ENZYMIC COAGULABILITY, ACID PRODUCTION AND CURD CHARACTERISTICS OF BOVINE MILK CONTAINING SOY PROTEIN ISOLATE

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ABSTRACT

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By

Shamsdokht Shams

Whole milk, skim milk, and dried milk ingredients were prepared in different formulae with addition of soy protein to study enzyme coagulability of these systems. Rennet and Emporase (a milk coagulating enzyme elaborated by Mucor pusillus) were used in this study. Coagulation of milk systems containing soy isolate was impaired, depending on the level of soy protein added.

Coagulation was improved by addition of calcium chloride. Good coagulation was obtained on a system containing 0.5 or 1 percent soy protein and 3.6 mM Ca^{++} (o.4g $CaCl_2$).

The curd tension was lower in samples coagulated by Emporase than by rennet, also the curd tension was lower in systems containing whole milk than skim milk. Cheddar cheese was manufactured from the soy-milk protein system containing 0.5 or 1 percent soya protein and compared to appropriate controls throughout 3 month curing. The curing room temperature was held at 50° F and relative humidity was kept at 95 percent. During the curing of the cheese, the pH, moisture and percent fat was determined

monthly. The cheese containing soya protein developed a bitter taste which made the cheese less acceptable than controls.

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Shamsdokht Shams

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To my Parents

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INTRODUCTION

A deficiency in protein supply is one of the most important food problems encountered in many developing countries of the world. Although animal products are the most desirable protein sources, they are more expensive than vegetable proteins. There is a constant demand to develop new sources of protein, with emphasis on plant sources. Recently interest has increased in the nutritive value of soybeans and other sources of plant proteins which may be used in the human diet to partially replace or extend animal proteins. For many years, soy milk products have been available as dried, canned or liquid products for feeding babies who are allergic to cow's milk and for others, such as vegetarians and certain religious groups, who do not desire animal protein. Tufu, or soybean curd, while practically unknown in the United States, has been an important food in the orient for centuries. Such characteristics as low cost, high protein and calcium content, a desirable texture, and many uses have contributed to its popularity.

The historical precursor of the vegetable-based cheese analogs is the Chinese food "sufu" which dates back to the era of the Hang Dynasty (179-122 B.C.). In Western

food science, interest in making cheese analogs from vegetable sources, mainly from soybeans, gained some momentum during the middle 1960s. Schroder and Jackson worked on soy cheese both for texture and flavor improvement.

This project was undertaken to investigate the possibility of coagulation, the formulas containing milk and soy protein and also to manufacture a cheddar cheese using these formulas.

LITERATURE REVIEW

Soybean

The soybean has been utilized for many centuries by Chinese and Japanese in the preparation of a great variety of fresh, fermented and dried food products which form an indispensable part of their diet. The numerous food preparations of the soybean not only give flavor and relish but supply to a very considerable extent the protein that in the diet of the Western people is furnished largely by meats. Some of the soybean products are eaten at every meal by rich and poor alike especially in interior of oriental countries where seafood is not obtainable (Piper and Morse, 1923).

Soybeans containing approximately 40 percent protein offer an excellent source of this vital nutrient. In addition, they rank at the top of the vegetable protein list. Soybeans contain the essential amino acids necessary for growth, except for deficiency of methionine, which may be added as a supplement (Anonymous, 1972; Badenhop and Hackler, 1973). If acceptable soybean products can be produced in large enough quantities, they can contribute toward alleviating the tremendous protein shortage in the world today. The shortage of protein in diets of

millions of people throughout Asia, Africa, and South America is extremely serious and contributes to the high infant mortality which sometimes reaches 50 percent of all children before age 15 (Steinkraus et al., 1962).

Soybeans and products derived from them, according to Bailey et al. (1935) have played a major role for many centuries as a source of protein in the diet of millions of people in Asia. Rakosky (1975) reported that although soybeans and their products have been used as foods by the orientals for centuries, it was not until the turn of the present century that the Western world recognized soybeans for their human food value. Nelson et al. (1971) stated that the taste and odor, often described as painty, beany or bitter, is often objectionable to Western taste. At first it was the oil that gained wide acceptance; later, the meal was recognized for its high protein content and consequent value in various food systems.

Food Value of the Soybean

The food value of the soybean in comparison with other legumes is indicated in Appendix 1 (Piper and Morse, 1923). Osborne and Mendel (1917) conducted rather extensive investigations with the proteins of the soybean in comparison with other proteins. In diets containing either soybean meal or commercial cake as the sole source of protein used together with fats and "protein free milk," several broods of young rats were produced and the young

grew normally on the same diet.

Amino Acid of Soybean

The essential amino acid content of soy protein and the amino acid pattern for hen's egg protein recommended as a reference protein of good nutritional quality by FAO-WHO Expert Groups is given in Appendix 2. Most of the amino acid levels in the soy proteins are equal to or exceed the level in egg proteins with one exception. The sulfur amino acids are low, and as a result the protein scores for the soy protein are low as compared to egg proteins. The lower score of isolates as compared to flour and concentrates results from loss of amino acids in the whey protein during isolation. It is therefore necessary to supplement with methionine when isolates are the sole source of protein as is being done with infant formulas (Wolf, 1975).

Correlation of Amino Acid Index with Nutritional Quality of Several Soybean Fractions

In all studies reported here, a certified, dark varitety of soybeans was used in preparation of soy fractions. Figure 1 shows graphically the steps involved in the fractionation of soybeans.

The crude protein, lipid and moisture contents of freeze-dried soybeans are shown in Appendix 3. The results of the amino acid composition of the soybean fraction are reported in Appendix 4 as g of amino acid per 16 g nitrogen.

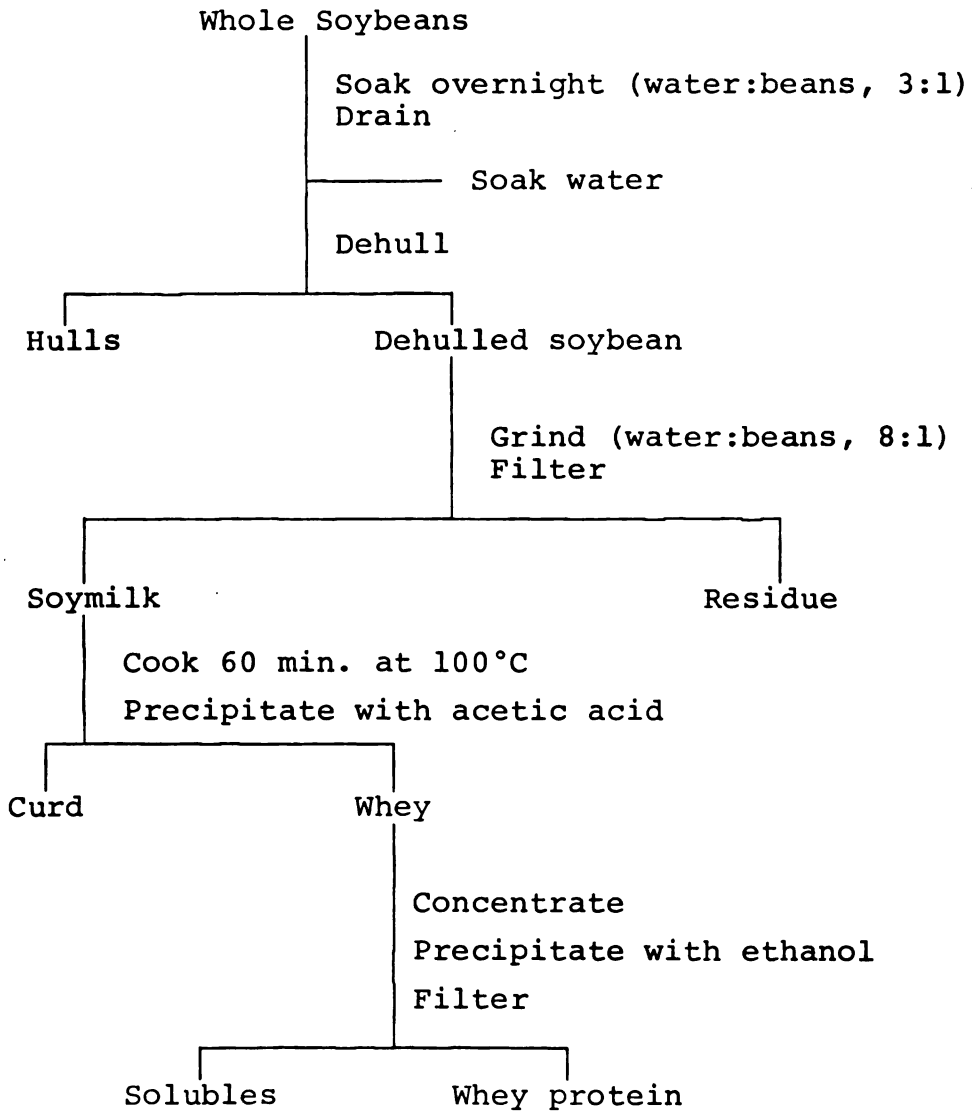

Figure 1. Steps Involved in the Fractionation of Soybeans

Comparison of nutritional quality of soybean fractions as measured by protein efficiency ratio (PER), essential amino acid index (EAAI), requirement index (RI), and available lysine are listed in Appendix 5 (Hackler et al., 1967).

Impact of Vegetable Proteins on Dairy Products

A deficiency in protein supply is one of the most important food problems encountered in many developing countries of the world. Although animal products are the most desirable protein sources, they are more expensive than vegetable protein (Fujimaki et al., 1968).

Recently, interest has increased in the nutritive value of soybeans and other sources of plant proteins which may be used in the human diet to partially replace or extend animal proteins. Soybeans and their products continue to receive special emphasis because they can be grown economically under a wide range of soil and climatic conditions (Hang and Jackson, 1967a). Jonas (1974) stated that the major problem with soy-based dairy analogs is flavor. Some of the off-flavor constitutents can be removed or reduced in intensity, but even after careful processing, soy off-flavors remain.

The following is a compilation of the various milk-like beverages made from non-dairy proteins and using predominately plant raw material such as soybeans, peanuts or coconuts for the source of protein.

Milk Analogs

Milk Analogs Based on Coconut Protein

Coconuts as raw material have been suggested for milk analogs by Dendy and Timmins (1973). Traditionally, coconut is dried to produce copra and the oil is then obtained from copra by expression or solvent extraction methods. The residual product containing 18-25 percent protein is too fibrous for human food use; consequently, it is sold as an animal food supplement. The possible routes of utilization of coconuts for human food is coconut milk, coconut cream, and coconut protein isolate. However, to obtain these products a wet cocount milling process had to be developed which differs essentially from the copra route (Jonas, 1974). Steinkraus et al. (1968) developed flavored soya and coconut milks for the Philippine market. Hagenmaier et al. (1974) reported that a 12 percent solution of coconut skim milk is an acceptable beverage.

Milk Analogs Based on Peanut Protein

Use of peanut protein for preparation of nutritious beverages dates back to 1950. This early work is reviewed by Swaminathon and Parpia (1967). Large scale development work followed, using recommendations of the Central Food Technological Research Institute, Mysore, India. This group saw in peanut milk an excellent extender for the high fat buffalo milk available in India. "Miltone," a

toned peanut protein milk product was placed in the highlights of the Institute of Food Technology Award in 1971 (Anonymous). The Central Food Technological Research Institute of Mysore, India was honored by developing a new dairy product that could double the supply of milk for the children of India. Miltone has all the properties of milk and could be used in the same way as milk (Jones, 1974). In 1973 (Anonymous), Miltone was being produced in two cities in India, with two more plants being planned.

Milk Analogs Based on Soybean Protein

Soybean milk is said to have been originated by the Chinese philosopher, Whai Waim Tze, long before the Christian era and is known to the Chinese under the name of Fu Chaing or bean curd sauce. This is not only used in the fresh state by the Chinese, but is also condensed in the same manner as cow's milk. It also forms the basis of the various kinds of bean curd or vegetable cheese which are so extensively used throughout the orient. Attempts have been made at various times to place this bean milk on the market in European countries and America but without much success (Piper and Morse, 1923).

The earliest attempts to produce a soy milk are reported in the Western literature in a German patent issued to Gossel (1911); this was followed by other patent applications in Great Britain and in the U.S. Dehulled soybeans were soaked in water, mashed or milled, and the slurry

filtered. Nutritional fortification of the soymilk analogs was considered and nonfat milk powder, calcium salts and vitamins were suggested for use. Flavor improvement was thought to be necessary and proteolytic enzymes were suggested for this purpose in 1921. In the next decades, efforts to debitter soybean proteins were reported by Japanese and German sources (Jonas, 1974).

The infant food industry moved into the direction of soy milk to supply infants allergic to cow's milk with nutritionally acceptable substitutes. Further work along the same lines, but directed toward mass feeding of infants by UNICEF in Indonesia, was done in the 1950s. Large scale production of liquid soy milk was carried out in the Far East in numerous locations in 1965. Dried soy milks were marketed in Japan (Swamimathan and Parpai, 1967).

In the U.S., Hand et al. (1968) researched the preparation of soya milk. An acceptable bland soya milk analog was produced by Wilkens (1967). Wilkens ground dehulled soybeans with water between 80-100°C and maintained the beans at this temperature for 10 minutes to completely inactivate lipoxidase. This development was adapted to the Philippine soy milk market by Steinkraus et al. (1968). Growth and activity of lactic acid bacteria in soya milk was investigated very thoroughly by Angels and Marth (1971).

The status of soya milk technology, processing,

economics, nutrition and organoleptic properties were summarized by Bourne (1970). The mechanism of protein solubilization in dried soy milk preparation was studied by Fukushima and Van Buren (1970). This was followed by extensive process studies conducted by Khaleque et al. (1970). Their finding indicated that it is possible to produce a sterilized fluid canned soya milk concentrate up to 15 percent solids. Removal of fatulence--causing oligosaccharides from soy milk was proposed by Sugimoto and Van Buren (1970) who used a galactosidase and invertase enzyme preparation obtained from Aspergillus saitoi. Chien (1974) treated soybean with yeast to hydrolyze these oligosaccharides.

The most significant soya milk commercial venture was the introduction of heat sterilized, bottled soya milk under the trade name "Vitasoy" in Hong Kong by Lo (1968). This project was the first successful commercial effort for promotion of soya milk. Results were satisfactory from both financial and nutritional points of view.

Preparation of Soy Milk

The preparation of soybean milk was also described by Schroder and Jackson (1971 and 1972). Dry, mature soybeans were steamed for periods ranging from 0-30 minutes. Steaming beans for 20 minutes under atmospheric conditions resulted in bland flavored milk, but a considerable time was required for drying the beans. A three-minute steam

treatment was sufficient to loosen the hulls, with the advantage of a short drying time. The hulls were then cracked in a cereal grinder and pneumatically removed. The dehulled beans were blended for five minutes in a Waring blender with water at 80-100°C; 540 g of beans were added to five liters of water. The resulting milk was steamed for 30 minutes to destroy and remaining anti-digestive factors in accordance with the finding of Wilkens et al. (1967). This method is simple, fast and produces a bland product without caramelization or loss of protein solubility.

The soybean off-flavor problem is mainly associated with volatile compounds. Preliminary experiments utilizing gas chromatography was reported by Wilkens et al. (1967) who demonstrated that the volatiles responsible for off-flavor were derived only from soy milk prepared from whole fat soybean. Soy milk prepared from soybeans which were fat-extracted prior to hydration exhibited fewer volatile constituents.

Also, Wagner (1972) recommended a process for preparation of milk analogs by comminuting soybeans or other legumes in the presence of edible acid which resulted in a reduction of off-flavor compounds.

Steinberg et al. (1973) prepared soy milks based on a new technology. In this process, undesirable flavor development was prevented by a rapid blanching operation. This research indicated that the "beany" off-odors and

flavors are not inherent in the beans, but are induced by enzymes when ruptured cells are moistened. A simple heat treatment of beans before crushing was found to inactivate the enzyme and thereby prevented formation of undesirable flavors. Also the destruction of the enzyme lipoxidase by heat generated during the blanching (Nelson et al., 1971), during the extraction process, eliminates most of these problems.

Composition of Soybean Milk

The composition of vegetable milk will vary with the variety of beans used and with the method of manufacture. This milk is quite comparable to animal milk as shown in Appendix 6. The properties of soybean milk may be said to be similar to those of animal milk. Acids, rennet, pepsin and certain salts will precipitate the proteins as with true milk (Piper and Morse, 1923).

Mechanism of Milk Coagulation

The casein micelle is an aggregate of a number of different caseins. The major types of protein molecules invalued are α_s-casein, β-casein, κ-casein, and γ-casein (Whitaker, 1972). Rose et al. (1970) reported that calcium-sensitive α_s caseins account for 45 to 55 percent of skim milk protein, and along with the β-caseins (25 to 35 percent of skim milk protein) and κ-casein (8 to 15 percent of skim milk protein) make up nearly all the protein in the caseinate micelles of milk.

Milk clotting occurs in two separate phases; a primary phase in which the enzyme attacks κ-casein to destroy its stability capacity, and a secondary nonenzymatic phase in which the destabilized system clots in the presence of calcium ion (Ernstrem, and Wong 1974). The early theories of Hammarsten (1877) that attempted to explain the clotting of milk by rennin were based on the belief that casein was "homomolecular." He proposed that rennin converted native casein into a new form called paracasein, which clotted in the presence of calcium ion. Later this theory was more accurately described by MacKinley and Wake (1971) as

$$\kappa\text{-casein} \longrightarrow \underset{\text{(insoluble)}}{\text{para-}\kappa\text{-casein}} + \underset{\text{(soluble)}}{\text{macropeptide}}$$

Waugh and Von Hippel (1956) demonstrated the κ-casein functions in a significant way to stabilize caseinate micelles against precipitation, and that the stabilizing action of κ-casein is specifically destroyed by rennin. Zittle (1961) verified the stabilizing effect of κ-casein on calcium-sensitive α_s and β-caseins. Delfour et al. (1965) reported that addition of rennin to milk at pH 6.5 and 30°C bring about a rapid hydrolysis of a single phenylalanylmethionyl bond in κ-casein. The splitting of the peptide bond in κ-casein destroys the stability of the micelle which then dissociates the aggregates to form a clot. The initial step is the only enzyme-catalyzed step; the others are nonenzymatic.

Milk Clotting Enzymes

Whitaker (1972) reported that not all proteolytic enzymes are equally good in clotting milk for cheese production. Only a few other even approach the acceptability of rennin in the production of cheese. He stated that these other enzymes include porcine pepsin and two fungal proteinases from Endothia parasitica and Mucor pusillus.

Abdel-Fattah et al. (1972) reported that all proteolytic enzymes can clot milk, but rennin is the main milk-clotting enzyme. A rennin enzyme or rennin substitute, suitable for cheese making, is characterized by a high milk-clotting activity and low proleolytic action. The author indicated that enzymes occur in animal tissues and higher plants as well as in microorganisms. Although much is known about enzymes of animal and higher plant origins, little is known about microbial milk-clotting enzymes. Attention has, therefore, recently focused on the production of milk-clotting enzyme from microbial sources for use as rennin substitutes. Successful attempts to use microbial milk-clotting enzymes have been made by Ş rinivasan et al. (1962) and Toshihiko and Shuji (1969).

A study by Richardson et al. (1967) shows that a fungal rennet produced from M. pusillus is satisfactory for cheese manufacturing.

Influence of Calcium Chloride on Milk-Clotting Activity

It has been indicated by Ernstrom (1974) that the precipitation of paracasein is very sensitive to small variation in the calcium ion concentration near the concentration necessary for coagulation. Pyne (1955) stated that difference in calcium ion concentration was a decisive factor in renneting variation.

Krishnamurthy et al. (1973) found that milk-clotting activity of all the enzymes increased with an increase in the concentration of calcium chloride in the substrate. The influence was highest in the case of animal rennet. On addition of 0.15 percent $CaCl_2$ to milk the clotting activity of animal rennet increased by 54 times over the original activity. On the other hand, the coagulation of milk by the enzyme of M. pusillus lindt was more affected by Ca^{++} than veal rennet according to Richardson et al. (1967).

Cheese Analogs Based on Soybean

Hofner (1959) stated that soybeans offer excellent possibilities as a source of a large quantity of high quality protein suitable for use in many ways as human food. According to Steinkraus et al. (1962) soybeans in their whole, unmodified form are relativey indigestible and have never been highly acceptable as a food. In Asia, where soybeans have been consumed for centuries, they are extracted, fractionated or fermented before eating.

Miller (1943) reported that people in Asia obtain 90 percent and possibly even more soybean protein in the form of soybean curd or tofu. Fresh tofu contains 94-90 percent moisture, 5-8 percent protein, 3-4 percent fat and about 2-4 percent carbohydrate (Piper and Morse, 1923). According to Smith, et al. (1960), fresh, commercial tofu has an approximate composition of 6 percent protein, 3.5 percent fat, 1.9 percent carbohydrate, 0.6 percent ash, and 88 percent water. Smith (1958) stated that tofu is a very suitable product for making fermented cheese and is, in fact, used for this purpose in China. Calcium chloride is frequently used to coagulate the curd, occasionally magnesium chloride and even a concentrated fraction of sea salt has been used in China (Smith, 1949).

Preparation of Tofu

Tofu was prepared by Wang (1967) and the procedure was as follows: Soybeans were mashed, soaked and ground with water when the soluble proteins, along with some lipids and other soluble constituents, were extracted by water. So-called soybean milk was the result after removing the residue by filtering. The milk was then heated to boiling to inactivate growth inhibiters and remove some of the beany flavor. The curdling process was initiated by the addition of salts, such as calcium sulfate, magnesium sulfate and chloride. After this material was pressed to remove excess whey, a soft but firm cake-like product

results which can then be cut into cubes of desired sizes. This product is commonly known as tofu.

Sufu

Sufu is the only traditional fermented product of bean curd. It is a soft cheese-like product made from cakes of soybean curd (tofu) by action of mucaroceaous fungi. According to Wang, et al. (1970), the mold used in this fermented has to have certain qualifications. The mold must have white or light yellowish-white mycelium to ensure that the final product has an attractive appearance. The texture of mycelial mat should also be dense and thick so that a firm film will be formed over the surface of the fermented tofu cakes to prevent any distortion in their shape. Of course, it is also important that the mold growth does not develop any disagreeable odor, astringent taste or mycotoxin. Wai and Hesseltine (1968) confirmed that Actinomucor elegans, Mucor hiemalis, Mucor silvaticus and Mucor subtilissimus passed all these characteristics and can be used to make sufu, having a good quality. The flow sheet provided by Hesseltine and Wang (1970) shows the steps in preparation of sufu (Figure 2).

Digestibility of Soybean Curd

According to the results of digestion experiments by Oshima (1905), the digestibility of the nutrients in bean curd was found to be high. As much as 95 percent of the protein is digested and about the same portion of fat.

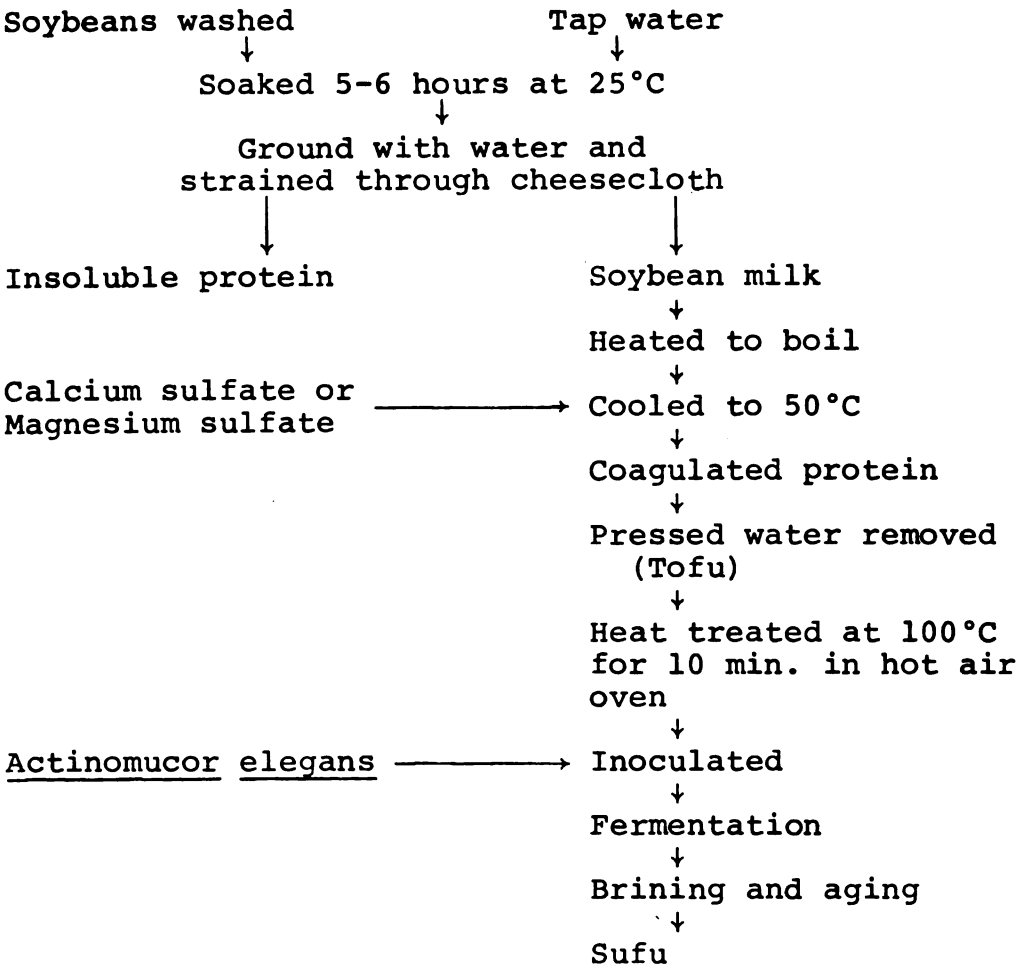

Figure 2. Flow Diagram for the Production of Sufu

The carbohydrates were found to be somewhat less digestible when the tofu was eaten alone, but when eaten with rice, about 99 percent digestibility was reported.

Preparation of Soybean Cheese Using Lactic Starter Organisms

Studies on the preparation of soybean cheese using acetic acid, calcium sulfate and starter organisms were made by Hang and Jackson (1967a).

This study indicated that there were variations in the recovery of protein. Approximately 12-13 percent more protein was recovered by the use of acetic acid than by either of the other two methods. Although the yield of protein by the other two methods was less, the resulting cheese was superior in body and texture. The use of calcium sulfate, which is traditionally a method of precipitation, results in a high concentration of calcium in the cheese. Other salts of calcium, for example, calcium chloride, could be used for this purpose.

As was reported by the author, moisture content and hardness of the soybean cheese prepared by the three methods showed marked variations. The soybean cheese from lactic fermentation was the hardest and contained the least moisture. The softest cheese, and also the one containing the most moisture, was produced by using calcium sulfate. Hang and Jackson (1967a) concluded that a satisfactory cheese could be prepared by using a lactic acid fermentation of soybean milk. The resulting cheese contains

less moisture than that produced by calcium sulfate precipitation and has a better body and texture than cheese produced by either acetic acid or salt precipitation.

Effect of Addition of Rennet Extract and Skim Milk

Other research by Hang and Jackson (1976b) was undertaken to reduce the time from addition of starter to cutting the curd.

Skim milk, when added to soybean milk together with rennet extract and starter bacteria, reduced the coagulation time from just over three hours to two and one-half hours. They concluded that this is probably a result of the action of the rennet extract on the skim milk.

Effect of Mold Ripening and Increasing Concentrations of Skim Milk Solids

Soybean cheese was prepared from blends of skim milk powder and soybean milk by Schroder and Jackson (1971) in this experiment, and skim milk powder contributed 0, 25, 50 and 75 percent of total dry weight. They concluded that the amount of skim milk had little effect on the flavor of the finished cheese, due to the dominating effect of the beany flavor of the soybeans. Similarly, the skim milk had little effect on the texture of the finished cheese, indicating that only a small amount of fibrous matter from the soybeans is necessary to impart a mealy texture to the product. Mold ripening resulted in desirable changes in texture, but these were offset by

the development of bitter flavors.

Lundstedt et al., (1973) claimed that a "blue cheese" with the same flavor, taste, and blue veins as Roquefort cheese, could be made from a soybean curd which is prepared from soy milk fortified with milkfat and nonfat milk solids by inoculation with P. roqueforti and preferably also S. diacetilactis. Mold ripening only required a two-week period for full flavor development.

Economic Aspects of Filled and Imitation Cheese

According to Vernon (1976), any substitute product must have a reason for being: economy; conveneince; short supply of the natural product; additional flexibility in the use or storage of the product; or, in the case of certain food products, dietary consideration.

1. Economy

Compared to some foods, dairy products are expensive. Substitute products can generally be produced at a substantially lower cost.

2. Convenience

Dairy substitutes may be in powdered form, providing extended shelf-life without refrigeration, or liquid, in which they can be handled in fresh or frozen form, providing longer storage times with complete safety.

3. Dietary Considerations

A considerable controversy continues over the effect of saturated and polyunsaturated fats on serum cholesterol

levels in the blood. Although the matter is by no means resolved, some medical authorities feel that consumption of animal fat products such as ice cream, whole milk, butter and cheese should be restricted. Most substitutes utilize partially hydrogenated vegetable oils, which are much lower in saturated fats than the animal fats of similar plasticity.

EXPERIMENTAL METHODS

Materials

1. Enzymes.

Rennet and "Emporase" (derived from pure culture fermentation by Mucor pusillus) were purchased from Dairyland Food Laboratories, Inc. Waukesha, Wisconsin 53186. Prior to use, the enzyme was diluted with 20 volumes of water.

2. Dehydrated Milk.

Nonfat dry milk: purchased from Land O Lakes Creamery, St. Paul, MN.

Lactose: purchased from McKesson, Robbin Co.

3. Soy Protein - "Edipron" - Ralston Purina Company, St. Louis, Mo.

4. Calcium chloride, analytical reagent.

5. Milk, skim and whole milk obtained raw from the M.S.U. Dairy Plant.

6. Cheese culture: a mixed strain Str. lactis culture, propagated by the M.S.U. Dairy Plant.

Enzyme Clotting Effect

I. Seven batches of synthetic milk, based on 3.2 percent protein (soy isolate and casein) were prepared from dried components (Table 1) and water. The batches

were pastuerized in a laboratory can heater at 145°F for 30 min. and cooled to 50°F in a refrigerated water bath. Four hundred ml samples were taken from each batch for evaluation of enzyme coagulability. These samples were heated to 89°F and different concentrations of enzyme, (rennet or Emporase) were added, mixed thoroughly and incubated at 89°F for 40-90 minutes. Curd tension was measured as grams, using a curd tension meter (Submarine Signal Co.). The protein content of these solutions was kept constant at 3.2 percent.

Table 1. Composition of Synthetic Milk Substrates Used in Enzyme Studies

Solution (No.)	Component Used in Synthetic Milk Substrates				
	Soy Isolate	NFDM	Na_2PO_4	Lactose	Total Solids
	%	%	%	%	%
1	1.00	6.00	0.10	1.90	9.00
2	1.50	5.50	0.10	1.90	9.00
3	2.00	3.00	0.15	3.85	9.00
4	2.50	2.00	0.15	4.35	9.00
5	3.00	0.60	0.20	5.20	9.00
6	3.50	0	0.20	5.30	9.00
7	0	8.90	0.10	0	9.00

II. Two series of milk samples were prepared as follows:

A. Varying amounts of soy isolate (0-3.5%) were dispersed in each of seven batches of raw skim or whole milk; the total amount of protein in batches therefore

varied between 3.2 and 6.7 percent.

B. Seven batches of synthetic milk, based on 3.2 percent protein (soy isolate and casein) were prepared from soy isolate and skim or whole milk (Table 2).

Table 2. Composition of the Soy Protein - Cow Milk Formulations Used in Enzyme Studies

Formulae (No.)	Component Used in Formulae			
	Soy Isolate	Skim or Whole Milk	Distilled Water	Protein
	g	g	g	%(w/w)
1	20	3375	605	3.2
2	40	2750	1210	3.2
3	60	2150	1815	3.2
4	80	1500	2420	3.2
5	100	875	3025	3.2
6	120	250	3630	3.2
7	128	0	3872	3.2

Four hundred ml samples were taken from each batch for the enzyme studies. These samples were warmed to 89°F, 0.1 to 0.5 ml of enzyme (rennet or Emporase) were added, mixed thoroughly and incubated at 89°F for 40 minutes. After coagulation curd tension was measured.

III. The samples described in Part I and II of this section were also used in the following experiment. Four hundred ml samples were again taken from each batch, were heated to 89°F, different amounts of calcium chloride (1.8 - 5.7 mM), and 0.2 ml of rennet or Emporase, were added to them. These samples were mixed thoroughly, incubated at 89°F for 40-90 minutes, and curd tension

measured on the coagulated samples.

Lactic Acid Development in Milk Systems Containing Soy Isolate

Formulae 1, 2 and 3 shown in Table 2 were taken for the following tests. Skim milk and simulated milk ultrafiltrate (SMU) (containing 4.8 percent added lactose and 3.2 percent soy isolate) were used as controls. SMU was prepared according to Jenness and Koops (1962).

1. Four hundred ml samples of the above formulae and controls were heated to 90°F and 5 percent starter culture and 0.4 g calcium chloride were added, mixed thoroughly and incubated at 90° for 10 hr.

2. Four hundred ml samples of the above formulae and controls were heated to 79°F and 1 percent starter culture 0.4 g calcium was added and were incubated at 74° for 16 hr.

At hourly intervals 9 g of each sample were taken and diluted with 10 ml of distilled water. These samples were titrated with 0.1 ml sodium hydroxide to the phenolphthalein end point and the percentage of total acid was calculated as lactic acid. Hydrogen ion activity was also determined by measuring the pH each time a sample was taken for titration.

Preparation of Natural, Cheddar-Type Cheese from Bovine Milk Containing Soy Isolate

Cheese of varying composition was made from the systems listed in Table 3.

Table 3. Composition of Formulae Used in Making Cheese

Formulae (No.)	Component Used in Formulae					
	Soy Isolate	Whole Milk	Lactose	Distilled Water	$CaCl_2$	Cream 40%
	lbs	lbs	lbs	lbs	lbs	lbs
A[1]	1.73	290	2.75	51.50	0.085	0
B[2]	1.73	290	2.75	51.50	0.085	4
C[3]	3.45	237	5.00	99.55	0.085	8
D[4]	3.45	237	5.00	99.55	0.085	4

[1]Formula contains 0.5 percent soy isolate, 4.8 percent lactose, 3 percent fat and 3.2 percent protein.

[2]Formula contains 0.5 percent soy isolate, 4.8 percent lactose, 3.5 percent fat and 3.2 percent protein.

[3]Formula contains 1 percent soy isolate, 4.8 percent lactose, 3.5 percent fat and 3.2 percent protein.

[4]Formula contains 1 percent soy isolate, 4.8 percent lactose, 3 percent fat and 3.2 percent protein.

Preparation of Samples

Soy isolate, lactose, calcium chloride and distilled water were mixed thoroughly and the mixture was heated to 160°F. Then the mixture was homogenized at 4,000 psi (3500 and 500) in a two stage Manton-Gaulin homogenizer, Model 75M3-5TBS and cooled to 50°F. Raw whole milk was added to this homogenized mixture and the blend was then pasteurized at 147°F for 30 min. Control cheeses were also prepared for each experiment using fresh pasteurized bovine milk.

Cheese was made in 40 gallon double wall vats in the

dairy processing laboratory. The pasteurized "milk" was heated to 88°F in the vat and starter culture was added as follows: for vats A and B, 5 lbs., 2 1/2 oz; C and D 10 lbs. Twelve ml cheese color was added to each lot at this stage. The milk was held until the acidity had increased 0.02 percent; this required approximately 30 min. Rennet was added as following for samples A, B, and C, 100 ml; and D, 120 ml, and controls, 40 ml. The samples were held at 88°F for 30-40 min. until the sample obtained a firmness suitable for cutting. The curd was cut with cheese knives (3/8 inches) and cooked with continuous agitation for one hour and 20 min. at 100-104°F. (The cooking temp for sample containing soy protein was higher than controls). Then the whey was drained and the curd was fused and cut for cheddaring. The slabs were turned every 15 min. and the drained whey was examined for total acidity. When the acidity reached 0.55-0.65 percent, the cheddared slabs were milled. The milled curd was salted (2 percent), allowed to stand until the salt was dissolved and placed in 10 lb. hoops. The hoops were pressed at 20 psi pressure for 40 min and 40 psi pressure over night. Next morning the cheese was taken out of the press and the cheese cloth was removed. The cheese was placed in plastic bags (Cryovac bags) and was vacuum packed. The bagged cheese was immersed in 190°F water for a few seconds to shrink the bag. The cheese was placed in a curing room for later examination. The curing room was maintained at a relative

humidity of 95 percent and temperature of 50°F.

ANALYTICAL PROCEDURES

Moisture

Moisture content of cheese samples was determined by the vacuum oven method described by the Association of Official Analytical Chemists for cheese (AOAC, 1975).

pH

The pH measurements were made with a Leeds and Northrup Digital pH/mv meter equipped with a glass electrode. The pH was determined to the nearest one-hundredth of a pH unit.

Percent Fat

Percent fat of cheese samples was determined by the method described by the Association of Official Analytical Chemists for cheese (AOAC, 1975).

RESULTS AND DISCUSSION

The Effect of Milk Coagulating Enzymes on Aqueous Synthetic Milks Reconstituted from Dry Ingredients

The solutions shown in Table 1 (Experimental Methods) were treated with coagulating enzymes to initially ascertain the effect of enzymes on reconstituted milks containing additional soy protein isolate. All samples failed to coagulate with 0.1 to 0.5 ml rennet or Emporase after 40 min incubation. The time of incubation was extended to 75 and then to 90 min; still no sign of clotting was found in the samples. The amount of enzymes were doubled, but no coagulation was observed. Different amounts of calcium chloride (1.8 to 5.77 mM) were added to samples, and no clotting was found after 40 to 90 min of incubation. The failure to coagulate may be due to high heat treatment of the fluid milk from which the nonfat dry milk (NFDM) had been manufactured. As was reported by Kannan and Jenness (1961), the high heat treatment may impair the clottability of milk by enzymes. It was indicated by these authors that the chemistry of this effect has not been entirely clarified but appears to involve interaction of κ-casein and β-lactoglobulin as well as insolubilization of calcium phosphate. Coulter and Jenness (1973) also stated that the heat shock

reduced the strength of gel structure (clot) formed after rennin action and retarded its shrinkage (syneresis).

Curd Tension of Skim Milk or Whole Milk and Skim Milk or Whole Milk Containing Soy Isolate Following Treatment with Coagulating Enzymes

The curd tension of skim milk containing various amounts of soy isolate is shown in Table 4. The coagulation of samples was impaired, depending on the level of soy protein added. Samples containing zero percent soy isolate showed a good curd tension. When 0.5 percent soy isolate was added, the curd tension dropped from 20 to 40 g to 0 to 12.5 g.

Samples containing 1 percent soy isolate showed very slight clotting, and more than 1 percent soy isolate resulted in no coagulation. The result of this test could be compared and evaluated with the result of Dordevic and Carie (1969). These investigators conducted a research on feasibility of manufacturing cheese from milk with addition of soya bean flour. They found that with increasing concentration of the flour, the coagulation time increased, and the milk failed to coagulate when the concentration exceeded 4 percent. When 0.04 percent of $CaCl_2$ was added, the milk coagulated even when the flour content was increased to 5 percent. The data in Table 5 show that the curd tension will increase with addition of calcium chloride to the samples. All samples containing 0.5 percent to 3.5 percent soy isolate showed a marked improvement in curd

Table 4. Curd Tension of Skimmilk Containing Various Amounts of Soy Isolate Coagulated by Different Enzyme Extracts

Amount of Enzyme (ml)	Curd Tension (Grams) of Skimmilk Containing Various Percentages of Soy Isolate Coagulated by Different Enzyme Extracts							
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
				RENNIT				
0.10	20.0	0	0	0	0	0	0	0
0.15	25.0	7.5	0	0	0	0	0	0
0.20	25.0	10.0	0	0	0	0	0	0
0.25	30.0	10.0	2.5	0	0	0	0	0
0.35	35.0	10.0	5.0	0	0	0	0	0
0.50	40.0	12.5	5.0	0	0	0	0	0
				EMPORASE				
0.10	7.5	0	0	0	0	0	0	0
0.15	10.0	0	0	0	0	0	0	0
0.20	17.5	0	0	0	0	0	0	0
0.25	20.0	0	0	0	0	0	0	0
0.35	25.0	7.5	0	0	0	0	0	0
0.50	35.0	7.5	2.5	0	0	0	0	0

Table 5. Curd Tension of Skimmilk Containing Various Amounts of Soy Isolate and Ca^{++} Coagulated by 0.2 ml of Different Enzyme Extracts

Amount of Ca^{++} (mM)	Curd Tension (Grams) of Skimmilk Containing Various Percentages of Soy Isolate Coagulated by 0.2 ml of Different Enzyme Extracts							
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
				RENNIT				
1.40	45.0	25.0	25.0	17.5	20.0	10.0	0	2.5
1.80	70.0	25.0	30.0	17.5	20.0	12.5	7.5	12.5
2.16	75.0	25.0	35.0	20.0	35.0	20.0	14.0	20.0
2.52	80.0	30.0	35.0	30.0	35.0	30.0	27.5	25.0
2.88	85.0	35.0	37.5	30.0	35.0	30.0	30.0	25.0
3.24	85.0	35.0	40.0	35.0	35.0	35.0	35.0	35.0
3.60	90.0	35.0	45.0	35.0	35.0	35.0	35.0	35.0
				EMPORASE				
1.40	30.0	10.0	10.0	7.5	7.5	10.0	5.0	5.0
1.80	45.0	10.0	10.0	10.0	7.5	10.0	5.0	5.0
2.16	55.0	10.0	10.0	10.0	10.0	12.5	10.0	10.0
2.52	65.0	10.0	12.5	12.5	10.0	20.0	20.0	15.0
2.88	65.0	12.5	15.0	15.0	15.0	20.0	20.0	15.0
3.24	70.0	15.0	15.0	15.0	15.0	22.5	22.5	17.5
3.60	75.0	20.0	20.0	20.0	20.0	25.0	25.0	20.0

strength after addition of calcium chloride. As is seen in this table, the higher the amount of Ca^{++}, the firmer the curd would be. This was shown with both rennet and Emporase.

Data listed in Tables 6 and 7 show the curd tension of enzyme coagulated whole milk with or without addition of Ca^{++}. Similar results were obtained for the curd tension of whole milk as with skim milk (Tables 4, 5, 6 and 7).

With an increase in calcium chloride, the curd tension increased in both whole milk and skim milk samples containing soy isolate, and virtually all samples showed coagulation (Tables 5 and 7).

Schultz and Ashworth (1974) found that major factors influencing rennet curd strength are pH, calcium ion concentration and casein content. They indicated that there is an optimal percent of calcium which will yield a maximum curd tension in milk. According to Ernstrom (1974), the precipitation of paracasein is very sensitive to small variations in the calcium ion concentration near the concentration necessary for the coagulation.

Comparing Tables 4 and 5 with 6 and 7 showed that all formulae made with whole milk resulted in lower curd tension than samples made with skim milk. Fahimi (1951) reported that skim milk, both from cows and buffaloes, took a longer time to coagulate than whole milk. He suggested that the fat globules in whole milk could act as particles

Table 6. Curd Tension of Whole Milk Containing Various Amounts of Soy Isolate Coagulated by Different Enzyme Extracts

Amount of Enzyme (ml)	Curd Tension (Grams) of Whole Milk Containing Various Percentages of Soy Isolate Coagulated by Different Enzyme Extracts							
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
				RENNIT				
0.10	10.0	0	0	0	0	0	0	0
0.15	15.0	5.0	0	0	0	0	0	0
0.20	17.5	7.5	0	0	0	0	0	0
0.25	25.0	7.5	0	0	0	0	0	0
0.35	30.0	10.0	2.5	0	0	0	0	0
0.50	35.0	10.0	5.0	0	0	0	0	0
				EMPORASE				
0.10	7.5	0	0	0	0	0	0	0
0.15	12.5	0	0	0	0	0	0	0
0.20	15.0	0	0	0	0	0	0	0
0.25	20.0	0	0	0	0	0	0	0
0.35	27.5	2.5	0	0	0	0	0	0
0.50	27.5	5.0	0	0	0	0	0	0

Table 7. Curd Tension of Whole Milk Containing Various Amounts of Soy Isolate and Ca^{++} Coagulated by 0.2 ml of Different Enzyme Extracts

Amount of Ca^{++} (mM)	Curd Tension (Grams) of Whole Milk Containing Various Percentages of Soy Isolate Coagulated by 0.2 ml of Different Enzyme Extracts							
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
				RENNIT				
1.40	35.0	15.0	15.0	20.0	20.0	7.5	0	0
1.80	37.5	15.0	15.0	20.0	20.0	10.0	2.5	2.5
2.16	40.0	17.5	17.5	22.5	20.0	15.0	5.0	5.0
2.52	40.0	17.5	17.5	2.5	20.0	20.0	7.5	17.5
2.88	45.0	17.5	17.5	25.0	22.5	22.5	15.0	20.0
3.24	45.0	17.5	17.5	25.0	22.5	22.5	20.0	20.0
3.60	55.0	17.5	20.0	25.0	25.0	25.0	22.5	22.5
				EMPORASE				
1.40	25.0	10.0	15.0	12.5	15.0	7.5	0	0
1.80	27.5	10.0	15.0	12.5	15.0	10.0	2.5	2.5
2.16	30.0	10.0	15.0	15.0	15.0	15.0	7.5	5.0
2.52	30.0	10.0	17.5	20.0	15.0	20.0	15.0	15.0
2.88	32.5	12.5	17.5	20.0	20.0	20.0	20.0	20.0
3.24	35.0	12.5	17.5	22.5	20.0	22.5	20.0	20.0
3.60	40.0	15.0	17.5	25.0	20.0	25.0	22.5	22.5

promoting the aggregation of the precipitating paracasein micelles in a manner analagous to seed material which may promote crystallization. Hamdy and Edelsten (1970) suggested that in whole milk the fat content is higher and casein content, therefore, is lower than in skim milk. This means that rennet had a bigger amount of substrate (casein) to coagulate in 50 ml skim milk than in 50 ml whole milk. However fat globules may serve as "point of weekness" in the gel structure and this may well account for the lower curd tension of milk containing dispersed fat.

Sanders et al. (1936) stated that homogenization of whole milk causes a marked softening of rennet curd without changing the rennet coagulation time and that homogenization of skim milk has no effect upon rennet curd tension or rennet coagulation time. These results indicate that the curd-softening effect of homogenization is probably due entirely to the increased number of fat globules dispersed in the gel structure, thus hindering the subsequent aggregation of the casein micelles. As further evidence in support of this assumption, it is shown that the rennet curd tension of whole milk is considerably lower than that of the same milk after fat separation, although the rennet coagulation time is not decreased by the removal of the fat.

The enzyme Emporase resulted in a slightly softer curd than rennet in both skim and whole milk samples. Samples coagulated with rennet had a curd tension 15 g

higher than samples clotted with Emporase, the enzyme harvested from Mucor (Tables 4, 5, 6 and 7). This work agreed with work done by Richardson et al. (1967) and Melachouris and Tuckey (1968) reported that fungal rennet solutions standardized with the standard rennet assay appeared to be weaker when tested in cheese milk.

The Effect of Enzymes on Fluid Substrates Containing 3.2% Protein

The formulae 1 through 7 shown in Table 2 (Experimental Methods) were used for these studies. All samples showed insignificant curd tension when treated with either enzyme and results have not been recorded here. However, when calcium chloride was added to these systems, the strength of the coagulum increased. Data presented in Tables 8 and 9 show the curd tension of system after addition of calcium chloride. The best coagula were observed in the system containing 0.5 or 1 percent soy isolate and when 3.6 millimoles (mM) of Ca^{++} was added; samples in Tables 8 and 9 with lower protein content (3.2%) resulted in lower curd tensions than samples with higher percentage of protein, 3.2 to 6.7% (Tables 5 and 7).

Sanders et al. (1936) found that the percentage of casein has an influence on the rennet curd tension and upon the firmness of rennet curd in the cheese process. Later work done by Schultz and Ashworth (1974) supported this work. They found that 4 to 5 percent increase in total protein content (without additional calcium)

Table 8. Curd Tension of Fomulae 1-7 Made with Skim Milk Containing Various Amounts of Ca^{++} Coagulated by 0.2 ml of Different Enzyme Extracts

Amount of Ca^{++} (mM)	Curd Tension (Grams) of Formulae 1-7 Coagulated by 0.2 ml of Different Enzyme Extracts						
	1	2	3	4	5	6	7
			RENNIT				
1.80	20.0	10.0	0	0	0	0	0
2.52	27.5	17.5	7.5	0	0	0	0
3.24	35.0	22.5	15.0	0	0	0	0
3.60	37.5	25.0	20.0	0	0	0	0
5.77	50.0	32.5	20.0	0	0	0	0
			EMPORASE				
1.80	15.0	10.0	0	0	0	0	0
2.52	25.0	15.0	0	0	0	0	0
3.24	27.5	20.0	0	0	0	0	0
3.60	30.0	22.5	10.0	0	0	0	0
5.77	32.5	25.0	20.0	0	0	0	0

Table 9. Curd Tension of Formulae 1-7 Made with Whole Milk Containing Various Amounts of Ca^{++} Coagulated by 0.2 ml of Different Enzyme Extracts

Amount of Ca^{++} (mM)	Curd Tension (Grams) of Formulae 1-7 Coagulated by 0.2 ml of Different Enzyme Extracts						
	1	2	3	4	5	6	7
			RENNIT				
1.80	15.0	7.5	0	0	0	0	0
2.52	25.0	20.0	0	0	0	0	0
3.24	30.0	22.5	7.5	0	0	0	0
3.60	32.5	25.0	15.0	0	0	0	0
5.77	40.0	30.0	17.5	0	0	0	0
			EMPORASE				
1.80	12.5	7.5	0	0	0	0	0
2.52	17.5	15.0	0	0	0	0	0
3.24	25.0	20.0	0	0	0	0	0
3.60	30.0	22.5	7.5	0	0	0	0
5.77	32.5	25.0	10.0	0	0	0	0

increased curd tension 25-30 percent and increased protein content of 18-20 percent raised curd tension almost 50 percent. These workers also noted that a combination of added casein plus extra calcium produced a much stronger curd tension than casein alone.

Development of Acidity

The data in Table 10 show the development of acidity in samples 1, 2 and 3 from Table 2 and also skim milk and SMU when a Str. Lactis culture was added to each of these systems. The SMU had developed a lower acidity at the end of 10 hr incubation compared to other samples. As the level of soy isolate increased, the total acidity decreased slightly; the skim milk developed the highest acidity. The pH of the samples is also shown in Table 11. With the exception of the SMU's system, the samples reached essentially identical pH values at the end of 10 hr. Hang and Jackson (1967b) made a cheese using soy bean milk and skim milk. They found that the bacteria in the cheese containing skim milk would appear to multiply a little more rapidly than the one containing only soy bean milk. This could possibly be attributed to the presence of more highly stimulating substances in the skim milk. They concluded that it is also possible that the skim milk had a stimulating action on the growth of the starter bacteria, resulting in a more rapid utilization of fermentable substrate and increased acid production.

Table 10. Development of Acidity in Different Formulae Inoculated with 5% Culture and 3.6 mM Ca^{++} and Incubated at 90°F

Time (hr)	Percentage Acidity in Various Formula at Different Times of Incubation				
	Control				
	Skimmilk	SMU[a]	1[b]	2[c]	3[d]
	(%)	(%)	(%)	(%)	(%)
0 (before starter)	0.24	0.09	0.17	0.16	0.16
0 (after starter)	0.26	0.15	0.20	0.19	0.20
1	0.29	0.16	0.22	0.22	0.22
2	0.32	0.19	0.26	0.25	0.25
3	0.41	0.23	0.35	0.33	0.36
4	0.54	0.25	0.48	0.47	0.49
5	0.77	0.26	0.69	0.68	0.69
6	1.00	0.32	0.88	0.81	0.78
10	1.17	0.45	1.00	0.94	0.89

[a]Simulated milk ultrafiltrate containing 4.8% added lactose and 3.2% soy isolate.

[b]Skimmilk containing 0.5% soy isolate.

[c]Skimmilk containing 1.0% soy isolate.

[d]Skimmilk containing 1.5% soy isolate.

Table 11. pH Measured in Different Formulae Inoculated with 5% Culture and 3.6 mM Ca^{++} and Incubated at 90°F

Time (hr)	pH Measured in Various Formulae at Different Times of Incubation				
	Control				
	Skimmilk	SMU[a]	1[b]	2[c]	3[d]
0 (before starter)	6.40	6.40	6.65	6.60	6.60
0 (after starter)	6.35	6.30	6.60	6.60	6.55
1	6.20	6.25	6.40	6.45	6.50
2	5.90	5.95	6.09	6.10	6.20
3	5.60	5.70	5.80	5.75	5.90
4	5.20	5.40	5.35	5.40	5.40
5	4.70	5.35	4.80	4.85	4.89
6	4.40	5.10	4.60	4.60	4.60
10	4.35	4.85	4.35	4.35	4.30

[a]Simulated milk ultrafiltrate containing 4.8% lactose and 3.2% soy isolate.

[b]Skimmilk containing 0.5% soy isolate.

[c]Skimmilk containing 1.0% soy isolate.

[d]Skimmilk containing 1.5% soy isolate.

As was indicated by Wang (1974), different samples of milk have different buffering capacities, depending on the concentration of casein, salts and other components, including acids and which are only partly dissociated at the normal pH of milk. Table 12 shows the development of acidity in the soy systems and controls with different amounts of starter and incubated for different periods of time. The pH of these samples is shown in Table 13. The results are similar to those reported in earlier experiments using higher concentrations of starter culture (Tables 10 and 11).

According to Wang (1974), the rate and extent of acid development influences the texture and subsequent ripening of the cheese. Cheese made with excessive acid development, especially that made from over-ripe milk, is likely to have so-called acid defects, i.e., bitter flavors, a "short" brittle body that frequently becomes pasty and a tendency to form splits or cracks. Thus one can appreciate that the proper development of acid by lactic acid forming becteria is essential during manufacture and subsequent ripening of cheese.

The Preparation of a Cheddar-Type Cheese from Bovine Milk Containing Added Soy Isolate

The cheddar-type cheese and appropriate controls were prepared according to accepted commercial practices. Changes in pH and moisture and total crude fat were determined initially and during ripening.

Table 12. Development of Acidity in Different Formulae Incubated with 1% Culture and 3.6 mM Ca^{++} and Incubated at 74°F Overnight

Time (hr)	Percentage Acidity in Various Formulae at Different Times of Incubation				
	Control				
	Skimmilk	SMU[a]	1[b]	2[c]	3[d]
	(%)	(%)	(%)	(%)	(%)
0 (before starter)	0.24	0.09	0.16	0.16	0.16
0 (after starter)	0.24	0.09	0.18	0.16	0.16
4	0.24	0.11	0.18	0.17	0.18
16	1.13	0.29	1.00	0.94	0.82

[a]Simulated milk ultrafiltrate containing 4.8% added lactose and 3.2% soy isolate.

[b]Skim milk containing 0.5% soy isolate.

[c]Skim milk containing 1% soy isolate.

[d]Skim milk containing 1.5% soy isolate.

Table 13. pH Measured in Different Formulae Inoculated with 1% Culture and 3.6 mM Ca++ and Incubated at 74°F Overnight

Time (hr)	Percentage Acidity in Various Formulae at Different Times of Incubation				
	Control				
	Skimmilk	SMU[a]	1[b]	2[c]	3[d]
	(%)	(%)	(%)	(%)	(%)
0 (before starter)	6.40	6.40	6.60	6.60	6.60
0 (after starter)	6.30	6.35	6.55	6.55	6.55
4	6.10	6.20	6.35	6.30	6.30
16	4.35	5.40	4.40	4.40	4.40

[a]Simulated milk ultrafiltrate containing 4.8% lactose and 3.2% soy isolate.

[b]Skim milk containing 0.5% soy isolate.

[c]Skim milk containing 1% soy isolate.

[d]Skim milk containing 1.5% soy isolate.

Changes in pH During Ripening

The representative sample of cheese was analyzed at 1, 30, 60 and 90 days of the ripening period. The pH value for four different samples of cheese is listed in Table 14. In all samples, there was a gradual increase in pH during ripening. The cheese containing the larger amount of soy isolate was more acidic. These results agree with Schroder and Jackson (1971).

Changes in Moisture Content During Ripening

The moisture content of cheese was determined by a vacuum oven method described by the Association of Official Analytical Chemists (1975). The moisture content of cheese was measured at 1, 30, 60 and 90 days. Moisture content of cheese is important because of the legal standard established for cheddar cheese, Cheddar cheese may contain $\leq$ 39 percent water.

Changes in the moisture content of the soy cheese and whole milk cheese (control) during ripening are shown in Table 15. In all samples there was a gradual loss of moisture during ripening. The cheeses with a higher amount of soy isolate had a higher moisture content than the whole milk sample. The whole milk curd probably facilitated whey drainage to a greater extent than did the curd containing soy protein. This result agrees with findings of Schroder and Jackson (1971).

The cheese containing soy isolate had a moisture

Table 14. Change in pH During Ripening of Cheese Containing Various Amounts of Soy Isolate and Fat Compared to Control

Time (days)	pH of Cheese During Ripening Period				
	Control	A[1]	B[2]	C[3]	D[4]
1	5.12	4.98	5.03	4.71	4.72
30	5.13	5.06	5.07	4.72	4.82
60	5.19	5.12	5.14	4.83	4.98
90	5.28	5.20	5.22	4.91	5.02

[1]Formula contains 0.5% soy isolate, 4.8% lactose, 3% fat, and 3.2% protein.

[2]Formula contains 0.5% soy isolate, 4.8% lactose, 3.5% fat, and 3.2% protein.

[3]Formula contains 1% soy isolate, 4.8% lactose, 3.5% fat, and 3.2% protein.

[4]Formula contains 1% soy isolate, 4.8% lactose, 3% fat, and 3.2% protein.

Table 15. The Moisture Content of Cheese Containing Different Amounts of Fat and Soy Isolate Compared to Control During Ripening

Time (days)	Moisture of Cheese Containing Varying Amounts of Soy Isolate and Fat				
	Control	A[1]	B[2]	C[3]	D[4]
	(%)	(%)	(%)	(%)	(%)
1	38.88	48.14	48.19	51.97	50.91
30	37.56	47.94	47.52	49.81	49.63
60	36.91	47.01	46.92	48.95	48.91
90	36.01	46.81	46.01	47.98	47.86

[1]Formula contains 0.5% soy isolate, 4.8% lactose, 3% fat, and 3.2% protein.

[2]Formula contains 0.5% soy isolate, 4.8% lactose, 3.5% fat, and 3.2% protein.

[3]Formula contains 1% soy isolate, 4.8% lactose, 3.5% fat, and 3.2% protein.

[4]Formula contains 1% soy isolate, 4.8% lactose, 3% fat, and 3.2% protein.

content which was greater than legal maximum for cheddar cheese. Alteration in the cooking procedure and/or curd size would probably be necessary to lower moistrue levels. However, at present there are no standards for a "synthetic" or soy based cheddar-type cheese.

Change in Fat Content During Ripening

The fat content of the cheese was measured by a method described by the Assoication of Official Analytical Chemists (1975). The change in fat during ripening period of 3 months is shown in Table 16 and reflects the gradual loss of moisture. Samples containing higher percent of soy isolate showed a lower percent fat in comparison with controls. This may be due to the lack of clot formation by the systems containing soy isolate. The curd containing soy protein was weaker than the control, therefore, it did not hold the fat in the curd and some fat was lost in the whey at the time of manufacturing. There was more fat in the whey of curd containing 1 percent soy isolate than the one with 0.5 percent soy isolate during manufacturing.

Cheddar cheese should contain a minimum of 30.5 percent fat. The samples containing soy isolate show a fat content lower than this level. Once again, alteration of the conventional cheese process might be necessary to better control fat content. As ripening progressed, the body of the cheese containing soy isolate gradually softened and became less elastic; its flavor became mild.

Table 16. Fat Content of Cheese Containing Various Amounts of Soy Isolate and Fat During Ripening Period

Time (days)	Fat Content of Cheese Containing Various Amounts of Soy Isolate and Fat				
	Control	A[1]	B[2]	C[3]	D[4]
	(%)	(%)	(%)	(%)	(%)
1	30.56	21.36	27.11	23.97	17.36
30	31.43	22.28	26.52	24.76	17.28
60	30.81	21.62	28.01	24.61	18.62
90	32.76	21.50	27.91	23.91	17.50

[1]Formula contains 0.5% soy isolate, 4.8% lactose, 3% fat, and 3.2% protein.

[2]Formula contains 0.5% soy isolate, 4.8% lactose, 3.5% fat, and 3.2% protein.

[3]Formula contains 1% soy isolate, 4.8% lactose, 3.5% fat, and 3.2% protein.

[4]Formula contains 1% soy isolate, 4.8% lactose, 3% fat, and 3.2% protein.

These changes are probably the result of the solubilizing action on the soy protein by the proteolytic enzymes present in the rennet extract. This observation agreed with data reported by Hang and Jackson (1967b).

Bitter flavors developed in the cheese containing soy isolate during the ripening period. These bitter flavors may be due to the formation of peptides during degradation of the soy protein. According to Schroder and Jackson (1971), the breakdown is probably from the action of the rennet extract. This process would be accelerated by the low pH from the production of lactic acid by the starter bacteria.

SUMMARY AND CONCLUSIONS

The effects of milk coagulating enzymes (rennet and the enzyme extracted from Mucor pusillus--"Emporase") were studied on milk systems containing soy isolate and the following observations were made:

1. Coagulation of milk system containing soy isolate was impaired, depending on the level of soy protein added.

2. Coagulation was improved by addition of calcium ion ($CaCl_2$) to the system.

3. Good coagulation was obtained on a system containing 0.5 or 1 percent soy protein and 3.6 mM Ca^{++} (0.4g $CaCl_2$ /400 ml).

4. The curd tension was lower in samples coagulated by Emporase than by rennet.

5. The system made with whole milk showed a lower curd tension and softer curd compared to systems made with skim milk.

6. When starter culture was added to this system, all samples developed acidity. As the level of soy isolate increased, total acidity decreased slightly; the skim milk developed the highest acidity.

7. The cheddar cheese with whole milk containing 0.5 or 1 percent soy isolate and appropriate controls were

prepared according to accepted commercial practices.

8. Changes in pH and moisture and total crude fat were determined initially and during ripening of the cheese.

9. There was a gradual increase in pH during ripening. The cheese containing the larger amount of soy isolate was more acidic than the controls.

10. In all samples there was a gradual loss of moisture during ripening. The cheeses with a higher amount of soy isolate had a higher moisture content than the whole milk sample.

11. Samples containing higher percent of soy isolate showed a lower percent fat in comparison with controls.

12. The cheese containing soy protein developed a bitter taste during period of ripening which made the cheese less acceptable than the control cheese.

13. Further experiments are needed to control the proteolysis and prevent the formation of bitter peptides, and manufacture a more palatable product.

APPENDICES

APPENDIX 1

The Food Value of Soybean in Comparison with Other Legumes

Food	Protein (%)	Fat (%)	CHO (%)	Ash (%)	Water (%)	Calories (%)
Soybean	34.0	16.8	33.7	4.7	10.8	1,970
Navy bean	22.5	1.8	59.6	3.5	12.6	1,605
Peas	24.6	1.0	62.0	2.9	9.5	1,655
Cow peas	21.4	1.4	60.8	3.4	13.0	1,590
Lentils	25.7	1.0	59.2	5.7	8.4	1,620
Lima bean	18.1	1.5	65.9	4.1	10.4	1,625
(Mexican) Frijoles	21.9	1.3	65.1	4.2	7.5	1,695
Peanuts	25.8	38.6	24.4	2.0	9.2	2,560
Eggs	14.8	10.5	--	1.0	73.7	720
Beef steak	18.6	18.5	--	1.0	61.9	1,130
Pork chops	16.9	30.1	--	1.0	52.0	1,580
Cottage cheese	20.9	1.0	4.3	1.8	72.0	510
Rice	8.0	2.0	77.0	1.0	12.0	1,720
Corn	10.0	4.3	73.4	1.5	10.8	1,800
Oats	11.8	5.0	69.2	3.0	11.0	1,720
Wheat	12.2	1.7	73.7	1.8	10.6	1,750
Buckwheat	10.0	2.2	73.2	2.0	12.6	1,600
Rye	12.2	1.5	73.9	1.9	10.5	1,750

Reference: Piper, C.V. and W.J. Morse, 1923. The Soybeans McGraw-Hill, New York.

APPENDIX 2

Essential Amino Acid Pattern for Soybean and Hen's Egg Protein

Amino Acid	Flour	Concentrate	Isolate	Egg Protein
Isoleucine	119	115	121	129
Leucine	181	188	194	172
Lysine	161	151	152	125
Total "Aromatic" A.A.	209	220	227	195
Phenylalanine	117	125	134	114
Tyrosine	91	95	93	81
Total Sulfur A.A.	74	73	60	107
Cystine	37	40	34	46
Methionine	37	33	27	61
Threonine	101	100	93	99
Trypotophan	30	36	34	31
Valine	126	118	120	141
Protein Score	68	68	56	100

Reference: Wolf, W.F. and J.C. Cowan. 1975. Soybean as a Food Source. CRC Press, Cleveland.

APPENDIX 3

The Crude Protein, Lipid, and Moisture Contents of Fraction of Soybean

	Crude Protein (N x 6.25) (%)	Lipid (%)	Water (%)
Soybean, dehulled	44.3	25.8	0.8
Residue	23.1	17.3	4.5
Soy milk	50.0	28.5	3.0
Curd	51.5	36.0	2.5
Whey protein	55.0	4.0	7.2
Soak water	18.6	2.0	3.6
Hull	15.4	6.3	9.6

Reference: Hackler, L.R., B.R. Stillings and R.J. Polimeni. 1967. Correlation of amino acid indexes with nutritional quality of several soybean fractions. Cereal Chem. 44:639.

APPENDIX 4

Amino Acid Composition of Soybean Fractions

	Residue	Soy Milk	Curd	Whey Protein	Hulls	Soak Water
	g/16gw	g/16gw	g/16gw	g/16gw	g/16gw	g/16gw
Lysine	5.09	6.05	5.85	8.84	5.98	2.90
Histidine	2.57	2.53	2.35	3.45	2.41	1.71
Arginine	6.19	7.16	6.97	8.39	5.27	5.29
Aspartec Acid	11.5	11.0	11.1	11.2	10.0	11.6
Threonine	4.10	3.87	3.66	5.20	3.52	3.26
Serine	4.90	4.84	4.91	4.40	5.12	3.02
Glutamic Acid	15.6	16.9	17.8	22.1	14.2	9.15
Proline	4.82	4.37	4.88	4.05	5.10	3.60
Glycine	4.21	3.92	3.93	4.52	6.11	3.08
Alanine	3.97	3.94	4.02	4.31	3.97	3.73
Valine	5.01	4.78	4.71	3.18	4.61	3.06
Cystine	2.34	1.61	1.71	2.74	1.38	2.51
Methionine	1.02	1.44	1.35	2.17	1.01	0.50
Isoleucine	4.53	4.79	4.89	2.88	3.89	2.46
Leucine	8.02	7.89	8.00	3.96	6.72	4.21
Tyrosine	3.00	3.90	3.72	3.82	3.16	2.44
Phenylalanine	4.93	4.87	4.83	2.00	3.95	3.18
Tryptophane	1.64	1.53	1.08	1.77	1.31	0.64

Reference: Hackler, L.R., B.R. Stillings and R.J. Polimeni, 1967. Correlation of amino acid indexes with nutritional quality of several soybean fractions. Cereal Chem. 44: 639.

APPENDIX 5

Comparison of Nutritional Quality of Soybean Fractions as Measured by PER, EAAI, RI and Available Lysine

	Residue	Soy Milk	Curd	Whey Protein	Hulls	Soak Water
PER	2.71	2.11	2.20	1.93	--	--
EAAI	75.2	75.1	72.8	70.8	66.2	70.6
RI	85.5	85.9	84.7	81.6	78.9	82.1
Available Lysine	3.73	5.33	4.58	7.01	4.00	1.98

Reference: Hackler, L.R., B.R. Stillings and R.J. Polimeni. 1967. Correlation of amino acid indexes with nutritional quality of several soybean fractions. Cereal Chem. 44:639.

APPENDIX 6

Comparison of Soybean Milk Compared with Cow's Milk (Compiled from Various Sources)

Kind of Milk	Water (%)	Protein (%)	Fat (%)	Carbo-hydrate (%)	Other Substance (%)	Ash (%)
Soybean	92.00	3.70	2.00	1.80	--	0.50
soybean	80.00	4.95	2.97	1.34	--	0.44
Soybean	89.25	3.15	3.10	3.02	1.02	0.45
Soybean	92.50	3.02	2.13	0.03	1.88	0.41
Cow	86.06	3.05	4.00	5.00	1.19	0.70

Reference: Piper, C.V. and W.J. Morse. 1923. The Soybean. McGraw-Hill, New York.

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