EFFECTS OF PROCESSING AND STORAGE
ON NUTRITIONAL QUALITY
OF SOYBEAN CURD

by

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INTRODUCTION

Increasing demands on the world food supply have encouraged agricultural researchers to concentrate their efforts on commodities with the greatest potential for meeting human requirements for energy and nutrients. Presently, soybeans are considered the premier oilseed crop and most abundant source of vegetable protein in the world (Smith, 1981). The United States' soybean crop accounts for more protein and fat in our food economy than any other source (Smith and Circle, 1978).

Soybean production dates back to 14th century B.C. during the Shang Dynasty (Yung-Shung, 1981). Soybeans were one of the main crops of the age. Currently, the United States is the major producer of soy and soybean products, and production is still on the rise. Each year the United States' number one cash crop increases 1.75-2.25 billion bushels (Hopkins, 1983). This remarkable growth has encouraged research efforts to increase utilization of soybeans (Smith, 1981). Ray (1981) recently described soy as the world's miracle food. Soybean crops provide the world with its most economical, high quality protein available to meet current demand.

This indispensible farm commodity formerly was considered fit only for farm animal consumption and until recently has been slighted as human food (Hopkins, 1983). But in the advent of staggering population growth and limited resources, Western civilization seriously is exploring the benefits of soy-based products since they can provide nutritious, well-balanced meals (Dutra de Oliveira and dos Santos, 1981; Langsdorf, 1981). Two statistics point out the reason for this increased interest in vegetable proteins, and specifically, soybeans. An acre of land produces about 43 lbs of animal protein annually. The same acre of land will generate 450 lbs of plant protein in the form of soybeans (Hartmann, 1966).

Furthermore, world production of wheat, corn and rice in 1975 was more than twice the total production of major food legumes. The legume crop of one-half the size generated 7% more lysine when compared to cereal grains. This supports the essential coupling of these two commodities to synergistically upgrade the nutritive value of both soy and grain crops.

Legumes are an economical source of protein. One unit of animal protein requires a ten-fold increase in energy expenditure to equal one unit of legume protein. Also, the nitrogen fixing legumes require less energy for protein production than cereal grains (Rockland and Radke, 1981).

Dry beans make an important contribution to the diet of those unable to afford milk and other expensive animal products (Carpenter, 1981). Government and national agencies continue to support the implementation and utilization of soy products as they work to eliminate world hunger in developing countries (Carpenter, 1981). There also is a growing consumer awareness in the United States about the nutritional advantages of soy-based products (Casey, 1979).

The nutritional importance of soybeans, a prominent legume, as a member of the meat, poultry, fish and bean group has been overshadowed in the past by the popularity of animal products. The Dietary Guidelines published by the United States Department of Agriculture and the Department of Health, Education, and

Welfare (USDA/HEW, 1985) suggest that consumers avoid excessive intake of total fat, saturated fat, and cholesterol, as well as sugar and sodium. The guidelines also recommend increased consumption of complex carbohydrates. In view of this publication and increased knowledge of human nutritional requirements, the nutritional contributions of soybeans should be emphasized.

Objectives for the current study were: 1) To study effects of traditional (hot-grind) and Rapid Hydration Hydrothermal Cooking extraction methods on amino acid, protein, carbohydrate, ash, thiamin and riboflavin contents of soymilk and soybean curd; 2) To investigate effects of using selected chemical preservatives in immersion solutions to extend the shelf life of tofu produced from traditional (hot-grind) soymilk; and 3) To study effects of using chemical preservatives in the immersion solutions on selected nutrients in soybean curd.

REVIEW OF LITERATURE

Physicochemical properties of soybean proteins

Soybean proteins consist of a group of proteins that cover a broad range of physical and chemical properties. The major proteins are globulins which are insoluble at their isoelectric points. Heat treatment also can render them insoluble.

These proteins have been separated by the use of ultracentrifugation. Wolf (1972) developed a typical ultracentrifugation pattern for water-extractable proteins from defatted soybean meal. The meal contained four major fractions presented in Table 1. Fractions were separated based on their sedimentation rates.

Table 1 - Ultracentrifuge fractions of soybean proteins from defatted soybean meal[a]

Protein fraction	Percentage of total	Components	Molecular weight
2S	22	Trypsin inhibitors	8,000 21,500
		Cytochrome C	12,000
		2.3S globulin	18,200
		2.8S globulin	32,000
		Allantoinase	50,000
7S	37	Beta-amylase	61,700
		Hemagglutinins	110,000
		Lipoxygenases	108,000
		7S globulin	186,000 - 210,000
11S	31	11S globulin	350,000
15S	11	_	600,000

[a] Wolf (1972)

The 2S fraction contains several trypsin inhibitors, cytochrome C, two globulins and allantoinase. The 7S fraction is about one-third of the total soybean protein and it contains beta-amylase, hemagglutinins, lipoxygenase and 7S globulin. The 11S fraction makes up another one-third of the protein and the 11S globulin is the principle component of this fraction. The 7S and 11S proteins share a number of chemical properties. And finally, one-tenth of the soy protein is the 15S fraction. This globulin has not been isolated or characterized.

<u>7S protein</u>. Because of the different properties and physical behavior of these globulins, several researchers have investigated the two principle storage proteins, the 7S and 11S fractions. These proteins have many physicochemical properties that may have practical significance in food applications.

The 7S fraction contains lipoxygenase, hemagglutinins, beta-amylase, and predominantly the 7S globulin. Beta-conglycinin, the major 7S globulin, undergoes dissociation into subunits when heated in distilled water (pH 6.8) (Iwabuchi and Shibasaki, 1982). The 7S fraction also dimerizes into low and high ionic strength solutions. The 7S globulin contains two to three cystine groups per mole of protein; therefore, the number of sulfhydryl and disulfide residues is less than found in the 11S globulin (Yamagishi et al., 1982).

Iwabuchi and Shibasaki (1982) also studied the effect of ionic strength on reconstitution of salt-free heat denatured beta-conglycinin. The protein fraction was heated in a boiling water bath (99 °C) for five min and cooled quickly. Standard disc electrophoresis using a 6.5% polyacrylamide gel showed the heat-induced dissociation of the beta-conglycinin fractions (alpha, alpha-1 and beta). Separation patterns also showed a gradual reassociation of the subunits with an increase in ionic strength (0.01, 0.05 and 0.1 M NaCl). The results supported their hypothesis about the nature of the forces involved in intersubunit association. During heating, electrostatic repulsions may play an important role in preventing thermal aggregation of dissociated subunits. Moreover, the finding that heated subunits could be converted back to beta-conglycinin by addition of salt demonstrates that electrostatic bonds assist in association of these subunits. Hydrophobic interactions probably have an important role in redefinition of quaternary structure.

11S protein. Cooling an aqueous extract of soybean meal precipitates the cold-insoluble 11S fraction. The 11S protein fraction has several important physicochemical properties. First is its ability to form disulfide-linked polymers under relatively mild conditions of precipitation by cooling, dialysis or acidification (Wolf and Smith, 1961). Depolymerization of this disulfide polymer can be accomplished by the application of mercaptoethanol, sulfite ion and sodium borohydride. This depolymerization increases its solubility and decreases turbidity of the solution. Dissociation is dependent upon pH and ionic strength of the solution suggesting involvement of hydrogen bonds and van der Waal's interactions. Sulfhydryl-blocking reagents can prevent reassociation of the 11S components.

Koshiyama et al. (1981) investigated the 11S protein reaction during changes in ionic strength and heat denaturation. The protein fraction was heated to temperatures between 50° and

100°C for one hour in 0.1 and 0.5 ionic strength potassium phosphate and sodium chloride buffers, respectively. (Ionic strength of buffers was calculated from the equation $\mu = \sum rZ^2/2$). The researchers found denaturation temperature was dependent on ionic strength. Denaturation occurred between 70° and 80°C in the 0.1μ buffer and between 80° and 90°C in the 0.5μ buffer. The researchers concluded the ionic strength prevented dissociation of the quaternary structure and, therefore, stabilized the structure against denaturation. Hydrophobic bonding seemed to contribute to stability of the quaternary structure at 0.5u ionic strength.

The physicochemical differences between the 7S and 11S fractions produce distinct, well-documented variations in their functional properties. For example, soy proteins readily form gels with heat application (Table 2), but 7S and 11S fractions differ in gelation behavior (Saio et al., 1974; Saio et al., 1975; Utsumi et al., 1982).

Table 2 - Effect of heat on some physical properties of soy protein[a]

	Heating temperature (°C)				
Property	80	100	120	140	160
Subunit structure	dissociation unfolding	------------	------------	degradation	
Solubility	decrease - precipitation	------------	------------	increase in solubility	
Viscosity	increase	--decrease	------------	decrease	
Hydration	increase	------------	decrease		
Gelation (following heating)	regular	----hard-fragile	-------	soft elastic	--sol

[a] Saio et al. (1975) as modified by Kinsella (1979)

Both fractions can form heat-induced or calcium-induced gels. However, heat-induced gels from the 11S globulin fraction exhibit higher tensile and shear strength and greater water-holding capacity than those produced from the 7S globulin. Continued investigations on the physicochemical properties of the 7S and 11S proteins and the unfractionated soy proteins are being encouraged because they all display different properties that can be extrapolated to a great number of food applications.

Functional properties of soybean proteins

Utilization of soy products extends to every part of the globe. In Japan, soybean protein is popular as a less expensive protein source. Various types of soy products (textured, structured, paste and powdered) are used as extenders, as well as for other functional properties they impart (Kanda, 1981). Through processing, soybeans can yield a broad spectrum of products for numerous food applications. Processors can select the desired product characteristics and formulate soy protein to meet product specifications (Hafner, 1964). Functional properties of soy protein preparations in food systems are presented (Table 3) as well as factors governing those functional properties (Table 4).

Table 3 - Functional properties of soy proteins in food systems[a]

Functional property	Mode of action	Food system
Solubility	Protein solvation, pH dependent	Beverages
Water absorption and binding	Hydrogen-bonding of HOH, entrapment of HOH, no drip	Meats, sausages, breads, cakes
Viscosity	Thickening, HOH and binding	Soups, gravies
Gelation	Protein matrix formation and setting	Meats, curds, cheese
Cohesion-adhesion	Protein acts as adhesive material	Meats, sausages, baked goods, pasta products
Elasticity	Disulfide links in gels deformable	Meats, bakery
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna, soup, cakes
Fat adsorption	Binding of free fat	Meats, sausages, donuts
Flavor-binding	Adsorption, entrapment, release	Simulated meats, bakery
Foaming	Forms stable films to entrap gas	Whipped toppings chiffon desserts, angel cakes
Color control	Bleaching by lipoxygenase	Breads

[a] Kinsella (1979)

Table 4 - Factors governing functional properties of food proteins[a]

Intrinsic	Process treatments	Environmental factors-Food-system-components
	Heating	Water
	pH	Carbohydrates
Composition of protein(s)	Ionic strength	Lipids
Conformation of protein(s)	Reducing agents	Salts
Mono-or multi-component	Storage conditons	Surfactants
Homogeneity-heterogeneity	Drying	Flavors
	Physical modification	Oxidation/ Reduction status
	Chemical modification	pH

[a] Kinsella (1979)

Effect of heat treatment on nutritional value of soy protein

Many processes are employed to utilize efficiently soybean protein. During processing most commodities will undergo slight to significant declines in nutritive value. This decline often is attributed to the application of heat treatment. Heat treated soybeans, on the other hand, have a higher biological value in comparison to raw soybeans (Hayward et al., 1936).

Early research by Osborne and Mendle (1917) noted that soybeans would not support the growth of rats unless soybeans were cooked in a steam bath. Innumerable studies have been undertaken to explain the beneficial effects of heat treatment on nutritional value of soybean protein.

Mitchell et al. (1945) discussed the importance of commercial processing on nutritive value of soybean protein. Digestibility and biological value of raw and heat treated soybeans were determined by the nitrogen metabolism method (Mitchell and Carman, 1924). An experimental diet contained 12.5-13.1% total protein that was adequate for maximum growth. Statistical differences were found between autoclaved and raw soybean meal in digestibility and biological value. Further research was stimulated to investigate the mechanism(s) producing the increase in nutritive value.

Mitchell and Smuts (1932), in a paired-feeding study on young growing rats, suggested that cystine was the amino acid limiting biological use of soybean protein. To test this hypothesis, raw and heated soybean protein diets were supplemented with 0.25% cystine. Rats showed a significant increase in weight gain in both cases. Heated soybean inherently had an improved availability of cystine.

With the understanding that cystine additions to raw soybeans increased the biological value and digestibility of protein, it appeared desirable to obtain data on sulfur balances as well. Johnson et al. (1939) used rats fed 18% raw or heated soybean protein to determine total sulfur in the rations, feces and urine. Sulfur was absorbed to the same degree on all rations, but retention of sulfur varied greatly. Rats fed heated soybeans retained approximately 40.1% sulfur, and rats fed raw soybeans retained 16.6% sulfur. All rats on heated soybean diets excreted less sulfur in their urine. In summary, about 2.5 times as much sulfur and 1.8 times as much nitrogen was retained by rats fed heated diets when compared to rats fed raw diets.

Other researchers felt raw soybeans supplied a sub-optimal level of available methionine. Almquist et al. (1942) considered the diversified function of methionine in animal tissues. The researchers suggested the methionine necessary for growth could be affected by the presence or absence of dietary choline, creatine and cystine. Chicks were fed a diet consisting of raw or heated soybean meal which provided 20 g crude protein per 100g diet. It was concluded that lack of available methionine was the major growth-limiting factor in unheated soybean proteins. Chicks without methionine supplements gained 2-3% per day. Adding choline or cystine caused little or no increase in rate of weight gain, but addition of methionine caused a substantial increase in rate of weight gain.

Furthermore, the unsupplemented heated soybean diet was capable of producing a weight gain similar to the standard. When the heated diet was supplemented with methionine, weight gains increased to the level of the standard. The amino acid content of heated soybean protein otherwise was adequate to meet the needs of the growing chick.

Another hypothesis investigated by Kwong and associates (1962) suggested that reduced nutritive quality of raw soybeans was a result of excessive excretion of amino acids in endogenous nitrogen losses in the feces. Since methionine is the first limiting amino acid, it was chosen to make a direct comparison of fecal loss of methionine to total nitrogen losses. This analysis evaluated the question of excessive fecal loss of methionine from raw soybean diets. Male weanling rats were fed a raw or heated soybean diet. Food intake and fecal collections were conducted for five consecutive days. Fecal nitrogen and methionine were determined and percent absorption was calculated. Heated soybean diets showed a definite increase in net nitrogen absorption, while unheated soybean diets had a depressed nitrogen and methionine absorption. But the researchers felt increased fecal nitrogen may have represented endogenous losses rather than a digestion or absorption problem with raw soybeans. This decision was based in part on the fact that an unheated soybean diet did not cause a decrease in absorption of free methionine. Percentage absorption of nitrogen and methionine did not decrease as the level of unheated soybeans was increased. Consequently, it was concluded that losses of endogenous nitrogen and methionine in feces did not contribute to low nutritive value of unheated soybean products.

Work by Westfall and Hauge (1948) suggested that trypsin inhibitor in raw soybean was the critical factor. With this in mind, Kwong and Barnes (1963) investigated the influence of raw soybeans and trypsin inhibitors on tissue utilization of methionine and cystine in rats.

DL-methionine-2-C^{14} was given to rats by stomach tube or by intraperitoneal injection. Rats were pan-fed a raw or heat treated soybean diet. After two weeks, food was withheld for 16 hr and a dose of trypsin inhibitor was given by stomach tube to half of the rats that had received the heated soybean diet. All rats were given DL-methionine-C^{14} two hr later. Then rats were placed in metabolism units and expired CO_2 was collected. A greater rate of $C^{14}O_2$ production in the first 3-4 hr was found in rats fed unheated soybeans or heated soybeans with a dose of trypsin inhibitor. A possible explanation for increased output of $C^{14}O_2$ caused by the trypsin inhibitor could be the result of increased conversion of methionine to cysteine.

To test this hypothesis, rats were fed heated soybean diets and one-half of the rats were given a 0.3% L-cystine supplement. Trypsin inhibitor and DL-methionine-2-C^{14} were administered as previously described. Rate of $C^{14}O_2$ production increased with trypsin inhibitor and no cystine supplement. This increase did not occur with addition of a cystine supplement . This suggested the increase in $C^{14}O_2$ resulted from an increased conversion of methionine to cysteine.

If methionine is the first limiting amino acid in soybean products its supplementation should give a greater increase in weight gain. This belief is supported by the fact that the cystine content of raw and heated soybeans is the same (Kwong and Barnes, 1963). An experiment was designed to test the hypothesis that the raw soybean is of lower nutritional value because of a defect in cystine utilization. Kwong and Barnes (1963) fed two groups of rats unheated soybean diets supplemented with either a 0.3% DL-methionine or 0.3% cystine. Both groups showed the same weight gain. Therefore, they concluded methionine utilization was not impaired when an unheated soybean diet was used because the cystine supplement produced the same growth response observed with methionine supplementation.

Increased conversion of methionine to cysteine currently is recognized to be related to metabolic impairment caused by the trypsin inhibitor. An increase in the cystine requirement was found with raw soybeans. This suggested that a metabolic defect may be responsible for decreased rate of growth in rats fed unheated soybeans. Therefore, the apparent increase in nutritive value of soybeans through the application of heat results from the inactivation of trypsin inhibitor and not from an altering of the soybean protein.

Researchers successfully illustrated this point by observing that trypsin inhibitors cause hypertrophy of the pancreas and an accompanied increase in the general secretion of pancreatic juices. Further work on the hyperactive pancreas suggested that growth depression was related to the loss of essential amino acids in the feces (Lyman and Lepkovsky, 1957). Pancreatic hypertrophy caused an increased production of many enzymes which require large amounts of sulfur. This is a major concern because of the inherently low levels of methionine and cystine in soybeans.

However, Liener et al. (1949) showed that adding a level of trypsin inhibitor to the heat-treated soybean meal at the same level of activity as the raw soybean did not decrease the protein efficiency ratio (PER). This result was found with or without a methionine supplement. Researchers concluded the PER increase is attributable to more than just inactivation of the trypsin inhibitor. They concluded some other factor, unrelated to the trypsin inhibitor, must be inhibiting growth in animals fed raw soybeans.

Kakade et al. (1972) compared the PER values for several soybean varieties with their corresponding trypsin inhibitor activity. The researchers found no correlation between these two parameters. More definitive work was done by extracting the soybean protein to remove the trypsin inhibitor (Kakade et al., 1973). They found the trypsin inhibitor and nutritionally adequate soybean protein in its undenatured state together contributed most growth inhibition. They estimated the trypsin inhibitor alone accounts for approximately 40% of the growth impairment with raw soybeans.

The extract of protein without the inhibitor also was subjected to in vitro digestion. An increase in digestibility was observed in the sample with the inhibitor removed. However, its digestibility did not approach that of heated soy flour extract. This suggested unheated soybeans contain proteins not susceptible to enzymatic breakdown prior to heat treatment. Trypsin inhibitor and the resistant character of the soy protein act synergistically to inhibit protein digestibility according to these workers.

Nutritional value of soymilk

Soy products first were used by Western civilization as a substitute for cow's milk without compromising the nutrient needs of lactose intolerant infants (Aguilera and Lusas, 1981; Dutra de Oliveria and dos Santos, 1981). Soymilk is also of interest for infant feeding in many developing countries due to the scarcity of cow's milk and the desire to overcome widespread protein deficiencies (Hackler et al., 1965; Sugimoto and Van-Buren, 1970).

Soymilk provides essential nutrients to the human body (Chen and Snyder, 1983). Composition of soymilk, cow's milk and breast milk is presented in Table 5, and proximate composition of soymilk is shown in Table 6. When adjusted to the same water content as cow's milk, soymilk contains 52% more protein, 12% less calories, 24% less fat (48% less saturated fat), 16% less carbohydrate and no cholesterol (Chen, 1983).

Chen (1983) compared breast milk and soymilk, also. Soymilk contains 214% more protein, 16% less calories, 19% less fat and 47% less carbohydrate than breast milk. In addition, soymilk contains 15 times as much iron as cow's milk. However, soymilk contains only 52% as much calcium when compared to breast milk and 18.5% as much calcium as cow's milk.

Table 5--Composition of soymilk, cow's milk and breast milk[a]

Item/100 g	Soymilk	Cow's Milk	Breast Milk
Water, g	88.60	88.60	88.60
Calories	52.00	59.00	62.00
Protein	4.40	2.90	1.40
Fat	2.50	3.30	3.10
Carbohydrates	3.80	4.50	7.20
Ash	0.62	0.70	0.20
Calcium, mg	18.50	100.00	35.00
Sodium	2.50	36.00	15.00
Phosphorous	60.30	90.00	25.00
Iron	1.50	0.10	0.20
Thiamine	0.04	0.04	0.02
Riboflavin	0.02	0.15	0.03
Niacin	0.62	0.20	0.20

[a] Chen (1983)

Table 6 - Soymilk composition[a]

Composition	g/100 g
Protein	3.30
Fat	2.15
Carbohydrates	3.60
Minerals	0.35
Cal/100 g	44.00

[a] Chen (1983)

Numerous researchers have investigated nutritional adequacy of soymilk. Shimoda et al. (1926) determined the vitamin B content of soymilk. These researchers made the early observation that soymilk provides a substantial amount of B-vitamins to the diet of young pigeons. Miller (1945) and Miller et al., (1952) made further analyses to quantitate the nutrient composition of soymilk.

Chang and Murray (1949) fed young albino rats a 10 % protein diet of either dried soymilk, dried soybeans or whole milk powder as a standard. Animals on the soymilk diet ate more and, therefore, had higher weight gains in growth studies. However, the PER for whole milk was higher than that for soybeans or soymilk powder. Thiamin, riboflavin and nicotinic acid concentrations were determined on the diets. Soymilk was a better source of these nutrients than the beans.

Hackler et al. (1963) continued this exploration of nutritional value of soybean protein products, and their results for dehulled dried soybeans and soymilk powders are of particular interest. Male Hotzman rats were fed a series of protein concentrations (10, 20 and 30%) using casein as a standard for comparison. At the 10% protein level the soymilk, soybeans and casein had PER values of 2.11, 2.51, and 2.86, respectively. When protein was fed at the 20 and 30% levels PER values were similar to those obtained at the 10% level. Therefore, the researchers concluded an increased consumption of soy protein products was not deleterious to the health of the animals.

Recently, Dutra de Oliveria and dos Santos (1981) tested the utilization of soymilk for the treatment of children with kwashiorkor and marasmus. Clinical and biological tests indicated children fed soymilk generally had the same enhanced nutritional status as those children fed cow's milk. Furthermore, these researchers studied introduction of a soy-vegetable mixture or cow's milk into the childrens' local Brazilian diet. Results disclosed nitrogen balance was better in the diet supplemented with the soy mixture. Thus, foods commonly consumed by low socioeconomic groups could be improved nutritionally through soy product supplementation.

Soymilk extraction methods. Soymilk has a long history of popularity in China. However, in Japan few people consume it because of off-flavors developed during traditional processing techniques (Fukuskima, 1981). Recently, techniques for extraction of soymilk have been developed that yield a product with more desirable sensory qualities. Because of these technological advances Japan has seen a phenomenal increase in sales of soymilk. In 1975 soymilk sales grossed 2.9 million dollars. This figure rapidly rose to 25 million in 1981, and then doubled to 50 million in 1982. The projected figure for soymilk sales in Japan is 300 million dollars by 1990 (Chen, 1983). Chen (1983) stated several factors for increasing consumption across the globe: flavor improvements, low cost, no lactose, highly digestible, nonallergenic, cholesterol free and a lowering of serum cholesterol, highly nutritious, simple technologies, highly versatile, protein sufficiency and finally, easy storage of the raw material. Of these stated advantages, a few are of particular interest when considering the processing techniques utilized in the production of soymilk. These would include 1) simple technologies, 2) flavor improvements, and 3) lowering oligosaccharide contents.

Numerous methods exist for extraction of soymilk. The Chinese and Japanese utilize the traditional Oriental process (Fig. 1), with variation occurring only in heat application before or after filtration. Both processes require little capital investment and equipment needs are minimal. However, these processes demand heavy manual labor and the resulting soymilk has a beany flavor.

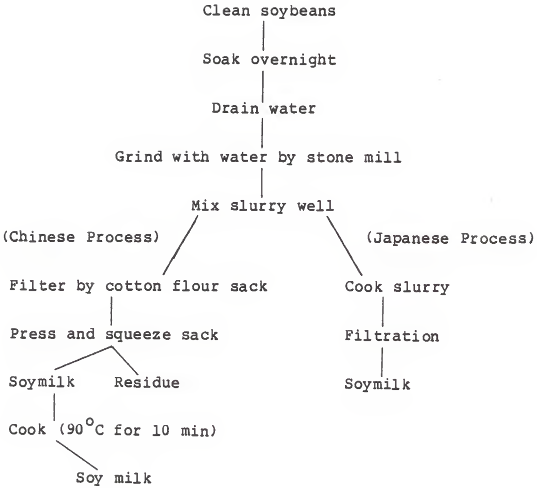


Fig. 1 - Soymilk extraction by the traditional Oriental process[a]

[a] Chen (1983)

Consequently, the hot water grind (Cornell) and whole soybean (Illinois) processes were developed.

The hot water grind process (Fig. 2) was developed by a team at Cornell University. Wilkens et al. (1967) discovered that the application of steam or boiling water during grinding of soybeans would yield a fairly bland soymilk. The Cornell process inactivates the enzyme lipoxygenase which is responsible for the development of the beany off-flavor in soymilk. This process also recovers 78% of the protein from ground soybeans.

The whole soybean or Illinois process (Fig. 3) also was developed to eliminate the objectionable beany flavor of soymilk. The Illinois University group (Nelson et al., 1976) found that a boiling water blanch following soaking, but prior to grinding, effectively inactivated lipoxygenase producing a soymilk with a bland flavor. Furthermore, this process produces a high protein recovery (99.5%) and high soymilk yield (89.5%). This procedure removes a majority of the undesirable oligosaccharides when compared to other methods. One of the unfortunate disadvantages of this method is the necessity of utilizing expensive homogenizers to produce a smooth, stable emulsion.

Much of the recent scientific research has been prompted by new soymilk technologies. Specific areas of concern are 1) improving soymilk's nutritional quality by optimizing cooking conditons to increase yield of soymilk, 2) reducing soybean off-flavors to suit Western preferences, and 3) reducing oligosaccharide concentrations.

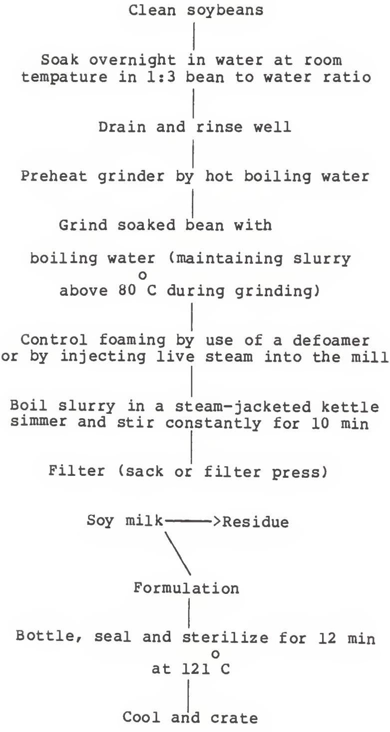
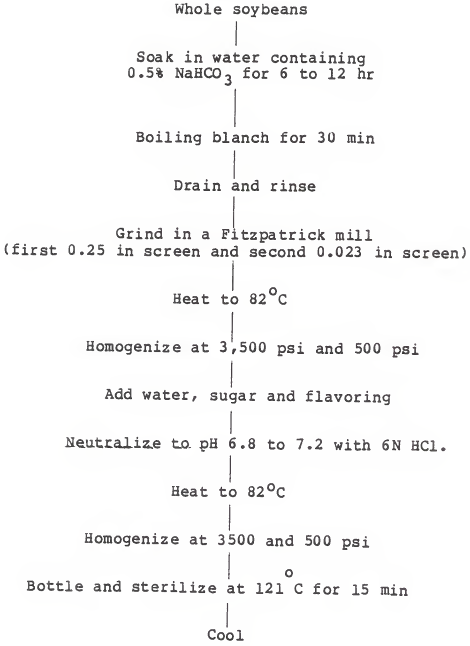


Fig. 2 - Soymilk extraction by the Hot Water Grind Process (Cornell)[a]

[a] Chen (1983)



Whole soybeans

Soak in water containing
0.5% $NaHCO_3$ for 6 to 12 hr

Boiling blanch for 30 min

Drain and rinse

Grind in a Fitzpatrick mill
(first 0.25 in screen and second 0.023 in screen)

Heat to 82°C

Homogenize at 3,500 psi and 500 psi

Add water, sugar and flavoring

Neutralize to pH 6.8 to 7.2 with 6N HCl.

Heat to 82°C

Homogenize at 3500 and 500 psi

Bottle and sterilize at 121°C for 15 min

Cool

Fig. 3 - Soymilk extraction by the Whole Soybean Process (Illinois)[a]

[a] Chen (1983)

Effects of processing on yield and quality of soymilk. Hackler et al. (1965) investigated the effect of heat treatment on nutritional value of soymilk protein using weanling rats. They found cooking time and temperature are both important in processing of soymilk. Two temperatures (93 °C and 121 °C) were compared over a range of time exposures. Processing soymilk at 121 °C for five min was found to produce the highest PER, 2.24 versus a maximum PER of 1.96 for soymilk heated at 93 °C for 60 min. Further investigation showed with adjustments in processing time similar PER values can be obtained at the two different processing temperatures. These results indicate the PER of heat-processed soymilk is both time and temperature dependent.

Further soymilk investigation by Hackler and Stillings (1967) examined the amino acid composition of heat-processed soymilk and its relationship to protein quality. Cooking samples at 93 °C for as long as four hr had no significant effect on amino acid composition, essential amino acid index (EAAI) or requirement index. When the PER and EAAI were compared, a high correlation existed between the two measurements once the best PER had been achieved.

Secondly, soymilk was heat-processed at 121 °C and analyzed for amino acid composition. Decreases in the PER value and amount of available lysine were noted when soymilk was heated at 121 °C for 32 min. Also cystine and tryptophan decreased as the cooking time increased from 0-120 min. The critical relationship between time and temperature was supported again. Consequently, heat processing conditions must be chosen to produce a soymilk with the highest possible nutritional quality.

Lo et al. (1968) studied the effects of soaking soybeans prior to soymilk extraction on chemical composition and yield of soymilk. Soybeans were soaked for 24 and 72 hr in three times their weights of water at 1°C, and processed into soymilk. As the soaking time increased, greater amounts of water-soluble solids were found in the soak water. Researchers suggested metabolic changes in soybeans during soaking caused the observed decline in protein content from 43% in the non-soaked control to 38% for the 24 hr soak to 36% for the 72 hr soak. Fat also decreased from 24% to 19% in the 72 hr soak. And carbohydrate contents were found to increase during soaking (23%, control; 24%, 24 hr soak; 26%, 72 hr soak). However, comparison of soymilks prepared from soybeans soaked overnight (about 16 hr) and soybeans soaked 72 hr generally showed few differences.

Wilkens and Hackler (1969) soaked whole soybeans for 1, 3 and 5 hr at water temperatures of 25-65°C. Hydrated beans then were ground with water at 100°C and filtered to produce soymilk. Unsoaked (control) dehulled soybeans were ground with water extraction temperatures varying from 0-100°C and filtered. Evaluations of soaked soybeans exhibited a decline in total solids with increased soaking times and increased temperatures. Overall, the effect of soaking primarily was leaching of water-soluble carbohydrates into the soaking water. Consequently, a concentration of protein and lipid in the soymilk resulted. Increasing soaking temperatures severely reduced soymilk yield.

Increasing water temperatures during grinding of unsoaked soybeans showed the carbohydrate fraction decreased and the lipid fraction increased at tempertaures between 60°-70°C. Protein recovery slightly decreased at temperatures of 70°C or greater. With extraction temperatures greater than 70°C the insoluble carbohydrate fraction was hydroscopic and its subsequent swelling inhibited filtration of soluble solids. Therefore, a decline in total solids was observed at temperatures above 70°C. This decline could have practical importance on the functional properties and protein recovery of the resulting milk.

Further investigations considered application of various solutions during the soaking period and their effects on the composition of the resulting soymilk. Khaleque et al. (1970) soaked soybeans in water (no additive), 0.4 M Na_2CO_3 or 0.2 M NaOH for 8, 12, 18 or 24 hr at room temperature (17-21°C). Soaked soybeans were dehulled and ground into soymilk. Proximate composition and amino acid composition were determined on all soymilks. Composition of soymilks from beans soaked for 24 hr in water or 0.4 M sodium carbonate and 12 hr in 0.2 M sodium hydroxide were compared. Overall composition of soymilks prepared from water or sodium carbonate soaked beans showed no appreciable differences. Milk extracted from beans soaked in sodium hydroxide, however, contained lower amounts of protein, fat, carbohydrate and ash when compared with the other two milks.

Protein recovery was observed to be higher in the soymilk produced from the 24 hr sodium carbonate soak, but the sodium hydroxide soak significantly reduced protein recovery. Pretreatment with sodium carbonate for 24 hr and sodium hydroxide for 12 hr had no significant effect on amino acid composition when compared to the control.

Johnson and Snyder (1978) investigated the effects of three different processing techniques on yield and composition of soymilks. The three different processes employed are outlined in Table 7. In addition to processing variations, different soybean:water ratios were studied. Samples were lyophilized prior to proximate analyses.

Table 7 - Outline of processes used for the preparation of soymilk[a]

Process A	Process B	Process C
Soak soybeans in 0.5% $NaCHO_3$ solution for 18 hr	Soak soybeans in tap water for 18 hr	Soak soybeans in tap water for 18 hr
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

[a] Johnson and Snyder (1978)

Solids yield was greatest for process C, intermediate for process B and least for process A. Differences observed were attributable to variations in heat application. Generally, heating decreased solids yield and heating prior to grinding decreased solids yield more than heating during grinding of soybeans. Process A yielded a soymilk with less protein and more lipid than the other two processes. The authors concluded that poor protein recovery was caused by denaturation of protein from the heat treatment. Carbohydrate and ash values were similar for all three milks. Carbohydrate contents of all milks were considerably lower than the original beans which indicated a failure to extract all the carbohydrate from the bean cell walls.

When soybean:water ratios were varied during soymilk production, processes A and C showed similar increases in percent soymilk yields. But process B exhibited a lower percentage soymilk yield in all ratio variations. Researchers suggested this decrease resulted from water lost during the hot-grind procedure and from soymilk lost in the precipitate during centrifugation.

In all three processes, the percent solids in soymilk increased as soybean:water ratios decreased. Conversely, percent solids yield increased as the ratio of soybeans:water increased. (Solids yield was determined as the percent of solids in the original soybeans recovered in the milk). This finding can be explained by considering the two processes occurring as the beans are pulverized. The first process would be extraction of soluble solids, and the second, a grinding of large particles into smaller pieces that remain in solution following centrifugation. Researchers stated that the first process would be more effective with an increase in the volume of water, but the second process would be enhanced by a decrease in the volume of water.

Effects of processing on soymilk flavor. Hand et al. (1964) stated that the most important criterion for soymilk acceptance was flavor. Development of off-flavors in soymilk during processing has been attributed to degradation of lipids by enzymatic oxidative reactions. The enzyme lipoxygenase, present in soybeans, catalyzes the oxidation of polyunsaturated fatty acids. This can occur only when the soybean cell wall structure is disrupted. During the grinding of soybeans to extract the milk the enzyme and substrate interact producing off-flavors. Shutte and Van Den Ouweland (1979) presented a summary of various off-flavors that develop in soy protein materials (Table 8).

Many scientists have examined the effects of preprocessing conditions on flavor of soymilk. Wilkens et al. (1967) studied the effects of temperature of grinding on oxidative off-flavor development in soymilk. Researchers ground soaked and unsoaked whole soybeans with water at temperatures between 20-100 °C. Following filtration, sample volatiles were analyzed by gas chromatography. The authors found the rancid flavor in soymilk was caused by the volatile fraction. Formation of volatiles decreased with an increase in grinding temperatures indicating thermal inhibition of enzymatic activity. When 100 °C water was employed, gas chromatographic profiles indicated few differences

Table 8 - Off-Flavors in Soy Protein Materials[a]

Off-flavor	Compounds responsible	Precursors	Prevention/removal
Bitter taste	?	Phosphatidyl cholines	Alcohol extraction
Sweet taste	Sucrose	---	Water extraction
Green, grassy odors	Carbonyl compounds	Polyunsaturated fatty acids + lipoxygenase	Hexane extraction Heating intact beans
Cooked soybean odors	p-vinyl phenol p-vinyl guatacol	p-Choumaric acid ferulic acid (lignin)	Low heat treatment Alcohol extraction
Burnt flavor	Ketones, aldehydes furans, sulfur compounds pyrazines	Amino acids + carbohydrates	Low heat treatment Water extraction
Catty odors	4-methyl-4-mercapto-2-pentanone	Acetone + hydrogen hydrogen sulphide	Solvent removal
Fusel note	Long chain alcohols	---	Solvent removal

[a] Shutte and Van Den Ouweland (1979)

between soymilks produced from soaked and unsoaked soybeans.

Khaleque et al. (1970) extracted soymilks prepared from beans soaked in 0.5 M Na_2CO_3, 0.5 M $NaHCO_3$, 0.2 M NaOH, 0.5 M Na_2SO_4, 0.5 M Na_5SO_3, 0.5 M Na_2HPO_4, 0.5 M Na_3PO_4, 0.8 M NaCl or 0.5 M $Na_2S_2O_5$ + NH_4OH solutions. A control (no additive) was used when samples were presented to the taste panel. Panelists evaluated the intensity of the beany flavor. Solutions of 0.5 M Na_2CO_3 and 0.2 M NaOH produced a reduction ($P<0.01$) in beany flavor. Furthermore, soybeans soaked in water for 24 hr, 0.4 M Na_2CO_3 for 24 hr, and 0.2 M NaOH for 12 hr then were ground using a series of temperatures above 70 ^oC. A room temperature grind was used as a control. Unexpectedly, there were no significant differences between the intensity of beany flavor and the variations in grinding temperature or soaking solutions. Consequently, the investigators stated the failure to observe an increase in the beany flavor after cold grinding in the control as well as the experimental variables suggested there were more significant factors, other than lipoxygenase activity, responsible for the development of the beany flavor.

Johnson and Snyder (1978) studied the effectiveness of inactivating lipoxygenase prior to grinding by heating whole hydrated soybeans. Soybeans were steeped in water for different time and temperature combinations. Two min at 100 ^oC was adequate to inactivate the enzyme. When the temperature was reduced to 60 ^oC, 2.5 hr were required for inactivation to occur.

<u>Effects of soymilk processing on flatus production</u>. Soymilk contains considerable amounts of the oligosaccharides stachyose and raffinose and trace amounts of verbascose. These

alpha-D galactopyranosyl sugars are fermented by microorganisms in the intestine caused by humans' lack of alpha-galactosidase (E.C. 3.2.1.22) in their digestive tracts. The anaerobic fermentation of these carbohydrates in the ileum and colon causes the release of high concentrations of carbon dioxide and hydrogen gases (Rackis et al., 1970; Rackis, 1981). Consequently, reduction or removal of these sugars from soy products has been the topic of several research projects.

Sugimoto and VanBuren (1970) investigated the use of an enzyme preparation from *Aspergillus saitoi* to reduce the flatus production of soymilk. The researchers were able to produce a purified preparation of alpha-galactosidase and invertase with a simple molecular sieving procedure. Thin-layer chromatographic profiles showed additions of small amounts of the enzyme preparation resulted in complete hydrolysis of the galacto-oligosaccharide in soymilk.

Further research was conducted by Thananunkul et al. (1976) on *Mortierella vinacea* which produced alpha-galactosidase. The enzyme preparation successfully hydrolyzed some of the oligosaccharides in soymilk. Results showed a substantial increase in raffinose content from hydrolysis of stachyose. But the researchers noted the stachyose content in soymilk is three times that of raffinose. This would explain increased raffinose content following enzyme treatments. This reduction of stachyose and subsequent increase in raffinose has practical application, since stachyose induces greater flatus production than raffinose.

Innovations in soymilk processing. Hand et al. (1964)

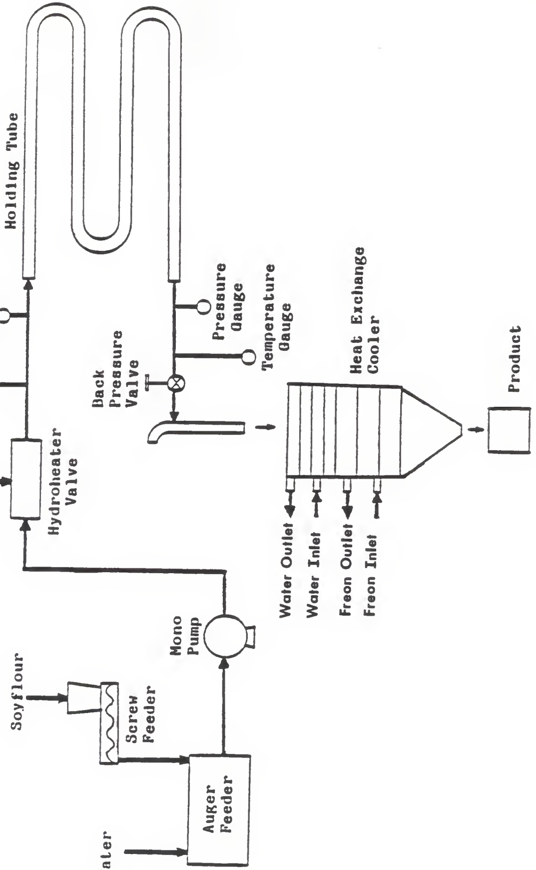
investigated yield and quality of soymilk produced directly from whole soybeans without utilizing a water-extraction step. Soybean hulls were loosened, whole beans were heat treated and dehulled. Beans were ground in a comminuting mill, slurried with water at 140°F, homogenized and spray-dried. This process increased yield of soymilk and reduced labor costs.

Johnson (1978) and Johnson et al. (1981) developed a steam-infusion cooking method to extract a high quality, bland soymilk. Whole soybeans were ground into a flour and fed into a Penick Ford laboratory jet cooker. Soy flour was slurried with water and heated in the jet cooker for variable times (0-34 sec) at temperatures of 121, 132, 143 or 154°C. Soy flour was slurried with water and cooked at 99°C for 5-60 min as a simulated, traditional control Cooked jet and traditional slurries were collected, quickly cooled and centrifuged. Percentages for soymilk fraction yields, solids yield and protein yields were determined. In general, the use of soybean flour at temperatures of 121-154°C produced greater yields. Maximum solids recovery was 86%; maximum protein recovery was 89.5%; and maximum yield of soymilk was 90%. The authors offered several explanations for the high yields associated with the steam infusion process. Increased yields from this stable emulsion probably were the result of optimum heat exposure and dissociation of protein bodies by the steam-shear force.

Further research on steam-infusion extraction of soymilk was conducted by Hung (1984) who used a modified Penick Ford laboratory continuous jet cooker (Fig. 4). Soymilk was processed under Rapid Hydration Hydrothermal Cooking (RHHTC) at temper-

Fig. 4- Modified Penick Ford laboratory jet cooker[a]

[a] Hung (1984)


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

atures of 152, 154 and 157°C for approximately 35 sec. The cooked slurry was cooled quickly, collected and centrifuged. Yields of fractions, solids and nutritional quality of the RHHTC and traditional control soymilks were determined. The yield of the soymilk fraction and solids increased as RHHTC temperatures increased. Highest yields were reported for the 157°C soymilk. No marked differences in amino acid composition of the soymilks were noted. PER values for both extraction methods showed the RHHTC method produced a soymilk with higher nutritive value. This method seems to have strong potential for production efficiency and profitability which could have major impact on the soybean industry.

Quality of soybean curd

Nutrient composition of soybean curd. Soybean curd generally is considered a highly digestible and nutritive product (Schroder et al., 1973). Hayowitz et al. (1981) evaluated a typical serving of tofu for nutrient content. A 130-g portion provides 91 calories, 20% of the Recommended Daily Allowance for protein and calcium, 26% for magnesium, 22% for phosphorous and 12% for iron. Calcium and magnesium contents vary with the coagulant used during production.

Smith (1981) reported that the ideal soybean would be high in protein, high in oil, low in indigestible oligosaccharides and low in anti-nutritional factors. By processing soybeans into tofu these four criteria can be satisfied. Tofu contains substantial concentrations of protein and fat, small quantities of oligosaccharides, and with proper processing techniques, low levels of antinutritional factors.

During tofu processing, soybean curds and whey are separated. Nutrients are sacrificed when the whey is discarded, specifically soluble proteins and B-vitamins. The removal of whey also is desirable since a majority of the soluble oligosaccharides are lost in the whey. This substantially reduces tofu's flatus production (Fukushima, 1981). Furthermore, Miller et al. (1952) observed large losses of B-vitamins when soymilk was processed into tofu. The whey pressed out of the soybean curd contained more B-vitamins than the curd itself, and leaching continued as the block was allowed to stand. Overall, the authors reported that approximately half of the water-soluble vitamins were lost in the whey released from the curd.

Early work on the biological value (BV) of tofu was conducted by Chang and Murray (1949). Soy protein product BV was determined by the rat growth method. Growth of rats on the soymilk diet was greater than growth observed in rats on the tofu diet. The soymilk also was found to contain greater amounts of B-vitamins when compared to the tofu.

Hackler et al. (1963) determined growth and PER values for several soybean fractions. Diets containing full-fat flour, soymilk, soybean residue from water extraction, soybean curd and whey fractions were fed to male weanling rats at protein levels of 10, 20, and 30%. Generally, results for all dietary protein levels were similar. Surprisingly, the residue fraction contained the highest quality protein. Soymilk and tofu diets were found to be inadequate when compared with the residue and soy flour diets. As expected, poor growth and PER values were observed with the whey protein diets.

Schroder et al. (1973) also evaluated the nutritional adequacy of soybean curd. Weanling rats were fed diets containing casein or soybean curd as the sole source of protein. Rats fed the tofu test diets consumed less feed, had lower weight gains and had lower PER values than rats fed the casein test diet. This overall decline could be attributable to the limiting amino acids in soybeans.

Further investigations were conducted by Gillette et al. (1978). Commercially processed isolated soy protein, soy flakes, soybean nuts and tofu were studied, and bovine nonfat dry milk (NFDM) was used as a control. Weight gain coefficients for male weanling rats were determined. The tofu diets had a weight gain coefficient of 65% when compared to the NFDM diet. This finding has nutritional importance if tofu is used as a dietary replacement for bovine milk or cheese.

Shelf life and microbiological studies. Researchers have documented a limited shelf life for tofu (Dotson, 1977; Pontecorvo and Bourne, 1978; Andres, 1985). Temperature fluctuations during processing and transportation and initial microbial loads cause variations in the spoilage rates of tofu.

Dotson et al. (1977) measured changes in the immersion solutions of tofu during three days of storage at 15 ^{o}C. Optical density, pH and viable cell counts of the solutions were monitored as indirect methods of spoilage rates of tofu. In general, pH and optical density decreased and viable cell counts increased with time. The authors concluded these changes were primarily from the presence and multiplication of lactic acid bacteria. To date, microbiological standards have not been set for the bacterial safety of tofu (Rehberger et al., 1984).

Rehberger et al. (1984) studied microbiological quality of soybean curd. Four different brands of tofu were purchased within 24 hr after the shipment had arrived at retail outlets. During collection of samples, the authors noted inconsistent storage practices in the stores. Microgiological analyses performed immediately on the tofu included total aerobic plate counts, psychotrophic counts, yeast and molds, coliforms, sporeformers and coagulase-positive staphylococci tests. If the package provided a manufacturer's pull date, then samples were held until the pull date before they were analyzed. A substantial number of the samples analyzed immediately had total counts greater than 10^6 colony forming units (CFU)/g. Also, more than half of the samples had confirmed coliform counts greater than 10^3 CFU/g. Greater than 10 CFU/g were reported for all other microbiological tests. Samples held until the manufacturer's pull date contained higher total and psychotropic counts when compared with samples analyzed immediately following purchase. The authors concluded the high microbial counts indicated that tofu is an excellent medium for microbial growth. The presence of such high microbial counts shortly after delivery suggested production and distribution problems. High initial counts in tofu are a great concern. Processors must improve sanitation during manufacture and retailers must provide more consistent refrigeration of this product.

Lim (1984) also evaluated the microbiological quality of commercially available soybean curd. All samples were purchased within 24 hr after delivery at the retail outlets. Initial bacterial loads of immersion solutions ranged from $10^4 - 10^8$ CFU/ml. Several of the samples had counts greater than 10^8 CFU/ml within four days of storage at 4-7 ^oC, and all six samples had 10^8 CFU/ml after six days of storage. Furthermore, no differences were found between water-packed and pasteurized water-packed tofu.

Aulisio and coworkers (1983) documented foodborne Yersiniosis associated with the consumption of commercially processed tofu. The soybean curd was contaminated with _Yersinia enterocolitica_. The authors found that the processing water was the source of one of the two strains identified. Concern about the microbiological safety of tofu increased following this Yersiniosis outbreak. The tofu manufacturer associated with this contaminated product now uses chlorinated water and surface sterilizes the tofu blocks with boiling water (Anon., 1983).

Some researchers have concentrated their efforts on improvement of the microbiological quality of tofu during storage. Pontecorvo and Bourne (1978) developed a simple method for the extension of the shelf life of tofu in tropical areas. Soymilk was extracted and coagulated with lemon juice. Three preservation techniques were investigated. These included addition of table salt (1 and 5% NaCl), 10% lemon juice, or methyl (0.1%) and propyl (0.1%) parabens to immersion solutions. Sodium chloride and lemon juice were combined for some treatments (3% NaCl + 5% lemon juice; 5% NaCl + 5% lemon juice; 3% NaCl + 10% lemon juice; 4% NaCl + 10% lemon juice or 5% NaCl + 10% lemon juice). Smoking the soybean curd for 4, 8, 12, 24, 36 or 48 hrs or a combination of smoking and an immersion solution was studied. Tofu samples were stored at room temperature (24-25°C) or 37°C for up to 15 days. Immersion solutions were changed weekly, and the control (water) was changed daily. Panelists evaluated flavor, aroma, mouthfeel, texture, color and general appearance.

Tofu immersed in the 4% NaCl + 10% lemon juice solution maintained fresh odor and flavor acceptability for 10 days and were judged acceptable after 15 days at 24° and 37°C. Judges found the use of NaCl or lemon juice alone produced a product unsuitable for consumption. Parabens also were unacceptable because they produced medicinal odors and flavors during storage. As smoking time increased, the flavor, odor and general preference of samples increased. Some surface mold growth occurred on samples smoked for 4, 8, 12 and 24 hr. However, samples smoked for 36 and 48 hr showed no surface molding following 10 days of storage.

Coupling of smoke treatment and immersion solution synergistically improved microbiological quality of the stored tofu. Samples smoked for 24 hr and immersed in 4% NaCl + 10% lemon juice had a shelf life of 9-10 days at both 24° and 37°C. Overall, microbiological examinations of fresh and stored soybean curd showed that after 10 days of storage the tofu was safe for human consumption.

More recently, a new tofu packaging technique has been developed enabling an "everfresh" product to be stored at 25°C for up to 3 months. This method utilizes high temperature-short time (HTST) heating for soymilk of approximately 10% solids. The milk is heated to 131°C for 1 sec, cooled, aseptically packaged and heated to coagulate the protein (Fukushima, 1981). HTST processing has been successfully introduced into the dairy industry.

Lim (1984) evaluated the effects of the addition of selected chemical preservatives to the immersion solutions on shelf life of soybean curd. Tofu was made on a laboratory scale and immersed in solutions containing no additive; 0.5% acetic acid (AA); 0.1% citric acid (CA); 0.15% potassium sorbate (PS); 0.005% tertiary-butyl-hydroquinone (TBHQ); 0.15% PS + 0.5% AA: 0.15% PS + 0.1% CA; or 0.15% PS + 0.005% TBHQ. Immersed samples were stored at 10-15 °C for up to 23 days. Viable cell counts and lactic acid bacteria counts were monitored during storage.

After 23 days of storage, AA or PS + AA were effective in controlling microbial growth. These two treatments maintained bacterial populations less than 10^6 CFU/ml. Immersion solutions of CA or PS + CA were effective antimicrobial agents up to 13 days of storage. However, the remaining treatments did not successfully inhibit microbial growth with bacterial loads of 10^9 - 10^{10} CFU/ml after 23 days of storage. Lactic acid bacteria increased substantially as storage time increased in all immersion solutions except PS + AA.

Sensory evaluation of tofu showed the AA or PS + AA immersed samples had greatest total flavor intensity up to nine days of storage. However, these samples also were judged as having an acidic odor and sour taste. In general, all samples were scored lower in tofu flavor when compared with fresh tofu regardless of chemical additive and storage time.

Soybean curd processing

Preparation of soybean curd. Tofu is a highly gelatinous curd derived from soybean milk. The popularity of tofu has risen and it is now considered one of the most popular soybean products (Sri Kantha, 1983; Rehberger et al., 1984). Soyfood industry observers suggest that tofu is quickly becoming the "yogurt of the 1980's" (Leviton, 1982). Industrial interest in tofu production and sales has led to extensive research on the processing and sensory qualities of tofu.

Tofu production by the traditional method involves three main steps: extraction of soymilk, coagulation of protein and pressing the curds into a cohesive block (Fig. 5). Silken tofu does not require the final step.

Dry soybeans are rinsed, soaked overnight at room temperature, drained and ground with water. Heat treatment may be applied before or after filtration to inactivate antinutritional factors, reduce off-flavors and improve curd formation. The coagulant is added to soymilk producing curds and whey, and the curds are pressed to remove excess whey (Wang, 1984).

Coagulant type and concentration. Coagulation of soybean proteins into tofu is the most critical factor affecting yield and quality of soybean curd. However, this process is not well understood (Wang, 1984).

Early work by Circle et al. (1964) suggested that there was an ionic protein-protein interaction during coagulation by calcium sulfate. Separation and characterization of soybean protein fractions has allowed researchers to probe further into

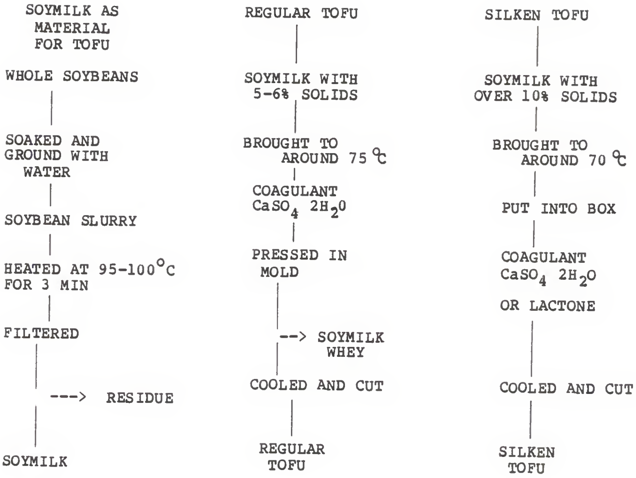


Fig. 5 - Manufacturing processes for regular and silken tofu[a]

[a] Fukushima (1981)

the factors affecting soybean curd formation.

Saio et al. (1967) and Appurao and Rao (1975) also suggested calcium coagulation of soybean proteins was caused by cross-linking between protein molecules by the calcium ion. However, the site of this cross-linking is still under debate.

Specific protein fractions also have been studied for their contributions to soybean protein gel formation. Saio et al. (1969) reported differences in tofu gel hardness when 11S and 7S purified protein fractions were used. The 11S protein isolated from soybean meal produced a much harder gel than the 7S fraction. Ratios for 7S:11S protein fractions may vary with different soybean varieties. Therefore, Saio and her coworkers postulated that soybean variety could affect tofu texture. Skurry et al. (1980) compared 15 varieties of soybeans and found no significant correlations between the 7S:11S protein ratios and tofu quality. However, the researchers found tofu quality was heavily dependent on the calcium concentration.

Several calcium salts can be used in tofu production. Saio (1979) found that $CaCl_2$, which is soluble in water, had a faster curd formation ability than $CaSO_4$. The author found the optimum concentration of calcium salt to be approximately 0.02N.

Lu et al. (1980) investigated the use of various precipitating agents for tofu production. Soybean milk was coagulated by the addition of either $CaSO_4 \cdot 2H_2O$, $CaCO_3$, $CaHPO_4$, $Ca(CH_3COO)_2$, $Ca(OH)_2$, $C_{12}H_{22}CaO_{14}$, $CaCl_2 \cdot H_2O$ or $C_6H_{10}CaO_6 \cdot 5H_2O$. Two noncalcium compounds, glucono-delta-lactone (GDL) and acetic acid, also were examined. Coagulants successfully precipitated soy protein at concentrations ranging from 0.05-0.5%. In all cases, soy protein began to coagulate when the pH dropped to approximately 6.0. Some coagulants at specific concentrations did not precipitate the protein. The authors concluded this failure to form a precipitant was primarily pH dependent and not caused by coagulant concentration.

Wang and Hesseltine (1982) reported that both ionic concentration (0.01-0.08M) and the coagulant type ($CaSO_4$, $CaCl_2$, $MgSO_4$ and $MgCl_2$) affected the gross weight and the moisture content of tofu. Researchers suggested that quality of tofu is dependent particularly on type and concentration of the coagulant. Their results advocated the use of $CaSO_4$ for highest quality soybean curd. The authors found the proper amount of calcium sulfate should be dispersed in water equal to 10% of the volume of soybean milk with a final concentration of 0.02M $CaSO_4$ in the soymilk.

Innovations in soybean curd processing. Tofu's nutritional composition and simple preparation techniques suggest that more highly mechanized procedures could be found for large-scale production (Smith et al., 1960). Fukushima (1981) reviewed recent developments in tofu processing. Silken tofu production has been adapted readily to industrial processing methods. Soymilk containing about 10% solids can be mixed with GDL and placed in 250-300 ml plastic containers and sealed. The container is then heated in a water bath at 80-90 °C for 40-60 min. The coagulant is activated by heat and the milk produces a soft, fragile curd.

A mass production system for tofu has been developed. Traditionally. soybean curd production can be labor intensive.

But this system utilizes a continuous process of coagulation, pressing, cutting and packaging in large moving box frames. Silken and regular tofu also may be made from instant powdered soymilk. The processor hydrates the powder and proceeds with the production scheme.

One recent innovation in soybean curd processing is called "bagged tofu". Soymilk and a calcium salt are placed in a plastic bag, sealed and submerged in hot water for about 1 hr. This heat treatment is necessary for proper curd formation and it also sterilizes the tofu. Whey remains in the bag and may be removed by the consumer (Smith and Circle, 1978).

MATERIALS AND METHODS

Soymilk extraction

Traditional method. Soymilk was extracted according to the procedure of Lim (1984) with modifications. Whole certified seed grade Williams soybeans (700 g) were washed and soaked in 2.8 L tap water at ambient temperature (22°-25°C) for 10 hr. Beans were rinsed with tap water and drained. Soybeans were weighed into seven portions. Hot tap water (300 ml) was added to each portion and blended for two min on high with an Osterizer blender. Blended portions were combined with hot tap water (2.8 L) in a heavy cast aluminum utensil (capacity 19 L). The puree was transferred slowly to a cloth filter bag (Tofu Kit, Soyfoods Center). The soymilk was allowed to drain with occasional stirring of the residue. When drainage slowed, 700 ml of boiling water were poured into the filter bag and stirred. The filter bag was squeezed for one min to express the remaining soymilk. Soymilk yield was measured in ml and the residue was discarded. Total solids were determined following AOAC (1985) procedures. A predetermined quantity of the resulting soymilk (3 L) was heated to 100°C and simmered for seven min. The above procedure was repeated seven times per replication to obtain sufficient quantity.

Rapid Hydration Hydrothermal Cooking method. Whole soybeans were dehulled and ground slowly through a 0.51-mm screen using a Fitz hammermill (Fitz Patrick Co., Elmhurst, Illinois). Soybean flour was stored at 4°-7°C until needed for soymilk production. The modified Penick and Ford laboratory continuous jet cooker (Penick and Ford Limited, Cedar Rapids, Iowa) was used to produce soymilk under RHHTC at a temperature of 121°C and 70 psi.

The resulting soymilk was quickly cooled to 5°-10°C by a conduction heat exchange cooler. Approximately 57 L of soymilk were extracted for each of two replications. The soymilk was stored at 4°-7°C overnight. Yields of the soymilk fraction (Johnson and Synder, 1978) and solids (AOAC, 1985) were determined. All remaining soymilk was centrifuged 10 min in 500-ml centrifuge bottles at 2200 rpm developing 1100 x g centrifugal force for 10 min (International Equipment Co., Boston, Mass.). The supernatant (soymilk) was decanted and the residue was discarded.

Soybean curd preparation

Food grade calcium sulfate dihydrate (Charles B. Crystal Corp., Inc.) was used as the coagulant. The procedure of Lim (1984) was modified to standardize the amount of soymilk used for tofu production. RHHTC soymilk (2.5 L) was heated to 80°C in a cast aluminum utensil and a dispersion of 13.7 g calcium sulfate (0.60%, 0.04M) in 250 ml water were added. Traditional soymilk (3 L) was cooled to 80°C and 10.7 g (0.32%, 0.02M) calcium sulfate dispersed in 315 ml water were added. Tofu blocks were weighed immediately after immersion for 15 min in cold tap water.

Treatments

Each block (19.7 x 10.6 x 10 cm) of tofu was divided into three pieces (6.85 x 10.6 x 10 cm) and each was placed into a white plastic food tray (GFW-26., Gage Industries, Inc.) with an immersion solution.

Traditional tofu. The following immersion solutions were used:

1. Sterilized distilled water (no additive)
2. 0.5% Acetic acid (AA) (Fisher Scientific)
3. 0.15% Potassium sorbate (PS) (Pfaltz and Bauer, Inc.) + 0.5% AA

Samples were covered with aluminum foil and stored at 4-7 °C until analyzed for moisture, microbiological, amino acid, protein, carbohydrate, ash, thiamin and riboflavin contents. Each block was assigned randomly a storage period (0, 3, 7, 10, 14, 17 or 21 days) and each of the three pieces randomly was assigned a treatment (type of immersion solution).

Rapid Hydration Hydrothermal Cooking tofu. Five blocks of tofu were prepared from each extraction of soymilk. One-third of each block was chosen from random positions for analyses. These samples were immersed in sterilized, distilled water (no additive) and analyzed immediately for amino acid, protein, carbohydrate, ash, thiamin and riboflavin contents.

Microbiological analysis of traditional tofu

Viable cell counts were monitored during the 21 day storage period. One ml serial dilutions of soybean curd immersion solutions were plated using Total Plate Count Agar (Difco) according to specifications and standard methods of SUP, APHA and AOAC, as stated in the Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory

Procedures (1977). Plates were incubated at 32 °C for 48 hr.

Moisture content

Moisture content was determined on days 0, 3, 7, 10, 14, 17 or 21 of storage according to AOAC (1985) methods. A 1x1x10 cm slice of tofu was made approximately 3 cm from the uncut edge of the piece. If the sample was to be taken from the end of a block, then the uncut end was removed prior to sampling.

Chemical Analyses

B-Vitamins. Thiamin and riboflavin contents were measured after 0, 3, 7, 10, 14, 17 or 21 days of storage using a Coleman Jr. Photofluorometer following the procedure of Freed (1966). Approximately 40 g of each sample were used following the sampling plan previously described for moisture content.

Proximate composition: Traditional extraction. All soymilk and tofu samples were freeze-dried prior to analyses. The seven traditional soymilk extractions from each replication were pooled because of speed and cost considerations which produced one sample per replication for analyses. Tofu samples from replication one and two were pooled for each storage period and tofu samples from replications three and four were pooled for each storage time. Protein content, expressed as percent nitrogen, was calculated from the total amino acid composition using a conversion factor of 5.71 (Heidelbaugh et al., 1975; Morr, 1981; Morr, 1982). Fat content was determined using AOAC procedures (1985). Carbohydrate content was calculated by difference. Samples were ashed following AOAC procedures (1985).

Proximate composition: Rapid Hydration Hydrothermal Cooking extraction. Soymilk and tofu samples from the two RHHTC replications were freeze-dried prior to analyses for the components described in the previous section.

Amino acid composition: Traditional extraction. Fresh soymilk samples were analyzed for amino acid composition and tofu samples were assessed following 0, 7, 14 or 21 days of storage using a Dionex D-300 kit single column amino acid analyzer. The samples were hydrolyzed for 31 hr at 100 °C with p-toluenesulfonic acid. Norleucine was used as an internal standard.

Amino acid composition: Rapid Hydration Hydrothermal Cooking extraction. RHHTC samples of soymilk and tofu were analyzed immediately after preparation using a Dionex D-300 kit single column amino acid analyzer. The samples were hydrolyzed for 31 hr at 100 °C with p-toluenesulfonic acid. Norleucine was used as an internal standard.

Experimental design and analysis. Seven large blocks of traditional soybean curd were prepared and sectioned into three small blocks (approximately 330 g each). Each large block randomly was assigned a storage period. The three slices also randomly were assigned an immersion solution (sterilized, distilled water, AA, or PS + AA) and stored at 4-7° until analyzed. This procedure was replicated four times.

Five large blocks of tofu were prepared from RHHTC soymilk for each of two extractions. Each block was sectioned into three small blocks and one of the sections was chosen randomly for immediate analysis.

The data were analyzed using Statistical analysis systems (SAS)-analysis of variance when the experiment was balanced and by SAS-general linear models when the experiment was unbalanced.

For balanced cases, means were computed and compared by the least significant difference procedure when the F-values from analysis of variance were significant. For unbalanced cases, least squares means were computed and compared using the P-Difference option within the least squares means option in the SAS-general linear model.

RESULTS AND DISCUSSION

Processing soymilk into soybean curd

Marked differences in nutrient composition were observed when soymilk was processed into tofu (Table 9) regardless of processing method.

Proximate composition. In general, the precipitation of soymilk proteins causes a marked increase in protein and fat, and a decline in carbohydrate contents. Proximate compositions for traditional and RHHTC soybean curd are presented in Table 9.

Proximate composition of traditional tofu was similar to previously reported data (Chang and Murray, 1949; Smith et al., 1960; Schroder and Jackson, 1972). In this study, processing of traditional soymilk into tofu produced an increase in protein ($P<0.0001$) and fat ($P<0.001$), and a decline ($P<0.0001$) in carbohydrate and ash contents.

Coagulation of soy proteins during tofu production is pH dependent. The addition of calcium sulfate causes the pH to shift toward the isoelectric point of soybean protein (pH = 4.5), producing minimum solubility and maximum precipitation of proteins (Sarker and Khaleque, 1978). Following precipitation of protein, curds and whey separate. The whey fraction contains water soluble or dispersible proteins and carbohydrates (Del Valle, 1981). This fractionation of water soluble and insoluble components of soymilk supports the observed changes in nutrient composition during tofu processing in this study.

In contrast to traditional tofu, the proximate analysis of tofu from RHHTC soymilk showed a decline ($P<0.0001$) in

Table 9 - Proximate composition[a] and thiamin and riboflavin contents[b] of traditional and RHHTC soymilk and soybean curd

Measurement	Traditional			RHHTC		
	soymilk[c]	soybean curd[d]	Significance of F-value	soymilk[e]	soybean curd[f]	Significance of F-value
Protein (%)	30.4	57.4	****	56.1	36.3	****
Carbohydrate (%)	45.5	9.5	****	36.6	45.9	**
Fat (%)	18.1	27.9	***	2.1	11.8	**
Ash (%)	6.0	5.1	****	5.3	5.9	***
Thiamin (µg/g)	11.9	4.1	****	15.1	6.6	****
Riboflavin (µg/g)	3.9	2.0	****	3.6	2.3	**

[a] Moisture free basis

[b] Moisture free, fat free basis

[c] Each value is a mean for 28 determinations

[d] Each value is a mean for 12 determinations

[e] Each value is a mean for 2 replications

[f] Each value is a mean for 10 determinations

significant at the 1% level; * significant at the 0.1% level; ****significant at the 0.01% level protein content when compared to the RHHTC milk. This variation may be caused , in part, by different calcium sulfate concentrations. The RHHTC milk required a 0.04M calcium sulfate concentration for adequate curd formation without producing off-flavors and textural problems, whereas the traditional soymilk required a 0.02M calcium sulfate concentration. Wang and Hesseltine (1982) found as the Ca or Mg salt concentration used to produce soybean curd was increased above 0.04M, a lower percentage of nitrogen was recovered.

Thiamin and riboflavin contents. Thiamin and riboflavin contents of traditional tofu decreased (P<0.0001) when soymilk was processed into tofu (Table 9). The declines in thiamin and riboflavin contents in this work agreed closely with previous work by Chang and Murray (1949). Miller (1945) also observed a low retention of thiamin during soybean curd processing. Miller et al. (1952) investigated retention of nutrients in commercially prepared soybean curds. The authors found large losses of B-vitamins occurred during tofu production. Further analyses showed the whey contained higher amounts of B-vitamins when compared to the curd. Consequently, it was concluded the water soluble B-vitamins were leached into the whey during final steps of tofu processing. When RHHTC soymilk was processed into soybean curd similar findings were observed.

Amino acid composition. Methods for tofu processing caused variations in amino acid composition (Table 10). Traditional tofu showed a favorable response to processing with declines in only two amino acids, threonine (P<0.05) and methionine (P<0.01). Precipitation of soymilk proteins resulted in no

Table 10 - Amino acid composition[a] of traditional and RHHTC soymilk and soybean curd before storage

	Traditional			RHHTC		
Measurement	soymilk[b]	soybean curd[c]	Significance of F-value	soymilk[d]	soybean curd[e]	Significance of F-value
Aspartic acid	4.1	4.5	ns	6.4	5.2	**
Threonine	1.5	1.1	*	2.2	1.7	**
Serine	2.0	2.4	**	3.1	2.4	***
Glutamic acid	5.9	7.1	ns	10.4	7.6	**
Proline	1.8	9.4	****	12.8	2.4	****
Glycine	1.5	1.6	ns	2.3	1.9	**
Alanine	1.5	1.7	*	2.5	1.8	***
Half cystine	0.44	0.40	ns	0.69	0.47	***
Valine	1.1	1.3	***	2.0	1.2	****
Methionine	0.52	0.48	**	0.60	0.55	**
Isoleucine	1.1	1.1	ns	1.8	1.3	***
Leucine	2.5	2.6	ns	3.9	3.1	**
Tyrosine	1.5	1.4	ns	2.0	1.7	**
Phenylalanine	1.7	1.8	ns	2.5	1.9	****
Histidine	1.2	1.4	ns	1.7	1.6	ns
Lysine	2.1	3.0	**	4.2	2.8	***
Arginine	2.4	6.5	****	8.8	3.2	****
Ammonia	0.36	1.2	****	1.4	0.44	****

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[a] Grams amino acid per 100g protein corrected to 100% recovery of protein, moisture free basis

[b] Each mean represents 4 determinations for 4 pooled replications

[c] Each mean represents 6 determinations for 4 pooled replications

[d] Each value is a mean for 2 replications

[e] Each value is a mean for 10 determinations

*significant at the 5% level; **significant at the 1% level; ***significant at the 0.1% level; ****significant at the 0.01% level; ns, not significant

significant changes or higher concentrations of all amino acids than found in the corresponding traditional milk.

Soymilk from both extraction methods had higher threonine ($P<0.05$) and methionine ($P<0.01$) contents when compared to their respective soybean curds. These finding are in accordance with those of Del Valle (1981). But in contrast to his report, lysine ($P<0.01$) and valine ($P<0.001$) contents in this study were lower in the traditional soymilk when compared to the corresponding tofu.

Observable differences were found in amino acid composition of tofu when compared to the soymilk used for its production. All amino acids, except histidine, had moderate to extreme declines in amino acid content when RHHTC soymilk was processed into tofu. This finding supports the observed decrease in protein content when RHHTC soymilk was processed into soybean curd.

Soymilk processing

Total solids and yield of solids in soymilk. Mean total solids values for traditional and RHHTC soymilks were 8.00 and 10.72%, respectively. RHHTC method produced a 52.77% solids yield in soymilk.

Proximate composition. Soymilks from both extraction methods had different proximate compositions (Table 11) from those previously reported for traditional soymilk (Chang and Murray, 1949).

RHHTC soymilk had a higher ($P<0.0001$) protein content, as expected, when compared with traditional soymilk. Johnson et al. (1981) reported the utilization of direct steam-infusion

Table 11 - Proximate composition[a] and thiamin and riboflavin contents[b] of traditional and RHHTC-processed soymilk and soybean curd before storage

	Soymilk			Soybean curd		
Measurement	Traditional[c]	RHHTC[d]	Significance of F-value	Traditional[e]	RHHTC[f]	Significance of F-value
Protein (%)	30.4	56.1	****	57.4	36.3	****
Carbohydrate (%)	45.5	36.6	***	9.5	45.9	****
Fat (%)	18.1	2.1	****	27.9	11.8	***
Ash (%)	6.0	5.3	****	5.1	5.9	****
Thiamin (μg/g)	11.9	15.1	****	4.1	6.6	****
Riboflavin (μg/g)	3.9	3.6	ns	2.0	2.3	ns

[a] Moisture free basis

[b] Moisture free, fat free basis

[c] Each value is a mean for 28 determinations

[d] Each value is a mean for 2 replications

[e] Each value is a mean for 12 determinations

[f] Each value is a mean for 10 replications

***significant at the 0.1% level; ****significant at the 0.01% level; ns, not significant cooking facilitated maximum protein recovery because of optimum heat exposure and dissociation of protein bodies by steam shear force.

In contrast, fat content was less (P<0.0001) in the RHHTC soymilk. Extraction by the rapid method at 121°C may not produce a stable fat dispersion or emulsion. Lo et al. (1968) also noted a decrease in extraction of fat into soymilk when a NaOH soak had been employed. The authors suggested there was a direct interference with diffusion of small molecules as well as larger protein molecules.

RHHTC processing also produced a soymilk with a lower (P<0.001) carbohydrate content when compared with traditional milk. Carbohydrate content of RHHTC soymilk was not quantitated by previous researchers (Johnson et al., 1981; Hung, 1984). However, it is understood that soaking of soybeans prior to traditional soymilk extraction allows leaching of soluble carbohydrates from the beans (Wilkens and Hackler, 1967). The observed difference between the two soymilks may result from a failure in the RHHTC extraction procedure to remove soluble carbohydrates from bean cell walls. Secondly, soy flour used for the RHHTC procedure was made from dehulled beans and this may have affected the amount of carbohydrate available for extraction from the soybean flour (Sarker and Khaleque, 1978). Ash values ranged from 5.3 - 6.0% for the two soymilks and are reported in Table 11.

Thiamin and riboflavin contents. RHHTC soymilk contained more (P<0.0001) thiamin than traditionally processed soymilk (Table 11), which agrees with observations made by with Osborne and Voogt (1978). Thiamin was noted to be one of the most unstable vitamins to moist heat treatment. Differences in the current study were attributable to heat exposure for a shorter period of time with the rapid extraction method.

In this study, no significant differences were found in riboflavin contents between the two soymilks. This supports the observation that riboflavin is not affected adversely by heat treatment (Osborne and Voogt, 1978).

Amino acid composition. Amino acid composition of traditional and RHHTC soymilks is presented in Table 12. Values for both soymilks are lower than those previously reported (Hung, 1984). These differences may be attributed to variations in extraction procedure and heat processing between the investigations

Varying degrees of significant differences were observed between traditional and RHHTC soymilk amino acid compositions. RHHTC soymilk contained greater amounts of all amino acids than traditional soymilk. Higher values for methionine (P<0.001) and cystine (P<0.0001) in the RHHTC soymilk are of particular interest since they are the limiting amino acids in soybean products.

Hackler and Stillings (1967) reported processing time and temperature were critical factors affecting amino acid composition of soymilk. These investigators processed soymilk at 121°C for up to 120 min. RHHTC soymilk processed at 121°C for 35 sec had greater retention of methionine (P<0.001) and cystine (P<0.0001) than traditional soymilk held at 100°C for 7 min. Hackler and Stillings (1967), however, found soymilk processed at 121°C for 0-120 min declined in soymilk amino acid contents.

Table 12 - Amino acid composition[a] of traditional and RHHTC soymilk and soybean curd before storage

Amino acid	Soymilk			Soybean curd		
	Traditional[b]	RHHTC[c]	Significance of F-value	Traditional[d]	RHHTC[e]	Significance of F-value
Aspartic acid	4.1	6.4	***	4.5	5.2	ns
Threonine	1.5	2.2	***	1.1	1.7	**
Serine	2.0	3.1	****	2.4	2.4	ns
Glutamic acid	5.9	10.4	***	7.1	7.6	ns
Proline	1.8	12.8	****	9.4	2.4	****
Glycine	1.5	2.3	****	1.6	1.9	**
Alanine	1.5	2.5	****	1.7	1.8	ns
Half cystine	0.44	0.69	****	0.40	0.47	ns
Valine	1.1	2.0	****	1.3	1.2	ns
Methionine	0.52	0.60	***	0.48	0.55	**
Isoleucine	1.1	1.8	****	1.1	1.3	*
Leucine	2.5	3.9	****	2.6	3.1	*
Tyrosine	1.5	2.0	****	1.4	1.7	**
Phenylalanine	1.7	2.5	****	1.8	1.9	ns
Histidine	1.2	1.7	*	1.4	1.6	ns
Lysine	2.1	4.2	****	3.0	2.8	ns
Arginine	2.4	8.8	****	6.5	3.2	****
Ammonia	0.36	1.4	****	1.2	0.44	***

[a] Grams amino acid per 100g protein corrected to 100% recovery of protein, moisture free basis

[b] Each mean represents 4 determinations for 4 pooled replications

[c] Each value is a mean for 2 replications

[d] Each mean represents 6 determinations for 4 pooled replications

[e] Each value is a mean for 10 determinations

*significant at the 5% level; **significant at the 1% level; ***significant at the 0.1% level; ****significant at the 0.01% level; ns, not significant

Differences in holding times during processing help explain higher amino acid levels in RHHTC soymilk.

Soybean curd processing

Proximate composition. Table 11 presents proximate composition of tofu produced from traditional and RHHTC soymilks. Tofu made from RHHTC milk had a lower ($P<0.0001$) amount of protein when compared to traditional tofu. Conversely, the tofu from RHHTC milk also had a higher ($P<0.0001$) level of total carbohydrates. Traditional soybean curd contained higher ($P<0.001$) amounts of fat and lower ($P<0.0001$) ash values.

Thiamin and riboflavin contents. Tofu from RHHTC-produced soymilk contained greater ($P<0.001$) amounts of thiamin than traditional tofu. No differences were noted in riboflavin contents between traditional and RHHTC-produced tofu.

Amino acid composition of freshly prepared soybean curd produced from traditional and RHHTC soymilk. Amino acid composition of tofu from traditional and RHHTC soymilks is presented in Table 12. RHHTC-produced tofu contained higher quantities of glycine ($P<0.01$), methionine ($P<0.01$), threonine ($P<0.01$), tyrosine ($P<0.01$), isoleucine ($P<0.05$) and leucine ($P<0.05$) than the traditionally processed tofu. In contrast, traditional tofu contained more ($P<0.0001$) arginine and proline. than RHHTC-produced tofu. Generally, differenes between soybean curds produced from the two extraction methods were less pronounced than differences observed between the two soymilks.

Yield of soybean curd

Soybean curd yield was greater ($P<0.0001$) when RHHTC-produced soymilk was used (Table 13). Lu et al. (1980) reported

Table 13 - Soybean curd yield from traditional and RHHTC-produced soymilks[a]

	Soymilk		
Measurement	Traditional[b]	RHHTC[c]	Significance of F-value
Tofu yield (g/l)	414.4[d]	688.0[e]	****

[a] Means within a row followed by a common letter are not significantly different ($P \leq 0.05$)

[b] Each value is a mean for 28 determinations

[c] Each value is a mean for 10 determinations

****significant at the 0.01% level

yields of 225 ± 140 g/L for traditionally processed tofu when 0.25 ± 0.08% $CaSO_4$ was used. The level of $CaSO_4$ used in the present study was 0.32% which is also in the range of the amount used by Lu and co-workers (1980) to produce a similar yield (Table 13). Observed differences in tofu yields from different extraction methods may be attributed to the two $CaSO_4$ concentrations required for precipitation. In direct contrast to results reported in Table 5, Wang and Hesseltine (1982) observed a slight decrease in gross weight of tofu when $CaSO_4$ concentrations were increased from 0.02 to 0.04M. Also contrary to these results, Appurao and Rao (1975) observed at higher calcium ion concentrations, precipitation decreased and the protein became soluble again.

Observed contradictions in previous work could have been caused by limited solubility of $CaSO_4$, and uncertainties of actual ionic concentration (Wang, 1984). Furthermore, it has been suggested the duration of heat treatment will affect tofu yield. Researchers have found an increased processing times for soymilk reduced tofu yield (Wang and Hesseltine, 1982). Such findings would support the observed increase in gross tofu weight prepared from RHHTC-produced soymilk.

<u>Microbiological analysis of traditional tofu immersion solutions</u>

Least square means for logs of viable counts per ml are shown in Figure 6, and viable cell counts expressed as CFU/ml and Log no./ml are found in the Appendix (Table A-1).

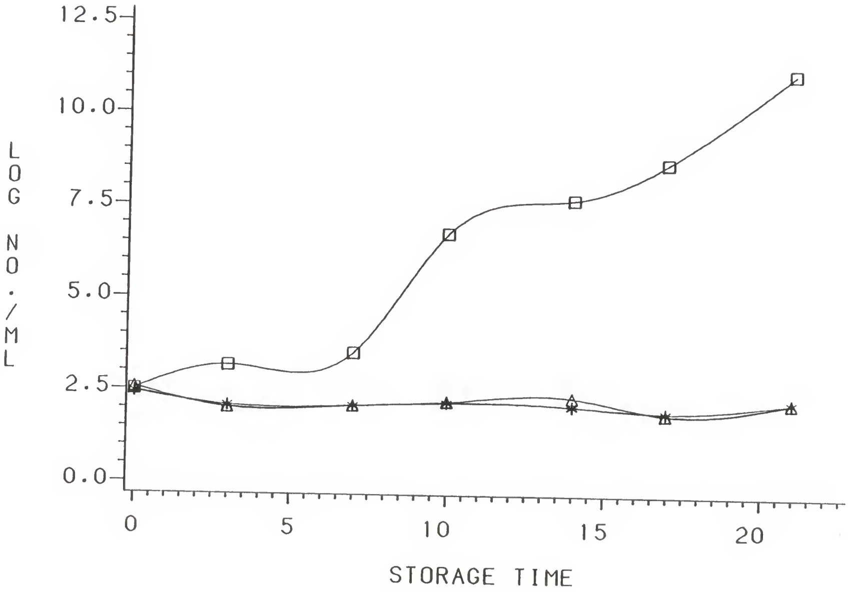
All immersion solutions had initial bacterial loads of 10^2 CFU/ml before storage. These values are lower than initial loads of commercially prepared tofu reported by Rehberger et al.

Fig. 6 - Effect of storage on least squares means of viable counts for immersion solutions of tofu

⊟—⊟—⊟ Sterilized, distilled water

——* 0.5% Acetic acid

△—△—△ 0.15% Potassium sorbate + 0.5% Acetic acid


LOG NO./ML
12.5
10.0
7.5
5.0
2.5
0.0
0
5
10
15
20
STORAGE TIME

(1984). High initial bacterial loads may be the result of contamination during processing, packaging or storage.

Immersion solutions of AA or PS + AA were effective in controlling bacterial growth for 21 days storage. Tofu immersed in these solutions retained less than 10^3 CFU/ml in their soaking solutions.

Lim (1984) studied the addition of AA or PS + AA to immersion solutions of tofu stored for 23 days at 10-15 ^{o}C. Tofu stored under these conditions had approximately 10^4-10^5 CFU/ml in the immersion solutions following 23 days of storage. Sterilized, distilled water for tofu immersion and storage at 4-7 ^{o}C markedly increased in bacterial growth after 7 and 17 days of storage with a final bacterial load of 10^{11} CFU/ml. These results paralleled findings by Lim (1984).

Storage of traditional soybean curd

Thiamin and riboflavin contents. Thiamin content in traditionally processed tofu declined ($P \leq 0.05$) after 7, 10 and 14 days of storage (Table 14). Loss of thiamin during storage may be ascribed to leaching of this water-soluble vitamin into immersion solutions (Bender, 1978) Generally, sterilized, distilled water (control) for immersion maintained greater amounts of thiamin during storage when compared with immersion solutions containing AA or PS + AA. This is contrary to Bender's (1978) observation that thiamin was most stable in acidic media. According to previous work by Lim (1984), AA or PS + AA immersion solutions maintained pH values ranging from 4.25-5.08 during 23 days of storage.

Riboflavin contents of tofu were more stable during storage

Table 14 - Thiamin and riboflavin contents[a] of traditional soybean curd before and after storage[b]

Days of Storage	Treatment	Thiamin (ug/g)	Riboflavin (ug/g)
0	Control	4.3c	2.0c
	AA	4.1d	1.9cd
	PS + AA	3.8d	2.0d
3	Control	4.1c	2.0e
	AA	4.0d	1.9ef
	PS + AA	4.4d	1.8f
7	Control	4.1c	1.6e
	AA	3.9d	1.7ef
	PS + AA	4.0d	1.6f
10	Control	4.0e	1.8e
	AA	3.4f	1.7ef
	PS + AA	3.1f	1.6f
14	Control	3.5g	1.7e
	AA	3.1h	1.7ef
	PS + AA	3.2h	1.7f
17	Control	2.7i	1.8e
	AA	2.8j	1.6ef
	PS + AA	2.6j	1.6f
21	Control	3.4i	1.8e
	AA	2.7j	1.6ef
	PS + AA	2.6j	1.7f
Significance of F-value			
	TRT	**	*
	ST	****	****
	TXS	ns	ns

---

[a] Moisture-free, fat-free basis

[b] Each value is a mean for 4 replications

[c] Means within a column followed by a common letter are not significantly different ($P \leq 0.05$)

*Significant at the 5% level; **significant at the 1% level; ****significant at the 0.01% level; ns, not significant than thiamin contents with the only decrease ($P \leq 0.05$) occurring after 3 days of storage. This decline also is attributed to leaching of the vitamin into the immersion solution. However, failure to observe the same incremental decline as noted with thiamin retention may have been caused by differences in solubilities of the vitamins (Freed, 1966; Osborne and Voogt, 1978). Differences in riboflavin contents were noted among treatments. Contrary to previous reports (Freed, 1966; Bender, 1978; Osborne and Voogt, 1978), the control maintained higher levels of riboflavin throughout storage when compared to the PS + AA treatment. No differences were found between tofu immersed in the control or AA solution.

Proximate composition. Proximate composition of traditional soybean curd before and after storage is presented in Table 15. Protein content remained stable up to 14 days of storage, after which it decreased ($P \leq 0.05$). Carbohydrate content in soybean curd increased ($P \leq 0.05$) following 14 days of storage, whereas fat content remained unchanged during storage. No differences were found with respect to treatment for protein, carbohydrate and fat contents. Ash values were affected by type of immersion solution and storage with values ranging from 4.7-5.6%.

Amino acid composition. Table 16 presents the amino acid composition of traditional soybean curd before and after storage. Immersion solutions had no effect on amino acid composition of tofu before or after stoarge. Most declines in amino acids occurred following 7 or 14 days of stoarge. Threonine, isoleucine, cystine, and methionine had no losses throughout the

Table 15 - Proximate composition[a] of traditional soybean curd before and after storage[b]

Days of Storage	Treatment	Protein (%)	Carbohydrate (%)	Fat (%)	Ash (%)
0	Control	57.1c	9.5c	27.8c	5.6c
	AA	59.6c	7.6c	28.0c	4.8d
	PS + AA	55.6c	11.6c	27.9c	5.0d
7	Control	61.3c	4.4c	29.2c	5.1d
	AA	58.9c	7.4c	29.0c	4.7e
	PS + AA	62.3c	3.5c	29.6c	4.6e
14	Control	59.1c	5.5c	30.0c	5.5f
	AA	57.7c	9.1c	27.9c	5.2g
	PS + AA	58.4c	6.1c	30.1c	5.4g
21	Control	33.7d	32.0d	28.9c	5.3c
	AA	38.3d	25.6d	31.1c	4.9d
	PS + AA	34.6d	30.6d	29.7c	5.1d
Significance of F-value					
	TRT	ns	ns	ns	**
	ST	**	**	ns	*
	TXS	ns	*	ns	ns

[a] Moisture-free basis

[b] Each mean represents 2 determinations for 4 pooled replications

[c] Means within a column followed by a common letter are not significantly different ($P \leq 0.05$)

*significant at the 5% level; **significant at the 1% level; ns, not significant

Table 16 - Amino acid composition[a] of traditional soybean curd before and after storage[bc]

Amino acid	Days of Storage												Significance of F-value		
	0			7			14			21					
	Treatments														
	Control	AA	PS+AA	Control	AA	PS+AA	Control	AA	PS+AA	Control	AA	PS+AA	TRT	ST	TXS
Aspartic acid	4.3c	4.5c	4.6c	4.5c	4.7c	4.8c	4.1d	4.2d	4.1d	3.9d	3.6d	3.8d	ns	*	ns
Threonine	1.1c	1.2c	1.2c	1.5c	1.3c	1.6c	1.1c	1.1c	1.1c	1.3c	1.3c	1.3c	ns	ns	ns
Serine	2.3c	2.4c	2.5c	2.2c	2.4c	2.3c	2.1c	2.3c	2.2c	1.9d	1.8d	1.9d	ns	*	ns
Glutamic acid	6.8c	7.1c	7.3c	7.0c	7.3c	7.4c	6.4d	6.8d	6.5d	5.5d	5.2d	5.5d	ns	*	ns
Proline	8.8c	9.7c	9.7c	8.9c	9.1c	9.4c	8.4c	8.9c	8.5c	1.7d	1.6d	1.7d	ns	***	ns
Glycine	1.5c	1.6c	1.6c	1.6c	1.6c	1.7c	1.4d	1.5d	1.5d	1.4d	1.3d	1.4d	ns	**	ns
Alanine	1.6c	1.8c	1.8c	1.8c	1.8c	1.8c	1.6d	1.6d	1.6d	1.4e	1.4e	1.5e	ns	**	ns
Half cystine	0.38c	0.42c	0.42c	0.38c	0.40c	0.40c	0.36c	0.38c	0.37c	0.40c	0.37c	0.42c	ns	ns	ns
Valine	1.3c	1.4c	1.4c	1.3c	1.4c	1.4c	1.2d	1.2d	1.2d	1.1d	1.0d	1.2d	ns	*	ns
Methionine	0.46c	0.48c	0.49c	0.44c	0.50c	0.46c	0.44c	0.46c	0.44c	0.46c	0.44c	0.48c	ns	ns	ns
Isoleucine	1.1c	1.1c	1.1c	1.2c	1.2c	1.3c	1.0c	1.0c	1.0c	1.1c	1.0c	1.2c	ns	ns	ns
Leucine	2.5cd	2.7cd	2.7cd	2.7c	2.7c	2.9c	2.4d	2.5d	2.4d	2.4d	2.3d	2.5d	ns	*	ns
Tyrosine	1.3c	1.4c	1.5c	1.4c	1.4c	1.4c	1.2d	1.3d	1.2d	1.3cd	1.3cd	1.4cd	ns	*	ns
Phenylalanine	1.7c	1.8c	1.7c	1.7c	1.8c	1.8c	1.6d	1.6d	1.6d	1.7d	1.5d	1.7d	ns	*	ns
Histidine	1.3c	1.4c	1.4c	1.2c	1.3c	1.3c	1.2c	1.3c	1.3c	1.1d	0.99d	1.0d	ns	*	ns
Lysine	2.9c	3.1c	3.1c	3.0c	2.9c	3.2c	2.8c	2.9c	2.9c	2.0d	1.8d	2.1d	ns	**	ns
Arginine	6.2c	6.6c	6.6c	6.2c	6.2c	6.5c	5.9c	6.2c	6.0c	2.2d	3.9d	2.3d	ns	**	ns
Ammonia	1.2c	1.3c	1.2c	1.1c	1.1c	1.1c	1.1c	1.2c	1.1c	0.33d	0.28d	0.30d	ns	**	ns

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[a] Grams amino acid per 100g protein corrected to 100% recovery of protein, moisture free basis

[b] Each mean represents 2 determinations for 4 pooled replications

[c] Means within a row followed by a common letter are not significantly different ($P \leq 0.05$)

*significant at the 5% level; **significant at the 1% level; ***significant at the 0.1% level; ns, not significant

21 days of storage.

CONCLUSIONS

Based on the conditions of this study, the following conclusions can be made:

1. Thiamin and riboflavin contents were reduced when traditional and RHHTC soymilks were processed into soybean curd. Traditional soymilk produced tofu with higher protein, fat and ash contents and lower carbohydrate content than the original milk. RHHTC-produced soymilk, however, yielded tofu with lower protein content and higher carbohydrate, fat and ash contents when compared to the corresponding soymilk. Methods for soybean curd processing also caused variations in amino acid composition.

2. RHHTC-produced soymilk had higher thiamin and protein contents and lower riboflavin, carbohydrate, fat and ash contents when compared to the traditional milk. Soybean curd produced from traditional soymilk had lower thiamin, riboflavin, carbohydrate and ash values while protein and fat contents were higher than tofu produced from RHHTC soymilk. RHHTC and traditional soymilks had greater variation in amino acid composition than their corresponding soybean curds.

3. Tofu produced from RHHTC soymilk had greater soybean curd yields than tofu produced from traditional milk.

4. Immersion solutions of AA or PS + AA were effective in controlling bacterial growth at 4-7 ^{o}C for 21 days.

5. Storage of traditional soybean curd produced declines in thiamin and riboflavin contents. Proximate composition was altered during storage with a decline in protein and an increase in carbohydrate contents at 21 days of storage. Immersion solutions had no effect on amino acid composition of tofu before or after storage. Most declines in amino acid content occurred following 7 or 14 days of storage.

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Table A-1 - Total counts in immersion solutions of traditional soybean curd before and after storage[ab]

Days of storage	Treatment	Total counts (CFU/ml)	Total counts (Log no./ml)
0	Control	4.4×10^{2}	2.5c
	AA	3.2×10^{2}	2.4d
	PS + AA	4.0×10^{2}	2.5d
3	Control	1.8×10^{3}	3.2c
	AA	1.8×10^{2}	2.1d
	PS + AA	1.2×10^{2}	2.0d
7	Control	1.3×10^{4}	3.5c
	AA	1.6×10^{2}	2.1d
	PS + AA	1.4×10^{2}	2.1d
10	Control	1.2×10^{7}	6.7e
	AA	1.9×10^{2}	2.2f
	PS + AA	1.9×10^{2}	2.2f
14	Control	2.6×10^{9}	7.6e
	AA	1.3×10^{2}	2.1f
	PS + AA	2.2×10^{2}	2.3f
17	Control	7.2×10^{8}	8.6e
	AA	1.6×10^{2}	1.9f
	PS + AA	7.7×10^{2}	1.9f
21	Control	2.4×10^{11}	11.1g
	AA	4.6×10^{2}	2.2h
	PS + AA	5.6×10^{2}	2.2h
Significance of F-value			
	TRT		****
	ST		****
	TXS		****

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[a] Each value is a mean for 4 replications

[b] Means within a column followed by a common letter are not significantly different ($P \leq 0.05$)

****significant at the 0.01% level

EFFECTS OF PROCESSING AND STORAGE
ON NUTRITIONAL QUALITY
OF SOYBEAN CURD

by

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Abstract

Soymilk was extracted by traditional and Rapid Hydration Hydrothermal Cooking (RHHTC) methods. Soymilk processed by the RHHTC method had higher thiamin and protein contents when compared to traditional soymilk ($P<0.0001$). Processing of soymilks into soybean curd reduced riboflavin (traditional, $P<0.0001$; RHHTC, $P<0.01$) and thiamin ($P<0.0001$) contents. Traditionally produced tofu had higher protein ($P<0.0001$) and fat ($P<0.001$) contents while carbohydrate and ash contents were decreased ($P<0.0001$). RHHTC-produced tofu had lower protein content ($P<0.0001$) and higher carbohydrate ($P<0.01$), fat ($P<0.01$) and ash ($P<0.001$) contents when compared to its soymilk. Soymilks had greater variation in amino acid composition than the corresponding soybean curds. RHHTC-produced tofu had greater ($P<0.0001$) soybean curd yields than traditionally produced tofu.

Traditionally processed tofu was immersed in sterilized, distilled water, 0.5% acetic acid (AA), or 0.15% potassium sorbate (PS) + 0.5% AA, and analyzed immediately or after storage for up to 21 days. Immersion solutions (AA or PS + AA) had little effect on nutritional quality during storage, but effectively reduced ($P<0.0001$) viable cell counts. Storage of soybean curd produced declines ($P\leq0.05$) in thiamin and riboflavin contents. After 14 days of storage, protein content decreased and carbohydrate content increased ($P\leq0.05$). Declines ($P\leq0.05$) in amino acid contents for traditionally processed tofu occurred following 7 or 14 days.