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Title Effect of Processing Methods on the Chemical and Physical Properties of Soybean Milks

Abstract approved
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In the present study, processed soymilks prepared by various manufacturing methods were examined for quality by physical and chemical methods. Viscosity, pH, soluble solids, total solids, fat and protein content were used as indicators of quality. The principal factor investigated was the protein content as measured by the dye-binding procedure.

The manufacturing variables studied were soaking and blending times, size of beans, sprouting of the beans, drying temperatures for powdered soymilk, and hull-on and hull-off beans. The laboratory method giving optimum quality comprised soaking the beans for four hours at 20°C, blending with boiling water for eight minutes and filtering hot. Large and medium sized beans, with the hulls removed, gave the highest quality soymilks. Pilot plant procedures were developed by scaling up the laboratory methods.

Heat treatments were applied to soymilks with no added sugar, 5% and 10% sucrose, 5% lactose and 5% glucose. Heat treatment had little effect on the protein content of soymilks with no added

sugar or with 5% and 10% added sucrose as measured by the dye-binding method. Browning was observed in the lactose and glucose added samples as a result of prolonged heating. The dye-binding capacity of the protein in soymilk varied with the sugar level, the type of carbohydrate and the length of the heating period. The most noticeable change due to heat treatment was an increase in viscosity.

Soymilks were spray dried in a Anhydro Spray Dryer. Both drying temperature and drying time had an effect on the quality of the dried milk. The sample dried at the higher temperature was brown in color and gave lower test values for all the quality factors investigated.

The Kjeldahl method for protein was compared with the dye-binding method and the results showed that the dye-binding method was suitable for estimating the protein content in soymilk.

Varieties of soybeans investigated were Merit and Kanrich with the Merit soymilks being of higher quality.

EFFECT OF PROCESSING METHODS ON THE CHEMICAL AND PHYSICAL PROPERTIES OF SOYBEAN MILKS

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INTRODUCTION

The soybean has been used as a basic human food in China for at least 5,000 years. The beans are consumed as fresh, fermented or dry food products after processing by traditional methods. In the United States, soybeans are used mainly as an animal feed and as a source of an edible oil.

As the so called "beany flavor" in the soybean milk is strange to many people in the western world, the demand for soymilk has been from those allergic to animal milk. This is not true in the oriental countries where the people are accustomed to soymilk and the beverage is well accepted by the public. In Hong Kong, the manufactured soymilk is marketed under the trade name, "Vita Milk", and the cost is lower than Coca-Cola or other beverage drinks.

Though the soybean is classed among the leguminous plants, it differs from other legumes in containing far more protein and fat than ordinary peas or beans.

Scientific studies and physicans' findings have emphasized the fact that the soybean contains vitamins in substantial quantities and is a valuable source of the B complex vitamins. Soybean protein contains the essential amino acids and is therefore a complete protein which satisfies the human requirement for protein metabolism.

Babies fed soybean milk up to the age of six to nine months are quite comparable to healthy nursing infants in weight increase.

(In preparing soymilks by the traditional oriental method, the soaked soybeans are ground with water in a stone mill in small quantity lots. The slurry is filtered through a coarse cloth and the filtrate is recovered as milk. After boiling, the milk is ready to drink, customarily with added sugar. In the western countries, the milk is usually prepared by dispersing in water a mixture of soy flour, vitamins, carbohydrates and a flavoring compound.)

Processing methods have an effect on the nutritional value of soymilks. Lack of proper processing may give rise to unpleasant digestive responses. The most important factor is the heat treatment. There are several "antibiological factors" present in the raw soymilk which are heat labile.

Soybean protein is improved by moderate cooking since the nutritive value is higher in properly heat-treated soybean meals used as animal feed. Very little is known about the fundamental nature of the changes in the protein as a result of heating.

Being a high-quality and low-cost protein source, soybean products should find a market as a human food. There is a need for both research and sales promotion before the public in the Western world comprehends the nutritional value of soybeans or vegetable proteins in general.

Dr. Miller of the International Nutrition Laboratory said, "The more I work with soybean, the more I am struck by the broadening opportunity offered in the food field." (13, p. 82)

The purpose of this experimental work was to develop a method of preparation of soymilk and to evaluate its quality by physical and chemical tests, especially the dye-binding method of estimating protein content.

LITERATURE REVIEW

History of the soybean

Origin of the soybean. The botanical name of soybean is Glycine hispida or more commonly called Glycine max (55, p. 33; 13, p. 8; 19, p. 1). Its first description was in the Ben Tsao Gang Mu, the ancient materia medica, which dates back to 2838 B. C. in the reign of the Chinese Emperor Shen Nung (19, p. 2). For 5,000 years the soybean has been one of the essential foods of China supplying a major share of the protein in many Oriental diets.

Varieties and cultivation. There are more than 1,500 varieties of soybean (19, p. 3), with a growing period ranging from 75 to 200 days. Different varieties are distinguished according to the size and color of the seeds, the end use and the length of the growing season. Generally, soybeans can be classified into two major types, namely the commercial field type and the edible garden type (12, p. 126).

The beans are mostly round in shape, like ordinary peas, with considerable size variation within and between varieties. The color of the seeds may be yellow, green, brown or black and a striped or spotted combination of all these colors (19, p. 3). Generally speaking, the yellow beans are higher in protein and fat when compared to green or black seeded varieties (19, p. 13).

The soybean does not require a specific kind of soil and will grow in most soils if properly treated (55, p. 57). The plants prefer a warm climate, are remarkably drought resistant and suffer little injury from excessive moisture (19, p. 9). Soybeans are often grown in rotation or combination with other farm crops such as corn, cowpeas or Sudan Grass to get the advantages of a balanced ration and larger yields (55, p. 79-84).

The soybean in the Western World. The soybean was first introduced to Europe by a German botanist, Engelbert Kaempfer, in 1712 (13, p. 8; 19, p. 2). The first soybeans entered America in 1804 when a Yankee Clipper ship returned from China and brought several bags of soybeans as a reserve food supply (13, p. 8). In the year 1829, Thomas Nuttal (13, p. 9; 55, p. 39) grew a variety with red flowers and brown seeds in the botanic garden at Cambridge, Mass. In 1911, soybeans were imported to this country from Manchuria by the Pacific Oil Mills in Seattle but not until 1915 were soybeans grown in United States for use by the mills (13, p. 14; 54, p. 188).

Soybean crops in the United States. The soybean has changed the mid-western agriculture more than any other crop in recent years. This change can be seen in the increasing production of soybeans over these years.

In 1924, one-half million acres produced about 5 million bushels of soybeans in the United States. By 1940, 78 million bushels were produced on 5 million acres. In 1958, the acreage increased to 24 million and the production of soybean to 575 million bushels. Compared to 1924, the 1958 acreage had increased 50 times and production increased 11,500 percent (43).

Nutritional value of the soybean

Soybeans are known for their high nutritive value. The average chemical composition of soybeans is as follows (40, p. 139; 4, p. 444):

Composition	%
Moisture	8.0
Ash	4.6
Fat	18.0
Fiber	3.5
Protein	40.0
Pentosan	4.4
Sugars	7.0
Starch-like substances by diastase	5.6
P_2O_5	1.7
K_2O	2.3
CaO	0.5
MgO	0.5
Weight per 1000 seeds, grams	150.0

Soybeans contain substantial quantities of the B complex vitamins such as thiamine, niacin and riboflavin and certain enzymes which will be discussed separately.

Protein. Protein is the constituent in soybeans which makes them so valuable in human nutrition. The protein content of soybeans is higher than that of meat, egg, wheat, milk and many other protein foodstuffs (12, p. 7; 31). While it is generally considered that animal proteins are superior to vegetable proteins, soybean proteins resemble animal proteins in the essential amino acid content and other chemical properties. The following table is a comparison of the amino acid content in soybean oil meal, milk, egg, beef and wheat flour calculated as the percentage of amino acid in the sample (12, p. 8).

	Extracted Soybean Oil Meal	Whole Milk	Whole Egg	Beef Loin	Patent Wheat Flour
Crude protein	48.52	3.7	13.70	21.65	12.6
Arginine	3.50	0.12	0.85	1.35	0.49
Histidine	1.12	0.086	0.33	0.18	0.21
Isoleucine	2.68	0.22	0.80	1.13	0.78
Leucine	3.65	0.34	1.23	1.86	
Lysine	2.83	0.23	1.03	1.96	0.17
Methionine	0.65	0.10	0.45	0.54	?
Phenylalanine	2.32	0.19	0.66	0.91	0.27
Threonine	1.95	0.18	0.64	0.97	---
Tryptophane	0.82	0.05	0.21	0.26	0.18
Valine	2.59	0.25	0.93	1.15	0.20

Glycinin, which was the name given by Osborne and Campbell (49) to the chief fraction of protein in soybeans, represents 75% of the total soy protein (6, p. 243). Four different fractions namely glycinin, a phaseolin which is more soluble than glycinin, an albumin-like protein and a proteose were separated by salt-extraction by Osborne and Campbell (49). Jones and Csonka (34) precipitated five fractions from the salt-soluble proteins by using ammonium sulfate. Briggs and Mann (6, p. 245) using a phosphate buffer at pH 7.6 found at least seven electrophoretically distinct proteins in which glycinin was about 75% of the total protein and the composition of glycinin was dependent upon the preparation method. Four fractions from the extractable globulins having sedimentation constants of 2, 7, 11 and 15S by ultracentrifugation were discussed by Wolf and Smith (80, p. 12) in a review article on soybean proteins. The 7S and 11S components of the glycinin fraction accounted for 70% of the total soybean protein. Water will extract over 95% of the total nitrogen present in the soybeans (6, p. 255).

The isoelectric point of glycinin ranges from pH 4.1 to 5.4 as reported by Jones and Csonka (34), Briggs and Mann (6, p. 255), Osborne and Campbell (49) and Howath (31). Tadokoro and Toshimura (79, p. 517) found that the isoelectric point was highest in glycinin and lowest in legumelin.

Glycinin in soybeans is similar to casein in cow's milk in amino acid content and in coagulation by acid or alkali (12, p. 9). The main chemical difference is that the valine content in casein is higher than that in glycinin. The following table is a comparison of soybean glycinin and milk casein (54, p. 198). The lysine content of glycinin in this table is low.

Amino Acid	Soybean Glycinin %	Milk Casein %
Glycine	0.87	0.45
Valine	0.68	7.20
Leucine	8.45	10.50
Proline	3.78	6.70
Phenylalanine	3.86	3.20
Aspartic acid	3.89	1.40
Glutamic acid	19.46	15.55
Tyrosine	1.86, 4.55	4.50
Arginine	5.12	3.81
Histidine	1.39	2.50
Lysine	2.71	5.95
Tryptophane	1.94-2.84	1.50
Cystine	0.74-1.45	0.25
Methionine	1.84	3.25-3.53

In digestibility comparisons of soybean protein with the protein of cow's milk, the cow's milk was more rapidly hydrolyzed by trypsin and less rapidly hydrolyzed by pepsin (54, p. 199).

Individual amino acid assays of soybean protein by different workers vary and this variation is perhaps due more to the methods employed than to the amino acid distribution of the protein fractions. For example, the lysine contents reported by different workers (54, p. 198) were 2.71, 3.34, 5.77, 6.08 and 9.06 percent. The amino acid content does vary between soybean varieties and this factor needs further investigation.

Osborne and Mendel (50, p. 372) and Johnson et al. (33) showed that solvent extraction did not impair the nutritive value of the soybean protein. This is important in the utilization of solvent extracted meals.

Heat has an important role in improving the nutritional value of soybean protein (15, p. 3; 50, p. 372; 33; 38; 44, p. 58-69; 58, p. 255; 78). Experiments in rat feeding showed that rats fed cooked soybeans obtained better growth than those fed on raw soybeans (50, p. 372; 28, p. 231; 52). The degree of heating required for nutritional improvement varies for the different soybean products. Either under or over heating may give a lower than optimum nutritional value. Underheating does not inactivate the toxic substances or denature the proteins to a point where they can be completely digested (61), while overheating will result in a deficiency of lysine (24; 36; 70), methionine and leucine (15, p. 4). Mitchell and Smuts (46, p. 280) and Shrewsbury and Bratzler (62) and others (20; 24 ; 42) reported that there was a deficiency of cystine and other sulfur-containing amino acids in the raw soybean and that the cystine in the raw soybean could not be utilized by the body, although it was absorbed. The two sulfur-containing amino acids, cystine and methionine, are more available after heat denaturation of the soybean protein (18; 27; 33; 42; 54, p. 202). This might be due to the unfolding of the SH and S-S groups that normally face the interior of the coiled molecule. Many workers (24; 51, p. 30-31; 58, p. 256) found that moist heat had a greater beneficial effect on the quality of soybean protein than did dry heat. Evans and Butts (16) stated that dry heat will cause a binding of lysine so that it can not be utilized by the animal. Probably there are two types of inactivation of lysine, namely destruction of lysine and binding of lysine. Bound lysine cannot be hydrolyzed by enzymes but will be by acids.

Franek (21) explained the changes during the application of moist heat as a consecutive reaction; native protein ⟶ denaturated protein ⟶ protein reacting with carbohydrates. The first reaction takes place below 100°C and the second reaction will occur at higher temperatures. He proposed that the denaturation at temperatures below 100°C was a structural change rather than a chemical interaction and that the carbohydrate reaction was accompanied by the destruction of basic amino acids. The denaturated protein was thought to be better utilized than either the native protein or the protein reacted with the carbohydrate.

Riesin et al. (59), Klose et al. (35) and Hou et al. (29) have shown an increased liberation of the essential amino acids by heat, with prolonged heating resulting in a deficiency of methionine, lysine and leucine. Patton et al. (53) showed a loss of lysine, arginine, tryptophan and histidine when soy globulin was boiled in 5% glucose for 24 hours but no loss of these amino acids occurred during boiling in water. This indicated that the carbohydrates native to the soybean do not play a part in the loss of amino acids upon boiling. Browning did occur in samples with no added sugar boiled longer than three hours. About 50% of lysine in the soybean protein was destroyed by autoclaving when 20% sucrose was added (16). The results of Hou et al. (29) showed that the cooking of soybeans increased the nutritive value of the soybean by releasing the essential amino acids. No amino acid destruction was found on prolonged boiling (12 hours).

Fat. Soybeans contain 13.5 to 24.0% lipid materials (41, p. 52). Over 95% of the fatty materials are in the cotyledons (4, p. 448; 41, p. 52). The oil or fat extracted from the seed consists principally of saturated and unsaturated fatty acids with some related lipid materials such as phosphatides and sterols.

Keimatsu (70, p. 520) found that about 80% of the total lipids were unsaturated fatty acids and 12% were saturated fatty acids. Among the unsaturated fatty acids, half of them were the isomer of linoleic acid, 15% were linoleic acid and 35%, oleic. Kaufmann and Dollear *et al.* (41, p. 78) working with different varieties of soybeans found the linolenic acid varied from 3-12%.

Soybean oil contains varying amounts of free fatty acids (41, p. 64). However, Meissl and Bocker (12, p. 17) found no free fatty acid in their studies.

Soybean oil contains sufficient lecithin to be a commercial source of this phosphatide. The amount of lecithin including cephalin and inositol was about 3% (12, p. 18-19) and American-grown soybeans are reported to contain from 2.0 to 3.82% phosphatides calculated as lecithin (40, p. 149). It was found that the lecithin in soybean is identical to that in egg yolk (12, p. 19). The oil also contains a fair amount of fat-soluble vitamins.

In the unsaponified fraction of the oil, there are some sterols mainly phytosterol, a mixture of several sterols which can be crystalized, hydrocarbons, alcohols and ketones (41, p. 74). Among the phytosterols are sitosterol and stigmasterol which are used to synthesize hormones (12, p. 20). Soybean oil is subject to flavor reversion (12, p. 73; 32, p. 1153) and therefore the keeping quality of the oil is considered to be poor. Upon exposure to light, the oil undergoes changes resulting in off odors.

Despite the reported defects, large quantities of soybean oil are used for edible purposes in margarines and other foods.

Vitamins. The fat-soluble vitamins or precursors of vitamins A, D, E, and K, and the water-soluble vitamins, C and the B complex, are present in soybeans in various amounts (12, p. 31; 50, p. 374).

The carotene in the soybeans is highest in the green stage and drops to a minimum when the beans are mature. Soybeans consumed as dry mature seeds are not considered as a good source of vitamin A (12, p. 31).

Many workers (11; 12, p. 34-37; 37, p. 57) indicated that soybeans are a good source of the vitamin B complex especially thiamine, riboflavin and niacin. Vitamin C, as well as riboflavin and niacin, increased during germination of the seed and thiamine showed alternate increases and decreases through a 54 hour germination period (76; 12, p. 38). Thus, the sprouting of soybeans will increase the vitamin content.

Vitamin E, the essential factor for reproduction, and the blood-clotting vitamin K are also found in soybeans (12, p. 32).

Carbohydrates, minerals and enzymes. Piper and Morse (55, p. 109) reported that the total carbohydrates in soybeans ranged from 22-29%. O'Kelly and Gieger (48) reported that the various soluble carbohydrates varied from 17.74% to 30.18%. The total sugars, calculated as sucrose, ranged from 2.70 to 11.97% as reported by Cartter and Hopper (9, p. 46). The carbohydrates in largest quantities are galactans, 4.86%; pentosans, 4.94%; cellulose, 3.29%; and dextrins, 3.14% as shown by Street and Bailey (71).

The starch content of the soybeans is high in the immature seeds and decreases as the beans mature. The starch content is very low in the matured beans and was found to be less than 2% of the nitrogen free extract (4, p. 455). Street and Bailey (71) stated that the thoroughly ripened seeds are free from starch. The large variation in the amounts of carbohydrates reported in soybeans is mainly due to variety and maturity factors.

The ash of soybeans has an alkaline reaction and runs from 4-5% (12, p. 30). Calcium, phosphorus and iron are high in soybeans as compared to other cereals and legumes and these elements are readily available for growth. Chlorine is deficient in the soybean (40, p. 141).

A number of enzymes are present in the soybeans; amylases and diastases, proteases, lipases, urease, oxidase and peroxidase and an anti-tryptic factor and other toxic substances (32, p. 1278; 71; 77). Westfall and Hauge (77) concluded that the trypsin inhibitor was the major cause of the poor utilization of the raw soybean protein.

Most of the enzymes can be inactivated readily by heat. Urease activity and trypsin inhibitor have been used to determine whether the soybean products have been properly heat treated (5; 10; 61; 66; 77). The inactivation of trypsin inhibitors and urease by heat has been found to be rapid.

Utilization of the soybean as human food

Commercial uses in the United States.

The use of soybeans as commercial foods in the United States has approximately a 30 year history. Most extensive and important uses are soybean flour, grits, protein concentrates, isolated soy-proteins and soybean oil. On the dry weight basis, protein concentrates contain about 70% protein whereas isolated soy protein exceeds 90% protein (26).

Soy flour and grits are used interchangeably. These products are good sources of Ca, P, the Vitamin B complex and protein. The tocopherols present in the soybean possess antioxidant properties and thus prevent or retard the development of rancidity in foods where soy flour or grits are added (26).

Wheat flour being deficient in lysine can be balanced by adding soy flour which contributes an abundance of lysine (26). Soy flour and grits are used in the baking industry to improve the crust of bread, retain moisture and improve flavor. Cookies, crackers, cake mixes and cereal products are superior in nutritive quality when soy flour and grits are added. Other important uses of soy flour are macaroni and spaghetti products, baby foods, meat loaf and sausage stuffings.

Oils from the soybean are used in shortening, margarine and salad oils (68). Around 85% of the soybean oils are used in foods with the rest going into nonfood uses such as paint and varnish (12, p. 74).

Soymilk. Soymilk has been used as a drink in China for thousands of years. It is said to have been originated by the Chinese philosopher, Whai Nain Tze, long before the Christian era (55, p. 228).

Soymilk is made by grinding the soaked beans with water and filtering this slurry through a coarse cloth. The filtrate, after boiling and with added sugar, is ready to drink.

There are numerous references reporting the results of infant and child feeding studies with soymilk in China during the Chinese-Japanese War (65). Children receiving soymilk gained more weight than those not receiving the milk. During World War II, soymilk was fed to under-nourished children in Germany (12, p. 14) and the conclusion was that soymilk is a complete substitute for cow's milk. There seems to be no doubt that soymilk has the nutritive value to support normal growth.

Dr. Harry W. Miller of the International Nutrition Research Foundation, U.S.A., spent more than half a century in China observing the method of preparation and the results of feeding soy products to humans. His observations date back 36 years to the time when soymilk was served to the patients, nurses and staff in Shanghai Sanitarium and later to infants and children in the Shanghai Clinic and Hospital. The results were reported in the China Medical Journal of 1937 (82, p. 152).

1. <u>Feeding experiments</u>. There are several reports of soymilk feeding tests both in China and the United States (20; 57, p. 1222; 60; 72). The results of early experiments carried out in China were reported in Chinese medical journals. Large scale feeding of children during 1937-39 in refugee camps was successful (65). In China, soymilk has been successfully employed as a food for growing infants.

Tso (72) prepared a milk from soaked soybeans which contained 4.4% protein, 1.8% fat, 1.5% carbohydrates, 0.018% Ca and 0.057% P. This milk was fed to six infants ranging from birth to a few weeks old and the weight gain results were comparable to healthy infants fed with cow's milk. He concluded that soymilk is comparable to cow's milk in Vitamin A and richer in Vitamin B. The digestibility of soy protein fed in the form of soymilk was 89.6%

as recorded by Cahill et al. (8). The average biological value of soybean protein in a commercial soybean milk was 95.3% in which the protein of whole egg was used as a standard.

The clinical laboratory of the Children's Hospital in Columbus, Ohio, carried out a feeding experiment on 17 infants fed on soymilk and nine infants fed on cow's milk. The soymilk babies gave a higher performance for average growth in length and in grams of hemoglobin but the cow's milk babies had a higher average weight gain at the end of a three months feeding test (82, p. 155).

Ruhrah (60), one of the pioneer investigators of the use of soymilk in infant feeding, found that this vegetable milk was not only valuable in treating diabetes but also found its use beneficial in summer diarrheas, in other intestinal disturbances and in marasmus. Sinclair (64) came to the same conclusion and stated that soymilk has the necessary pabulum to nourish the sick child.

In this country, non-animal infant foods are almost entirely based on powdered soymilk. Fomon (20) used a formula, provided in cans as a concentrated liquid, to feed four infants, four to 6½ months of age, for a period of 38 to 73 days. The results showed that the rate of weight gain and retention of nitrogen were normal as compared to normal full-time infants of similar ages fed human milk.

Soymilks have high nutritional value both for children and adults. While this is less important in normal nutrition in the United States where there is an abundant supply of cow's milk, it is most important to those allergic to animal milks. The low cost of soymilks make them eminently suited to the poor and underdeveloped countries.

2. Commercial production of soymilk in United States and the problem of acceptance. The commercial product based on soymilk is a spray-dried soymilk with added fat, vitamins and sugar (31; 40, p. 998; 82, p. 152). An example of the ingredients contained in a commercial soymilk, Mull-Soy, manufactured by the Borden Co. is as follows: water, soy flour, soy oil, sucrose, dextrose, dextrins-maltose dextrins, tri-calcium phosphate, calcium hydroxide, monoglycerides, potassium iodide, vitamins A, D and B_{12}, sodium ascorbate, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride and niacin (82, p. 166). Other commercial products are Loma Linda's Soyalac and Mead Johnson's Sobee (45).

The most important acceptance problem for the soymilk in this country is the characteristic flavor. The flavor can be changed by processing or by adding flavoring compounds. Though this seems to be the major problem encountered in the consumer acceptance of soymilk in the United States, it is not the case in most Oriental countries where soybean has a history of human utilization.

Uses of soybean in the Orient other than milk. The Chinese and Japanese are the people who have used the soybean as a food most extensively over a long period of time. Many of the ways to use the soybean as a food are unknown to most of the Occidentals.

These uses can be grouped into three major categories, namely fresh, dried and fermented food products. The most common foods are soy sauce, soybean curd, fermented bean cake and sprouted beans and many additional products derived from these which have different names.

The dried and fermented soybean products have a long shelf life. These products are valuable to people unable to purchase animal proteins.

Soy sauce is the best known Oriental sauce in this country. There are many kinds of soy sauce for different uses such as one for cooking and another for table use. The main difference is in the quality. Soy sauce in Chinese and Japanese cookery may be compared to the use of salt in Western recipes.

One of the products worthy of mention is the soybean curd. The curd can be produced as a fresh, dried or ferment food product. The nutritional value is higher than most of the other soybean products. Soybean curd is a precipitate formed from soymilk by the addition of magnesium chloride, calcium chloride, vinegar or condensed sea water. The excess water is drained away leaving a solid cake or cheese. The curd can be consumed fresh, deep-fat fried, preserved as a dried product or made into a fermented food similar to cheese. These products are high in protein and minerals. Another product from the soymilk is the dried bean curd (skin of the milk). When soymilk is heated, a protein skin is formed on the surface. When the skin becomes relatively thick, it can be removed in the form of a sheet or stick. Successive skins are removed one layer after another, and sun dried. The dried product is yellow and shinny in appearance and has good storage stability.

Sprouted beans are used as a vegetable by the Oriental. A few studies have been made on the sprouted beans (23; 76; 81). The amino acid, lysine, was found to decrease during the germination period and Wu and Fenton (81) showed a retention of only 36% of the lysine on the eighth day of sprouting. Several workers found that the α-amino acids and peptones are decomposed in the gernimating seeds and ammonia released as a result of splitting the α-amino groups. The sprouts are a good source of ascorbic acid, carotene, riboflavin and niacin. All of the above vitamins increase during germination with a decrease or no change in thiamine (76). Due to the high vitamin content, sprouted beans are recommended for use as a vegetable.

Dye-binding method for protein estimation

The Kjeldahl method for the determination of protein content of milk and milk products is a standard procedure. Because of the time consuming nature of the digestion, distillation and titration steps, the test is not ideal for routine analysis.

Dye-binding was first investigated in 1944 by Fraenkel-Conrat and Cooper (22) as a possible protein test. The polar groups in proteins bind dyes of the opposite charge forming insoluble protein-dye precipitates. In a buffer at pH 2.2 the acid dye, Orange G, combined stoichiometrically with basic groups such as the free amino group, the imidazole group of histidine or guanidino group of arginine where the groups are in a dissociated or a free state. In 1957 this method was extended to the determination of protein in milk by Ashworth and Seals (2) in this country. Udy (73) applied this principle to the estimation of the protein content of wheat and flours. Recently, Moran, Jensen and McGinnis (47) used dye-binding in soybean and fish meal as an index of quality after processing.

The two dyes generally used are Amido Black (Buffalo Black) and Orange G. Saturation of the protein was accomplished much faster with Amido Black than with Orange G and Amido Black forms a more dense dye-protein complex (14; 74). Dolby (14) found that with a less pure sample of dye, less dye was bound per unit weight of protein. He concluded that Amido Black, though reacting with protein in the same molar ratio as Orange G, gave a more sensitive indication of protein content. Herrington at Cornell University reported that Amido Black 10B by E. Merck in Germany is the purest Amido Black they have found (The Dye-Binder, Cornell University, Oct. 1962).

Methods of preparation of soymilk

The methods of preparing fresh soymilk mentioned in the literature are basically the same. The basic method should consist of the following four steps: soaking, grinding, filtration and heating. The amount of added water can be varied to suit the end use of the product. For soymilks consumed fresh, one pound of dry beans to one gallon of water is often used.

A traditional Chinese method of preparing the soymilk was described by Piper and Morse (55, p. 228-229). The soaked beans are ground in a horizontal stone mill comprising two pieces of flat circular stone, one on top of the other. The upper stone is equiped with a wooden handle on the side and is turned against the lower one while the beans and water are fed into a hole near the edge of the upper stone. The milk is filtered through a piece of cheese cloth and diluted three fold with water. After boiling, the milk is ready to drink.

The stone mill grinding operation was recommended by Dr. Miller (82, p. 151) who considered grinding to be better than the cutting action of other mills.

In this country, Hand et al. (82, p. 157) at Cornell University, Geneva, New York, used the following method to prepare soymilk. The beans were soaked overnight with water (3:1) and were ground through the 0.023" screen of a Rietz disintegrator using one gallon of warm water (140^{o}-150^{o}F) to one pound of dry beans. The slurry was filtered through a plate filter. The milk was run through a heat exchanger and was held at 121^{o}C (250^{o}F) for ten minutes.

The soaking times mentioned in the literature were usually overnight or several hours (11; 55, p. 229). Smith and Beckel (65) gave ten hours of soaking in summer and as much as 24 hours in winter, whereas Miller (82, p. 150) indicated eight hours for whole beans and two hours for dehulled beans. Chang and Murray (11) mentioned a method used in France in which the soybeans were ground without previous soaking.

MATERIALS AND METHODS

Materials

The two varieties of soybeans used were the Merit and the Kanrich.

The Merit variety, obtained from the Western Farmers Association in Seattle, Washington, was grown in Quincy, Washington. The grade on the soybeans was as follows: No. 3 yellow soybean, the test weight per bushel was 55 pounds and the moisture was 11.5%. These soybeans were grown in the fall of 1962 and harvested early in 1963.

Not much information was obtained for the Kanrich variety. It is one of the so-called vegetable varieties obtained from Strayer Seed Farms at Hudson, Iowa, and was recommended by the American Soybean Association in Hudson, Iowa, for the manufacture of soymilk.

Processing methods

The ancient Chinese wet process was adopted as the basic process in this experiment. The beans were washed free from dirt and then soaked in tap water. About one gallon of water was added to each pound of dry beans including both the soaking and blending operations. The soaked beans were ground with water to a slurry in a Waring Blendor and filtered through cheese cloth. The filtrate was recovered as milk. Dry weights of 54.3 gm of beans were used for each sample except for the pilot plant production. Each milk sample was heated to boiling in a one liter beaker using a gas stove.

Soaking study of the bean. The variables in the soaking study were the temperature of the soaking water and the soaking time. The water temperatures used were room temperature (about 20°C), 40°C and 100°C. The soaking periods were 2, 4, 6, 8, 16 and 32 hours for the beans soaked at room temperature and 40°C. The beans in 100°C water were cooked for 5, 10, 20 and 30 minutes.

Blending study. The soaked beans were ground in a Waring Blendor with boiling water. In this study, 54.3 gm of beans (ca 1/8 pound) were used for each sample. Three hundred milliliters of boiling water were added to the beans and the sample ground at medium speed (Variac set at 80) for various times. The slurry was filtered hot through a triple layer of cheese cloth. The remaining amount of water was used to wash the blendor and added to the residue. The total water added was 473 ml (1/8 gallon) including the water absorbed by the beans during soaking and added before and after blending.

The blending times used were 2, 4, 6, 7, 8 and 10 minutes.

Batch production in the pilot plant. In the pilot plant studies , a Fitzpatrick Comminuting Machine, Model D, was used. Five pounds of beans were soaked for four hours and fed into the hammermill in small quantities with the addition of boiling water.

The No. 2 screen (1/16 in. diameter holes) and the highest speed was used in grinding the beans. The slurry was reground in the mill a second time before filtration. The filtration was done by hand using cheese cloth and moderate pressure. The proportion of bean to total water was one pound of beans to one gallon of water. The soymilk was used for the heat treatment studies.

Sprouting of the beans. One pound of dry Merit beans were soaked in tap water for ten hours and spread out in a wooden box lined with a polyethylene sheet containing a few layers of paper towel at the bottom of the box. The beans were covered with a piece of cloth and a few layers of paper towel. The beans were sprinkled with tap water four or five times a day for periods of two and three days in the dark. The temperature of the room was 22^{o}-26^{o}C.

The sprouted beans were hand-sorted and used in making the soymilk.

Powdered Soymilk. An Anhydro Laboratory Spray Dryer made in Copenhagen, Denmark, was used to dry the soymilks. In this drying system, the liquid enters through the top of the atomizer and passes down to a atomizer wheel. Due to the centrifugal speed of the wheel, the liquid sprays into the drying chamber as an extremely fine, uniform mist. The hot air mixes continuously with the atomized liquid and the volatile contents of the liquid evaporate so that the non-volatile part remains as fine, solid particles of powder.

The powder is carried by the air current to the bottom of the chamber where it is removed by means of a pneumatic discharger which sucks the dry air and powder out of the chamber to a cyclone separator. The powder is separated from the air and is collected in a container placed under the cyclone and the purified air is discharged through the suction fan.

The heater can heat the incoming air to any desired temperature up to 300°C.

Two inlet temperatures were used during the drying process. The higher one was approximately 190°C and the lower one 160°C. Three samples were obtained namely high and low temperature and a browned sample collected from inside the drying chamber.

Heat treatments of the soymilk. The milk was filled in 307 x 409, C-enamel cans, sealed under 28 inches vacuum and subjected to different times of heat treatment. The canned milks were processed for a period of 2, 4, 8, 32 and 64 minutes at 116°C (240°F) in a steam retort and for 0.5, 1, 2, 4. and 8 minutes at 100°C (212°F) in a water bath.

Analytical procedures

pH: A Beckman Zeromatic pH meter was used for measuring the pH of the soymilks. The milks were at room temperature.

Viscosity: The Calab Capillary Viscometer was used to determine the viscosity of the soymilks. The time required for water to descend to the calibration line was 12.8 seconds at 20°C. All samples were run at 20°C and the viscosity reported as the flow time in seconds.

Soluble Solids: A Basuch & Lomb refractometer was used to read the soluble solids as degrees Brix. The soymilk samples were first centrifuged at 2000 rpm for ten minutes in a Universal, Model UV, centrifuge. The refractive index of the filtrate was read and expressed as the weight percentage of dissolved sucrose (degrees Brix).

Total Solids: Approximately five grams of soymilk were weighed in an aluminum dish on an analytical balance. The milk was put on top of a 82°C (180°F) water bath until dry. The dish was then dried in a vacuum oven at 70°C for 14 hours.

Babcock Test for Fat: The fat of the soymilk was run by the Babcock method for cow's milk (3, p. 191). The digestion of the soymilk by the sulfuric acid seemed incomplete since pieces of curd were noticed in the neck of the Babcock bottle.

Urease Test: A modified Caskey-Knapp method (61) was used. Two milliliters of soymilk were used in each test. The absorbance was measured at 430 $m\mu$ by a Bausch & Lomb Spectronic 20 colorimeter. The urease activity was expressed as mg/l of urea decomposed.

Kjeldahl Method for Protein: The standard Kjeldahl method was used to determine the total nitrogen content of soymilk, whole beans, hulls and powdered milk.

Approximately 5 gm of milk, 1 gm of powdered milk, 2-3 gm of hull and 0.5 gm of whole beans in a course powder form were weighed to the nearest milligram on an analytical balance and transferred to Kjeldahl digestion flasks. Ten grams of catalyst (anhydrous Na_2SO_4 with 200 mg $CuSO_4 \cdot 5H_2O$ per 10 gm Na_2SO_4) and 25 ml of concentrated sulfuric acid were added. The digestion was continued for about 20 minutes after the mixture was colorless. The digestion periods for the above samples were between 40 and 60 minutes.

For the distillation, 50 ml NaOH (1:1) were added to each sample and 40 ml of saturated boric acid were put in the receiving flask. Titration was done with 0.1 N HCl using a special indicator of methyl orange (1.0 gm methyl orange, 1.4 gm xylene cyanole made up to 500 ml with 50% ethyl alcohol).

Dye-binding Method for Protein: The modified method of Fraenkel-Conrat and Cooper (22) was used. Amido Black 10B by E. Merck of Germany and citric acid buffer were used in making up the dye solution. One gram of dye, weighed to the nearest milligram, was dissolved in one liter of citric acid solution containing 21 gm of reagent grade citric acid. The dye solution should stand for at least four hours before use. This solution will have a pH of approximately 2.2.

The soymilk samples were warmed up to 20°C and mixed thoroughly. One milliliter of milk was pipetted into a 1-oz plastic bottle containing 25 ml of dye solution. The sample was well mixed by hand and held at room temperature overnight. The bottle containing the dye-protein precipitate was centrifuged in a Babcock Tester, Model TD 24H, Testrite by Cherry-Burrell at 733 rpm for five minutes. One milliliter of clear supernatant fluid was pipetted into a 200 ml volumetric flask and diluted with distilled water to volume. The optical density of the diluted sample was determined at 615 mμ in a 1 cm cuvette with Coleman Junior Spectrophotometer, Model 6A, using distilled water as a blank.

Color: The color of the processed soymilk was measured by the Hunter Color and Color Difference Meter using the L scale. The sample container was a 4 x 3 x 2 inch plastic box. A NBS white plate was used to standardize the instrument. Results were recorded as L, a_L and b_L values.

RESULTS AND DISCUSSION

The effect of processing methodology on the quality of soymilks was studied using dye-binding, total solids, soluble solids, viscosity, pH and fat as an indication of the quality of the processed milks.

Since soymilk is prepared from the dry mature soybeans, soaking, grinding and heating are important unit operations that affect the quality of the milk.

Two varieties of soybean, Merit and Kanrich, were studied with the major work being done on the Merit variety. There are so many varieties of soybeans developed to serve different uses that it is impossible to say which particular variety is best suited for soymilk production. It is true that with the same raw material, a better quality product can be obtained by suitable processing methods.

Soaking Study

It is important that an adequate soaking period be given to the dry beans in order to get complete grinding. The ratio of the bean to water added in preparing a soymilk is one pound of dry bean to one gallon of water. A dry-bean sample size of 54.3 gm (ca one eighth of a pound) was used. The total water added was 473 ml (one eighth of a gallon) corrected for the water absorbed by the bean as a result of soaking. All samples were ground for five minutes.

The beans approximately doubled in weight as the soaking period reached four hours. Longer soaking time increased the amount of absorbed water slightly. The results of the quality tests mentioned before are found in Tables 1 and 2.

For the beans soaked in 20°C tap water, there was no difference in the pH of the milks. The viscosity of the milks tended to decrease as the soaking time increased. Total solids and soluble solids increased and then decreased as the soaking time increased. There was no change in percentage of fat. Little difference in dye-binding was obtained from the samples soaking for 2, 4, and 6 hours. The dye-binding results for protein are the mean of three trails for each sample. Total solids and soluble solids were optimum for the 2, 4 and 6 hour soaking periods at 20°C.

For the beans soaked in 40°C water, there were no definite trends in the viscosity, total solids and soluble solids but the amount of dye bound by the sample decreased as the soaking time increased.

Soymilks prepared from the cooked beans resulted in low quality milks when compared to the soaked bean milks. The most characteristic change in the unit operations for the cooked bean samples was the difficulty in filtration. The resulting milks were relatively thin in consistency.

The nutritional value of soybeans is improved by cooking because the proteins are denatured resulting in higher digestibility. Though the cooked beans have improved nutritional value, these

Table 1. Soaking Study of Merit Variety

Soaking time, hrs.	pH	Viscosity sec.	Total Solids %	Soluble Solids °B	Fat %	mg dye bound/ ml of milk
Soaking water 20°C						
0	6.64	48.3	8.19	8.5	1.4	17.8
2	6.62	45.2	8.23	9.6	1.4	18.7
4	6.63	31.8	8.33	9.5	1.4	18.5
6	6.64	37.2	8.29	9.5	1.4	18.3
8	6.65	25.2	7.82	8.7	1.4	17.9
16	6.64	22.8	7.78	8.8	1.3	18.7
32	6.64	22.3	7.35	8.3	1.3	16.9
Soaking water 40°C						
2	6.64	26.8	7.68	8.5	1.1	18.7
4	6.61	30.5	8.11	9.0	1.3	18.2
6	6.57	21.6	7.27	8.1	1.3	16.6
8	6.54	25.9	7.17	7.8	1.2	16.1
16	6.52	22.9	7.12	8.0	1.4	16.4
32	6.43	37.8	6.97	5.4	1.4	16.1
Soaking water 100°C						
1/12	6.72	19.9	6.65	6.0	0.1	13.8
1/6	6.72	18.5	6.14	5.5	0.1	13.3
1/3	6.74	15.5	4.72	4.5	0.1	8.1
1/2	6.72	14.4	3.56	3.3	0.1	5.7

Table 2. Soaking Study of Kanrich Variety

Soaking time, hrs.	pH	Viscosity sec.	Total Solids %	Soluble Solids °B	mg dye bound / ml of milk
Soaking water 20°C					
0	6.54	15.0	6.61	6.8	12.2
2	6.54	17.0	7.51	8.5	15.3
4	6.56	17.4	7.65	8.8	16.1
5	6.60	17.1	7.63	8.6	15.6
6	6.60	15.9	7.54	8.4	15.6
12	6.62	16.9	7.72	8.6	16.2

beans are not suitable for use in soymilk production since the denatured proteins and other nutrients are less extractable by water.

Comparing the results of the samples for the three soaking temperatures, the highest quality milks were prepared from beans soaked in tap water for four hours at room temperature (20°C).

For the Kanrich variety (Table 2), only one soaking temperature, 20°C, was used. The changes in the test values followed the same pattern as those of the Merit variety.

In comparing the Merit variety to the Kanrich variety for making soymilk, a superior quality soymilk was obtained from the Merit beans based on the above tests. Both varieties showed higher protein content as the soaking time reached four hours.

Blending Time Study

The grinding operation is an important unit operation. During grinding, the soluble nutrients are released to the solution, and other constituents, such as the proteins, are colloidally dispersed. The resulting suspension is an opaque, white liquid closely resembling cow's milk in appearance and consistency.

The old-fashioned, oriental stone mill is ideally suited for the grinding operation. The slow stream of water extracts completely the soluble nutrients from the torn and mashed beans. This method may be better than the cutting operation of other mills, however, grinding by the stone mill is time consuming.

In the laboratory, the Waring Blendor was used for preparing small samples. For large quantities, a Fitzpatrick hammermill was used. The better the grinding operation, the more nutrients the milk will contain. This can be observed from the results in Table 3.

Table 3. Blending Study of Merit Soybeans

Blending time, min.	pH	Viscosity sec.	Total Solids %	Soluble Solids °B	Fat %	mg dye bound/ ml of milk
			Beans soaked for 4 hours at 20°C			
2	6.54	18.4	7.06	8.2	1.0	17.4
4	6.54	21.7	7.52	9.0	1.3	18.7
6	6.54	24.5	7.76	8.8	1.4	17.7
7	6.52	25.3	7.93	9.1	1.4	18.7
8	6.55	29.1	8.08	9.9	1.2	19.0
10	6.54	26.7	7.93	9.4	1.3	18.3
			Beans soaked for 2 hours at 20°C			
2	6.55	19.4	6.60	7.8	0.7	15.5
4	6.55	21.2	7.02	7.9	1.2	16.6
6	6.55	24.1	7.61	8.6	1.3	18.7
7	6.55	28.0	7.88	8.8	1.4	18.5
8	6.55	28.0	7.84	8.7	1.3	18.0
10	6.55	29.4	7.81	8.6	1.3	18.1

Samples soaked for 2 hours and 4 hours were subjected to various blending times. There was little difference in pH values. The viscosity, total solids, soluble solids and the amount of dye bound had a tendency to increase as the blending time increased. This is due to the completion of the grinding operation. There is an indication that the blending operation may be overdone, since most of the test values showed decreases at the longest time. The samples soaked for 4 hours gave higher results, a trend which was also indicated in the soaking study.

Size of the Beans

The Merit variety, as purchased, consisted of mixed sizes of beans. The beans were graded into three sizes with the aid of a pea grader.

Among the small-sized beans, a small portion was not penetrated by water, regardless of the length of the soaking period. Undoubtedly, the composition of the seed coats of these particular beans differed from the normal composition.

All samples were soaked in 20°C water for 4 hours and blended for eight minutes in the Waring Blendor.

The results of total solids, viscosity and dye-binding are shown in Table 4.

Table 4. Comparison of soymilks from different sizes of Merit soybeans

Size of bean	Absorbed water %	Viscosity sec.	Total Solids %	mg dye bound/ ml of milk
Large, >10/32" diameter	118.4	33.6	8.13	21.0
Medium, 9/32" to 10/32" diameter	100.5	30.0	8.17	19.8
Small, < 9/32" diameter	89.3	29.2	7.84	17.4

The large and medium sized beans gave higher viscosities, total solids and amounts of dye bound than did the smaller beans. The medium-sized beans were used for the rest of the milk preparation studies.

Soymilks from Sprouted Beans

Merit beans were soaked in tap water for ten hours and allowed to germinate in the dark for periods of two and three days. A small portion of the beans did not sprout and the well-sprouted portion was about 70% of the total lot. Well-sprouted beans were hand picked and weighed into 100 gm lots. There were approximately 28 gm of sprouts in every 100 gm of well-sprouted beans. Assuming that the dry weight of 100 gm of sprouted beans was 50 gm, the total added water was calculated and added to the sample.

The results reported in Table 5, show that the sprouted beans were not suitable for making high protein content milks. The sprouted-bean milks were poor in appearance, water thin, and had a raw vegetable odor.

Table 5. Soymilks from germinated beans

	Water added ml	pH	Viscosity sec.	Total Solids %	Soluble Solids °B	mg dyebound/ ml of milk
		Germinated 2 days				
100 gm beans and sprouts	423	6.53	13.4	5.70	6.2	13.7
72 gm cotyledons	305	6.60	13.2	6.10	6.8	15.1
		Germinated 3 days				
100 gm beans and sprouts	423	6.42	13.1	4.65	4.8	10.7
72 gm cotyledons	305	6.43	13.4	5.85	6.8	14.4

Most of the quality test values for soymilk decreased as the germination period increased. The amino acid, lysine, decreases due to germination of the seed (81). The low dye-binding capacity may be explained by the disappearance of lysine and other free amino groups.

Though the germinated seeds have no advantage over the soaked beans in the preparation of soymilks, sprouted beans are a good source of vitamin C, carotene, riboflavin and niacin.

Heat Treatment of the Soymilks at 116°C (240°F)

Heat processing is a necessary treatment to improve the nutritional value of soybean milks. Either under or over heating lowers the quality of the soymilks. In this experiment soymilks, with and without added sugars, were heated for various lengths of time at 116°C in 307 x 409, C-enamel cans in a steam retort.

One gallon of water was added to each pound of dry beans. The samples were soymilks without added sugar, with 5% and 10% sucrose, with 5% lactose and with 5% glucose. The results are shown in Table 6.

The viscosity showed a definite increase as the heating time increased. The hydration and uncoiling of the protein molecules due to heat denaturation increased the consistency of the soymilk. Samples with added sugar showed slightly higher initial viscosities.

The pH of the soymilks ranged between 6.5 and 6.6, except for the lactose sample heated for 64 minutes and the samples with added glucose. As the heating period increased to 64 minutes, the

Table 6. Heat treatment of soymilks at 116°C (240°F)

Heating time, min.	pH	Soluble Solids °B	Total Solids %	Viscosity sec.	mg dye bound/ ml of milk
			Samples with no added sugar		
0	6.60	7.4	8.24	19.5	21.8
2	6.60	9.0	8.25	22.5	21.7
4	6.55	9.4	8.29	23.1	21.6
8	6.60	9.4	8.26	24.0	21.7
32	6.58	9.4	8.27	28.1	21.7
64	6.60	9.4	8.18	40.0	21.6
			Samples with 5% added sucrose		
0	6.60	13.0	13.00	20.7	21.5
2	6.54	13.6	12.80	23.7	21.5
4	6.60	13.8	12.80	24.3	21.6
8	6.60	13.8	12.90	25.5	21.3
32	6.55	13.5	12.78	31.8	21.5
64	6.50	13.6	12.84	49.8	20.8
			Samples with 10% added sucrose		
0	6.55	18.0	17.17	22.2	20.8
2	6.50	18.0	17.16	23.7	20.8
4	6.60	18.0	17.11	24.3	20.3
8	6.60	18.0	17.20	25.5	20.4
32	6.60	17.8	17.14	31.8	20.3
64	6.50	17.8	17.14	49.8	20.3
			Samples with 5% added lactose		
0	6.50	12.9	12.43	20.7	20.8
2	6.50	13.5	12.37	23.7	20.8
4	6.50	13.6	12.32	24.6	21.3
8	6.58	13.8	12.61	26.3	20.6
32	6.60	13.8	12.30	34.1	20.5
64	6.25	13.0	12.35	70.2	20.3
			Samples with 5% added glucose		
0	6.45	12.8	12.25	20.4	20.8
2	6.45	13.0	12.23	24.0	21.3
4	6.40	13.4	12.14	26.1	20.3
32	6.30	13.4	12.18	33.3	20.1
64	6.00	12.8	12.13	61.5	19.2

pH of the samples with lactose and glucose decreased. Since both lactose and glucose are reducing sugars, upon heating, the reducing groups react with the free amino groups of the protein. One of the principal changes is browning as is shown in Figures 1 and 2. Acidic compounds are formed including some organic acids, thereby decreasing the pH.

There was no definite trend of change in total solids and the changes were small within the same group of samples. The soluble solids (^{o}B) showed a definite increase due to the heat treatment in the samples without sugar. Heat apparently solublized a portion of the insoluble solids dispersed in the soymilk. Small increases in the soluble solids were obtained in the lactose and glucose series but the values dropped to the original level as the heating time increased to 64 minutes. The 10% sucrose samples showed no initial increase in soluble solids but did decrease upon prolonged heating. All the changes were small compared to the Brix changes in samples without added sugar.

The dye-binding values for protein did not change in the samples without added sugar and showed little change in the 5% sucrose samples. The amount of dye bound decreased slightly in the samples with 10% sucrose, 5% lactose and 5% glucose. The 5% glucose sample heated for 64 minutes bound the least amount of dye. In a unit weight, glucose has more reducing groups than lactose and thus can react more completely with the free amino groups.

Figure 1. Hunter L values of the processed soymilks at 116°C (240°F)

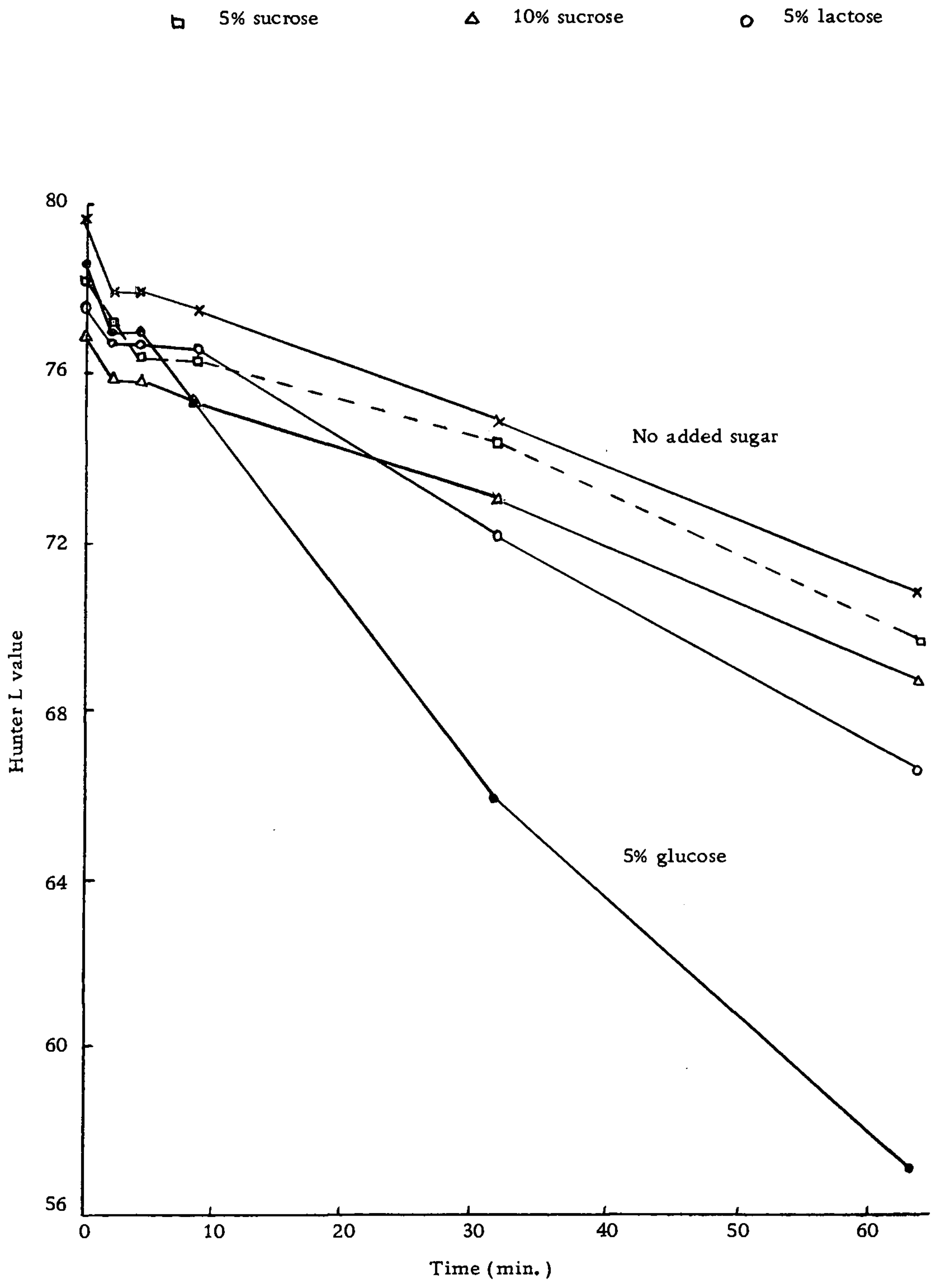
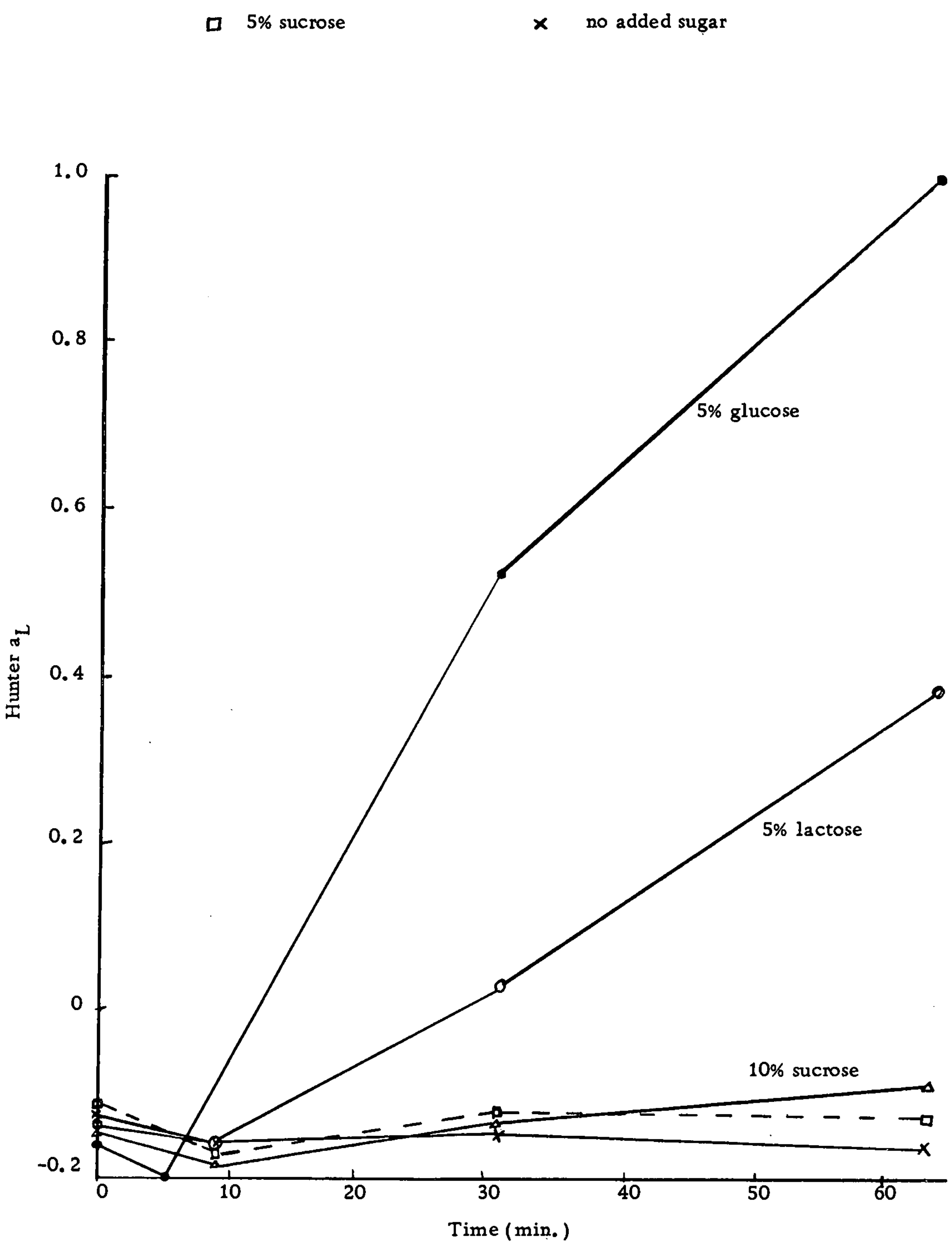


Figure 2. Hunter a_L values of the processed soymilks at 116°C (240°F)



A visual browning occurred in the long-heated sugar-added samples. This color change was measured quantitatively by the Hunter Color and Color Difference Meter. In Figures 1 and 2, L and a_L values were used to indicate the color change. A decrease in L value shows a darkening of color and an increase in a_L value indicates an increase of redness. The L and a_L values of a standard white plate are 85.6 and -0.4, respectively.

Heat Treatment of the Soymilks at 100°C (212°F)

In this batch, five pounds of dry beans were made to a final volume of five gallons of soymilk. The soymilk was canned in 307 x 409, C-enamel cans vacuum sealed at 28" vacuum and heated at 100°C in a water bath for various times. The temperature of the soymilk was measured at a point 3/4 inch from the can bottom on the central axis using a Taylor dial thermometer mounted through a rubber stopper in the can top.

There were no significant changes in total solids or dye-binding in this heat treatment study as shown in Table 7. As the heating time increased, the viscosity and percent soluble solids increased.

Upon heating, the hydration of the protein molecule increases and the SH, S-S groupings are exposed as a result of the uncoiling of the protein chain. An increase in viscosity is one of the physical changes denoting protein denaturation.

Table 7. Soymilks Heated at 100°C (212°F)

Heating time, min.	C deg.	Total Solids %	Soluble Solids °B	Viscosity sec.	mg dye bound/ ml of milk
			Samples with no added sugar		
0	10.0	6.87	6.6	18.2	18.0
0.5	22.2	6.90	6.6	18.1	17.8
1	37.8	6.90	6.6	18.4	17.7
2	66.7	6.86	7.1	18.4	17.7
4	87.5	6.85	7.5	18.8	17.9
8	96.0	6.85	8.0	20.4	17.7
			Samples with 5% added sucrose		
0	10.0	11.97	12.2	19.4	17.2
0.5	22.2	12.15	12.2	19.7	17.4
1	37.8	12.14	12.2	19.6	17.4
2	66.7	12.14	12.4	19.7	17.2
4	87.5	12.13	12.8	19.9	17.4
8	96.0	12.10	12.8	21.5	17.2

Moderate heating improves the nutritive value of soybean proteins without affecting the essential amino acid composition and total protein nitrogen content. Heat not only improves the digestibility of protein but liberates the sulfur-containing amino acids such as cystine and methionine. The other important function of heat treatment of soymilks is the destruction of toxic substances present in the raw beans.

The urease test used in this study was a modification of Schramm and Aines (61) colorimetric method. Two milliliters of soymilk were used for each test. The results in Table 8 are expressed as the percentage inactivation of urease based on the activity in the soymilk sample receiving no heat treatment.

The soluble solids increased slightly as the temperature of the soymilk increased to 60°-$70^{\circ}C$. This was due to the extraction and some hydrolysis of the polysaccharides present in the suspension.

Table 8. Urease Activity of Soymilks Heated at $100^{\circ}C$ ($212^{\circ}F$)

Heating time min.	mg/l urea decomposed	% inactivation
0	148.0	0.0
1	143.5	3.0
4	51.0	65.5
8	9.0	94.0
12	9.0	94.0

Spray-dried Soymilk

The soymilk samples dried in the Anhydro A/S spray dryer were blended in a Waring Blendor with the addition of water at the rate of 10 ml to each gram of powder.

The high temperature sample and the sample taken from inside the dryer had a definite brown color. Soymilks from the brown powders settled out readily upon standing and centrifugation.

Results of the various tests are shown in Table 9.

In this study, the two factors of temperature and time of drying were involved. From the results, it can be seen that the holding time in the drying chamber at about $100^{\circ}C$ affected the quality more than a temperature increase. The results of the various tests on low and high temperature samples showed small differences except for the color change.

Table 9. Soymilks Dried at Various Temperature and Reconstituted

Sample	Temp °C inlet	Temp °C outlet	Viscosity sec.	Total Solids %	Soluble Solids °B	mg dye bound/ ml of milk	pH
Low temp	168	99	17.4	7.78	7.4	19.2	6.47
High temp	194	118	17.6	7.64	6.4	18.2	6.45
From dryer	--	--	15.2	7.44	4.6	15.1	6.38

	Hunter readings L	Hunter readings a_L	Hunter readings b_L
Low temp	79.1	0.0	+1.99
High temp	69.9	+0.29	+2.50
From dryer	59.6	+0.75	+2.33

The dye-binding test for protein varied among the three samples with the low temperature sample binding the greatest amount of dye. The sample from inside the drier bound less dye. This is due to the long heating period inside the drier. The soluble solids decreased as the temperature of drying increased and markedly decreased with time of exposure to elevated temperatures. Total solids and pH of the soymilk also decreased with increased temperature and drying time.

The changes occurring during the drying operation at the high temperature and for the long time period were accompanied by browning. The L and a_L values indicate the nature of this change in color as compared to the white plate with readings of L = 85.6, a_L = -0.4, b_L = 0.5. The decreased L values show a darkening of color, positive a_L values increased redness and positive b_L values increased yellowness of the soymilk.

Kjeldahl Method for Protein as Compared to the Dye-binding Method

Three samples of soymilk from the same batch were given different heat treatments and triplicate determinations by both the Kjeldahl and dye-binding methods were run. The dye-binding values are the means of three determinations.

Results were shown in Table 10.

Table 10. Relationship of Kjeldahl Protein and Dye-binding Capacity

Sample	Kjeldahl protein %	mg dye bound/ ml of milk	Factor Kj/dye binding
No heat	3.94	19.9	0.198
	3.94	20.3	0.194
	3.98	20.3	0.196
Heat to boiling	4.00	20.3	0.197
	4.04	20.0	0.202
	3.95	19.8	0.199
Heat in boiling water bath for 4 minutes	4.00	20.3	0.197
	3.92	20.3	0.193
	4.01	20.8	0.193
Grand mean	3.975	20.22	0.197
Standard Deviation	0.039	0.270	0.0028
Coefficient of variation	0.981%	1.335%	1.42%

According to the results of the Kjeldahl and dye-binding tests, the heat treatment had little effect on the protein content and so the results of all nine samples were used to calculate the standard deviation and coefficient of variation of the tests.

Assuming one milliliter of milk is equivalent to one gram, the factor 0.197 can be used to convert dye-binding readings as mg dye bound/ml of milk to percent protein based on the Kjeldahl method.

Soymilks from Hull-on and Hull-off Beans

The hulls of the beans were removed by hand after a 4-hour soaking period. All of the cotyledons and germs were recovered. The milks were prepared by the same method using the dilution ratio of 54.3 gm of dry beans to 473 ml of water.

Table 11. Comparison of Soymilks from Hull-on and Hull-off Beans

Sample	pH	Soluble Solids °B	Total Solids %	Viscosity sec.	mg dye bound/ ml of milk
Merit, hull-off	6.52	9.6	8.46	19.1	21.4
hull-on	6.52	8.6	7.86	29.5	19.0
Kanrich, hull-off	6.54	9.0	7.85	16.3	17.2
hull-on	6.45	9.0	7.61	19.5	16.0

It is interesting to note that the soymilks made from the hull-off beans were superior in appearance and taste to the hull-on milks. The most noticeable changes were in the flavor and smoothness of the milk. The beany flavor was less intense in the hull-off milks.

The hulls of the Merit variety had a greater effect on milk quality than did the Kanrich hulls based on the above tests. After soaking, the hulls of the Kanrich variety were softer and easier to remove than those of the Merit variety. There was little difference in the milks of the Kanrich variety whether the hulls were on or off. It was noticed that the hulls of the Kanrich variety impart a bitter taste to the milk.

When the hulls of the soybeans were removed, the cotyledons received a more complete grinding resulting in a much finer suspension. This finer grinding will bring more of the nutrients into solution in the milk. On the other hand, constituents in the hull like minerals may bind a small amount of protein or other soluble solids in the milk prepared from the whole beans.

As a whole, milks prepared from hull-on beans of Merit variety were superior to the Kanrich hull-off milks.

Protein Content of Hulls, Whole Beans and Powdered Soymilk by the Kjeldahl Method

The hulls and cotyledons of the soybeans were separated by a F No. 4 Quaker City Corn Mill grinder. Whole beans were ground to fine particles by running through the grinder several times. The protein content of hulls, whole bean powder and powdered milk were determined by the Kjeldahl method using a sample size of approximately 3 grams for the hull, 0.5 gm for the whole beans powder and one gram for the milk powder. The results are shown in Table 12.

A higher protein content was obtained from the Merit whole beans by the Kjeldahl method. This was also indicated by the dye-binding method for estimating the protein content in soymilks made from Merit beans.

Table 12. Protein Content of Hulls, Whole Beans and Powdered Soymilk by Kjeldahl Method

Sample		Protein, % dry weight basis
Hull	Merit	9.99
	Kanrich	9.18
Whole bean	Merit	44.14
	Kanrich	42.21
Powdered milk		
Low temp	Merit	49.10

The hulls of both varieties contained an appreciable amount of nitrogen but the milks made from hull-on beans had lower dye-binding capacities. This suggested that the proteins presented in the hulls were not extractable by hot water and/or that the other constituents may bind some of the proteins in the soymilk.

SUMMARY AND CONCLUSIONS

The processing methodology and quality of soymilks were studied in laboratory and pilot plant. The quality tests used were pH, viscosity, soluble solids, total solids, fat and protein content. The application of the dye-binding method for estimating soymilk protein and the effect of processing method on the protein content of these milks were the principal factors investigated.

Dry whole soybeans soaked for four hours in tap water at 20°C, blended for eight minutes in the Waring Blendor and filtered hot gave a soymilk of high quality.

Large and medium sized beans with the hulls removed were the best raw product to use in making soymilks.

Soymilks made from cooked or sprouted beans were of poor quality with the longer cooking and sprouting times giving the lower quality test values.

Browning was observed in soymilks with 5% added glucose and 5% added lactose as the heating time reached 32 minutes at 116°C (240°F). A slight change in color was also noticed in the sample with 10% added sucrose heated for 64 minutes at the same temperature. Heating had no harmful effect on the destruction of protein as measured by the dye-binding method. An increase in viscosity and the development of a fishy flavor occurred during prolonged heating.

Both drying temperature and drying time had an effect on the quality of spray dried soymilk. A brown colored powder was obtained when the soymilk was dried at 194°C inlet temperature and all the quality test values except viscosity were lower.

Dye-binding method was comparable to the Kjeldahl for estimating the protein content in soymilk. A factor of 0.197 can be used to convert dye-binding readings from mg of dye bound/ml of milk to percentage protein based on the 6.25 Kjeldahl factor.

Higher quality soymilk was obtained from the Merit variety soybeans than from the Kanrich variety.

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