THE EFFECT OF SOYBEAN PRE-TREATMENTS ON THE QUALITY OF SOYMILK

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BIOGRAPHICAL SKETCH

The author was born on August 23, 1941, in Medan, North Sumatra, Indonesia. He is the fifth child from nine children of the couple of Iskander Manullang and Kesia br. Hutapea. He received his elementary education at Methodist English School and Sekolah Rakyat No. 5, in Medan, from 1949 to 1955. He acquired his secondary education at Sekolah Menegah Pertama Negeri I from 1955 to 1958 and Sekolah Menegah Atas Negeri I Teladan from 1958 to 1961 both are in Medan.

He enrolled at Faculty of Veterinary Medicine, Bogor Agricultural University, in 1961. In 1966 he was also working as assistant at Faculty of Mechanization and Agricultural Product Technology. In 1970 he completed his degree of D.V.M. from Faculty of Veterinary Medicine, Bogor Agricultural University.

He was awarded a SEARCA Scholarship in 1974, which enabled him to earn the degree of Master of Science in Food Science from the University of the Philippines at Los Baños.

The author is married to Sonny Satyawan with whom he has three sons, Lambok Aldy, Sandy Win, and Rio Reinhardt.

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ABSTRACT

Monang Manullang, University of the Philippines at Los Baños, March 1977. The Effect of Soybean Pretreatments on the Quality of Soymilk. Major Professor: Prof. Priscilla Chinte Sanchez.

Three factors, namely hulling, cooking time, and the fermentation time were investigated for their effect on the quality of soymilk. Analysis of variance showed no significant differences between hulled and unhulled treatment on the water soluble protein, pH of soymilk after processing, and general acceptability of soymilk formulated with 20 ppm vanilla. However, total protein content, amino acid nitrogen, viscosity, pH of soymilk before processing, and general acceptability of soymilk formulated with 20% fresh milk are significantly affected. Treatment of unhulled soybean shows higher values than hulled soybean.

There is relationship between cooking time and total nitrogen content, water soluble protein, and viscosity. Regression analyses showed that the total protein content, water soluble protein and viscosity decreased and reached the minimum value when soybean was cooked between 10 to 20 minutes, but increased again as heating continued to 30 minutes.

The relationship between cooking time and total protein content of unhulled and hulled soybeans was defined by the equation: $Y = 2.86 - 0.15X + 0.004X^2$ with $r^2 = 0.8824$ and $t_1 = -8.1822$**; $t_2 = 7.7835$**, and $Y = 2.86 - 0.21X + 0.006X^2$ with $r^2 = 0.8878$ and $t_1 = -8.3813$**; $t_2 = 7.9340$**, respectively.

The relationship between cooking time and the water soluble protein from unhulled and hulled soybeans was defined by the equation $Y = 2.39 - 0.198X + 0.0056X^2$ with $r^2 = 0.8868$ and $t_1 = -8.0814$**; $t_2 = 7.3421$**, and $Y = 2.44 - 0.196X + 0.0052X^2$ with $r^2 = 0.9086$ and $t_1 = -9.3906$**; $t_2 = 8.0297$**, respectively.

The relationship between cooking time and the viscosity from unhulled soybean was defined by the equation $Y = 13.64 - 0.8198X + 0.0302X^2$ with $r^2 = 0.7007$ and $t_1 = -3.3498$**; $t_2 = 3.9818$**.

Linear regression analysis showed that amino acid nitrogen increased with fermentation time as defined by linear regression equation $Y = 1.1299 + 0.011X$ with $r^2 = 0.6599$ and $t = 4.4099$**.

**Significant at 1% level.

The general acceptability of soymilk formulated with 20 ppm vanilla from hulled treatment decreased with fermentation time as defined by the equation $Y = 5.49-0.1293X + 0.0011X^2$ with $r^2 = 0.9536$ and t_1 =-9.0207**; t_2 = 5.7340**.

Likewise, the general acceptability of soymilk formulated with 20% fresh milk from hulled soybeans decreased with fermentation time as described by the equation $Y = 4.89-0.1251X+ 0.0012X^2$ with $r^2 = 0.8384$ and t_1 = -5.4154**; t_2 = 3.9979**.

**Significant at 1% level.

INTRODUCTION

More than half of the World's population is suffering from hunger or malnutrition. In many developing countries the shortage of food is responsible for protein deficiency.

Soybean provides an important source of protein in man's diet in many parts of the world. Before it is consumed the protein in soybean may be subjected to a variety of processes which may ultimately affect its nutritional value. Fermentation is one such process applied to produce numerous products from soybeans.

Fermented foods comprises an important component of man's diet especially in Southeast Asia, the Near East and parts of Africa. In many instances the final products serve as sources of proteins, calories, and some vitamins (Van Veen and Steinkraus, 1970).

In general, fermented foods are more nutritious than the raw ingredients. This certainly holds true for fermented soybean products such as tempeh, miso, and natto. Tempeh, an Indonesian fermented food is used as a possible source of low cost protein for children's feeding programs in underdeveloped countries because of its nutritional value (Autret and Van Veen, 1955). This soybean product could be more easily digested than unfermented soybean. Another advantage of the fermented soybean is its excellent protein quality (Van Veen and Schaefer, 1950). More so, the nutritive value of one lot of freeze-dried tempeh is equivalent to skim milk and is much higher than unfermented soybeans (Gyorgy, 1961).

An increase of Protein Efficiency Ratio (PER) of tempeh might be attributed to better availability of amino acids liberated from the soy protein during fermentation and to the better digestibility of tempeh due to the increase of water soluble solids and nitrogen as suggested by Steinkraus (1960 and 1965).

Soymilk is another product from soybeans. It has been given considerable attention as an economical high protein food suitable for overcoming malnutrition of infants in the developing countries. This product is available as bottled drinks and in powdered form. The latter preparation has some advantages of easier transport and preservation along with centralized large scale production (Fukushima and Van Buren, 1970). However, considering the increase in the nutritive value brought about by the fermentation of soybeans, it seems possible to include this process as a pre-treatment in the preparation of soymilk.

Soymilk could be made more acceptable as a drink for infants to help alleviate the malnutrition problem. Moreover, its nutritive value could be increased by modifying the processing method. Since this product would cater to the need of this particular consumer, then, its processing method has to be modified so as to convert the proteins to more easily digestible components.

OBJECTIVES OF THE STUDY

This study was conducted at the Department of Food Science and Technology, University of the Philippines at Los Baños, with the following objectives:

a) to develop a method of soybean pre-treatments for soymilk production, and

b) to compare the product in (a) with the soymilk prepared by the conventional method, using chemical analysis and sensory evaluation.

REVIEW OF LITERATURE

Brief History of Soybean

Soybean (Glycine max (L) Merril) has been known since ancient history. Legend tells us of the first use of soybeans for food when a caravan laden with gold and gems, returning to an eastern Chinese country was attacked by bandits. The caravan took refuge in a cave and was nearing starvation when a servant, upon eating beans from a vine-like plant, recovered his vigor and induced others also to eat the bean, which sufficed as food until the bandits were driven away. This bean, supposedly the wild soybean, was thus established as a food in China.

The first Chinese records which mention soybean written in the books PEN TS'AO KONG MU. In 2838 B.C., Emperor SHANG-NUNG published the books PEN TS'AO KONG MU, which describe the plants of China, including a description of the soybean. Soybeans were mentioned frequently in later Chinese records and have been considered China's most important legume.

Soybeans were reported to be one of the "MU KU" or sacred grains of China, which included also rice, wheat barley, and millet, and were considered essential for the existence of the Chinese race. The soybean has many different names depending on the country where it is grown and used. It is generally reported that its name derived from the Chinese "Chiang-Yin" which means soy sauce in Japanese. It should be pronounced "Sho-Yu" in the Japanese language. Rather, recent names include soya bean, soja bean, soy, so-ya, Chinese pea, Manchurian pea and soia among more than 50 other names throughout the Orient (Smith and Circle, 1972).

Physical and Chemical Properties

Molecular Structure and Conformation

The range of molecular sizes of soybean proteins can be demonstrated by ultra centrifugation. There are 4 major fractions resolved; the fractions are designated as 2S, 7S, 11S and 15S based on their sedimentation rates.

2S Globulin

According to Smith and Circle (1972) at least five proteins have been isolated and shown to be constituents of 2S fraction of water extractable proteins: Bowman-Birk trypsin inhibitor, cytochrome C, Kunitz trypsin inhibitor, and two 2S globulins.

Bowman-Birk Inhibitor

Ultra violet difference spectral studies indicate that the two tyrosine residues are accessible to solvent in the native state, high concentrations of urea or guanidine hydrochloride cause no changes attributable to exposure of buried phenolic groups. Its high cystine content, however, suggests that the molecule is extensively crosslinked and therefore has a definite three-dimensional structure (Steiner and Fratali, 1969)

Kunitz Trypsin Inhibitor

This protein possesses a single polypeptide chain crosslinked by two disulfide bonds. Intrinsic viscosity, fluorescence polarization and other physical properties indicates that the inhibitor is compact, low in asymmetry, and rigid structure (Steiner and Fratali, 1969).

Nakamura et al. (1975) studied the trypsin inhibitors in Japanese soybeans and found the new trypsin inhibitor to have an isoelectric point ranging from pH 4.70 to 4.75.

7S Globulin

The molecules are folded compactly even though large regions of disordered (non-helical) structure occur.

Ultraviolet differential spectrum in urea solutions showed that the tyrosine residues are buried in the interior of the molecule while the tryptophan residues are accesible to solvent. Fukushima (1968) proposed that hydrophobic bonding is important in maintaining the tertiary structure.

The 7S globulin consists of hemagglutinin, lipoxygenase, β-amylase and 7S globulin.

Hemagglutinin

Practically nothing is known about the structure of this glycoprotein. The absence of cystine cross-links suggests that the molecule may be fairly flexible and subject to conformational changes under milder conditions than are required of other molecules such as the trypsin inhibitors (Smith and Circle, 1972).

Lipoxygenase

This enzyme appears to consist of two sub-units of 58,000 molecular weight. Dissociation into sub-units occurs with guanidine hydrochloride or sodium dodecylsulfate (Steven et al., 1970).

11S Globulin

Fukushima (1968) mentioned that optical rotary dispersion and infra red measurements suggest that the secondary and tertiary structure of 11S protein are similar to the structure of the 7S globulin. The 11S protein contain a little, if any, α- helical structure but may consist of anti parallel β-structure and disordered regions. Ultraviolet differential spectral studies indicated that the tyrosine and tryptophan residue of the 11S protein are buried in hydrophobic regions of the molecule.

15S Globulin

Although this protein has not been isolated and characterized, there is evidence that it also has a quarternary structure (Wolf and Briggs, 1958).

Solubility and Denaturation of Soybean Protein

Kirk and Othmer (1953) mentioned that the solubility of protein are influenced by pH, by the concentration of salt present, by temperature and by the presence of other proteins components owing to the possibility of interaction such as salt or complex formation.

Wolf and Cowan (1975) described that the majority of soy proteins are globulins. This class of proteins is insoluble in water in the region of their isoelectric points but will dissolve on the isoelectric state when salts such as sodium or calcium chloride are added. If the pH is above or below the isoelectric point, a globulin will also dissolve in aqueous solutions in the absence of salts. Insolubility in the isoelectric pH region can be eliminated by hydrolyzing the protein with pepsin to a much lower molecular weight than of the original proteins. The pH solubility relationship of soybean proteins is similar to the pH dependency of solubility for casein which has an isoelectric point 4.6. Consequently, soy isolates can often substitute for casein and caseinates in food products.

Soybean proteins are sensitive to most of the ordinary conditions known to denature the proteins. Of primary interest to the food technologists are the effects of moist heat and the extremes of pH.

Beckel et al. (1942) and Belter and Smith (1952) mentioned that denaturation of soybean proteins by moist heat is well known and has long been used to eliminate anti-nutritional factors (believed to be protein) in soybean meals and flours used in animal feeds and food for humans. Even though heat is a common physical treatment given to most food either during processing or cooking, surprisingly little is known about the reaction that soybean protein undergoes when it is heated. Early studies dealt with the effects of heat and moisture on defatted meal and were primarily concerned with measurements of the amounts of soluble protein remaining after heating.

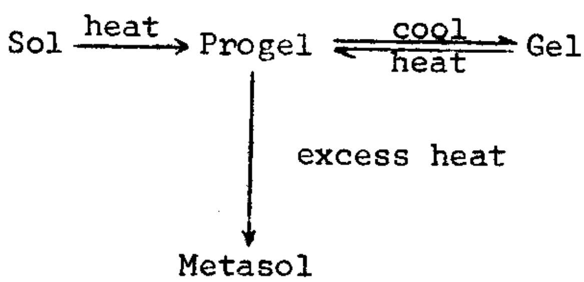
Changes in extractability of the different protein components as a function of heating time are reported by Shibasahi et al. (1969).

Fukushima (1959a) reported that when soybean meal and an equal weight of water were autoclaved above 100°C, the water soluble protein decreases to a minimum and then increases again as treatment is continued.

Likewise, heating at 100°C with greater amounts of water, solubilizes large amounts of protein (Fukushima, 1959b). Circle et al. (1964) studied the effect of heat on aqueous dispersion of commercially prepared soybean globulins, and found gels formed when protein dispersion of 8% or higher were heated for 10 to 30 minutes at 70°C. In the concentration range of 8 to 12% the gels broke down when heated at 125°C. This behavior may be related to resolubilization of protein observed by Fukushima (1959a) when meal is heated at 100°C for prolonged time with excess water or at temperature about 100°C.

Catsimpoolas and Meyer (1970) proposed that heating soybean globulin at a concentration greater than 8% converts them to a progel state that gels on cooling. Gel to progel conversion is reversible on heating. Excessive heat or additional chemicals such as disulfite cleaving agents, form a "Metasol" state that does not gel.

Sol-progel-gel transition can be shown as follows:



On heating, the sol is irreversibly converted to the progel which in turn gels on cooling. The gel is reversibly converted to the progel by reheating. During conversion from sol to progel, viscosity increases as temperature is raised until a maximum is reached. At higher temperature the viscosity decreases as a result of irreversible conversion to a metasol state that does not gel on cooling (Wolf and Cowan, 1975).

Nutritional Aspects of Soybeans

Protein

The soybean is best known for its high protein content. The yield of protein from soybeans, weight for weight, is approximately twice that of meat; four times that of eggs, wheat, and other cereals, five or six times that of bread; twice that of lima and navy beans, walnuts, filberts and most other nuts; and twelve times that of milk. Not only is the protein of the soybean higher in percentage than that of the other legumes; but, it is also a much superior quality.

The quality of a protein is dependent upon the variety and amount of its constituent building blocks called amino acids. Those proteins which can supply sufficient amounts, the kinds of amino acids required by the body for building and repairing of tissues are called complete proteins. The incomplete proteins are those which lack these essential amino acids or contain them in insufficient amounts. These amino acids are called essential amino acids because the body is unable to synthesize them and they must be furnished by the foods (Chen and Chen, 1962).

Proteins of high biological value for growth such as egg, milk, and other foods usually provide about 40% of the total nitrogen (N) in the form of essential amino acids; while relatively poor quality protein provides only about 25% of the total nitrogen in the form of essential amino acids N (Fisher, 1974). Block, R. J. and R. W. Weiss present the amino acid composition as cited by Smith et al. (1974) in Appendix Table 1.

Carbohydrate

Soluble carbohydrate. The carbohydrates of the soybean have not been studied seriously as a potential source of food or feed. Their principal utilization is in animal feeds where they contribute some calories to the diet, especially for ruminants, since they are able to use the polysaccharides.

The principal sugars of soybean are the disaccharide sucrose, $C_{12}H_{22}O_{11}$, the trisaccharide raffinose,

$C_{18}H_{32}O_{16}$ and the tetrasaccharide stachiose, $C_{24}H_{42}O_{21}$. A pentosaccharide verbascose, $C_{30}H_{52}O_{26}$ has been found in very minor amounts.

Glucose or other reducing sugars are present in green or immature beans in substantial amounts, but, they disappear as the beans approach maturity and the occurrence of glucose in mature soybean is questionable. The alcohol extract of defatted soybean meal has been reported to contain sucrose, raffinose, stachiose, fructose, galactose, rhamnose, arabinose and glucoronic acid.

Starch is frequently reported in immature beans but is seldom found in mature beans (Smith and Circle; 1972). Because of low starch content the soybean is especially valuable as the basis of foods for persons requiring a low starch diet, and is occupying an important place in the dietary needs of the diabetic population (Chen and Chen, 1962).

Insoluble carbohydrate. The major polysaccharides that can be extracted from soybean seed are arabinan and arabinogalactan (Smith and Circle, 1972).

Fat

Not only is soybean rich in high quality protein but it is also unique in its high fat content of 20%. The fact that the soybean oil contains 52% linoleic acid in which butter contains none, renders it exceptionally valuable as a fatty food, since this acid cannot be synthesized by warmblooded animals. In fact, this acid (and few other unsaturated fatty acids) in the human diet is so important that it has occasionally been referred to as "Vitamin F." These acids are also called "essential fatty acids." The oil in the soybean also contains a large amount of lecithin and fair amounts of fat soluble vitamins. The phosphatide or lecithin (including cephalin and inositol) content of the soybean is over 3%. This amount is more than that found in any other plants and is equal to that found in eggs. The lecithin in the soybean is identical with that in egg yolk.

Lecithin is an important constituent of all organs of the human body and especially of the nervous tissue, the heart and the liver. The soybean, therefore, is a good nerve and brain food. In addition to the phosphatides, crude soybean oil also contains a group of organic substances known as the sterols.

These sterols are found in the unsaponified fraction of the oil. Unlike animal fats whose sterol is cholesterol, (the chief offender for arteriosclerosis, heart disease and kidney stones) soybean oil contains phytosterol which produces no known harmful effects on the human body (Chen and Chen, 1962).

Available Energy

Owing to its high fat content, the soybean is a food that supplies the body with large amounts of energy. The energy available for metabolism (metabolic energy) from soybean may be calculated from its content of carbohydrates, fat, and protein, taking into account the digestibility of each of these as well as the heat of combustion. Except under conditions of extreme caloric deprivations, the protein would not be expected to be utilized to any significant extent as a source of energy. The amount of energy theoretically available from soybean protein may be calculated by multiplying the protein content by the factor 3.47 cal. per gram (Watt and Merril, 1963).

The carbohydrate material may have been removed during processing and will be in the range from 22-29% for the whole soybean. The USDA Handbook assumes the carbohydrate of soybeans to be almost completely digestible (97%) and bases its caloric calculation for soybean and soybean products on a factor of 4.07 cal. per gram.

In the cases of soybean milk, soybean curd and protein isolates, this factor may be applicable since the preparation of these products involves the removal of most of the insoluble and indigestible carbohydrates (Liener, 1972).

Nutritional Aspects of Fermented Soybean

Studies on the nutritive value of fermented soybean with *Rhizopus oligosporus* (tempeh) as reported by Murata et al. (1967) indicated no large differences in protein and ash content between fermented soybean (tempeh) and unfermented soybeans but the extractable matter increased somewhat after fermentation. There was a slight increase in fiber during fermentation which might be due to the growth of the mold. The amount of free amino acids increased progressively during fermentatation from 1 to 85 times. The fat content of tempeh was slightly lower than that of soybean but the amino acids value was noticeably higher.

Riboflavin, vitamin B_6, nicotinic acid and panthothenic acid increased during fermentation although thiamine was little altered.

The amino acid contents of tempeh were affected by fermentation time and heat processing (Stilling and Hackler, 1965). Steaming of tempeh for 2 hours or less had no effect on the amino acid contents. The effect of fermentation on amino acid composition is presented in Appendix Table 2.

Smith et al. (1964) studied the nutritive value of tempeh in relation to processing. They showed that the loss of solids and proteins in hulling, soaking, washing, and cooking soybean before fermentation did not reduce the nutritive value of either cotyledons or full fat grits (chips) which are used to make tempeh. Since pancreatic hypertrophy did not occur in rats fed with tempeh the heat used in normal preparation is sufficient to destroy the factors in raw soybeans responsible for poor growth and pancreatic hypertrophy. Methionine supplementation of tempeh significantly increased the rate of rat growth and protein efficiency ratio.

Van Veen and Steinkraus (1970) studied the nutritive value and the wholesomeness of fermented foods and claimed that the keeping quality of fermented food is increased and cooking times are often decreased. The organoleptic characteristics of fermented food are generally improved when compared to the raw ingredients. The digestibility of certain foods such as tempeh, ontjom and bongkrek is much improved compared to the raw ingredients.

Wang et al. (1970) cited in his personal communication with Djien that Rhizopus oligosporus produces an antibacterial agent. Because this organism also has the unique characteristic of fast growth, there is probably little chance for bacteria to gain ground before the tempeh fermentation is complete. He purposely inoculated the tempeh with different amounts of Escherichia coli, B. mycoides, Pseudomonas pyocyanea, Proteus sp. or P. cocovenenans along with Rhizopus oligosporus in making tempeh and the results indicated that the fermentation is not intervened by the presence of inoculated bacteria.

He also commented that pre-fermentation during soaking or addition of acid to the soaking water may not be very important in the process of tempeh fermentation.

Soybean Milk

Soybean milk or vegetable milk or "Fu Chang" in Chinese is reported to have been developed and used in China before the Christian era by the philosopher Whi Nain Tze, who is credited also with the development of tofu (Piper and Morse, 1943). The tofu and yuba are closely associated with soymilk since the preparation of the milk is the first step in the processing of tofu and yuba (Smith and Circle, 1972).

Soybean milk has been of considerable interest to nutritionist's as a possible substitute for cow's or human milk particularly in the feeding of infants allergic to animal's milk or where cow's milk may be either too expensive or unavailable. Soybean milk and cow's milk have approximately the same protein content (3.5-4.0%) and a composition of the amino acid of soybean and milk protein shows a fairly close correspondence. The main deficiency of soybean protein as compared with the protein of cow or human milk lies in the content of the S-containing amino acids.

The effect of presoaking soybeans in solution of various chemicals for the reduction of the beany flavor in soymilk has been reported by Khaleque et al.

(1970). Of the chemicals used, sodium carbonate and sodium hydroxide had a significant effect on the reduction of beany flavor in soymilk. Soymilk prepared from beans presoaked in carbonate contained more protein and had a higher viscosity than milk prepared from beans presoaked in sodium hydroxide and was easier to process. There was no significant difference in the amino acid pattern of the proteins in the soymilk prepared by the three methods.

Bourne et al. (1976) studied the effect of sodium alkali and salts on pH and flavor of soymilk. The taste panel showed greater acceptability for soymilk adjusted to pH 7.0 to 7.5 with NaOH but noted a soapy flavor and gave lower score for milk with pH greater than 7.5. Soymilk adjusted to pH 7.0 to 7.5 with Na_2CO_3 or $NaHCO_3$ was disliked by the panel. The reason could be due to the increase in sodium ion concentration rather than the change in pH.

Soymilk preparations with Na_2CO_3, $NaHCO_3$, $NaNO_3$, Na_2SO_4, Na acetate and Na citrate additions added at the same levels as the amount of NaOH required to raise the pH to 7.2 were given approximately the same scores by the panel as the NaOH-treated sample, even when the pH was not in the range of 7.0 to 7.5. This evidence supports the theory that the sodium ion concentration is the effective mechanism in improving the flavor of soymilk rather than the change in pH.

Puertollano et al. (1970) studied the effect of changes in the formulations of soymilk on its acceptability to Filipino children and described that soymilk containing 7% sugar was given a significantly higher acceptability scores than the soymilk containing 5% sugar. Sugar levels of 9% and 11% had a higher (but not significantly different) in acceptability than 7%. A standard formula containing 7% added sugar and 20 ppm of a commercial essence of vanilla was selected on account of high sustained acceptability and reasonable cost.

Hackler and Stilling (1967) studied the amino acid composition of heat-processed soymilk and its correlation with nutritive value by using two processing temperatures, 93° and 121°C. They found that soymilk heat processed for as long as 4 hours at 93°C caused no significant changes in amino acid composition. On the other hand, decreases in some of the amino acids (especially cystine) were observed when the processing temperature of 121°C was used. The amino acid composition of soymilk processed from 15 to 40 minutes at

93°C is shown in Appendix Table 3, and soymilk processed at 121°C is shown in Appendix Table 4.

MATERIALS AND METHODS

The soybean used in the whole experiment was similar to those used by the "Philsoy" manufacture bought from the Cubao supermarket with label US 100. The fungus used in the pretreatment, Rhizopus oligosporus (Saito), was obtained from the Food Microbiology laboratory, Department of Food Science and Technology, UPLB

Three factors such as hulling (A), cooking time (B) and fermentation time (C) were evaluated for their effects on the quality of soymilk. The soymilk produced from unhulled, uncooked and unfermented soybeans served as the control or the standard.

Preparation of Inoculum

The preparation of the inoculum was described by Hermana et al. (1972); that is, steamed rice was inoculated with Rhizopus oligosporus and incubated until the spores had fully matured. The mold culture was dried in the oven at temperature from 45 to 50°C and then ground to a powder form. The amount of inoculum used is 10 g/250 g soybean.

Preparation of Fermented Soybean

Two hundred fifty grams of whole soybean were soaked in water for 8 to 12 hours. The seed coat was retained (unhulled) or removed by hand (hulled) depending on the treatment, and the beans were later steamed under pressure, 1.06 kg/cm^2 at 121°C, for 0, 10, and 30 minutes and then drained.

The beans were inoculated with *Rhizopus oligosporus*, wrapped with banana leaves and then fermented at 35° to 37°C for as long as 0, 24, 48, and 72 hours. The procedure is shown schematically below:

Dry soybean

↓

Soak in excess water 8-12 hours

↓

Steam under pressure 1.06 kg/cm^2, 121°C

↓

Drain well

↓

Inoculate with *Rh. oligosporus*

↓

Wrapped with banana leaves

↓

Fermented at 25°-37°C

↓

Fermented Soybean
(tempeh)

Soymilk Processing

The method used in the production of "Philsoy" was adopted except that the raw material used is fermented soybean. The method as described by Bourne et al. (1976) is shown in the scheme below:

Fermented soybean
↓
Blendor in boiling water, minimum temp. 80°C
↓
Filter ⟶ Insoluble residue
↓
Formulate
↓
Bottling
↓
Sterilization
↓
Soymilk

Fermented soybean was disintegrated in a commercial blendor for 10 minutes at high speed with the addition of 2.5 liters (1:10) of boiling water. The slurry was filtered with cheese cloth while the temperature was maintained at 80°C. The insoluble residue was discarded and the soymilk was divided into 2 parts. One was formulated by adding 7% sugar and 20 ppm of commercial essence of vanilla and another was formulated with 20% of fresh milk.

Approximately 210 ml of each sample were poured into a 7-fl. oz. bottle, sealed with a crown seal, and sterilized in an autoclave at 1.06 kg/cm^2 and at 121^{o}C.

Analysis and Determination

To determine the effect of hulling, cooking time and fermentation time soymilk samples were subjected to chemical analysis of total protein content, water soluble protein, amino acid nitrogen, viscosity, pH and sensory evaluation. The detailed procedures for chemical analysis are presented in the Appendix A.

Sensory Evaluation

The sensory evaluation of the soymilk involved 12 nontrained Indonesians as members of the taste panel. Twenty five ml of soymilk from each treatment were served using Philsoy as the control. A 7-point hedonic scale (7 = like strongly and 1 = dislike strongly) was used to score the soymilk samples.

Statistical Analysis

The sensory evaluation scores were treated statistically by analysis of variance using the completely randomized design (CRD). The effect of cooking time and fermentation time was determined by multiple regression analysis (MRA) (Steel and Torrie, 1960). Analysis and data computations were done at the UPLB Computer Center.

RESULTS AND DISCUSSION

Composition of Raw Soybean

The chemical analysis of the raw soybean used (U.S. 100) is presented in Table 1.

Table 1. Chemical analysis of soybean U.S. 100

Total protein content	35.92%
Moisture	9.53%
pH	6.4

The total protein content, the moisture content and the pH are typical of soybeans suitable for soymilk production.

Effect of Pretreatment

The analyses of variance for the total protein content, water soluble protein, amino acid nitrogen, viscosity, pH of soymilk before and after processing, general acceptability of soymilk formulated with 20 ppm vanilla and 20% fresh milk are shown in Appendix Tables 5 to 12.

a. Hulling. The chemical, physical and sensory characteristics of soymilk from hulled and unhulled soybeans are shown in Table 2.

The analysis of variance showed that the total protein content, amino acid nitrogen, viscosity, pH of soymilk before and after processing and general acceptability scores of the soymilk formulated with 20% fresh milk, are higher for those derived from unhulled soybeans than those from the hulled soybeans. These results can be explained by the worked of Smith et al. (1964), which shows that dehulling, soaking, washing, cooking and fermenting steps employed in the preparation of tempeh all contribute to the loss of meal constituents. The hull fraction, when removed after soaking from Harosoy soybeans, accounted for nearly 13% of the whole bean and contained 4.64% nitrogen on a dry basis.

The hulls, when separated by hand from Harosoy soybeans represent 7.9% of the whole bean and contain 1.87% nitrogen. Processing losses for two methods of tempeh production can be seen in Appendix Table 13.

Because the hull fractions contain a certain percentage of nitrogen then the total protein content of unhulled soybean is greater than the hulled ones.

Table 2. Chemical and sensory characteristics of soymilk from hulled and unhulled soybeans.

Characteristics	Hulled	Unhulled	F
1. Total protein content (%)	2.13	2.33	128.84**
2. Water soluble protein (%)	1.56	1.61	3.62 ns
3. Amino acid nitrogen (mg/ml)	1.38	1.53	135.36**
4. Viscosity (cp)	13.41	17.77	148.36**
5. pH before processing	6.66	6.77	9.03**
6. pH after processing	6.50	6.55	3.37 ns
7. General acceptability of soymilk formulated with 20 ppm vanilla	3.04	3.11	0.13 ns
8. General acceptability of soymilk formulated with 20% fresh milk	2.87	3.13	2.91**

**Significantly different at 1% level

ns = Not significant

The high protein content of soymilk from unhulled soybean could be the main cause for the observed increase in viscosity. Temperature and duration of heating are another factors affecting viscosity and gel formation of soymilk. These results confirmed the findings of earlier workers (Watanabe et al., 1962; Saio et al., 1968).

The effect of hulling on the amino acid content of soymilk shows that unhulled soybeans resulted in greater amino acid content than hulled soybeans due to the lose of amino acid nitrogen during soaking and peeling, once the soybeans peeled by hand, the swollen soybeans received a pressure caused the released of amino acid nitrogen into the soaking water and this was discard before cooking the soybeans.

General acceptability scores of soymilk from unhulled soybeans formulated with 20% fresh milk is greater than those from the hulled soybeans. For more discussion on general acceptability refer to the effect of fermentation time on the general acceptability of soymilk.

b. Cooking time. Even though heat is a common physical treatment given to most foods either during processing or cooking, suprisingly little is known about the reaction that soybean protein undergoes when it is heated.

Chemical and sensory characteristics of soymilk subjected to different cooking times are shown in Table 3.

Soybean treatment with different cooking times shows that the total protein content, water soluble protein, amino acid nitrogen, viscosity, pH of soymilk before processing and general acceptability of soymilk formulated with 20 ppm vanilla decreased at 10 minutes cooking but increased again as heating continuous to 30 minutes.

The relationship between cooking times and total protein content of the hulled and unhulled soybeans was significantly different at 1% level, shown in the equation $Y = 2.86-0.21X + 0.006X^2$ with $r^2 = 0.8878$ and $t_1 = -8.3813$; $t_2 = 7.9340$, and $Y = 2.86-0.15X + 0.004X^2$ with $r^2 = 0.8824$ and $t_1 = -8.1822$; $t_2 = 7.7835$, respectively (Figure 1).

Both curves show that hulled soybeans is more sensitive to cooking time than the unhulled soybeans, in respect to the total protein content. The curves show that prolonged heating of the soybean from 10 to 20

Table 3. Chemical and sensory characteristics of soymilk from soybean subjected to different cooking times

Characteristics	Cooking times (minutes)			HSD
	0	10	30	0.05
1. Total protein content (%)	2.86^{a}	1.60^{c}	2.22^{b}	0.05
2. Water soluble protein (%)	2.42^{a}	0.99^{c}	1.35^{b}	0.08
3. Amino acid nitrogen (mg/ml)	1.43^{b}	1.40^{b}	1.54^{a}	0.04
4. Viscosity (cp)	16.21^{b}	12.65^{c}	17.70^{a}	1.06
5. pH before processing	6.77^{a}	6.65^{b}	6.73^{ab}	0.11
6. pH after processing	6.46^{b}	6.55^{ab}	6.57^{a}	0.09
7. General acceptability of soymilk with 20 ppm vanilla	3.34^{a}	3.23	2.66^{b}	0.57
8. General acceptability of soymilk formulated with 20% fresh milk	3.26^{a}	3.18^{a}	2.56^{b}	0.45

Note: Means superscripted by the same letter are not significantly different at 5% level.

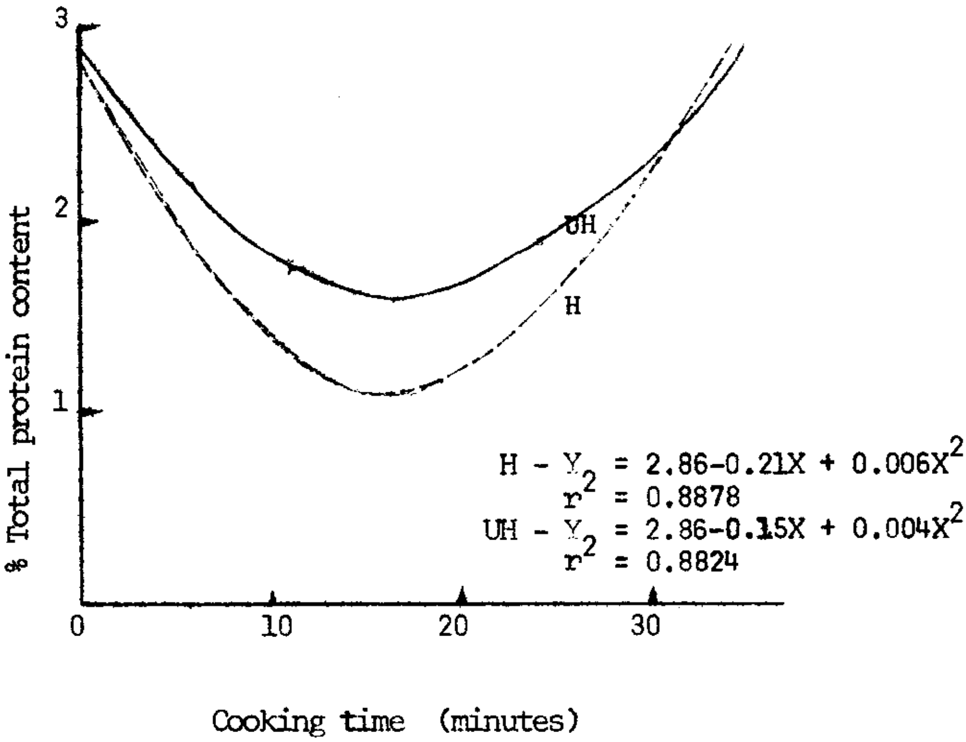


Figure 1. Relationship between cooking time and total protein content of soymilk from hulled (H) and unhulled (UH) soybeans.

minutes caused a fast drop in protein content, indicating a rapid rate of protein denaturation.

Shibasahi et al. (1969) claimed that hydrogen and hydrophobic bonds appeared to be the major bonds responsible for the protein insolubilization of heat denatured soy protein. Prolonged heating for 30 minutes increased again the total protein content. The mechanism of this phenomenon is not yet known since soybeans are known to be sensitive to moist heat.

The relationship between cooking time and water soluble protein of soymilk from hulled and unhulled soybeans was significantly different at 1% level as defined by the equation $Y = 2.44 - 0.196X + 0.0052X^2$ with $r^2 = 0.9253$ and $t_1 = -9.3956$; $t_2 = 8.0297$, and $Y = 2.39 - 0.198X + 0.0056X^2$ with $r^2 = 0.8868$ and $t_1 = -8.0814$; $t_2 = 7.3421$ respectively (Fig. 2). Both curves show similar trends. The minimum of water soluble protein content of hulled and unhulled soybeans was reached when soybeans were subjected to cooking between 10 to 20 minutes, only to increase again as heating continued to 30 minutes.

This behavior is similar to that found by Fukushima (1959). Fukushima heated the soybean in an autoclave above 100°C and found that the water

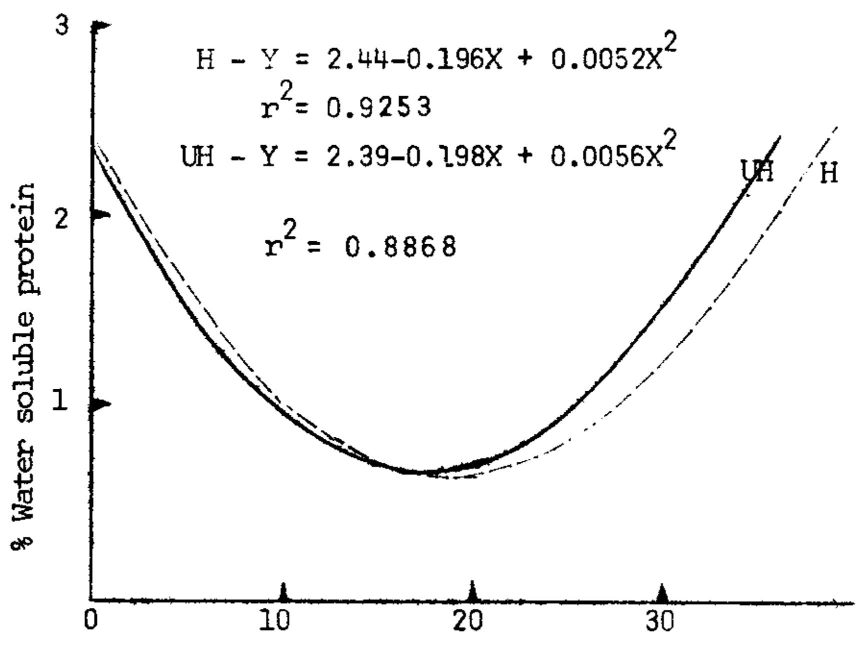


Figure 2. Relationship between cooking time and water soluble protein content of soymilk from hulled (H) and unhulled (UH) soybeans.

soluble protein decreased to a minimum and then increased again as heating continued. However, he did not explain the cause of this behavior.

It is not yet clearly established whether this decrease is due to the denaturation of water soluble protein or due to the difference in interaction energy between protein molecules. Joly (1965) described that the interaction energy between molecules of any protein varies when the protein undergoes denaturation. As a consequence of such a variation of interaction energy, the state of dispersion of the molecules in the solution may change. Indeed, any condition which decreases the interaction between water molecules and protein molecules decreases the solubility of the protein.

The relationship between cooking times and viscosity of soymilk from unhulled and hulled soybeans are defined by the equation $Y = 18.64-0.8198X + 0.0302X^2$ with $r^2=0.7007$ and $t_1 = -3.3498$; $t_2 = 3.9818$, and $Y = 14.20-0.3547X + 0.0118X^2$ with $r^2 = 0.2912$ and $t_1 = -1.7566$; $t_2 = 1.8892$, respectively. The equation for the hulled soybean treatment is not significant at 1% level (Fig. 3).

The curves show that heating the soybean from 10 to 20 minutes caused decreased in the viscosity soymilk. Observation of soymilk during filtration

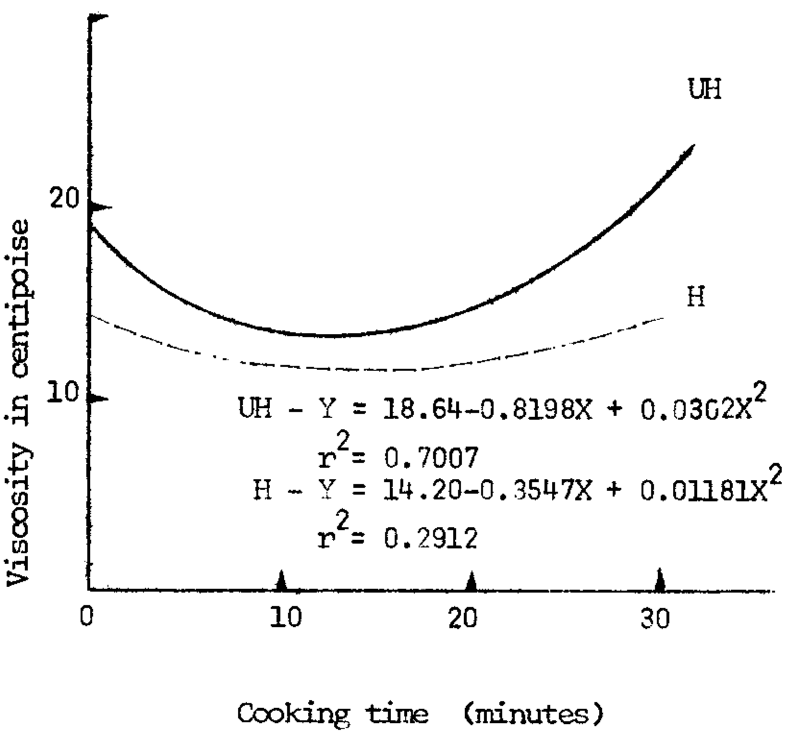


Figure 3. Relationship between cooking time and viscosity of soymilk from hulled (H) and unhulled (UH) soybeans.

shows that 10-minute heat treatment of soybeans resulted in longer time to filter due to the gel formation. The formation of gel might be attributed to several reasons. Soybeans have been subjected to heat during cooking resulting in the disintegration with hot water. Maintenance of this temperature on soymilk before sterilization has the effect of decreasing the viscosity and the total protein content. Markley (1950) discussed that there are several factors which affect the viscosity of solutions such as concentration of the dispersed phase, hydration, temperature, size of particle, hysteresis and electrolytes.

Chemical and sensory characteristics of soymilk from hulled or unhulled soybeans and different cooking times are shown in Table 4.

The relationships between cooking times and general acceptability of soymilk from unhulled soybeans formulated with 20 ppm vanilla and 20% fresh milk resulted in a highly significant difference with the equation: $Y = 3.667-0.0017X$ with $r^2 = 0.5042$, and $t = -3.1891$ and $Y = 3.6597-0.0016X$ with $r^2 = 0.3479$ and $t = -2.3099$, respectively (Fig. 4).

These curves show that as cooking time progresses the general acceptability decreases. This result can

Table 4. Chemical and sensory characteristics of soymilk from hulled or unhulled soybeans subjected to different cooking times.

Characteristics	Cooking times (minutes) 0	10	30	HSD 0.05
1. Total protein content(%)				
Hulled	2.86^{a}	1.41^{e}	2.11^{c}	
Unhulled	2.86^{a}	1.79^{d}	2.33^{b}	0.09
2. Water soluble protein(%)				
Hulled	2.44^{a}	1.00^{d}	1.23^{c}	
Unhulled	2.39^{a}	0.97^{d}	1.47^{b}	0.14
3. Amino Acid Nitrogen(mg/ml)				
Hulled	1.42^{bcd}	1.37^{cd}	1.36^{d}	
Unhulled	1.44^{b}	1.42^{bc}	1.72^{a}	0.06
4. Viscosity (cp)				
Hulled	14.20^{c}	11.83^{d}	14.19^{c}	
Unhulled	18.64^{b}	13.46^{cd}	21.21^{a}	1.84
5. pH before processing				
Hulled	6.79^{ab}	6.71^{bc}	6.49^{d}	
Unhulled	6.75^{bc}	6.60^{cd}	6.97^{a}	0.19
6. pH after processing				
Hulled	6.52^{bc}	6.62^{b}	6.35^{d}	
Unhulled	6.40^{cd}	6.47^{cd}	6.79^{a}	0.15
7. General acceptability of soy milk formulated with 20 ppm vanilla				
Hulled	3.13^{ab}	2.84^{ab}	3.16^{a}	
Unhulled	3.56^{a}	3.63^{a}	2.16^{b}	0.99
8. General acceptability of soy milk formulated with 20% fresh milk				
Hulled	3.11^{abc}	2.58^{cd}	2.92^{bcd}	
Unhulled	3.41^{ab}	3.78^{a}	2.19^{d}	0.78

Note: Mean supercripted by the same letter are not significantly at 5% level.

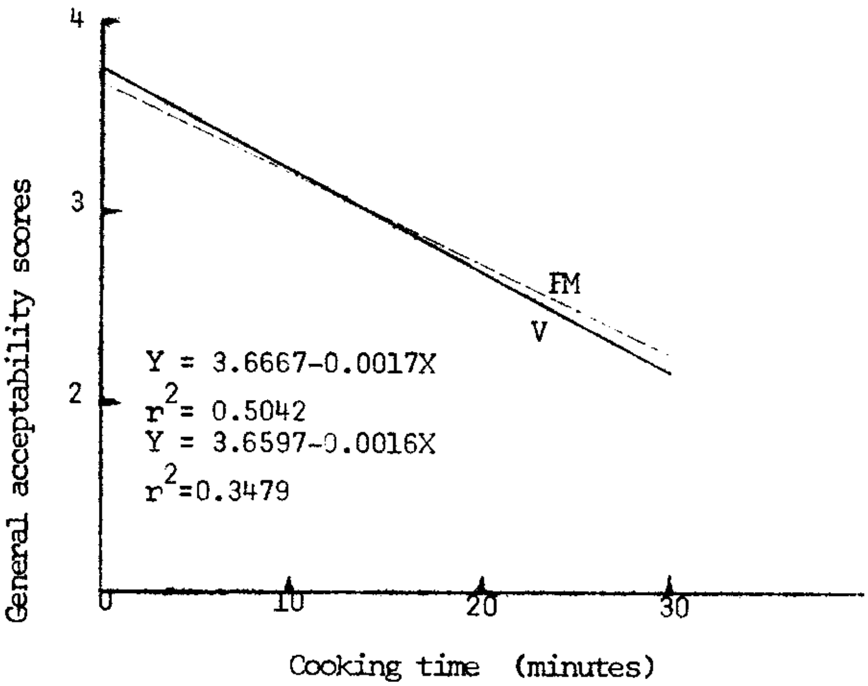


Figure 4. Relationship between cooking time and general acceptability of soymilk formulated with 20 ppm vanilla, 20% fresh milk from unhulled (UH) soybeans.

be explained by the action of heat on the protein of soybeans releasing the ethyl α-D-galactopyranoside and L - tryptophan which cause the bitter taste. These two bitter components have been isolated by Honig et al. (1971).

c. Fermentation times. The effect of fermentation times on the chemical, physical and sensory characteristics of soymilk is presented in Table 5.

Regression analyses showed no significant effect of fermentation time on the total protein content, water soluble protein, viscosity, pH before and after processing and the general acceptability of milk formulated with 20 ppm vanilla from the hulled and unhulled soybeans. However, the amino acid nitrogen increases with fermentation time as described by the equation: $Y = 1.1299 + 0.011X$ with $r^2 = 0.6599$ and $t = 4.4049$, and $Y = 1.1937 + 0.0052X$ with $r^2 = 0.2564$ and $t = 1.8566$ for soymilk from unhulled and hulled soybeans, respectively (Fig. 5). The increase of amino acid nitrogen in the soymilk from hulled soybeans was not statistically significant.

The amino acid nitrogen is quite low at zero fermentation time, however, it rose rapidly with

Table 5. Chemical and sensory characteristics of soymilk from soybeans subjected to different fermentation times

Characteristics	Fermentation times (hours)				HSD 0.05
	0	24	48	72	
1. Total protein content (%)	2.15^{b}	2.24^{a}	2.23^{a}	2.28^{a}	0.07
2. Water soluble protein (%)	1.41^{b}	1.62^{a}	1.62^{a}	1.69^{a}	0.10
3. Amino acid nitrogen (mg/ml)	1.03^{c}	1.51^{b}	1.65^{a}	1.63^{a}	0.05
4. Viscosity (cp)	15.64^{a}	16.27^{a}	16.61^{a}	13.75^{b}	1.35
5. pH before processing	6.70^{ab}	6.71^{ab}	6.64^{b}	6.82^{a}	0.14
6. pH after processing	6.50^{ab}	6.53^{ab}	6.47^{b}	6.61^{a}	0.11
7. General acceptability of soymilk formulated with 20 ppm vanilla	4.68^{a}	2.87^{b}	2.46^{b}	2.31^{b}	0.72
8. General acceptability of soymilk formulated with 20% fresh milk	4.63^{a}	2.73^{b}	2.28^{b}	2.35^{b}	0.57

Note: Means superscripted by the same letter are not significantly different at 5% level.

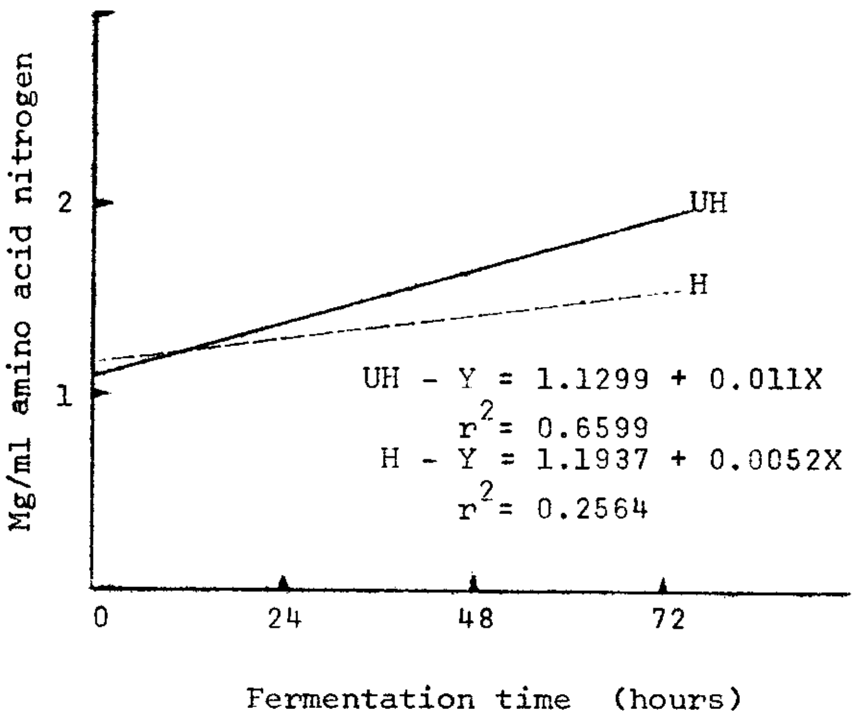


Figure 5. Relationship between fermentation time and amino acid nitrogen of soymilk from hulled (H) and unhulled (UH) soybeans.

fermentation time probably due to the production of proteolytic enzyme by <u>Rhizopus oligosporus</u>. Degradation of the protein to amino acid nitrogen is required by the mold to utilize amino acid nitrogen for its rapid growth. These results are quite similar to those found by Murata et al. (1967) although they found that the amount of individual amino acid increased from 1 to 85 times.

The relationship between fermentation time and the general acceptability of soymilk is best described by the quadratic equation: $Y = 5.49 - 0.1293X + 0.0011X^2$ with $r^2 = 0.9536$ and $t_1 = -9.0207$; $t_2 = 5.7340$ for soymilk formulated with 20 ppm vanilla, and $Y = 4.89 - 0.1251X + 0.0012X$ with $r^2 = 0.8384$ and $t_1 = -5.4154$; $t_2 = 3.9979$ for soymilk formulated with 20% fresh milk both from hulled soybeans (Figure 6). These equations show statistical significance at 1% level. Both curves of soymilk formulated with 20 ppm vanilla and 20% fresh milk show similar trend, reaching the minimum value at 48 hours fermentation which increased again at 72 hours fermentation (Table 6).

Observations on the analyses of variables such as viscosity, pH after processing and amino acid nitrogen would help explain the results. When the

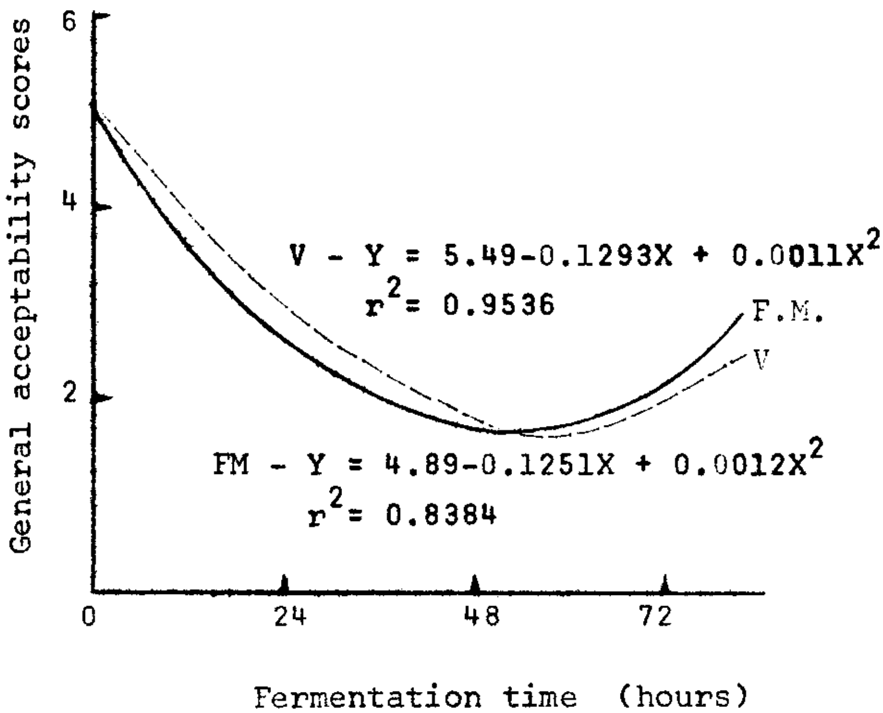


Figure 6. Relationship between fermentation time and general acceptability of soymilk from hulled (H) soybeans formulated with 20 ppm vanilla (V) and 20% fresh milk (FM).

Table 6. Chemical and sensory characteristics of soymilk from hulled (H) or unhulled (UH) soybeans subjected to different fermentation times.

Characteristics	Fermentation times (hours) 0	24	48	72	HSD 0.05
1. Total protein content (%)					
Hulled	2.21^{bc}	1.98^{d}	2.18^{bc}	2.37^{c}	0.11
Unhulled	2.11^{c}	2.50^{a}	2.28^{b}	2.43^{a}	
2. Water soluble protein (%)					
Hulled	1.44^{cd}	1.60^{bc}	1.66^{b}	1.55^{bcd}	0.17
Unhulled	1.39^{d}	1.64^{b}	1.58^{bc}	1.84^{a}	
3. Amino acid nitrogen (mg/ml)					
Hulled	1.05^{c}	1.51^{b}	1.52^{b}	1.46^{b}	0.08
Unhulled	1.01^{c}	1.52^{b}	1.78^{a}	1.80^{a}	
4. Viscosity (cp)					
Hulled	13.60^{cde}	13.24^{de}	15.11^{cd}	11.67^{e}	2.27
Unhulled	17.68^{ab}	19.29^{a}	18.28^{a}	15.84^{bc}	
5. pH before processing					
Hulled	6.77^{bcd}	6.88^{ab}	6.42^{e}	6.58^{de}	0.23
Unhulled	6.63^{cde}	6.54^{de}	6.85^{abc}	7.06^{a}	
6. pH after processing					
Hulled	6.59^{ab}	6.68^{a}	6.26^{d}	6.45^{bc}	0.19
Unhulled	6.40^{cd}	6.37^{cd}	6.68^{a}	6.76^{a}	
7. General acceptability of soymilk formulated with 20 ppm vanilla					
Hulled	5.55^{a}	2.86^{bc}	1.97^{c}	1.80^{c}	1.22
Unhulled	3.82^{b}	2.87^{bc}	2.95^{bc}	2.82^{bc}	
8. General acceptability of soymilk formulated with 20% fresh milk					
Hulled	5.02^{a}	2.22^{c}	2.10^{c}	2.13^{c}	0.96
Unhulled	4.24^{a}	3.23^{b}	2.46^{bc}	2.57^{bc}	

Note: Means superscripted by the same letter are not significantly different at 5% level.

general acceptability reached the minimum value at 48 hours fermentation, the amino acid nitrogen and viscosity reached the maximum value of 1.52 mg/ml and 15.11 centipoise, respectively, while pH dropped to a minimum value of 6.26. As mentioned earlier the amino acid nitrogen component is one among other factors responsible for the bitter taste.

The bitter taste appears when the soybean is fermented due to the release of protease-liberating peptides or amino acids (Murray and Baker, 1952). The nature of the protease (Pelissier and Manchon, 1976), ethyl -α-D-galactopyranoside (Honig et al., 1971), soybean phospholipids, lipid oxidation (Sessa et al., 1949), aldehyde and ketones compound also contributed to the bitter taste.

Fresh milk and Philsoy have been shown to have a viscosity of 12.0 centipoise. Lower or higher value was shown to affect the general acceptability.

Bourne et al. (1976) mentioned that pH of soymilk adjusted to near neutrality showed greater acceptability. In this experiment the pH of soymilk reached the minimum value 6.26. Thus, the general acceptability decreased.

The relationship between fermentation time and the general acceptability of soymilk formulated with 20% fresh milk and 20 ppm vanilla of unhulled soybeans appears linear, as described by the equation: $Y = 3.9975 - 0.0242X$ with $r^2 = 0.3537$ and $t = -2.3395$, and $Y = 3.55 - 0.0121X$ with $r^2 = 0.1199$ and $t = -1.1671$, respectively (Fig. 7).

The amino acid nitrogen of unhulled soybeans increased as the fermentation time increases with values of 1.01 mg/ml initially to 1.80 mg/ml after 72 hours. This is related to the decrease in general acceptability as fermentation proceeds due to the bitter taste produced.

When fermentation as pretreatment is used for production of soymilk, the color of the soymilk changes from slightly brown to brown as the fermentation proceeds; also, the bitter taste would appear with fermentation.

Change in color or formation of the brown color in soymilk is a result of a non-enzymatic (Maillard) reaction. As mentioned earlier, during fermentation the amino acid nitrogen increased and the formulation of soymilk requires 7% sugar, both of these factors

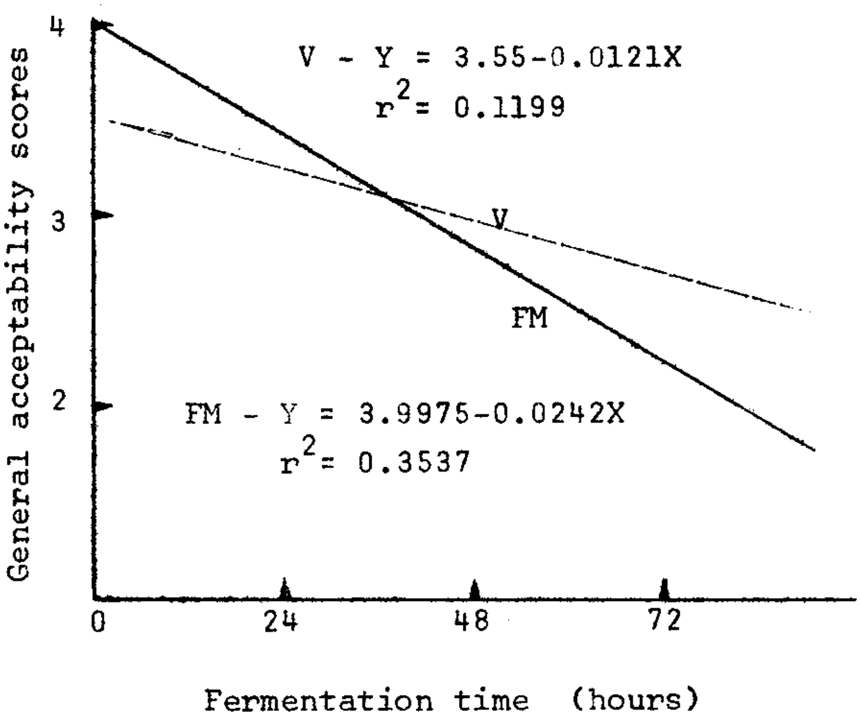


Figure 7. Relationship between fermentation time and general acceptability of soymilk from unhulled (UH) soybeans formulated with 20% fresh milk (FM) and 20 ppm vanilla (V).

with the addition of heat stimulate the "Maillard" reaction. These changes lead to a decrease in nutritional value (Smith et al., 1964; Hackler et al., 1967).

The bitter taste appears when soybean is fermented. This observation is most probably due to the protease-liberating peptides or amino acids. Murray and Baker (1952) mentioned that enzymic casein hydrolysates possessed a bitter taste of great intensity. Reed (1966) mentioned that the bitterness is due to an intermediate product of protein hydrolysis and the degree of hydrolysis has an estimate of the degree of bitterness.

Further explanation about the bitter taste is discussed by Felissier and Manchon (1976) saying that αS1-casein hydrolysates were more bitter than β casein hydrolysates and the nature of the protease utilized has a real influence on the development of bitterness.

Another components that contribute to the bitter taste are soybean phospholipids and lipid oxidation products (Sessa et al., 1969); aldehydes and ketones have been implicated to produce upleasant flavor in soymilk.

Interactions

The analysis of variance for the total protein content, water soluble protein, amino acid nitrogen, viscosity, pH of soymilk before and after processing and sensory evaluation of soymilk formulated with 20% fresh milk indicates that the interactions among the factors such as hulling soybeans, various cooking times and various fermentation times show high significant differences.

Table 6 shows the chemical and sensory characteristics of soymilk from hulled or unhulled soybeans and different fermentation times.

The chemical and sensory evaluation characteristics of soymilk treated with different cooking times and different fermentation times are broughtout in Table 7.

The mean values of total protein content of soymilk determined from hulled or unhulled soybeans under different fermentation times are presented in Table 8.

Hulled soybeans without cooking and fermentation for 72 hours had the highest total protein content, followed by the protein content of unhulled soybean without cooking but fermented for 72 hours.

Table 7. Chemical and sensory characteristics of soymilk from soybeans subjected to different cooking times and different fermentation times

Characteristics		Fermentation times (hours) 0	24	48	72	HSD 0.05
1. Total protein content (%)						
Cooking time (mins.)	0	2.61^{b}	2.93^{a}	2.89^{a}	2.61^{a}	
	10	1.76^{f}	1.61^{g}	1.47^{g}	2.10^{g}	0.15
	30	2.10^{e}	1.61^{de}	1.57^{c}	1.47^{cd}	
2. Water soluble protein (%)						
Cooking time (mins.)	0	2.32^{b}	2.62^{a}	2.17^{b}	2.57^{a}	
	10	0.82^{f}	0.99^{ef}	1.09^{de}	1.04^{def}	0.23
	30	1.10^{de}	1.24^{d}	1.59^{c}	1.47^{c}	
3. Amino acid nitrogen (mg/ml)						
Cooking time (mins.)	0	0.78^{f}	1.53^{c}	1.76^{a}	1.64^{b}	
	10	0.97^{e}	1.35^{d}	1.65^{b}	1.63^{bc}	0.10
	30	1.32^{d}	1.66^{ab}	1.54^{c}	1.63^{bc}	
4. Viscosity (cp)						
Cooking time (mins.)	0	16.82^{ab}	18.00^{ab}	17.67^{ab}	13.20^{cd}	
	10	12.93^{cd}	11.82^{d}	13.47^{cd}	12.38^{d}	3.01
	30	17.17^{ab}	18.98^{a}	18.96^{a}	15.69^{bc}	
5. pH before processing						
Cooking time (mins.)	0	6.78^{ab}	6.78^{ab}	6.56^{b}	6.95^{a}	
	10	6.51^{b}	6.64^{b}	6.68^{ab}	6.78^{ab}	0.30
	30	6.81^{ab}	6.71^{ab}	6.68^{ab}	6.72^{ab}	
6. pH after processing						
Cooking time (mins.)	0	6.50^{bc}	6.50^{bc}	6.33^{c}	6.53^{abc}	
	10	6.38^{bc}	6.50^{bc}	6.55^{abc}	6.76^{a}	0.25
	30	6.62^{ab}	6.58^{ab}	6.53^{abc}	6.53^{abc}	
7. General acceptability of soymilk formulated with 20 ppm vanilla	0) 10) 30)	not significantly different				
8. General acceptability of soymilk formulated with 20% fresh milk	0	4.40^{ab}	2.71^{ed}	2.97^{cd}	2.95^{cd}	
	10	4.99^{a}	3.33^{bc}	2.10^{cd}	2.32^{cd}	1.27
	30	4.51^{ab}	2.15^{cd}	1.77^{d}	1.79^{d}	

Note: Means superscripted by the same letter are not significantly different at 5% level.

Table 8. Mean values of total protein content of soymilk (6.25 x % N).

	Cooking times (minutes)	Fermentation times (hours) 0	24	48	72
	0	2.57[ed]	2.87[ab]	2.93[a]	3.05[a]
Hulled	10	1.86[hij]	1.21[k]	1.29[k]	1.27[k]
	30	2.19[fg]	1.84[ij]	2.33[ef]	2.09[gh]
	0	2.65[bc]	2.99[a]	2.83[ab]	2.96[a]
Unhulled	10	1.66[j]	2.01[ghi]	1.64[j]	1.86[hij]
	30	2.02[ghi]	2.49[cde]	2.36[def]	2.46[cde]

HSD (0.05) = 0.24

Note: Means superscripted by the same letter are not significantly different at 5% level.

Unhulled soybeans without cooking but subjecting it to fermentation for 24 hours yielded also the best total protein content.

The mean values of water soluble protein content of soymilk produced from hulled or unhulled soybeans under different cooking and fermentation times are presented in Table 9.

Hulled soybeans without cooking, fermented for 24 hours yielded the highest water soluble protein content followed by hulled and unhulled soybeans without cooking but fermented for 72 hours, and then followed by unhulled soybeans without cooking and fermented for 24 hours.

The mean values for the amino acid nitrogen of soymilk in hulled or unhulled soybeans subjected to different cooking and fermentation times are brought in Table 10.

Unhulled soybeans cooked for 30 minutes and allowed to ferment for 72 hours yielded the highest amino acid nitrogen followed by the same treatment except that fermentation time was shortened to 48 hours.

The other treatments on unhulled soybeans such as cooking for 10 minutes and fermentation for 72

Table 9. Mean values of water soluble protein content of soymilk (6.25 x % N).

	Cooking times (minutes)	Fermentation times (hours) 0	24	48	72
	0	2.30^{bcd}	2.70^{a}	2.19^{cde}	2.57^{ab}
Hulled	10	0.84^{k}	0.97^{jk}	1.23^{hij}	0.97^{jk}
	30	1.63^{ijk}	1.12^{ijk}	1.55^{fgh}	1.01^{ijk}
	0	2.34^{abcd}	2.53^{abcj}	2.14^{de}	2.56^{ab}
Unhulled	10	0.81^{k}	1.02^{ik}	0.96^{jk}	1.11^{ijk}
	30	1.03^{ijk}	1.36^{ghi}	1.63^{fg}	1.84^{ef}

HSD (0.05) = 0.37

Note: Means superscripted by the same letter are not significantly different at 5% level.

Table 10. Mean values of total amino acid nitrogen of soymilk (mg/ml).

	Cooking times (minutes)	Fermentation times (hours) 0	24	48	72
Hulled	0	0.74^{k}	1.51^{fgh}	1.74^{bcd}	1.69^{cde}
	10	1.14^{j}	1.35^{hi}	1.65^{cdef}	1.36^{hi}
	30	1.26^{ij}	1.66^{cdef}	1.16^{j}	1.34^{i}
Unhulled	0	0.83^{k}	1.54^{efg}	1.78^{abc}	1.60^{def}
	10	0.81^{k}	1.34^{i}	1.65^{cdef}	1.90^{ab}
	30	1.39^{ghi}	1.66^{cdef}	1.91^{a}	1.92^{a}

HSD (0.05) = 0.17

Note: Means superscripted by the same letter are not significantly different at 5% level hours and without cooking but subjecting soybeans to fermentation for 48 hours resulted in lower value than the first two discussed earlier.

The most favorable treatment was 30 minutes cooking and fermentation for 72 hours on unhulled soybeans.

The mean values on the viscosity of soymilk produced under various treatments of either hulled or unhulled soybeans at different cooking and fermentation times are given in Table 11.

Unhulled soybeans cooked for 30 minutes and fermented for 24 hours had the highest viscosity reading followed by cooking for 30 minutes and without fermentation on the same materials.

However, production of the highest viscosity of soymilk is not always indicative of the best treatment. The analysis of fresh milk and Philsoy showed viscosity reading of 12.00 centipoise. Treatment combinations involving unhulled soybeans, cooked for either 10 or 30 minutes and fermented for 24 hours and hulled soybeans subjected to cooking for 10 minutes and fermentation for 48 hours resulted in most suitable viscosity comparable to the viscosity of fresh milk and Philsoy.

Table 11. Mean values of viscosity of soymilk (centipoise).

	Cooking times (minutes)	Fermentation times (hours)			
		0	24	48	72
Hulled	0	14.03 efgh	16.09 cdefg	15.33 cdefgh	11.33 gh
	10	12.71 gh	11.64 gh	12.00 gh	10.97 h
	30	14.04 efgh	12.00 gh	18.00 bcdef	12.71 h
Unhulled	0	19.60 bcd	19.91 bc	20.00 bc	15.07 defgh
	10	13.14 gh	12.00 gh	14.93 defgh	13.77 gh
	30	20.30 b	25.96 a	19.91 bc	18.67 bcde

HSD (0.05) = 4.79

Note: Mean superscripted by the same letter are not significantly different at 5% level.

The mean values of the pH of soymilk before and after processing of either hulled or unhulled soybeans at different cooking and fermentation times are presented in Tables 12 and 13.

Subjecting unhulled soybeans to cooking for 30 minutes and fermentation for 72 hours produced the most suitable pH of soymilk followed by treatment of hulled soybeans with cooking for 10 minutes and fermentation for 72 hours and treatment of hulled soybeans with cooking for 30 minutes and fermentation for 48 hours. Treatment of unhulled soybeans with cooking for 10 minutes and fermentation for 48 hours produced the 4th most suitable soymilk product.

The mean values of general acceptability of soymilk formulated with 20% fresh milk derived from hulled or unhulled soybeans subjected to different cooking and fermentation times are brought out in Table 14.

Hulled soybeans subjected to cooking for 30 minutes without fermentation produced the highest general acceptability of soymilk formulated with 20% fresh milk.

The other treatments such as 10 minutes cooking without fermentation on unhulled soybeans and no

Table 12. Mean values of pH of soymilk before processing.

	Cooking times (minutes)	Fermentation times (hours) 0	24	48	72
Hulled	0	6.78 bcdefgh	7.07 ab	6.50 cdefghi	6.79 bcdefg
	10	6.67 bdefgh	6.98 abc	6.35 fghi	6.83 bcdef
	30	6.85 abcde	6.51 cdefghi	6.41 efghi	6.10 i
Unhulled	0	6.78 bcdefgh	6.49 defghi	6.62 abcdefgh	7.10 ab
	10	6.35 cghi	6.30 hi	7.00 b	6.73 bdefgh
	30	6.76 bcdefgh	6.83 bcdefg	6.94 abcd	7.33 a

HSD (0.05) = 0.48

Note: Means superscripted by the same letter are not significantly different at 5% level.

Table 13. Mean values of pH of soymilk after processing.

	Cooking times (minutes)	Fermentation times (hours) 0	24	48	72
	0	6.52^{bcde}	6.75^{bc}	6.22^{ef}	6.60^{bcde}
Hulled	10	6.55^{bcde}	6.79^{abc}	6.30^{def}	6.85^{ab}
	30	6.71^{bc}	6.50^{bcde}	6.27^{def}	5.91^{f}
	0	6.48^{bcde}	6.25^{ef}	6.43^{cde}	6.45^{cde}
Unhulled	10	6.20^{ef}	6.20^{ef}	6.80^{abc}	6.67^{bcd}
	30	6.52^{bcde}	6.67^{bcd}	6.80^{abc}	7.16^{a}

HSD (0.05) = 0.40

Note: Means superscripted by the same letter are not significantly different at 5% level.

Table 14. Mean values of general acceptability of soymilk formulated with 20% fresh milk.

	Cooking times (minutes)	Fermentation times (hours)			
		0	24	48	72
Hulled	0	4.72^{ab}	2.53^{cde}	2.78^{bcde}	2.40^{cde}
	10	4.38^{abc}	2.28^{de}	1.64^{e}	2.03^{e}
	30	5.96^{a}	1.86^{e}	1.88^{e}	1.97^{e}
Unhulled	0	4.09^{abcd}	2.89^{bcde}	3.16^{bcde}	3.50^{bcde}
	10	5.59^{a}	4.37^{abc}	2.57^{cde}	2.60^{cde}
	30	3.05^{bcde}	2.44^{cde}	1.67^{e}	1.60^{e}

HSD (0.05) = 2.02

Note: Means superscripted by the same letter are not significantly different at 5% level.

cooking and no fermentation of hulled soybeans resulted comparably acceptable soymilk. For economic reason, the no cooking, no fermentation treatment seemed preferable.

CONCLUSION

This investigation shows that pretreatments of soybean prior to soymilk production results in the following advantages and disadvantage. The advantage that occured to various treatments are as follows:

Hulled soybeans without cooking and subjected to fermentation for 24, 48, and 72 hours resulted in the increase of total protein content from 2.87%, 2.92%, and 3.05%, respectively.

Hulled soybeans without cooking and fermented for 24 hours resulted in the highest value of water soluble protein of 2.70%.

Likewise hulled soybeans without cooking and subjected to fermentation for 24, 48, and 72 hours resulted in soymilk with high amino acid nitrogen with value of 1.51, 1.74, and 1.69 mg/ml., respectively.

It is noted that high values for the total protein content, water soluble protein and the amino acid nitrogen, will improve the digestibility and protein efficiency ratio. However, the determination of digestibility and protein efficiency ratio was not included in this study.

Hulled soybeans cooked for 30 minutes but without fermentation yielded soymilk which when formulated with 20% fresh milk had moderate acceptability. However, unhulled soybeans receiving cooking treatment for 10 minutes without fermentation resulted in soymilk, which when formulated with 20% fresh milk would have moderate acceptability.

Pretreatment of soybeans becomes disadvantageous to soymilk production when the treatment results in the production of bitter taste factor which necessarily lowers general acceptability.

GLOSSARY

1. BONGKREK. An Indonesian fermented food product made from coconut press cake and fermented with Rhizopus sp.

2. FU CHANG (Soybean Milk). When prepared by the Oriental technique, it is a suspension of ground soybeans in water in that soaked soybeans are finely ground and mixed with water and the resultant mass poured into cheese cloth through which the liquid phase passes and is recovered as the milk; in the western world and other areas where it is becoming more widely used as food for infants that are suffering from malnutrition and for individuals afflicted with certain allergies, diabetes and other diseases associated with diet, soymilk is made as a dispersion of soy flour or soy protein isolate in water with added vitamins, minerals, carbohydrates, and in many instances, a flavoring compound.

3. FULL FAT GRITS. The screened, graded product obtained from high quality, sound, clean, dehulled soybeans, usually ground to conform granulations in terms of majority percent through standard screens.

4. MISO. Made from soaked, steam-heated soybeans which are inoculated with cultures of micro-organisms grown on rice or barley and then allowed to ferment; one method of fermentation is called the "natural brewing process" in which the soybeans are allowed to ferment for approximately 9 months; the other method is known as "the quick brewing process" and the material is produced in a short time by reducing the length of time for processing (heating) the soybeans and the fermentation; a sweet type or a salty type may be produced; used as a soup which is mixed with tofu or vegetables or seaweed, for preserving fish, meat and vegetables and for various kinds of Japanese-style cooking with vegetables, meat or fish.

5. NATTO. A fermented soy product produced in Japan in essentially the same manner as tempeh, except that a different organism is used to bring about the desired effect on the soy protein (*Bacillus natto*).

6. ONTJOM. Indonesian fermented food product from peanut press cake and fermented with *Neurospora sitophila*.

7. PANCREATIC HYPERTROPHY. A kind of disease that shows increase in pancreas size when fed with soybean contain trypsin inhibitor.

8. PHILSOY. The Philippines soymilk made by the Department of Food Science and Technology University of the Philippines. The process involves soaked the soybeans 8-12 hours, grind using hot water filtered formulated with 7% sugar, 20 ppm vanilla and sterilized.

9. SOY SAUCE (Sho Yu). A hydrolysate soybeans protein mixed with wheat flour, resulting from the action of molds, yeasts and bacteria as prepared by the Oriental method; the fermentation or enzymatic action is permitted to progress for up to 1 1/2 years, at which time the extract is heated and processed to produce the liquid for edible uses; may also be prepared by chemical (acid) hydrolysis of soybean protein; used as a seasoning in the preparation of foods and as a table condiment.

10. TEMPEH. A fermented soybeans food product developed in Indonesia, in which soybeans are soaked overnight and then cooked for a short time; the cooked soybeans are inoculated with the fungus <u>Rhizopus sp</u>. and allowed to ferment

FOR 18 to 48 hours to permit optimum growth of the mycelium of the organism; the product is roasted, cooked in soup or fried in oil; may also be sliced and dried.

11. TOFU. A cheese-like product made from soymilk; the protein is precipitated by the use of calcium sulfate or a comparable coagulating agent and then placed into molding boxes or forms and allowed to cool; when the curd has properly cooled, it is cut into approximate sized portions for cooking; may be eaten seasoned with shoyu, put into miso soup or cooked with miso, vegetables, fish and meat; also used in western-style cooking as a component of omelets, croquettes, soups, stews or in the fried form; a method for making soft tofu has been recently introduced, a process for which American-grown soybeans are especially well suited.

12. YUBA. The name given to the protein film that forms on the surface of soymilk when it is heated nearly to the boiling point; the film is removed and dried, requiring soaking in water before it is used as food; eaten in the cooked form with appropriate seasoning.

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APPENDICES

APPENDIX A

APPENDIX A

Method of Chemical Analysis

1. Total Nitrogen

Transfer 1 ml. of soymilk to the digestion flask. Add 1.9 $\pm$ 0.1 g. K_2SO_4, 50 $\pm$ 10 mg. HgO and 2.0 $\pm$ 0.1 ml H_2SO_4. Add boiling chips which pass through the No. 10 sieve. Digest $\pm$ 1 hour, cool and add the minimum volume of distilled water to dissolve solids.

Transfer digesting and boiling chips to distillation apparatus and rinse 5 to 6 times with distilled water. Place 125 ml. Erlenmeyer flask containing 10 ml. standard H_3BO_4 (4%) solution and 2-4 drops of the incator, methyl red-methylcne blue under the condensor with tip extending below the surface solution. Add 8-10 ml. NaOH-Na2S2O3 solution. Collect 50 ml. distillates and dilute to 75 ml. Titrate with − 0.01N HCl to the grey end point or first appearance of violet. Make blank determination.

$$\%N = \frac{(\text{mlHCl}-\text{ml blank}) \times \text{Normality} \times 14.007 \times 100}{\text{mg sample}}$$

2. pH

Standardize the pH meter with buffer pH 4.0 and 7.0. Place the soymilk sample in a 125 ml beaker and measure exactly using Zeromatic Beckman pH meter.

3. Viscosity

The soymilk viscosity is measured by using Brookfield Viscometer model RVF 100.

Place 250 ml of soymilk into a 300 ml beaker, using a No. 2 spindle and speed of 50 rpm. Let the spindle rotate a minimum of 10 times until the needle is stable and can be read. The viscosity will be multipled by the correction factor 8.0.

4. Water Soluble Protein

To determine the water soluble protein, 250 ml. soymilk were placed in the centrifuge for 15 minutes with 1200 rpm. The solution was filtered by using a Buckner funnel with asbestos pad. The filtrate will be used for determination of the water soluble protein and the amino acid nitrogen. Total water soluble protein is determined by using the method in 1.

5. Amino Acid Nitrogen

Amino acid nitrogen is measured using the Van Slyke apparatus. Measure 2.96 ml. acetic acid glacial and 11.8 ml. $NaNO_3$, mix together and shake. Fill 2 ml soymilk in another tube. Let 1 ml. of soymilk combine with the acetic acid glacial and $NaNO_3$. Shake for 10-minute at 100 rpm. After mixing the gas N2 is produced and mixed with $KMNO_4$ and then shaken for 1 minute at 100 rpm. Read the volume of nitrogen gas. Convert the volume of gas nitrogen produce by using the Table edited by Department of Agricultural Chemistry Kyoto University. During the analysis the temperature and atmospheric pressure should be constant.

APPENDIX B

Appendix Table 1. Amino acid composition of soybean protein

Amino Acid	Wt. % A.A/Total Protein
Arginine	7.0
Histidine	2.5
Lysine	6.6
Tyrosine	3.2
Tryptophan	1.2
Phenylalanine	4.8
Cystine	1.2
Methionine	1.1
Serine	5.6
Threonine	3.9
Leucine	7.6
Isoleucine	5.8
Valine	5.2
Glutamic acid	18.5
Aspartic acid	8.3
Glycine	3.8
Alanine	4.5
Proline	5.4

*Block R-J and R. W. Weiss, (1956).

Appendix Table 2. Amino acid compositions (g/16 g/N) in nutritive indexes of processed and fermented soybeans and fermentative mold*

Item	Soybean oil meal	Raw soybeans	Length of soybean fermentation (hrs)					*Rh. oligosporus*
			0	12	24	36	72	
Lysine	6.24	6.08	5.92	5.88	5.79	5.51	5.54	4.07
Histidine	2.63	2.50	2.53	2.46	2.47	2.27	2.46	1.52
Arginine	7.53	7.13	7.07	6.73	6.57	6.14	6.17	2.47
Aspartic	11.00	11.30	11.10	10.80	10.60	10.60	10.50	4.82
Threonine	3.71	3.76	3.82	3.76	3.73	3.71	3.62	2.56
Serine	5.86	5.67	5.92	5.80	5.55	5.81	5.55	2.95
Glutamic	17.60	16.90	17.10	16.40	15.70	16.10	15.80	4.72
Proline	5.03	4.86	5.10	4.87	4.80	4.54	4.52	1.96
Glycine	4.11	4.01	4.07	3.97	3.91	3.92	3.86	2.68
Alanine	4.23	4.23	4.30	4.20	4.27	4.34	4.29	2.77
Valine	4.43	4.59	4.81	4.58	4.51	4.48	4.43	3.41
Cystine	1.61	1.70	1.70	1.61	1.56	1.61	1.55	1.03
Methionine	1.15	1.22	1.30	1.35	1.34	1.28	1.25	0.89
Isoleucine	4.47	4.62	4.83	4.78	4.65	4.65	4.59	3.22
Leucine	7.64	7.72	8.07	7.98	7.69	7.78	7.65	4.61
Tyrosine	3.38	3.39	3.50	3.56	3.63	3.58	3.48	1.85
Phenylalanine	4.78	4.84	5.02	4.96	4.96	4.92	5.06	2.89
Tryptophane	1.23	1.24	1.03	1.03	1.32	1.29	1.23	0.50
Ammonia	1.86	1.85	1.87	2.00	2.22	2.04	2.68	4.37
Glucosamine	0.22	0.19	0.20	0.39	0.65	0.89	1.23	2.73
E. A. A. I.	73.30	74.40	74.40	73.60	74.60	73.50	72.80	46.20
Req. Index	83.00	84.00	84.40	83.30	83.20	81.80	81.90	53.90

*Stilling and Hackler (1965)

Appendix Table 3. Effect of processing of soy milk at 93°C on amino acid composition.*

	Minutes				
	15	30	60	120	240
	g/16g,N	g/16g,N	g/16g,N	g/16g,N	g/16g,N
Lysine	6.20	6.25	6.20	6.20	6.01
Histidine	2.54	2.59	2.57	2.51	2.53
Arginine	7.77	7.81	7.90	7.66	7.64
Aspartic acid	11.80	11.80	12.00	11.90	11.60
Threonine	3.84	3.87	3.95	3.85	3.79
Serine	5.22	5.30	5.27	5.23	5.32
Glut. acid	18.70	19.00	18.80	18.60	18.20
Proline	5.00	5.01	4.96	5.11	5.08
Glycine	4.23	4.25	4.28	4.24	4.16
Alanine	4.40	4.41	4.44	4.43	4.32
Valine	4.85	4.85	4.95	4.80	4.86
Cystine	1.70	1.72	1.74	1.76	1.70
Methionine	1.39	1.39	1.38	1.38	1.45
Isoleucine	5.10	5.10	5.12	5.14	5.07
Leucine	8.25	8.29	8.28	8.18	8.14
Tyrosine	3.86	3.95	3.94	3.90	3.81
Phenylalanine	5.17	5.13	5.11	5.11	5.08
Tryptophane	1.33	1.44	1.47	1.40	1.32
E.A.A.I	77.80	78.70	78.90	78.20	77.30
R. I.	86.60	86.70	86.90	86.70	86.70

*Hackler and Stilling (1967)

Appendix Table 4. Effect of processing soy milk at 121 on amino acid composition.*

	Minutes						
	0	5		20	40	60	120
	g/16g.N						
Lysine	5.45	5.60	5.54	5.46	5.60	5.56	5.47
Histidine	2.44	2.45	2.44	2.40	2.47	2.37	2.40
Arginine	7.20	7.10	7.14	7.12	7.17	7.23	2.40
Aspartic acid	11.00	10.80	10.70	10.80	11.20	11.10	11.00
Threonine	3.56	3.48	3.50	3.61	3.55	3.57	3.62
Serine	4.84	4.82	4.80	4.83	4.88	4.72	4.72
Glu. acid	16.70	16.80	17.00	17.00	17.10	16.70	10.40
Proline	4.87	4.93	5.03	5.00	4.82	4.85	4.82
Glycine	4.00	4.02	3.99	4.04	4.05	3.91	3.99
Alanine	4.12	4.15	4.19	4.25	4.29	4.16	4.26
Cystine	1.70	1.57	1.62	1.62	1.44	1.28	1.17
Methionine	1.46	1.44	1.48	1.45	1.44	1.47	1.45
Isoleucine	4.87	4.86	4.88	4.85	4.89	4.84	4.89
Leucine	8.10	8.08	8.08	8.05	8.01	7.80	8.02
Tyrosine	3.85	3.89	3.88	3.84	3.86	3.86	3.88
Phenylalanine	5.22	5.10	5.24	5.22	5.16	5.06	5.23
Tryptophane		1.46	1.48	1.42	1.45	1.26	1.28
E.A.A.I	76.90	76.10	76.50	76.30	75.30	73.50	73.50
R.I.	85.20	84.50	85.10	84.80	83.20	83.70	83.00

*Hackler and Stilling (1967)

Appendix Table 5. Analysis of variance for total protein content

Source of variation	Degrees of Freedom	Sum of squares	Mean squares	F-values
Factor A	1	0.74	0.74	128.84**
Factor B	2	18.94	9.47	1657.39**
Interaction AB	2	0.45	0.22	39.27**
Factor C	3	0.14	0.05	8.02**
Interaction AC	3	0.95	0.32	55.23**
Interaction BC	6	0.87	0.14	25.35**
Interaction ABC	6	0.54	0.09	15.87**
Error	48	0.27	0.01	
Total	71	22.89		

C.V. = 3.39%

** = Significant at 1% level

Appendix Table 6. Analysis of variance for water soluble protein

Source of variation	Degrees of Freedom	Sum of squares	Mean squares	F-values
Factor A	1	0.50	0.05	3.62^{ns}
Factor B	2	26.50	13.25	965.45**
Interaction AB	2	0.30	0.15	10.97**
Factor C	3	0.78	0.26	18.85**
Interaction AC	3	0.38	0.13	9.16**
Interaction AB	6	1.17	0.20	14.26**
Interaction ABC	6	0.42	0.07	5.12**
Error	48	0.66	0.01	
Total	71	30.26		

C.V. = 7.39%

ns = Not significant

** = Significant at 1% level

Appendix Table 7. Analysis of variable for amino acid nitrogen content

Source of variation	Degrees of freedom	Sum of squares	Means squares	F-values
Factor A	1	0.38	0.38	135.36**
Factor B	2	0.26	0.13	46.35**
Interaction AB	2	0.43	0.22	76.93**
Factor C	3	4.61	1.54	549.32**
Interaction AC	3	0.47	0.16	56.06**
Interaction BC	6	1.09	0.18	65.17**
Interaction ABC	6	0.72	0.12	42.70**
Error	48	0.13	0.00	42.70**
Total	71	8.09		

C.V. = 3.63%

** = Significant at 1% level

Appendix Table 9. Analysis of variance for pH of soymilk before processing

Source of variation	Degrees of Freedom	Sum of squares	Mean squares	F-values
Factor A	1	0.21	0.21	9.03**
Factor B	2	0.16	0.08	3.46*
Interaction AB	2	1.24	0.62	26.22**
Factor C	3	0.30	0.10	4.23**
Interaction AC	3	2.28	0.76	32.20**
Interaction BC	6	0.45	0.07	3.17*
Interaction ABC	6	1.22	0.20	8.61**
Error	48	1.13	0.02	
Total	71	7.00		

C.V. = 2.29%

* = Significant at 5% level

** = Significant at 1% level

Appendix Table 10. Analysis of variance for pH of soymilk after processing

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-values
Factor A	1	0.06	0.06	3.38^{ns}
Factor B	2	0.15	0.07	4.48**
Interaction AB	2	1.33	0.67	40.83**
Factor C	3	0.19	0.06	3.85**
Interaction AC	3	1.73	0.58	35.41**
Interaction BC	6	0.46	0.08	4.66**
Interaction ABC	6	1.35	0.22	13.79**
Error	48	0.78	0.02	
Total	71	6.04		

C.V. = 1.96%

ns = Not significant

** = Significant at 1% level

Appendix Table 11. Analysis of variance for sensory evaluation scores of soymilk formulated with 20 ppm vanilla

Source of variation	Degrees of Freedom	Sum of squares	Mean squares	F-values
Factor A	1	0.09	0.09	0.13ns
Factor B	2	6.38	3.19	4.78*
Interaction AB	2	10.75	5.38	8.06**
Factor C	3	64.68	21.56	32.31**
Interaction AC	3	22.38	7.46	11.18**
Interaction BC	6	5.28	0.88	1.32ns
Interaction ABC	6	6.82	1.14	1.71ns
Error	48	32.03	0.67	
Total	71	148.40		

C.V. = 26.53%

ns = Not significant

* = Significant at 5% level

** = Significant at 1% level

Appendix Table 12. Analysis of variance or sensory evaluation scores of soymilk formulated with 20% fresh milk

Source of variation	Degrees of Freedom	Sum of squares	Mean squares	F-values
Factor A	1	1.20	1.20	2.91ns
Factor B	2	7.14	3.57	8.67**
Interaction AB	2	11.16	5.76	13.54**
Factor C	3	66.18	22.06	53.57**
Interaction AC	3	7.51	2.51	6.10**
Interaction BC	6	6.82	1.14	2.76*
Interaction ABC	6	6.92	1.15	2.80*
Error	48	19.77	0.41	
Total	71	126.72		

C.V. = 21.40%

ns = Not significant

* = Significant at 5% level

** = Significant at 1% level

Appendix Table 13. Processing losses for two methods of tempeh production*

Materials and Procedure	Losses of solid		Losses of nitrogen		Protein content (N X 6.25)[a]
	Harosoy %	Hawkeye %	Harosoy %	Hawkeye %	Hawkeye %
Whole Soybeans					43.0
Full fat meal					47.5
Dehulling and soaking[b]	12.6	9.5	8.0	3.9	-
Cooking	11.0	14.0	10.0	3.0	51.7
Fermentation	3.4	1.0	1.7	0.8	53.1
Total Loss	27.0	24.5	19.7	7.7	-
Dehulled Chips					44.7[c]
Soaking and Cooking[d]	38.0	-	31.4	-	48.6
Fermentation	5.0	-	2.0	-	50.0
Total Loss	43.0	-	33.4	-	-

*Smith et al. (1964)

[a]Dry basis

[b]Hulls separated by hand from both varieties of soybeans represent 7.9% of the whole bean and contain 1.87% nitrogen. Hulls separated mechanically account for 9.5% of the bean and contain 2% nitrogen.

[c]Harosoy variety

[d]Soaking losses are about 8%.