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I. Progress at Geneva

A. Engineering Studies

1. Spray drying of soy milk whey

The soy milk beverage developed in this laboratory is made by the following procedure: Whole soybeans are soaked for 2 hours in 0.06 N NaOH at 50°C. After draining to remove the excess alkali, the soaked beans are ground with hot distilled water in a ratio of 1:10 (w/w) in a Rietz disintegrator equipped with a screen having 0.023-inch diameter holes. Steam is also injected into the grinding chamber to insure a temperature of 195°F or higher in the discharged slurry. This is filtered in a plate and frame filter, and the filtrate then is either canned or dried as a soy milk beverage.

During the present report period, experiments were initiated on the preparation, acceptability, redispersibility, and nutritional quality of spray-dried soy whey using several drying temperature schedules in a small spray dryer.

Attempts were made to dry soy milk whey at 400°F with outlet temperatures ranging from 210°F down to 120°F in a pilot scale dryer using a 2-fluid nozzle. Efforts to achieve outlet temperatures of 140°F and lower by restricting the air flow through the drying chamber and using relatively high liquid feed rates were unsuccessful. The retention time was too short for complete drying of the droplets, and the air flow was insufficient for conveying the product from the drying chamber to the cyclone separator.

Some of the difficulties encountered were due to an inadequate knowledge of air velocities in the dryer and methods of controlling them, and appreciation of the degree of liquid and air nozzle pressures required to maintain the liquid feed rate within critical limits. Before pursuing further studies on the effect of spray drying conditions on nutritive quality of dried milks, a system was developed for monitoring the air flow in the dryer, determining the minimal velocity required to convey the product to the cyclone and the relationship between dry particle size and air velocity for good separation in the cyclone. More information is required on the interaction between liquid and air nozzle pressures, feed characteristics, evaporation rates in the dryer, and particle size in the dried product.

2. Total solids measurement

The first step in the preparation of soy milk by the wet process is to grind soaked beans with boiling water in the presence of live steam. The solids content of the resulting slurry is dependent upon the accuracy of metering the beans and water into the grinder. Accurate feeding of the

soaked beans is sometimes difficult because a mucilaginous exudate retards their movement through a vibratory feeder unless precautions are taken to insure continued wetting of the beans. For this reason, the solids content of the slurry should be determined frequently to maintain uniformity of composition and efficiency of grinding. Control of the solids content is also of importance in the final formulation of soy milk prior to bottling or canning and for concentration prior to drum or spray drying.

A rapid method was developed for measuring the total solids contents of slurries. A 10-gram sample of liquid is dried within 16 minutes at a temperature of 325°F in an Ohaus moisture balance under standardized conditions developed in this study. The solids contents of the sample must be adjusted so that they are less than 10%. The standard deviation of the method was found to be ± 0.2% compared to ± 0.06% for the conventional vacuum oven procedure.

3. Particle size determination

A knowledge of particle size is of value in the operation of a spray dryer since particles 5 microns or less in diameter are difficult to collect in a cyclone separator. The size of the particles in the dry powder is closely related to the size of the droplet sprayed in the drying chamber, and, hence, a knowledge of the particles in the product is of help in achieving optimal atomization consistent with adequate evaporation of the water. The presence of a large percentage of fine particles in a powder makes it more difficult to disperse in water because the finer particles rehydrate faster causing lumps or clots which contain unwetted product in the center. While this disadvantage of fine particles can be overcome by sieving and agglomeration, it is generally desirable to have as large particles as possible in a spray dried product.

The literature on particle size measurement is extensive, and the nature of various methods has been reviewed by the Analytical Methods Committee of the Society for Analytical Chemistry (Classification of Methods for Determining Particle Size, *The Analyst*, 88: 166-186, 1963). The basic method of particle size measurement is based on microscopic examination. This procedure is tedious because a large number of particles must be measured in order to obtain data that are representative of the size distribution in the sample.

Screening is a common method for determining the particle size distribution, but the experimental milk powders formed balls on an 80-mesh sieve, probably due to the presence of fat. At first, it was thought that this agglomeration of particles on the screens was due to absorption of moisture from the air, but the same difficulty was encountered when the screening was done in an atmosphere of 10% relative humidity. For this reason,

attention was turned to the development of a microscopic method for measuring particle size in spray drying studies. In the future, this method will be used as a reference procedure in checking particle distributions obtained in a more rapid sedimentation test.

The size of particles in two experimentally spray dried soy milks and a commercial soy protein isolate powder were determined microscopically. Samples of these powders were dispersed in a white mineral oil containing a dispersant to overcome clumping of the particles. A drop of this suspension was placed on the surface of a hemocytometer which was viewed through a binocular microscope equipped with a mechanical stage and a Filar micrometer eyepiece calibrated with a stage micrometer.

The particles ranged in size from 1 to 80 microns and had an average size of 8 to 10 microns. One of the experimentally dried soy milks came from the collection drum below the cyclone of the spray dryer, while the other was collected from the walls of the cone at the base of the drying chamber. The particles from the collection drum were generally larger than those from the cone and more closely resembled in size those found in the commercial protein isolate powder.

4. Heat penetration

A heat penetration study was conducted to determine the theoretical minimum process time for soy milk in #10 cans. This work was done at Geneva to develop information for application in the Philippines where it is planned to use larger containers to economize in the distribution of soy milk to Philippine school children.

B. Biochemical Studies

1. Mechanisms of insolubilization of soy milk proteins during drying. The role of disulfide and hydrophobic bonds.

In the last semi-annual progress report the influence of chemical and physical factors during or before drying on the redispersibilities of dried soy milk proteins was reported. Our recent experiments dealt with the mechanisms of the insolubilization of soy milk proteins during drying.

Insolubilization through the formation of the intermolecular -S·S- bonds during drying was examined. When various concentrations of -SH blocking agents, such as N-ethylmaleimide and p-chloromercuribenzoate, were added to heated soy milk (100°C, 20 minutes) before drying, the protein redispersibilities increased abruptly with the increase of reagent concentrations and reached a plateau at 2×10^{-4} mol of both blocking agents. This indicated that the 2×10^{-4} mol of the -SH groups of soy milk proteins were related

to the insolubilization of the proteins through intermolecular -S•S- bond formation, and 32% of the total soy milk proteins were insolubilized through these mechanisms.

We reported previously that the redispersibilities of soy milk proteins decreased rapidly with the heating time before drying, reaching a minimum at 5-10 minutes heating. Then further heating caused the redispersibilities to increase again with the much larger effects at 120°C than at 100°C. However, when the -SH blocking agents were added to these soy milks before drying, the redispersibility curves changed to uncomplicated ones in which redispersibility decreased steadily with heating time to reach a minimum. Here the redispersibilities were larger at 100°C than at 120°C. From these curves we can calculate separately the percentage of the proteins insolubilized through the -S•S- polymerization and through the other causes during drying. The results of the calculation showed that about 35% of the total soy milk proteins were insolubilized through -S•S- polymerization, another 35% were insolubilized through the other cause, and the remaining 30% were not insolubilized in a short time heating (5-10 minutes) of soy milk before drying. The amounts of the proteins insolubilized through -S•S- polymerization reached maximum (about 35%) in this short time heating (5-10 minutes) before drying and thereafter decreased with the heating time, gradually at 100°C heating and more rapidly at 120°C, finally going to zero. On the other hand, the amounts of the proteins insolubilized through causes other than -S•S- polymerization increased steadily and reached a plateau in about 30-minutes heating. The values of the redispersibilities at the plateau are about 40% at 100°C heating and about 50% at 120°C heating. This insolubilization by causes other than -S•S- polymerization is considered to be intermolecular polymerization through hydrophobic interaction among the amino acid side chains of the proteins.

We had concluded previously that native soybean protein molecules are very rigid and compact, their hydrophobic amino acid side chains located inside the molecules, and their hydrophilic amino acid side chains located outside. Therefore, when soy milk protein is native, the hydrophobic interaction does not occur during drying. Further, in raw soy milk, the free -SH groups which are located on the surface of each native protein molecule are considered to be inactivated during the production of soy milk through oxidation by contact with air. Accordingly, insolubilization through -S•S- polymerization during drying does not occur in unheated soy milk. In heated soy milk, however, the three-dimensional structure of a soy milk protein molecule is broken, and, as a result, the active free -SH groups and hydrophobic amino acid side chains are exposed from the inside of a molecule leading to insolubilization through both -S•S- and hydrophobic bonds. In the heating of soy milk the increase of time and elevation of the temperature increases the exposure of the hydrophobic groups, leading additional amounts

of proteins to insoluble intermolecular polymers through hydrophobic interaction. On the other hand, the free -SH groups, once exposed by heating, are inactivated by oxygen that may be present in water during heating. This inactivation of free -SH groups proceeds very rapidly at 120°C and more slowly at 100°C. The observed decrease of the insolubilization through -S•S- polymerization with heating time of soy milk can be explained by the inactivation of the exposed free -SH groups. In fact, the shaking of soy milk with oxygen gas during the heat treatment of soy milk led to greatly increased redispersibilities after drying. In this case, the further increase of the redispersibilities by the addition of the -SH blocking reagents to the oxygen gas-treated soy milk was not observed, indicating that all the exposed -SH groups were inactivated by oxygen.

From these results it is concluded that the insolubilization of the soy milk proteins during drying occurs both through the intermolecular -S•S- polymerization and through the intermolecular hydrophobic interaction. Thus, the complicated phenomena observed on the redispersibilities of dried soy milk could be explained very reasonably by these mechanisms.

2. Quality control tests for fat and specific gravity

With the development and introduction of new food products, quality control tests are indispensible and must be developed to evaluate experimental effects. During the past six months, a test has been developed for fat in soy beverage. Standard specific gravity determinations by several methods were found to be in close agreement.

The following modification of the Babcock test for milk fat proved acceptable for soy fat analysis:

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 per cent fat:

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

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:

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

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

C. Flavor Studies

1. Soybean milk volatiles

The isolation and identification of volatile components of soy milk produced under conditions favoring maximum enzymic and autoxidative off-flavor development has been summarized in a manuscript to be submitted to the *Journal of Agricultural and Food Chemistry*.

Approximately 80 peaks were obtained by gas chromatography, and some of these consisted of two or more compounds. Definite identifications of 41 compounds were made, and 13 others were identified tentatively. Hexanal comprised about 25% of the volatile sample. The odors of the identified compounds were described by an untrained panel. The majority of compounds possessed odors that did not fit the concept of a milk or milk-like product. The typical green-beany flavor of soy is probably derived from a mixture of many compounds.

2. Coconut meat volatiles

The isolation and identification of coconut volatiles showed 18 compounds, the majority of which were described by pleasing terms such as fruity, nutty, buttery, citrus-like, etc. The specific coconut aroma came from the lactones, delta-octalactone, delta-decalactone, and delta-undecalactone.

The presence of numerous pleasing flavor compounds in coconut confirms the advantage of blending coconut with soybean milk to improve consumer acceptance.

3. Non-protein nitrogen fraction of soy milk

A study has been initiated to determine the non-protein nitrogen content of soy milk and the affect of alkaline soaking conditions on this fraction. Studies will center on the free amino acids and other low-molecular weight, ninhydrin positive, chromatographable compounds. Since low molecular weight peptides have been reported to contribute to flavor, such an investigation is essential.

D. Nutritional Studies

1. Alkaline extracted soy milk

In an earlier report it was noted that as the pH of the extracting solvent (NaOH) increased (pH = 6.55, 7.37, 8.04, and 9.18) there was a decrease in the utilization of the protein in both the insoluble and soluble fractions. A further decrease in PER occurred when the soy milk was heat-processed for 10 minutes at 121°C. However, the PER value for the pH 7.37 soy milk appeared to be extremely low; therefore, a second experiment was designed to evaluate this specific point. The PER values obtained in the second experiment confirmed our hypothesis concerning the low value previously observed for the pH 7.37 soy milk. As the pH of the extracting solvent increased, protein utilization decreased. The average PER values for the two experiments were 2.41, 2.18, 2.20, and 1.70, respectively. Since earlier work in our laboratory, as well as in others, has shown that any change in the cystine content of soy products would be indicative of a decrease in utilization of the protein, cystine analyses were performed on the samples to check this specific point. Cystine was found to decrease (1.66, 1.41, 1.37, and 1.15 g/16g N, respectively) with an increase in pH of the extracting solvent. As a matter of fact, the changes observed in cystine agree with the changes observed in protein utilization by weanling rats. Available lysine data indicate that pH has no discernible effect on lysine availability. Results of niacin analyses indicate that the use of alkaline soaking solutions increases niacin availability.

2. Dry-roasted soybeans

The nutritional evaluation of dry-roasted soybeans is presently being completed, and a publication is in preparation. The protein-efficiency-ratio values of the roasted samples were all higher than those of the non-roasted control samples. The PER values of the roasted samples ranged from 1.87 to 0.99 and reflected the degree of roast, i.e., PER values were inversely related to roasting temperature. Amino acid analyses revealed that lysine availability is affected by roasting. According to the literature, cystine is heat-labile, but apparently it was not affected by the dry-roasting process. Cystine analyses are being repeated in order to be certain of this finding.

II. Progress at Los Baños

A. Pilot Plant Development

The Food Science pilot plant is now in active use for the preparation of both fresh and sterilized bottled soy and soy-coconut milks. Additional equipment on order includes the following:

1. A temperature-humidity controlled circulating hot air dryer.

2. A Fitzpatrick grinder.

With the above additions, the pilot plant will be able to undertake many more research problems in the food field. For example, it will permit studies on the production of quick-cooking, dehydrated foods.

B. Soy Milk Investigations

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.

No significant differences were found among the flavors of soy milks produced from Davao, Hsieh-Hsieh, and Taichung soybeans using a hot (170°F) water grind.

Comparing soy milks produced by grinding the soybeans with hot water (170°F) 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 (170°F). This more intense flavor effect in the Waring blendor may be caused by the beating with 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 per cent of the panel preferred 0.15% sodium bicarbonate over 0.1% sodium bicarbonate.

Sodium bicarbonate can be 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 per cent 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.

C. Coconut Studies

Research on fermentation as a method of separating coconut oil and protein has been completed.

Various patented and published methods of extracting oil from fresh 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 per cent 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.

D. Extension Activities

1. Peace Corps

Fifty additional Peace Corps volunteers have been given training in the manufacture of soybean milk, soybean curd, and tempeh. Some of the volunteers have spent time working on these products in our laboratory. Most of the volunteers report that soybean culture is expanding at the village level, and they want to teach the villagers how to use the soybeans for food. This is an effective way of extending information developed in the Cornell and UPCA laboratories under AID sponsorship.

2. 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 by Drs. Banzon and Steinkraus. As one result of the trip, the University of Illinois group at 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.

III. Publications

A. Published

Badenhop, A. F., and W. F. Wilkens. 1969. The formation of 1-octen-3-ol in soybeans during soaking. *J. Am. Oil Chem. Soc.* 46: 179-182.

Fukushima, D. 1968. Internal structure of 7S and 11S globulin molecules in soybean proteins. *Cereal Chem.* 45: 203-224.

Fukushima, D. 1969. Denaturation of soybean proteins by organic solvents. *Cereal Chem.* 46: 156-163.

Mattick, L. R., and D. B. Hand. 1969. Identification of a volatile component in soybeans that contributes to the raw bean flavor. *J. Agr. Food Chem.* 17: 15-17.

Puertollano, C. L. 1969. Separation of the oil and protein fractions in coconut (*Cocos nucifera* Linn.) by fermentation. Master of Science Thesis, University of the Philippines, College of Agriculture, Los Baños.

B. In Process

Badenhop, A. F., and L. R. Hackler. 1969. The effects of soaking soybeans in sodium hydroxide solution as a pretreatment for soy milk production. (Submitted to *Cereal Chem.*).

Fukushima, D. 1969. Enzymatic hydrolysis of alcohol-denatured soybean proteins. *Cereal Chem.* (in press).

Fukushima, D. 1969. High protein food products. *Proceedings of the Western Hemisphere Nutritional Congress II* (in press).

Steinkraus, K. H., L. T. David, L. J. Ramos, and J. Banzon. 1969. Development of flavored soymilks and soy-coconut milks for the Philippine market. (Submitted to *Philippine Agriculturist*).

Wilkens, W. F., and L. R. Hackler. 1969. The effect of processing conditions on the composition of soy milk. *Cereal Chem.* (in press).

Wilkens, W. F., and F. M. Lin. 1969. Gas chromatographic and mass spectral analyses of soybean milk volatiles. (To be submitted to *J. Agr. Food Chem.*).

IV. Papers Presented

A. Geneva

Fukushima, D., and J. P. Van Buren. Physical and chemical processing factors affecting redispersibility of dried soy milk proteins. AACC 1969 Annual Meeting, Chicago, Ill., April 27 to May 1.

B. Los Baños

Puertollano, C. L., J. Banzon, and K. H. Steinkraus. Separation of the oil and protein fractions in coconut (*Cocos nucifera* Linn.) by fermentation. To be presented by invitation at the September 1969 ACS meetings in New York and published in one of the Society's journals.