EFFECTS OF PROCESSING AND STORAGE ON SELECTED NUTRIENTS IN SOYMILK

by

KYUNG SOOK HONG

B.S. in Home Economics, Dongguk University

Seoul, Korea, 1979

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

FOOD SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

Approved by:

Martha B Stone

Major Professor

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ABSTRACT

Soymilk was extracted by traditional and Rapid Hydrothermal Cooking (RHHTC) methods. Bottled soymilk (240 ml) was placed in refrigerated storage (3-5°C) for 22 days and analyzed at predetermined times. Soymilk processed by the two methods showed no definite trends in amino acid composition after storage. Thiamine and riboflavin contents in soymilk processed by the RHHTC method were lower than in soymilk from the traditional extraction. No significant changes occurred in thiamine content during storage of soymilk produced by either method, although riboflavin content decreased slightly. Values for viscosity of soymilk processed by the traditional method were lower than those for RHHTC-processed soymilk, although total solids contents of the soymilks were similar. RHHTC-produced soymilk was darker, less green and more yellow than traditionally extracted soymilk. No significant changes in color values occurred after storage. No significant bacterial growth was evident for either extraction method after 8 days of storage. After 12 days of storage, the microbial load increased ($p<0.05$) for the RHHTC-extracted soymilk, while the traditionally produced soymilk maintained low bacterial growth after 22 days of storage.

INTRODUCTION

Soybeans are excellent sources of high quality, inexpensive protein. Because of their high protein content and low cost, soybeans may be featured prominently in the diets of people in protein-deficient countries (Bourne, 1970).

One of the simplest products made from soybeans is a water extract, which is usually called "soymilk" because of its milky appearance (Bourne, 1970). Soymilk is extensively used as a substitute for bovine or human milk in areas where bovine milk is expensive or unavailable and also for infants who are allergic to animal milk (Johnson et al., 1981). Soymilk usually contains from 1.5-3.0% protein and its profile of essential amino acids, except for methionine, is similar to that of cow's milk (Smith and Circle, 1972). Soybeans contain no lactose, the milk sugar to which many people are intolerant.

Although soymilk is a nutritious, low-cost protein food for people in developing countries, the unpleasant flavor of soymilk has been the principal obstacle to its widespread acceptance. It was found that the enzyme lipoxidase or lipoxygenase which is naturally present in the soybean was the cause of the flavor problem (Bourne, 1970).

Traditionally, soymilk is made by immersing soybeans in water for several hours until they are saturated and then grinding the beans with water. The slurry of beans and water is strained or filtered to remove the insoluble residue. The resulting milk is heated to boiling for 30 min to improve nutritional value and flavor (Smith and Circle, 1972; Smith, 1949).

Recently Johnson et al. (1981) developed a process to prepare soymilk from whole soybean flour. They reported up to 86% solids and 90% protein yields using a continuous steam-infusion cooking process at 154°C, neutral pH, and a holding time of 34 seconds. Use of the rapid-hydration hydrothermal cooking (RHHTC) technique minimized off-flavor development and the resulting milk tasted bland (Johnson et al., 1981).

In this study the effects of processing and storage on amino acids, thiamine and riboflavin of soymilk were analyzed to provide a better understanding of differences in nutritional quality that might be caused by differences in heating in traditional and rapid-hydration hydrothermal cooking processes.

REVIEW OF LITERATURE

Soybeans first appeared as a domestic food crop in the eastern half of North China about the 11th century B.C. (Hymowitz, 1970). Soybeans were mentioned first in American literature in 1804 by Mease who encouraged their cultivation in Pennsylvania (Dies, 1942). However, cultivation of soybeans in the United States did not occur until after World War I. Food oil shortages at that time prompted the development of U.S. production of soybean processing plants.

In the late 1940's and early 1950's the U.S. became the world's leading producer of soybeans (Dies, 1942). In 1975 41.4 million metric tons of soybeans were produced in the U.S. which was 66% of the world total (Anonymous, 1976).

Beginning in the late 1930's, the high protein soybean meal by-product from oil extraction was used as animal feed (Smith and Circle, 1972). Currently soybean meal, soy flour, soy protein concentrates, soy protein isolates, textured and modified soy proteins are marketed and used in food products.

Considerable interest in protein from soybeans has developed for several reasons. Soybeans can be grown in a variety of soils and under a wide range of climate conditions. The yield of edible protein is of high nutritional quality (Mustakas et al., 1971). The proximate composition of soybeans and seed parts is shown in Table 1.

Raw soybeans are unpalatable because of their bitter characteristic. They have a low nutritive efficiency because of the presence of trypsin inhibitor(TI), hemagglutenins and other undetermined factors (Mustakas et al., 1971). Soybeans are an excellent source of low-cost protein. However, flavor of processed soybeans is not as acceptable to people in the Western world as it is to Asians. Beany flavors and indigestible components are obstacles that must be overcome before there is more widespread use of soybeans (Eley, 1968). Soybean foods generally are consumed after they have been subjected to some degree of heat treatment. Heat processing enhances palatability and acceptance and improves the nutritive quality of these

Table 1 – Composition of soybeans and seed parts[a]

Fraction	Protein(Nx6.25) (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Whole bean(100%)	40	21	34	5
Seed coat(8%)	9	1	86	4
Cotyledon(90%)	43	23	29	5
Hypocotyl(2%)	41	11	44	4

[a]Moisture-free basis

Source : Kawamura, 1967

foods (Angeles and Marth, 1970). Factors such as lipoxygenase are associated with oxidized, painty, off-flavor compounds. Trypsin inhibitors, which can reduce nutritional quality of the protein, are relatively heat labile. Adequate heat treatment has been found to overcome both problems (Rackis et al., 1970).

History of soymilk

Soymilk, traditionally a Chinese staple produced for many centuries in the home or on the village level, is growing in popularity as a beverage throughout the world. Historically, soymilk production can be divided into four major periods (Shurtleft and Aoyagi, 1984).

Ancient times to 1899. The earliest reference to soymilk appeared in China in the Lun Heng by Wang Chung. The first reference by a European to the use of soymilk as a beverage was in 1866 by the Frenchman, Paul Champion. The first reference to soymilk in the United States or in England was by Trimble in 1896.

1900-1952. Scientific interest developed in soymilk, its nutritional value, and its use for feeding infants in China or those allergic to cow's milk in the west. Li Yu-Ying, a Chinese citizen living in Paris, was the first real soymilk pioneer in the west. In 1910 he started the world's first soy dairy and also was granted the first patent (British) for soymilk production. In 1916 or 1917 soymilk was first produced commercially in the United States by J.A. Chard, Soy Products in New York City. It was called Soy Lac. In 1929 Mead Johnson produced the world's first commercial soy-based infant formula, Sobee. In 1939, Harry Miller began making Soya Lac filtered soymilk in the United States at Mt. Vernon, Ohio.

1953-1965. In the early 1950's soymilk entered the modern era. Pioneering work was done by K.S.Lo with Vitasoy in Hong Kong and by Yeo Hiap Seng in Singapore. Soymilk was marketed in bottles like soft drinks in both locations in 1953 and 1954, respectively. In 1956 Worthing Foods introduced instant soya meal, the world's first soymilk made from soy protein isolate. Then in 1965 Mead Johnson introduced Prosobee the first true infant formula based on soy protein isolates. It was a great improvement over the earlier product based on soy flour, and this quickly led to a vast worldwide expansion of nutritionally balanced formulas for infants that were allergic to cow's milk.

1966 to the 1980's. In 1966 three scientists at Cornell University discovered the reason that traditional soymilk has what many Westerns describe as a "beany" flavor. They also discovered a process to eliminate this flavor. Another breakthrough came in 1967 in Singapore, where soymilk was first packaged in tetra pack cartons. This made it possible to market soymilk in a colorful, disposable container that gave a shelf life of 6 months or more without refrigeration.

By the mid-1970's all of these factors caused the world to make a strong commitment to manufacturing and marketing soymilk. By the early 1980's soymilk consumption through East Asia was skyrocketing, making it one of the region's fastest growing and most highly regarded food products. Soymilk was unquestionably the world's fastest growing soy food.

In recent years, large-scale production has evolved along with commercial marketing of soymilk. An overview of the world soymilk market on a country-by-country basis, is shown in Table 2. Only countries for which there are reasonably good statistics are listed.

Soymilk extraction methods

Traditional method. Traditional soymilk is processed from whole dry soybeans. Beans are soaked in cold water overnight and ground with added water to form a slurry. Additional water is added to the slurry to obtain the desired concentration (usually a bean : water ratio of 1 to 8 by weight), and filtered to remove the insoluble residue. Then the slurry is boiled 20-30 min to improve nutritional value and flavor. Approximately 60% of the whole soybean is recovered in milk made by this process (Smith and Circle, 1978).

Erickson (1983) showed that enzymes can improve the yield of soymilk in production when compared to the traditional procedure. Best results were obtained with a neutral proteinase, "Neutrase", which increased protein and solids yields from 33 and 42%, respectively, to 73 and 66%, respectively, when added at 0.5% .

The acceptance of soymilk produced by the traditional

Table 2 — Overview of world soymilk production in 1983

Rank	Country	Soymilk production (Million liters)	Population (Millions)	Consumption Yearly Per Capita (liters)	Annual Growth (%)
1	Taiwan	210.0	19	11.1	30
2	Hong Kong	39.1	5.2	7.5	10
3	Singapore	11.2	2.5	4.7	15
4	S. Korea	67.0	42	1.60	60
5	Malaysia	21.4	14	1.53	-
6	Japan	131.8	120	1.10	101
7	Thailand	50.0	50	1.00	-
8	USA	9.6	232	0.04	-
	Total	548.3			50

Source: Shurtleft and Aoyagi, 1984.

process has been limited in non-Oriental 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 the undesirable flavors and odors are caused by the lipoxygenase enzyme system. When soybeans containing active lipoxygenase are ground in the presence of sufficient moisture and oxygen the enzyme acts on unsaturated fatty acids, primarily linoleic and linolenic (Wolf and Cowan, 1975). The ultimate result of enzyme activity is the formation of numerous volatile lipid-decomposition products, some of which are related to the beany, painty flavors of soymilk (Wolf and Cowan, 1975; Wilkens and Lin, 1970; Mattick and Hand, 1969).

Numerous modifications of the traditional process have been reported to improve flavor. In recent years, several different processes have been developed.

<u>Hot water extraction</u>. Hot water extraction of soybean solids for soymilk was developed by a group at Cornell University (Bourne, 1970). This was accomplished by taking beans that had been soaked 5 to 10 hours at room temperature and grinding with boiling water. The critical condition during grinding was maintenance of a temperature of at least 80°C in the grinder at all times. After grinding, the slurry was filtered and the filtrate held at boiling for 30 - 60 min to inactivate the trypsin inhibitor. The beverage was then formulated with sugar and flavoring. The final product was reported to be

free from lipoxygenase induced off-flavors. Heating the product after grinding or heating the beans before grinding was reported to be unsatisfactory for this process. Lipoxygenase was too active for post-grinding heating to be of any value in prevention of off-flavors. Heating the beans before grinding altered the protein in such a manner that it would be filtered out resulting in low protein recovery.

Al-Kishtaini (1971) observed reduction of yield with temperature above 75°C and stated that lipoxygenase activity was present in soymilk extracted below 75°C. Lo et al. (1968) and Wilkens and Hackler (1969) found that maximum yield of soymilk and solids was achieved by extraction in the range of 50 to 75°C. At grinding temperatures above 75°C, significant reduction in yield occurred. This was attributed to reduced filtration caused by protein gelation.

Bourne et al. (1976) reported that soymilk made correctly by a boiling-water grind process was free of strong beany flavor and had a faint, pleasant cereal-like flavor. They studied the effects of adding various sodium salts on pH and flavor of soymilk. They found that sodium hydroxide treated soymilk at pH adjusted to 7.5 was more acceptable than alkali-free soymilk or soymilk treated with sodium bicarbonate or sodium carbonate at similar pH's. The investigators concluded that acceptability was not directly related to pH and hypothesized that sodium ion concentration was responsible for differences in acceptability.

Acid extraction. Al-Kishtaini (1971) produced a bland soymilk by extracting soaked dehulled soybeans at 25°C with water adjusted to pH 3.0 or below. Lipoxygenase inactivation at low pH's was shown to produce maximum yields. Lao (1971) found that extraction at pH 2.0 resulted in soymilk free of paint flavor, but with a slight beany flavor. He indicated that this flavor was inherent to soybeans and not the result of lipoxygenase activity. There were disadvantages of this process, also. A salty off-flavor was detected in the beverage and the suspension stability was not as good as soymilk produced by hot water extraction process (Al-Kishtaini, 1971).

Alkaline extraction. Al-Kishtaini (1971) prepared soymilk at alkaline pH (9.5) and 25°C. Compared to acid extraction, lipoxygenase was irreversibly inactivated at acid pH; however, at alkaline pH the soymilk showed high lipoxygenase activity. It was postulated that at pH 9.5 the enzymes responsible for breakdown of intermediate products of lipoxygenase-catalyzed reactions were inactivated so that the volatile compounds responsible for oxidized flavors were not formed (Hung, 1984). Yields were 75% total solids, 80% protein and 80% fat by this extraction, which was better than the other extraction methods discussed thus far.

Non-extraction methods. Non-extraction processes include the whole soybean or the cotyledon without filtering insoluble materials. The major advantage of non-extraction processes is higher total solid yields and higher recovery of protein from soybeans.

Hand et al. (1964) developed two similar methods for making soymilk. Prior to soaking cotyledons, the soybeans were heated in dry air at 104°C for ten min and steamed. They were dehulled and cotyledons were soaked in water overnight (3:1), steamed for 45 min at 100°C and ground in a hammer mill. The 16% solids slurry was homogenized at 2000 psi, and spray dried. For another procedure the soybeans were steamed at 100°C for 45 min, dried for 10 min at 104°C, dehulled, and ground. The slurry was adjusted to 10% solids with 60°C water, homogenized at 2000 psi, and spray-dried. Nelson et al. (1975) indicated that both procedures produced soymilks that had poor mouthfeel and suspension stability.

Lo (1971) studied soy beverages containing full fat soy flour that were made by flaking, extruding, and grinding cotyledons into a flour. The flour was dispersed in water with carrageenan added as a stabilizing agent. The suspension was cooked, homogenized at 8000 psi and bottled. Nelson et al. (1975) reported that soymilk made by this process had excellent suspension stability and good mouthfeel.

Nelson et al.(1976,1975) developed a process for preparation of soymilk from the whole soybean or soybean cotyledons. Whole beans were soaked and then blanched in boiling 0.5% sodium bicarbonate ($NaHCO_3$) solution until the desired tenderness was achieved. Cotyledons were blanched in 0.25% $NaHCO_3$ without prior soaking to inactivate lipoxygenase and the anti-nutritional trypsin inhibitor. Hydrated beans were ground with tap water, heated, and homogenized at 82°C and 3500 psi. By this method, about 89% of the whole soybean and 95% of the protein were dispersed in the beverage. A disadvantage of this process was the high beverage viscosity.

Direct steam-infusion cooking methods. Johnson et al. (1981) prepared soymilk from whole soybean flour by a continuous steam-infusion cooking process at 154°C, neutral pH and a holding time of 34 seconds. Solids yield was 86% and protein yield was 90% with this method (Table 3). They explained their high yields as a result of optimum heat treatment and extreme shear encountered in steam-infusion and flashing since a certain amount of heat is required to dissociate the protein bodies leading to increased solubility and emulsification.

Problems with soymilk

Lipoxygenase. Much research has been devoted to identifying and solving the off-flavor problems associated with soy products. The extent to which undesirable flavors are inherent to the bean and those produced during processing have not been resolved completely. Generally, it is accepted that soy products generate significant off-flavors during processing, particularly when nonheat-treated soy flour contacts water.

The major objectionable flavor that has been described as "green bean-like" flavor is not present in the original intact raw soybean but develops immediately upon maceration. A number of researchers (Nelson et al., 1976; Nelson et al., 1971; Wilkens et al., 1967) believe that the flavor is produced when the

Table 3 - Properties of soymilk processed by steam-infusion cooking temperatures to inactivate 92-93% original TI activity

Cooking conditions			Soymilk		
pH	Temp (°C)	Time (sec)	Fraction yield (%)	Solids yield (%)	Protein yield (%)
6.7	99	3600	72.1	61.2	73.2
6.7	121	280	74.1	66.41	76.6
6.7	132	165	77.1	71.6	77.9
6.7	143	100	88.0	84.0	88.0
6.7	154	40	90.2	86.0	89.4
9.5	99	150	76.6	66.2	77.2
9.5	121	80	71.6	63.2	72.3
9.5	143	40	80.5	75.3	80.6

a % soymilk fraction yield= $\frac{\text{wt of supernatant}}{\text{wt of cooked slurry}} \times 100$

b % solids yield=

% soymilk fraction yield x $\frac{\text{\% solids in supernatant}}{\text{\% solids in soy slurry}}$

c % protein yield=

% soymilk fraction yield x $\frac{\text{\% protein in supernatatant}}{\text{\% protein in soy slurry}}$

Source: Johnson et al., 1981.

cell tissue is disrupted or damaged in the presence of moisture. The resulting off-flavor is caused by lipoxygenase activity and occurs almost instantaneously. Nelson et al.(1976,1971) believe lipoxygenase activity occurs during grinding of dry soybeans.

Mustakas et al. (1969) stated that lipids in cracked and dehulled soybeans rapidly oxidize after activation of the lipoxygenase system by increasing the moisture to 20%. Steaming, blanching, and toasting of raw soybeans prior to grinding or flaking are effective methods of inactivating lipoxygenase and are employed commercially (Johnson, 1978). However, those methods have their disadvantages and are not amenable to all food applications. Functional properties may be altered, protein solubility and dispersibility decreased, color darkened, and a typical "nutty" flavor developed. These changes are incompatible with most food applications (Wolf and Cowan, 1975).

Trypsin inhibitors. Osborne and Mendel (1917) were first to observe that raw whole soybeans had to be cooked for several hours before they would support the growth of rats. Nutritional quality increased with heat treatment until a point was reached where significant protein degradation occurred. This increased feeding efficiency has been attributed to two factors: increased susceptibility to proteolytic enzymes and inactivation of trypsin inhibitors (Osborne and Mendel, 1917).

Soy protein cannot be digested completely until the interior tertiary structure is destroyed as with heat treatment. The nutritional quality of properly heat-treated soybean is also inversely proportional to the TI content (Liener, 1969; Borchers et al., 1948). Rackis (1974) showed that less soybean-trypsin activity is needed to cause pancreatic hypertrophy than to retard growth in a rat being fed raw soybean meal. TI was inactivated with heat in whole soybeans at 20% moisture within 15 min by atmospheric steaming (Albrecht et al., 1966). Borchers et al. (1947) showed that 28% of the native TI of soybean meal remained after 4 hrs of dry oven heat at 275°F (135°C) while all inhibitors were inactivated after steaming for 90 min. Heating the milk at 200°F (93°C) for 30-75 min or at 250°F (121°C) for 5-10 min can destroy 90% of the native TI activity according to several researchers (Hand et al., 1967; Hackler et al., 1965; Van Buren et al ., 1964). Hackler et al. (1965) showed also that maximum PER of soymilk was obtained where 90% of the TI had been destroyed at either 210 or 250°F (99 or 121°C).

Traditionally, soymilk is heat-treated for approximately 60 min at pH 6.6, 99°C to reduce TI activity by about 92% to improve nutritional value. Johnson et al. (1981) evaluated a continuous direct steam infusion process facilitating aseptic processing with shorter heat exposure times and elevated temperatures. Maximum recovery occurred at the same point as did adequate inactivation of TI at 154°C for 34 sec at pH 6.7.

Nutritive value of soymilk

Utilization of soybeans offers potential opportunities for alleviating present and imminent world-wide shortages of food. The soybean outranks any other natural source in its plentiful and inexpensive supply of calories and protein. The nutritive quality of soybean protein is the best of those available from plant sources and is inferior to animal protein only because it is deficient in sulfur-containing amino acids (Angeles and Marth, 1970).

Mittal et al. (1976) studied comparative chemical analysis of soymilk vs cow's milk which is summarized in Table 4. Soymilk compared favorably with cow's milk in respect to protein. The average content of total solids in soymilk (6.12%) was lower than that of cow's milk (12.81%). This might be related to the virtual absence of carbohydrates in the former (Mittal et al., 1976). Webb and Johnson (1965) reported that lactose can be considered as the only carbohydrate in milk and is the predominant solid in milk, except in the case of some cows that may produce more fat than lactose. The average amount of lactose is about 4.9%. Chang and Murray (1949) stated that the amount of carbohydrate in soymilk is about 2.4% with 9% total solids content.

A comparative study of the amino acid composition of soy proteins and milk proteins (Table 5) shows a fairly close resemblance except for sulphur containing amino acids. Soymilks can be beneficially utilized by incorporating them with concentrated soy proteins to provide beverage of high protein quality (Patil and Gupta , 1981; Khaleque and Wallace, 1975; Hackler and Stillingo, 1967).

Table 4 - Chemical constituents of soymilk vs cow's milk[a]

	Cow's milk		Soymilk	
Total solids(g/100 ml)	12.81	± 0.7483	6.13	± 1.8970
Protein(g/100 ml)	3.28	± 0.0234	4.00	± 0.0225
Fat(g/100 ml)	3.74	± 0.1480	1.69	± 0.1180
Total Ash(g/100g)	0.7234	± 0.008	0.3961	± 0.056
Calcium(mg/100ml)	203.3	± 6.67	78.1	± 7.260
Phosphorous(mg/100ml)	137.47	± 11.20	39.54	± 1.014
Iron(ug/100ml)	15.5	± 1.94	32.4	± 4.40
Copper(ug/100ml)	3.1	± 1.94	6.9	± 0.84

[a]Based on 8 replicates

Source: Mittal et al., 1976.

Table 5 - Composition of amino acids in soymilk and cow's milk

Amino Acid	Soymilk		Cow'milk
	(1)	(2)	(2)
Lysine	6.25	6.17	7.8
Histidine	2.59	2.10	2.3
Arginine	7.81	7.75	3.2
Aspartic Acid	11.8	-	-
Threonine	3.87	2.81	4.6
Serine	5.30	-	-
Glutamic Acid	19.0	-	-
Proline	5.01	-	-
Glycine	4.25	-	-
Alanine	4.41	-	-
Valine	4.85	4.94	6.9
Cystine	1.72	1.35	0.9
Methionine	1.39	1.15	2.4
Isoleucine	5.10	4.61	6.4
Leucine	8.29	7.78	9.9
Tyrosine	3.95	3.62	5.1
Phenylalanine	5.13	4.73	4.9
Tryptophan	1.44	1.43	1.4
Total sulphur containing amino acid		2.50	3.3
Total aromatic amino acid		8.35	10.0

1)Processed soymilk at 93°C for 30 minutes.
Data from Hackler and Stillings (1967).

2)Data from Khaleque and Wallace (1975).

Soybeans are an excellent source of low-cost protein. However, the soybean flavor is not as acceptable to the Western world as it is to the Asians. Beany flavors and indigestible components are obstacles to more widespread use of soybeans (Eley, 1968). Soybean foods generally are consumed after they have been subjected to some degree of heat treatment. Heat processing enhances palatability and acceptance and also improves the nutritive quality of such foods (Angeles and Marth, 1970). Factors such as lipoxygenase, associated with oxidized, painty, off-flavor compounds and trypsin inhibitors, which can reduce nutritional quality of protein, are relatively heat labile and heat treatment has been found to overcome them (Rackis, 1974).

However, heat treatments are ineffective against other undesirable factors such as phytic acid and oligosaccharides. The major oligosaccharides in soybeans are sucrose (4-8% dry basis), raffinose(1-2% d.b.) and stachyose(5-8% d.b.) (Cegla and Bell, 1977; Smith and Circle, 1972). The two larger oligosaccharides, raffinose and stachyose, are the causative factors for flatulence and the uncomfortable feeling often experienced upon ingestion of soybean products. This effect apparently is due to the lack of galactosidase activity in the human digestive tract, and hence the oligosaccharides pass into the large intestine where they are anaerobically fermented to produce gas (Omosaiye et al., 1978; Rackis et al., 1970).

Many attempts have been made by food scientists to treat soybeans or its products to remove or degrade these indigestible oligosaccharides. Mital and Steinkraus (1975) attempted to degrade oligosaccharides by lactic acid bacteria. Enzyme treatments have also been used. Sugimoto and Van Buren(1970) used commercial alpha - galactosidase to hydrolyze oligosaccharides to their component sugars. Rackis et al.(1970) has suggested that antibodies and certain phenolic acids can inhibit flatus activities, but it is unlikely that such additives would be approved for human use (Omosaiye et al., 1978).

Omosaiye et al. (1978) removed up to 96% oligosaccharides by a two-stage ultra-filtration process. They also reported that their final product assayed 60% protein, 35% fat and 0.6% oligosaccharides(d.b.).

MATERIALS AND METHODS

Preparation of soy flour

Certified seed grade Prize soybeans were purchased from the Department of Grain Science and Industry at Kansas State University. Cleaned beans were passed through a rough corrugated, differential speed roller. The spacing between the two rollers was adjusted to approximately 2/3 of the diameter of the soybeans for cracking the beans and separating the hull from the cotyledons. As the soybeans discharged from the roller mill, most of the cotyledons broke away from the hull. In some instances cotyledons were still encased within hulls. An input dehuller was utilized to separate the cotyledons from the surrounding hull.

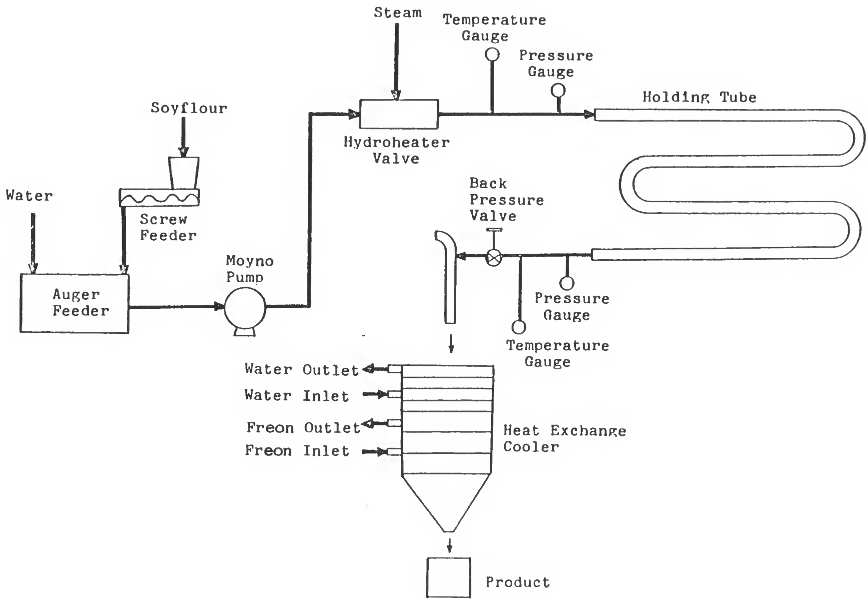
A Kice separator (Kice Metal Product Co., Wichita, Kansas) was used to separate the hulls from the cotyledons by air aspiration. Most of the hulls, along with some hypocotyls and fine particles of cotyledons, were separated from the cotyledons. This separation was repeated 3-4 times. Dehulled beans were ground using a Fitz hammermill equipped with a 0.51 mm screen. Grinding was done at a slow rate to prevent accumulation of beans in the cutter compartment, which would cause overheating and reduce the solubility of the resulting flour.

Rapid Hydration Hydrothermal Cooking(RHHTC)

The modified Penick and Ford Laboratory continuous jet cooker (Penick and Ford Limited, Cedar Rapids, Iowa) was used to process soymilk by RHHTC (Figure 1). Average residence time was 33-35 seconds, depending upon the back pressure in the system (about 65-70 psi) and using continuous operation with a 37 foot fold tube.

Soybean flour was fed continuously into the hopper of the Moyno-pump along with tap water. Mixing of the water and soybean flour was conducted in as small a surge volume as possible. Pumping distance from the slurrying tank to the hydroheater was shortened to 40 cm. Contact time of soybean flour with water was held to a minimum before cooking. The flour and water were adjusted to give about 9-10% solids in the cooked slurry by varying speeds of the screw feeder and water flow rate. The Moyno pump was set at 40 rpm.

Figure 1. Modified hydrothermal jet cooker and heat exchange cooler


Steam
Temperature Gauge
Pressure Gauge
Holding Tube
Soyflour
Water
Screw Feeder
Hydroheater Valve
Back Pressure Valve
Moyno Pump
Auger Feeder
Pressure Gauge
Temperature Gauge
Water Outlet
Water Inlet
Freon Outlet
Freon Inlet
Heat Exchange Cooler
Product

The cooked slurry discharged from the flash unit into a conduction heat exchange cooler. Flashing the product through a pressure release provided instantaneous cooling to 99°C. The cooked slurry was cooled from 99°C to 5-10°C within 15-17 seconds. All cooked slurries were stored at 4°C until bottling.

Traditional extraction

Nine hundred grams certified seed grade Prize soybeans were washed thoroughly and soaked in 4.2 l tap water at room temperature for 10-11 hours. After soaking the beans were rinsed with water and drained. Hydrated beans were weighed into nine portions. Each portion was blended for 1 min with 450 ml boiling water in an Osterizer blender.

The puree was poured directly into cooking pots containing 1.5 l boiling water and held at boiling point until all nine portions were added. The procedure used during blending and filtering prevented enzymatic activity and reduced flavor and color changes. The puree was heated and poured into a cloth filter bag (Tofu Kit, Soy Food Center). The filter bag was pressed repeatedly against a colander to express as much soymilk as possible. Boiling water (500 ml) was poured over the contents in the filter bag and pressure was applied again.

Bottling for storage

Fresh soymilk was poured into clear, glass bottles (240 ml capacity) and screw caps were adjusted. Four replications were used in the experimental design for each extraction method. Ten bottles of soymilk (240 ml) were prepared for each replication, randomly placed in refrigeration storage (3-5°C) for 22 days and analyzed for amino acids, thiamine, riboflavin and microbial counts at predetermined times.

Total solids in soymilk

To monitor total solids content initially and during storage, 2.5-3 g soymilk were weighed into aluminum pans (5-cm diameter) and heated at 80°C until a consistent weight was obtained. Samples were cooled in a dessicator and weighed. Total solids were calculated as the percentage residue remaining (AOAC, 1984).

Composition of soymilk

Composition analysis was made on the freeze-dried soymilk powder extracted by the traditional method. Protein content was determined by a microkjeldahl method (AOAC, 1984) using a nitrogen to protein conversion factor of 6.25. Fat and ash contents were determined for selected samples by AOAC (1984) procedures. Raffinose and stachyose contents were determined by HPLC during preliminary work.

Nutritional assay

Amino acid composition. Soymilk samples (100 ml) were freeze-dried overnight and ground finely. After grinding the samples were pooled to make composite samples for each extraction method. Samples were analyzed for total amino acid composition using a Dionex D-300 kit single column amino acid analyzer.

Thiamine and riboflavin. Thiamine and riboflavin in soymilk were determined by the fluorometric method of Freed (1966). Soymilk (40 g) was added with 75 ml of 0.1 N HCl. After autoclaving 15 min at 15 lb pressure, samples were cooled to 50°C and combined with 5 ml of freshly prepared enzyme. Samples were incubated in a water bath 45-50°C overnight and cooled. Then samples were diluted to 200 ml and filtered. Samples were frozen until they were analyzed for thiamine and riboflavin.

Viscosity of soymilk

Viscosity of soymilk was determined with a Brookfield viscometer (Brookfield Engineering Laboratories, Stoughton, MA.) Samples from four replications for each extraction method were pooled and mean values were reported. Pooled samples were held at room temperature 3-4 hrs until consistent readings could be obtained. Determinations were made at 60 rpm using a No. 2 spindle. Brookfield readings were converted to viscosity in centipoise (cps).

Color measurement

Color was measured by a HunterLab Spectrophotometer (Model S54P-5). Soymilk samples were placed in optically clear cells and HunterLab L, a, and b-values were obtained. Instrumental values were reduced to the color functions, hue angle ($\tan^{-1}b/a$)

and saturation index $[(a^2+b^2)^{1/2}]$. Color values were determined on fresh soymilk samples and at subsequent testing periods after storage.

Total microbial counts

Bottles of soymilk were removed from refrigerator storage for microbial analysis at predetermined times. One ml serial dilutions of soymilk were plated in Standard Plate Count Agar according to Standard Methods for the Examination of Dairy Products (Marth, 1978). Colonies were counted after incubation at 32°C for 48 hours.

Statistical analysis

To test differences in the methods, two sample t-test were performed on the response variables, for each of the time periods for which both methods were measured. Results which indicated differences significant at the five percent and one percent significance levels are noted in the tables. Ninety five percent confidence intervals for the means are also tabled for each treatment and time period.

Analysis was performed using the Statistical Analysis System (SAS) software package.

RESULTS AND DISCUSSION

The overall objective of this research was to determine the effects of processing methods and storage on nutritional quality. The investigation was divided into six parts: 1) composition of soymilk; 2) composition of amino acids in soymilk and changes during storage; 3) effect of processing on thiamine and riboflavin in soymilk extracted by RHHTC and traditional methods; 4) measurement of apparent viscosity of samples extracted by both methods; 5) measurement of color of samples extracted by both methods and 6) microbiological analysis of soymilk during storage.

Composition of soymilk

Freeze-dried soymilk powders obtained by both extraction methods were characterized by analysis for protein, crude fat, and crude ash and data are presented in Table 6. Raffinose and stachyose in traditionally extracted soymilk were measured by HPLC during preliminary work and values are presented on a moisture-free basis in Table 6.

Effects of processing on amino acid composition of soymilk

The amino acid composition of soymilk processed by traditional and RHHTC methods are shown in Tables 7 and 8, respectively. Values for soymilks produced from the two extraction methods were similar for aspartic acid, serine and

Table 6 - Composition of freeze-dried soymilk samples[a] extracted by traditional and RHHTC methods[b]

	Protein (N x 6.25),%	Fat +%	Carbohydrate Raffinose %	Carbohydrate Stachyose %	Ash %
Traditional	50.60	22.4	0.99	2.7	5.54
RHHTC[c]	49.30	21.1	-	-	5.05

[a]Moisture-free basis

[b]Each value is a mean for two determinations.

[c]Source : Hung (1984)

Table 7 – Grams of amino acids per 100g protein corrected to 100% recovery, protein basis, in soymilk[a] extracted by traditional method.

	Days of storage					
	1	16	22	25	28	Mean value
Aspartic acid	12.50	12.75	12.63	12.12	11.87	12.37±0.37
Threonine	4.12	4.15	4.10	3.90	4.05	4.06±0.10
Serine	6.01	6.14	5.99	5.88	5.76	5.96±0.14
Glutamic acid	18.34	18.77	18.66	17.98	17.44	18.24±0.54
Proline	6.19	6.25	5.77	7.26	7.22	6.54±0.67
Glycine	4.47	4.59	4.59	4.39	4.36	4.48±0.11
Alanine	4.76	4.78	4.70	4.62	4.59	4.69±0.08
Half cystine	1.19	1.16	1.20	1.19	1.31	1.21±0.06
Valine	3.18	3.18	3.21	3.16	3.27	3.20±0.04
Methionine	1.49	1.39	1.46	1.47	1.76	1.51±0.14
Isoleucine	2.97	2.96	2.96	2.90	3.03	2.96±0.05
Leucine	7.06	7.16	7.19	6.89	6.92	7.04±0.14
Tyrosine	4.04	4.02	4.22	4.21	4.41	4.18±0.16
Phenylalanine	4.71	4.95	5.36	5.31	5.45	5.16±0.31
Histidine	4.50	3.82	4.49	4.61	4.86	4.46±0.39
Lysine	5.99	5.52	6.06	5.90	5.97	5.89±0.21
Ammonia	1.92	1.90	1.81	1.79	1.66	1.82±0.10
Arginine	6.55	6.49	5.59	6.43	6.07	6.23±0.40

[a] Each sample represents four replications pooled for each storage period.

Table 8 - Grams of amino acids per 100g protein corrected to 100% recovery, protein basis in soymilk[a] extracted by RHHTC method.

	Days of storage					
	1	12	16	19	22	Mean value
Aspartic Acid	12.36	12.34	12.74	12.35	12.21	12.40±0.20
Threonine	4.24	4.05	4.18	4.02	4.02	4.10±0.10
Serine	5.97	5.92	6.12	5.81	5.86	5.94±0.12
Glutamic Acid	18.13	17.91	18.65	18.13	17.89	18.14±0.31
Proline	6.95	6.13	6.33	6.08	6.58	6.41±0.36
Glycine	4.56	4.42	4.58	4.51	4.44	4.50±0.07
Alanine	4.77	4.71	4.87	4.73	4.66	4.75±0.08
Half Cystine	1.25	1.19	1.17	1.20	1.20	1.20±0.03
Valine	3.31	3.24	3.24	3.25	3.16	3.24±0.05
Methionine	1.60	1.51	1.45	1.53	1.56	1.53±0.06
Isoleucine	3.07	2.96	2.96	3.01	2.90	2.98±0.06
Leucine	7.22	7.15	7.20	7.14	7.09	7.16±0.05
Tyrosine	4.13	4.24	4.04	4.23	4.14	4.16±0.08
Phenylalanine	5.05	5.43	4.98	5.50	5.36	5.26±0.23
Histidine	3.93	4.88	3.83	4.91	4.83	4.48±0.55
Lysine	5.43	6.20	5.50	6.34	6.16	5.93±0.43
Ammonia	2.21	1.93	1.88	1.87	1.93	1.96±0.14
Arginine	5.81	5.80	6.28	5.39	6.03	5.86±0.33

[a] Each sample represents four replications pooled for each storage period.

glutamic acid after one day of storage. Values for histidine, lysine, and arginine appeared lower when the RHHTC extraction method was used. However, soymilk processed by the two methods showed no definite trends in amino acid composition after storage.

Previous investigations (Bandenhop and Hackler, 1973; Rios Iriarte and Barnes, 1966; Taira et al., 1965; Stillings and Hackler, 1965) on the effect of heat processing on amino acid composition of soy products have shown that cysteine, lysine, tryptophan, arginine, serine, and histidine are prone to heat destruction. Johnson (1978) found no significant differences in these amino acids when the process temperature was increased and cooking time decreased to yield equivalent trypsin inactivation at each process temperature.

Most cereal grains are limited in their biological value by their lysine content and this amino acid is susceptible to destruction during heat processing . Bread, for example, loses approximately 10-15% of its available lysine during baking (Rosenberg and Rohdenburg, 1951).

Hackler et al. (1965) reported that cooking soymilk 1-6 hr at 93°C had no adverse effect on protein efficiency ratios in weanling rats, or on available lysine content. Heating soymilk for 32 min at 121°C, however, produced a definite decrease in protein efficiency ratio, and an indication that available lysine was declining. The drop in available lysine was greater after the soymilk had been heated 40 min at 121°C. These results show that the protein efficiency ratio of heat-processed soymilk is dependent upon both time and temperature treatment.

Hung (1984) reported no significant changes in amino acid composition as a result of processing method. Soymilk made by the RHHTC method had a higher reactive lysine value than that of the traditional soymilk samples although the total lysine was nearly the same. In vitro digestibility and in vivo protein efficiency ratio of soymilk products showed soymilk made by RHHTC process had higher nutritive value than samples made by the traditional method. Regression analysis of these data showed an excellent correlation between PER and in vitro digestibility. This suggests that the action of steam infusion and flashing under the RHHTC process results in improved protein nutritive quality favoring enzyme attack.

Mitchell et al. (1945) recognized that the most important agent modifying the nutritive value of food proteins during processing was heat. Several years later, Hayward (1959) stated that one of the most common causes of quality variance in soybean products is heat--either too much or too little. Their investigation was undertaken to learn the extent of variations in the nutritional quality of protein in a water extract of soybeans caused by differences in heat treatment. Although heat usually causes some destruction of protein under certain conditions, heat can maintain the nutritive value by inactivating enzymes that would otherwise cause losses. Heat may improve the nutritive value by liberating nutrients from otherwise unusable complexes. Improvement in the nutritive value of soybeans after mild heat treatment has been recognized (Bender, 1966).

Effects of processing vary with many other factors, such as the type of food, the duration and severity of the process, and the size and condition of the portion of food. These factors help explain why some research reports appear to be contradictory.

Effects of processing on thiamine and riboflavin of soymilk

As shown in Tables 9 and 10, some differences occurred between the two samples extracted by the traditional and RHHTC methods during storage. After the first day of storage, thiamine and riboflavin concentration were 14.69 and 6.06 ug/g, respectively, from the traditional extraction, and 11.48 and 5.53 ug/g with RHHTC method. Thiamine and riboflavin contents in soymilk processed by RHHTC method were lower than soymilk from traditional extraction. The T-values were higher for thiamine (Table 9) than for riboflavin (Table 10). Thiamine and riboflavin contents seem to be more critical when processing at higher temperatures for short times than when lower temperatures and long time are used. Chang and Murray (1949) found that soymilk contained 11.8 ug/g thiamine and 3.7 ug/g riboflavin on a moisture-free basis.

There were no significant changes in thiamine content during storage of soymilk produced by either method. Thiamine values fluctuated throughout the storage period and no definite trends were prevalent.

Table 9 – Thiamine content of soymilk during storage[a]

Storage day	Traditional (ug/g)	RHHTC (ug/g)	T-value
1	14.69±0.16	11.48±0.73	-13.61***
4	14.58±0.06	11.43±1.21	-8.20**
8	14.70±0.45	11.73±0.80	-10.34***
12	14.64±0.35	11.37±0.80	-11.90***
16	14.51±0.48	11.60±0.64	-11.52***
19	15.07±0.32	11.60±0.70	-14.31***
22	14.76±0.16	11.50±0.54	-18.87***
25	14.58±0.16		
28	14.75±0.35		

**$P < 0.01$

***$P < 0.001$

[a]Each value is a mean for four replications.

Table 10 – Riboflavin content of soymilk during storage[a]

Storage day	Traditional (ug/g)	RHHTC (ug/g)	T-value
1	6.06±0.06	5.53±0.35	-4.83*
4	6.28±0.27	5.39±0.48	-4.92**
8	6.11±0.22	5.35±0.41	-5.15**
12	6.10±0.27	5.21±0.51	-4.39**
16	6.09±0.16	4.80±0.76	-5.31*
19	5.90±0.06	5.09±0.54	-4.86*
22	5.92±0.16	5.03±0.45	-5.82**
25	5.86±0.13		
28	5.73±0.13		

* $p < 0.05$

** $p < 0.01$

[a]Each value is a mean for four replications.

Thiamine is one of the more labile vitamins and can suffer considerable decreases during food processing. It is stable to acid, even at temperatures up to 120°C, but unstable at neutral and alkaline pH values. Even the mild alkalinity of natural water can result in the destruction of thiamine. For example, thiamine in boiled rice was reported to be unstable to oxygen, destroyed by sulfur dioxide, and unaffected by light (Bender, 1966).

Toasting, for periods of 30-70 sec, has been shown to result in a loss of 10-30% of the thiamine present in bread (Downs and Meckel, 1943). Losses of thiamine in meat during cooking vary with the size of the cut, fat content, and other factors but averages about 15-40% in broiling, 40-50% in frying, 30-60% in roasting and 50-75% in canning (Harris and Von Loesecke, 1960). No losses of thiamine and riboflavin occurred in chilling poultry and storage time had no effect on the retention of these vitamins when subsequently cooked (Bender, 1966). Milk loses 10% thiamine when spray-dried, 20-30% when roller-dried, 3-20% in pasteurization and 30-50% in sterilization (Harris and Von Loescke, 1960).

Riboflavin is heat stable in acid solution and in the presence of mild oxidizing agents, but is very sensitive to light at neutral and alkaline pH. In neutral solution, it is moderately heat stable. About 50% of the riboflavin can be destroyed in 2 hr by exposure to bright sunlight, and 20% can be lost on a cloudy day (Harris and Von Loesecks, 1960). Riboflavin is converted into lumiflavin and this destroys vitamin C (Harris and Von Loesecks, 1960).

Effects of processing on viscosity of soymilk

Significant differences existed in apparent viscosity between samples extracted by traditional and RHHTC methods. Mean values for viscosity of soymilk processed by the traditional method (12 cps) were lower than viscosity (212 cps) of RHHTC-processed soymilk, although total solids contents of the soymilks were similar (8.0%, traditional; 9.3%, RHHTC).

According to Hung (1984), apparent viscosity of RHHTC processed soymilk had a high positive correlation with fiber content of the soymilk. Protein and fiber absorb water during RHHTC-processing which is visually reflected in thickening and concurrent increased viscosity. Urbanski et al.(1982) showed that the small fiber fraction in food contributes more to viscosity than the larger protein fraction. Foster and Ferrier (1979) reported that the presence of soybean hulls in a soy beverage caused higher apparent viscosity than was observed in beverages from dehulled cotyledons.

Preliminary work for the current investigation indicated that although the total solids content of soymilk processed by traditional method was higher, the apparent viscosity was lower than that of soymilk produced by RHHTC. Possible explanations for these results are that the dehulling process did not completely remove all of the hull and/or the high temperature of processing by RHHTC might alter the protein structure to produce an increased viscosity.

Effect of processing on color of soymilk

HunterLab values for soymilk are presented in Table 11 and the Appendix (A-2, A-3 and A-4). HunterLab L, a, and b -values were different for the two extraction methods after one and 16 days storage. However, no significant differences were attributable to storage.

Johnson (1978) reported that at 210°F (99°C), the Hunter L-value of soymilk decreased from 77.1 to 73.0 at pH 6.6 over 60 min cooking time. When Johnson used color of soymilk cooked at 210°F (99°C) for 60 min as a criterion of protein quality, soymilk could be RHHTC-processed for about 600 sec at 250°F (121°C), 400 sec at 270°F (132°F), 210 sec at 290°F(143°C), and 90 sec at 310°F (154°C) before equivalent chemical browning occur red.

Hunter L-values indicate the lightness or darkness of samples. Higher Hunter L-values produced by traditional extraction indicates lighter color and less browning. In this experiment, it seems that factors other than browning (such as dehulling) may have contributed to color differences that were evident between the two methods. As previously mentioned the RHHTC produced soymilk may have contained some components of the hulls. These particles contain pigments which may have caused lower HunterLab L-value. Hue angles and saturation indexes

Table 11 - HunterLab values for soymilk after 1 and 16 days storage

	Day 1	Day 16
L-value		
Traditional	81.460	81.318
RHHTC	79.465	79.543
T-value	-22.772***	-17.680***
a-value		
Traditional	-2.240	-2.240
RHHTC	-1.423	-1.405
T-value	10.201**	10.555***
b-value		
Traditional	12.345	12.328
RHHTC	13.213	13.280
T-value	6.457***	6.139***
Hue angle		
Traditional	-1.390	-1.391
RHHTC	-1.463	-1.465
T-value	-10.852**	-11.605***
Saturation index		
Traditional	12.547	12.530
RHHTC	13.290	13.355
T-value	5.744**	5.407**

** $p < 0.01$

*** $p < 0.001$

(Table 11, A-5 and A-6 in the Appendix) were different for the two extraction methods after one and 16 days storage. However, there were no significant differences during storage by either method. HunterLab a or b values represent redness-greenness or yellowness-blueness, respectively, as separable attributes of a color stimulus. They are used repeatedly by food scientists and are not as meaningful as hue angle and saturation index (Little, 1975). A plot of Hunter a versus b joined with a line to the center (Figure 2) to form an angle, O, with the horizontal axis is used as a measure of hue. The saturation index (SI) is indicated by the length of that line (Setser, 1984).

Microbiological analysis of soymilk

Least square means for log numbers of viable counts per ml are shown in Fig 3, and viable counts expressed as log numbers per ml and as bacteria colony forming units(CFU)/ml are found in Tables A-7 and A-8 in the Appendix.

Soymilk extracted by either method (traditional or RHHTC), had an initial bacterial load of 10^1 CFU/ml after one day of storage. The initial bacterial load may come from bacterial contamination during the cooling and bottling process after heat treatment. There was no significant bacterial growth for either method after 1,4 or 8 days storage. After 12 days of storage, the microbial load was significantly increased to 10^7 for the RHHTC extracted soymilk, but by 22 days of storage, the traditionally produced soymilk was still maintaining low

Figure 2. Hunter a, b plot showing the basis for reduction of data (Little, 1975).

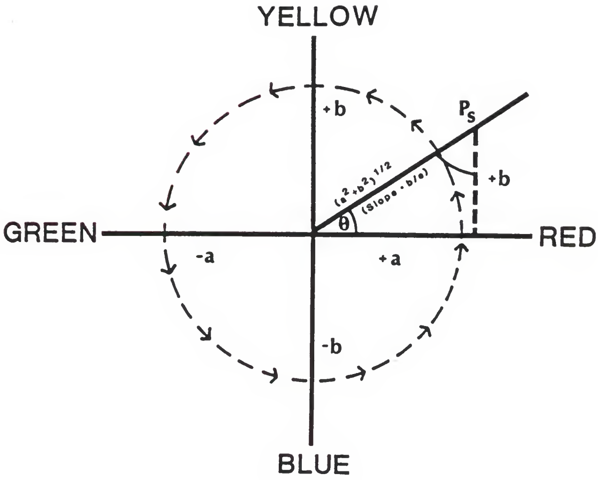
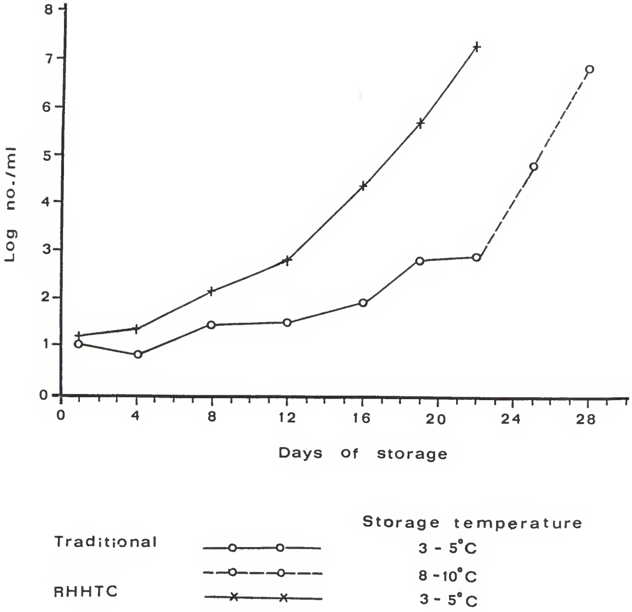

YELLOW
+b
P_s
+b
$(a^2+b^2)^{1/2}$
(Slope = b/a)
θ
GREEN
RED
-a
+a
-b
BLUE

Figure 3. Effect of storage on least square means of viable counts for soymilk extracted by traditional and RHHTC methods


Log no./ml
0
1
2
3
4
5
6
7
8
0
4
8
12
16
20
24
28
Days of storage
Storage temperature
Traditional
3 - 5°C
8 - 10°C
RHHTC
3 - 5°C

bacterial growth (10^3). Storage temperature was increased from 3-5°C to 8-10°C for traditional soymilks to facilitate bacterial growth. Angeles and Marth (1970) stated that many lactic acid bacteria can grow in soymilk and that acid production in this substrate is possible. According to Lim (1984) the lactic acid bacteria increased markedly as storage days increased in immersed tofu samples. The increse in lactic acid bacteria may have contributed to decomposition of soybean protein in the tofu. Fujii et al. (1978) speculated that protein decomposition is soybean curd also affected pH. However, pH of soymilk was not measured in the current study. General agreement was found with Angeles and Marth (1970) who studied effects of heating soymilk for different times at different temperatures (unheated, 60, 80, 100 and 120) on the subsequent activity of lactic acid bacteria. Responses in the medium heated at 80°C were similar for the various time exposures and, for most organisms, were the lowest exhibited. Responses in soymilk heated at 100°C for short durations in medium heated at 80°C, but for longer times at 100°C caused increases in lactic culture activity. Heating soymilk at 120°C had the same effect as extended heating at 100°C. The effect of heating at 120°C did not seem to be time-dependent; responses at the different time exposures were nearly similar. There is general agreement that certain heat treatments of milk benefit culture growth, whereas others result in development of inhibitory properties (Speck, 1962).

Available evidence indicates that beneficial effects of heating cow's milk result from destruction of heat sensitive naturally-occurring inhibitory substances, as well as protein denaturation and the accompanying presence of optimum conditions for free sulfhydryls (Greene and Jezeski, 1957; Henningson and Kosikowski, 1957; Auclair and Hirsch, 1953). A reduction of volatile sulfides upon further heating creates conditions more favorable for culture growth (Greene and Jezeski, 1957).

CONCLUSIONS

From this study, it can be concluded that:

1. The amino acid values of soymilk processed by traditional and RHHTC methods were similar for aspartic acid, serine and glutamic acid after one day storage. Values for histidine, lysine, and arginine were lower when the RHHTC extraction method was used. However, soymilk processed by the two methods showed no definite trends in amino acid composition after extended storage.
2. Thiamine and riboflavin contents in soymilk processed by RHHTC-method were lower than soymilk traditionally extracted. There were no significant changes in thiamine content during storage of soymilk produced by either method, although riboflavin content decreased slightly.
3. Mean values for viscosity of soymilk processed by the traditional method were lower than viscosity of RHHTC-processed soymilk, although total solids contents of the

soymilks were similar.

4. HunterLab L, a, b, hue angles and saturation index-values were different for the two extraction methods after one day and 16 days storage. RHHTC-produced soymilk was darker, less green, and more yellow than traditionally extracted soymilk. However, no significant differences were attributable to storage.

5. There was no significant bacterial growth for either method after 1,4 or 8 days storage. After 12 days of storage, the microbial load was significantly increased for the RHHTC extracted soymilk, but by 22 days of storage, the traditionally produced soymilk was still maintaining low bacterial growth.

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APPENDIX

Table A-1. Calculation of thiamine and riboflavin

1. Calculation of thiamine:

$$\frac{U-UB}{S-SB} \times \frac{1}{5} \times \frac{25}{V} \times \frac{100}{\text{wt of sample}}$$

Where U= deflection of unknown

UB= deflections of the standard

S= deflections of standard blank

V= volume of solution used for the adsorption on decarso

The factor 1/5 converts the reading to ug per ml instead of ug per 5 ml aliquot. Since the final volume of eluate is 25 ml, the factor 25/v corrects for volume change during adsorption and elution.

2. Calculation of riboflavin:

$$\frac{A-C}{B-A} \times \frac{\text{riboflavin increment}}{\text{10 ml aliquot}} \times \text{dilution factor} \times \frac{1}{\text{sample}}$$

where A= 10 ml filtrate + 1 ml water

B= 10 ml filtrate + 1 ug riboflavin

C= Blank of filtrate

Table A-2. HunterLab L-values for soymilk during storage[a]

Storage day	Traditional	RHHTC	T-value
1	81.46±0.10	79.47±0.25	-22.77***
12		79.72±0.54	
16	81.32±0.29	79.54±0.16	-17.68***
19		79.51±0.10	
22		79.48±0.57	
25	81.40±0.00		
28	81.40±0.16		

*** $P<0.001$

[a]Each value is a mean for four replications.

Table A-3. HunterLab a-values in soymilk during storage[a]

Storage day	Traditional	RHHTC	T-value
1	-2.24±0.03	-1.42±0.25	10.20**
12		-1.38±0.29	
16	-2.24±0.13	-1.41±0.22	10.56***
19		-1.37±0.22	
22		-1.47±0.35	
25	-2.22±0.06		
28	-2.22±0.06		

** P<0.01

*** P<0.001

[a]Each value is a mean for four replications.

Table A-4. HunterLab b-values in soymilk during storage[a]

Storage day	Traditional	RHHTC	T-value
1	12.35±0.13	13.21±0.41	6.46***
12		13.38±0.41	
16	12.33±0.13	13.28±0.48	6.14***
19		13.26±0.41	
22		13.19±0.41	
25	12.40±0.19		
28	12.35±0.16		

*** $P < 0.001$

[a]Each value is a mean for four replications.

Table A-5. Hue angles for soymilk during storage[a]

Storage day	Traditional	RHHTC	T-value
1	-1.39±0.00	-1.46±0.03	-10.85**
12		-1.47±0.03	
16	-1.39±0.00	-1.47±0.03	-11.61***
19		-1.47±0.03	
22		-1.46±0.03	
25	-1.39±0.00		
28	-1.39±0.00		

** P<0.05

*** P<0.001

[a]Each value is a mean for four replications.

Table A-6. Saturation indexes for soymilk during storage[a]

Storage day	Traditional	RHHTC	T-value
1	12.55±0.00	13.29±0.03	5.74**
12		13.45±0.03	
16	12.53±0.00	13.36±0.03	5.41**
19		13.33±0.03	
22		13.27±0.03	
25	12.59±0.03		
28	12.54±0.16		

** $P<0.05$

[a]Each value is a mean for four replications.

Table A-7. Microorganisms in soymilk extracted by traditional and RHHTC methods

Storage day	Rep 1	Rep 2	Rep 3	Rep 4
	---Traditionally extracted soymilk---			
1	1.0×10^{1}	0.9×10^{1}	1.3×10^{1}	1.2×10^{1}
4	0.3×10^{1}	1.0×10^{1}	0.7×10^{1}	1.0×10^{1}
8	2.0×10^{1}	4.5×10^{1}	2.6×10^{1}	0.0
12	4.2×10^{2}	3.1×10^{1}	0.8×10^{1}	1.2×10^{1}
16	2.0×10^{1}	1.3×10^{2}	4.0×10^{1}	4.6×10^{2}
19	2.1×10^{2}	9.4×10^{2}	4.3×10^{3}	2.4×10^{2}
22	4.1×10^{2}	2.1×10^{3}	2.2×10^{3}	2.8×10^{2}
25	4.9×10^{4}	8.1×10^{5}	6.1×10^{5}	1.1×10^{4}
28	9.1×10^{6}	1.1×10^{7}	1.2×10^{7}	3.6×10^{6}
	---RHHTC-processed soymilk---			
	Rep 1	Rep 2	Rep 3	Rep 4
1	2.6×10^{1}	2.0×10^{1}	1.7×10^{1}	0.7×10^{1}
4	2.0×10^{1}	1.5×10^{2}	1.0×10^{1}	1.0×10^{1}
8	2.0×10^{2}	1.1×10^{2}	1.0×10^{3}	2.1×10^{1}
12	1.6×10^{3}	1.2×10^{3}	1.1×10^{2}	1.0×10^{3}
16	1.1×10^{5}	6.8×10^{4}	1.4×10^{4}	4.1×10^{4}
19	9.4×10^{5}	1.1×10^{6}	4.6×10^{6}	2.2×10^{4}
22	3.1×10^{8}	3.3×10^{7}	2.4×10^{7}	1.1×10^{6}

Table A-8. Least squares means[a] of viable counts after storage for soymilk extracted by traditional and RHHTC methods

storage day	Traditional	RHHTC	T-value
1	1.04	1.20	1.25[ns]
4	0.83	1.37	1.77[ns]
8	1.46	2.17	1.69[ns]
12	1.52	2.83	2.78**
16	1.92	4.41	5.62**
19	2.83	5.75	5.02**
22	2.93	7.36	7.97**
25	4.86		
28	6.91		

** $P < 0.05$

[a]Each value is a mean for four replications.

EFFECTS OF PROCESSING AND STORAGE ON SELECTED NUTRIENTS IN SOYMILK

by

KYUNG SOOK HONG

B.S. in Home Economics, Dongguk University

Seoul, Korea, 1979

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ABSTRACT

Soymilk was extracted by traditional and Rapid Hydrothermal Cooking (RHHTC) methods. Bottled soymilk (240 ml) was placed in refrigerated storage (3-5°C) for 22 days and analyzed at predetermined times. Soymilk processed by the two methods showed no definite trends in amino acid composition after storage. Thiamine and riboflavin contents in soymilk processed by the RHHTC method were lower than in soymilk from the traditional extraction. No significant changes occurred in thiamine content during storage of soymilk produced by either method, although riboflavin content decreased slightly. Values for viscosity of soymilk processed by the traditional method were lower than those for RHHTC-processed soymilk, although total solids contents of the soymilks were similar. RHHTC-produced soymilk was darker, less green and more yellow than traditionally extracted soymilk. No significant changes in color values occurred after storage. No significant bacterial growth was evident for either extraction method after 8 days of storage. After 12 days of storage, the microbial load increased ($p<0.05$) for the RHHTC-extracted soymilk, while the traditionally produced soymilk maintained low bacterial growth after 22 days of storage.