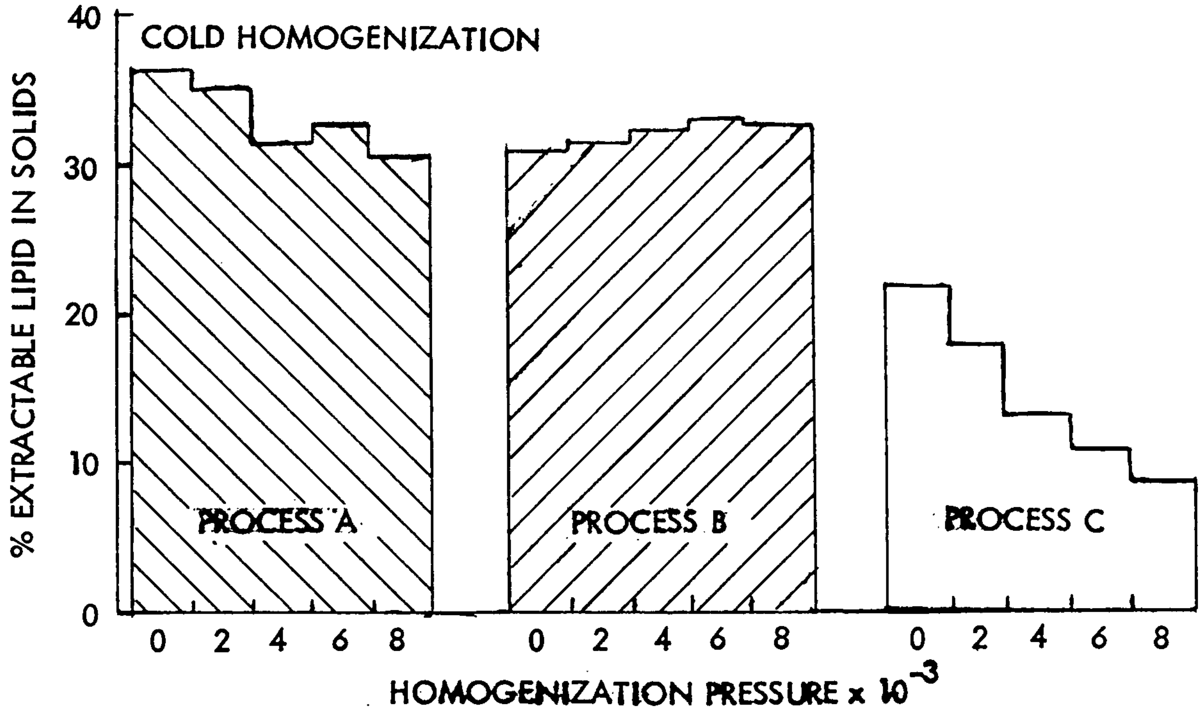
Lipid

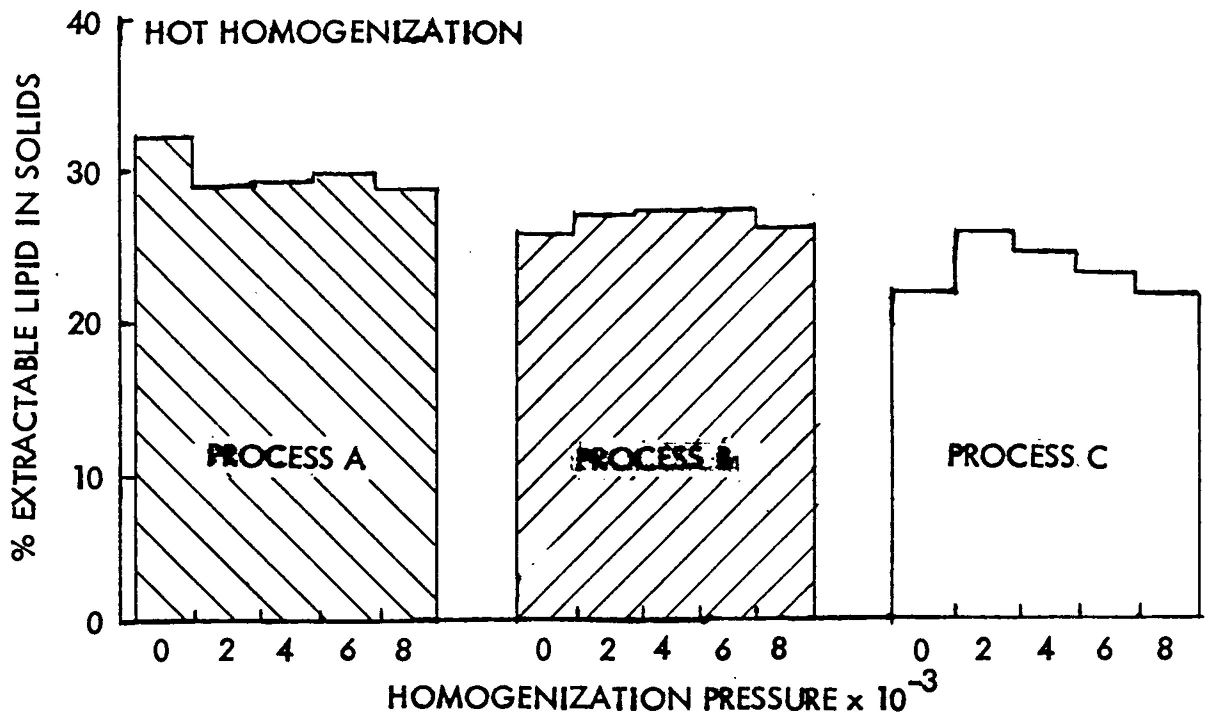
One of the principal uses for homogenization has been for the emulsification of lipids in dairy products.

Lipid as a percent of solids decreased by 4-5% with increasing homogenization pressures in process A using both cold and hot homogenization (Fig. 11), however since the solids content nearly doubled, a net increase in the percent lipid of the soymilk, from 1.5% to 2.7%, at 0 and 8000 psi respectively, did occur.

Nelson et al. (1976) found no extractable lipid, in soymilk made in a manner similar to process A, using hexane extraction with a Soxhlet extraction apparatus. This may have been due to the addition of sucrose to the soymilk and further homogenization to blend a palatable soymilk. Shultz et al. (1968) found addition of sugars to egg yolk before drying aided in the retention of the foaming ability upon rehydration of the egg yolk. This is believed to be due to sugars replacing the water of hydration of the lipoproteins during drying preventing the release of free lipids, which inhibit foaming ability. The sucrose may be acting in the soymilk in a similar way to coat the lipid during freeze drying and prevent its extraction by hexane.

Figure 11. Effect of homogenization pressure and temperature upon extractable lipid prepared by three different processes


COLD HOMOGENIZATION
% EXTRACTABLE LIPID IN SOLIDS
0
10
20
30
40
PROCESS A
PROCESS B
PROCESS C
0 2 4 6 8
0 2 4 6 8
0 2 4 6 8
HOMOGENIZATION PRESSURE x 10^-3


HOT HOMOGENIZATION
% EXTRACTABLE LIPID IN SOLIDS
0
10
20
30
40
PROCESS A
PROCESS B
PROCESS C
0 2 4 6 8
0 2 4 6 8
0 2 4 6 8
HOMOGENIZATION PRESSURE x 10^-3

The percent of lipid extracted in soymilk made by process C and homogenized cold decreased from 21% of the total solids at 0 psi to 8.5% at 8000 psi (Fig. 11). The extractable lipid in the high temperature homogenization series increased to a maximum of 25% at 2000 psi, then decreased to 21% at 8000 psi.

The decrease in extractable lipid at both temperatures can probably be attributed to increased emulsification of the lipid at higher homogenization pressures. The increase in lipid at 2000 psi with hot homogenization of the soy slurry is probably due to cell breakage and release of spherosomes, coupled with a decrease in the emulsification properties of the protein caused by the higher temperatures. The higher pressures were able to compensate somewhat for the decrease in emulsification due to heating, resulting in lower crude lipid extracted by the hexane. The emulsification of the lipid with increasing pressures in the samples homogenized cold may be responsible for the great decrease in the lipid extracted with higher homogenization pressures.

The hexane extractable lipid in soymilk made by process B remained nearly the same percent of solids at both temperatures of homogenization and all pressures used (Fig. 11).

The heat used in preparing soymilk is an important consideration in the percent lipid of the solids in the resultant soymilk. The highest percent lipid was found in those samples receiving the greatest heat treatment. This is due both to the lowering of other constituents of the solids, mainly protein, and to the increased emulsification of the lipid in samples receiving less heat treatment which lowers their extractability by hexane.

Ash

The ash content of the soymilk as a percent of solids remained fairly constant at about 5.5%, varying from 4.54 to 6.13%, in the three processes and at both temperatures and all homogenization pressures used (Tables 4, 5, and 6).

Carbohydrate

The carbohydrate content varied inversely with the sum of the protein and crude lipid content of the solids. This is to be expected since the carbohydrate content was determined by difference. The accuracy of the values reported in Tables 4, 5 and 6 is dependent upon the accuracy of the values obtained for the other constituents. In the case of the crude lipid, these values may be excessively low in some instances such as the cold homogenization at higher pressures in process C due to increased emulsification of the lipid and the inability of hexane to extract these lipids.

CONCLUSIONS

The processing conditions in preparing soymilk are important factors in determining the yield of soymilk, the constituents of the solids and the flavor of soymilk.

The primary factor limiting acceptability of soymilk is flavor. The use of heat to eliminate the off-flavors associated with soymilk can also result in decreased yields and lowered nutritional quality of the soymilk as reflected by the protein content.

Soaking soybeans is commonly used to soften the beans for more efficient grinding. Increasing the temperature of the soak water results in a faster hydration rate, while soaking in $NaHCO_3$ solution decreased the hydration rate at 21 C compared to a tap water soak at the same temperature.

Blanching soybeans was an effective method to eliminate the green, beany flavor in soaked soybeans. Shorter times of heating were sufficient with higher heating temperatures, with 100 C for 2 min the only effective temperature to eliminate both the green, beany flavor and to prevent development of bitter tastes during overnight refrigeration.

The ratio of soybeans to water during grinding had little effect upon the solids composition of soymilk but did cause changes in the concentration of solids in the soymilk. Increasing the water to soybean ratio was found to produce higher yields of soymilk, however the ratio of solids recovered in the soymilk from the soy slurry remained fairly constant, dependent only upon the process used in preparing the soymilk.

The absorbance of dilutions of soymilk at 400 nm can be used for reliable estimates of the solids content of soymilk, however changes in processing conditions will require the preparation of new standard curves for accurate estimates since the process changed both the slope of the curve and placement of the curve upon the graph.

Sonication will increase the solids content of soymilk that has been heated prior to grinding, but this may not prove to be of much practical value at present due to the relatively small gain of solids in the soymilk in comparison to the high energy and equipment requirements that would be necessary.

A 30 min boiling of the soybeans prior to grinding greatly reduced both the yield and the solids content as well as the percent protein of the solids in the soymilk. These losses can partly be corrected for by homogenization.

Much of the loss in solids and percent protein of the solids may be due to the fixation of the protein bodies by heating prior to grinding of the soybean. The protein bodies appear resistant to disruption during grinding, causing a large proportion of the protein to be lost into the precipitate upon centrifugation. The protein bodies in unheated soybeans on the other hand are easily disrupted. Homogenization appears to be an efficient method to disrupt the protein bodies causing a release of their protein content which remains in solution.

This phenomenon is an example of the deficiency of the term "denaturation" for describing certain protein changes. The general definition for protein denaturation is any irreversible change in the quaternary, tertiary or secondary structure of the protein and is generally associated with loss of protein solubility and function. The protein in the protein bodies does not appear to be denatured in the usual sense of the word when heated prior to grinding. Instead, the protein bodies are fixed and grinding is not sufficient to disrupt the structures. Homogenization may be solubilizing the protein by simply disrupting the protein body structure causing a release of the protein into the solution.

The use of homogenization to increase yields is most effective in systems where the solids content is low because of heating prior to grinding and is less effective when recovery is already high such as when unheated soybeans are used to prepare soymilk.

The use of hot homogenization would allow the use of lower homogenization pressures while still maximizing soy yields and solids recovery. The use of 70-75 C for the hot homogenization would allow for in-line processing of soymilk coupled to the pasteurization step in processing.

Processing conditions, especially the heat used in preparing soymilk, can greatly alter the soymilk. An understanding of how these conditions change the soymilk should allow the processor to adapt his product for the specific functional requirements of the consumer.

Further work needs to be done with protein bodies to better understand the effect of heat and how best to keep this source of protein soluble during processing. Also, lipid extraction with several different solvents should be done, and the lipid should be characterized to better understand the effect of processing on soymilk lipids.

LIST OF REFERENCES

A.A.C.C. 1969. A.A.C.C. Approved Methods. 7th ed. American Association of Cereal Chemists, St. Paul, Minn.

Albrecht, W. J., G. C. Mustakas, and J. E. McGee. 1966. Rate studies on atmospheric steaming and immersion cooking of soybeans. Cereal Chem. 43:400-407.

Al-Kishtaini, S. F. 1971. Methods of preparation and properties of water extracts of soybeans. Ph.D. Thesis. University of Illinois, Urbana, Ill.

Anderson, R. A. 1976. Acid sensitive proteins affect flavor. J. Food Sci. 41:293-296.

A.O.A.C. 1970. Official Methods of Analysis. 11th ed. Assoc. Offic. Agr. Chemists, Washington, D.C.

Arai, S., H. Suzuki, M. Fujimaki, and Y. Sakurai. 1966. Studies on flavor components in soybean. Part 2. Phenolic acids in defatted soybean flour. Agr. Biol. Chem. 30:364-369.

Arnold, J. B., J. D. Summers, and W. K. Bilanski. 1971. Nutritional value of heat treated whole soybeans. Can. J. Anim. Sci. 51:57-65.

Badenhop, A. F., and L. R. Hackler. 1970. The effects of soaking soybeans in sodium hydroxide solution as a pretreatment for soymilk production. Cereal Sci. Today 15:84-88.

Badenhop, A. F., and L. R. Hackler. 1973. Methionine supplementation of soymilk to correct cystine loss resulting from an alkaline soaking procedure. J. Food Sci. 38:471-473.

Badenhop, A. F., and W. F. Wilkens. 1969. The formation of 1-octen-3-ol in soybeans during soaking. J. Am. Oil Chem. Soc. 46:179-182.

Baker, E. C., and G. C. Mustakas. 1973. Heat inactivation of trypsin inhibitor, lipoxygenase and urease in soybeans: Effect of acid and base additives. J. Am. Oil Chem. Soc. 50:137-141.

Birk, Y., and A. Gertler. 1961. Effect of mild chemical and enzymatic treatment of soybean meal and soybean TI on their nutritive and biochemical properties. J. Nutr. 75:379-387.

Bohak, Z. 1964. Nε-(DL-2-amino-2-carboxyethyl)-L-lysine, a new amino acid formed on alkaline treatment of proteins. J. Biol. Chem. 239:2878-2887.

Bourne, M. C., E. E. Escueta, and J. Banzon. 1976. Effect of sodium alkalis and salts on pH and flavor of soymilk. J. Food Sci. 1:62-66.

Brookwalter, G. N., K. Warner, R. A. Anderson, G. C. Mustakas, and E. L. Griffin, Jr. 1975. Fortification of dry soybean-based foods with DL-methionine. J. Food Sci. 40:266-270.

Catsimpoolas, N., T. G. Campbell, and E. W. Meyer. 1968. Immunochemical studies of changes in reserve proteins of germinating soybean seeds. Plant Physiol. 43:799-805.

Christopher, J. P., E. K. Pistorius, and B. Axelrod. 1970. Isolation of an isozyme of soybean lipoxygenase. Biochim. Biophys. Acta 198:12-19.

Christopher, J. P., E. K. Pistorius, and B. Axelrod. 1972. Isolation of a third isozyme of soybean lipoxygenase. Biochim. Biophys. Acta 284:54-62.

Cowan, J. D., J. J. Rackis, and W. J. Wolf. 1973. Soybean protein flavor components: A review. J. Am. Oil Chem. Soc. 50:426A-444A.

Decock, A. 1974. Soy protein isolates in hypoallergic infant formulations and humanized milks. J. Am. Oil Chem. Soc. 51:199A-200A.

DeMan, J. M., D. M. Stanley, and V. Rasper. 1975. Composition of Ontario soybeans and soymilk. J. Inst. Can. Sci. Tech. Aliment. 8:1-8.

Dieckert, J. W., and M. C. Dieckert. 1976. The chemistry and cell biology of the vacuolar proteins of seeds. J. Food Sci. 41:475-482.

Fujimaki, M. 1969. Fundamental investigation of proteolytic enzyme application to soybean protein in relation to flavor. University of Tokyo, Japan, Dept. Agr. Chem., U.S. Dept. Agr., Project No. UR-All(40)8.

Fujimaki, M., M. Yamashita, S. Arai, and H. Kato. 1970. Plastein reaction. Its application to debittering of proteolyzates. Agr. Biol. Chem. 34:483-484.

Gini, B., and R. B. Koch. 1961. Study of a lipohydroperoxide breakdown factor in soy extracts. J. Food Sci. 26:359-364.

Gossens, A. E. 1974. Protein foods - flavors and off-flavors. Food Eng. 46:59-60.

Greuell, E. H. M. 1974. Some aspects of research in the application of soy protein in foods. J. Am. Oil Chem. Soc. 51:98A-100A.

Grosch, W., and G. Laskawy. 1975. Differences in the amount and range of volatile carbonyl compounds formed by lipoxygenase isoenzymes from soybeans. J. Agr. Food Chem. 23: 791-794.

Guss, P. L., T. Richardson, and M. A. Stahmann. 1967. The oxidation-reduction enzymes of wheat. III. Isoenzymes of lipoxidase in wheat fraction and soybean. Cereal Chem. 44:607-610.

Hackler, L. R., D. B. Hand, K. H. Steinkraus, and J. P. VanBuren. 1963. Comparison of the nutritional value of protein from several soybean fractions. J. of Nutr. 80: 205-210.

Hamad, N., and J. J. Powers. 1965. Inhibition and pectic content of canned dry lima beans. Food Technol. 19:216-220.

Hand, D. B. 1967. Formulated soy beverages for infants and preschool children. Proc. Int. Conf. Soybean Protein Food, Peoria, Ill., ARS-71-35.

Hansen, L. P., ed. 1974. Vegetable protein processing. Noyes Data Corp., Park Ridge, N.J.

Khaleque, A., W. R. Bannatyne, and G. M. Wallace. 1970. Studies on the processing and properties of soymilk. I. Effect of preprocessing conditions on the flavor and composition of soymilks. J. Sci. Fd. Agric. 21:579-583.

Koide, T., and T. Ikenaka. 1973. Studies on soybean trypsin inhibitors. 3. Amino-acid sequence of the carboxy-terminal region and the complete amino-acid sequence of soybean trypsin inhibitor (Kunitz). Eur. J. Biochem. 32:417-431.

Ku, S. 1972. Extraction of oligosaccharides in processing whole soybeans. Ph.D. Thesis. University of Illinois, Urbana, Ill.

Liener, I. E. 1953. Soyin, a toxic protein from the soybean. J. Nutr. 49:527-539.

Liener, I. E. 1958. Pages 83-92 in A. M. Altschul, ed. Processed plant foodstuffs. Academic Press, New York, N.Y.

Lo, W. Y., K. H. Steinkraus, D. B. Hand, L. R. Hackler, and W. F. Wilkens. 1968a. Soaking soybeans before extraction as it affects chemical composition and yield of soymilk. Food Technol. 22:1188-1190.

Lo, W. Y., K. H. Steinkraus, D. B. Hand, W. F. Wilkens, and L. R. Hackler. 1968b. Yields of extracted solids in soymilk as affected by temperature of water of various pretreatments of beans. Food Technol. 22:1322-1324.

Mattick, L. R., and D. B. Hand. 1969. Identification of a volatile compound in soybeans that contributes to the raw bean flavor. J. Agr. Food Chem. 17:15-17.

Mital, B. K., and K. H. Steinkraus. 1976. Flavor acceptability of unfermented and lactic fermented soymilks. J. Milk Food Technol. 39:342-344.

Mustakas, G. C., W. J. Albrecht, G. N. Brookwalter, V. E. Sohns, and E. L. Griffin, Jr. 1971. New process for low cost, high protein beverage base. Food Technol. 25:534-540.

Nelson, A. I., M. P. Steinburg, and L. S. Wei. 1976. Illinois process for preparation of soymilk. J. Food Sci. 41:57-61.

Noyes, R. 1969. Protein food supplements. Food processing review No. 3. Chemical Process Reviews. Noyes Development Corp., Park Ridge, N. J.

Odani, S., and T. Ikenaka. 1973. Studies on soybean trypsin inhibitors. VIII. Disulfide bridges in soybean Bowman-Birke proteinase inhibitor. J. Biochem. 74:697-715.

Osborne, T. B., and L. B. Mendel. 1917. The use of soy bean as food. J. Biol. Chem. 32:369-387.

Piper, C. V., and W. J. Morse, eds. 1923. The soybean. McGraw-Hill Book Co., Inc., New York, N.Y.

Pomeranz, Y., ed. 1976. Advances in cereal science and technology. American Assoc. of Cereal Chemists, Inc., St. Paul, minn.

Rackis, J. J. 1972. Pages 158-202 in A. K. Smith and S. J. Circle, eds. Soybeans: Chemistry and technology. Vol. 1. AVI Publishing Co., Westport, Conn.

Rackis, J. J. 1974. Biological and physiological factors in soybeans. J. Am. Oil Chem. Soc. 51:161-174.

Rackis, J. J., D. J. Sessa, and D. H. Honig. 1967. Isolation and characterization of flavor and flatulence factors in soybean meal. Proc. Int. Conf. Soybean Protein Food, Peoria, Ill., ARS-71-35.

Restrepo, F., H. E. Snyder, and G. L. Zimmerman. 1973. Calcium activation of soybean lipoxygenase. J. Food Sci. 38: 779-782.

Rice, E. E. 1970. Nutritive value of oilseed proteins. J. Am. Oil Chem. Soc. 47:408-413.

Sessa, D. J., K. Warner, and D. H. Honig. 1974. Soybean phosphatidylcholine develops bitter taste on autoxidation. J. Food Sci. 39:69-72.

Sessa, D. J., K. Warner, and J. J. Rackis. 1976. Oxidized phosphatidylcholine from defatted soybean flakes taste bitter. J. Agric. Food Chem. 24:16-21.

Shultz, R. J., H. E. Snyder, and R. H. Forsythe. 1968. Co-dried carbohydrates effect on the performance of egg yolk solids. J. Food Sci. 33:507-513.

Smiley, K. L., D. E. Hensley, and H. J. Gasdorf. 1976. Alpha-galactosidase production and use in a hollow-fiber reactor. Appl. Environ. Microbiol. 31:615-617.

Smith, A. K., and S. J. Circle, eds. 1972. Soybeans: Chemistry and technology. Vol. 1. AVI Publishing Co., Westport, Conn.

Snyder, H. E. 1973. A simple technique for inhibiting production of green, beany flavor in soybeans. Korean J. of Food Sci. and Technol. 5:33-35.

Steinkraus, K. H., L. T. David, L. J. Ramos, and J. Banzon. 1968. Development of flavored soymilks and soy/coconut milks for the Philippine market. Phil. Agr. 52:268-276.

Sugimoto, H., and J. P. Van Buren. 1970. Removal of oligosaccharides from soymilk by an enzyme from Aspergillus saitoi. J. Food Sci. 35:655-660.

Tombs, M. P. 1967. Protein bodies of the soybean. Plant Physiol. 42:797-813.

Tsui, E. Y. 1947. Design of a small electric mill for soybean processing. M.S. Thesis. Iowa State University, Ames, Ia.

Turner, R. H., and I. E. Liener. 1975. The effect of selective removal of hemagglutinin on the nutritive value of soybeans. J. Agric. Food Chem. 23:484-487.

Van Buren, J. P., K. H. Steinkraus, L. R. Hackler, I. El Rawi, and D. B. Hand. 1964. Heat effects on soymilk. Indices of protein quality in dried soymilks. Agr. Food Chem. 12: 524-528.

Wagner, J. R., A. C. Olson, R. Becker, and S. Kon. 1975. Process for increasing digestibility of legume seeds. U.S. Pat. 3,876,807.

Wallace, G. M., W. R. Bannatyne, and A. Khaleque. 1971. Studies on the processing and properties of soymilk. II. Effect of processing conditions on the trypsin inhibitor activity and the digestibility in vitro of proteins in various soymilk preparations. J. Sci. Food Agric. 22:526-531.

Wang, L. C. 1975. Ultrasonic extraction of proteins from autoclaved soy flakes. J. Food Sci. 40:549-551.

Weisburg, S. M. 1974. Nutritional experience with infant formulas containing soy. J. Am. Oil Chem. Soc. 51:204-207.

Wilding, M. D. 1970. Oilseed proteins. Present utilization patterns. J. Am. Oil Chem. Soc. 47:398-401.

Wilding, M. D., D. E. Alden, and E. E. Rice. 1968. Nutritive value and dietary properties of soy protein concentrates. Cereal Chem. 45:254-259.

Wilkens, W. F., L. R. Mattick, and D. B. Hand. 1967. Effect of processing method on oxidative off-flavors of soymilk. Food Technol. 21:86-89.

Wolf, W. J. 1967. Trypsin inhibitors, hemagglutinins, saponins and isoflavones of soybeans. Proc. Int. Conf. Soybean Protein Food, Peoria, Ill., ARS-71-35.

Wolf, W. J. 1970. Scanning electron microscopy of soybean protein bodies. J. Am. Oil Chem. Soc. 47:107-108.

Wu, A. C. M., R. R. Eitenmiller, and J. J. Powers. 1975. Effect of fluctuating temperature treatment on milk coagulation and inactivation of soybean trypsin inhibitor. J. Food Sci. 40:1171-1173.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. Harry E. Snyder for his guidance, help and constructive criticism during the progress of this investigation.

Thanks are also due to David Moon and Young Sook Chang for their assistance and interest. Furthermore, I am indebted to Dr. Peter Riley and the Chemical Engineering Department for allowing me to use their laboratory scale homogenizer.

Also, the author wishes to express his appreciation and gratitude to his wife, Jean, and daughter, Tracey, for their patience, understanding and assistance during my tenure at I.S.U.