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The effects of financial, human, and social capital on the perception of financial well-being

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Production of a cheese-like product using soy protein and milk fat

by

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TABLE OF CONTENTS

INTRODUCTION

The functional versatility of soybean meal has made it a ubiquitous ingredient in food products, ranging from simple chocolate to complicated meat or cheese analogues. Soy protein especially has been used extensively in the preparation of meat and imitation meat products. As studies have indicated that the consumption of soy foods confer many health benefits such as lowering levels of cholesterol, incidences of heart diseases and cancer, many food products with soy incorporated have been introduced into the market. In Asia, the nutritional qualities of soybeans and products derived from them have long made them a major source of protein for millions of people. In Europe and the United States, soy products are enjoying increasing interest, as health studies promote soy protein consumption (Visser and Thomas, 1987). Major developments in soy-based dairy substitutes have been taking place in the United States. One such soy-based food is soy cheese.

Soy cheese products are available in the market, but consumption is limited due to their off-flavor and mushy texture. Numbers of attempts have been made to produce cheese-like food based on soy protein from blends of soy and cows' milk. These attempts have shown that the addition of soy protein increases the moisture content and off-flavors of the soy cheese. The cheese moisture content and off-flavors increase with increase in soy protein.

The objectives of this research were:

1. Production of a cheese-like product with an acceptable flavor and texture based on soy protein and milk fat by using traditional cheese making methods
2. Exploitation of the advantage of lipoxygenase-null soy white flakes in making soy cheese.
3. Possible production of a cheese-like food based on soy, that is acceptable to people used to a western diet, thus encouraging soy protein consumption.

LITERATURE REVIEW

Versatile functionality, nutritional value, immediate availability, and price have made soybean and soy protein products a widely used ingredient in foods. In Asia, the nutritional qualities of soybeans and the products derived from them have long been a major source of protein for millions of people (Visser and Thomas, 1987). "Soy proteins are unique among plant proteins by virtue of their relatively high biological value and essential amino acid content and are widely used in meat preparation" (Rani and Verma, 1994). Vitamins and minerals, such as calcium and iron, are also present in soy in substantial amounts. In recent decades, as numerous studies revealed soy's strong potential for lowering blood cholesterol levels and the incidence of heart disease, cancer and other chronic diseases, many food products incorporating soy have been introduced in the market (Visser and Thomas, 1987). In the United States, major developments on dairy substitutes by soy protein are taking place.

Although soy used to be regarded as an adversary to the dairy industry, it has been incorporated into a number of new products present in the market. Of course, it took a long time for the conjunction of soy and dairy products. In the beginning, the dairy industry regarded soy products as a danger to refrigerated dairy products. These days, attempts by both dairy manufacturers and soy processors have shown that dairy and soy-containing ingredients can coexist in products leading to successful formulations, that take advantage of their health benefits. The critical point in this process has been the identification of food applications where the coupling of dairy and soy ingredients can take place (Dahm, 2000). As widely known, soy-containing foods often have a characteristic beany, grassy or roasted off-flavors and grainy texture that are major impediments to their acceptance in Western cultures (Granata and Morr, 1996). To overcome such obstacles, efforts have been made over the past decades to improve the formulation of soy products to make it more acceptable as ingredients in yogurt, cheese and creamers.

Cheeses are considered a way of conserving selected constituents of milk. However, in developing countries where the milk supply is relatively limited, other sources of milk for cheese production are necessary. Soybean, with its high protein content and other nutritive values, makes it an excellent and economic substitute (Rani and Verma, 1995, 1996).

A number of attempts in making cheese-like foods based on soy protein have been made in the past four decades. One of the earlier attempts was to prepare soy cheese using a lactic starter culture (*Streptococcus thermophilus*), acetic acid, calcium sulfate, rennet extract, and skim milk by Hang and Jackson (1967). Cheeses prepared by using acetic acid, calcium sulfate, and lactic starter organisms to coagulate the soy protein gave "cheeses" with major differences in yield, moisture content, and hardness. Although precipitation with acetic acid yielded the highest protein content by 12-13%, the body and texture of the cheeses produced by the other two methods were superior. The moisture content of the soy-cheeses was the lowest and their textures hardest using lactic acid fermentation while the most moist and softest cheeses resulted from calcium sulfate precipitation. Acetic acid fermentation yielded cheeses of intermediate moisture and hardness. Thus, Hang and Jackson concluded that "a satisfactory cheese could be prepared by using a lactic acid fermentation of soybean milk". In succeeding experiments, they reduced the time of gel formation in soy cheese preparation by adding skim milk and rennet extract. The reduction in coagulation time from just over 3 to 2.5 hr was attributed to the reaction of rennet extract on the skim milk. An improvement in the flavor of the finished product was also claimed as a benefit of including skim milk in the cheese, which possibly resulted from the products formed by the enzymatic decomposition of casein as well as soybean proteins.

Hang and Jackson (1967) examined the cheeses they made for proteolytic and acid- and alkali-producing species, but found only acid-producing bacteria in all of the samples. Bacteria of prime importance during the ripening process of most cheese produced from cow's milk, Lactobacilli, were not found in any of the soy cheeses. The content of water-soluble nitrogen was constant during the ripening of the cheeses made from soybean milk and starter culture. Hang and Jackson (1967) concluded that starter bacteria, *S. thermophilus*, did not posses proteolytic activity and that its main function was acid production.

A substitute for "Ras", a hard Egyptian cheese, was reported by El-Ella (1980) to have been made successfully from soymilk by mixing soymilk with *Streptococcus lactis*, along with an emulsion of 30% good quality ripened "Ras" cheese solids, and calcium lactate. A cheesy flavor was obtained during a three-month ripening period. Products made from soymilk had greater moisture content than those made from cows' milk. Increasing the ratio of soymilk to cows' milk in blends also resulted in elevated moisture content. On the other hand, the total nitrogen as well as the fat content were lower in cheese made from soymilk and cow-soy blends. After a comparison of body, texture, and color was made for cheeses prepared with soymilk, cows' milk and blends of the two milks, El-Ella concluded that "from a technological point of view, soy milk could be used successfully in the manufacture of a cheese substitute. However, flavor and body, as well as texture and color, were improved by increasing the percentage of cow's milk" (El-Ella, 1980).

A study on rennet coagulated curds from 80% raw milk and 20% soy protein solutions from defatted soybean meal were investigated by Lee and Marshall (1981). Both the unfractionated soy protein solution and a 11S soy protein-rich fraction were tested. The rennet coagulated curds from milk and soy protein solutions were generally soft, inelastic and mealy. The addition of $CaCl_2$ at 0, 10, 20, 40mM did not result in much improvement in the curd texture. However, the rennet-coagulated curd from the blend of milk and the 11S soy protein-rich fraction was higher in solid yields and harder in texture. Although soy protein was able to coagulate with milk protein in the rennet curd formation, the water holding capacity increased proportionally with the addition of soy protein and loosened the casein curd microstructures, causing excess losses of milk fat. To further study and understand the interactions between soy protein and milk protein and improve the texture of cheese-like foods containing soy proteins, Lee and Marshall examined the differences in microstructure of various milk protein curds caused by adding soy protein using scanning electron microscopy (SEM). Either heat-denatured soy protein or native soy protein was added to the various milk proteins and coagulated. Addition of soy protein to processed cheese, milk curd and caseinate was destructive to curd microstructure and texture. Heat-denatured soy protein, which had stronger intermolecular interactions, was observed to be more destructive than native soy protein.

The effect of soymilk supplementation on the coagulation time and losses of milk components in whey were investigated by Rani and Verma (1994). Calf and microbial rennet were used for their cheese manufacturing. In the comparison of cow's milk, soymilk, and their blends, with the exception of protein content, the contents of total solids, fat, acidity, and ash were greater in cows' milk than in soymilk. The addition of soymilk to cows' milk increased the coagulation time of blends. The yield of cheese increased with an increase in the proportion of soy solids in the blend. The moisture, titratable acidity, soluble protein, and free fatty acids of cheese made from cows' milk-soy milk blends increased with the proportion of soy solids.

In another study done by Rani and Verma (1996) on cheddar cheese made from cows' milk and cows'-soy milk blends coagulated by calf and microbial rennets, the moisture retention in the cheese again was greater with increased soy solids. They reported that the moisture content decreased with ripening time and that the decrease was inversely related to soy solids. The protein, fat, and salt contents were greater in cheeses made with soy and were constant during ripening. The titratable acidity, soluble proteins and free fatty acids increased in proportion to the soymilk in the blends. During ripening, the soluble proteins and fat contents showed little change but titratable acidity varied.

Further studies on rennet-coagulated Cheddar cheeses made from cows' and soymilk blends during eight months of ripening was done by Singh and Verma (1996). They noted that a higher proportion of soy solids gave a lower hardness and that hardness increased during the initial four months of the ripening, followed by a decrease. The same trend was noted for the rheological parameters of springiness, gumminess, and chewiness, while the reverse was noted by cohesiveness.

The development of a soy cheese spread that was acceptable as a regular cheese substitute was studied by Singh and Mittal (1994). The soy cheese spread was developed by blending soy and milk solids. Dehulled and preboiled soybeans, sodium chloride fortified soy protein concentrate, cream and/or skim milk powder were mixed into slurries in six formulations, pasteurized, and inoculated with 5% *Streptococcus lactis* and 0.01% rennet. The mixes were incubated at 30°C with daily agitation and pH adjustments to pH 5.3 for 8 days. The stabilization of pH served as an index of the end of ripening period. The resulting soy cheese spreads contained about 35% solids, 18% fat, 11% protein, 2% sodium chloride, and 3% ash. It was observed that the elimination or decrease in soy protein concentrate reduced bitter flavor, and the addition of skim milk powder improved flavor characteristics of the soy cheese spread.

The effects of processing conditions, starter cultures and rennet on the flavor of the soy cheese spread were investigated in a separate study by Santosh and Singh (1985). Daily agitation, use of lactic cultures, use of rennet, and maintenance of pH 5.3 were essential in the flavor development. The addition of starter cultures and rennet resulted in relatively higher final concentration of soluble protein, which was further enhanced by pH adjustments. Sour character was prevented by pH 5.3 maintenance. Soy slurries with mixed cultures tended to deviate from the normal course of ripening quite often, resulting in off-flavor. Soy slurries with single culture inoculation tended to produce a soy cheese spread with a fresh flavor and clean taste.

The use of yeasts and fungus as starter cultures for making cheese-like foods based on soy protein were also investigated. Kawaguchi et al (1982) investigated various yeasts for their suitability in the production of soy cheese foods. *Saccharomyces fragilis* was found to produce an acceptable mild-flavored curd that had smooth texture with small pores. Kim et al (1995) investigated soybean cheese made from the addition of cows' milk to soybean curd inoculated with a fungus *Actinomucor elegans*. A good quality soy cheese was made after soaking the curd in salt/ethanol mixture for brining and 8-day storage. Total nitrogen decrease in curd was observed during storage, while total nitrogen in brining solution increased. Free amino acids content of the soy cheese was observed to increase due to proteolytic activity of the fungus. With the exception of moisture content, increases were observed in crude protein, carbohydrate, fat, and ash contents of the soy cheese.

Few successful attempts have been made in making an acceptable cheese-like food based solely on soybean or soy protein. Therefore, it is our objective to make a cheese-like food based on soy protein and milk fat that is acceptable in flavor and texture to western palates, thus encouraging soy protein consumption.

MATERIALS AND METHODS

White soy flakes

Two types of soy flakes were used: regular white soy flakes (Cargill, Minneapolis, MN), and lipoxygenase-null white soy flakes (POS Pilot Plant Corp., Saskatchewan, Canada).

Preparation of milk fat

USDA Grade AA sweet unsalted cream butter (Crystal Farm, MN) purchased from a local grocery store was melted by heating in a standard microwave oven and centrifuged in 250-ml centrifuge bottles for 15 min at 612 x *g*. After centrifugation, the clear oil phase was recovered while still warm.

Preparation of soymilk as culture medium

Soymilk-based medium was made by mixing 9 g of regular white soy flakes (defatted) (Cargill, Minneapolis, MN) with enough water to make a 9% soy solution. The solution was then stirred for 1.5 hr before centrifuging in 250-ml centrifuge bottles at 612 x *g* for 15 min. The supernatant (soymilk) was dispensed in 10-ml aliquots in test tubes and autoclaved for 20 min at 121°C.

Preparation of cultures

Streptococcus thermophilus AC2, *Lactobacillus delbreuckii* var. *bulgaricus* AR2, *Propionibacterium shermanii* P19, and *Lactobacillus casei* were obtained from the culture collection in the Department of Food Science and Human Nutrition at Iowa State University. A 1% inoculation each of *S. thermophilus* and *L. delbreuckii* var. *bulgaricus* were grown in 10 ml of sterile 9% (w/v) reconstituted non-fat dry milk (Carnation Co., Los Angeles, CA) at 40°C for 24 hr. *P. shermanii* was inoculated in 1% in sodium lactate broth and incubated at 30°C for 48 hr. *L. casei* was inoculated in MRS broth at 1% level and incubated at 30°C for 48 hr. Subsequent transfers were made for each culture at 1% inoculation into appropriate medium to obtain 18-hr active cultures for the production of soy cheese.

To make the soy-cheese, *S. thermophilus* was transferred into sterilized 9% soymilk medium before use, whereas the *L. delbreuckii* was transferred to sterilized 9% soymilk enriched with 1 % Swiss cheese liquid whey.

Processing of soy cheese using the dry method

Regular white soy flakes (37.4 g), molten milk fat (22.8 g), sterilized water (50 g), provolone cheese whey (1 % v/w total cheese weight), salt (sodium chloride, 1% w/w total cheese weight), sodium nitrite (1 ppm v/w total cheese weight) and 1% (v/w total cheese weight) starter culture mix containing of *S. thermophilus* (70%) and *L. delbreuckii*(30%) were mixed manually into a dough paste in a mixing bowl. The dough was incubated at 42°C until the pH dropped to pH 5.2. The fermentation was carried out either with or without moist carbon dioxide gas passing thought the headspace of the fermentation container. When pH 5.2 was reached, the supply of carbon dioxide gas, if used, was discontinued. The soy cheese was ripened at 6-7°C in sealed container for a total of six weeks; however, periodically, they were taken out to make necessary pH adjustments.

Processing of soy cheese using the wet method

Soy cheese was prepared from soymilk extracted from regular white soy flakes, with milk fat homogenized into it. The milk was pasteurized (95°C, 7 min) and coagulated by fermentation (42°C, 6 hr) with *S. thermophilus* and *L. delbreuckii.* The fermented curd gel was cut into small pieces and cooked to expel whey. The soy cheese curds were collected by draining through two layers of cheesecloth and cheese paper, and pressed to remove additional whey. The pH of the soy cheese was adjusted to pH 5.0-5.4 using saturated aqueous sodium carbonate solution, and ripened anaerobically for six weeks. The detailed soy cheese make and storage procedure is shown in Table 1.

Flavor modification and variations. In order to make soy cheese more acceptable to western palates, several types of soy cheese were made varying in their flavors in randomized order. To achieve flavor modification and variation, the following additives were added either individually or in combination at the time of the addition of table salt and sodium nitrite: 5% (w/w) yeast (Natural autolyzed yeast extract, Givaudan Roure Flavors, Cincinnati, OH), 0.087% (w/w) lipase (Kid Goat Lipase, Chr Hansen, Inc.,

Table 1. Procedure for manufacture of soy cheese using the wet method.

Operation	Time	Temperature (°C)	Process
Soymilk extraction		room	Approx. 9% (w/v) soy flake-water solution was stirred for 1.5 hr, and centrifuged in 250-ml centrifuge bottles at 612 x *g* for 15 min. The soymilk supernatant was collected, and 1% (v/v) Swiss cheese whey was added.
Pasteurization	7 min	95°C	Pasteurized milk was collected, cooled, and stored in cold room overnight at 5°C.
Homogenization		55±5°C	5% (v/v) molten milk fat was homogenized into warmed pasteurized milk (70°C). The homogenizer (Gaulin-16M, Everett, MA) was set at 3000psi at first stage, and 500 psi at second stage.
Inoculation		45°C	1% (v/v) total of *S. thermophilus* (0.7%) and *L. delbreuckii* (0.3%).
Fermentation	5-6 hr	42°C	Until pH dropped to about pH4.6
Cutting			With spatula
Cooking	30 min	50°C	Water bath
Diluting	30 min	45°C	Sterilized water was used to wash and dilute the curd from 9% to 7%
Draining		room	
Pressing	16-20 hr		Water filled 4L beaker
pH adjustment			Adjustment to pH 5.3 with saturated sodium carbonate, while blending with KitchenAid blender.
Addition of salt & nitrite			1.5% (w/w) salt, 1 ppm (v/w) sodium nitrite solution blended in.
Initial packaging			Blended soy cheese transferred into glass beaker and sealed with paraffin.
Cold incubation	2 weeks	6-7 °C	
pH adjustment			Adjust to pH 5.3 with saturated sodium carbonate. Mold removed if discovered.
Final packaging			pH stabilized soy cheese transferred to cheesecloth pouch and waxed by dipping in paraffin.
Cold incubation	4 weeks	6-7 °C	

Milwaukee, WI), 6.6μL/100g cheese of protease (Neutrase, Novo Nordisk Biochem, North America, Inc. Franklin, NC), 5% (w/w) hydrogenated vegetable protein (Natural HVP-Litesate, Givaudan Roure Flavors, Cincinnati, OH), 1% (v/w) *P. shermaii* culture, and 1% (v/w) *L. casei* culture. The amount of added salt was adjusted to 1% (w/w), when adding 0.5% (w/w) of either hydrolyzed vegetable protein and yeast extract, which contained 50% salt. A control soy cheese was prepared which did not include the addition of any flavor modifying compounds.

Once the fermentation was complete, soy cheeses were transferred to glass beakers, and sealed with layers of hot paraffin wax before incubating at 6-7°C for two weeks. The pH of the soy-cheese, which typically dropped during this period, was measured and adjusted to pH 5.2 until stabilized using a saturated aqueous sodium carbonate solution. The pH-stabilized soy cheeses were removed from the glass beakers, placed into cheesecloth pouches and sealed by dipping in paraffin. The pouched cheeses were ripened anaerobically at 6-7°C for six weeks.

Sensory analysis of soy cheeses made using the wet method

A preliminary sensory analysis was carried out using hedonic technique on eight soy-cheese samples containing the various flavor modifications discussed earlier. The analysis evaluated both the flavor and the texture attributes of the soy cheeses in a randomized experimental design. Ten untrained panelists (9 Caucasians, and 1 Asian) accustomed to western (American) diets were selected from faculty, staff, and students in the Department of Food Science and Human Nutrition at Iowa State University. The scorecard is shown in Appendix A. Scores ranged from +3 for "like very much" to –3 for "dislike very much".

The samples (5 g) were weighed into individual paper cups. The eight varieties of soy cheeses which included a control, and cheeses with flavor modification by protease, lipase, yeast extract, hydrolyzed vegetable proteins, *L. casei,* and *P. shermanii* were presented for evaluation. The result of the preliminary sensory evaluation was used to determine a narrower range of flavor additives to be used in soy cheese making on a larger scale.

A similar hedonic evaluation was conducted on a consumer scale using 30 panelists with four varieties of soy cheese. The scorecard is shown in Appendix B. Scores ranged from 7 for "like very much" to 1 for "dislike very much". The panelists were people accustomed to western (American) diets, selected from faculty, staff, and students in the Department of Food Science and Human Nutrition at Iowa State University. The four varieties included the control, and three soy cheeses modified by protease, *L. casei*, lipase, and hydrolyzed vegetable protein in specific combinations.

Chemical analysis

The moisture, fat, salt, protein, and pH of the soy-cheese were determined in duplicate. Moisture was determined according to method #926.08 (AOAC, 1998). Fat in cheese was determined according to the Pennsylvania Test for Fat from Laboratory Manual: Methods of Analysis of Milk and Its Products (Milk Industry Foundation, 1952). Salt was determined by using a DiCromet Salt Analyzer (Model DSA-1000, Diamond Crystal Salt Company, St. Clair, MI) using 10 g of prepared soy-cheese sample blended with 100-ml distilled water and filtered through a coffee drip filter paper. Protein was determined by the micro-Kjeldahl methods according to #955.04 (C) and #954.01 (AOAC, 1990), with cupric selenite dihydrate as a catalyst. The pH was measured with a pH meter (Accumet AR15, Fisher Scientific Company, Pittsburgh, PA) calibrated with buffers of pH 7.0 and pH 4.0. The water activity, a_w, was measured by a water activity meter (AQUA Lab CX-2, Decagon Devices, Inc., Pullman, WA).

RESULTS AND DISCUSSION

Cheese culture cultivation

For soy cheese production, active 18-hr cultures of *S. thermophilus* and *L. delbreuckii* var. *bulgaricus* grown in soymilk medium were used. The *S. thermophilus* grew well in soymilk medium, but the *L. delbreuckii* did not. Absence of a fermentable carbohydrate source in soymilk for *Lactobacillus* species accounted for this (Hang and Jackson, 1967). However, addition of either 1 % (w/v) lactose or yeast extract did not give significant growth of *L. delbreuckii* in the soymilk. The addition of 1% (v/v) of either provolone or Swiss cheese whey enabled the *Lactobacillus* species to survive well in soymilk after multiple transfers.

Lipoxygenase null soy flakes

The presence of lipoxygenase enzymes in soybean has been considered one of the main causes of off-flavors in soymilk and products made from them (Wilson, 1995). Thus, the initial hypothesis was that using lipoxygenase-null white soy flakes (LNWSF) (POS Pilot Plant Corp., Saskatchewan, Canada) to make the soy cheese would produce a product with an acceptable flavor. To test this hypothesis, a taste comparison on soymilk at pasteurization temperatures 50, 60 and 70°C prepared from both LNWSF and white soy flakes were conducted. An informal taste test was carried out with the help of two experienced panelists. They agreed that for both kinds of soy flakes, soymilk pasteurized at

60°C had the least flavor. However, raw beany flavor remained even after pasteurization at 70°C. Raw-beany flavor was particularly strong in the soymilk of regular white soy flakes.

The initial challenge of making an acceptable cheese-like product from soy was investigated using regular white soy flakes, since LNWSF was available in limited quantity. The soy cheese made from regular white soy flakes had an acceptable bland flavor and almost no beany or off-flavor. Essentially all of the off-flavor of soymilk was lost when it was homogenized with molten milk fat, and fermented with the starter cultures at 42°C for 6 hours (or till the pH dropped to 4.6). Since it was determined that a soy cheese product with acceptable flavor could be obtained from regular white soy flakes, the LNWSF was not used in subsequent experiments.

Processing of soy cheese using the dry method

Soy cheese was prepared from regular white soy flakes, molten milk fat, sterilized water, Swiss cheese whey, salt (sodium chloride), sodium nitrite, and starter cultures by mixing them manually into dough.

The dry method had the advantage that the moisture content of the cheese could be controlled, and a fairly firm body could be achieved. Also the portion of the soy flakes that were not soluble in water could be incorporated into the soy cheese, improving its yield. The immediate challenges with the dry method included grainy texture, surface yeast growth (pink patches), and pH control during fermentation.

To improve the grainy texture of soy cheese made by the dry method, initially regular white soy flakes sieved through mesh 14 and 28 screens were collected and used to make the product. However, graininess remained. During processing by the "wet method" protease treatment of the soy cheese caused a noticeable decrease in grainy texture of the cheese, so applying the protease treatment in the processing of soy cheese by the "dry method" might also alleviate the grainy texture problem.

The growth of an unidentified red yeast on the surface of the soy cheese, which made the product appeared to have pink patches, was another problem encountered with this method. Addition of potassium sorbate (0.25% w/w of soy flake used) to the dough did not retard the yeast growth. The solution was the exclusion of oxygen from exposed surfaces of the product. This was achieved by covering the major surfaces with aluminum foil during incubation, however, this still allowed the edges to turn pink. An alternative solution was to pass a continuous supply of moist carbon dioxide gas into the headspace of the fermentation container. The carbon dioxide gas was moistened by bubbling it through water to prevent drying of the soy-cheese surface. The pink surfaces caused by yeast growth during the fermentation period was prevented by this treatment, but reappeared upon the absence of the carbon dioxide gas during the ripening period. Theoretically, the carbon dioxide treatment could be continued during the ripening period, but it would still allow color change due to yeast growth onto the surface of the product if it were exposed to air, as it would be during use by consumers. Possibly the yeast could be destroyed by irradiation treatment, or other preservation additives, but these techniques were not explored.

The time required to reach the desirable pH 5.2, during fermentation of soy cheese made by the dry method, varied considerably ranging from 15 to 25 hours. Processing of the soy cheese by the dry method used a small amounts of water; thus, small fluctuations in the water activity may have been responsible for the pH fluctuation during fermentation. This variation made production of soy cheese by this method very inconvenient.

Soy-camembert cheese was made by inoculating the surface of fermented soy cheese (made by the dry method) with *Penicillium candidum*, (New England Cheesemaking Supply Company, Ashfield, MA) and a six week ripening incubation at 6-7°C. Molds other than the expected white and grayish *P. candidum* flourished on the cheese surface during ripening, therefore this method was abandoned.

Processing of soy cheese using the wet method

Soy cheese was prepared from fat-free soymilk extracted using regular white soy flakes, with molten milk fat homogenized into it. The milk was pasteurized (95°C, 7 min) and coagulated by fermentation (42°C) with *S. thermophilus* and *L. delbreuckii*. The fermented curd was cut into small pieces and cooked to expel the whey. The curds were then collected by draining through 2 layers of cheesecloth and cheese paper, and pressed overnight to remove additional whey. The soy cheese was adjusted to pH 5.0-5.4, and allowed to ripen anaerobically for six weeks at 6-7°C.

Various soymilk concentrations were tested for suitability in curd coagulation. Soymilk of 7, 9 and 12% solids produced from regular white soy flakes were processed and fermented on small scales, according to the wet method. The curd yield from 9% soymilk was firm enough to cut and expel the whey. The curd gel produced from 7% soymilk was soupy and too soft to cut, whereas the curd produced from 12% soymilk was too firm and integrated to expel whey. Thus, 9% soymilk was used for soy cheese production via the wet method.

Soy curd coagulation also varied with the amount of milk fat homogenized into the soymilk. Batches of soymilk were homogenized with milk fat at 5, 10 and 20% respectively, and processed according to the wet method of cheese making. Curd coagulation was not achieved with 20% milk fat, possibly because the protein was bound too tightly to the fat to integrate and gel. Coagulation of the curd was achieved with 5 and 10% milk fat, with 5% yielding a gel that was firm enough to cut, whereas that of the 10% was too soft and creamy, and easily mashed. Therefore 5% milk fat addition was used in all soy cheese making via the wet method.

Surface yeast growth was initially a problem with this method as with the dry method. In earlier experiments, the pasteurization temperature and time for was at 63°C for 30 min, which is standard during cheese processing. We had wished to use as low a temperature as possible to minimize soy protein denaturation. Addition of potassium sorbate (0.25% w/w of soy flake used) to soymilk prior to pasteurization did not prevent the yeast growth. To overcome the red yeast problem, various higher temperatures and shorter times for pasteurization were tried, and it was found that pasteurizing at 95°C for 7 min solved the yeast growth problem as well as providing an 80% reduction in trypsin inhibitor activity as shown by Bai Yong (1997).

Another problem encountered with this method was the pH drop during cold fermentation at 6-7°C. Although sodium carbonate was used to adjust the pH after the elevated-temperature fermentation, further pH drop occurred during the ripening stage at the refrigerated temperatures. The cultures used were not expected to grow at these low temperatures. This problem was partially solved by diluting the original soymilk from 9 to 7 % solids by adding water to the soy curd after cooking to wash out fermentable sugars. The required pH for a safe cheese incubation and good flavor production is in the range of 5.2 to 5.4. After pH adjustment to 5.2 with saturate sodium carbonate solution, the pH of the soy cheese tended to drop back to pH 4.80 during the first two weeks of cold ripening stage. Therefore, the pH had to be adjusted once or twice during the early stages of the ripening period until it remained constant in the required range. It was observed that the addition of *L. casei* to soy cheese for flavor modification lowered the pH more throughout the ripening period even after several pH adjustments, which was also observed by Trépanier et al (1992). Possibly the *L.casei* was able to utilize carbon sources in the curd not available to the starter cultures.

Mold growth was another major problem encountered during the ripening period when the soy cheeses were stored in glass beakers and sealed with layers of paraffin wax. Air leaks in the paraffin seal on the side of the glass beaker was the cause of the problem. To correct this, the soy cheese samples were transferred from their initial package in the glass beaker after the final pH adjustment into a cheesecloth pouch, and sealed by dipping the entire product in wax.

The slight grainy texture problem of the soy cheese with wet method was solved by addition of Neutrase (a protease), resulting in a very smooth texture of the product. The amount of protease in the mix is critical, since, an excess imparted a soupy texture to the soy cheese.

In order to optimize the flavor of soy cheese during the ripening period (six weeks at 6-7°C), the following components were added individually or in combination at the salt and sodium nitrite addition step: yeast, hydrolyzed vegetable protein, lipase, Neutrase (protease), *P. shermanii*, and *L. casei*. The addition of yeast extract and hydrolyzed vegetable protein was added to provide more amino acids for flavor generation. Protease was added to shorten the ripening period and add flavor by further break down of soy proteins and hydrolyzed vegetable proteins to provide more amino acids for flavor formation (Griffith and Hammond, 1989; Trépanier et al, 1992). Lipase was added to aid the release of short chain fatty acids that enhance cheese flavors (Kilcawley et al, 2001). *L. casei* grows frequently during ripening of cheese without initial addition of such cultures. It was observed to be one of the dominant flora in many matured cheeses and may be responsible for some flavor generation (Trépanier et al, 1992; Swearingen et al, 2001). Thus, it was added to enhance the flavor of the soy cheese. Propionicbacteria were observed to be responsible for the sweet flavor in Swiss cheese as well as propionic acid and acetic acid (Biede and Hammond, 1979). Thus, the addition of *P. shermanii* was tried in the soy cheese to impart desirable flavors.

Sensory analysis

Preliminary sensory analysis. Table 2 shows the results of the preliminary sensory analysis (using 10 panelists) of soy cheeses processed by the wet method with various additives: yeast, hydrolyzed vegetable protein, lipase, Neutrase (protease), *P. shermanii*, and *L. casei*. The preliminary sensory test was conducted mainly to see which flavor modifying compounds imparted desirable flavors so that the possibilities of improving the soy cheese flavor with such compounds could be explored. Thus, no statistical analysis was conducted on the results of the preliminary sensory test.

Table 2. Preliminary Sensory Evaluation of Soy Cheese Made By Wet Method.

Cheese samples with Flavor Modification	Flavor [a]	Texture [a]
Control	10	4
Hydrolyzed Vegetable Protein	7	9
Yeast extract	-3	8
Lipase	2	4
Protease	3	10
L.casei	7	1
P. shermanii P19	7	4
P. shermanii P19 at room temperature for 1 week	0	-1

[a] Sum of scores for individual treatments

The results show that the soy cheeses with the most preferred flavors were the control, and cheeses containing hydrolyzed vegetable protein, *L. casei*, and *P. shermanii*. The samples with the most preferred texture were of the soy cheeses with added hydrolyzed vegetable protein, yeast extract, and protease. From the results of the preliminary sensory analysis, we selected the additives: protease, hydrolyzed vegetable protein, *L. casei*, and lipase to be made on a larger scale for a consumer sensory panel. Although the score for the soy cheese with lipase addition scored low in both the flavor and the texture evaluation, it was included because it seemed to produce a flavor typical of cheeses such as parmesan or romano.

Consumer sensory analysis. A hedonic sensory evaluation on four soy cheeses processed by the wet method with various additives were conducted (Table 3). The four soy cheeses were (1) protease, *L. casei*, lipase and hydrolyzed vegetable protein, (2) protease, *L. casei* and hydrolyzed vegetable protein, (3) protease, *L. casei* and lipase, and (4) control. The panelists (n=30) were asked for their flavor and texture preferences. In

Table 3. Consumer Sensory Evaluation of Soy Cheese Made By Wet Method.

Cheeses	**Flavor** [a]	**Texture** [a]
1 (Protease + *L. casei* + HVP + lipase)	4.97^{b}	5.66^{b}
2 (Protease + *L. casei* + HVP)	4.10^{c}	4.24^{c}
3 (Protease + *L. casei* + lipase)	4.82^{ac}	5.41^{b}
Control	4.59^{bc}	5.07^{b}

[a] Means of 30 panelists. Means within columns followed by the same superscript are not significantly different ($p \leq 0.05$).

general, the average scores of the soy cheeses were close to 5, which is moderate in the scale; suggesting that the soy cheeses were acceptable in flavor and texture to the panelists, but not with too much excitement. There was no significant difference between the control soy cheese verses the other treatments in terms of flavor (Table 3). This indicates that soy cheese with acceptable flavor and texture without food additives was possible. Within the three treatments of additives, treatment 1, which contained protease, *L. casei*, lipase and hydrolyzed vegetable protein, was significantly different in flavor preference from sample 2, which contained every element in sample 1 except lipase. Similarly, no significant difference in the texture was found between samples 1 and 3, and control. However, sample 2 was significantly different from the others (Table 3). These results suggest lipase is essential if the benefits of the other additives in sample 1 are to be enjoyed.

Slightly acidic flavors were detected in most cheese samples by the panelists. Acidic flavors are common in fermented soy cheeses according to many previous studies (Hang and Jackcon, 1967, Abou El-Ella, 1980, Rani and Verma, 1994). Slight bitterness also was detected in some soy cheese samples, which is probably due to fermentation products of *S.*

thermophilus. Santosh and Singh (1985) noted that the use of *S. thermophilus* in soy cheese fermentation tended to give sweetish-bitter flavor.

In both the preliminary and consumer sensory evaluation, nearly all the panelists that were chosen were accustomed to American diets because we wished to make a product acceptable to Americans. For discrimination of the small difference found in our treatments, the consumer panel should involve more people, but this was not possible because of the limitations in time and sample quantities. However, the results we obtained can be use as an indication of the level of product acceptance of our soy cheeses.

The wet method of making soy cheese produced acceptable products, as indicated by the sensory test results. Even though the panelists did not object strongly to the texture, a disadvantage of the wet method was that it was impossible to reduce the moisture of the soy cheese below 65%, so the product had a texture more like a cheese spread than a firm cheese.

Chemical characteristics of soy cheeses from wet method

Results in Table 4 represent the salt and compositional variation in the wet processing method for regular type soy cheese (control) and that made with curd dilutions after the cooking step. As discussed earlier, the dilution step was used to ameliorate the problem of pH drop during the ripening period at 6-7°C for a total of six weeks. There was a small but significant increase in moisture in the final product made by curds that were washed (to

Table 4. Chemical Analysis of Control Soy Cheese-like Products Made By Wet Method.

Cheeses	Moisture (%)[a]	Fat (%)[a]	Salt (%)[a]	Protein (%)[a]	a_w [a]	pH
Control A (diluted after draining)	66.51^{bc}	23.00^{b}	1.23^{b}	13.02^{b}	0.99^{b}	5.10
Control B (diluted before draining)	67.78^{b}	22.00^{b}	1.20^{b}	13.17^{b}	0.99^{b}	4.98
Control C (undiluted)	66.26^{c}	21.00^{b}	1.20^{b}	8.66^{c}	0.99^{b}	4.80

[a] Means of two duplications. Means within columns followed by the same superscript are not significantly different ($p \leq 0.05$).

dilute) after cooking. There were no significant changes in salt, water activity, or the fat composition. However, dilution before or after draining produced soy cheeses with significantly higher protein content than soy cheese made without dilution step. The pH ranged from 4.80 to 5.10, indicating that diluting of the soy curds did help to wash out carbon sources available for culture fermentation, thus slowing acid production.

Results of chemical analysis of the cheeses produced using the wet method for consumer sensory evaluation are presented in Table 5. The moisture contents in the soy cheeses ranged from 67.52% to 71.48%, which was very close to the 66.60% to 69.10 %

Table 5. Chemical Analysis of Soy Cheese-like Products Made By Wet Method.

Cheeses	Moisture (%) [a]	Fat (%) [a]	Salt (%) [a]	Protein (%) [a]	a_w [a]	pH
1 (protease + *L. casei* + HVP + lipase)	68.81 ^{b}	22.00 ^{b}	1.23 ^{b}	9.15 ^{b}	0.99 ^{b}	4.98
2 (protease + *L. casei* + HVP)	71.48 ^{c}	19.50 ^{c}	1.20 ^{b}	9.39 ^{b}	0.99 ^{b}	4.70
3 (protease + *L. casei* + lipase)	70.38 ^{d}	20.00 ^{bc}	1.18 ^{b}	9.50 ^{b}	0.99 ^{b}	5.03
Control	67.52 ^{e}	22.00 ^{b}	1.20 ^{b}	8.79 ^{b}	0.99 ^{b}	5.15

[a] Means of two duplications. Means within columns followed by the same superscript are not significantly different ($p \leq 0.05$).

moisture range reported by Hang and Jackson (1967) in their soy cheeses made from whole soybeans and starter cultures. The water activity of the soy cheeses was 0.99 at 24°C. The pH ranged from 5.15 to 4.70. The lower pH was typical of cheese made of soymilk, or those with the incorporation of soy protein in milk or of soy-cow milk blends (Rani and Verma, 1995; Singh and Verma, 1995). The fat contents ranged from 20.00% to 23.00% of total cheese weight. Fat analysis using the Mojonnier method did not yield good results for the soy cheese from the wet method. The Pennsylvania Fat test (modified Babcock test) for cheese gave better results, probably due to the digestion of fat with sulfuric acid which could "release" the fat more completely. The end recovery of fat in the soy cheese samples is 93%. The 7% loss of fat probably occurred during homogenizing since no fat was found in the whey. The salt contents ranged from 1.18% to1.23% of the total cheese weight. The protein contents ranged from 8.79% to 9.50% of the total cheese weight. The end recovery of protein in the soy cheese samples was only 50%. The other 50% of protein probably was in the whey, which was not analyzed. Chemical analysis was not done on the soy cheese for the preliminary sensory evaluation.

Chemical analysis of only one soy cheese from the dry method was done on fat, moisture and pH. The fat content was 19.09%, the moisture content was 48.61%, and the pH at 5.2. The fat and moisture contents of the soy cheese were close to the original amount used to make the cheese. Due to the unsuccessful attempts to make soy cheese via the dry method, this method was not explored further.

SUMMARY

Soy cheese production using dry and wet methods were explored. The dry method included cheese preparation from regular white soy flakes, molten milk fat, sterilized water, Swiss cheese whey, salt, sodium nitrite, and *S. thermophilus* and *L. delbreuckii* manually mixed into dough and ripened for six weeks at 6-7°C. In the wet method, soy cheese was prepared from fat free soymilk extracted from regular white soy flakes with molten milk fat homogenized into it. The milk was pasteurized and then coagulated by fermentation with *S. thermophilus* and *L. delbreuckii*. The fermented curd gel was cut, cooked and pressed to expel whey. The collected soy curds were adjusted to pH 5.0-5.4, and ripened anaerobically for six weeks at 6-7°C.

The dry method used very little water, and its low water activity may have been responsible for its inconsistent fermentation time, which made the production of soy cheese by this method very unreliable.

Sensory evaluations were conducted on soy cheeses produced by the wet method. Most of the soy cheeses were acceptable to the panelists. All soy cheeses had pleasant, yet bland flavors and were essentially free of the beany flavors associated with soy products. Flavor modification of the soy cheeses can be achieved by adding hydrolyzed vegetable protein, protease, lipase, and additional microorganisms. Soy cheeses produced by the wet method contains about 68% moisture, 21% fat, 9% protein, 1.2% salt,

pH 5, and a water activity of 0.99. The moisture content is much greater than that of typical cheese, so its texture is more that of a cheese spread than a hard cheese such as Swiss or Cheddar. The wet method of making soy cheese produced acceptable products.

Thus, a possible new type of healthful soy-based product may be available to populations used to western diets, and those who enjoy the flavor of mild cheeses. This product provides a new option for increasing soy protein consumption and a healthier diet.

FUTURE IMPROVEMENTS

For the wet method of soy cheese processing:

1. Addition of nonstarter lactic acid bacteria (NSLAB) from milk cheeses. It has been observed that increase in NSLAB in Cheddar cheese during ripening period corresponds to the development of positive and negative flavors and textures. The dominant NSLAB found in Cheddar cheeses are from the genus *Lactobacillus*. Common heterogeneous *Lactobacillus* population in the cheese matrix includes *L. casei, L. plantarium, and L. brevis*. In adjunct Cheddar cheeses, certain adventitious NSLAB contribute to positive flavor development (Swearingen et al, 2001).
2. Ultrafilter the soymilk prior to homogenization to concentrate soy protein solids, and filter out most of the carbohydrate sugars. Most of the remaining carbohydrates can be used up in fermentation, eliminating the need to dilute the soy curds by washing,. This may also help prevent drastic pH drops during ripening period in cold incubation when using the wet method.
3. Shelf–life studies of the soy cheeses.

For the dry method of soy cheese processing:

1. Irradiation of the soy flakes to prevent yeast growth.
2. Addition of protease to improve the texture soy cheese.

APPENDIX A. PRELIMINARY SENSORY EVALUATION SCORE CARD

Indicate on the scales below your degree of liking or disliking of the samples.

Sample 1

Flavor	Texture
_____ Like Very Much	_____ Like Very Much
_____ Like Moderately	_____ Like Moderately
_____ Like Slightly	_____ Like Slightly
_____ Neither Like Nor Dislike	_____ Neither Like Nor Dislike
_____ Dislike Slightly	_____ Dislike Slightly
_____ Dislike Moderately	_____ Dislike Moderately
_____ Dislike Very Much	_____ Dislike Very Much

Sample 2

Flavor	Texture
_____ Like Very Much	_____ Like Very Much
_____ Like Moderately	_____ Like Moderately
_____ Like Slightly	_____ Like Slightly
_____ Neither Like Nor Dislike	_____ Neither Like Nor Dislike
_____ Dislike Slightly	_____ Dislike Slightly
_____ Dislike Moderately	_____ Dislike Moderately
_____ Dislike Very Much	_____ Dislike Very Much

Sample 3

Flavor	Texture
_____ Like Very Much	_____ Like Very Much
_____ Like Moderately	_____ Like Moderately
_____ Like Slightly	_____ Like Slightly
_____ Neither Like Nor Dislike	_____ Neither Like Nor Dislike
_____ Dislike Slightly	_____ Dislike Slightly
_____ Dislike Moderately	_____ Dislike Moderately
_____ Dislike Very Much	_____ Dislike Very Much

Sample 4

Flavor	Texture
_____ Like Very Much	_____ Like Very Much
_____ Like Moderately	_____ Like Moderately
_____ Like Slightly	_____ Like Slightly
_____ Neither Like Nor Dislike	_____ Neither Like Nor Dislike
_____ Dislike Slightly	_____ Dislike Slightly
_____ Dislike Moderately	_____ Dislike Moderately
_____ Dislike Very Much	_____ Dislike Very Much

Sample 5

Flavor

_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Neither Like Nor Dislike
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much

Texture

_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Neither Like Nor Dislike
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much

Sample 6

Flavor

_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Neither Like Nor Dislike
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much

Texture

_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Neither Like Nor Dislike
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much

Sample 7

Flavor

_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Neither Like Nor Dislike
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much

Texture

_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Neither Like Nor Dislike
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much

Sample 8

Flavor

_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Neither Like Nor Dislike
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much

Texture

_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Neither Like Nor Dislike
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much

Comments:

____

APPENDIX B. CONSUMER SENSORY EVALUATION SCORE CARD

Indicate on the scales below your degree of liking or disliking of the samples.
Please put additional comments on the back of the page.

Sample 1

Flavor	Texture	Comments
_____ Like Very Much	_____ Like Very Much	
_____ Like Moderately	_____ Like Moderately	
_____ Like Slightly	_____ Like Slightly	
_____ Neither Like Nor Dislike	_____ Neither Like Nor Dislike	
_____ Dislike Slightly	_____ Dislike Slightly	
_____ Dislike Moderately	_____ Dislike Moderately	
_____ Dislike Very Much	_____ Dislike Very Much	

Sample 2

Flavor	Texture	Comments
_____ Like Very Much	_____ Like Very Much	
_____ Like Moderately	_____ Like Moderately	
_____ Like Slightly	_____ Like Slightly	
_____ Neither Like Nor Dislike	_____ Neither Like Nor Dislike	
_____ Dislike Slightly	_____ Dislike Slightly	
_____ Dislike Moderately	_____ Dislike Moderately	
_____ Dislike Very Much	_____ Dislike Very Much	

Sample 3

Flavor	Texture	Comments
_____ Like Very Much	_____ Like Very Much	
_____ Like Moderately	_____ Like Moderately	
_____ Like Slightly	_____ Like Slightly	
_____ Neither Like Nor Dislike	_____ Neither Like Nor Dislike	
_____ Dislike Slightly	_____ Dislike Slightly	
_____ Dislike Moderately	_____ Dislike Moderately	
_____ Dislike Very Much	_____ Dislike Very Much	

Sample 4

Flavor	Texture	Comments
_____ Like Very Much	_____ Like Very Much	
_____ Like Moderately	_____ Like Moderately	
_____ Like Slightly	_____ Like Slightly	
_____ Neither Like Nor Dislike	_____ Neither Like Nor Dislike	
_____ Dislike Slightly	_____ Dislike Slightly	
_____ Dislike Moderately	_____ Dislike Moderately	
_____ Dislike Very Much	_____ Dislike Very Much	

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