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DEVELOPMENT OF SOY-BASED FOODS OF HIGH NUTRITIVE VALUE FOR USE IN THE PHILIPPINES

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Cornell University
New York State Agricultural Experiment Station
Department of Food Science and Technology
Geneva, New York

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Final Report

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SUMMARY

With the ultimate goal of producing soy based foods of high nutritive value for use in the Philippines, research was carried out along several approaches: (1) Development of a simple procedure for manufacture of a bland soy beverage and other products that could be easily produced without the use of elaborate and expensive equipment; (2) Introduction of the soy beverage to selected groups of Philippine school children; (3) Engineering studies on unit operations in soy product manufacturing, (4) Nutritional evaluation of the products and the nutritional effects of the unit processes involved, (5) Basic laboratory research on physical and chemical qualities of soy products and components to lay a scientific basis for improvement of the products and unit processes involved in their manufacture.

Soy Product Development

A satisfactory soy beverage process was developed to the pilot plant stage using a boiling water grind preceded by soaking the beans in dilute alkali. This extraction procedure resulted in a bland product with less "beany" flavor than is encountered in previously described procedures. Further pilot plant studies involved the concentration and spray drying of the product. These processes might appeal to large food processing companies.

Development of other products is described in less detail: Soy-coconut milk blends, soybean curd (Philippine tokau), tempeh (a mold-fermented product), a soy-coconut candy, and roasted soybeans.

Soy Beverage Acceptability

Soy milks were found to be generally liked by Philippine children, when the beverages were sweetened with sugar and vanilla flavored. Coconut blends and chocolate flavoring also found acceptance, and are valuable in masking any residual soy flavor.

Variety tests showed 14 of 16 soybean varieties tended to give a satisfactory beverage flavor.

Nutritional Studies

Since soybeans are high in lysine compared to cereal grains, and the cereal grains are higher in sulfur-containing amino acids, a combination of soy and cereals give a complementary nutritional effect of genuine value to the Philippine diet.

Methionine supplementation is suggested as a means of increasing the protein quality of the products.

Chemical and Physical Investigations

Ethyl vinyl ketone was identified as the principle compound responsible for the raw, green bean odor, generally associated with soy beverages. Experimental evidence suggests that the compound is formed by enzyme action during aqueous extraction of soybeans. The use of boiling water, introduced during this investigation, prevents its formation. Hexanal is another major component of the disagreeable aroma, and is oxidized to hexanoic acid, a harsh, fetid-smelling compound found in defatted soy flour.

With a view toward eventual development of a reconstitutable powdered soy product for beverage purposes, considerable attention was given to redispersibility of dried soy proteins. In order to obtain basic knowledge of this functional property, various physical and chemical processing factors were investigated, including temperature and time of heating before drying, the effect of chemical disulfide-splitting agents, and the effect of emulsifier and chelating agent additions.

Extensive studies on the addition of sugars before drying showed that the sweetened milks showed much improved redispersibility. No differences in effect were observed in comparing glucose, fructose, galactose, sucrose, and maltose. Lactose, perhaps because of its lower solubility, was not so effective as the other sugars.

Enzyme studies showed the possibility of producing an oligosaccharide-free soy beverage. This may be of practical significance if allegations concerning these sugars as flatulence factors are true.

INTRODUCTION

Protein deficiency is the most prevalent and serious nutritional problem in developing countries, and the Philippines is no exception. The primary need is for protein supplementation and the most obvious potential sources of inexpensive protein are of plant origin. Soybeans have a greater potential than any other plant material for the production of protein-rich foods. Soybeans grow in a wide variety of climates and their high yields result in a low-cost protein. Experimental trials of soybeans grown at the University of the Philippines College of Agriculture have been promising, showing excellent yields and climatic adaptability.

The research program described in this report was undertaken to develop processes for manufacturing soy-based foods on a scale and of a simplicity appropriate to the socio-economic requirements of relatively small population centers in countries such as the Philippines. Flavor, acceptability, economy, and nutritional value were of primary concern.

A major purpose of the contract was to establish a pilot plant at the College of Agriculture of the University of the Philippines, where Philippine personnel could be trained in food research and manufacturing.

RESULTS IN THE PHILIPPINES

Personnel

Cornell University supplied two visiting professors of food science to serve as project directors during the duration of the project. During this period, Cornell also supplied two short term consultants. Also, the Department of Food Science and Technology, Cornell University, Geneva, New York, faculty supplied continuing basic research and advice to their Philippine representatives and their Filipino counterparts. In addition, two faculty members of the University of the Philippines were given special training in pilot plant operation and maintenance at Cornell University and returned to the University of the Philippines to strengthen its Food Science and Technology activities. Many other students, both undergraduate and graduate as well as faculty, took one or more courses from the visiting professors in subjects related to food science, technology, and food processing.

The Food Science Pilot Plant

A major assignment of the overall project was the establishment of a food science pilot plant to be used for the production of soy or soy/coconut based beverages and other protein-rich products. Since a new Food Science Building was scheduled to be built for occupancy in 1971, it was necessary to establish a temporary pilot plant. At the same time, the visiting professors, with help and advice from the Cornell University faculty, advised their Filipino counterparts on many phases of the design for the new building.

A temporary pilot plant was first established by renovating the rear third of a large lecture auditorium in the Chemistry Building. The steam boiler, air compressor, water softener and auxiliary equipment furnishing power to the pilot plant were housed in a small room erected beside the pilot plant.

The following pieces of equipment were installed in the pilot plant:

1. Rietz disintegrator
2. Moyno pump
3. Shriver filter press
4. Lee steam-jacketed kettle
5. Manton-Gaulin homogenizer
6. Dixie retort
7. No. 2 and No. 10 can closing machines
8. Morehouse mill
9. Colloid mill
10. CeCoCo cereal breakers, flour grinders and related equipment
11. Motorized carborundum grinder

While the above pieces of equipment (with the exception of the CeCoCo grinders) were supplied with U. S. AID funds, the Department of Food Science and Technology at Cornell University also supplied a variety of pieces of surplus equipment which increased the usefulness and efficiency of the University of the Philippines pilot plant.

The pilot plant was especially designed for research on soybean and soy/coconut milks.

Basic processing studies were carried out at Cornell University and this information was supplied to the University of the Philippines through the visiting professors.

The most important development in the production of soy milks was the use of a procedure variously described as the "boiling water" or the "hot grind" process. In this procedure, the soaked soybeans are ground with boiling water. Since the beans are not at the temperature of boiling water when added to the water, the actual grinding temperature is generally 80°C or higher, but this is sufficient to inactivate the lipoxidase which produces undesirable off-flavors in soybeans ground with water at low temperatures.

This basic improvement in soy milk processing was adopted in the Philippines and used with minor variations in all studies.

Laboratory Studies on Soy Milk

Initially three varieties of locally produced soybeans were used to make soy milk: Hsieh-Hsieh and Taichung grown at Los Baños and Davao produced in Mindanao.

Soy milks were prepared by several procedures. Soybeans were soaked overnight in 3 X their weight of water. The beans were drained and the amount of water absorbed during the soaking calculated. They were then ground for 5 minutes in a Waring blendor with water added in the proportion of 10 X the weight of dry beans allowing for the amount of water taken up during the soaking. Beans were ground with water at 100-110°F or ground with added boiling water - minimum temperature 77°C. The milks were filtered through a Buchner funnel using coarse filter paper. Milks with and without added flavoring (3% sucrose and 0.1% salt) were filled into 7 or 8-ounce soft drink bottles, capped, and sterilized at 15 pounds steam pressure for 10 minutes. Soy milks were also prepared from unsoaked beans using the same proportions of water. The following preliminary results were obtained with Filipino taste panels:

(a) Soybean milks from unsoaked beans were rated unacceptable with or without flavoring.

(b) Soy milk from soaked beans extracted with water at temperatures of 100-110°F were rated unacceptable with or without flavoring.

(c) Unflavored soy milks produced by the "hot grind" extraction method were found to be unacceptable.

(d) Soy milks prepared by the "hot grind" extraction method and flavored with 3% sugar and 0.1% salt (W/V) were rated acceptable.

(e) There did not seem to be any significant difference in the flavor of soy milks prepared with Taichung, Hsieh-Hsieh, or Davao soybeans.

Soy milks were prepared from soaked beans by the "hot grind" extraction method. The soy milks were flavored with 6% sugar with and without 0.1% salt. Although all samples were rated acceptable, a majority of the taste panel preferred the samples containing salt. Salt was not added to all subsequent soy milks, however, because of a tendency on the part of some soy milks to coagulate in the presence of salt during sterilization.

Seventy-five (75) sixth grade students from two elementary schools in two barrios near Calauan in Laguna were given soy milk samples to drink. The soy milks were prepared from soaked beans using the hot hydration method and contained 5% sugar. Thirty-four (34) or 43% of the children found the soy milk acceptable, while forty-one (41) found the samples were not sweet enough.

Addition of 9% sugar resulted in 82% of the children indicating a liking for it. Addition of 0.1% vanilla with 9% sugar increased the percentage of school children liking the product to above 90%. With 0.1% pure vanilla extract added, the percentage of sugar could be decreased to 7%, and still the soy milk received an above 90% "like" rating. Addition of chocolate (5%) or coconut milk (10%) resulted in the soy milks being rated as "like" by 96 to 97% of the school children. This represents as high a degree of acceptance as any new product can achieve.

The laboratory investigations were concerned with soy milk sterilized in 7-oz. soft drink bottles for 12 min. at 121°C with and without coconut milk as an adjunct. Samples were supplied in various flavors to approximately 335 elementary students in four schools to determine acceptability.

Students in some barrio schools, such as Sta. Ana, were much more critical, especially when coconut milk, a flavor with a low prestige value in the coconut-producing areas, was incorporated. Mothers also at Sta. Ana were more critical of the soy milk flavor.

The results indicated that the variously flavored soy milks were very acceptable to the children when the milks were given to them. The question that naturally followed was whether the children would buy them if they were made available to the school canteens and the children had to pay for the milks in competition with soft drinks and other snack items. Two highly acceptable soy milk formulations were tried. The first contained 9% sugar and 0.1% pure, vanilla extract, a low-cost flavoring combination. The second contained 9% sugar, 0.1% pure vanilla extract, and 5% chocolate. This formulation cost about 2 times the first formulation.

It was found that children, particularly in the private school (Maquiling), did buy the sterilized, bottled soy milk in reasonable quantities. In the public schools, however, most of the bottled soy milk was sold to teachers, as the majority of the children did not bring enough money to school each day to purchase a whole bottle of milk costing 15 or 20 centavos.

At this point, it had become very clear that we needed some economic data regarding the amount of money children bring to school each day. There is no need to manufacture products which sell beyond the economic means of the student consumers. A survey of the amount of money brought to school by students in four schools was made. It was found that the majority of the students brought less than 10 centavos (2.5¢ U. S.) to school per day. From 12 to 24% of the students brought no money to school on a given day.

In addition, the results showed that at barrio schools, such as Sta. Ana, higher percentages of the children (27 to 44%) brought no money, and the majority of the students brought 5 centavos (1.5¢ U. S.) or less per day. Getting nutrition supplements to this group of students requires that supplements either cost 5 centavos or less, or that they be subsidized.

Additional taste studies were undertaken primarily with selected panels within the Department of Agricultural Chemistry. These panels had the purpose of substantiating flavor differences, if any, among soybean varieties and among various processes of making soy milks.

Comparing soy milks produced by grinding the soybeans by the boiling water method (minimum temperature 77°C) and with cold water (90°F), it was found that 50% of the panel preferred soy milks prepared by the hot grind, while 33% preferred milk made by grinding the beans with water at room temperature.

Two-thirds or more of the panel members preferred the flavor of soy milks made from Davao, Taichung, or Hsieh-Hsieh soybeans ground through the Rietz in preference to those ground in the Waring blendor. All samples were ground with hot water (minimum temperature 77°C). This more intense flavor effect in the Waring blendor may have been caused by incorporation of more oxygen.

The taste panel was unanimous in preferring soy milks prepared from Taichung soybeans soaked in dilute alkali (0.1% NaOH) above those soaked in water. In contrast, only 50% of the panels preferred soy milks prepared from Hsieh-Hsieh soybeans soaked in dilute alkali above those prepared from the soybeans soaked in water.

The taste panel was unanimous in preferring soy milk containing 0.15% $NaHCO_3$ over those containing no sodium bicarbonate. The panel found 0.2% sodium bicarbonate too strong. Eighty-three percent of the panel preferred 0.15% over 0.1% sodium bicarbonate.

Sodium bicarbonate was added to the grinding water or to the final milk. It was found that 50% of the taste panel preferred soy milks in which the sodium bicarbonate was added during the grinding. Thirty-three percent of the panel could not tell a difference in the milks.

Comparing regular "hot grind" soy milk containing 9% sucrose and 0.1% vanilla with the soy milk made by the regular formula, but containing 10% coconut milk (v/v), and regular soy milk containing 5% Hershey chocolate syrup, the taste panel gave an average score of 6.3 (like slightly) for the regular soy milk, 7.8 (like very much) for the soy milk containing 10% coconut milk, and 7.5 (like very much) for the chocolate soy milk. These ratings are based upon the scale of 9 (like extremely) to 1 (dislike extremely). The findings reflect the fact that coconut milk or chocolate mask any residual soybean flavor. Inasmuch as coconut milk costs about the same as soybean milk, while the cost of the chocolate alone equals the cost of all other ingredients, it is obvious that coconut milk is the choice for covering residual soybean flavor for economical soy milks processed in the Philippines.

Based upon the laboratory studies and preliminary studies in the Los Baños food science pilot plant, the following process evolved for production of soy milks with flavors acceptable to the majority of the Filipinos who had tasted the milks:

1. The beans were hydrated in dilute alkali (1 g sodium hydroxide/liter) for 4 hr. at room temperature (approximately 30°C).
2. The beans were drained and washed with fresh water.
3. The beans were ground with boiling water at a temperature above 77°C in a ratio of 1:10 (dry beans (w) to water (v) allowing for the water absorbed during hydration). The beans are ground in a Waring blendor for 5 min.
4. The soybean milk slurry was filtered under vacuum through a standard milk filter pad.
5. Various flavoring ingredients and/or coconut milk were incorporated in the soy milk.

Coconut milk was prepared by removing fresh coconut meat, grinding it in a Waring blendor with water (100 ml water/100 g coconut meat), and filtering the milk through cloth.

Coconut milk was combined with soybean milk in proportions of 1:4, 1:9, and 1:19 (coconut milk to soy milk, v/v).

The results of the laboratory and preliminary pilot plant studies on soy milks with flavors acceptable to Filipinos were published: Steinkraus, K. H., L. T. David, L. J. Ramos, and J. Banzon. Development of Flavored Soymilks and Soy/coconut Milks for the Philippine Market. Phil. Agriculturist. LII:268-276. 1968.

Pilot Plant Studies on Soy Milk Production

Soy milks were produced in the pilot plant on a larger scale than was possible in the laboratory. As many as 900 - seven ounce bottles of soy milk were prepared and sterilized each day.

For larger scale production, certain economies were introduced:

1. Vanilla essence which could be added in quantities of from 20 to 40 ppm was used instead of 0.1% pure vanilla extract.
2. Chocolate powder was used instead of Hershey's chocolate syrup.
3. Only unmarked bottles were used.

During the laboratory studies, unmarked bottles were used only when the soy milk was being test-marketed.

The "Boiling Water" or "Hot Grind" process was used in all cases to insure that grinding occurred at temperatures 80°C or above. Grinding was done principally in the Rietz disintegrator using an 032 screen. Soy milks were filtered through a Shriver filter press with cloth filters. A milk filter pad had been used in the laboratory.

All sterilization was done in a Dixie Retort with automatic controls. In the laboratory studies, sterilization was done in a large pressure cooker. Sterilization times in both cases were 12 minutes at 121°C.

Acceptability of Soy Milks Produced in the Pilot Plant

A large scale acceptability study of different soy milk formulations was conducted at the Anos Elementary School, near Los Baños, using approximately 400 school children with ages ranging from 7 years to 13 years. Each child was given a 7-fl. oz. bottle of soy milk at each session, and they scored the soy milk on a 7 point "Smiley" scale. The "Total like" score ranged from 77.2 to 91.8% depending on formulation and temperature of serving. The acceptability increased as the sugar content was raised from 5% to 11% and it decreased again when the sugar content was increased to 13%. Serving the soy milk cold gave a higher acceptability than serving the same formulation at ambient temperature. On the basis of these tests, a standard formula containing 7% sugar and 20 ppm of a commercial essence of vanilla was selected for soy milk. A very acceptable chocolate formulation was developed using the formula: 9% sugar, 1% cocoa powder, .05% salt and 100 ppm of commercial vanilla essence. This formulation had higher acceptability than regular soy milk. These results indicated that there is practically no difference in soy milks produced in the laboratory and in the pilot plant as far as acceptability and flavors are concerned.

Many groups of adults were served standard formula soy milk under less vigorous conditions than the Anos school test and asked to score it. Acceptability ranged from 79% to 96%.

Effect of Alkali Soaks and Alkali Addition on Flavor Acceptability of Soy Milks

Basic research at Cornell University on the effect of adding sodium hydroxide to the soak water (Badenhop, A. F. and L. R. Hackler. Effects of Soaking Soybeans in Sodium Hydroxide Solution As Pretreatment for Soy Milk Production. Cereal Science Today. 15:84-88. 1970.) showed that soaking soybeans of Harasoy 63 variety in 0.05 N sodium hydroxide yielded a boiling water grind soy milk with a pH of 7.37 which was judged by a 14 member taste panel as having a better flavor than the water soaked control soy milk at the 1% level of confidence. Based upon this conclusion, soy milks prepared in the laboratory at Los Baños were soaked in 0.1% sodium hydroxide solution which is about half the concentration used by Badenhop and Hackler. It was found that Filipino taste panels preferred soy milks made from Taichung variety of soybeans when they were soaked in the alkali. When the soy milks were made from Hsieh-Hsieh soybeans, about one-half the tasters preferred the soy milks made from beans soaked in alkali and about one-half preferred the soy milks made from beans not soaked in alkali.

Subsequently, in pilot plant studies on soy milk, the value of alkali soaks in improving flavor was questionable. Therefore, an intensive study was made of the effect of alkali additions on the pH and flavor of soy milk. Sodium hydroxide, sodium carbonate and sodium bicarbonate were used to raise the pH of the soy milk. The pH titration curve for each alkali was determined and samples ranging from pH 6.60 (control) to 8.0 were prepared for flavor and acceptability evaluation.

The sodium hydroxide gives a steep rise in pH, sodium carbonate a moderately steep rise and sodium bicarbonate a very slow increase in pH. Sterilization of soy milk in bottles for 12 min. at 250°C caused a decrease in pH of pH-elevated samples, but the control (no alkali added) was unaffected. The decrease becomes greater as pH elevation prior to sterilization becomes higher.

Results of the taste panel evaluation showed that at 5% level of significance (F-test), there is an increased acceptability for sodium hydroxide treated soy milk in the pH range of 7.00 to 7.50. Further increase in pH, however, produced soy milk with a peculiar "soapy" flavor which caused the acceptability scores to decline.

The study also showed that in the same pH range, sodium carbonate or sodium bicarbonate added to soy milk causes an unfavorable flavor in the soy milk. This is in contrast to laboratory studies wherein Filipino taste panels showed unanimous preference for soy milk containing 0.15% $NaHCO_3$ over those containing no bicarbonate. On the basis of all of these studies, the regular soy milk formula now has 0.02% sodium hydroxide added to it.

Variety Trial

Sixteen varieties of soybeans were obtained from the Philippine Bureau of Plant Industry and fourteen varieties from the Agronomy Department of the U. P. College of Agriculture for a variety evaluation study. Each variety of soybean was made into soy milk using a standard procedure. The soy milks were submitted to an experienced taste panel for flavor evaluation.

Results to date show that 14 out of 16 varieties of soybeans make a soy milk of satisfactory flavor while 2 score significantly lower. The raw soybeans have ranged from 28% to 36% protein and 18% to 21% fat. The soy milks range from 2.4% to 3.5% protein and 1.0% to 1.2% fat. The insoluble residue ranges from 18% to 20% protein.

Tests On Soy Milk By Commercial Firms

300 bottles of regular formula soy milk were supplied to Pepsi Cola (Philippines) for a marketing and acceptability study. Pepsi-Cola concluded that there is a potential market for this kind of beverage and that the degree of acceptance, while not overwhelming, indicates reasonable acceptance of the product.

Storage Stability of Soy Milk

Bottles of soy milk were stored at ambient tropical temperature and taste tested regularly. After 6 months storage, the taste panel detected a slight loss of flavor of the stored sample in a paired comparison with freshly made soy milk. On these grounds, it was concluded that bottled soy milk has a shelf life of 6 months in the tropics.

After 16 months storage, the soy milk made in the pilot plant, still had a pleasant flavor with no beany or rancid flavor notes, but the cereal-like flavor became weak. The color became slightly darker. There was separation of "cream line" about 3/8" thick in the top of the bottle, but no breaking of the emulsion or free oil. There was a 1/2" thick layer of what appears to be concentrated soy milk at the bottom of the bottle. Both layers were easily re-incorporated into the soy milk by gentle shaking.

The results of acceptability studies using soy milks produced in the pilot plant have been submitted to Phil. Agriculturist for publication as the following manuscript:

Puertollano, C. L., M. C. Bourne, J. Banzon, and J. C. Melgar. Effect of Changes in Formulation of Soymilk on Its Acceptability to Filipino Children.

Other Soybean Products

Soy-Coconut Candy

It was observed that a popular snack item in the school canteens was ice candies costing 5 centavos each. These are flavored sugar water frozen in plastic bags. They contain no protein. Our soy milks were placed in similar narrow plastic bags (60 ml/bag), frozen, and offered for sale in the school canteens. It was found that they sold well. In the barrio school (Balayhangin), offered in direct competition with commercial ice candies, the soy milk candies outsold the commercial product.

An excellent candy was developed by the following procedure: 100 g ground whole soybeans, 100 ml coconut milk, and 100 g sugar are mixed and brought to a boil. The mixture is boiled with constant stirring until the sugar is carmelized. Thirty (30)-g pieces are individually wrapped in waxed paper. The candy contains approximately 20% protein. Each piece, therefore, contains nearly as much protein as a 7-oz. bottle of soy milk. It represents an alternate way of getting protein to children in a form liked by children.

Soybean Curd (Philippine Tokua)

Soybean curd is offered for sale on most Philippine markets. It is a product that is well known and accepted by the Filipinos. A related product is tahu which is a softer soybean curd used for dessert. Tokua and tahu are apparently produced by the Chinese manufacturers using traditional procedures. Most Filipinos seem to be unfamiliar with the methods of manufacturing tokua and tahu. Therefore, in all our soy milk studies, we have taught the Filipinos how simple it is to manufacture soybean milk and to prepare tokua by heating the soy milk to boiling and precipitating the curd with magnesium or calcium salts.

Indonesian Tempeh

Tempeh prepared by fermenting dehulled, soaked, partially cooked soybeans with a mold belonging to genus *Rhizopus* is a standard article of the diet sold in every market in Indonesia. It appears to be unknown among the Filipinos. Project personnel and students have been taught the traditional and laboratory methods of manufacturing tempeh. Sliced thin, dipped in salt brine solution, and deep-fat fried, it has been well accepted by all Filipinos who have tasted it.

In the traditional processing of tempeh, the soybeans are dehulled after soaking by rubbing them between the hands and floating the detached skins off the water. The CeCoCo grinders have been adjusted so that the whole dry soybeans can be dry dehulled following treatment for 15 minutes in a hot air oven at 200°F to shrivel the cotyledons. The detached hulls are then removed in the CeCoCo hand winnower.

Tempeh was produced by both traditional and by improved laboratory procedures. The traditional process in which the fermenting bean mass is wrapped in wilted banana leaves would be most easily adapted to small cottage industry in the barrios.

Full-fat Soy Flour By the USDA (Peoria) Laboratory

Using the CeCoCo grinders, full-fat soy flour was prepared. Following pre-cooking of the soaked soybeans, the beans are supposed to be sun dried. This has proved to be about a 2-day process when the days are sunny. This is a step when the beans can easily spoil. Therefore, some effort has been made to design and build a simple, hand-operated dryer which could be heated over an open fire or with charcoal.

A gallon metal can was drilled with 150 1/8-inch holes on the sides and ends to allow escape of water vapor. The can was equipped with a handle so that it could be revolved over the fire. It was found that pre-cooked soybeans could be dried in about 2 hours at temperatures of about 70-80°C (158-176°C) in this simple dryer. A 50-gallon drum could easily be adapted for a larger capacity drying chamber.

Quick-Cooking Mungo Beans

At the request of Dr. R. W. Engel, of the U. S. AID/Virginia Polytechnic Project and the Philippine National Nutrition Program, experiments were conducted on the production of a "quick-cooking" mungo bean which could be used in feeding studies with infants, toddlers, and mothers at Mothercraft Centers being set up on an experimental basis in various parts of the Philippines. Since this was an opportunity to help a nutrition project closely related to our interests, both by way of basic support and general objectives, we undertook the task.

Mungo beans are available in nearly all markets in the Philippines. They are familiar to the Filipinos and used in various recipes. The fact is, however, that there is generally an insufficient supply to permit an expanded use of mungo beans in various nutrition programs. They are more readily available than soybeans, and the Filipinos know how to use mungo beans. Filipinos, in general, are not as familiar with soybeans. However, the protein content of mungo is about 25% versus 40% in soybeans.

Mungo beans, like most other beans, require a rather long cook to make them palatable. At the Mothercraft Centers, where the U. S. AID/Virginia Polytechnic-Philippine National Nutrition Program are feeding mixtures of mungo, rice, and dried pulverized fish to infants, children, and mothers, one of the problems is the long cook required for the mungo beans. Therefore, it was valuable to have a pre-cooked, dehydrated mungo bean that could be added to the mixtures and would require very little, if any, additional cooking.

To this end, following discussions with Dr. R. W. Engel, mungo beans were subjected to two processes: (1) toasting in dry oven at 250°F for 1 hour followed by dry grinding, and (2) soaking (4 hours), draining, boiling in water for 30 minutes, draining, drying in hot air (180 to 200°F for 2-1/2 to 3 hours), and dry grinding. These powders were incorporated in the diets at one Mothercraft Center near Santa Cruz, Laguna. It was found that some of the infants developed diarrhea when the mungo bean flours were incorporated in the soups without additional cooking.

Therefore, mungo beans were subjected to increased cooks as follows: (1) Mungo beans were pre-soaked to double weight and then toasted by drying in an oven at 250°F until dry--approximately 3 hours followed by dry grinding. (2) Mungo beans were pre-soaked to double weight, drained, boiled in water for 1 hour, drained, and dried in a hot air oven at 200°F until dry (approximately 2-1/2 hours), and dry ground. In this second experiment, the toasted mungo beans were definitely toasted as shown by a slight darkening which was not present in the first experiment. Boiling time was doubled with the pre-cooked mungo beans. One hour boiling was about the maximum cook in water that could be followed without causing disintegration of the bean structure. Cooking could still be further increased with a steam process. However, these beans were acceptable to the infants.

Coconut Milk Beverages

Coconut milk was prepared by shredding the fresh coconut meat and pressing it with added water in the proportion of 1:1 (W/V). The coconut milk was filtered through a cloth filter. It was bottled in 8-ounce soft drink bottles with and without 5% added sucrose and sterilized in the bottle for 10 minutes at 250°F.

It was found that the coconut protein coagulated when heated, somewhat like dilute milk-egg custard. Although coagulation occurred, upon shaking, the curd tended to redisperse into a milk. The flavor of the coconut milk containing 5% sucrose was excellent, sweet, and definitely coconut in character. Mixed with soybean milk 1:9 (V/V), it imparted a pleasant coconut flavor to the resulting milk. As little as 1 part coconut milk to 19 parts soybean milk introduced a pleasant coconut flavor masking the usual soybean flavor.

In higher concentrations, the coconut milk could be used as pudding.

Coconut Protein Studies

Fermentation as a method of separating coconut oil and protein was investigated.

Various patented and published methods of extracting oil from coconut meat by fermentation were studied, and the best features were combined to devise a better process. Processing conditions were optimized wherever possible.

The factors which must be controlled to break the emulsion and liberate the oil were investigated.

It was found that coconuts available commercially, which vary rather widely in maturity and length of storage before processing, varied in their response to fermentation processing. Approximately 60% of the milks produced from individual coconuts showed a breaking of the emulsion when fermented under controlled conditions. Forty percent failed to break indicating that some factor(s) responsible for the coconut milk emulsion stability remained uncontrolled during fermentation. This probably demonstrates why no patented or published method for the wet processing of coconut oil through fermentation has thus far succeeded commercially.

It was found that grinding conditions exerted a profound effect upon the stability of the coconut milk emulsion. The best method of grinding coconut meat with water in the initial extraction was the shearing type action produced in a Waring blendor. Extractions using a Rietz disintegrator (023 screen) produced an emulsion that failed to break with fermentation. The optimum dilution for rapid fermentation of coconut milk and separation of the oil and protein was found to 1:1 (w/v) coconut meat:water. *Lactobacillus plantarum* produced better fermentation with more rapid separation of oil than *Lactobacillus delbrueckii*. The fermentation progressed best under microaerophilic conditions at 40°C. The fermentation was successful in breaking the emulsion at a relatively broad range of pH and titratable acidity.

Concentrated Coconut Water

Fresh coconut water in 40-gallon lots was obtained through the cooperation of Franklin Baker Company at San Pablo City. The coconut water was concentrated at about 130°F in a Mojonnier vacuum evaporator at the General Milk Company pilot plant in Manila. In the first experiment, the coconut water with initial solids content of 3.7% was concentrated to 57% solids. Other analyses of the concentrated coconut water were as follows:

Titratable acidity (as lactic)	- 1.75%
Ash	- 6.55%
Crude protein	- 1.02%
Crude fat	- 1.72%
Color	- light yellow

The first batch received no heat until it was concentrated.

A second batch was prepared in which enzymes and microorganisms were destroyed prior to concentration by heating the coconut water to 190°F. Analytical data on the second batch were as follows:

Total solids	- 82%
Crude protein	- 1.79%
Crude fat	- 1.22%
Ash	- 9.00%
Color	- brown

The second batch, despite its higher solids content, was more fluid than the first.

Both lots of concentrated coconut water were bottled in 8-ounce soft drink bottles. It was found that both lots would remain free of microbial growth without further heat treatment.

Samples of concentrated coconut water were sent to nine industrial firms in the U.S.A. specializing in antibiotic and other pharmaceutical manufacture and to the Northern Regional Research Laboratory (Peoria) for evaluation as to its value as a fermentation stimulator.

It was found that coconut water concentrate could be used in many industrial fermentations in place of corn steep liquor. It appeared to be a specific stimulator in the production of bacterial polysaccharides such as xanthan.

The Future of Soymilk Research at Los Baños

The U.P. College of Agriculture intends to continue the research on soy milk and other foods from soybean because they believe that soybean foods can help to bridge the protein gap in the Philippines. The College is vigorously promoting the growing of soybeans among farmers in the Philippines at the present time. The College Administration has, therefore, established project UPCO #151 to enable the Los Baños part of this research contract to continue. This new project has been funded at the rate of 27,000 pesos per annum until June 30, 1971 which is the end of the current fiscal year. This funding is sufficient for the project in Los Baños to continue at its present level with the exception of the salary of the visiting professor, (Dr. M. C. Bourne). The Administration would like the project to continue past June 1971, but this will depend on what funds are available and how the new food technology building and pilot plant at Los Baños are set up and staffed.

Extension Activities

Peace Corps

Several hundred Peace Corps volunteers were given training in the manufacture of soybean milk, soybean curd, and tempeh. Some of the volunteers spent time working on these products in the food science laboratory. Most of the volunteers reported that soybean culture is expanding at the village level, and they wanted to teach the villagers how to use the soybeans for food. This was an effective way of extending information developed in the Cornell and UPCA laboratories under AID sponsorship.

AID India and Southeast Asia

Results of the soybean/coconut milk protein food research were extended to India (at the request of the U.S. AID-University of Illinois Soybean Project) and to Malaysia, Ceylon, and Indonesia. The U.P. Agricultural University at Pant Nagar, Dist. Nainital, India, have decided to build the first synthetic cow's milk plant in the world based upon soybean protein. The Indian population knows cow's milk, and any vegetable-based milk to be successful must, insofar as possible, duplicate the organoleptic characteristics of cow's milk. This will be a real test to determine whether or not animal milks can be successfully simulated for the commercial market.

Demonstrations of soybean processing and soybean technology were made to Indian women, both housewives and home demonstration agents, at Pant Nagar in the period June-July, 1969. They were taught how to make and utilize soybean milk, soybean curd, soybean cotyledons, soybean residue, Indonesian tempeh, and soybean nuts. The procedure used was to demonstrate the production of these various products in the morning sessions and then invite the participants to return for an afternoon session during which time they actually prepared the products. The Indian women displayed remarkable ingenuity in adapting the various soybean products to Indian taste using varieties of spices and flavorings available in India. Soybean milk was flavored with almond extract and cardamon. The resulting soy milk was lacking the usual soy milk flavor and appeared to be highly acceptable to the Indians. The soybean residue was processed by heating it with ghee and added sugar to make a confection. Soybean cotyledons were incorporated in a number of tasty Indian dishes. The net result was that the University of Illinois project hired a number of the Indian women to work on recipes. A book will be published within the next year on the use of soybeans in Indian cooking.

Dr. Bourne made a trip through countries in Southern Asia in April 1970 in order to visit teaching and research institutions in food technology. Demonstrations and lectures on soy milk were given in a number of locations. A formal seminar, followed by a demonstration and sample tasting was given at the University of Pant Nagar, Uttar Pradesh, India, and at the Ford Foundation in New Delhi, India.

An informal seminar, followed by a demonstration of the boiling water grind technique, was given at the Institute of Food Research & Product Development, Kasetsart University, Bangkok; the Applied Scientific Research Corporation of Thailand, Bangkok and the Lakpahana Training School, Mailapitiya, Ceylon. An informal seminar without demonstration was given to the Institute of Medical Research, Kuala Lumpur; Bogor Agricultural College, Indonesia; Medical School and Nutrition Institute of the University of Indonesia, Djakarta; Yeo Hiap Seng Ltd. and Cold Storage Creameries Ltd. in Singapore. The Cold Storage Creameries have utilized the boiling water grind technique to market a line of flavored soy milks in Singapore.

A one day meeting at Los Baños was held January 29, 1971 in order to acquaint senior executives in industry and in Government of the potential that soy milk has for increasing protein intake in the Philippines, and to determine whether any obstacles remain to obstruct the full commercialization of soy milk in the Philippines.

ENGINEERING AND PROCESSING STUDIES

In a previous report(1) from this laboratory, a process was described for the wet milling of soybeans and the extraction of the slurry for the production of fresh, canned or dried soy milk to serve as a protein supplement for children in under-developed countries. Since the issuance of that report, a number of questions have arisen regarding increased product yields through finer grinding, other methods of removing non-nutritive solids and the feasibility of using lower temperatures in the spray drying of the milk. Further, it had been found in this laboratory that the objectionable beany flavor usually associated with soy milk could be avoided if the beans were ground with water at a temperature of 200°F or higher. However, up to the time of this study, the experimental work had been confined to grinding the soaked beans with hot water in a Waring Blendor and it remained to translate this finding into manufacturing practice.

Grinding

Formerly a Rietz Disintegrator was used for grinding soaked beans with water (1:10) at room temperature. This disintegrator is an inclined hammermill having fixed knives that are surrounded by a perforated screen. The beans and water are fed into the eye of the grinding chamber and the solids are retained until ground

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(1) Pilot Plant Studies in Soy Milk
Hand, D. B., Steinkraus, K. H., Van Buren, J. P., Hackler, L. R., el Rawi, J., and H. R. Pallesen. Food Technol. 18, 139-142, 1964.

sufficiently fine to pass through the screen, or forced through a secondary discharge opening. Approximately 7% of the original dry bean solids were lost through the secondary discharge and more solids were lost through the foam generated during comminution. This foam amounted to nearly 22% on a volume basis or, 24% on a solids basis. However, by reducing the bean-water ratio to 1:4, the foam problem was eliminated and the solids loss through the secondary discharge was greatly reduced. The slurry, however, had a pronounced beany flavor.

When boiling water was used in place of cold water as a means of rapid enzyme inactivation during grinding, the beany flavor was reduced but a stable foam was produced and the openings in the screen soon plugged. The foaming problem was overcome by injecting steam directly into the grinding chamber and the screen stoppage was alleviated changing the screen from one having 0.023" diameter holes to one having 1/16" diameter perforations. The heat liberated by the condensing steam insured the discharge of a slurry having a temperature uniformly over 200°F. The use of the coarser screen necessitated a secondary grinding step prior to filtration. An electric boiler was used to generate the pure steam added to the mill.

The hot slurry from the Disintegrator contained large particles ranging from 650 μ to 2000 μ in diameter, and in order to reduce their size, the slurry was subjected to a secondary grinding in a colloid mill equipped with carborundum discs. Most of the slurry from the stone mill passed through a No. 325 mesh (44μ) screen. Feeding the slurry to the stone mill under a pressure of 8 - 10 psi more than doubled the output of the mill, reduced the danger from heat damage due to friction between the stones and increased the uniformity of grind.

Two other mills have been used for the primary grinding of soaked soybeans. The Bauer single disc or attrition type mill when operated at a high speed and with fine ribbed plates having a dam at the periphery produced a uniformly finely ground slurry with little foam and seems to be capable of handling a wide range of bean to water ratios. The retention time in the grinding chamber was extremely short and a holding tube may be needed after the grinder to insure sufficient time for enzyme inactivation. A wiped film heat exchanger might also be used after the Bauer mill to heat the slurry to a high temperature for a short time prior to cooling for secondary milling. The Disperser-Mixer type of Bauer Disc mill has a screw feed and provision in the hollow shaft of the screw for injection steam and water through concentric tubes into the center of the grinding chamber.

The other type of mill used for the primary grinding of soaked beans was the Comitrol cutter made by the Urschel Laboratories. Such a cutter equipped with a Microhead comminuted a relatively thick mixture of 1 part of beans to 3 parts of water. Wet sieving of the slurry showed the particle size to be much smaller than that obtained with the Rietz Disintegrator. Replacement of the

Microhead in the Comitrol with a Homogenizing head, permitted further reduction of the particles in the slurry from the Microhead. Sieve analyses of the slurry from the Homogenizing head showed that particle reduction was equivalent to that achieved in the stone mill.

A major problem encountered in the production of a bean slurry of uniform solids content was delivering soak soybeans at a controlled rate into the primary grinder. A vibratory feeder with rheostat control has been used most extensively in this study but it has been difficult to maintain a desired flow of beans. The difficulty is due to a mucilaginous exudate forming on the surface of beans after removal from the soak tank. This coating retards the movement of the beans along the trough of the vibratory feeder. No solution has been found to this problem other than reducing the time between draining and loading into the feeder. A vibratory screw feeder was tried but the bean discharge rates were not significantly more uniform. In addition, propulsion by the screw resulted in considerable breakage of the beans creating opportunities for enzymic generation of the beany flavor before heating in the grinder. The vibratory screw feeder was not of a sanitary design and with the nutrients available in soybeans for microbial growth, avoidance of spoilage would be difficult.

Liquid-Solids Separation

It may be advantageous to separate the liquid and fine particles from the coarser materials after the primary grinding step in the disc mill and then recycle the coarse particles through the disc mill as a means of increasing the yield of soy milk. For this separation, a Bauer Hydrasieve, which is a stationary inclined screen, was used in preliminary tests with a slurry having 16% solids. The screen blinded and the liquid separation was not good. However, with a more dilute solution and a coarser screen, a suitable separation might be affected. Future work on this method of separation must involve protein analyses to determine if less valuable carbohydrate components can be discarded by screening.

For the final separation of solids from the milk, a plate and frame filter has been used in the past with a Nylon cloth. This equipment is used extensively for the clarification of liquids but its value as a means of controlled separation is doubtful because of the changing porosity of the filter medium as solids accumulate on the cloth. Moreover, unless great care is taken in cleaning the cloths, proteinaceous deposits will gradually change the porosity. Such deposits can also serve as a medium for microbial growth.

As an alternative method for the final separation of large particles, centrifugation has been considered. The successful use of a centrifuge for this application depends on the density of the particles, size of the particles, centrifuge speed and retention time in the centrifuge. Of these factors, particle density seemed to be the most important and in the absence of published information, a method was developed for measuring density by the pycnometer method using xylene. The density of the soy particle was found to be 1.25 which should be sufficiently different from the aqueous medium to permit rapid separation by centrifugation.

Spray Drying

Attempts were made to dry soy milk at 400°F with outlet temperatures ranging from 210°F down to 120°F in a pilot scale dryer using a 2-fluid nozzle. The dryer was arranged for cocurrent drying in a chamber 42" in diameter x 12' high. The limited diameter of the spray chamber necessitated the use of a narrow angle spray pattern produced by the 2-fluid nozzle. Unfortunately, 2-fluid nozzles have a long "throw" spray pattern which limits the residence time in a dryer of this height. Consequently, a high air temperature must be maintained in order to complete evaporation before the product stream enters the cyclone. The minimum air discharge temperature that could be used consistent with the collection of a dry product was around 200°F. The size of the dried particles ranged from 1 to 80 microns and the average was 8 to 10 microns. This is much smaller than the 75 to 150 micron range found in many commercial spray dried products.

Since these trials several types of single fluid nozzles giving a narrow spray pattern have been located and these may provide the desired residence time in the dryer so that lower air temperatures may be used. The base of the drying chamber is also being modified to permit more rapid cooling of the dried particles and the collection of the majority of the product without the use of a cyclone. This would avoid erosion and their disintegration in the cyclone. Particle size is also governed by the viscosity of the feed, and in the work done to date, the solids contents of the feed has been limited to 16%. Higher concentrations have gelled during preparation. In more recent studies on concentration of soy milk, concentrations of 25% have been attained without gelatin. The concentrated milks have not been spray dried as yet.

Concentration of Soy Milk

In early studies on the concentration of soy milk, a wiped film evaporator was used and relatively high temperatures (250°F) were used in the heating shell. The greatest concentration that could be attained without gelation of the concentrate was around 16% solids. More recently in a falling film type of evaporator, it has been possible to concentrate the milk from 7 to 22% solids. This has been done by limiting the jacket temperature to 150°F or less, and maintaining sufficient vacuum to keep the product temperature at 85°F or less. Higher jacket temperatures caused a rapid fouling of the evaporator tube and resulted in a sharp decrease in evaporation rate. Soy milk is a pseudoplastic fluid and on concentration becomes very viscous and thixotropic. It appears to be more sensitive to heat damage than cow's milk.

Equipping of Processing Laboratory at University of the Philippines

In order to demonstrate the feasibility in the Philippines of preparing a nutritive beverage having an acceptable flavor from soybeans, a number of pieces of equipment were shipped to the University at Los Baños for use by a member of this Department who was stationed at the College of Agriculture. This involved the careful selection of units that would facilitate the production of canned or bottled soy milk at the rate of 1/2 gallon per minute. The equipment was received at the pilot plant in Geneva from the manufacturers and the necessary accessory controls installed before crating for overseas shipment. Manuals for the operation of the various units were also prepared. Two members of the University of the Philippines staff came to Geneva and were trained in the operation and maintenance of the equipment.

NUTRITIONAL STUDIES

COMPLEMENTARY AND/OR SUPPLEMENTARY EFFECT OF VARIOUS FOOD PROTEINS

The protein in soy milk is recognized as being of high quality. Thus, its incorporation into the diets of people in the underdeveloped countries is almost certain to improve the quality of their dietary protein. Also, the potential value of soy milk in upgrading dietary protein quality by a complementary effect of proteins should not be overlooked. Therefore, a study has been made of the possible complementary and/or supplementary effect of soy milk protein on various Filipino foods. The criterion used for evaluating has been the modified essential amino acid index (EAAI) (H. H. Mitchell, 1954). This procedure compares the proportion of each essential amino acid in a protein to that in egg protein. It does not indicate the availability of the amino acids. For example, animal proteins are more readily digested in the gastrointestinal tract than are plant proteins; this would not be indicated by the EAAI values.

The calculated EAAI values are shown in Tables 1 and 2. Since soy milk is high in lysine in comparison to cereal grains, it supplements and improves the dietary protein quality. On the other hand, the cereal grains are higher in the sulfur amino acids than is soy milk, thus a complementary effect exists between them. This complementary effect of proteins partially accounts for recommendations that people should consume a wide variety of foods.

The incorporation of soy milk or soybean products into the

Filipino diet would improve considerably the quality of their dietary protein. Obviously, this is easier said than accomplished as food habits and customs are difficult to alter.

Table 1

The EAAI values of various foods singly and in combination with soy milk[1]

		combinations with soy milk		
		3:1	1:1	1:3
	percentage of soy milk			
	100	75	50	25
soy milk	80.0			
coconut	52.8	74.9	68.7	59.5
rice	76.6	80.5	79.9	78.7
corn	70.3	78.8	76.9	73.8
corn, FAO	65.1			
sweet potato	78.2	80.8	80.6	79.8
fish, cured	80.9			
mung beans	62.6			
peanut	60.0			

1 The amino acid values for the food sources were taken from the following:

soy milk: L. R. Hackler and B. R. Stillings, Cereal Chemistry, Vol. 44, 1967.
coconut: analysis of coconut sample CG1, L. R. Hackler.
rice: L. R. Hackler, page 37 - HN 4686.
corn: USDA, H. E. Rsch Rep. No. 4, 1957, page 34, item 146.
sweet potatoes: USDA, H. E. Rsch Rep. No. 4, 1957, page 38, Item 167.
mung beans: FAO (No. 24) 1970. Item 66, pg. 54, cystine (MB) & tryptophan (MB).
cured fish: FAO (No. 24) 1970. Item 360, pg. 128, tryptophan (MB).
corn: FAO (No. 24) 1970. Item 9, pg. 38.
peanut: FAO (No. 24) 1970. Item 57, pg. 52.

Table 2

The EAAI values for various food combinations

Proportion of protein from each source	EAAI	Source of Protein
1:1:4	77.7	SM:Corn:Rice
1:1:1:1	72.0	SM:Corn:Rice:Coconut
1:1:1:1	73.9	SM:Corn:Rice:Coconut:Sweet Potato
1:4:1	78.8	SM:Corn:Rice:Coconut:Sweet Potato
1:4:1	74.9	SM:Corn:Rice:Coconut
1:1:1:1:1	74.0	SM:Corn:Rice:Coconut:Sweet Potato
4:1	80.4	SM:Corn:Rice:Coconut:Sweet Potato:Fish
4:1	76.5	SM:Corn:Rice:Coconut:Sweet Potato:Fish:Mung Bean
1:4:1	76.1	SM:Corn:Rice:Coconut:Sweet Potato:Fish:Mung Bean
1:4:1	77.6	SM:Corn:Rice:Coconut:Sweet Potato:Fish:Mung Bean

EFFECT OF SOAKING SOYBEANS IN NaOH SOLUTIONS ON FLAVOR AND NUTRITIONAL VALUE

In an effort to improve the flavor and acceptability of soy milk, several studies were conducted with NaOH additions during the soaking step. The initial study involved the preparation of soy milk samples which had final pH's of 6.55, 7.20, and 8.41. These samples were submitted to the 14 members of the soybean research group for evaluation. All members of the group preferred the most alkaline sample. Samples of soy milk were also prepared at five different pH values ranging from 6.56-8.85. These were heat processed at 250°F for 10 minutes to determine the effect of processing on acceptability. The milks were found to hold up quite well under process, the most marked change being a slight darkening of the color.

Two samples were also prepared for amino acid and Kjeldahl nitrogen analysis. Both samples were prepared using a preliminary soaking treatment of 1-1/2 hours at 50°C, followed by thorough rinsing and a hot extraction with distilled water. One sample was soaked on .085 N NaOH (pH 12.7), and the other was soaked in distilled water. The resulting milks had pH's of 7.86 and 6.56, respectively. Both samples of milk were found to contain 7.06% Kjeldahl nitrogen. Amino acid analysis of the two samples indicated that the two samples were very similar in amino acid composition. However, cystine and tryptophan were not measured in this study.

Since the improvement in flavor noted by the research team was an important observation, another study was conducted to evaluate the effect of soaking soybeans in sodium hydroxide solution as a pre-treatment for soy milk preparation. Four (4) samples were prepared by soaking soybeans in sodium hydroxide solutions of 0, .048, .076, and .097 normality. Soybeans were soaked for 2 hr. at 50°C, then rinsed well with distilled water prior to grinding in a Rietz disintegrator. Sufficient water was used to obtain a 1:10 (bean:water) ratio. The slurry was then filtered. The filtered milk samples had pH's of 6.55, 7.37, 8.04, and 9.18, respectively. Filtration rate was observed to decrease with increasing pH. Hunter _L_ value was also observed to decrease indicating a darker product with increasing pH. The flavors of the samples were evaluated by a 14-member taste panel. Results indicated that the sample with a pH of 7.37 was significantly better than the others at the 95% probability level. The panel was also asked to determine the difference in mouthfeel. Although the results of this panel were not significant at the 95% probability level, the ratings given by the panel did improve with increasing pH.

The samples of soy milks used in the taste panel evaluation as well as the residues from the alkali treated soybeans, were evaluated nutritionally by amino acid analyses and rat feeding experiments. Soy milks of four different pH's were each divided into two parts, and one of the samples of each pH was canned and processed for 10 minutes at 250°F. The resulting 12 samples (eight milks and four residues) were incorporated as the protein source into diets at a level of 10% protein and were fed to groups of ten male weanling rats for a period of 28 days. In addition to the 12 treated samples, a casein control was also fed to a group of rats in order to calculate adjusted protein efficiency ratios.

The protein efficiency ratio, (PER) values are shown in Table 3. The data indicated that increasing the pH of soy milk above 8.04 causes a marked drop in the utilization of soy milk protein for growth. On the other hand, the protein in the residue (water insoluble fraction) appears to be unaffected by the addition of NaOH during the soaking step. The cystine values (Table 3) decrease as the pH of the soy milk is increased. Since the sulfur amino acids are first limiting in soybean protein, this explains the PER values observed in the soy milk samples.

The PER values for the residues showed little apparent affect of pH. The non-heat-processed samples showed an improvement in nutritional value of the protein with increasing pH of the milk. The improvement would appear to be attributable to the decline in trypsin inhibitor values with increasing pH. The percent trypsin inhibitor retention values represent the inhibiting activity of the samples in preventing trypsin to digest gelatin and is expressed as a percent of the activity of ground, whole soybeans. The values for the non-heat-treated milk clearly show that raising the pH of the milk diminishes the trypsin inhibitor activity until, at pH 9.18, the activity is very near that of a heat-treated sample.

The complete amino acid spectrums of the samples show that the effect of pH is minimal, with the exception of cystine.

Table 3. The effect of NaOH on various attributes associated with nutritional quality of soy milk and residue

Sample Description	pH	Trypsin Inhibitor Retained %	Cystine g/16gN	Protein Efficiency Ratio[a]
Milk, raw[b]	6.55	68.1	1.74	1.44
Milk, heat-processed	6.55	7.6	1.66	2.41
Residue	--	20.0	1.48	2.11
Milk, raw	7.37	60.0	1.72	1.60
Milk, heat-processed	7.37	5.9	1.41	2.18
Residue	--	20.0	1.37	2.13
Milk, raw	8.04	45.0	1.56	1.75
Milk, heat-processed	8.04	5.0	1.37	2.20
Residue	--	10.0	1.01	2.14
Milk, raw	9.18	17.3	1.30	1.88
Milk, heat-processed	9.18	2.7	1.15	1.70
Residue	--	6.4	1.32	2.09

[a] Adjusted to a casein standard with a PER of 2.5

[b] The term "raw milk" means milk which was not heat-processed for 10 min. @ 121°C.

Soaking soybeans in a sodium hydroxide solution of approximately 0.05N is a desirable pretreatment prior to a high-temperature grinding operation for the production of soy milk with an improved flavor. This procedure will produce soy milk with an approximate pH of 7.4 which was judged by a panel of 14 members to have a significantly better flavor than a water-soak control treatment at the 1% level of significance.

Alakaline conditions were found to render trypsin inhibitors more heat labile and, therefore, easier to destroy during heat processing.

A disadvantage of alkaline treatment of soybeans prior to soy milk production appears to be partial destruction of the amino acid, cystine, which is reflected in low PER values obtained for the soy milk samples at the higher pH's.

Another study was conducted to evaluate the use of methionine supplementation for improving the nutritive value of alkaline extracted soy milk. Samples of soy milk of four different pH's (6.50, 7.21, 7.81, and 8.97) were prepared by soaking soybeans in water and three concentrations of NaOH solution (0.050 N, 0.075 N, 0.100 N). Half of each sample was heat-processed for 10 minutes at 121°C. Amino acid analyses revealed that cystine was the only amino acid which showed a consistent decrease with increasing pH in both the unprocessed and processed samples. Animal feeding studies using the soy milk as the protein source indicated that supplementation with 0.35% L-methionine improved nutritive value in both the heat-processed and non-processed samples. The PER of the non-processed samples was improved by approximately 50% at each pH. This uniform improvement is believed to be the result of an interaction between trypsin inhibitor and the destruction and unavailability of cystine. The effect of added methionine on the PER of the heat-processed samples was consistent at pH 6.50, 7.21, and 7.81 producing increases of 28, 32, and 27%. At pH 9.0, however, an increase of 92% was observed which is probably a reflection of both destruction and unavailability of the sulfur-containing amino acids. In no case was methionine supplementation alone found to be as effective as a combination of heat processing and methionine supplementation for improving the nutritive value of soy milk.

FLAVOR AND NUTRITIONAL ATTRIBUTES OF ROASTED SOYBEANS

The simplest and most direct form of roasting process is often termed "dry roasting". It involves heating of the product in air and/or a mixture of gases from the heat source. Dry roasting is employed in the coffee and cocoa processing industries, to a considerable extent in the peanut processing industry, and to some extent in the tree-nut processing industry. Another type involves the heating of the product in an oil or fat and is often termed "oil roasting" or "deep-fat frying." Oil roasting is used extensively in the peanut and tree-nut processing industries, as well as in other industries, such as the potato chip processing industry.

Dry Roasting Studies

The flavor attributes of dry roasted soybeans was initiated with the construction of a laboratory size, motor driven, electrically heated roaster which could accommodate a sample size of approximately 200 grams of beans. The roaster is a closed drum type that allows the trapping of volatiles emanating from the roasting beans. A collection system for the volatiles was also constructed which could be attached directly to the roaster. The collection system consists of a water jacketed condenser and collection flask and a glass wool column soaked with carbon disulfide. The water condenser is used for the condensation of the volatiles which are steam distilled over with the inherent moisture from the beans. The glass wool column is placed above the condenser to trap the volatiles which would otherwise escape through the condenser in the form of aerosols. The resulting condensates and carbon disulfide extracts were analyzed and evaluated by gas chromatography and mass spectrometry.

Mass spectra have been obtained from nine different gas chromatographic peaks. The identity of three of the peaks have been confirmed by comparison to standards. These three compounds are: (1) 2-methyl pyrazine, (2) 2,5 dimethyl-pyrazine, (3) 2,6 dimethyl pyrazine. A fourth peak with a parent mass of 122 could be a trimethyl pyrazine and fifth peak also with a parent mass of 122 could be a pyrazinaldehyde, possibly 5-methyl pyrazine-2-carboxaldehyde.

A small commercial sample roaster was obtained for use in other phases of the dry roasting work. This is an open flame gas fired unit.

Preliminary tests indicated that some pretreatment of the soybeans was necessary in order to prepare a product without a hard texture. Therefore, a study was conducted in which soybeans of four moisture levels were prepared. Namely 43, 47, 53, and 59% moisture. In addition, three final temperatures of roast were used (170, 180, and 185°C).

As expected, the color of the samples within each moisture level darkened as the end point roasting temperature increased. An interesting point to note is that the longest soak (59% moisture) time produced samples considerably darker than the three other moisture levels. Also, as the moisture level increased, the texture of the beans softened. In other words, less force was necessary to crush or puncture the roasted soybeans.

In the roasting process, the temperatures attained are considerably higher than needed to destroy trypsin inhibitors and may indeed be detrimental to the nutritional quality of the protein. For this reason, the previously described samples were fed to rats and protein efficiency ratios determined. Also, the amino acids were determined by liquid chromatography ion exchange procedures.

Total amino acid analyses indicated that the effect of roasting on the amino acid composition of soybean protein is confined mainly to the destruction of total and available lysine, cystine, and tryptophan. Even these amino acids were not consistently affected by the degree of roast. Total lysine content was found to be inversely related to degree of roast in all but the three samples roasted containing 59% moisture. Available lysine, however, was inversely related to the degree of roast in the samples from all 4 moisture levels. Cystine content was inversely related to degree of roast in the samples roasted from 43 and 48% but not those from 53 and 59% moisture. Tryptophan content was inversely related to degree of roast in the samples from all 4 moisture levels.

In order to show the combined effects of the roasting process on the essential amino acids, the essential amino acid index (EAAI) was also calculated according to the method of Mitchell (1954). These values for the 15 samples indicated that the EAAI is inversely related to the degree of roast in all but the three samples with the highest initial moisture content.

Following in order of heat lability are available lysine, total lysine, cystine, and histidine, which decreased by 31, 17, 15, and 6%, respectively. Since the most heat-labile amino acids are all essential, or semi-essential, the effect of roasting is reflected in the EAAI values calculated. The EAAI drops from 68.4 for the 170°C samples to 67.7 for the 180°C samples and 63.8 for the 185°C samples. Finally, a comparison of the average EAAI of all 12 roasted samples with that of the 3 unheated samples shows a decrease from 71.4 to 66.5 due to the roasting process.

The results of the animal feeding studies indicate that PER is inversely related to the degree of roast. This relationship was noted for samples roasted from moisture contents of 48 and 53% respectively. Samples roasted from 43% moisture, showed no change in PER between the 170°C and 180°C roasts. For both samples, the

PER was 1.51. The PER dropped to 1.17 for the sample roasted to 185°C. The only other deviation from inverse relationship was noted in the sample roasted to 185°C from 59% moisture. This sample produced a PER of 1.73, a higher value than that obtained from the 180°C roast of the same initial moisture. The explanation for this high value may be the abnormally low protein content of the diet used for measuring the PER value. The average PER values for all samples roasted to each of the three degrees of roast were 1.70, 1.46, and 1.28 for the 170, 180, and 185°C end points respectively.

The PER values for the 12 roasted samples averaged 1.48 as opposed to an average PER of 0.61 for the unroasted samples. While the roasting process improves the nutritive value of soybeans by destroying the activity of trypsin inhibitor, the resulting PER values are not as high as those obtained by more gentle heating. PER values greater than 2.00 are easily obtained for soybean protein that has been properly heat treated (autoclaving at 121°C for 5-10 minutes) in the presence of sufficient moisture (Hackler _et al_., 1965). Such a treatment will achieve the desired destruction of trypsin inhibitor activity without significant destruction of the essential amino acids. The higher temperatures attained during the roasting process result in the destruction of some of the essential amino acids. The loss of essential amino acids contributes to the lowering of the PER value.

Based on these results on the quality and acceptability of roasted soybeans, it is apparent that the dry roasting process is a simple method of producing an edible food from soybeans. Roasted soybeans could serve as a high protein snack food or be used as a valuable protein-enriching adjunct in the candy industry. Another possibility is the grinding of roasted soybeans with the addition of sufficient oil to produce a peanut butter analog.

When the similarity of roasted soybeans to roasted peanuts is contrasted with the raw product cost ratio (ca. 1 to 5), extensive product development using roasted soybeans as an ingredient would seem to merit considerable attention.

Oil Roasting Studies

For the oil roasting work, a preliminary survey of different soak times and temperatures was conducted in order to find the proper pre-treatment required to produce a desirable product. Soak temperatures of 100, 90, 70, and 50°C were used initially. After soaking, the beans were dehulled by passing them through a Quaker City Mill (Model F No. 4) which had the grinding plates spaced far enough apart to effect a loosening of the hulls from the beans while producing a minimum of damage to the cotyledons. In the oil roasting work, dehulling was desirable in order to avoid the deposition of oil between the hull and the cotyledons. After the hulls were loosened from the cotyledons, they were separated by a water flotation procedure. The coytledons were then deep-fat fried in corn oil at 190°C. A small deep-fat fryer (Sunbeam, Model TC F-6) was used for the initial work, and a Hotpoint, Model HK-3, deep-fat fryer was used for the preparation of the larger samples.

Texture measurements were made on the samples of beans which were soaked at the various temperatures for increasing periods of time. All samples were roasted for 3 minutes at 190°C. The test was conducted using an Instron texture measuring device employing a round, flat-faced punch 1/16-inch in diameter to puncture individual cotyledons. The results show that the higher soak temperatures produce a softening effect on the roasted product in much less time than do the lower temperatures. From the texture values, it would appear that a short soak at 100 or 90°C would be the ideal pre-treatment. Such pre-treatments were found to cause considerable difficulty in dehulling, and the texture of the finished product, although soft, was found to become mealy when chewed, causing it to be quite objectionable to a screening panel of four people who sampled the product from the various pre-treatments. Based on the results of the screening panel, a soak time of 2 hours at 70°C was used in the subsequent experimentation. Such a soaking treatment produced a hydration ratio of approximately 2.30 or a moisture content of approximately 60 percent. This pre-treatment produced a significant decrease in the bulk density from approximately 0.7 for the raw product to approximately 0.4 for the finished product.

An experiment was next conducted using roasting times of 0, 2, 3, 4, and 5 minutes. A zero cooked sample was prepared from soaked beans that were freeze-dried for use as a control. Hunter L values were determined on the roasted soybeans and the results show that there was an increase in darkness of the beans subjected to the longer frying times. The sample roasted for 4 minutes was judged to be superior in flavor by the screening panel.

The volatile flavor components of deep fat-fried soybeans have been isolated, fractionated, and identified with the techniques of gas chromatography and mass spectrometry. Several carbonyls, aromatic compounds, pyrazine and pyrrole derivatives have been identified. These compounds are similar to those isolated from roasted peanuts and, thus, account for the "peanut butter-like" aroma of deep fat-fried soybeans.

Although the nutritional quality of deep fat-fried soybeans has been discussed in some published reports, no research has been conducted to find out at what point optimum nutritional quality would occur as a result of deep fat frying (oil roasting). Therefore, the previously discussed samples (roasted for 0, 2, 3, 4, and 5 min.) were fed to rats to determine protein efficiency ratios.

The adjusted PER values indicate that the nutritional quality of the protein was improved initially by deep fat frying due to inactivation of trypsin inhibitors, while cooking for as long as 5 minutes resulted in a marked depression in protein utilization. The results on trypsin inhibitor (TI) retention shows that only 47% of the initial TI activity remained in the zero deep fat-fried sample. Thus, 2 hours of hydration at 70°C inactivates 53% of the TI.

The soybeans that were deep fat fried for 2 minutes had a PER value that was significantly higher than any of the other values. The sample cooked for 5 minutes had a significantly lower PER value.

The amino acid values for the deep fat-fried samples are helpful in explaining the decreased utilization of protein as measured by protein efficiency ratios. Cystine, tryptophan, and lysine decreased with increased cooking time. Since neither lysine nor tryptophan is the first limiting essential amino acid in soybeans, it would seem logical to assume that the decreased utilization of the protein from the deep fat-fried soybeans was due to an initial decrease in cystine. Cystine data appear to be valuable in predicting an over heat-processed soybean product, while TI is of value for an under-cooked soybean product.

Deep fat-fried soybean cotyledons that are properly heat-processed represent a nutritious food product. As the protein is of better quality than peanut protein, they should be evaluated as a possible snack item by the food industry.

UTILIZATION OF THE RESIDUE FROM SOY MILK MANUFACTURE

Potential uses of the residue (insoluble fraction) remaining after the preparation of a water extract (soy milk) from soybeans is one of the concerns of food companies looking at the possible acceptance of soy milk.

Two possibilities immediately come to mind; one is to sell the material as an animal feed and, secondly, to process it into a food product suitable for human consumption. The residue is bland, thus it could be flavored to suit various tastes.

A major stumbling block to the use of the residue as an animal feed or as a food for man is the presence of enzyme inhibitors in the uncooked material which interfere with the utilization of its protein. Once the inhibitors have been destroyed, the material may be dried for animal consumption or processed into suitable food for humans.

Therefore, some studies have been conducted to monitor the loss of trypsin inhibitors (TI) in the residue. The first experiment was conducted to follow the elimination of TI in the residue heat processed at 212°F. The results are shown in Table 4.

Table 4. The destruction of TI in soy milk preprared by either a cold or hot grind procedure and then processed at 212°F

Processing Time, Minutes	TI Retention, % Cold Grind	Hot Grind
0	45	30
15	18	14
30	13	11
45	8	6
60	4	>1
90	2	>1
120	>3	>1

Earlier studies with soy milk have indicated that a TI retention of less than 20% is necessary before one can expect significant utilization of soy milk protein. We consider 10% TI retention as a safer level and one ch will not interfere with protein utilization as measured by PER. The results in Tables 4 and 5 indicated that heat processing the residue, either for 5 minutes at 250°F or

Table 5. The destruction of TI in soy milk prepared by either a cold or hot grind procedure and then processed at 250°F.

Processing Time, Minutes	TI Retention, % Cold Grind	Hot Grind
0	45	30
5	--	8
10	--	9
20	>8	>8
40	>1	>1
80	>1	>1

for 45 minutes at 212°F will decrease the amount of TI retained to 8%. At that level, good utilization of the protein could be expected. It should be pointed out that it is necessary to process the residue in thin layers in order to have good heat penetration.

Residue has been fed to rats and the PER values indicate that the protein quality is good (PER values of 2.1 and higher have been obtained). Thus, its potential usefulness as either an animal feed or human food is well documented. Its physical and chemical characteristics make the residue suitable for processing into a breakfast cereal similar to "All-bran". This has been tried successfully in our laboratory.

NUTRITIONAL VALUE OF SEVERAL SOYBEAN VARIETIES

Questions arise periodically concerning possible differences in the nutritional value of the protein from different soybean varieties. This is an important question that needs to be answered, since soy products are being advocated as a potential aid in improving the nutritional status of children. Furthermore, protein quality and general nutritional status may have an impact on their mental and physical development. Therefore, it seemed desirable to evaluate the utilization of protein from several soybean varieties by weanling rats. Varieties were selected that may be adaptable for growing in other countries, as well as two special use varieties of the large seeded type.

The varieties studied and the PER values obtained are shown in Table 6. Disoy and Kanrich are the two large seeded varieties that have special uses. Statistical analysis of the PER values indicate that the Hood variety is significantly lower than all the other values. Also, Kanrich is significantly lower than Lee but not different from Disoy, Harosoy, Bragg and Hampton. These results show that biological evaluation is necessary in determining protein quality and that protein content, as measured by Kjeldahl, is of limited value. Since varietal differences were observed in the utilization of protein, one could speculate that flavor of the soybean extracts might also be different, thus some knowledge of the variety to be used is important.

Table 6. PER Values for Several Soybean Varieties

	Adjusted PER*
Field Varieties	
Bragg	$2.10^{bc} \pm .08$
Hampton	$2.10^{bc} \pm .10$
Harosoy	$2.07^{bc} \pm .06$
Hood	$1.78^{a} \pm .05$
Lee	$2.14^{c} \pm .03$
Special Use Varieties	
Disoy	$2.00^{bc} \pm .03$
Kanrich	$1.96^{b} \pm .06$

* PER values having the same letter superscript are not statistically different at a probability level of 5%, while those with different letter superscripts are statistically different (PER values were adjusted to a Casein standard = 2.50)

CHEMICAL AND PHYSICAL INVESTIGATIONS

IDENTIFICATION OF A VOLATILE COMPONENT IN SOYBEANS THAT CONTRIBUTES TO THE RAW BEAN FLAVOR

The raw bean flavor of soybeans has been recognized as a major flavor defect when soybeans are used as food for human consumption. The major objectionable flavor has been described as "green bean-like". This flavor is not present in the original intact raw, whole soybean but develops immediately after maceration of the bean. The formation of the compound probably results from enzymatic action, since its appearance is rapid and only in the unblanched product. Other food products are known to form characteristic flavors by enzymatic action following maceration - i.e., horseradish and onions.

The characterization of the component responsible for the flavor defect and an insight into its mechanism of formation should aid the development of soybean products more palatable for human consumption.

Sample Preparation

A sample of 350 grams of soybeans (var. Clark) was ground in a Waring Blendor (Model CB-4) with 2800 ml. of distilled water. The initial grinding was accomplished at the low speed for 30 seconds, immediately followed with the high speed for 1.5 minutes. The initial temperature of the mixture was 25°C; however, because of the heat generated during the grind, the final temperature was between 34° and 35°C.

The gas chromatogram of the head space sample shows the peak which exhibited the green bean odor. This peak was located using the "sniffer". The odor imparted by the remaining peaks was bland and could be described as "aldehyde-like." The area of the peak exhibiting the raw bean odor was small in relation to the other components eluted. However, the odor was very strong and penetrating.

When this peak was subjected to mass spectrometer analysis, a parent peak of m/e 84 was observed, while the base peak was present at m/e 55. The base peak suggests a vinyl ketone ($CH_2=CH-\overset{+}{C}=O$). The difference between the base and parent peaks indicates that the remainder of the molecule was an ethyl group (m/e 29). The mass spectrum for ethyl vinyl ketone was obtained with an authentic sample. A comparison of the spectra of the unknown and the known compounds indicates that the compounds are identical. The retention times of the unknown and the authentic ethyl vinyl ketone further substantiate the identity of the compound.

Next, soybeans were ground as described above, but the temperature of the water was 90°C. The raw bean odor was not observed during grinding or in the final slurry. When this slurry was subjected to vacuum distillation and gas chromatographic analysis, the ethyl vinyl ketone peak was eliminated. Soybean milk prepared in this manner had no raw bean odor. When ethyl vinyl ketone was added to this milk to give a concentration of 5 p.p.m., the raw, green bean odor was judged to be present.

The green bean flavor is not the only flavor which contributes to the total raw bean flavor of soybean milk. Other components formed during the oxidation of lipoxidase have an effect on the over-all total raw bean flavor. However, compared to the green bean flavor, ethyl vinyl ketone, they are relatively mild in odor intensity. These data coincide with the results of Hill and Hammond which attribute the flavor imparted in the early stages of the autoxidation of soybean oil to mixtures of pentanal and ethyl vinyl ketone.

The theories of autoxidation of unsaturated fatty acids, together with the action of lipoxidase in soybean milk suggest a possible mechanism of formation by the oxidation of linolenic acid.

During the soaking of soybeans at temperatures around 50°C prior to grinding and milk preparation, an unsaturated alcohol, 1, octene-3-ol forms through an enzymatic process. This alcohol has an odor described as mushroom-like, musty and earthy. It had previously been found to be the cause of mushroom flavor in oxidized dairy products. The threshold for organoleptic detection in soy milk is about 1 ppm, while it can be produced in beans soaking for 6 hours to levels of 40 p.p.m., yielding milk containing 4 p.p.m.

When soybean milk is prepared by a cold water extraction, a great array of volatile compounds are formed which produce a rancid-beany flavor in the product. These compounds have been separated by gas chromatography and analyzed by gas chromatographic retention time and mass spectrometry. The largest component was identified as hexanal which comprised about 25 weight percent of the collected volatiles. Other components which have been identified or tentatively identified are listed below. Those with appreciable flavor impact are indicated with an asterisk.

Pentane	1-octen-3-ol*
1,7-octadiene	2-octen-1-ol*
Pentanal*	2-octenal*
Decane	2,trans-4,cis-heptadienal*
Diphenyl ether*	2,trans-4,trans-heptadienal*
Hexanal*	Benzaldehyde*
1-penten-3-ol*	Nonanal*
Heptanal*	1-octanol
2-heptanone*	2-nonenal*
2-hexenal*	2,trans-4,cis-nonadienal*
n-propylbenzene	2,trans-4,trans-nonadienal*
5-hydroxymethyl furfural*	Hexanoic acid*
3-octanone*	Heptadecane
2-octanone*	2,trans-4,cis-decadienal*
Octanal*	2,trans-4,trans-decadienal*
Vinyl amyl ketone*	Heptanoic acid*
n-butylbenzene	Benzathiazole*
Hexanol	Gamma-nonalactone*
3-octanol	Propyl furan*
2,4-hexadienal*	Pentyl furan*

Hexanal comprised about 25 wt. % of the volatile sample. Fujimaki et al. reported approximately 10 p.p.m. of hexanal in raw soybean. With an extremely low threshold, hexanal is probably one of the major compounds contributing to the disagreeable aroma of soybean milk. On exposure to air, hexanal could be oxidized to hexanoic acid which possesses a harsh, fetid odor. Hexanoic acid was found in defatted soyflour and raw soybean.

Hexanol, possessing a harsh, grassy odor at high concentration, was considered one of the alcohols which contributed to the green-beany odor of raw soybean.

2-Hexenal, known as leaf aldehyde, possesses a pronounced odor of green foliage. t-2-hexenal could be derived from cis-3-hexenal during soybean milk preparation and volatile extraction.

Both 1-penten-3-ol and 1-octen-3-ol were reported in reverted soybean oil. 1-Octen-3-ol could be formed during the soaking of soybeans in water as pretreatment for soybean milk preparation. This compound has a moldy odor with the threshold between 0.5 and 1.0 p.p.m. in soybean milk.

Amyl vinyl ketone is identified in a soybean product for the first time. This compound has been found in oxidized milk fat and the decomposition product of corn oil.

2-Pentyl furan was described as possessing a characteristic beany or grassy odor, and predominantly responsible for the reversion flavor of soybean oil.

Not all of the compounds identified in this investigation possess a disagreeable odor. For example, it is well recognized that benzaldehyde possesses a cherry or almond-like aroma; octanol, nononol, and 1,1-diethoxypropane have an odor slightly reminiscent of roses; most of the aliphatic methyl ketones possess fruity aromas; 2,4-decadienal was reported as having potato chip-like odor and γ-nonalactone possesses butter or coconut-like aroma. However, these compounds seem to occupy a very small portion of the total volatile flavor components of soybean milk.

The majority of the identified compounds possessed undesirable odors. Several compounds could be described as beany.

Since no effort was made to relate the concentration of each identified compound occurring in the soybean milk, and probably a great number of compounds are still unidentified, the flavor reconstruction to form characteristic odors of soybean milk remains to be solved. No single peak in gas chromatograms could approximate the overall off-flavor of soybean milk, and it may be concluded that the typical green-beany flavor of soy can probably be ascribed to a mixture of many compounds.

The rancid odor of distillates disappeared after treatment with 100 ml of 2,4-DNPH. After removal of the precipitate and redistillation of the treated distillate, a distillate was obtained absent of rancid odor. The GLC profile of this distillate showed the removal of practically all peaks. One substantial peak remained after this treatment which had a retention time identical to one of the main components obtained in the hot soak procedure of soy milk preparation.

The contribution of carbonyls to the rancid off-flavor in soy milk is established and is known to be derived from autoxidation of the lipid fraction. An oxidative enzyme system, most likely lipoxidase, is the primary catalyst for the oxidation of the fat in soybeans as evidenced by treatments which are known to inactivate enzyme activity. The best evidence for proof of lipoxidase catalyzed autoxidation arises from the observations of rancid off-flavor formation immediately following grinding of the beans with water. A non-enzymic autoxidation should have a considerable induction period and would not be expected to produce the quantities of volatiles obtained by the methods of soy milk preparation in this study.

Soy milk prepared in an oxygen free atmosphere at 25°C exhibited few volatile components provided the milk was heated to 95°C shortly after grinding in water. The odor of the sample was not noticeably "beany". The general description assigned to the milk was cooked "cereal-like". A similar description was given to samples of beany milk from which the volatile components had been removed by distillation.

Hydrogen sulfide was found to be evolved from heated soy milk. Position identification was made by trapping the hydrogen sulfide in lead acetate, and then liberating the sulfide with acid and confirming identity by gas chromatography. Other sulfur compounds were also detected but not identified.

Recently some Japanese workers reported the isolation of a bitter peptide thought to contain pyroglutamic acid (PCA). Since PCA may contribute to organoleptic properties of soybean products, investigations have been undertaken to determine its presence in soy milk. Thus far, no detectable quantity of PCA in either a control or oven-heated soy milk has been found. It is possible that the extraction procedure was not appropriate for splitting PCA from a soy peptide, however, the procedure used was adequate for extracting PCA from beet puree.

Other research was conducted to determine the dipeptides present in soy milk since they may be responsible for the bitter, astringent taste. Three large samples of soy milk have been prepared in the pilot plant for use in flavor studies, as well as chemical studies, on the possible role of peptides in soy flavor.

Thus far, elution times and color ratios of 39 dipeptides have been determined by automated column chromatography procedures. Subsequent analysis of six picric acid supernatants revealed four peaks of significant size which were eluted in the dipeptide range. It appears that the quantity of each of these eluted compounds is dependent upon the processing procedures used in the preparation of soy milk. Published information from other laboratories has shown the presence of two dipeptides in soybeans. Both of these previously isolated dipeptides have a sour to astringent flavor. No mention has been made in the literature concerning the possibility of other dipeptides. If these compounds and the processes by which they are released can be identified, we may be able to improve the flavor of soy milk.

DETERMINATION OF SOY MILK FAT

An important component of soy milk is the fat and a rapid method of its measurement would be of value in quality control. A method has been developed, based on a modified Babcock test used for cow's milk, utilizing a surface active agent to help secure a clean fat column.

The method is as follows:

Equipment and Reagents

Pipette T.C. 17.6 ml - T.D. 17.5 ml

Babcock test bottle 18 grams - 8%

Centrifuge - capable of being heated to 130-140°F

Sulphuric acid - Sp. Gr. 1.82 at 68°C. Can be prepared from H_2SO_4 Sp. Gr. 1.84 by mixing 965 ml H_2SO_4 and 43 ml H_2O

Alkyl dimethyl benzyl ammonium chloride - 17% soln.

Procedure

1. Pipette into the Babcock test bottle, 17.5 ml of soy beverage.

2. Add 16 ml of the H_2SO_4 soln. (prepared by the addition of 2.7 ml of 17% soln. of alkyl dimethyl ammonium chloride to 100 ml H_2SO_4 Sp. Gr. 1.82. This reagent should be prepared fresh daily.).

3. Mix by a rotary movement for approximately 1 minute.

4. Centrifuge in the heated centrifuge at 130-140°F at 3,200 rpm for 5 minutes.

5. Add hot water (over 160°F) to bring the fat near the top of the bulb (almost to the base of the neck).

6. Centrifuge for 2 minutes in the heated centrifuge.

7. Add hot water (over 160°F) to bring the fat up to the calibrated neck of the bottle.

8. Centrifuge for 1 minute in the heated centrifuge.

9. Temper in a hot water bath (130-140°F) for 5 minutes. Make sure the fat column is below the surface of the tempering water bath.

10. Read as described for a Babcock test to the nearest 0.1% and estimate to the 0.01%.

11. Tare a 25-ml beaker, add 17.5 ml of soy beverage to the beaker, and weigh. Calculate the weight of 17.5 ml.

12. To calculate the percent fat:

$$\text{\% fat in soy beverage} = \frac{(\text{\% fat read on column})(\text{wt. of 17.5 ml})}{18.0}$$

This test is based on the Connecticut Method (H. L. Wildasen and E. O. Anderson, "Improved Babcock Test for Analyzing Homogenized Milk," Storrs Agr. Expt. Station Bulletin 287, 1952).

To test the precision of the method, a sample of soy beverage was tested. Ten replicates were employed. The following results were obtained:

$$\bar{X} = 1.56$$
$$S\underline{y} = \pm 0.031$$
$$Sy = \pm 0.01$$

To test the accuracy of the method, the quality control method was compared to the methanol:chloroform extraction of soy milk. The water was removed from the soy milk under vacuum and the fat extracted using $CHCl_3$:MeOH (2:1 v/v). The results compared favorably $\pm$ 0.1% fat.

Further, the specific gravity at 15°C was determined for the soy milk by the Westphal balance, hydrometer, and weighing a known volume.

	Sp. Gr. @ 15°C
Westphal balance	1.013
Hydrometer	1.011
Weight of known volume	1.011

EFFECT OF PROCESSING METHODS ON OFF-FLAVORS OF SOYBEAN MILK

The purpose of this study was to investigate the effect of various processing techniques and conditions on the development of volatile off-flavors during the production of soybean milk. Previous investigations on soy milk processing indicated that flavor was the most detrimental factor to acceptability of this product.

Soybean milk has been prepared for hundreds of years in the orient by a standard method of soaking beans in water for several hours, followed in order by grinding with water, filtration, and cooking for about 30 min. Although this conventional process method is relatively simple, the techniques and conditions have not been thoroughly investigated for producing a soy milk with a bland or desirable aroma.

The soy milk off-flavor problem is mainly associated with volatile compounds. Preliminary experiments, utilizing gas chromatography instrumentation, demonstrated that the volatiles responsible for off-flavor were derived only from soy milk prepared from whole fat soybeans. Soy milk prepared from soybeans which were fat extracted prior to hydration exhibited few volatile constituents by gas chromatographic analysis. The extracted fat fraction from soybeans, after exposure to air or oxygen, developed rancid off-flavors and produced complex chromatograms similar to that of soy milk from whole fat beans. The soy milk preparations which contained the fat were subjectively described as beany and rancid, while soy milk from fat-extracted beans had a comparatively bland, cereal-type odor. The rapid formation of rancid flavors occurred almost instantly during the grinding of soybeans with water and was shown to be due to oxidation of polyunsaturated fats catalyzed by lipoxidases.

It was postulated that soy milk could be produced with a minimum of off-flavor development by developing techniques and conditions involving a high-temperature, rapid-hydration grinding process that would inactivate the lipoxidase enzyme and yet attain protein extraction to produce an acceptable whole fat soy milk.

Soymilk Preparation

A ratio of 10 parts water to 1 part dehulled Clark soybeans was used for soy milk preparation in both laboratory and pilot plant investigations. Soybeans were soaked overnight in 25-30°C water, and the soak water drained. The amount of fresh water added to attain a 10:1 ratio was then calculated. Soaked and unsoaked beans were ground with water at temperatures between 20-100°C in a Waring blendor or on a pilot plant scale with a Rietz Model RA-4-K53 disintegrator.

After grinding for 5 min. in a Waring blendor, the milks were passed through a heavy-duty milk filter. Larger quantities prepared with the disintegrator were either passed through a milk filter or a plate and frame filter press after a 10-min. hold at 90-98°C.

The rancid flavor of soy milk resides in the volatile fraction. This defect can be corrected either by removing volatiles as a final step in processing or by preventing their formation. Since soy milk is composed of both fat and aqueous phases, difficulties can arise in attaining satisfactory flavor levels by stripping the volatiles in the final phases of the processing operation. Preventing volatile off-flavor formation offers a more feasible solution.

Water Temperature and Formation of Volatiles

The decrease in formation of volatiles found with higher water temperatures at grinding indicated thermal inhibition of the oxidative rancidity flavor mechanism attributable to lipoxidase enzyme activity. Precautions were taken to eliminate escape of volatiles, so that differences in the volatile present did not represent a distillation loss to atmosphere at the higher temperatures. The Waring blendor top was fitted with a gasket seal. The only procedure which resulted in any volatile losses occurred during the rapid transfer of the milk from the blendor to the distilling flask and all such transfers occurred at milk temperatures below 85°C. The formation of volatiles was inversely related to water temperature at the initiation of grinding.

Gas chromatography profiles representative of soy milks produced in the pilot plant operation with 20, 80, and 100°C water in the grinding operation confirmed the laboratory scale experiments on the relation of water temperature at grinding to volatile formation.

Heat Inactivation and the Grinding Operation

Gas chromatography of the flavor components yielded profiles demonstrating that heat inactivation of lipoxidase must be accomplished concomitant with the grinding operation with water at a minimum temperature of 80°C. Following the initial grinding, heating in the jacketed kettle for 10 min. at 93°C would irreversibly inactivate lipoxidase. The differences in the profiles reflected the importance of water temperature during the initial seconds of grinding. Heating at a minute or two after a cold water grinding cannot repair the flavor damage that occurs during and immediately following a cold water grinding operation.

The distillable volatile content of the processed soy milk decreased with storage at 20°C. Since most of these volatiles are carbonyls, their decrease may possibly be attributed to a carbonyl-amine reaction.

Inactivation of Lipoxidase

Attempts were made to inactivate lipoxidase prior to the hot-hydration extraction procedure. The Gas Chromatograph profiles of soy milks prepared with 25°C water were used as an indication of lipoxidase inactivation. Treatments with dry heat at 100°C for periods up to 30 min. on whole or dehulled beans had little effect on lipoxidase activity or protein extractability with water.

Dry heat at 200°C for 15 and 30 min. considerably lowered the lipoxidase activity, with 30 min. showing the greatest reduction in activity. At the same time, protein extractability with water was drastically reduced. After 30 min. at 200°C, a roasted bean odor became perceptible. Subjective evaluation of the odor rising from the beans during heating indicated that only a very minor amount of volatiles was released on dry heating, none of which were considered of a typically rancid nature or beany in character.

Beans soaked at various temperatures for 5 hr. and ground at 100°C resulted in a lower solids yield at soak temperatures above 25°C when compared with unsoaked beans ground at 100°C. At soak temperatures of 70°C and higher for 5 hr., the yields of replicate samples had a greater variability and pointed to further reduction in filtrable solids.

When water at 100°C was employed in the grinding process, there were few differences observed in gas chromatography profiles between beans soaked for 18 hr. at 25°C and unsoaked beans. However, at 80°C grinding, the soaked beans produced a larger quantity of volatiles than unsoaked beans. Apparently, the low temperature soak treatments brought the lipoxidase system to a potentially more active state at the time of grinding as evidenced by the 80°C grinding process. The soaking treatment does not offer any advantages over unsoaked beans in a high temperature, rapid hydration grinding process in terms of volatile off-flavor formation.

A temperature of 80°C may be considered as the borderline or the lowest effective limit for preventing significant volatile development. NDGA (nordihydroguaiaretic acid) was beneficial in the rapid-hydration grinding operation between 60 and 80°C. These results would confirm the work of Blain et al. and Siddiqui et al. that NDGA is an effective antioxidant where the lipoxidase system is active in catalysis of the oxidation of cis-cis 1,4-pentadiene fatty acids or esters. At hydration temperatures below 60°C it was not an effective antioxidant, under present Food and Drug Administration tolerances, due to the overwhelming catalytic effect of the natural lipoxidase content of soybeans.

VOLATILE FLAVOR COMPONENTS OF COCONUT MEAT

Coconut, the kernels of nuts of the coconut palm (*Cocos nucifera*), and its oil has been produced in the tropics and subtropics from the earliest times. Large amounts of coconut and coconut products are utilized for human consumption in the areas of production. Desiccated coconut meat, known as "shredded coconut" in the United States, is used as an ingredient in confectionery and bakery products.

Coconut possesses a pleasant, characteristic aroma which appears to be an acceptable flavor to most people. Wolf studied the organoleptic properties of grated coconut. Allen reported the presence of methyl ketones (C_7, C_9, C_{11}, C_{13}, C_{15}) and delta-lactones (C_6, C_8, C_{10}, C_{12}, C_{14}) in the volatile flavor constituents of coconut oil.

Coconuts were obtained from a local grocery store. 150 g of shredded coconut meat were ground in a Waring Blendor with distilled water (1:5, w/v) at low speed for 3.5 min. The slurry was distilled at 97°C under vacuum and the volatiles collected at 0°C. The volatiles were extracted from the combined distillates with redistilled CS_2 in a continuous liquid-liquid extractor.

Chromatographic separation of the volatile flavor of coconut meat gave approximately 32 peaks. Not all of the peaks are identified because of mass spectral sensitivity limitations combined with overlapping peaks. Definite identification of 15 volatile compounds was obtained. The authentic compounds of the identified volatiles were sampled in bottle by untrained panelists for odor description.

In addition to even-numbered delta-lactones and methyl ketones reported in coconut oil, 11 compounds, i.e., octanal, 2-heptanol, 2-octanol, 2-nonanol, 2-undecanol, hexanol, octanol, 2-phenylethanol, benzothiazole, delta-undecalactone, ethyl decanoate were for the first time found in coconut.

Relatively large amounts of delta-C_8 and -C_{10} lactones were present. Their characteristic, coconut-like aroma is responsible for the typical flavor of coconut meat.

Dimick et al. recently reviewed the occurrence and biochemical origin of aliphatic lactones in milk fat and proposed the existence of saturated fatty acid delta-oxidation in the ruminant mammary gland. Tang and Jennings found delta and gamma-C_8 lactones in apricot and pineapple; delta and gamma-C_{10} lactones in peach and apricot. Fioriti et al. reported both delta- and gamma-aliphatic lactones in highly peroxidized soybean and cottonseed oils but none of the lactones could be found in fresh, refined soybean oil. These lactones are postulated to arise from the high levels of hydroperoxides from the unsaturated fatty acids.

Delta-lactones but not gamma-lactones are found in coconut meat or its oil. Although catalytic pathways such as delta-oxidation of saturated fatty acids may be involved in the formation of delta-lactones in coconut, it is also possible that the precursors of delta-lactones may arise from the intermediate compounds involved in the biosynthesis of higher fatty acids, and specific enzymes may be involved.

A large amount of n-octanol with its lilac odor may also contribute significantly to the coconut meat flavor. The n-octanol probably arises from its acetate which serves as an intermediate compound in the biosynthesis of fatty acids and lactones in coconut. Kohashi et al. reported the preparation of n-octanol from the reduction of coconut-oil fatty acids at high temperature and pressure.

One of the distinct features of the coconut-meat flavor profile is the presence of secondary alcohols (C_7, C_8, C_9, C_{11}).

A small amount of benzothiazole was detected. It has been reported in butter oil, stale milk, and Swiss cheese. The origin and possible precursor of benzothiozole are not known.

EFFECT OF PHYSICAL AND CHEMICAL PROCESSING FACTORS ON THE REDISPERSIBILITY OF DRIED SOY MILK PROTEINS

Soy milk (SM) has been given considerable attention as an economical high protein food suitable for overcoming the malnutrition of infants in developing countries. Recently a limited quantity of bottled soy milk drinks has become commercially available in some areas. An alternative, soy milk as a dry powder, might have advantages of convenience and economy, because of easier transportation and preservation along with centralized large scale production.

One of the most important qualities desired for powdered soy milk is a high degree of redispersibility in water. In the manufacture of powdered soy milk, soy milk must be heated in order to inactivate the anti-nutritive factors and avoid off-flavors. However, when the powdered soy milk is prepared from the heated soy milk solution, large amounts of the proteins are insolubilized during drying, even when freeze dried.

In order to obtain basic knowledge to aid in solving this problem, various physical and chemical processing factors which influence the redispersibility after drying were systematically investigated.

Measurement of Redispersibility of Dried Soy Milk

10 ml. of soy milk were transferred into a 250 ml beaker and dried in a 50°C constant temperature room for 16.5 hours. This drying procedure was adopted as a convenient, reproducible method suitable for handling a large number of samples.

To the beaker containing the dried soy milk, water was added at the rate of 77 ml per gram of soy milk solids. After shaking for 2.5 hours at room temperature on a 170 rpm rotary shaker the liquid was filtered through milk filter paper (Agway Co., No. 87-0440). The total nitrogen of the filtrate was measured by the semi-micro Kjeldahl method. In the subsequent presentation it should be borne in mind that redispersibility and solubility refer to the state of the soy solids after the above procedure.

Effect of Temperature and Time of Heating Before Drying

The heat treatment of soy materials is necessary in order to destroy naturally occurring anti-nutritive factors. This beneficial effect of heating can be achieved by a variety of times and temperatures, keeping in mind that excessive heat treatment decreases the nutritional value. It is important, therefore, to examine the effects of various heating temperatures and times on the redispersibility of dried milk in order to obtain a product combining high nutritional value and high redispersibility. At very high temperatures coagulation occurred but there were limiting temperatures below which coagulation did not occur regardless of the heating time, and these limits were higher with decreased concentration of soy milk. For instance, the limiting temperatures for the 3.5, 5.0, 6.5, and 8.0% solids soy milk were about 130, 129, 127, and 126°C respectively. Further, it was found that preliminary heating of soy milk below the limiting temperature inhibited the coagulation which otherwise occurs in subsequent treatment above the limiting temperature. When heating was carried out under conditions not producing coagulation, the redispersibility decreased rapidly to a minimum at 10 minutes heating and then increased gradually at 100°C heating and more rapidly at 120°C heating. In other words, beyond a point in heating time, the redispersibility increased with a prolongation of the heating time. The redispersibility increased markedly with increased pH and with lowered solids concentrations of soy milk both during heating or just prior to drying suggesting that intermolecular interactions of the proteins both during heating and drying are related to redispersibility after drying.

Effect of Disulfide Splitting Reagents

It is known that the major soybean protein molecules have more than two free -SH groups per one molecule and can polymerize through the formation of intermolecular disulfide bonds when precipitated at the isoelectric point. In order to examine whether disulfide bonds are responsible for the insolubilization of dried soy milk proteins, various concentrations of disulfide bond splitting reagents were added to soy milk just prior to heating and drying. The results show that Na_2SO_3, cysteine, and 2-mercaptoethanol greatly increased the redispersibility of soy milk after drying. However, it should be noted that there was a maximum of redispersibility at very low concentrations of the reagents, beyond which the redispersibility decreased rapidly to a minimum value that is lower than the redispersibility without addition of reagents. Then, at still higher reagent levels, the redispersibility increased again to higher levels than before.

Since H_2O_2 is an oxidative disulfide bond splitting reagent its effect was examined. Protein redispersibility increased markedly as a result of its addition. Thus, the increase in the redispersibility by the addition of various kinds of disulfide bond splitting reagents showed that disulfide bonds were responsible for part of the insolubilization of soy milk protein during drying.

Effect of Emulsifier (Span 60 + Tween 60)

There was the possibility that a part of the insolubilization brought about by drying was due to the presence of non-polar groups on the protein molecules. If such was the case, the use of emulsifiers capable of complexing with the non-polar groups should increase the redispersibility of the dried product. Therefore the effects of emulsifier concentration and HLB value were examined. Redispersibility was increased by the addition of emulsifier to reach a maximum at a concentration around 0.6%. The effective range of the HLB value was found to be from 7.0 to 1. Generally, the degree of increased redispersibility brought about by emulsifiers was not so large.

Effect of Chelating Agent

Under certain conditions, some proteins are considered to polymerize through the intermolecular cross-linkage mediated by multivalent metals, but our results indicate that metals in soy milk were not important factors in the insolubilization of the soy milk protein during drying.

Soy milk (SM) heating conditions before drying had a large effect on the redispersibility of soy milk proteins after drying. Redispersibility decreased rapidly to a minimum at several minutes of heating at either 100°C or 120°C and then increased gradually at 100°C and rapidly at 120°C. Increased pH or decreased concentration of soy milk during heating increased the redispersibility after drying. The redispersibility was also greatly increased by decreasing the concentration of the heated soy milk, from which the soy milk was dried. The use of emulsifiers or EDTA had a slight effect on protein redispersibility. However, more effective reagents were disulfide bond splitting reagents, such as Na_2SO_3, cysteine, 2-mercapto-ethanol, and H_2O_2. The reducing reagents showed similar patterns in their relations between the added concentration and the redispersibility. For cysteine, e.g., there was a fairly large and sharp increase in redispersibility at 0.001 mol., then it dropped abruptly, later rising again to a maximum at 0.050 mol. The combined effect of cysteine and emulsifiers was additive, but the effect of cysteine was decreased by the addition of EDTA.

Quantitative studies of the insolubilization of soy milk (SM) during drying showed that polymerization took place through disulfide bonds, as indicated by the effects of sulfhydryl blocking agents, and through hydrophobic bonds, as indicated by the effect of sodium dodecyl sulfate. SM (7% solids) had 2-3 x 10^{-4} $\underline{M}$ active free -SH groups exposed on the surface of the molecules after a short time of heating, and these took part in polymerizations during the drying step leading to insolubilization of 35% of the total soy milk proteins. However, exposed active -SH groups could be inactivated by prolonged heating before drying, perhaps by the oxygen dissolved in the soy milk. On the other hand, the amounts of the proteins insolubilized through hydrophobic bonds increased evenly with the heating time before drying and reached a plateau when 40% to 50% of the total proteins were insolubilized through the hydrophobic bonds.

The heating and drying of soy milk (SM) gives rise to complicated differences in the solubility of the protein in the dried product. Short term heating resulted in more insolubilization during the drying step than did long term heating. These findings resemble those of Circle, et al., who found that the viscosity of soy bean protein dispersions reached a maximum and then fell off sharply with more severe heat treatment. An interpretation of the behavior of the total soy bean protein on the basis of the known characteristics of 7S and 11S soy proteins would be expected to have considerable validity because these two fractions make up most of the proteins of soybeans. It was previously concluded that the three-dimensional structures of the major soybean protein (7S and 11S) have more than two free -SH groups on their native molecular surface and are insolubilized through intermolecular disulfide bonds when they are brought to a precipitated state. The necessary and sufficient conditions for inter-molecular -S·S- polymerization are (a) the active free -SH groups on the surface of a molecule and (b) the distance between the molecules is small. When the proteins are kept in solution for a long time, the active -SH groups located at the molecular surface are inactivated, perhaps changed to intra-molecular disulfide bonds by the action involving the oxygen solubilized in the solution, because the long distance between the molecules reduces the opportunity for the formation of intermolecular disulfide bonds. In the case of the unheated SM insolubilization of raw SM proteins during the drying step was around 16% of the total protein in both the presence or absence of NEM (N-ethyl maleimide).

This suggests that there were not significant amounts of active -SH groups on the molecular surface of the unheated SM proteins. It is assumed that the active free -SH groups which had been located on the molecular surface had been oxidized to intra-molecular -S·S- bonds, mainly by the air introduced into SM by vigorous shaking during the initial extraction process, or by peroxides formed as a result of lipoxidase action, or they could have already formed intermolecular -S·S- bonds to an extent not yet sufficient to cause protein insolubilization. During the heating of SM the native three-dimensional structure of the proteins becomes disrupted, exposing -SH groups formerly buried inside the molecules. When such protein molecules came close together during drying, the proteins had the opportunity to become insolubilized through the formation of intermolecular disulfide bonds. However, when dilute SM was further heated, the active free -SH groups, once exposed by heat denaturation, apparently were gradually inactivated leading to a decrease in the possibility of insolubilization through -S·S- polymerization.

The inactivated products formed from the free -SH groups during heating might be intramolecular disulfide bonds, sulfenic acid (-SOH), sulfinic acid ($-SO_2H$), and sulfonic acid ($-SO_3H$) groups. The likelihood of intermolecular S-S polymerization was low since the dilute nature of the SM solution favored intramolecular reactions. Two possibilities for the oxygen donor are the oxygen dissolved in SM and the lipid peroxides produced from the soybean oil. However, the latter possibility may be unimportant because the speed of the inactivation was not influenced substantially by the lack of lipids. Therefore, the oxygen donor might be considered to have been the molecular oxygen dissolved in soy milk.

The possibility of insolubilization through covalent bonds other than the disulfide bond appeared low since almost all the proteins in the SM dried with the addition of NEM were solubilized by SDS (sodium dodecyl sulfonate) which does not split covalent bonds. Further, this solubility behavior with SDS suggests that the bonds responsible for the insolubilization through causes other than -S·S- polymerization were hydrophobic bonds, because SDS has both hydrophobic and hydrophilic portions in its molecules and can break the hydrophobic bonds among the molecules. When SM is not heated, the hydrophobic interaction among molecules occurs to only a small extent, because most of the hydrophobic groups of the native soybean proteins remain buried inside the molecules. In heated SM, however, the hydrophobic side chains of the major proteins can be exposed accompanying the break-down of the three-dimensional structure of the molecule. The intermolecular hydrophobic interaction may then occur among the resultant hydrophobic residues when the molecules come closer as a result of the evaporation of water during drying. Increased time and elevation of temperature during the heating of SM before drying increased the exposure of the hydrophobic groups, leading additional amounts of proteins to become insoluble intermolecular polymers through hydrophobic interaction. The pH in the solution is very important for the formation of the hydrophobic bonds among the molecules because of electrostatic repulsion between the molecules. With the increase of pH, each protein molecule increased its negative charge, and consequently, the mutual electrostatic repulsion increased. Thus, the formation of hydrophobic bonds was prevented or the formed hydrophobic bonds were broken, leading to an increase in the solubilized proteins. It is concluded that the insolubilization of the SM proteins during drying occurs mainly through intermolecular -S.S- polymerization and hydrophobic interaction. The complicated phenomena observed so far with regard to redispersibility of dried SM can be explained very reasonably by these mechanisms.

EVALUATION OF MONOSACCHARIDES, DISACCHARIDES, AND CORN SYRUPS AS DISPERSANTS FOR HEAT-PROCESSED DRIED SOY MILK PROTEINS

It has been found in many studies that sweetening of soy milk remarkably enhances its organoleptic acceptability. While the level of sugar depends upon the consumer preferences in any particular market area, almost all commercially available bottled soy milks are sweetened with cane sugar nearly equal in concentration to that of the soy milk solids. In some cases, the sugar supplementation may be helpful in overcoming calorie undernutrition.

During the course of an investigation on the effect of sugar on soy milk, our attention was called to the much better reredispersibility of powdered soy milk prepared from the sweetened milk. This work was undertaken to clarify the action of sugars on solubility of proteins in dried soy milk, and to investigate the possible application of corn syrups for the preparation of easily soluble and moderately sweetened soy milk powder.

The addition of glucose, fructose, and galactose prior to drying upon redispersibility showed no difference due to the type of sugar. Also, the amounts of sugar necessary to approach 100% redispersibility were almost the same for all three, about 12 g sugar per 100 ml of 8.0% (w/v) soy milk solids. No difference in the effects of sugars could be recognized between the times of the additions, before or after heating. The additions of these sugars after drying did not show any positive effect on the redispersibility of dried soy milk.

Sucrose and maltose performed equally well, and were comparable to the monosaccharides described above. However, lactose showed a much different behavior, and at no concentration did it give anything near 100% redispersibility. Since lactose itself did not show any effect of insolubilization on soy milk proteins before drying of soy milk, this may have been due to the lower solubility of this sugar, leading, perhaps, to sugar crystallization during the drying process.

From the practical viewpoint, maltose, or a mixture of maltose and sucrose, in various ratios might give a suitable organoleptic sweetness and full dispersibility to the dried soy milk at the same time, but experience indicates some technical difficulties usually accompany the spray-drying of these sugars.

Effects of corn syrups

Commercially available corn syrups are economical sweeteners. They have several advantageous properties in connection with the improvement of redispersibility of dried soy milk. First of all, they allow faster spray-drying rates than are possible with other conventional sugars such as dextrose, sucrose or maltose. Actually, lower-conversion syrups have been proved successful as drying aids for other sugars. Secondly, the great variety in their organoleptic sweetness allows arbitrary selection of corn syrups for sweetness independent of the amounts of sugars required for a full redispersion of soy milk solids.

A clear relationship between the dispersant efficiencies of corn syrups and their dextrose equivalent (D.E.) values was demonstrated in that as the D.E. increases so does the redispersibility level. At lower concentrations, the enzyme-converted type seems to be more effective than the acid-converted type, however, for each treatment, little difference exists between the corn syrups at 10 and 12% concentration levels.

Since no difference in the effects of sugars could be observed between the times of additions, before and after heating soy milk, the sugars may have had no great effect in preventing the aggregation of proteins during the heating such as was observed with an electron microscope by Kawaguchi, et al. Alternately, such aggregation of proteins may have had little effect upon the redispersibility of the dried product. The addition of sugar after drying was quite ineffective.

Sugars which show large solubilities in water and minimum tendencies of crystallization seem to be the most suitable for maintaining high dispersibility for soy milk solids. Judging from another experiment with a highly viscosious carbohydrate, crown gall polysaccharide, viscosity of sugar solution seemed not to be an important factor for the dispersant efficiency. One possible reason for the dispersant effects of sugars for soy milk solids may be simply due to a physical separation of the protein molecules. In other words, in a reduction of opportunity for contact and polymerization during or after drying.

Among sugars tested, enzyme-converted corn syrups seemed to be the most suitable materials since it is possible to regulate the organoleptic sweetness of soy milk by selecting an arbitrary D.E. or by mixing them with other sugars such as sucrose or dextrose.

REMOVAL OF OLIGOSACCHARIDES FROM SOY MILK BY AN ENZYME FROM *ASPERGILLUS SAITOI*

One of soybean's problems is its tendency to induce flatulence, often accompanied by an uncomfortable feeling of fullness and intestinal activity. Recently, it has been suggested by some investigators that the flatulence caused by soy products could be due to their relatively high contents of galacto-oligosaccharides, especially stachyose and raffinose. On the basis of this hypothesis, one can conclude that it should be possible to reduce flatulence in soy milk by removal or decomposition of these oligosaccharides.

In preliminary studies, it was found that considerable numbers of fungal strains belonging to the genus *Aspergillus* exhibited powerful abilities to produce galacto-oligosaccharide decomposing enzymes such as α-galactosidase (E.C.3.2.1.22) or invertase (E.C.3.2.1.26). Furthermore, some of the commercial enzyme products prepared from the same fungi contained considerable activities of both enzymes.

Properties of α-galactosidase

Since α-galactosidase can play a most important part in the hydrolysis of soy milk oligosaccharides, some properties of the α-galactosidase in the partially purified enzyme preparation were investigated. The preparation exhibited its optimum pH between 4.5 and 5.5, while it was almost inactive below pH 2.0 and above pH 8.0. The enzyme seemed to be stable from pH 4.0 to pH 8.0, however, it was sensitive under more acidic conditions. During 18 hr. storage in 0.1M glycine buffer, pH 2.5, at 30°C, about 95% of the activity disappeared.

The optimum temperature for the hydrolysis of melibiose was observed near 55°C. However, the enzyme itself was unstable at high temperatures. For instance, 45% of the enzyme activity was lost after the treatment at 60°C for 30 min. in 0.1M acetate buffer, pH 4.5, and it was completely inactivated by maintaining it at 70°C under the same conditions.

Kawamura (1954) determined the contents of oligosaccharides in soybeans by means of paper chromatography and reported the following composition: sucrose 3.7%, raffinose 1.0%, stachyose 3.2% and total 7.9%. In our present experiments, all of these oligosaccharides could be recognized on the thin layer chromatograms, and indicated the following contents on the dry basis of soy milk sample: sucrose 4.6%, raffinose 0.8% and stachyose 4.0% in terms of anhydrous material. In our work, using soy milk prepared from the same variety, a sugar spot, supposedly verbascose, could also be detected. Treating the soy milk with 16×10^3 units of the α-galactosidase preparation per gram of the solid soy material completely decomposed all of these oligosaccharides to their constitutive monosaccharides after a 3 hr. incubation period. During the earlier stage of the incubation, sucrose was decomposed very rapidly by the action of invertase, and parts of both raffinose and stachyose appeared to be hydrolyzed by the same enzyme to melibiose and manninotriose respectively.

These facts were also demonstrated in experiments using pure substrates, authentic raffinose, and stachyose.

Contamination of enzyme with protease activity causes many undesirable effects on soy milk such as coagulation, precipitation of protein, formation of bitterness and so on. Consequently, the enzyme preparation for the present purpose must be free from protease activity. Fortunately, the α-galactosidase from *Aspergillus saitoi* has an especially high molecular weight, therefore, the separation of it from protease(s) can be easily attained by means of a simple molecular sieving method. From another point of view, this fact indicates the possibility that the enzyme can be obtained economically as a by-product of purified protease production. In addition, the α-galactosidase from *A. saitoi* has a few advantageous characteristics. For instance, since the optimum pH enzyme activity is in the natural pH range of soy milk, there is no need to change the original pH value of soy milk. Furthermore, the enzyme itself is very stable in the same pH range, and it is not necessary to take any special care in its storage. Secondly, differing from some of α-galactosidases previously reported, the activity of the enzyme from A. saitoi is relatively insensitive to the presence of an end product, α-D-galactose, in the reaction mixture. The enzyme has an undesirable feature in that it is inhibited by some organic acids that are present in soybeans in considerable amounts.

Separate from the results described above, there is a fundamental question, namely, whether or not stachyose or raffinose are actually the main cause of flatulence of soy milk. In this connection, future physiological experiments with the oligosaccharide-free soy milk will be useful in testing this hypothesis.

AN ENZYMATIC PROCESS FOR A NUTRITIONAL BEVERAGE BASED ON SOYBEAN PROTEIN AND LEMON JUICE

Although soy milk is nutritious, it contains many kinds of unpleasant flavor components and also some flatulence factors which seem to inhibit significantly the world-wide acceptance of this beverage. It appears quite difficult to eliminate completely these undesirable factors, particularly the minor flavor constituents, from soybean or its products. For instance, it is said some unpleasant soybean flavor substances still remain, even in an isolated soy protein.

Recently Fujimaki *et al*. suggested that these contaminating flavor components might be efficiently removed by treating isolated soy proteins with some kinds of microbial acid-proteases. According to their reports, undesirable flavors, including bitterness, frequently observed in the soy protein hydrolysates obtained using other proteases, could not be recognized in the hydrolysates.

The actions of various kinds of commercially available enzyme preparations on soy milk and soy milk residue were examined. All of the proteinase preparations (Pepsin, Molsin, Trypsin, Pronase, Prozyme, Nagase, *Asp. sojae* proteinases, and Thermosin) and the macerating enzyme preparation (Macerozyme) produced a bitter taste in soy milk. However, little or no bitterness was produced from well-washed soy milk residue, when treated with Pepsin and Molsin. Cellulase and Pectinase preparations (Cellulase onozuka, Cellulosin, NBC Cellulase, and NBC Pectinase) did not produce any bitter taste. Soy milk was coagulated easily at neutral pH by all the proteinase preparations which show proteolytic activities.

Our experience has indicated that one could easily get an enzymatically modified soy protein which tasted almost bland and does not form any precipitate at pH 4.6 by treating commercial isolated soy protein with an acid-protease preparation from a higher fungus, *Trametes sanguinea*. This presents the possibility that one might prepare a clear citrus-flavored sour drink of high nutritional value without any addition of stabilizer.

Although the optimum pH for the digestion of soybean protein with the acid-protease from *T. sanguinea* lies at pH 2.5-3.0, it appears more practical to carry the reaction out at pH 3.3-3.5 because a great amount of lemon juice is needed to decrease the pH of the reaction mixture below 3.30.

In general, as the enzyme concentration increased, so did the yield of the soluble nitrogen in the hydrolysate. An enzyme level of 1,000 - 1,200 x 10^{-3} PU (at pH 3.5) seemed to be adequate for the maximum digestion of 100 g Promine D. Under these conditions, the soluble nitrogen in the hydrolysate indicated about an 88% yield. The use of higher concentrations of the enzyme resulted in a greater relative increase of formol nitrogen than of soluble nitrogen recovery. The existence of excess amounts of formol nitrogen in the hydrolysate gives a complicated meaty flavor to the hydrolysate.

Almost 100% of the water-soluble nitrogen was also soluble in 5% (^{w}/v) trichloroacetic acid. The formol nitrogen was below 14.5% of the solubilized nitrogen at 1,050 x 10^{-3} PU (at pH 3.5) enzyme concentration level per 100 g Promine D.

The enzymatic reaction usually proceeded for 8 hr. There seemed to be a slight increase in soluble nitrogen after 10 hr., the long time incubation often introduced a peptone-like aroma in the hydrolysate.

Gel-filtration of the hydrolysate indicated that the soluble fraction consisted mostly of relatively large size peptides together with a small amount of small size peptides or amino acids. This agrees with the hypothesis of Verma and McCalla that an enzymatic proteolysis under acidic conditions is apt to induce the formation of large quantities of polypeptides as the intermediates in the reaction. This might be the reason why the enzymatic hydrolysis obtained in the present method was almost entirely free from unpleasant flavors, including bitterness and meat-like taste. As a matter of fact, besides the flavor derived from lemon juice, the hydrolysate tasted almost bland. Much lower values of the % formol nitrogen, based on total solubilized nitrogen, of the hydrolysate than that of the usual enzymatic proteolysis also confirms the above result and assumption.

As a result of the enzymatic treatment, the viscosity of the isolated soy protein suspension decreased significantly. Furthermore, the rheological characteristic was dramatically changed from that of non-Newtonian, thixotropic fluid to that of a typical Newtonian solution.

The hydrolysate possessed a high buffer capacity.

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B. FAO Soybean Symposium

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