TECHNOLOGY OF SOYMILK AND SOME DERIVATIVES

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PROEFSCHRIFT

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Technology of soymilk and some derivatives

1 GENERAL INTRODUCTION.

It is a wellknown fact that the rapid increase of the population in the world creates difficult problems as regards the safeguarding of its food supply. Although a number of countries have the disposal of sufficient sources to warrant a good nutrition of its populations, too many other countries are not able to meet the food requirements considered as a minimum for the sustainment of life.

From the nutrients at play especially the proteins are indispensable for the building up and maintaining of the human body. In as far as the underfeeding refers to warm climates the role of fat is of less importance.

Although this publication is not meant as a nutritional study it may be useful to cite some figures which may serve to gain an understanding of the background of this work. VISCO (1955) of the Institut National de la Nutrition, Rome, made some calculations assuming a protein requirement of 1 gram/day per kg of body weight and an optimal ratio of 1:1 between animal and vegetable proteins. Calculated in this way the daily, individual requirement of total protein amounts to 60 grams and of animal protein to 30 grams. From literature it appears that the consumption of total protein in tropical countries is near to these 60 grams. However, from the most important sources of animal protein, meat and milk, the average daily consumption is only 9 and 9.3 gram respectively (world average). According to Visco this figure is even rather high and a better understanding might be obtained if the maximum and minimum consumption of animal proteins of the different countries are taken into account.

	Maximum consumption of animal proteins	Minimum consumption of animal proteins
Asia	17.8 g/day	1.6 g/day
Africa	26.1	5.7
Europe	69.5	7.7
South-America	72.3	16.1
North-America	60.5	20.-

From these figures it may be seen that even the maximum consumption of animal protein in Asia and Africa -where more than half of the world's population is accumulated- is below the daily required amount.

As it will not be easy to increase rapidly the production of animal proteins in underdeveloped countries the use of vegetable proteins of high nutritional quality should be emphasized.

One of the most important sources suited for this purpose is the soybean, of which the proteins are highly nutritive. Besides the cultivation of the soybean has many attractive points. First, the soil and climate requirements of the crop are not high. Second, the yield per unit of area is high, while third the protein content of the beans is very high. Lastly, the amount of labour necessary to raise the proteins is low in comparison with many other proteins.

LAGER (1945) quotes figures from the U.S. Dep. of Agric., Bureau of Economics (R.P.Christensen):

AVERAGE OUTPUT OF PROTEINS PER UNIT OF RESOURCES

	Pounds per acre	Pounds per 100 hr of labour
Milk, whole	39	89
Eggs	25	56
Beef cattle	7	45
Wheat, whole flour	90	1002
Potatoes	118	174
Peanuts	116	200
Soybeans	339	2821

In order to get a notion of the importance of the soybean we further mention some figures on the production and uses. The Yearbook of Food and Agriculture (1956) gives the following data.

PRODUCTION OF SOYBEANS IN 1955 (1000 metric tons)

Europe	-
North America	10320
Latin America	120
Near East	10
Far East	10350
Africa	50
USSR	?

In North America the greater part was produced by the United States (10166), while in the Far East China (9144), Japan (504), Korea and Indonesia produced the most soybeans.

According to BURTISS (1950) from prehistoric times to 1908, soybean production and trade in soybeans, soybean cake, and soybean oil were confined almost exclusively to eastern Asia. A second stage, extending from 1908 to 1939, was marked by large exports of soybeans and soybean oil from Manchuria to Europe, where the soybean was one of the leading raw materials used by the oilseed-processing industry. A third and present stage is marked by the pre-eminence of the United States in production and processing soybean for oil and meal. This phase began in 1940 when war disrupted the trade between Manchuria and Europe.

It has been estimated that in China 55% of the crop is used in food products such as bean sprouts, soybean milk, and soycurd ("tofu"). These foods supply a very considerable part of the protein in Chinese diets. Soybean curds, prepared in numerous forms, are roughly equivalent to meat in occidental menu. Ten percent of the Chinese output is fed to animals, 8% is used for seed, and most of the remaining 27% is crushed for oil and cake. The oil, in turn, is largely used for food, while most of the cake is returned to the soil as fertilizer. Most of the Manchurian export of soybean cakes were destined as fertilizers for the rice fiels in Japan, and the sugar cane plantations in Taiwan and Southern China.

Also in Japan food products derived from the soybean play an important part in the Japanese diet. The leading soybean product consumed in Japan is "miso" a fermented product made from soybeans and rice. Before the last war about 67% of the soybeans consumed in Japan was used for food products, 22% for oil and cake, and 11% for seed.

In Indonesia soybeans have been grown since antiquity, and are widely used by the population for food preparations. However, it has never been so important as in China, Korea or Japan. Introduction of selected high-yielding varieties from Taiwan increased the production of soybeans.

In EUROPE, except in the Danubian countries, soybean production has never attained commercial importance. About 1935 the Germans began a programme of encouraging large-scale plantings of soybeans in the Danubian countries, particularly Rumania and Bulgaria, by guaranteeing a specific price for the production for contracted acreage. This was part of the German effort to become less dependent on overseas sources for foodstuffs.

The leading world importer of soybeans between the two world wars was Germany. Beginning in 1934 the net imports into Germany declined materially, largely as a result of a drive for greater self-sufficiency in fats. The soybeans imported from the Balkan were of high quality and were used almost entirely in the manufacture of fullfat soybean flour and flakes for the German army.

After the war, the American army shipped substantial quantities of soybean flour (mostly lowfat) and moderate quantities of soybeans and soybean oil to western Germany.

Up to 1898 only eight varieties has been brought to the UNITED STATES. In that year the Department of Agriculture began to introduce new varieties from the East. By 1937 more than 10.000 selections, representing 2500 distinct types, had been imported and these introductions formed the basis of a vigorous program of varietal research and selection.

Varietal selection has tended to produce three distinct types of soybeans grown, for processing for oil, for hay, and for use as a vegetable. Hay varieties are usually distinguished by black or brown seeds, and are finer-stemmed and more leafy than the yellow-seeded varieties. Soybeans specially adapted for direct human consumption are known as edible or vegetable varieties. The most suitable varieties are those with strawyellow, greenish-yellow, or green seed. These varieties are generally easier to cook and more palatable than others. They may be used either green or mature. Vegetable varieties usually have larger seeds and contain more protein and less oil.

However, soybean meal for livestock is the major soybean protein product in the United States. In 1942-1947, 90 to 95% of the total output was used for feed. Since 1941, soybean flour has been the principal soybean protein product other than meal. After the end of the second world war large orders were placed by the United Nations Relief and Rehabilitation Administration for relief feeding in Europe and Asia.

From the foregoing it is clear that in some East and South East Asiatic countries the processing of soybeans for food purposes has been a well established habit from ancient times. In fact, in China the use of derivatives of the soybean originates from about 3000 B.C. In many other countries, however, the use of soyproducts is little known. Especially for the underdeveloped countries knowledge of the technology of these products is useful to meet the food demands of its growing populations.

Regarding the long history of the soybean it is not surprising that many soybean products have come into being. One of these products is soymilk, a foodstuff which has already a very long history. This milk is the starting material for some other products derived from it, such as soy cheese, soymilk powder, concentrated soymilk, etc. Although these products certainly are not new, they have hardly been studied (at least in European languages not many data on these products are known). As, moreover, extension of the use of soymilk and derivatives is desirable, it was considered important to undertake a study of the manufacture of soymilk and some derivatives in order to improve the quality, the yield, and to lower the cost price.

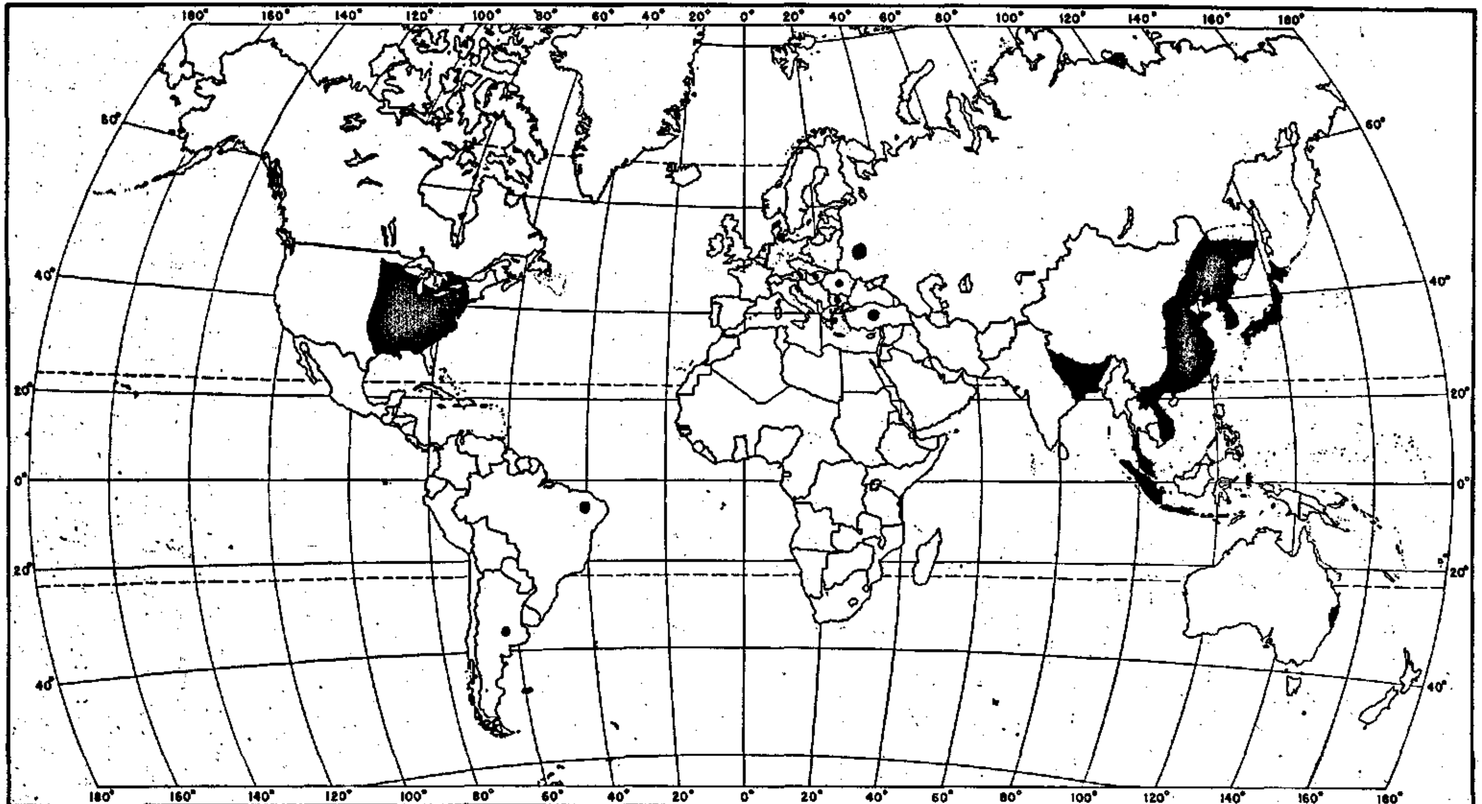


Fig 1. The black areas on this map show the principal soybean seed-producing countries of the world; after Morse.

2 THE SOYBEAN

plant: history, characteristics
seed : structure, composition, proteins

The early history of the soybean, like that of most important food crops, is lost in obscurity. According to MORSE (1950) ancient Chinese literature reveals that the soybean was extensively cultivated and highly valued as a food centuries before written records were kept. Many of the early writings record the advice of agricultural experts on soil preferences, proper time of planting, methods and rates of planting, the best varieties to plant under different conditions and for different uses, time to harvest, methods of storage, and utilization of the many varieties for different purposes. Some of this expert advice goes back as far as 2207 B.C., indicating that the soybean was perhaps one of the oldest crops grown by man.

The yellow- and green-seeded varieties were regarded as valuable foods for the use of mankind. In addition to the uses of the beans, many references were made during the centuries to the processes of preparing and the value of soybean sauce, soybean milk, soybean curd, soybean paste, and soybean sprouts, not only for food but also to treat various diseases and body ailments.

The soybean has been referred to as Glycine hispida (Moench) Maxim. The late Dr. Piper came to the conclusion that it must be named Soja max (L.) Piper. Other botanists still call it Glycine max (L.) Merrill. The name soya comes from the Chinese word "chiang-yiu" which means soy sauce, pronounced in Japanese as "show-yu" and contracted into "so-ya".

The soybean has its origin in eastern Asia and is believed to have been derived from Glycine ussuriensis, which grows wild throughout much of eastern Asia. A plant with characters between the wild and the cultivated species has been described by a Russian botanist as Glycine gracilis Skvortzov. There is little doubt as to the origin of the cultivated plant, since apparently *none* of the other wild plants found until now can possibly be its ancestor.

The cultivated soybean *plant* is an erect, branching summer legume, while the wild plant is a slender, twining variety. A complete series of forms connect both types of varieties. The varieties, which are very numerous, range in maturity from very early (about 75 days) to very late (200 days or more). With few exceptions, earliness is correlated with size, the tallest varieties being latest. Nearly all varieties are pubescent, that is the stems, leaves and pods are covered with tawny (brown) or gray hair. Several yellow-seeded varieties are entirely glabrous, that is lacking in pubescence.

The plant has trifoliate *leaves,* each compound leaf being made up of three leaflets. Occasionally varieties occur with four or five leaflets, especially some of the vegetable types and early Siberian types. In general the leaflet is ovate, lanceolate, but in some varieties it is narrowly lanceolate or almost linear.

The small, inconspicuous *flowers* borne in axillary racemes at the nodes are either white or purple. The flowers first appear at the base of the stem, then progressively toward the tip, the period of blooming not being over three weeks.

The *pods,* one to four inches in length, have colours ranging from straw-yellow over gray and brown to black. The beans in one pod numbe two to three; they are yellow, green or black in colour.

The soybean *seed* is a typical legumeseed. Its weight varies from about 2 g per 100 seeds to over 40 g per 100 seeds in certain vegetable varieties (W I L L I A M S, Markley 1950). Most varieties in common use weigh between 10 and 200 g per 100 seeds. Seed size is quite dependent on environmental conditions but relative sizes are quite constant. Soybeans vary in shape from almost spherical to elongated or flat.

The most extensive data on the microscopical structure of the soybean come from K O N D O (1912). He studied the anatomical structure of a number of varieties from China and Japan, and made the following observations.

The *seed coat* consists of four layers, a palisade layer, the column cells, the spongy parenchyma and the aleurone layer.

The epidermis is made up of palisade cells, 40-60 u in length and 10-20 u in width. The cellwalls are thick and the lumen becomes narrower in the direction of the surface of the seed coat. The walls themselves are always colourless. The colour of the seed coat is located in the lumen.

The columncells form the second layer. These cells are shaped like hourglasses, each end being hexagonal, and are a little longer than the palisadecells. Owing to the form of the cells there are large intercellular spaces. The cells and the intercellular spaces are empty. The hourglass cells separate readily from adjoining layers and from each other and by their characteristic form furnish a means of identifying soybeanmeal. The columncells are 27-70 u long and 16-27 u wide.

Below the columncells is a layer of spongy parenchyma consisting of about 7-8 rows of empty cells ranging in width from 40-60 microns. In a dry condition this layer is very thin, because the cells are much compressed radially.

The aleuronelayer consists of a single series of moderately thick-walled cells with dense protein (aleurone) contents. In a tangential section the aleurone cells are seen to be rectangular or polygonal. The other rows of cells are compressed into a colour-less, fiberlike layer.

The *hilum* is 2.5-4.0 mm long and 1.0-1.5 mm wide. It consists of a double layer of palisade cells, a fibrovascular bundle, a layer of starshaped cells, spongy parenchyma and an aleurone layer.

The palisade layer consists of two rows of cells, differing in colour. The cells of the outer layer are 59-67 u long and 7-10 u wide, those of the inner layer are 39-59 u long and 9-12 u wide. So the outer layer is somewhat thicker than the inner. Another difference is that outer layer is usually yellow in colour, while the inner layer is brown, green etc.

Below the palisade layer lies a layer of starshaped cells with thick walls. The cells contain a redbrown protoplasm.

In the middle of the hilum lies the fibrovascular bundle surrounded by the starshaped cells. The bundle consists of spindle-form tracheids with reticulated walls.

The spongy parenchyma and the aleurone layer have the same structure as already described for the seed coat. It is important to notice that in the spongy parenchyma there are two fibrovascular bundles.

The columncells are not present in the hilum, but near the hilum these cells are extraordinary long. In the vicinity of the hilum the columncells are 150-175 u long and 27-31 u in width and thus they are 2.5-4 times the lenght of the palisade cells. The further from the hilum the shorter the columncells become and at the flat side of the seed the columncells measure only 0.7-1.0 times the palisade cells.

The *embryo* consists of two seedleaves (cotyledons), the primary leaves (plumule) and the hypocotyle, whose root end (radicle) is at the micropyle. The seed leaves usually are flat and elliptical, the two primary leaves are ovoid and free of starch, while the rootlet is a little bit curved and perpendicular to the primary leaves.

The cotyledons ordinarily are yellow-coloured. The inner and outer epidermis consists of small cells, filled with small grains of aleurone. Below the inner epidermis are 2-6 rows of palisadecells, making up about one third of the cotyledons. The following rows consist of still longer cells and the last one or two rows are made up of isodiametrical cells directly below the outer epidermis. The palisade cells are 82-105 u long and 11-24 u in width, but in the middle of the tissue the cells measure 94-188 u in length and 24-42 u in width. These cells are filled with large grains of aleurone and fat. The aleurone grains are elliptical or polyhedral in form and very big (5-16 u). The Chinese varieties have bigger grains than the Japanese. The cells contain much oil, which is located between the aleurone grains.

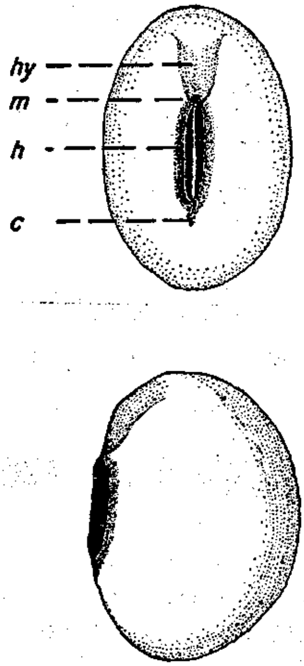
According to literature soybeans are starchfree. As regards this much disputed point KONDO, however, has found that some of the examined varieties do contain starch. The Chinese varieties and one Japanese variety were free of starch. The starch grains are small and irregular in form. The outer, about one third part of the cotyledons are free of starch and it is the inner part that contain the starch grains.

As to the chemical composition, Morse has quoted figures from LECHARTIER (1903), being the most extensive of its kind. For the weight of the stems, leaves and pods the ratio 25.45 : 40.18 : 34.37 was found while the dry plant proved to contain 11.96% of protein, of which 6.24% is present in the pods.
The different parts of the beans have the following composition.

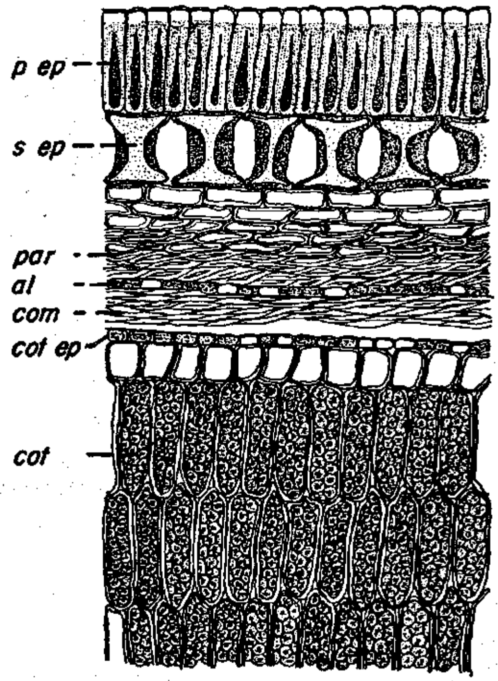
	Proportions of the seed	Dry matter	Nitrogenous substances	Carbohydrates	Fat	Ash
Entire seed	100 %	90.18	38.06	12.06	17.80	4.44
Cotyledons	90	89.43	41.33	14.60	20.75	4.38
Embryo	2	87.99	36.93	17.32	10.45	4.08
Seed coat	8	87.47	7.00	21.02	0.60	3.83

From these figures it may be seen that most of the protein is concentrated in the cotyledons.

As regards the chemical composition of the dry matter of the soybean, including seed coat, CARTTER and HOPPER (1942) made extensive studies on the average composition of 10 varieties of soybean grown during 5 years in 5 different localities. They arrived at the following average composition.



Drawing of Lincoln soybean seed showing: *h,* hilum; *c,* chalaza; *m,* micropyle; and *hy,* hypocotyl seen through the testa.



Cross section of soybean seed coat and portion of cotyledon showing: spermoderm which consists of *p ep* palisade cells of epidermis; *s ep* hourglass cells, and *par,* parenchyma, both of subepidermis; *al,* aleurone cells; and *com,* compressed cells of the endosperm; *cot ep,* cotyledon epidermis; and *cot,* aleurone cells of cotyledon.

Fig 2A. Structure of the soybeen seed (After Williams).

AVERAGE CHEMICAL COMPOSITION OF SOYBEANS OF TEN VARIETIES, ON A MOISTURE-FREE BASIS, GROWN IN FIVE LOCATIONS IN FIVE CROP YEARS.

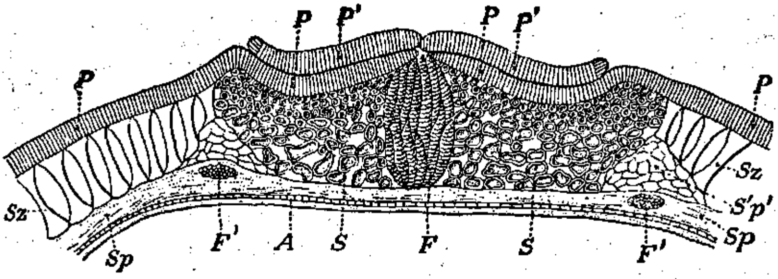
Crude protein	42.78%	Phosphorus	0.659
Crude oil	19.63	Potassium	1.67
Crude fibre	5.52	Calcium	0.275
Sugars (as sucrose)	7.97	Ash	4.99

Besides the wellknown fact that the soybean is an oilseed, the bean also proves to contain a very high percentage of crude protein, as may be judged from these figures.

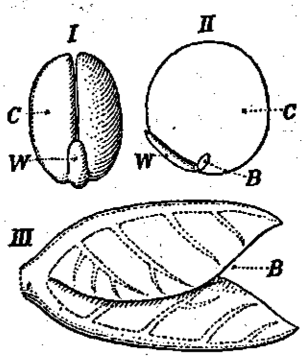
On comparison of the composition of the soyproteins with that of animal proteins (both in the form of milk) a marked resemblance is evident. We cite the following figures from the F.A.O. Nutritional studies No.8 (Brock and Autret, 1952).

	Cow's milk g per litre	Soybean milk g per litre
Arginine	1.26	2.82
Histidine	0.91	1.01
Lysine	2.625	2.40
Tryptophane	0.525	0.56
Phenylalanine	1.785	1.95
Methionine	0.840	0.56
Threonine	1.505	1.57
Leucine	3.434	2.93
Iso-leucine	2.625	2.09
Valine	2.52	2.00
Proteins	35.00	35.00

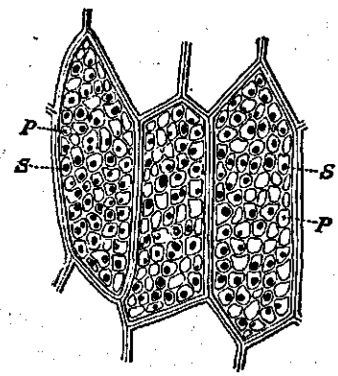
The authors conclude that the amino-acid content of soymilk is comparable to that of cow's milk, except in methionine. The methionine content of soymilk is, however, double that of the milk of the European mother and three times that of the milk of the African mother.



—Cross-section of hilum of a yellow soybean from China. *Pl*, outer palisade layer; *P*, inner palisade layer; *S*, asteroid parenchyma; *Sp*, spongy parenchyma; *F*, fibro-vascular bundle; *F*, fibro-vascular bundle of the testa; *A*, aleurone layer; *Sz*, hour-glass cells. (*After Kondo.*)



Embryo of a yellow soybean seed from Japan. I. Whole embryo from the ventral side. II. Half of embryo seen from the inner side. III. The two leaves of the plumule. *C*, cotyledons; *W*, hypocotyl; *B*, leaves of plumule. (*After Kondo.*)



Soybean. Cells of the cotyledon filled with fat, protein and starch; *P*, protein; *S*, starch. (*After Kondo.*)

Fig 2^B Structure of the soybean seed.

3 MANUFACTURE OF SOYMILK

3.1 Introduction.

The first statements in old Chinese literature on the processing of soybeans do not seem to relate to soymilk as such, but to "tou fu" a product derived from soymilk. As, however, the first necessary phase in making toufu is the preparation of soymilk in this chapter we draw attention to the first known reference to making toufu. This first statement is already found in the Han Dynasty Taoist work, Huai Nan Tsu, or writings of Liu An, a prince of Huai Nan who died in 122 B.C.(MORCE, Markley, 1950).

However, as has been mentioned before, the plant has been known since about 2800 B.C. and besides ancient literature reveals that the soybean was cultivated and highly valued as a food centuries before written records were kept. We may therefore safely assume that the processing of soybeans dates back to before the period of the Han Dynasty.

It is very likely that Chinese and Japanese workers already made extensive studies of the processing of soybeans. Data in languages accessible to European research workers did not appear earlier than the beginning of the 20th century however. Examples are the work of Inouye (1897), Katayama (1906), Li (1911) and others.

The soybean was first brought to the attention of Europeans by the German botanist, Kaempfer, who spent two years (1691-92) in Japan. Although he discussed in detail various food products prepared from the soybean, little interest was taken in the crop and it was not until the beginning of the twentieth century that the production of soymilk and derivatives was started on an industrial scale. This was done about 1910 near Paris and at Frankfurt in Germany (INST. INT. D'AGRIC., 1936). After the Second World War a soymilkpowder plant built by Miller for the Chinese Government was completed (SMITH, 1949), while in the United States another plant was located in Ohio (LAGER, 1945). Since then several other plants seem to have been built in Asiatic countries.

Soymilk may be described as a stable dispersion of water-dispersable constituents of the soybean. Depending upon the raw material and the method of manufacture the dry matter content is about 10%. This dry matter mainly consists of proteins, fats, and sugars.

In the following table some figures on the composition of soymilk are shown.

	Java Prinsen-Geerligs 1896	Japan Kata-yama 1906	China Chiu 1927	India De 1948	U S A Markley 1950	Human milk	Cow milk
Proteins	3.13 %	3.02 %	5.72 %	4.2 %	2.79 %	1.25 %	3.50%
Fats	1.89	2.13	2.44	3.4	2.22	2.50	4.00
Sugars	-	-	0.43	1.8	6.05	6.00	3.25
N-free extr.	-	1.88	-	-	-	-	-
Ash	0.51	0.41	0.73	0.75	0.94	0.25	0.75

Soymilk has a cream yellow colour and in a raw state it smells of raw beans. This beany odour disappears in cooking and then a typical flavour of cooked soymilk develops which is much appreciated in countries where soymilk and derivatives form an integral part of the diet. According to LAGER (1945) the handicap of unpalatability has been conquered by physical rather than chemical methods, so that the milk is sweet and rather nutlike.

Literature data concerning the pH of the milk are contradictory. According to BLOCH (1906) the milk is slightly alkaline, while PRINSEN GEERLIGS (1896) states that its reaction is mild acid. Since the milk can be prepared in many ways it is quite possible that the method of manufacture also influences the reaction of the milk. According to MATAGRIN (1939) the boiling point is about 101°C; the milk rises in boiling, forms a film and may cause incrustation.

The raw soymilk deteriorates rapidly. If raw soymilk is left at room temperature it separates into two parts. The lower part is whitish and resembles the curd of animal milk and the upper part has a transparant, greenish yellow colour (MONNIER, 1935). If the milk is boiled it can be preserved for a much longer time. It is only after 15 to 29 hours that symptoms of putrefaction appear.

Concerning the tuberculose-bacteria, MATAGRIN (1939) states: "Il garantit par rapport au produit animal un avantage d'extrême importance: l'absence certaine de bacille de Koch, dont la présence assez fréquente dans le lait de vache ménage de contagion tuberculeuse plus encore les animaux domestiques que les nourissons, puisque la stérilisation ou la pasteurisation se généralisent aujourd'hui dans l'allaitement artificiel".

On microscopic observation a large number of fat globules are visible in the milk. The smaller globules are in an animated Brownian movement. By far most of the fat globules are between 0.5 to 1 u in size; larger globules of from 10 to 12 u rarely occur.
MATAGRIN (1939) states that no starch grains are present in the milk, while the milk does not react to colouring methods of iodine dissolved in KJ, methyleneblue or rutheniumred.

Photo 1 is a microscopical photo of soymilk made by us.

3.2 Literature.

3.2.1 Methods of manufacture.

Data on the method of manufacture of soymilk as applied by the populations of several countries in East and South East Asia are very numerous. SMITH and BECKEL (1946) have compiled the existing literature on the preparation of soymilk. The references number 77, of which 33 are patents.

To illustrate these original manufacturing methods we will cite some authors.

According to PRINSEN-GEERLIGS (1896) on Java, the beans, after being soaked for 3 hours, are ground between two stones. The thick liquid is boiled after which the mass is filtered through a cloth.

BLOCH (1912) describes how in Indo-China the beans are ground after being soaked overnight. The grinding is performed by two millstones and adding a constant supply of water. Then the dispersion is filtered through a cloth.

LI YU-YING (1912) states that in China the beans are soaked for 18 to 24 hours after which they are ground with a suitable amount of water. The grinding is performed by two millstones of from 0.5 to 1 meter in diameter. The thick liquid is received in a tank and then filtered through a linen cloth.

In the report of SMITH (1948), who visited China, Korea, and Japan to study the processing of the soybean into food products, no principal changes are found in the methods of manufacture. He states that in China the beans are carefully washed and soaked in water for 6 to 7 hours in summer and 24 hours in winter. Then the beans are ground in a stone mill and the ground mass is filtered through a filter cloth. The ratio between water and beans is ten to one.

INOUYE (1884) states that in Japan the beans are soaked for about twelve hours and then ground between mill stones until a uniform mass is obtained. This mass is boiled for half an hour with three times as much water, after which it is filtered.

In the following short discussion we will consider the research work done on the manufacture of soymilk.

The work of CHIU (1927, Cornell) is more concerned with the physical and chemical properties of soymilk than with its manufacture. The physical part of his work deals with specific gravity, freezing point, electrical conductivity, viscosity, refractive index of serum, dialysis, pH, and keeping qualities. The chemical part deals with the general composition, effect of soaking composition of the fat, kind of proteins, composition of the serum, and action of ferments.

Chiu used two varieties of soybeans, Manchu and Black Eyebrow, and followed the Chinese method of preparing soymilk. The soaking periods were 2, 4, 6, and 8 hours respectively. Two hundred and fifty grams of beans were soaked in 1.25 l of water. Then the beans were ground in a stone mill, 1 l of water was added, and the beans were ground again until they were very fine. The milk was filtered through bags made of cloth. After separating the solid particles from the liquid the milk was received in containers.

The specific gravity proved to vary with the times of soaking: as the soaking time increased the specific gravity became higher. If the percentage of dry matter varied from 10.0 to 12.0% the specific gravity ranged from about 1.0295 to 1.0315.

The relative viscosity of the milk was calculated by dividing the time of flow of a definite volume of milk through a capillary by that of water at the same temperature (25°C). Viscosity ranged from 2.75 to 3.65 for 11.0 to 12.0% of dry matter. The variety Black Eyebrow consistently gave higher values than Manchu.

The pH determined at 26°C was 6.43 for Manchu after 6 and 8 hours soaking. For Black Eyebrow these values are 6.52 and 6.53 after 6 and 8 hours soaking. He came to the conclusion that soymilk is only slightly more acid than cow milk. Soymilk colours lithmus red.

The composition of the milk will vary with the varieties of beans used, the methods of manufacture, and the times of soaking. According to this worker there is a limit to the extraction, as it was not possible to extract more than 20% of the solids of the beans with water.

In making soymilk the beans have to be soaked in water to facilitate grinding. It has been shown that long soaking removes the unpleasant odour of the beans and increases the percentage of the total solids in the milk. If the water in which the beans are soaked is used to make the milk, the substances extracted by the water are retained in the milk. According to Chiu it is not advisable to soak the beans for more than 8 hours, as otherwise the loss of total solids extracted by the soaking water will be too great. The fat content increases with longer periods of soaking. After 2 and 8 hours the fat content of the milk from Black Eyebrow is 2.31 and 2.56%, and from Manchu 2.23 and 2.44%. For Black Eyebrow the protein content is 5.73 and 5.90% and 5.59 and 5.72% for Manchu.

Many trials were made for determining fat in soymilk by the Babcock method but they were not succesful. The failure seemed to be due to the nature of the solids not fat, and their reaction with the sulphuric acid. Solid substances were formed which prevented the fat from going into the neck of the testbottle so that it was not possible to take the reading. Experiments have shown that the fat in the milk could be determined without difficulty by the Roese-Gottlieb method.

In Russia HOROWITZ-WLASSOWA, OBERHARD, and GUTERMANN (1931) of the Moscow Central Biochemical Research Institute of the Food Industry have worked out the following procedure. The ground soybeans are soaked for eight hours and treated with steam of 100°C for desodorisation. This is followed by the extraction with water and the separation of the residue (still containing 17.85-19.25% fat, 0.53-0.58% protein and 0.14% carbohydrates). The emulsion is cooked for five minutes and then 0.25% NaCl, 0.5% glucose, 2% soyoil, and 0.02% vanille or 0.2% almond essence is added. After mixing the liquid is sterilized.

After addition of 10% sugar concentrated milk was obtained by evaporation at one fourth of the original volume. The composition was 11.76% protein, 7.64% fat, 42.12% sugar, 2.32% ash and 37.16% water.

From the same institute BOGATSKII, STOROZHUK, and MUROMTSEV (1933) have done work on preparation and grinding of soybeans, emulsifying, boiling, and desodorisation by blowing with hot air. The sterilisation was carried out for 15-20 minutes at 115-120°C.

In Leningrad VOSKRESENSKII and DOBRUININA (1935) made soymilk by soaking the beans for two hours and thoroughly emulsifying; according to these workers longer soaking gives greater fat extraction but a less stable emulsion. In commercial production the material may be hulled, whole beans, thoroughly ground and homogenized in water for at least one hour, then diluted with water and emulsified for at least 30 minutes. Pasteurization is at 80°C for 15 minutes.

At the Institut des Recherches agronomiques Hanoi, CASTAGNOL (1934) has developed a method of manufacture for preparing soymilk on a large scale. He succeeded in getting a product of constant composition if the following operations were applied.

1. Soaking the beans
2. Grinding with a millstone and using water 2½ times the weight of the beans.
3. Dilution of the mass with water 2½ times the weight of the beans
4. Filtration of the thick liquid by means of a basket centrifuge
5. Centrifuging the milk.

The milk prepared in this way is a white liquid resembling cow milk in appearance and containing about 5.5-6% dry matter. Castagnol has successively studied each of these operations.

Soaking. It was found that when the beans were soaked in water of 15°C 200 g of beans absorbed 215 and 239 g of water after 12 and 48 hours respectively. After 60 hours the water absorption was 239 g. No significant difference was found when the soaking was carried out in water of 30°C during 24 hours.

Grinding. On an industrial scale it will be advantageous to use a twin stone mill which permits a regular supply of water during the operation. The amount of water which gives the best results may depend on the type of mill.

Dilution. As it was not possible to filter the thick liquid obtained after grinding, another 500 g of water was added.

Basket centrifuge. According to Castagnol the use of filter presses is not advisable as the capacity of the filter presses diminishes very soon due to the accumulation of residual mass. The basket centrifuge is much more efficient as it can be easily cleaned. Besides working conditions are much more hygienic than when using the filterpress and large quantities can be handled with an apparatus of comparatively small capacity.

Centrifuging. The purpose of centrifuging is to discard the cellulose residue which amounts to about 0.12% in the liquid.

As regards the results obtained from 200 g of soybeans with a crude protein content of 33.80%, the yield is 954 g of milk of 2.91% crude protein. This means that the output of the most important nutritive substance, the crude protein, is only 44%. The other 56% is found as a loss in the industrial waste product. So in this respect the process needs improvement.

According to Castagnol there are two important points that from an industrial point of view need proper consideration.

1. The fermentation of the material
2. The improvement of the yield of crude protein.

Fermentation is an important factor. In order to avoid this the soaking period must be short and the temperature of the water low. Due to fermentation the acidity increases causing a coagulation of the proteins.

From the fermenting liquid a lactic acid bacteria could be isolated. A count of the bacteria in a sample of fresh milk showed that there are 15.000 to 20.000 bacteria per ml. The fresh milk has an initial acidity of 12.0 ml NaOH 0.1 n., and after 24 hours the acidity increased to 44.5 ml. At the end of 36 hours a strong odour developed due to a decomposition of the proteins; the acidity diminished to

8.6 ml. The protein was separated in a curdlike form and submerged in a serum. It was found that the curd contained the major part of the protein, only about one tenth being left in the serum in the form of amino-acids.

Castagnol concludes that there are two kinds of fermentation. Due to the first fermentation the milk becomes sour, but not enough to prevent the proteolytic fermentation which ultimately prevails. At the end there is an increase in amino acids and a lessening of the acidity because of its neutralisation by the decomposition products of the protein (ammonia in particular). The acid fermentation has precipitated the greater part of the proteins, while the nitrogen remaining in the whey originates from decomposition products of the proteines (pepton, aminoacids, etc.).

The improvement of the protein output. As has been shown only about 44% was extracted with the process used by Castagnol. A better kind of extraction would reduce the cost price of the milk. Castagnol investigated the influence of three factors which can be controlled under actual factory conditions.

1. The acidity of the water
2. The influence of Na-ions in different forms
3. The influence of the soaking period.

He concludes that the acidity of the water has little influence, while the Na-ions have much influence on the output of protein. However, objections must be made as to the accuracy of his work. He himself states: "Par suite du matériel tout à fait insuffisant pour obtenir une experimentation digne de valeur, les chiffres obtenus ne peuvent fournir qu'une indication et il serait indispensable d'opérer avec un matériel travaillant de facon régulière et sérieuse pour obtenir des renseignements plus précis".

The influence of the soaking period. In order to find out whether transformations taking place in the beans as a result of soaking could improve the extraction several batches of 100 g of beans were soaked for 12, 24, 36, and 48 hours. With the purpose of inhibiting fermentation, which might take place during soaking, the temperature of the water was maintained at 15°C. The output of protein was 34.5, 26.4, 26.7, and 9.9 respectively. Soaking for 48 hours gave a milk with casein which was centrifuged out of the liquid for a large part. This may explain the low content of protein in this milk.

The amount of aminoacids as determined by titration with NaOH N/10 is 28, 16, 26, and 25 ml respectively. So the amino acids pass through a minimum at 24 hours soaking and afterwards increases again. Notwithstanding all precautions taken the pH diminishes when the duration of soaking was lengthened. Therefore Castagnol concludes that for his working conditions twelve hours seem to give the best results.

In India soymilk was not much known until the great Bengal famine of 1943 when soymilk was produced by G U H A and D E from the Calcutta University for feeding a few hundreds of starving infants and toddlers. A majority of the subjects survived the crisis on a diet mainly consisting of soymilk.

In 1944 work on soymilk started on a systematic basis at the Indian Institute of Science, Bangalore. The method of manufacture was as follows. The beans were soaked overnight in water and then spread out in trays to germinate for two days to enhance the nutritive value of the protein as well as the vitamin content. After germination the skin was removed. The kernel was then heated for about ten minutes at 70°C with a dilute solution of baking soda (0.08%) or glycerol (1.08%) which extracted the bulk of the colouring matter and the bitter principle. The extracted water was removed and the kernel was washed with water and ground to a fine paste with the addition of calcium hydrogen phosphate. The paste was then treated with about three times the volume of water and heated to the boiling point for 15 minutes. The milk was filtered through the sieve. Two percent cane sugar and a small amount of salt were added to improve the taste. As a major part of the milk is used as sour curd it was found advantageous to add invert sugar by hydrolyzing cane sugar with dilute acid. About 25% of the original bean is wasted as residue that can be used as cattle feed or when dried can be mixed with wheat flour for preparation of bread and biscuits.

The composition of the milk was as follows.

Total solids	10.15 %	Vitamin A	750	I.U./litre
Protein	4.2 %	Thiamine	0.82	mg /litre
Fat	3.4 %	Riboflavine	1.1	mg /litre
Carbohydrates	1.8 %	Nicotinic acid	2.49	mg /litre
Salt	0.75 %	Ascorbic acid	21.6	mg /litre

As may be seen from the foregoing examples the methods of manufacture in countries where soymilk is made differ regionnally. There are however many operations in common, and these operations may be designated as the pretreatment of the material, the extraction of the proteins, the separation and operations for the improvement of the quality of the milk.

As to the researchwork done, the work of C H I U does not give information on the process of manufacture, as it mainly deals with the physical and chemical properties of the milk. C A S T A G N O L's work, although dealing with the process of manufacture does not represent a reliable study of how the process can be improved. The work done in Russia is difficult to evaluate, due to lack of information, while lastly the work of D E is more on the nutritional side.

In general, it can be said that especially data on the output of the manufacture of soymilk are very scare.

3.2.2 Dispersion of vegetable proteins from seeds.

In addition to the literature referred to, there exists extensive literature on the isolation of proteins from seeds in general. In the compilation of the literature on isolation and utilization of vegetable proteins (S M I T H, 1954) the number of references is 161. This literature mainly deals with the isolation of vegetable proteins for industrial purposes and has been partly read in an attempt to find useful indications for the manufacture of soymilk.

It appears that especially to the following five factors attention was paid:

1. particle size and other physical factors
2. H-ions
3. salts
4. denaturation
5. germination

To our work the first and the second factor are important. The literature on the role of salts for extraction of vegetable proteins is voluminous, but for our purpose it has less value as the use of salts in the manufacture of foodstuffs in general will not be considered. Denaturation of the proteins has an important influence on the choice of the starting material. Lastly, germination may be of influence on the nutritive value of the milk.

3.2.2.1 Particle size and other physical extraction factors.

The pretreatment determines the particle size which in turn has an important influence upon the amount of dispersable protein. Observations of this kind were made by B I S H O P (1929) who states that barley meal ground through a 1-mm sieve yields 25.0% of the original nitrogen on extraction. If the sieve opening was reduced to 0.5 mm the output amounted to 31.2%, while a coffee mill increased the yield again to 36.2%. S T A K E R and G O R T N E R (1931) also realized the importance of fine grinding and the importance of a standard particle size for comparative studies. They ground the meal of various seeds to pass through a 100 mesh-sieve before extraction.

N A G E L, B E C K E L, and M I L N E R (1938) considered some physical factors which influenced the amount of nitrogenous constituents dispersed in water from fat-free soybeanmeal. The factors investigated were the size of the particles, the solvent-meal ratio, temperature, time, and separation of the meal residue from the suspensoid.

The soybeans used were an Illini variety. They were cracked, flaked, and then extracted with petroleum ether. The fat-free meal was air dried at room temperature, and ground. In order to avoid the possibility of heat denaturation the temperature of the meal was always kept below 40°C. It was observed that the hulls were much harder to grind and that particles retained on the coarser screens largely consisted of hull fragments.

The extraction was carried out as follows. A weighed amount usually 2.5 g of the fat-free meal was shaken with distilled water at room temperature for 30 minutes on a mechanical shaker. The mixture was then centrifuged for 6 minutes at about 3000 r.p.m. and the liquid was carefully decanted or filtered into a Kjeldahl flask and analyzed for nitrogen. The residue was again shaken with another portion of water for an equal length of time and the liquid analyzed as before. This process was repeated as many times as was considered necessary (usually three).

Grinding to different particle sizes was obtained by means of a Wiley mill with sieves of 2, 1, and 0.5-mm openings. In addition two other kinds of meal were obtained by dry grinding the flakes with a porcelain ball mill with flint pebbles until all the material passed sieves of 100 and 200 mesh. Also in the ball mill 10 g of meal from through 100 mesh sieves was ground with 400 ml of water for 4½ hours. The other meals (2.5 g) were extracted with 100 ml of water. It required about 24 hours to grind a sample until it would all pass through the 100 mesh screen and 48 hours to reduce to the 200 mesh size. On triple extraction the solubility increased in this order with a decrease in particle size as would be expected. The amount of nitrogenous material dispersed increased from 80.9 to 92.6% except that the 200-mesh meal is slightly less soluble than the 100-mesh meal. The rather unexpected result with the 200-mesh meal can be explained by the fact that the long pounding in the ball mill (48 hours), necessary to reduce the meal to 200-mesh, caused a denaturation of the protein.

On the basis of the greatest amount of nitrogen extracted with a minimum of labour, the method of wet grinding in a ball mill is the most efficient. Although this procedure does not seem to have been employed in analytical studies on the extraction of seed proteins, it appears to offer distinct advantages for this purpose. A very thorough grinding is obtained and the liquid prevents local overheating and mechanical shock, both of which apparently cause denaturation.

Of the meal particle sizes studied the 100-mesh meal was the most satisfactory. It not only yielded a large amount of protein in dispersion but it was also much easier to throw down in centrifuging.

thus permitting a clear separation by decantation. This size of meal was used in subsequent work.

If 2.5 g of meal is extracted by increasing amounts of water there is nothing critical about the meal-water ratio as to the sum of the successive extractions. However, for the first extractions the extracted nitrogen ranges from 59.4 to 81.0% if the amount of water is increased from 25 ml to 100 ml. It is concluded that a water-meal ratio of 100 to 2.5 by weight is satisfactory for this extraction.

In the temperature range of 1.5^{o} to $45.0^{o}C$ the dispersed amount of nitrogenous constituents increases by 0.25% per degree centigrade.

The time of extraction, like the meal-water ratio, is not at all critical. The results indicate that 1 minute's shaking for each of three extractions will disperse as much protein as 3 hours for each extraction. However, the first extractions range from 71.9 to 81.1% if the extraction time is increased from 1 to 30 minutes.

It is clear that all the factors considered exert their own influence upon the extraction. This influence applies either to the sum of three extractions or to the first extraction.

3.2.2.2 H-ions concentration.

SMITH and CIRCLE (1938) investigated the effect of H-ions concentration of hydrochloric acid, sulphuric acid, oxalic acid, sodium hydroxyde, and calcium hydroxyde on the dispersion of proteins from the soybean (variety Illini).

Extracted flakes were ground in a Wiley mill through a 0.5 mm screen and 2.5 g of this meal and 100 ml of the dispersing solution were placed in a 250 ml bottle and shaken mechanically for 30 minutes. The dispersions were centrifuged for 6 minutes in a centrifuge developing a maximum relative centrifugal force at the bottle tip of 1975 times gravity. The temperature of the extracting solutions varied from 24^{o} to $26^{o}C$. The nonprotein nitrogen was not determined but reported by another worker to be 5.55% of the total nitrogen. Analytical data are reported without correction for moisture.

In general it can be said that the dispersion curve obtained consists of three parts: a maximum at low pH, a minimum at a higher pH (3.5-5.0), and a slightly sloping part above pH 6.5.

With hydrochloric acid a maximum dispersion of 83% is reached, and at pH 4.1-4.2 the minimum dispersion is about 8%. At pH 0.5 the amount dispersed with trichloro acetic acid is very low, 5.5%, and only in very concentrated acid does it rise again.

The acid and base form one continuous curve on which the amount of nitrogenous matter dispersed by water is a single point. The maximum amount of nitrogen dispersed by acid is slightly less than that dispersed by water, which disperses about 84 per cent.

The most efficient dispersion appears to be produced by sodium hydroxide. The dispersion curve for this base is slightly above that for calcium hydroxide which at higher concentrations point downward quite sharply; this behaviour indicates that larger quantities of lime might markedly decrease the amount of nitrogen dispersed.

Similar studies were undertaken with cottonseed (OLCOTT and FONTAINE, 1939), peanut (FONTAINE and BURNETT, 1944), and sunflower (SMITH and JOHNSON, 1948). The curve for peanuts resembles very much that for the soybean, while the dispersion curve of cottonseed is characterized by a very broad minimum (pH 3.0-7.0) preceded by a maximum and followed by an almost horizontal part. The curve for peanuts has a minimum at pH 3, followed by a sloping part and then a maximum at pH 11.1.

It appears from these sources that the pH has a most important influence on the extraction of soybean proteins as well as some other vegetable proteins. Therefore it will be necessary to have this factor well under control when manufacturing soymilk.

3.2.2.3 Salts.

Several workers studied the role of salts in connection with the isolation of proteins from seed. We mention the work of STAKER and GORTNER (1931) who studied the dispersion effect of aqueous solutions of inorganic salts on proteins from 28 different seeds and grains. Also SMITH, CIRCLE and BROTHER (1944) investigated the effect of 12 neutral salts on the peptization of nitrogenous constituents of soybean meal, while FONTAINE and BURNETT (1944) studied the salt influence upon the peptization of peanut proteins.

In general it may be said that a definite and systematic effect of the salts was found. Staker and Gortner proved that the peptization behaviour of various groups of related seeds appears to be rather distinct and characteristic for each group.

3.2.2.4 Denaturation.

The oil industry produces large amounts of low-fat, proteinrich waste products which might become available for the extraction of proteins. In such a case it should be taken into account that the various heat treatments to which the material is subjected may have caused a denaturation of the material. It is clear that such a denaturation, measured in terms of water-soluble proteins, will effect the yield of proteins unfavourably.

As has been pointed out by BECKEL, BULL, and HOPPER (1942) there are many stages in the process where denaturation may occur: a. During storage if the beans contain more than 13% water or during drying to reduce the water content below 13%. b. During heating to increase the ease of flaking. c. During the extraction in connection with the extraction temperature. d. During desolventizing by means of steam dryers. e. During heating before the beans are fed to the expeller where the heating is considerable.

These workers have studied the denaturation of defatted soymeal heated from 60° to 127°C at relative humidities from 0 to 100% for half an hour to two and a half hours. Above 80°C at 100% relative humidity the change in rate of denaturation is rapid up to 100°C, but afterwards the rate seems to approach a limit. Lowering the relative humidities causes the denaturation to decrease.

While this work was done under laboratory conditions BELTER and SMITH (1952) studied the denaturation of flakes obtained from five soybean oilplants. They mention the following unit operations in a solvent plant operation: weighing the beans; cracking, cleaning, and dehulling; conditioning the grits (heating to predetermined temperature and adjustment of the moisture content); flaking; extracting the oil; desolventizing; deodorizing; toasting or other heat treatment of the residual flakes. These manufacturing steps are divided into three groups; a. preparation of the beans for extraction, which involves the first four operations b. extraction of the oil from the prepared flakes and c. recovery of the residual meal in a usable form which requires the three remaining steps.

The dispersibility of the protein from the original beans may vary considerable, a factor which must be recognized in the interpretation of the results of an investigation of this nature. The nitrogen dispersion results are reasonably consistent from one plant to another and show that only a minor portion of the total denaturation effect in processing can be attributed to the preparation steps. In fact more variation is evident between the original beans than between the various preliminary operations. Experience has indicated that, under conditions normally existing in solvent extraction plants, the extraction with hydrocarbon mixtures such as those used commercially does not appreciably denature the protein. It is evident from this investigation that the major portion of the denaturation occurs in the final meal treating steps.

After oilextraction the dispersibility ranges from 78 to 81%, while after deodorizing this may be as low as 43%. Toasting may cause a further reduction in the dispersibility to about 7%.

After the desolventizer discharge the dispersibility is still very high. The authors state that if this type of meal is required for industrial use, the small amount of residual solvent can be removed by proper use of stripping gases without further significant denaturation.

The peptizability of the total nitrogen contained in several commercial hydraulic-press peanut meals was determined by BURNETT and FONTAINE (1944). They investigated the effect of pH upon the amount of nitrogen peptized in comparison with similar data of solvent-extracted meal. The meals prepared under relatively mild, controlled conditions are similar to those obtained for solvent-extracted meals. However, slightly smaller amounts of nitrogen are peptized in the pH range of minimum peptization and in the alkaline range than is the case with solvent-extracted meal. In contrast to this, the representative meal of unknown history exhibits a significantly lower degree of peptization over the pH range 4 to 10 than does solvent-extracted meal. Slightly more nitrogen seems to be peptized on the acid side of the minimum peptization point in hydraulic-pressed meals than in solvent-extracted meals.

They conclude that hydraulic-press meals, because of variations in the methods of processing, vary with respect to the peptizability of their nitrogen constituents; some are nearly equivalent to solvent extracted meal but others are definitely inferior in this respect. It is considered possible, by the use of properly controlled hydraulic-pressing procedures, to remove oil effectively from the peanut under conditions which result in a minimum of change in the peptization properties of the nitrogenous constituents of the resulting meal.

The influence of processing factors on the denaturation of proteins from peanut meals was also studied by FONTAINE, SAMUELS, and IRVING (1944). These workers subjected fullfat flakes and defatted peanut meal to temperatures from 80° to 118°C at 100 and 0% relatieve humidities for half an hour to two and a half hours.

The peptizability of the nitrogenous constituents of the heat-treated solvent-extracted meal does not differ materially from that of heat-treated fullfat flakes. It is significant that heating for as long as 2.5 hours at 100°C and 118°C and 0% R.H., or at 80°C and 100% R.H., results in only a slight change in the peptizability. At higher temperatures, however (118°C and 100% R.H.), the peptization values indicate that a complete denaturation of peanut protein is attained rapidly. In contrast to this, soybean protein shows considerable denaturation after 2½-hours heating at 120°C and 0% R.H. and also after 2½-hours heating at 80°C and 100% R.H.

It may be seen from these sources that if the residue from oil factories is to be used serious denaturation of the protein should be taken into account. However, it should be noted that the workers in this field consider it quite possible to extract the oil from the beans or the flakes in such a way that no significant denaturation occurs. This applies to the solvent extraction as well as to the hydraulic pressing processes. In view of the very large amounts of soybean meal from oilfactories which is either fed to animals or returned to the soil as fertilizer these residues can be considered as a huge, potential source of highly nutritive vegetable protein for human consumption.

3.2.2.5 Germination.

During germination many biochemical changes take place in the seed. VON OHLEN (1931) has given a review in which he states that most workers are of the opinion that the oil in oily seeds break down into glycerine and fatty acids during germination, and that carbohydrates are finally formed. Starch appears or increases in a large number of oily seeds during germination. Reducing sugar appears in the embryo and is usually quite abundant in the developing seedlings. Proteins change rapidly to a soluble form and asparagine appears in the seedling by the fourth or third day.

For the study of the first stages of germination Manchu soybeans were soaked for one hour in distilled water and were then germinated between wet paper towels.The older seedlings were grown in pots filled with quartz sand and kept in the dark. Von Ohlen studied the translocation and transformation of the reserve foods of mature soybeans during germination and growth of the seedlings in the dark. These reserve foods consist of a large amount of protein, oil, some non-reducing sugar, and a small amount of starch.

The first changes found were the appearance of reducing sugar, and an increase in starch. The amount of starch in the cotyledons increased until the fifth day and then remained nearly constant until the ninth day, after which there was a rapid decrease. The starch disappeared from the different parts in the following order; root, hypocotyl, epicotyl, and cotyledons. Reducing sugar disappeared from the seedling in the same order but two days later.

A marked decrease in the amount of oil in the cotyledons occurred by the fourth day; depletion began at the base of the cotyledons and progressed towards the opposite end; the palisade was depleted last.

Asparagine appeared in the hypocotyl on the third day. It gradually increased during the development of the seedling, until it was abundant in the hypocotyl on the twenty-ninth day.

The organically bound phosphorus and magnesium in the cotyledons gradually change to the inorganic forms, a considerable portion of which remains in the cotyledons. On the other hand most of the potassium disappears from the cotyledons.

Germination also effects the vitamin content of the soybean as has been shown by WAI, BISHOP, MACK, and COTTON (1946). They compared the vitamin content of mature, unsprouted soybeans of the Bansei variety with that of the same variety of soybeans after different periods of sprouting under controlled conditions. Since the usual practice in oriental countries is to use the hypocotyls and to discard the cotyledons, the relative distribution of vitamins was ascertained separately in these portions after 54 hours of germination, the optimum period from the point of view of quantity coupled with organoleptic properties.

Assays for carotene, thiamin, riboflavin, niacin, reduced and dehydro ascorbic acid showed increase except for thiamin through 54 hours of germination. Thiamin showed alternate increases and decreases throughout the germination period investigated. The cotyledons contained notably greater amounts of all vitamins for which the tests were made. The process of sprouting beans, therefore, increases the nutrient value of the product. The entire bean should be eaten, since the cotyledons, which frequently are not retained, are a richer source of vitamins than are the hypocotyls.

As may be seen germination of the soybeans brings about important changes in the composition of the beans. No doubt the yield figures for the extraction will be effected to a large extent by those changes, so that facts found for the extraction of the non-germinated beans may not be of direct application to the germinated beans.

3.3 EXPERIMENTAL PART

3.3.1 Scope of work.

From literature it appears that in countries where soymilk is produced the methods applied are not the same. From the foregoing it is easy to point out the existing differences. However, it is also possible to state that important points of resemblance exist. Due to this resemblance the process of manufacture can be distinguished in four stages.

The first stage, *the pretreatment,* serves to make the raw material as suited as possible for the extraction. For this purpose apparatuses for the reduction of the particle size are used. This operation may be combined with the following as an apparatus suited for the pretreatment may also be used as an extractor.

The second stage consists of *the extraction proper,* in which constituents of the soybean have to be brought into solution, emulsion or suspension as well as possible. Effort should be made to increase the output of the extraction as much as possible. No doubt this yield is influenced by the degree in which the bean particles are reduced in size.

The third stage consists of *the separation of the milk* from the residue. One should aim at withdrawing as much milk as possible from the residue.

The last stage consists of several operations aiming at increasing *the quality of the product* e.g. application of heat to increase the nutritional quality, increasing the preservability etc.

From literature it may also be seen that few good, and systematic studies have been carried out concerning the manufacture of the milk. Especially regarding the yield that can be reached the data are very scarce and -if available- not very reliable. As the yield of the extraction has an important influence on the cost price of the milk it is desirable to focus our study in the first place on this point.

In this work we are concerned with the first and second stage of the manufacture; the pretreatment and the extraction respectively. The separation operation has not been studied, as in tentative experiments no important problems presented themselves (at least when using our method of manufacture). The separation has only to be carried out in such a way that if the milk stands for a long time no sedimentation occurs. When a centrifuge is used this aim is reached without difficulties. The quality of the milk is not studied in this chapter.

The starting material of all our experiments was a commercial variety of soybeans, obtained from an oil-factory. Presumably the country of origin was America. The water content was about 10%, and therefore the beans were probably not dried artificially. From these soybeans soyflour was obtained by grinding the beans including seed coats.

The composition of the soybeans used was as follows (% of dry matter)

		crude fibre	11.60 %
crude protein	40.50 %	ash	5.64 %

Concerning the pretreatment separate studies were made with the whole, unground beans and the soyflour, while the most important extraction factors - the extraction ratio, pH, temperature, time and method of mixing - were studied.

For the experiments on the pretreatment a certain extraction method was adopted, while the experiments on the extraction stage were always preceded by a certain pretreatment.

3.3.2 *Design of experiments.*

From the pretreatment stage the influence of the degree of grinding on the yield of the extraction was studied. To this end the whole beans were first soaked for 16 hours and after grinding the extraction was carried out according to a certain method. In a first experiment coarse grinding was performed with a desintegrator (1.1), while in a next experiment the particle size was further reduced by means of a pebble mill (1.2). With a Braunmixer a higher degree of particle size reduction was obtained (1.3), while in the last experiment (1.4) of this series the same apparatus was used with a higher number of rotations per minute.

The standard extraction method is the extraction carried out at a 10% ratio, a pH 6.5, a temperature of 20°C, for 10 minutes, using a Vibromixer as an extractor. In experiments with the Braunmixer the use of the Vibromixer is omitted, as the Braunmixer has a sufficient extracting action. For the separation a centrifuge is used.

The second series consisted of six experiments. The starting material was obtained by grinding the dry beans. Except for experiment 2.2 the flour obtained was always soaked for 16 hours. The extraction was carried out as described above except that in experiment 2.5 and 2.6 the Braunmixer and the Ika Ultraturrax were used as extractors instead of the Vibromixer.

In a first experiment the beans were ground through a 1-mm sieve (2.1), after which flour of a finer particle size was obtained by using a sieve with 0.5-mm openings (2.2). In the following experiment the same type of flour was soaked before extraction (2.3) while attempts were made to obtain a further reduction in the particle size by means of wet grinding in a pebble mill with porcelain balls (2.4). In the last two experiments the Vibromixer was replaced by the Braunmixer and Ika Ultraturrax respectively.

The study of the extraction proper comprised the influence of five factors: the extraction ratio, pH, temperature, time, and method of mixing. The manufacture was carried out applying a certain pretreatment and a certain extraction method. In each series the only variable factor was the one to be investigated.

The pretreatment consisted in grinding of dry beans (seedcoats included) by means of a hammermill provided with a sieve of 0.5-mm openings. The flour thus obtained was soaked for 16 hours.

The third series consisted of experiments on the extraction ratio. Water was added to the flour to such an extent that the weight of the dry flour was successively 5, 10, 15, and 20% of the mixture of flour and water. The ratio was then called 5, 10, 15, and 20 respectively. Distilled water was used for the extraction at 20°C for 10 minutes.

The fourth series, experiments 4.1 to 4.4, concerned the pH. Enough alcali was added for the pH of the mixture to increase to 7.5, 8.5, and 9.5. In the experiment 4.1 no alcali was added. The ratio was 10%, using a temperature of 20°C and an extraction time of ten minutes.

The fifth series, experiments 5.1 to 5.4, were meant to study the effect of temperature. By means of a water-bath the flour-water mixture was brought to 20°, 30°, 40°, and 50°C. The extraction was carried out at a 10% ratio, a pH 6.5, for 10 minutes.

The sixth series, experiments 6.1 to 6.4, dealt with the influence of the extraction time, which was 2½, 10, 30, and 60 minutes respectively. The extraction was carried out at a 10% ratio, a pH 6.5, and a temperature of 20°C.

The seventh series, experiments 7.1 to 7.4, concerned the method of mixing. The Vibromixer is replaced by the Ika Ultraturrax. The number of revolutions was 1800, 2100, 2600, and 3000 rpm successively.

As regards the extent of this investigation it should be noted that this was limited on purpose, as the research programma would be too extensive if all possible combinations of the factors to be investigated had to be considered. In general it may be said that the investigation was carried out in such a way that a certain factor was variable while the other factors were kept constant.

For the experiments only the undamaged, whole beans were used, while possible other kinds of seeds were discarded. So there had been some kind of selection. If necessary, the beans were ground by a hammermill.

Each experiment was replicated once in order to check the reliability of the experimental results. Besides the determinations of dry matter, and crude protein were done in duplicate; the same holds for the acidity, pH, viscosity, and specific weight. Thus the values represented in the tables of this chapter are the averages of at least four determinations.

The following is a summary of the experiments carried out:

Series 1	Pretreatment whole beans	:desintegrator, ditto + pebble mill Braun (8000 rpm), Braun (12000 rpm)
Series 2	Pretreatment beans ground	:1-mm, 0.5-mm, 0.5-mm sieve 0.5-mm sieve + pebble mill ditto + Braunmixer ditto + Ika Ultraturra

Series 3 Extraction ratio	:	5,	10,	15,	20 %
Series 4 Extraction pH	:	6,5,	7,5,	8,5,	9,5 pH
Series 5 Extraction temperature	:	20,	30,	40,	50°C
Series 6 Extraction time	:	2½,	10,	30,	60 min.
Series 7 Method of mixing	:	1800,	2100,	2600,	3000 rpm

3.3.3 *Technique of experiments.*

A short description of the apparatuses used is given, after which the experiments are described.

1. *Desintegrator,* CONDUX type LV15m, PS 0.75, n 2900 (photo 2).
   In the desintegrator the beans are ground between two discs, both provided with metal teeth. The stationnary one forms the cover of desintegrator, while the other one is driven by an electromotor.
2. *Pebble mill,* material porcelain, shape cylindrical, inner diameter 12.5 cm, inner length 10 cm.
   The bowl is placed on a rotation installation and kept moving at ± 30 rpm.
3. *Braunmixer,* 250 Watt (photo 3 and 4).
   The cup has a loose bottom provided with an axis with a number of knives. The axis is driven by an electromotor and can be adjusted at two speeds (8000 and 12000 rpm.)
4. *Vibromixer,* type El, 40 Watt (photo 3).
   This vibrating mixer consists of a motor, a rod and a metal disc, 6 cm in diameter, provided with perforations placed in two concentric circles. The amplitude can be adjusted, but it is not indicated. The number of vibrations is 50 per second.
5. *Hammermill,* Peppink, type 261 D 20/2, PK 2, n is 2850.
   This mill is provided with 6 arms, 9.5 cm in length, and several sieves differing in sieve opening.
6. *Ika Ultraturrax,* type TMV 45, n 8000, V 220, A 1.8 (photo 3 and 5). The head of the mixer consists of four concentric rings provided with teeth. The inner and outer ring are driven by the rotor. The number of revolutions per minute is adjustable up to a maximum of 8000.
7. *Centrifuge,* Baird and Tatlock, type V 808.
   This centrifuge has four plastic tubes of 3 cm diameter, which can be filled with 50 ml of liquid. The distance from the axis of the centrifuge to the tip of the tubes in horizontal position is about 14 cm. The separation was carried out by centrifuging for five minutes at 3000 rpm.

The experiments were carried out as follows.

1.1 Desintegrator. 60 g of beans were soaked and then they were fed to the desintegrator. The bean particles left behind between the teeth were washed away with water and the wash water together with the ground, swollen beans was brought into an enamel can. Water was added until the ratio was 10%. As soon as an extraction temperature of 20°C was reached the rod of the Vibromixer was placed in the can axially and the extraction carried out for ten minutes. Then the separation of the milk by means of the centrifuge was effected at 3000 rpm for five minutes. The clarified suspension was weighed as well as the centrifuge tubes containing the residue.

1.2 Desintegrator and pebble mill. The ground beans from the desintegrator and the wash water were put into the pebble mill and the bean particles were further ground for three hours. Then this mass was brought in the extraction can together with the wash water from cleaning the mill and the pebbles. After adding more water to obtain the desired ratio, the extraction and separation were carried out as described above.

1.3 Braunmixer. The soaked beans were brought in the cup together with the required amount of water, and then ground for ten minutes at 8000 rpm. As the Braunmixer has also an extracting action, no other extractor was used. Then the separation was performed.

1.4 Braunmixer. See 1.3; the number of rpm was 12.000.

2.1 Hammermill. To the 60 g of soyflour soaked in the extraction can distilled water was added until the 10% ratio was reached. By means of a waterbath the temperature was brought to 20°C ± 1. Then the extraction and separation were carried out as described before. The experiments 2.2 and 2.3 were carried out in the same way, except that in experiment 2.2 the flour was not soaked.

2.4 Hammermill and pebble mill. After soaking the flour was put into the bowl together with not more than 125 ml of water and then ground for three hours by means of porcelain pebbles.
The contents were then put into the extraction can together with the washwater for cleaning the mill and pebbles, and water was added until the desired ratio was reached. Extraction and separation were carried out as before.

2.5 Braunmixer. The soaked flour and the required amount of water were put in the cup of the mixer and the extraction was carried out as usual. Before centrifuging the mixture, which had attained a temperature of about 35°C, was cooled. The number of rotations of the knives was 8.000.

2.6 *Ika Ultraturrax.* The soaked flour was extracted with the required amount of water at 3.000 rpm for 10 minutes. The temperature increased by 25 to 30°C, which made it necessary to cool the mixture before centrifuging.

3 *Extraction ratio.* These experiments were carried out in the same way as experiment 2.1. The different ratios were obtained by adding the required amounts of water to the 60 g of soyflour.

4 *Extraction pH.* As in experiment 2.1. The required pH was obtained by pipetting 11.25 N of alcali to the mixture (which was kept in constant movement by a mixer), while at the same time the pH was indicated by a pH-meter.

5 *Extraction temperature.* As in experiment 2.1. The required temperature was obtained by placing the extraction can in a water-bath.

6 *Extraction time.* As in experiment 2.1.

7 *Method of mixing.* As in experiment 2.1. In order to prevent the large increase in temperature the extraction can was placed in a large tank with water. The extraction was carried out at 1800, 2100, 2600, and 3000 rpm respectively.

All the suspensions and sediments (residues) were analysed for dry matter and crude protein content. In addition, the acidity, pH, viscosity, and specific gravity of the milk were determined. All analyses and determinations were done in duplicate, and carried out as follows.

Dry matter. From a weighing bottle milk was poured on a porcelain disc. In an oven the milk was dried at 100°C for two hours, after which the disc was cooled in an exsiccator containing silicagel. Then the disc was weighed.

About 2.5 g of the sediment in glass weighing bottles was dried at 100°C for two hours. After cooling in an exsiccator the bottles and their contents were weighed.

Crude protein. About five grams were put into a Kjeldahl-flask, adding 0.7 g HgO, 10 g K_2SO_4 and 25 ml H_2SO_4 96%. The destruction was carried out until the contents were colourless, after which the heating was continued for two hours. After cooling, 50 ml 8% $Na_2S_2O_3$-solution and 90 ml NaOH-solution were added.

Then the contents of the flask were put into a Parnas-Wagner apparatus. By means of steam the ammoniak was distilled and received in 50 ml boric acid. After adding 2% Tashiro-indicator to the boric acid it was titrated with 0.1 n of base.

Acidity. 10 ml of milk were titrated with 0.1 n NaOH, using 0.5 ml phenolphtaleine as an indicator. The titration was carried out until the colour was the same as that of 10 ml of the same milk to which 0.5 ml of 0.0005% fuchsine-solution had been added.

pH-determinations were carried out by means of an Electrofact pH-meter.

Specific gravity. This was determined by comparing the weight of soymilk in 5 ml pycnometers with that of distilled water of the same temperature (20°C).

Viscosity. A Hoppler viscosity meter was used for the determination of the viscosity. In principle it consists of an inclined glass tube through which a glass sphere falls down. The time needed by the sphere to pass from one point to another of the tube is measured. Around the tube water of the desired temperature (20°C) is circulated.

3.3.4 *Results, discussion, and conclusions.*

The method of calculating the yield of dry matter, is as follows. After determining the amounts of dry matter in the milk and the residue, the percentage of the original dry matter in the milk and in the residue was calculated. It should be noted that in general the sum of the yields thus calculated will not be 100%, due to analytical errors and loss of material.

From the amounts of crude protein found in the milk and the residue the percentages of the sum of the amounts of crude protein in suspension and residue were calculated. Evidently the sum of the yields calculated in this way is always 100%.

As an example of the reproducibility of the analyses of dry matter, crude protein, and the determinations of pH, acidity, viscosity and specific gravity, the original results and the averages of the experiments 3.2, 4.1 and 6.2 are mentioned.

	MILK						RESIDUE	
	dry matter %	crude protein from dry matter %	pH	acidity (ml)	viscosity (20°C)	specific gravity (20°C)	dry matter %	crude protein from dry matter %
3.2 *Extraction ratio*	7.59	47.83	6.59	1.48	1.936	1.0192	19.19	34.78
	7.58	47.46	6.58	1.44	1.924	1.0191	19.12	33.81
duplicate	7.68	46.60	6.59	1.44	1.838	1.0192	19.30	30.15
	7.63	46.52	6.57	1.42	1.805	1.0191	19.24	30.12
average	7.62	47.10	6.58	1.45	1.876	1.0192	19.21	32.22
4.1 *Extraction pH.*	7.70	43.19	6.53	1.63	2.095	1.0197	19.24	40.36
	7.69	43.16	6.52	1.55	2.093	1.0196	19.23	- -
duplicate	7.52	40.11	6.42	1.67	2.111	1.0192	19.51	28.46
	7.51	38.42	6.41	1.66	2.088	1.0190	19.50	25.53
average	7.61	41.22	6.47	1.63	2.097	1.0194	19.37	31.45
6.2 *Extraction time*	7.68	47.40	6.59	-	1.898	1.0199	20.10	31.43
	7.66	47.16	6.57	-	1.894	1.0197	20.03	31.91
duplicate	7.81	47.31	6.58	1.45	1.874	1.0195	20.95	28.82
	7.79	46.97	6.57	1.40	1.872	1.0195	20.75	28.72
average	7.74	47.21	6.58	1.43	1.885	1.0197	20.46	30.22

The reproducibility of the manufacture can be checked from the following examples of the yields of dry matter and crude protein.

	MILK			RESIDUE		
	weight (g)	% from orig. dry matter	% from orig. crude protein	weight (g)	% from orig. dry matter	% from orig. crude protein
3.2 *Extraction ratio*	418.47	58.45	65.75	119.65	42.21	34.25
duplicate	416.17	58.67	67.70	121.83	43.25	32.30
average	417.32	58.56	66.73	120.74	42.73	33.28
4.1 *Extraction pH*	422.40	59.85	61.07	114.81	40.66	38.93
duplicate	420.50	58.20	66.49	118.50	42.56	33.51
average	421.45	59.03	63.78	116.66	41.61	36.21
6.2 *Extraction time*	417.24	58.93	66.49	119.50	44.16	33.51
duplicate	425.63	61.14	69.92	112.35	43.13	30.08
average	421.44	60.04	68.21	115.93	43.65	31.80

It may be seen from these figures that the analytical determinations are reliable, and that by replicating each experiment from the beginning the method of manufacture proved to be reproducable.

In table 1 and fig 3, the results of the investigation of *the first stage, the pretreatment,* are summarized and represented graphically. The whole beans and the ground beans were studied in series 1 and 2 respectively.

The results of the experiments on *the second stage* of the manufacture, *the extraction proper,* are summarized in table 2 and represented graphically in fig 4. They contain the results of five series of experiments: *3)* extraction ratio; *4)* extraction pH; *5)* extraction temperature; *6)* extraction time, and *7)* method of mixing.

It should be mentioned that in studying the methods of pretreatment the method of working with each apparatus was not varied, that is to say, no attempts were made to obtain the optimal working method. Otherwise a systematic study of such factors as degree of filling, number of rotations would be necessary. Nevertheless an appreciable difference was obtained.

When considering the results of *the pretreatment of the whole beans* (table 1, series 1), it may be seen that the dry matter content of the milk shows a regular increase in the sequence: coarse grinding, coarse grinding followed by further grinding in a pebble mill, fine grinding (Braunmixer 8.000 rpm), and fine grinding (Braunmixer, 12.000 rpm). The desintegrator proved to be unsatisfactory under the conditions used. The result of further grinding with the pebble mill was rather disappointing and might have been better if the time of grinding was lengthened. But in that case the milk would already be coagulated due to souring. Therefore fine grinding with the Braunmixer was tried. The results obtained were comparatively favourable; increasing the number of rotations of the knives appeared to increase the dry matter content.

In the theoretical case that the grinding is so fine that no sediment is left on centrifuging, the dry matter content of the milk would be 10%. If however, part of the beans is not ground fine enough the dry matter content of the milk after centrifuging will be lower than 10%. The coarser the particles the greater the decrease in the dry matter content of the milk. So the figures in the column of the dry matter content of the milk form *a measure for the fineness of grinding.*

It is clear that if the dry matter content of the milk decreases the yield of the dry matter will decrease too. From the column "dry matter %" and "percentage of dry matter in suspension" it may be seen, however, that the yield decreases more than the dry matter content. It appeared that the yield decreased if the grinding became coarser. This may be ascribed to the fact that if the dry matter content decreases due to coarser grinding, the particles that are centrifuged out form a heavier and more voluminous sediment.
Thus the interparticle volume is increased and retains more milk- and consequently also more dry matter-than when there is less, more

TABLE 1. *Influence of the pretreatment on the extraction of proteins from the soybean.*

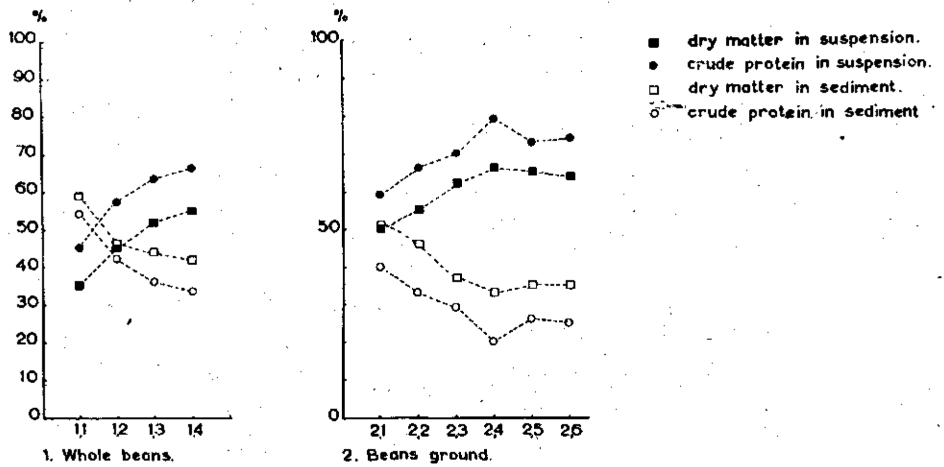
Extraction-conditions: extraction-ratio 10%, extraction-pH 6.5, extraction-temperature 20°C, extraction-time 10 min.

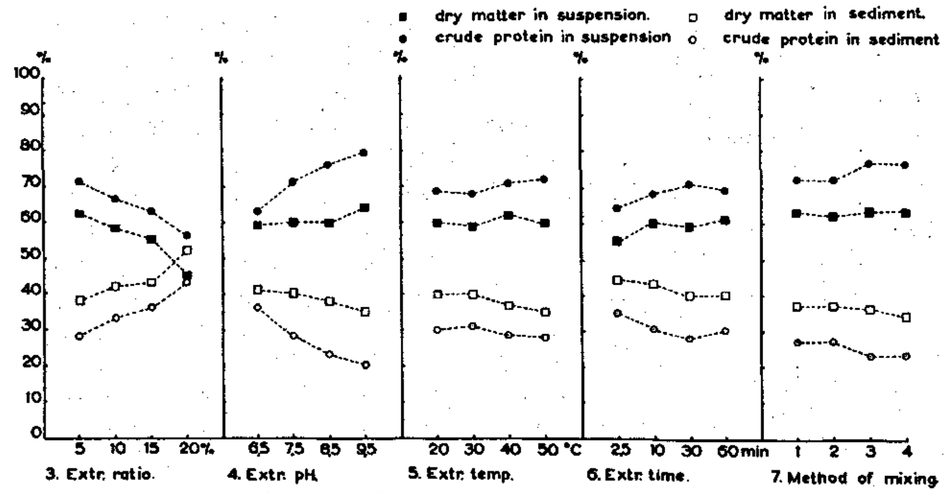
EXTRACTION-CONDITIONS: PRE-TREATMENT	EXTR. APPARATUS	weight (g)	dry matter (%)	crude protein from dry matter (%)	SUSPENSION: pH	acidity (ml)	viscosity (20°C cP)	specific gravity (20°C)	SEDIMENT: weight (g)	dry matter %	crude protein from dry matter (%)	% of original dry matter in suspension	% of original crude protein in suspension	% of original dry matter in sediment	% of original crude protein in sediment
1.1 Whole beans soaked, then ground with a desintegrator	Vibromischer	308.51	6.24	53.21	6.56	0.91	1.784	1.0139	221.76	14.58	38.34	35.44	45.31	59.51	54.69
.2 ditto, afterwards ground with a ball mill	Vibromischer	332.18	7.43	52.17	6.59	1.09	1.959	1.0164	197.56	12.77	38.60	45.47	57.15	46.47	42.86
.3 Whole beans soaked	Braunmischer (8.000 rpm)	356.26	8.03	47.51	6.60	1.29	2.962	1.0191	181.09	13.43	33.62	52.67	63.14	44.78	36.86
.4 Whole beans soaked	Braunmischer (12.000 rpm)	364.40	8.21	47.87	6.56	1.32	3.120	1.0196	172.18	13.42	30.99	55.10	66.58	42.55	33.42
2.1 Beans ground with hammermill (1 mm sieve) then soaked for 16 hours	Vibromischer	402.20	6.85	47.45	6.61		1.758	1.0181	134.18	20.32	31.65	50.42	59.64	51.20	40.36
.2 Beans ground with hammermill (0.5-mm sieve) not soaked	Vibromischer	402.73	7.43	50.78	6.57	1.35	2.468	1.0196	130.86	19.38	29.70	55.11	66.88	46.71	33.12
.3 Beans ground with hammermill (0.5-mm sieve) then soaked for 16 hours	Vibromischer	428.89	7.89	47.08	6.59	1.50	2.004	1.0195	109.27	19.40	31.47	62.32	70.44	37.80	29.57
.4 Beans ground with hammermill (0.5-mm sieve) then ground in a ball mill	Vibromischer	424.42	8.52	50.05	6.52	1.44	2.547	1.0198	107.61	16.76	26.20	66.62	79.43	33.32	20.57
.5 Beans ground with hammermill (0.5-mm sieve) soaked for 16 hours	Braunmischer	426.82	8.34	45.16	6.56	1.54	2.308	1.0201	111.15	17.55	30.63	65.89	73.10	35.85	26.90
.6 Beans ground with hammermill (0.5-mm sieve) and soaked for 16 hours	Ika Ultraturrax	426.62	8.37	46.01	6.54	1.49	2.339	1.0201	111.12	17.90	28.91	64.48	74.07	35.85	25.93

TABLE 2. *Influence of the extraction-conditions (ratio, pH, temperature, time and method of mixing) on the extraction of proteins from the soybean.*

Pre-treatment: unshelled soybeans ground with a hammermill (0.5-mm sieve) and soaked for 16 hours in distilled water.

	EXTRACTION-CONDITIONS					SUSPENSION							SEDIMENT						
	Extr.ratio	Extr.pH	Extr.temp.	Extr.time	Extr.with the aid of	weight (g)	dry matter (%)	crude protein from dry matter (%)	pH	acidity (ml)	viscosity (20°C, cP)	specific gravity (20°C)	weight (g)	dry matter (%)	crude protein from dry matter (%)	% of original dry matter in suspension	% of original crude protein in suspension	% of original dry matter in sediment	% of original crude protein in sediment
3.1	5 %	6.5	20°C	10 min.	Vibromischer	958.80	3.51	52.41	6.72	0.63	1.303	1.0089	121.60	17.33	33.03	62.02	71.68	38.75	28.32
2	10 %	6.5	20°C	10 min.	Vibromischer	417.32	7.62	47.10	6.58	1.45	1.876	1.0192	120.74	19.21	32.22	58.56	66.73	42.73	33.28
3	15 %	6.5	20°C	10 min.	Vibromischer	252.03	12.05	48.30	6.38	2.29	2.882	1.0300	104.94	22.59	35.03	55.96	63.78	43.65	36.22
4	20 %	6.5	20°C	10 min.	Vibromischer	155.39	16.06	48.38	6.38	3.27	6.778	1.0415	114.35	24.84	33.32	45.96	56.54	52.29	43.46
4.1	10 %	6.5	20°C	10 min.	Vibromischer	421.45	7.61	41.22	6.47	1.63	2.097	1.0194	116.66	19.37	31.45	59.03	63.78	41.61	36.22
.2	10 %	7.5	20°C	10 min.	Vibromischer	413.77	7.91	46.94	7.33	0.72	2.664	1.0200	124.85	17.82	26.88	50.30	71.90	41.00	28.10
.3	10 %	8.5	20°C	10 min.	Vibromischer	415.85	7.92	45.63	7.97	0.07	2.700	1.0204	122.38	17.12	21.75	60.67	76.77	38.59	23.24
.4	10 %	9.5	20°C	10 min.	Vibromischer	419.66	8.34	47.18	9.08	0.65	2.951	1.0212	118.80	16.19	21.76	64.43	79.96	35.42	20.05
5.1	10 %	6.5	20°C	10 min.	Vibromischer	431.92	7.58	51.66	6.54	1.46	1.889	1.0196	104.60	20.88	34.29	60.29	69.49	40.22	30.51
.2	10 %	6.5	30°C	10 min.	Vibromischer	428.55	7.51	52.13	6.50	1.43	1.878	1.0195	108.70	20.09	34.96	59.26	68.72	40.22	31.28
.3	10 %	6.5	40°C	10 min.	Vibromischer	429.28	7.86	51.36	6.50	1.51	1.916	1.0201	109.46	18.79	33.39	62.18	71.66	37.88	28.35
.4	10 %	6.5	50°C	10 min.	Vibromischer	429.85	7.65	51.87	6.49	1.50	1.914	1.0193	107.23	17.96	33.86	60.58	72.00	35.46	28.01
6.1	10 %	6.5	20°C	2.5 min.	Vibromischer	417.71	7.25	45.97	6.51	1.31	1.856	1.0193	122.95	19.50	31.80	55.73	64.62	44.15	35.38
.2	10 %	6.5	20°C	10 min.	Vibromischer	421.44	7.74	47.21	6.58	1.43	1.885	1.0197	115.93	20.46	30.22	60.04	68.21	43.65	31.80
.3	10 %	6.5	20°C	30 min.	Vibromischer	422.41	7.65	49.54	6.56	1.43	1.835	1.0185	115.04	19.72	29.10	59.82	71.05	40.85	28.92
.4	10 %	6.5	20°C	60 min.	Vibromischer	425.54	7.86	46.29	6.51	1.41	2.140	1.0198	114.75	19.45	29.86	61.63	69.95	40.92	30.05
7.1	10 %	6.5	20°C	10 min.	Ika Ultraturrax	418.78	8.19	46.30	6.44	1.73	2.674	1.0197	120.90	16.75	29.52	63.18	72.56	37.29	27.90
.2	10 %	6.5	20°C	10 min.	Ika Ultraturrax	413.78	8.15	41.99	6.38	1.65	2.766	1.0195	124.15	16.30	27.09	62.07	72.10	37.28	27.90
.3	10 %	6.5	20°C	10 min.	Ika Ultraturrax	415.40	8.31	44.86	6.47	1.73	2.841	1.0196	121.68	16.49	24.36	63.54	76.16	36.77	23.85
.4	10 %	6.5	20°C	10 min.	Ika Ultraturrax	415.00	8.29	45.59	6.40	1.84	2.886	1.0197	123.73	15.22	26.18	63.37	76.35	34.68	23.65


%
100
90
80
70
60
50
40
30
20
10
0
1.1 1.2 1.3 1.4
1. Whole beans.
100
50
0
2.1 2.2 2.3 2.4 2.5 2.6
2. Beans ground.
dry matter in suspension.
crude protein in suspension.
dry matter in sediment.
crude protein in sediment


dry matter in suspension.
crude protein in suspension
dry matter in sediment.
crude protein in sediment
%
100
90
80
70
60
50
40
30
20
10
0
5 10 15 20%
3. Extr. ratio.
6.5 7.5 8.5 9.5
4. Extr. pH.
20 30 40 50 °C
5. Extr. temp.
2.5 10 30 60 min
6. Extr. time.
1 2 3 4
7. Method of mixing

compact sediment of smaller particles with less interparticle space. So the figures for the yield of dry matter are determined by the fineness of grinding and the structure and quantity of the sediment formed by the bean particles. It may be said that they are *a measure for the quality of the method of manufacture.*

As regards the yield of protein it appeared that about half the amount of protein was obtained in the milk. As the grinding was more effective, more protein was found in the milk. All the yield figures for protein were higher than those for dry matter. As it is our aim to obtain a proteinrich food, it is a favourable fact that the soy-proteins are better dispersable in water than the other constituents.

The pH of the milk of these four experiments proved to differ very little from 6.6, while the acidity increased in the order mentioned earlier. This is evidently due to the increasing dry matter content of the milk, which is also indicated by the increasing values of the viscosity and the specific gravity.

The amounts of residue decreased in the same order and so did the dry matter and crude protein content, though not quite regularly. This decrease can be seen better from the values for the "percentage of original dry matter in sediment" and the "percentage of original crude protein in sediment".

Considering the results of each of the apparatuses we may state that the effect of *the desintegrator* was not satisfactory as only about one third of the dry matter and not yet half of the protein were found in the milk. This was already expected on visual observation as the swollen beans were not ground but torne asunder. Evidently in this way no sufficient reduction in particle size was possible. This may be considered to be the cause of the low yield.

Although a better result was obtained on *further grinding in a pebble mill* yet it was less marked than was expected. For a large part this may be ascribed to the fact the preceding grinding operation did not give enough reduction in particle size, in other words, we assume that the pebble mill used was too light for our purpose. Besides the presence of tough seed coats impede the destruction of the cell structure.

The Braunmixer gave reasonable results at 8.000 rpm. A little more than half of the dry matter and about two thirds of the crude protein were found in the milk. The reduction in particle size obtained was much better than with the desintegrator. As moreover the mixing intensity was appreciably higher than with the Vibromixer this higher yield was not surprising.

If the number of rotations was increased to 12.000 the yield of dry matter as well as that of crude protein was also further increased.

When evaluating the results of *the pretreatment of the ground beans,* i.e. *soyflour,* a regular increase in the dry matter content may be observed. The highest dry matter contents were reached when grinding was performed by a Braunmixer or Ika Ultraturrax, and also when further grinding was carried out with a pebble mill. The dry matter content in the second series was remarkably higher than in the first when corresponding experiments are compared.

The yield of milk was always higher in the second than in the first series. As a consequence of the higher dry matter content and the higher yield of milk the yield of dry matter in the milk was also higher in the second series.

The yield of crude protein increased with the increase in the yield of dry matter.

The pH and the acidity did not show important differences, while the specific gravity increased regularly. In general it may be said that the viscosity increased as the dry matter content of the milk increased.

The weight of the sediments did not differ much, except in the first experiment in which a larger sieve opening was used and in the second in which the flour was not soaked. Generally the dry matter content of the sediments was higher than in the first series.

As, however, the weights of the sediments in the second series were lower than in the first, the percentage of dry matter in the sediments in the second series was as a rule also lower in the second series. The same is true of the yield of crude protein in the sediments.

In contrast to the first series *the pebble mill* has given the best results. Evidently this is due to the fact that the pretreatment caused a sufficient reduction in material size, thus making it possible for the pebble mill to effect a further reduction in particle size.

On comparing the figures for the yield of dry matter of the *Braunmixer* and the *Ika Ultraturrax* with those of the *Vibromixer*, it appears that the figures for the Vibromixer are lower than those for the first two extractors. In the discussion of the pretreatment of the whole beans we assumed that the sediment retains less milk as the bean particles become smaller due to better grinding and so the milk yield increases. If we consider the milk yield of these three apparatuses we may see that the yields practically do not differ. This might be an indication that the favourable effect of the Braunmixer and the Ika Ultraturrax is due to a much more intensive method of mixing rather than to a further reduction in particle size.

It also appears that *the sieve opening* of the mill plays an important role. If the diameter was reduced from 1.0-mm to 0.5-mm the yield of the dry matter as well as that of the crude protein in the milk increased by about 10%. Besides the weight of the milk increased, which is in accordance with our assumption that this is a matter of the structure and the quantity of the sediment.

After centrifuging an unstable layer between the milk and the residue appeared in the centrifuge tubes if the flour was not soaked beforehand. In this layer the milk and the sediment are not well separated, so that then not only the milk yield but also the dry matter content of the milk decreases.

NAGEL et al. (1938) obtained a yield of 64.5, 69.7, 72.2, 81.0, 79.1, and 92.6% for the first extraction of defatted flakes using a ratio of about 2½% (1:40) for the pretreatment with a Wiley mill, hammermill with 2, 1, 0.5-mm sieve openings, porcelain ball mill and subsequent use of a 100 and 200 mesh sieve respectively, and wet grinding for 4½ hours in a ball mill.

With the 0.5-mm sieve they obtained 1.8% more protein than we did. This could have been expected since their ratio was much lower than ours (10%). In fact, their yield should have been higher. This was not the case, however, probably due to their mixing not being sufficiently intensive. Another point of difference was the condition of the starting material: while their flakes were defatted, our beans were not.

Enlarging the sieve opening from 0.5-mm to 1-mm decreased their yield with 2.5% only, where as in our experiment this decrease amounted to 7.5%. The different working method of processing used by us may account for this discrepancy.

With the ball mill these workers obtained 92.6% from the first extraction, while in our case this was only 79.4%. This may also be ascribed to a difference in the pretreatment and the extraction proper.

The results of *the extraction proper* are discussed hereafter.

The *extraction ratio* proved to have a decisive influence upon the composition of the milk, while also the yield was effected in a large degree. It appeared that the dry matter content of the milk increased as the ratio increased. In our experiments the dry matter content varied from 3.5 to 16.0%. Although as a matter of fact it is quite possible to lower the dry matter content still further we expect that increasing the dry matter content above 16-20% will not be easy as the separation of the milk from the residue will then become more difficult. It should be noted that the dry matter content tends to increase faster than the extraction ratio. This is due to the fact that the separation of the sediment by centrifuging is effected less efficiently as the viscosity of the flour-water mixture increases. As the ratio increased the milk yield as well as the yield of dry matter decreased.

While the percentage of protein from the dry matter remained nearly constant, the yield of protein in the milk decreased as the ratio increased.

The pH showed a decrease as the dry matter content of the milk increased. This is in accord with the pronounced increase in the acidity. Evidently the viscosity and the specific gravity also increased as the dry matter content increased.

The amount of residue tended to decrease. While the dry matter content of the residue increased with the ratio, the percentage of crude protein from the dry matter remained nearly constant.

Therefore as the extraction ratio increased, more dry matter and crude protein were found in the residue.

The *extraction pH* also had an important influence on the yield. The dry matter content appeared to increase if the pH was increased. This increase, however, was not very rapid, and therefore the yields of dry matter - the milk yield being about constant - increased only very slowly.

In contrast to this behaviour, the percentage of protein from the dry matter, showed a rapid increase. Consequently, and also due to the increasing dry matter content of the milk, the yields of protein in the milk increased appreciably with higher pH.

The increase in pH was accompanied by a decrease in acidity, while the viscosity and the specific gravity increased in value. Probably the increase in viscosity was not only caused by the increase in dry matter.

With the sediments the decreasing dry matter content and the decreasing percentage of protein of the dry matter contributed to a decrease in the yields of dry matter and crude protein in the sediment.

In the next series of experiments on *the extraction temperature* no pronounced differences between the results were found. At higher temperatures there was only a slightly higher yield of proteins.

With the exception of the first experiment *the extraction time* proved to have practically no influence on the composition of the milk and the yield. An extraction time of 2½ minutes, however, proved to be too short as both the milk yield and the dry matter content of the milk were higher with longer extraction times.

Also in the series of experiments of *the method of mixing* no significant differences in results were found either. However, when using the Ika Ultraturrax the dry matter content of the milk was appreciably higher than when using the Vibromixer. As in this series the protein content was at the same level as when using the Vibromixer, the yield of protein was comparatively higher. Evidently the acidity, specific gravity, and viscosity were then higher.

Now we shall compare the results of our stüdies on the second stage, the extraction, with those of other workers.

NAGEL et al. (1938) studied the amount of nitrogen extracted from defatted soyflour with *different amounts of water*. They obtained their ultimate results by triple extraction. For the sake of comparison, however, we are only interested in the results of the first extractions. They used amounts of water corresponding with about 10, 5, 2½, 1¼, and 0.6% flakes and obtained 59.4, 73.6, 81.0, 80.8, and 82.7% of nitrogen for the first extractions.

Their yield with 5% flakes, which is about the same as 50 ml of water, was 73.6%. Notwithstanding the difference in technique used, this result closely agrees with that obtained by us (71.7%). Although they used an extraction time much longer than ours, it is probable that the mechanical shaker used by these workers had not such a mixing intensity as our Vibromixer. This may account for the fact that in a concentrated mixture their yield decreased more than ours.

As far back as 1934 CASTAGNOL investigated the influence of the *acidity* on the yields of proteins. For pH 8, 7, 6, and 5 yields of 17.8, 18.4, 18.0, and 16.4% respectively were obtained. His conclusion was that in this range the pH has little influence upon the extraction. However, in view of the fact that these protein yield figures are very low his extraction process does not seem to have been carried out under good conditions. According to our figures in the pH range 6.5 to 8.5 there is already a difference of about 10%.

We shall therefore consider the work of SMITH and CIRCLE (1938) who investigated the extraction of nitrogenous material by acids and bases with and without salts. These workers obtained much better results than Castagnol.

It was found that the extraction with water gave only from 84.8 to 85.5% of the total nitrogen. With $Ca(OH)_2$ the amount of dispersed nitrogen is 89.2% at pH 9.8 and for NaOH this is 91.5% at pH 8.3. These figures are substantially higher than ours. It should, however, be borne in mind that the difference in yield is due not only to a difference in extraction method, but also to a difference in the method of calculation.

The effect of *the temperature* on the amount of nitrogen dispersed was also investigated by NAGEL, BECKEL, and MILNER (1938). At 1.5° and 45°C the percentage of nitrogen extracted was 71.4 and 83.1%. They found that in the range 15° to 35° the variation is about 0.25% of nitrogen per degree centigrade. As has been mentioned before, in our work no clear differences could be found in the range 20° to 50°C.

The same workers have also studied the effect of variation in *extraction time* on the amount of nitrogen extracted. For the first extractions 1, 7.5, 15, 30, 60, 120, 180, and 240 minutes gave yields of 71.9, 75.6, 78.0, 81.1, 80.7, 82.3, 83.6, and 83.9% of total nitrogen. In general this is in accord with our results.

After this discussion the question arises whether it might still be possible to obtain higher yields by other means than those investigated. As has been pointed out earlier in this discussion the figures for the dry matter content of the milk were comparatively higher than those for the yields of dry matter. We have argued that this was caused by the retention of the milk, and consequently of dry matter, in the residue. If it were possible to obtain this dry matter in the milk, the yield would increase appreciably. Then the yield would increase to a theoretical level that could be calculated from the extraction ratio and the dry matter content of the milk by means of the ratio.

$$\frac{\text{dry matter found (\%)}}{\text{extraction ratio (\%)}} \cdot 100\% = \text{(theoretical) total yield.}$$

That this is true we shall demonstrate by means of triple extraction. From soaked soyflour milk is prepared with the Braunmixer (ratio 10%, 20°C, pH 6.5, 10 min.) Then the residue is again extracted, but this time by means of the Vibromixer to avoid a possible grinding effect. The second residue is also extracted with ½ L water. From the dry matter obtained in each of the three milks the individual percentage of dry matter is calculated and after adding up these three yields the total yield becomes 78% of the original dry matter. The fact that the theoretical value (8.16 : 10).100% = 81.6% is not reached, can be ascribed to loss of material.

	Triple extraction of soyflour			*Triple extraction of soybeans*		
	dry matter (%)	yield of dry matter	yield of crude protein	dry matter (%)	yield of dry matter	yield of crude protein
1st extr.	8.16	59.7	71.5	7.82	53.6	64.7
2nd extr.	1.50	15.6	16.6	1.63	15.9	17.9
3rd extr.	0.29	2.6	3.6	0.39	3.6	4.7
		77.9 %	91.7 %		73.1 %	87.3 %

If the same is done with the soaked, whole beans we see that the theoretical value is not reached either. The difference between the theoretical value and the yield obtained is about 5%.

Nevertheless we believe that *the figures for the dry matter content of the milk from the first extraction may be considered as a measure for the total yield of dry matter.* This implies that by washing the residue twice it is possible to increase the yield to a much higher level.

From the table it appears that the protein yield figures are higher than those for the dry matter. This is mainly due to a difference in the first extraction. When considering the figures for total protein yield, we see that these yields amount to about 90%. For the flour the yield is higher than for the whole beans. Estimating the losses during the extraction of the flour at about 3%, the theoretical total yield of the proteins is about 95%. This means that the protein can be easily extracted nearly quantitatively. As it is our aim to obtain a proteinrich food by selective extraction, grinding should be carried out to such a degree that nearly all of the protein is dispersed.

After having discussed the results of our experiments, we arrive at the following conclusions.

1. The extraction experiments on the manufacture of soymilk are reproducible. Extraction of soaked flour under certain standard conditions gave milk containing 7.6-7.9% dry matter, about half of which was crude protein. The yields of dry matter and crude protein in the milk were about 59.0 and 68.0% respectively. Thus the proteins proved to be better dispersed than the rest of the dry matter.

2. Extraction of soaked flour yielded more milk than extraction of beans. With the flour as well as with the beans better grinding yielded more milk, and increased the dry matter and crude protein content of the milk. Thus the yield of dry matter and crude pro-

tein increased on better grinding.

3. The influence of the extraction ratio is important. If the ratio increased, the dry matter and crude protein content of the milk also increased. The yields of both decreased, however.

4. The extraction pH also had an important influence. However, if the pH was increased the dry matter content of the milk did not increase at the same rate as the crude protein content. At pH 9.5 the yield of crude protein was 80%.

5. The extraction temperature had little effect in the range 20^{o} to 50^{o}C.

6. After a certain minimum (in our case about 2½ minutes) the extraction time had little influence on the yield.

7. The rotation velocity of the Ika Ultraturrax had practically no effect on the extraction. However, the contents and yields of dry matter and crude protein were higher than when using the Vibromixer.

8. By washing the residue twice the total yields of dry matter and crude protein increased considerably and approached a theoretical value that -for the dry matter- can be derived from the percentage of dry matter of the milk and the extraction ratio. The total yield of crude protein obtained was about 90%; the estimated theoretical value is about 95%.

The recommendations resulting from this study on the manufacture of soymilk on a technical scale are given in the last chapter, the general discussion.

3.4 SUMMARY

Although long before the last war articles on soymilk appeared in medical journals, little work had been done on the technological side. In particular the data on the yield of the extraction were scarce and if available not very reliable. Therefore a new investigation of the yield of manufacturing soymilk was started.

The many methods of manufacture have four distinctly different stages in common: 1. the pretreatment, 2. the extraction, 3. the separation, 4. the improvement of the quality.

As the way in which the first stage, the pretreatment, is carried

out determines to a large extent the composition of the milk, this stage was studied on a laboratory scale in order to get an insight in the nature of the apparatus that should be applied on a technical scale. For this purpose several types of apparatuses were tested, using one extraction method and one separation method. Whole beans and also whole flour were used as starting material.

The second stage, the extraction, was studied to find out what factors might be adapted to get better yields with a given apparatus. The influence of five factors, the extraction ratio, pH, temperature, time, and method of mixing were investigated. Soyflour obtained with one and the same method of pretreatment was used.

4 PRESERVATION OF SOYMILK BY HEAT APPLICATION (STERILIZATION)

4.1 Introduction.

Soymilk is mainly composed of proteins, fats, and sugars. Therefore this product forms an ideal substrate for the growth of micro-organisms. When raw soymilk is left at room temperature it can be readily observed that after a shorter or longer time symptoms of decay will appear. This is especially evident at higher temperatures.

In the manufacture and distribution of soymilk such symptoms can not be allowed to develop as well as in any other field of the food industry. For this reason we have studied the preservation behaviour during storage of raw soymilk after application of heat.

4.2 Literature.

CHIU (1927) states that the general types of organisms that develop in milk are acid formers, gas producers, and putrefiers. In soymilk there are organisms acting on sugar producing alcohol and organic acids, those acting on proteins producing curdling of milk and evil smelling compounds, and those acting on the fat. The acidity of the milk was found to increase each day until the eighth day when the amount of acid produced checked the growth of the acid formers. Carbon dioxide was found due to the gas producers acting on the sugar. Ammonia was also found due to the action of the organisms on the protein. Each organism has an optimum temperature.

The symptoms of decay are described by MONNIER (1935). According to this worker after some hours the raw soymilk separates into two distinct parts: the lower part is whitish and resembles curd, while the upper part is a transparant greenish-yellow liquid.

By boiling for half an hour the colour becomes a little yellower, while the beanish odour is replaced by an agreeable flavour. The boiled milk is much stabler than the raw milk and may be preserved for 15 to 20 hours without "turning". After this period, however, the milk loses its homogeneity and decays. Contrary to the raw milk this decay results in curd particles floating in the whey.

The preservation by means of chemicals was studied. Sodium bicarbonate (63 g per liter) has given the best results; the flavour of the milk was improved by the addition of this compound; the milk was preserved for more than 54 hours. Benzoic acid, salicilyc acid and boric acid are impracticable as they cause the proteins to precipitate at the antiseptic dose.

As described in the chapter on the manufacture of soymilk CASTAGNOL (1934) has also given a description of the fermentation of the milk.

4.3 EXPERIMENTAL PART

4.3.1 Scope of work.

From the information available it appears that efforts have been made to improve the keeping qualities of the milk. This was mainly done either by the addition of chemicals or the application of heat.

As, however, in our opinion the addition of chemicals should be avoided because in practice a wrong application may easily cause injury to human health, we confined ourselves to a study of the preservation of soymilk by heat.

4.3.2 Design of experiments.

Before carrying out the sterilization experiments preliminary experiments on pasteurization in combination with cooling were set up. Raw soymilk in test tubes was pasteurized at 60°C for 30 minutes. Some of the tubes were stored at 20°C, while others were stored at 25°C. A number of tubes were immediately cooled after pasteurization. The pH was determined and a plate count was made.

For the sterilization experiments of our standard milk of about 8% dry matter three series of heat treatments were designed, using 101°C, 110°C, and 120°C as sterilization temperatures and 5, 10, and 15 minutes as holding times for each sterilization temperature. For each heat treatment nine cans were sterilized and then stored at 30°C. After one, six, and twelve months of storage each time three cans were examined visually as regards the condition of the can and the milk, the pH and acidity were determined, and a plate count was made of each milk.

A last group of sterilizations was carried out with milk of about 12, 16 and 24% dry matter. These milks were first preheated with the continuous sterilisator at 80°C for 45 sec and then evaporated with a Luwa film evaporator as described in the following chapter on evaporated milk. The sterilization in cans was carried out with a number of temperature-time combinations. Immediately after sterilization the cans were opened to see whether the milk had withstood the temperature-time combination applied. A number of cans were left at room temperature (20°C) to see whether they would spoil.

4.3.3 Technique of experiments.

The milk was prepared by extracting soaked beans with an Ultra-turrax in a 1 : 10 ratio. This method was found to be the most convenient on a pilotplant scale. After extraction the liquid was cooled to about 5°C by means of a cooling spiral through which cooled brine of some degrees below zero circulated. Then the cooled liquid was filtered with a basket centrifuge.

The milk was sterilized in cans of 0.35 liter. For each sterilization nine cans were put in the sterilizator and also one can provided with an opening which was closed by a rubber stop. Through this stop a thermocouple was placed in such a way that the end was in the centre of the can. The temperature at this place was registered by a Brown potentiometer.

The steam was lead into the sterilizator in such a way that in two minutes the required temperature was reached, after which the temperature was kept constant for the desired holding time. Then the steam supply was cut off and immediately water was fed into the sterilizator. During the sterilization process the temperature and time were observed, for which the average curves are shown in fig. 5.

After sterilization the cans were labelled and stored at 30°C. One, six and twelve months later from each heat treatment three cans were examined for the visual condition, pH, acidity, and plate count. The tins may be in good condition (+), a little blown up (-), or strongly blown up (x). For the milk the same symbols were used to designate that it was still in a good condition visually (+), flocculated but still milklike (-), or that a curd- and whey forming had taken place (x).

The pH and the acidity were determined as described in the former chapter, while the plate count was carried out as follows. One ml of milk is diluted with 9 ml sterilized, distilled water to 0.1; also a dilution of 0.01 and 0.001 are made. From each of these diluted milks 1 ml is mixed with bouillonagar substrate in Petri-dishes, after which the plates are stored at 37^{o}C for six days. The plates were prepared in duplicate. Then the number of colonies on each plate was counted.

Care was taken to prevent infection. Therefore the cans and also the opener were first burned with alcohol before opening and then the milk was poured out in a sterilized bottle. From this bottle the dilutions were made with sterilized pipets and plates.

4.3.4 Results, discussion, and conclusions.

Before carrying out the sterilizations a number of preliminary pasteurization tests were performed.

Raw soymilk was pasteurized in test tubes at 60^{o}C for 30 minutes and then stored at 20^{o} and 25^{o}C. Due to the pasteurization the initial number of bacteria decreased from 25.000/ml to below 1.000/ml. During storage the bacteria increased markedly, the pH decreased first and then increased after about a week. In general these observations are in accordance with those of Castagnol (1934).Our results are shown in the table below.

Plate-count and pH of stored, pasteurized soymilk (60^{o}C 30 min).

	Plate count		pH		
Number of days	20^{o}C	25^{o}C	Number of days	20^{o}C	25^{o}C
after pasteurization	935		1	6.59	6.49
	770		2	5.45	5.12
1	246.000	2.305.000	3	4.96	4.86
	220.000	2.430.000	4	4.80	4.53
3	76.000.000	31.600.000	5	4.22	4.53
	147.000.000	80.000.000	7	3.88	4.20
4	103.000.000	318.000.000	8	4.05	4.25
	137.000.000	1.297.000.000	9	4.25	4.22
5	557.600.000	600.000.000	10	4.35	4.21
	740.000.000	1.395.500.000	11	4.42	4.40
			12	4.43	4.35

If immediately after pasteurization the milk was cooled, after three and also after six days the pH and the physical appearance of the milk were still quite normal. However, determinations of the plate-count showed that-notwithstanding the low storage temperatures- a rapid development of the bacteria had taken place (see table below).

Plate-count and pH of pasteurized soymilk (60°C 30 min) in cold storage.

	pH		plate-count	
	3 days	6 days	3 days	6 days
10.5 °C	6.50	6.75	14.500	5.300.000
6 °C	6.45	6.45	3.600	1.550.000
3 °C	6.50	6.55	1.985	34.000

The results of sterilizing 8% soymilk are shown in table 3 and fig 5. As mentioned before the condition of the milk is designated as 1)good (+), 2)flocculated but still milklike (-), or 3)pronounced formation of whey and curd (x). The condition of the milk from the nine cans of a certain heat treatment is represented by horizontal lines in fig 5 and is obtained from table 3, column "Condition of milk". Above those lines the temperature course in the centre of the can is depicted.

A plate was considered sterile if less than 5 colonies were present. Then it was assumed that the plates were infected. This may also be seen from the fact that then the colonies were mostly located in the outer part of the plate.

Below are given some examples of sterile plates.

Number of colônies on plates after 6 days of incubation at 37°C

dilution / exp. no.	1/10	1/100	1/1000
5.7	-	1	1
	-	-	-
5.8	-	1	-
	-	-	1
5.9	-	1	2
	-	1	-
6.4	-	-	-
	-	1	-
6.5	-	-	-
	-	-	-
6.6	-	-	-
	-	-	-

TABLE 3 Condition of sterilized soymilk after 1, 6, and 12 months storage at 30°C.

Exp. no	Heat treatment	Storage	Plate count	Acidity	pH	Condition of can milk	
1.1	101°C 5 min	1 mnth		-	5.18	-	-
2				-	-	x	x
3				-	4.8	x	x
4		6				-	-
5						x	x
6						x	x
7		12				x	x
8						x	x
9						x	x
2.1	101°C 10 min	1 mnth		-	5.31	-	-
2				-	5.45	-	x
3				-	-	x	x
4		6	sterile	4.80	5.27	+	-
5			sterile	4.93	5.39	-	-
6			sterile	5.09	5.21	+	-
7		12		3.54	4.71	+	x
8				4.92	5.34	+	x
9				-	4.76	x	x
3.1	101°C 15 min	1 mnth		-	5.31	-	-
2				-	5.20	-	x
3				-	5.18	-	-
4		6	sterile	5.26	5.39	+	-
5			sterile	2.78	5.37	+	x
6			sterile	2.49	5.12	-	x
7		12	sterile	4.07	5.65	-	x
8			sterile	1.88	5.55	-	x
9			sterile	4.53	5.37	-	x
4.1	110°C 5 min	1 mnth	sterile	1.65	6.61	+	+
2			sterile	4.05	6.08	+	-
3			sterile	-	5.01	-	x
4		6	sterile	3.12	5.65	+	-
5			sterile	3.69	5.85	+	-
6			sterile		4.75	x	x
7		12	sterile	1.53	6.53	+	+
8			sterile	4.25	5.34	+	-
9			sterile	1.80	5.79	+	x

Exp. no	Heat treatment	Storage	Plate count	Acidity	pH	Condition of can milk	
5.1	110°C 10 min	1 mnth	sterile	1.51	6.60	+	+
2			sterile	3.79	6.31	+	-
3			sterile	1.64	6.65	+	+
4		6	sterile	1.77	6.43	+	+
5			sterile	2.38	5.79	+	x
6			sterile	2.06	5.79	+	x
7		12	sterile	4.46	5.50	+	-
8			sterile	4.19	5.58	+	-
9			sterile	4.00	5.51	+	-
6.1	110°C 15 min	1 mnth	sterile	2.19	6.51	+	+
2			sterile	1.96	6.32	+	+
3			sterile	1.73	6.20	+	+
4		6	sterile	2.64	5.79	+	-
5			sterile	2.64	6.51	+	+
6			sterile	2.64	6.50	+	+
7		12	sterile	1.72	5.84	+	x
8			sterile	1.42	6.56	+	+
9			sterile	1.64	5.53	+	x
7.1	120°C 5 min	1 mnth	sterile	1.51	6.49	+	+
2			sterile	1.52	6.49	+	+
3			sterile	1.61	6.47	+	+
4		6	sterile	1.51	6.56	+	+
5			sterile	1.49	6.54	+	+
6			sterile	1.28	6.55	+	+
7		12	sterile	1.52	6.58	+	+
8			sterile	1.44	6.59	+	+
9			sterile	1.52	6.58	+	+
8.1	120°C 10 min	1 mnth	sterile	1.75	6.49	+	+
2			sterile	1.56	6.69	+	+
3			sterile	1.56	6.67	+	+
4		6	sterile	1.41	6.55	+	+
5			sterile	1.49	6.57	+	+
6			sterile	1.49	6.56	+	+
7		12	sterile	1.51	6.59	+	+
8			sterile	1.46	6.58	+	+
9			sterile	1.44	6.58	+	+

Exp. no	Heat treatment	Storage	Plate count	Acidity	pH	Condition of can	milk
9.1	120°C 15 min	1 mnth	sterile	1.55	6.67	+	+
2			sterile	1.63	6.63	+	+
3			sterile	1.64	6.63	+	+
4		6	sterile	1.46	6.55	+	+
5			sterile	1.40	6.55	+	+
6			sterile	1.34	6.54	+	+
7		12	sterile	1.41	6.57	+	+
8			sterile	1.46	6.56	+	+
9			sterile	1.45	6.56	+	+

Milk: in good condition + Can: in good condition +

Milk: flocculated, still milklike - Can: blown up -

Milk: curd- and whey formation x Can: strongly blown up x

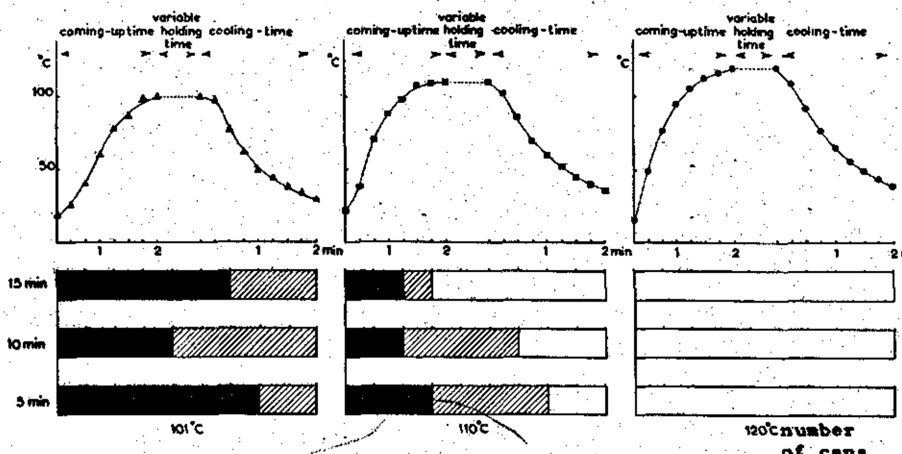


Fig 5. Sterilization of soymilk. Course of temperature in centre of cans. Condition of the milk.

☐ in good condition ▨ flocculated, still milklike

■ curd- and whey formation

It will be seen that the heat treatment at 100°C had no sterilizing effect, as using a holding time of 5, 10, or 15 minutes only resulted in spoiled milk. It is noteworthy that after 6 and 12 months no micro-organisms were found in the spoiled milk, though there is a pronounced decrease of the pH to 5.3 and a corresponding increase in acidity to 3.0 - 4.5 ml.

The sterilization at 110°C appears to be a transition temperature, because the number of cans of good milk increases as the holding time increases. It can be clearly seen that the well preserved milk has a pH of 6.5 - 6.7, whereas the pH of the spoiled milk is below 6. Thus the condition of the milk is expressed in terms of acidity as well. Also with this series no bacteria were found in the spoiled milk.

All of the three last heat treatments at 120°C gave favourable results. The values of the pH and acidity are in accordance with those of the unspoiled milk from the former three series.

The results of sterilizing soymilk with a higher dry matter content are shown below.

Dry matter	Sterilization conditions	Physical condition of milk after sterilization	Bacteriological condition of milk after one day (20°C)
12.4 %	120°C 5 min	normally flowing	good
12.4 %	120°C 15 min	normally flowing	good
16.4 %	120°C 15 min	gelatinized	partly spoiled
16.4 %	120°C 5 min	gelatinized	
16.4 %	110°C 5 min	gelatinized	
16.4 %	100°C 5 min	gelatinized	
24.3 %	120°C 5 min	gelatinized	spoiled

From this table it will be seen that 12.4 % milk can be safely sterilized at 120°C 15 min; the condition of the milk then remains good, while the heating is also bacteriologically effective. The 16.4 % milk, however, did not withstand the sterilization neither at 120°C 15 min nor at 120°C 5 min. Even sterilization at 100°C 5 min made a gel from the milk. This is not in accordance with the heat stability determination made on this milk. However, it might be possible that the use of a rotating sterilizator would have given more favourable results. It is important to note that for this milk sterilized at 120°C 5 min and even 15 min was not sufficient to prevent spoilage. This means that a more severe heat treatment must be applied when the dry matter content of the milk is raised. In view of our experience with the 16.4 % milk it is not surprising that the 24.3 % milk did not withstand the sterilization and that spoilage occurred at room temperature.

The following conclusions can be drawn:

1. Pasteurization of raw soymilk at 60°C 30 min considerably reduces the initial number of bacteria, but the milk will not keep even one day if left at room temperature (20-25°C). Pasteurization in combination with cold storage at 3-6°C may preserve the milk for about 3 days.

2. Sterilization of 8 % soymilk at 120°C 5 min is sufficient to preserve the milk for at least one year.

3. If the dry matter content of the milk is raised above 12 % the temperature-time combination regarded as sufficient for an 8 % milk will not suffice as a sterilization treatment.

4.4 SUMMARY

As soymilk is mainly composed of proteins, fats, and sugars it forms a good substrate for micro-organisms. In literature some data can be found indicating that the keeping qualities can be improved by application of heat. It is also known that attempts have been made to preserve the milk by chemical means. As in our opinion such methods might endanger human health, we only tried to obtain better keeping qualities by means of pasteurization and sterilization.

To this end a number of experiments were carried out. After preliminary pasteurization experiments -some of which combined with cold storage- nine temperature-time combinations for sterilization purposes were studied as to their influence on the keeping qualities of the soymilk after one, six, and twelve months storage at 30°C.

A number of tentative sterilizations of milks with higher dry matter contents, obtained by evaporation, were also carried out.

5 EVAPORATED SOYMILK

5.1 Introduction.

In chapter 3 we have seen that -if the same method of manufacture is applied- a product of constant composition can be obtained. We have also demonstrated that the dry matter content of the milk can be varied within rather wide limits by changing the extraction conditions, especially the extraction ratio.

If it is desired to manufacture soymilk with a high dry matter content there are in principle two possibilities. In the first place fresh milk with a low dry matter content can be concentrated by evaporation, and secondly one can try to make directly a milk with a high dry matter content by using less water for the extraction process. However, we have shown that with a one stage process the output strongly decreases as the dry matter content of the milk increases. The output becomes particularly low when one aimes at a dry matter content of 20% or more. Besides other difficulties arise. Thus if milk of more than 20% is to be manufactured, concentration by evaporation is inevitable.

Then the initial dry matter content to be chosen depends upon the total costprice of the evaporated milk. Starting with a very low dry matter content the output is high, but much water has to be evaporated. If milk with a higher dry matter content has to be concentrated less water needs to be evaporated, but the output of the extraction diminishes. The optimal, total costprice must lie somewhere between these two possibilities; this optimum can only be calculated in concrete cases. We estimate that it lies at about 8% dry matter. Therefore we started the manufacture of evaporated soymilk from raw milk of about 8% dry matter content.

Concentrating soymilk by means of evaporators is not an unknown process. However, some difficulties may arise during the process which require closer investigation. There are indications in literature that the viscosity of the evaporated product becomes so high at a relatively low dry matter content that the milk cannot flow any more. This phenomenon seems to be especially evident when the concentrated milk is cooled; the milk then seems more or less to solidify. It is clear that this may lead to serious troubles during evaporation and in further processing.

Another point of consideration concerns the further use of the evaporated milk. Evidently this should be preserved in some way to obtain reasonable keeping qualities. If preservation by sterilization is applied the milk should be in such a condition that it can withstand the sterilization conditions. If preservation is to be obtained by addition of sugar the viscosity of the concentrated milk should not be so high that this addition is not possible. If drying to milkpowder is chosen for preservation a not too high viscosity is also necessary.

From the foregoing it appears that on concentrating soymilk we should be interested particularly in the viscosity and the heatstability as a function of manufacturing conditions. It is also important to investigate to which dry matter content soymilk can be evaporated while retaining acceptable properties. The work concerning these factors is described in this chapter.

5.2 Literature.

The literature on evaporated soymilk is limited. Probably the first reference to this subject comes from KATAYAMA (1906) who observed that on direct evaporation of soymilk after addition of sugar the protein separated into small floccules. Therefore dipotassiumphosphate was added to prevent this separation. The author states that the solution could be concentrated in vacuo to a very thick liquid. Unfortunately the dry matter content reached was not mentioned.

Shortly after this communication a studygroup was founded in France (1910) with the aim to produce soymilk, evaporated soymilk andsoymilkpowder, but these efforts were abandoned for unknown reasons. On the other hand it is known that in Frankfurt the factory Soyama succeeded to manufacture fresh and concentrated soymilk (Inst. Int. d'Agric., 1936).

Also in Russia attention was paid to the concentrating of soymilk. HOROWITZ-WLASSOWA, OBERHARD, and GUTERMANN (1931) in Russia concentrated soymilk, after addition of 19% sugar, to one fourth of the original volume. The composition of the evaporated soymilk was 11.76% protein, 7.64% fat, 42.12% sugar, 2.32% ash, and 37.16% water.

The CENTRAL TECHNICAL INSTITUTE of TNO Netherlands (1955) carried out research on the viscosity of soymilk during concentration, the choice of the best type of evaporator, and the highest concentration attainable in the evaporator.

The milk was prepared by extraction of 6 kg of soybeans and 1.5 kg of peanuts with 63 kg of water. Thus 74% of the original dry matter, 79% of the protein, and 75% of the oil were extracted from the beans.

In the course of the preparation of soymilk the following heat treatments were applied. After soaking the beans were kept in 70°C water for 12 minutes. The extraction was carried out at the same temperature for 12 minutes after which the mixture was boiled for 20 minutes. The following filtering of the milk was done at 70°C during 12 minutes, after which the milk was cooled to 5°C.

Then the milk was concentrated in a Lurgi laboratory flash evaporator with forced circulation. After 70 minutes 20% dry matter was attained, but it was not possible to increase the percentage of dry matter further. The milk then had become so thick that the pump could not circulate it through the evaporator any more.

With a Kurt Herbert evaporator the milk could easily be concentrated to 20% dry matter. This type of evaporator has a natural circulation system of the liquid. However, if the concentrating was continued to 24% dry matter, the milk became a gel and clogged the pipes of the evaporator.

With a Luwa evaporator it was also found that the maximal concentration possible was only 20%. In this type of evaporator the liquid flows down a vertical pipe. A rotor forces the liquid to form a thin film against the inside of the pipe. The holding time is very short in comparison with the two other types of evaporators.

Measurements of the viscosity of the original milk showed that after five days at 5°C the viscosity was practically the same. Concentration to 16% dry matter did not show an important increase in viscosity, and at 18% the milk still flew very well. At 20%, however, the milk was very viscous, but it still flew if the milk was kept in constant movement. If not kept moving then milk of 18% solidified in one hour and milk of 20% in half an hour.

As regards the question whether it is possible to concentrate soymilk to a reasonable percentage of dry matter -e.g. 30%- we may see from literature that very little information is available. However, even in the few publications on this matter the conclusions are very contradictory. Horowitz-Wlassowa showed the possibility of concentrating soymilk, whereas T N O found that 20% dry matter should be considered as a limit to evaporation due to the high viscosity and the incrustation. In the literature, no data can be found on the second factor, the heat stability.

As very little or nothing is known about these two characteristics of soymilk and besides little can be said about the theoretical background of the viscosity and heat stability, it was found desirable to examine the literature on animal milk. Although we are quite aware of the fact that the animal and vegetable milk differ in composition it is probable that some lines may be found along which a research scheme may be developed.

The literature on this subject is very extensive and dates back to 1919. From this large number of publications we will mention only a few.

The relationships of concentration and time to the temperature of coagulation were studied by H O L M, D E Y S H E R, and E V A N S (1923). They found a decrease of about 1.50°C for every 1 percent increase in concentration of the milk. Between ten and sixty minutes the relation between the temperature to the time of coagulation proved to approximate very closely a logarithmic relation with respect to time. Milk of inferior quality had a decidedly lower coagulating temperature but the relationships of concentration and temperature seemed to be the same as for milk of good quality.

In the paper of D E Y S H E R, W E B B, and H O L M (1929) it is shown that the method of preheating the raw milk prior to evaporation influences the heat stability of the evaporated milk. Temperatures up to 70°C, applied for ten minutes, decrease the stability, while higher temperatures markedly increase it. Homogenization of the evaporated milk at pressures up to 4000 pounds per square inch affects its heat stability but slightly.

These workers point out that body of the evaporated milk should be of a creamy consistency. Their experiments indicate that the consistency attainable before coagulation begins and the heat stability are of a reciprocal nature. Those products of greatest stability had the lowest relative consistency at the time of coagulation. So milks of exceedingly high heat stability are not the most desirable from the standpoint of production of body.

Due to the observation that injection of high velocity steam in a stream of milk caused stabilization to the heat of sterilization WEBB and BELL (1942) designed experiments to study the influence of rapid heating on the heat stability of evaporated milk of 18% solids not fat. For the greater part the milk was heated to the desired temperature in 5 seconds, held 25 seconds and cooled to a temperature of less than 38°C in 4 seconds. The control samples were forewarmed at 95°C 10 min in a steamjacketed kettle, while the test samples were HTST heated over a range of temperatures from 101°C to 165°C.

It was found that the heat stability of evaporated whole milk of 26% total solids was generally twice and occasionally six times greater than that of the control samples. The relationship between the high forewarming temperature and the heat stability of the evaporated product differs with each milk.

The high forewarming temperature required to produce an evaporated milk of the desired viscosity may be within the limits of 2°C for one milk or within limits as wide as 60°C for another milk. Milks forewarmed to produce excessive high stability will be too thin, while those with too low stability will be rough after sterilization.

As the increase of the heat stability of concentrated milk by HTST forewarming may make possible the preparation and sterilization without coagulation of milks of solids concentrations greater than 26% WEBB, BELL, DEYSHER, and HOLM (1943) were thus able to investigate the effect of various degrees of forewarming upon the heat stability of milks of different solids concentrations.

In the range of solids concentrations investigated, 26 to about 33%, the stability increases as the holding time is lengthened for temperatures above 100°C to 150°C. Only when the HTST forewarming temperature was low or excessively high or when the holding period was too short, less stability was observed in the test than in the control samples. These control samples were heated in a steam-jacketed vessel at 95°C for 10 minutes.

Several experiments were also conducted to determine whether the stabilizing effect was derived from the heating temperature or from the rapid heating. It was found that rapid heating increased the stability of most milks above that imparted to samples heated slowly.

They also observed that the general heat stability relationships of milks subjected to different forewarming treatments were not affected by the development of acidity in the fresh milk or by the addition of stabilizing salts to concentrated milks; the difference in stability caused by acid or salts was one of degree.

The heat treatment can also be applied after concentration. WEBB and BELL (1943) heated the milk before and after concentration. HTST heating of the concentrated milk increased its heat stability to a maximum and then decreased it. With no holding time the stability increased with a rise in temperature up to the point of initial coagulation. Factors which controlled the attainment of maximum heat stability were the time and temperature of forewarming the raw and heating the concentrated milk and the solids content of the concentrate. The greatest heat stability was obtained when the raw milk was heated at 120°C for 4 minutes and the concentrate to 150°C with no holding period.

The authors point out that the most desirable heat treatment is not always the one that gives maximum stability, since the effect on the body and the colour must be considered.

Since the heat stabilities and concentrations of evaporated milks could be greatly increased by the use of high temperatures, this provided a means to prepare samples with a wide range of concentrations and stabilities. In this way it was possible for DEYSHER, WEBB, and HOLM (1944) to study the viscosity behaviour of evaporated milks during sterilization and storage as influenced by HTST heating before evaporation. Their results indicate how rapidly the viscosity of evaporated milks increased just before and after coagulation. Besides the body of the milk was shown to be greatly influenced by the heat stability of the milk. A reciprocal relationship between the heat stability and the viscosity after sterilization was noted. The highest viscosities were produced in milks of high concentration and low stability.

From their data it appears that the method of forewarming also influences the viscosity behaviour of the concentrated milk during storage. The viscosities from evaporated milks from 26 to 36 percent solids concentrations followed a well-defined pattern during processing and storage. Thickening occurred during the sterilization process. This was followed by a loss of body and a thinning early in the storage period. The low storage viscosity was maintained for various and unpredictable periods of time during which fat separation occurred. Late in the storage period some evaporated milks, especially those receiving light heat treatments and those with a high concentration of solids, began to show increases in viscosities even to the point of gelation.

Another example of the influence of heat on the viscosity of evaporated animal milk can be found in the publication of BELL, CURRAN, and EVANS (1944) who HTST heated the concentrated milk prior to canning.

If the milk was forewarmed, HTST sterilized after concentration and canned aseptically, the evaporated milks were of fairly satisfactory quality for only approximately 4 months when stored at 30°C. However, bried additional heating in cans at 115°C increased the storage life of these high-short sterilized milks.

The publications referred to are only part of the existing literature out of which we have chosen those articles which might be of importance to our work.

From the information on evaporated animal milk it may be seen that it is possible to influence the viscosity of the concentrated product by application of heat before or after evaporation. DEYSHER, WEBB, and HOLM (1944) demonstrated that the viscosity of the concentrated product after sterilization can be influenced by preheating. The viscosity behaviour during storage also depends on the method of forewarming the milks as they have shown. The work of BELL, CURRAN, and EVANS (1944) is another example of the influence of heat on the viscosity behaviour during storage. Besides it is an illustration of the fact that the influence of heat treatments has not only effect before evaporation but also after evaporation, in fact this influence applies to the sterilization process as well. Briefly we may say that the application of heat in the process of manufacturing evaporated animal milk results in changes of the viscosity after the sterilization process and during storage.

Returning to our own problem, the presence of a viscosity barrier on concentrating soymilk, we may expect that application of heat in some stage of the process will influence the viscosity behaviour of the concentrated product. Evidently it is not possible to predict what the results of such experiments will be, but considering the favourable results obtained with animal milk we may hope that this influence will be such as to make it possible to evaporate soymilk to at least 30% solids content.

In connection with our own work the results of DEYSHER, WEBB, and HOLM (1929) as regards the heat stability of animal milk are important. They have definitely shown, that it is possible to influence the stability of the evaporated product by variation of the heat treatment prior to evaporation. WEBB and BELL (1942) have demonstrated that if rapid heating in tubular heaters is applied, the stability of evaporated whole milk of 26% total solids is generally increased to twice that of the control samples. In this way it was possible for WEBB, DEYSHER, BELL, and HOLM (1943) to prepare a milk of 32% that could withstand sterilization. They also demonstrated that it is the velocity of heating, in other words the coming-up time, from which the stabilizing effect is derived. Also when the concentrated milk itself is heated WEBB and BELL (1943) found that the heat stability is influenced.

From this information it is clear that heat treatments have given favourable results as regards the heat stability of the concentrated animal milk. Therefore it seems justified to study the possible effect of the influence of heat on the stability of evaporated soymilk.

5.3 EXPERIMENTAL PART

5.3.1 Scope of work.

From the foregoing discussion of the literature it is evident that on concentrating soymilk difficulties may be expected, especially as regards the viscosity of the product. These difficulties seem to arise in particular when a dry matter content of 20% or higher is reached. Therefore a study of the influence of the concentration and other manufacturing conditions on the viscosity is desirable.

With cowmilk prior to evaporation a heat treatment is applied. It is known that this preheating or forewarming influences the viscosity. Possibly with soymilk preheating also has some influence on the viscosity.

From preliminary experiments we already knew that fresh soymilk has a comparatively low heat stability. If this heat stability should not become better on concentrating, or even become worse, then sterilization of the concentrated milk would be a difficult problem. From cowmilk it is known that the heat stability is improved on application of heat. On these grounds a study of preheating in connection with heat stability was desirable.

Also for other reasons heating the raw milk prior to evaporation is important; in the first place preheating lowers the initial number of micro-organisms; this is of importance when an evaporator is chosen in which the product remains a comparatively long time under conditions favourable for growth of micro-organisms, and also when the concentrated milk is used for the manufacture of sweetened, condensed milk or milkpowder. A low plate count is also favourable for the later sterilization process. Besides preheating is important because enzymes are inactivated which might harm the quality of the product later on. Among these enzymes one is undesirable from a nutritional point of view. This enzyme, the tryptic inhibitor, must be destroyed anyhow. Lastly, preheating is recommended as a method of desodorisation.

We might ask if the evaporation and the heat treatment could not be combined. In principle it is quite possible to evaporate at comparatively high temperatures and to adapt the holding time of the product to conditions most favourable for the viscosity, heat stability, and other factors mentioned. However, for a heat sensitive product as soymilk an evaporator is preferred with a short holding time and a low evaporation temperature. In such a type of evaporator the milk hardly undergoes a heat treatment. If such an evaporator is applied it will be necessary that a separate heating preceeds the evaporation. Then the advantage is that the heat treatment can be controlled more accurately.

Summarizing it was necessary to pay special attention to the process of preheating, and to its influence on the viscosity and heat stability of the evaporated milk.

For this purpose soymilk was prepared according to a uniform method recommended in the conclusions of the chapter on manufacture of soymilk. After preparation the milk was heated in various ways; this preheat treatment is described in the following chapter. Then the milk was evaporated under constant conditions; the evaporation was carried out at 40°C (about 50 mm mercury) by means of vacuum steam of 70°C. Samples were taken on which determinations of dry matter, viscosity, heat stability, acidity, and pH were made by three persons simultaneously so as to have the measurements made as soon as possible after sampling.

5.3.2 *Design of experiments.*

In actual practice the most common method of heating is batch heating in a vessel with a steam jacket. Such a vessel was used in our work. In our first heating series the lowest temperature applied was 60°C with a holding time of 30 minutes. It was not considered to use lower temperatures as this would make the heat treatment too much questionable as regards the destroying of micro-organisms.

In the discussion of the literature it was pointed out that WEBB and others (1943) have observed a definite effect of the holding time on the stability. To investigate whether this also holds for soymilk a second series was designed in which the same temperatures as in the first series were applied but using a holding time reduced to zero minutes theoretically. In practice it took about three minutes before all the milk had been transferred from the heating into the cooling vessel.

As has been shown by WEBB and others (1943) rapid heating increases the heat stability above that imparted to milk samples heated slowly. Such a rapid heating is only possible when the milk is heated continuously, that is to say, in a flowing condition. For this purpose a specially designed continuous, flash sterilizator was constructed by the machine factory of Stork (Holland). It consists of three concentric spirals of double pipes, the spirals successively representing the heating, the holding, and the cooling tube.

The original aim was to heat the milk at temperatures between 120° and 150°C with this flash sterilizator. However, when 120°C was applied the formation of incrustation was so strong that it was necessary to increase the steam pressure from 2 to more than 6 atm to keep the milk heated at 120°C. Besides after being used a second time the apparatus proved to be obstructed. In our opinion the rapid incrustation may be ascribed to the great temperature difference and the rapid heating.

On further experimentation with the sterilizator it appeared that temperatures lower than 100°C could be applied without too many difficulties.

In the third series of heat treatments, the lowest temperature applied was 70°C with a holding time of 45 seconds, while the highest temperature was 120°C 45 seconds. As it was also desired to investigate the influence of the holding time when the milk is heated rapidly in the fourth series this holding time is reduced to zero seconds theoretically. Actually, according to the construction data, the time needed by the milk to pass from the heating to the cooling tube is about one tenth of a second. Also in this case it was not considered to use lower temperatures than 70°C, as the meaning of such a heat treatment for the destroying of micro-organisms would become very questionable.

Summarizing we studied the properties of the evaporated soymilk as influenced by the following heating methods.

Series 1 Batch	Series 2 Batch	Series 3 Continuous	Series 4 Continuous
-	-	120°C 45 sec	-
-	-	-	100°C 0 sec
90°C 30 min	90°C 0 min	90°C 45 sec	90°C 0 sec
80°C 30 min	80°C 0 min	80°C 45 sec	80°C 0 sec
70°C 30 min	70°C 0 min	70°C 45 sec	70°C 9 sec
60°C 30 min	60°C 0 min	-	-

5.3.3 Technique of experiments.

In processing the milk to evaporated milk the first step was the preparation of the raw soymilk. This milk was heated by a steam-jacketed vessel or by the flash sterilizator, after which the milk was cooled to about 5°C. Then it was sent through a film evaporator, where it was evaporated at 40°C by means of 70°C vacuum steam. Samples of increasing dry matter content were taken and each sample was cooled. Determinations were made of the dry matter content, viscosity, heat stability, pH, and acidity.

In *preparing the milk* about 10 kg of dry beans were soaked overnight and extracted with water for half an hour in a 1 : 10 ratio by means of an Ultraturrax provided with knives. The mixture was cooled to about 5°C, after which it was sent through a centrifugal filter. As it was important for the milk to be completely free of small solid particles a second filtration was carried out. During the whole filtering process the milk was kept cool in order to prevent the growth of micro-organisms. The prepared raw milk was kept in the refrigerator at about 5°C.

Then the milk was heated either by a vessel described above or by the continuous flash sterilizator.

If the vessel was used (photo 6), the milk was kept in constant movement by a stirrer. This improves the heat transmission and avoided incrustation. After the required period of heating the milk flew into a container in which a cooling spiral and a mixer were placed. For heating 2 atm. steam was used, about 10 minutes was required to reach the desired temperature. It took about 30 minutes for the cooling operation to reach 20°C.

In the continuous sterilizator the coming-up time is 3-4 seconds. Also the cooling operation is very rapid, as it takes only 12 seconds to reach 20°C.

The sterilizator consists of three concentric spirals of double pipes (photo 7). In all three sections the milk flows through the inner pipe. In the inner spiral, the heating tube, the milk is heated by steam; in the outer spiral, the holding tube; the milk can be kept for 0, 15, 30, or 45 seconds at the temperature obtained; in the middle spiral, the cooling tube, the milk is cooled with water (fig 6).

The temperature is measured with thermocouples which are present in four places. The first is placed at the inlet of the soymilk, the second immediately after the heating tube, the third just before the cooling tube, and the last at the milk outlet (fig 7).

The data on the construction of the apparatus at 100 L/hour capacity -as given by the machine-factory- are summarized in the following table:

Flash-sterilizator: data for 100 L/hour capacity.

	length (m)	inner diameter (mm)	velocity (m/sec)	Re	K (Kcal/m^2.hr.^oC)
heating tube	5	5	1.4	12.000	2.500
holding tube	3.6÷7.2	12	1.4	--	--
cooling tube	12	12	0.28	7.000	1.000

After the heat treatment the milk was cooled with cold brine to about 5^oC, and then the milk was sent through the film evaporator.

The evaporation installation consists of a *film evaporator*, a *condensor*, a *vacuum system*, and a vessel for the production of *vacuum steam* (fig 8).

The lower longer part of the evaporator (fig 9) consists of the evaporator body which is surrounded by a steamjacket. The upper shorter part serves as a vapour liquid separator. Between these two parts the liquid is introduced tangentially into the evaporator body, where it is caught by a four-blade rotor extending from the top of the upper part to the bottom of the lower part. Due to the movement of the rotor, the liquid is thrown against the inner wall and flows down as a thin film. The rotor can be adjusted at three speeds. The advantage of this kind of evaporator is that the liquid comes only for a very short time into contact with the heating surface.

The evaporator body has the following dimensions:

inner diameter	60	mm
outer diameter	64	mm
length	490	mm
thickness of wall	2	mm
heating surface	0.0954	m^2

The diameter of the rotor varies from 5.67 - 5.77 cm. So the distance from the rotor to the heating surface varies from 1.0-1.5 mm. The three speeds of the rotor are

1) 1010 rotations per minute
2) 1470 " " "
3) 2120 " " "

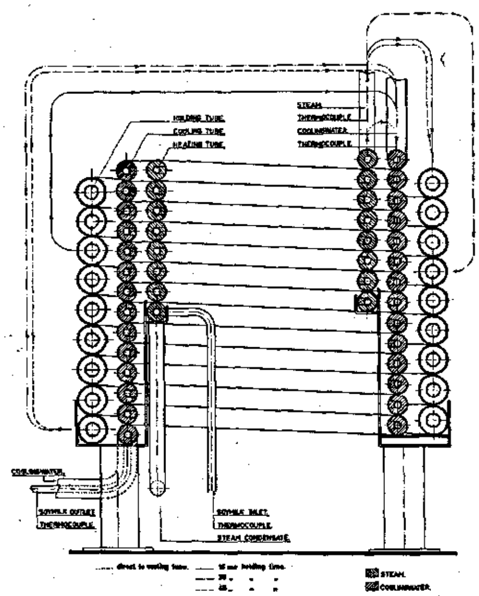


Fig 6. Stork double pipe, continuous, flash sterilizator.

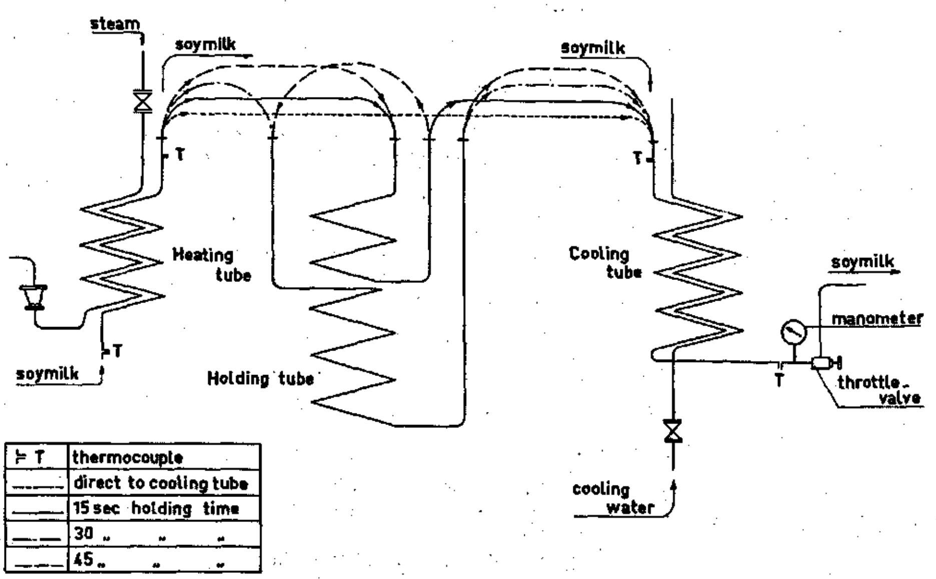


Fig 7. Flash sterilizator, position of thermocouples.

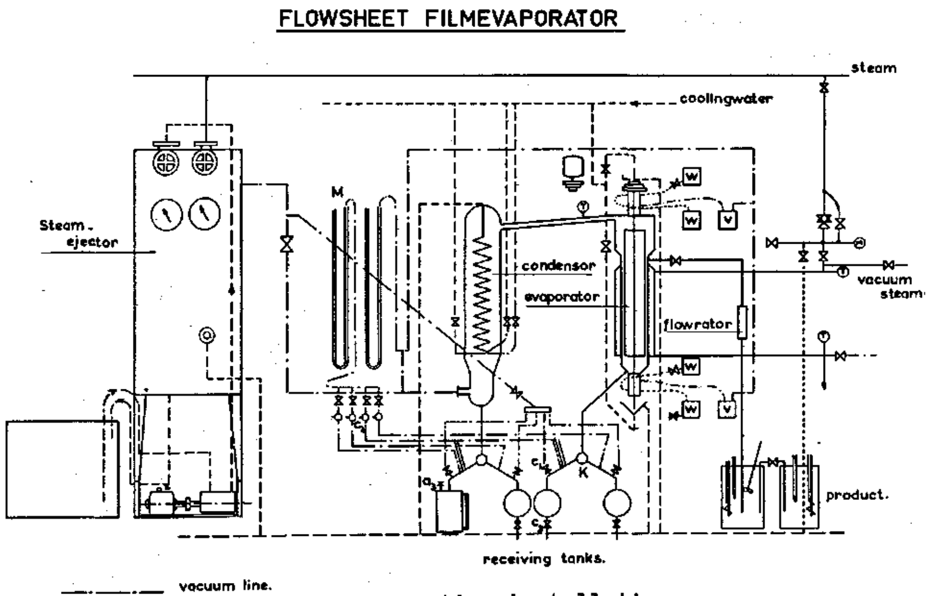


Fig 8. Evaporation installation.

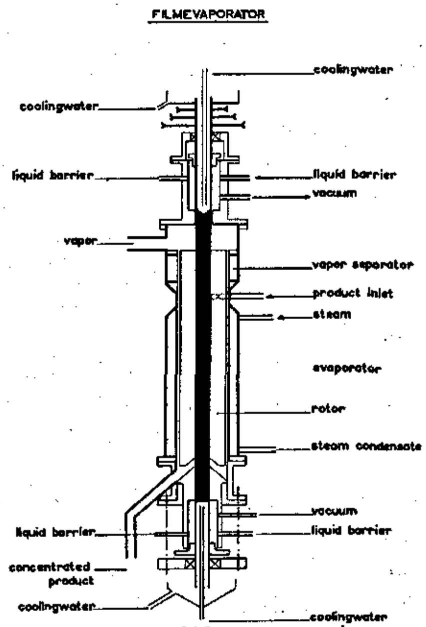


Fig 9. LUWA film evaporator.

From the vapour liquid separator the vapour comes through a connection pipe into the glass *condensor,* where it is condensed by three glass cooling spirals. Through these spirals water or cooled brine can be circulated. At its base the condensor is provided with two containers for the condensed vapour. The condensor is directly connected with the main vacuum system by the main vacuum pipe.

The vacuum in the evaporator is obtained by means of one water and two steam ejectors.The working pressures were 3 atm and 2.8 atm respectively. The vacuum is measured by a mercury manometer M and amounts to about 73 cm of mercury. The vacuum is kept constant by a leakage of valve A_3 on the top of the left container. Under working conditions the vacuum is adjusted to a constant vapour temperature which is measured by a mercury thermometer T inserted in the connection pipe between vapour separator and condensor. To insure the vacuum tightness a vacuum (V) and a waterlock (W) are applied around the upper and lower end of the rotating axle.

The evaporator body is heated by *vacuum steam* which is generated by a vessel with an own secondary vacuum system. The vacuum system is not connected with the main vacuum system. Due to an automatic heating regulation the vacuum steam had a constant temperature of $70^{o}C$. The secondary vacuum was about 53 cm of mercury.

The *actual operation* was as follows.The main vacuum system, water and vacuum locks were switched on,after which vacuum steam was generated. Then the rotor and condensor were switched on.

The cold milk, about $5^{o}C$, was sent into the evaporator body after having passed a flowrator.The milk was not sent into the evaporator at boiling temperature because from preliminary experiments it was known that this would cause trouble due to the foaming of the milk. However, if the temperature of the milk was lowered and the speed of the rotor increased the foam was completely suppressed. This flowrator was adjusted in such a way that the inlet amount was about 24 L/hour. The milk, flowing down as a thin,film, was heated by the steamjacket and the vapour escaped at a boiling temperature of $40^{o}C$. The milk has a strong tendency to foam and if the speed of the rotor is not high enough the foam will not be completely destroyed and passes into the condensor.Under the chosen circumstances the second speed of the rotor, 1470 r.p.m., was high enough to prevent this.

The concentrated milk was received by one of the two glass containers at the lower part of the evaporator. When this container C was filled,the milkflow was switched to the other container by means of tap K. Then the container C was shut off from the vacuum system by means of tap C_1, and air was admitted by tap C_2. By opening tap C_3

the milk flows out of the container. After emptying the taps C_3 and C_2 were closed and as tap C_1 was gradually opened the pressure of the main vacuum system was lowered until the required vapour temperature of 40^oC was reached again. In this way the glass containers were filled and emptied. The milk, which came out of the two glass containers, was immediately cooled with a cooling spiral through which cooled brine was circulated.

In this way the milk was sent a few times through the evaporator at the rate of about 24 L/hour, and during each pass a number of samples were taken. During the first pass some three to five samples with different dry matter content were taken by lowering the supply of milk to the evaporator. The greater part was evaporated under constant conditions and thus a first concentrate was obtained with a dry matter content of about 10%. After the first pass the milk was sent through the evaporator for a second time; the second concentrate had about 14% dry matter. Also during the second pass samples of increasing dry matter content were taken. If the viscosity barrier was not yet reached a third and fourth pass was effected and samples were obtained. Altogether the milk was evaporated two to four times until it hardly flows owing to the increased viscosity.

The samples obtained in this way were put in bottles, which were cooled in water of about 0^oC. The viscosity then increased due to the decrease in the temperature of the evaporated milk. The milks of low viscosity could be poured out easily, but with those of higher viscosity - though the milk was still flowing - the circular upper surface of the milk tended to retain its shape when the cylindrical bottle was kept sloping. When this happened such a milk was designated a gel, though the consistency of such a gel was not necessarily very high. This gelling occurred above 100 cP. The gels of the other samples with successively increasing dry matter content gradually became firmer until at last, keeping the milk bottle upside down, the contents did not flow out of the bottle but remained together like a very firm pudding.

The *viscosity* determinations were carried out with a Haake Viskowaage, (fig 10), which is a modification of the Hoppler viscosimeter. It consists of a standard with a fall tube and waterjacket. The standard carries a yoke which has a weight, rod, and metal ball on the left hand, a long needle in the middle, and a scale for weights on the right hand.

When the glass tube was filled with the evaporated milk, the ball was placed in its lowest position in the tube. By putting weights on the scale on the right the ball was pulled upward through the liquid and the needle moved over a marked scale. The time which the needle needs to pass between two points on the scale was observed. The determinations were carried out at 20°C. The water around the glass tube is circulated by a Hoppler thermostate, which keeps the water temperature at 20°C ± 0.05°.

The viscosity was calculated with the formula.

$n = F\ G\ T$ n = viscosity (cP)
F = rod factor (cm^{-2})
G = weight (g)
T = time (sec)

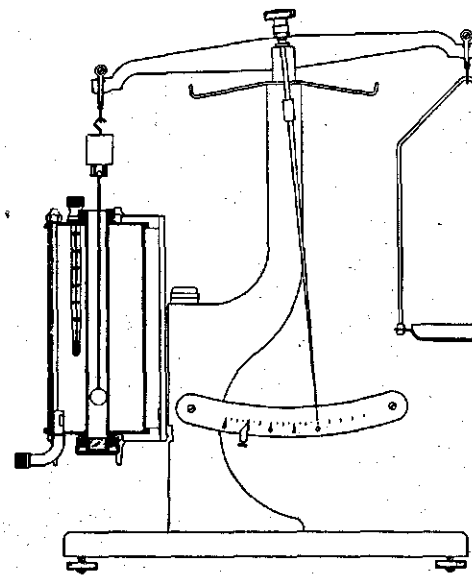


fig. 10 Viskowaage

The *heat stability* determinations were carried out in pyrex glass tubes open at one side. This side can be closed by means of a nut. The tube has the following average sizes: length 20.5 cm, outer diameter 1.2 cm; about 80% (= 8 ml) of the volume was filled with evaporated milk. The tubes were fastened to a movable rod by means of two clamps and after immersion in the 120°C paraffine bath kept in constant vertical movement as indicated in the following figure. This temperature of the paraffine was chosen because the sterilization of evaporated soymilk in cans would be carried out at 120°C. The tube ends were moved up- and downwards about 25 to 30 times a

minute.
A stirrer kept the paraffine in constant movement in the tank which at the front was provided with a glass window through which observations could be made. These observations were made in duplicate.

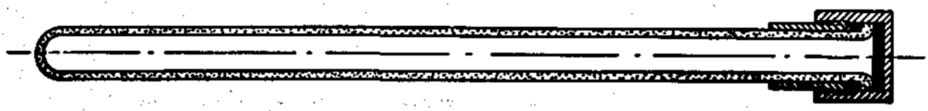


fig. 11 Pyrex glasstube

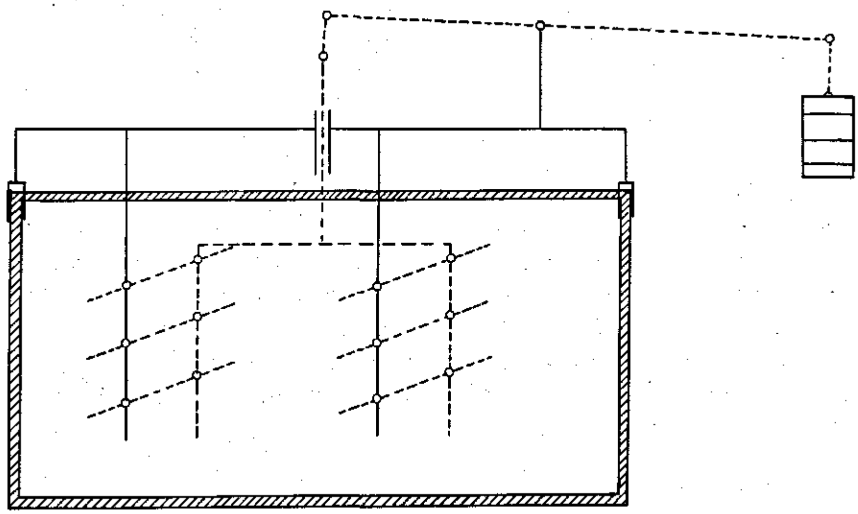


fig. 12 Paraffine tank

As soon as the tubes were immersed the time was marked with a stop-watch and the milk was kept flowing in the tubes. During the milk-flow small speckles appeared on the inner surface of the tubes; in the milk itself no solid particles could be observed as yet. Then the milk began to flow jerkingly (the flowline of the milk is not smooth any more) and when this point was reached the watch was stopped. The time between immersion of the tubes and this moment was considered as the heat stability. On further heating solid particles appeared throughout the milk and the milk solidifies.

5.4 Results, discussion and conclusions.

Before entering the discussion of the experimental results it is necessary to dwell upon the reliability of the determinations of the viscosity and the heat stability. To this end we cite some figures of viscosity determinations which were determined in triplicate.

Heat treatment 60°C 30 sec.

concentration	viscosity	concentration	viscosity
11.7 %	3.02 cP	21.3 %	17.88 cP
	3.18		17.80
	3.02		17.65
16.9 %	5.82	27.5 %	123.62
	5.72		119.72
	5.62		120.--
19.6 %	10.11	33.5 %	287.60
	10.18		271.60
	10.29		264.80

Roughly estimated up to 10 cP the variation is 0.2 cP, at 100 cP it is 2 cP, and at higher viscosities it becomes about 10 cP. At high viscosities the variation range may be even larger, but its meaning for the reliability of the viscosity curves is estimated to be small as the last part of the already very steep curve will not be influenced much by even comparatively large variations.

The heat stability determinations were carried out in duplicate, some of which are given below.

Heat treatment 70°C 45 sec.

concentration	heat stability	concentration	heat stability
10.8 %	61 min 6 sec	13.3 %	16 min 44 sec
	61 min 10 sec		16 min 40 sec
12.9 %	34 min 29 sec	16.1 %	8 min 0 sec
	34 min 9 sec		7 min 40 sec
13.9 %	18 min 6 sec	19.2 %	3 min 50 sec
	18 min 4 sec		3 min 45 sec

The results of our experiments are represented in table 4, 5, and 6, and fig 13-25.

The first four graphs, fig 13-16, deal with the heating experi-

TABLE 4 Influence of preheating on the viscosity and heat stability of evaporated soymilk.

SERIES I

Heat treatment	dry matter	viscosity	heat stability	pH	acidity (ml)
90°C 30 min	10.9 %	9.85 cP	60 m 15 sec	6.63	2.16
	11.4	10.21	46 m 45	6.67	2.49
	12.1	11.14	41 m 17	6.64	2.36
	13.1	21.03	33 m 52	6.62	-
	14.2	44.16	18 m 33	6.62	-
	14.4	43.60	18 m 39	6.67	-
80°C 30 min	14.4	5.38	23 m 17	6.56	
	15.5	6.28	8 m 18	6.56	
	16.3	7.35	7 m 53	6.56	3.34
	19.1	23.03	1 m	6.57	4.23
	19.5	26.09	2 m 48	6.54	4.13
	22.2	82.87	1 m	6.54	-
	gel 24.7	176.93	1 m	6.50	-
	gel 25.8	220.13	1 m	6.52	-
70°C 30 min	12.8	3.52	23 m 14	6.53	2.36
	14.0	4.48	8 m 58	6.53	2.45
	17.1	7.41	2 m 25	6.51	3.43
	19.2	11.56	1 m	6.49	3.47
	20.3	14.34	1 m 9	6.50	4.11
	20.8	22.28	1 m 7	6.50	3.91
	23.7	44.74	-	6.52	-
	25.3	80.81	-	6.50	-
	gel 25.4	102.57	-	6.52	-
60°C 30 min	11.7	3.07	36 m 42	6.41	3.06
	13.1	4.02	37 m 38	6.43	2.72
	13.4	3.71	22 m 34	6.43	2.97
	13.9	4.65	11 m 46	6.45	2.97
	15.9	6.01	5 m 38	6.45	3.51
	16.9	5.72	2 m 54	6.45	3.87
	18.2	8.92	2 m 42	6.44	3.95
	19.6	10.19	2 m	6.44	3.55
	21.3	17.78	-	6.45	4.60
	23.3	26.45	1 m 54	6.45	4.61
	27.4	131.37	0 m 25	6.43	-
	33.5	824.10	-	6.40	-

S E R I E S II

Heat treatment	dry matter	viscosity	heat stability	pH	acidity (ml)
90°C 0 min	12.8%	6.55 cP	31 m 30 sec	6.60	2.50
	12.9	6.37	33 m 46	6.60	2.55
	13.1	7.55	35 m 13	6.62	2.45
	13.2	7.67	32 m 19	6.58	2.83
	gel 16.5	64.68	4 m 38	6.62	-
	gel 17.2	99.87	2 m 56	6.58	-
	gel 18.5	286.98	-	6.60	-
80°C 0 min	13.7	4.21	48 m 53	6.59	2.50
	14.9	5.19	23 m 34	6.55	2.71
	16.5	6.77	7 m 29	6.53	2.93
	18.9	12.02	3 m 17	6.50	3.82
	22.6	36.32	1 m 30	6.50	4.43
	clumps 24.5	85.06	1 m 10	6.49	4.67
	clumps 26.1	110.24	1 m 4	6.50	-
	gel 29.1	234.93	1 m 10	6.46	-
70°C 0 min	20.0	15.80	1 m 27	6.42	3.80
	20.8	23.27	1 m 22	6.46	4.17
	23.1	29.17	1 m 4	6.44	4.69
	25.4	78.26	0 m 47	6.41	4.97
	26.2	64.99	1 m 4	6.42	-
	gel 28.5	174.00	0 m 32	6.40	-
	gel 30.0	260.70	0 m 41	6.41	-
	gel 32.9	563.85	-	6.32	-
	15.2		8 m 38		
	15.8		6 m 47		
	17.6		3 m 16		
60°C 0 min	20.2	9.97	2 m 24	6.50	3.82
	20.5	9.97	2 m 24	6.49	4.08
	21.3	11.31	1 m 58	6.50	4.51
	21.7	11.85	2 m 30	6.50	4.19
	22.7	14.74	2 m 6	6.50	4.44
	26.4	31.06	-	6.49	4.95
	30.4	66.65	0 m 30	6.42	-
	32.9	208.37	0 m 40	6.40	-
	clumps 33.6	157.45	0 m 30	6.43	-

SERIES III

Heat treatment	dry matter	viscosity	heat stability	pH	acidity (ml)
90°C 45 sec	12.4%	3.71 cP	87 m 56 sec	6.59	2.78
	15.0	4.74	17 m 33	6.53	3.51
	15.5	5.43	16 m 49	6.53	3.73
	17.4	7.95	9 m 36	6.49	4.17
	22.3	40.13	2 m 59	6.56	-
	22.3	41.64	2 m 57	6.51	-
	22.9	64.40	2 m 58	6.42	5.63
	gel 26.3	298.41	-	6.49	-
	gel 28.8	368.80	-	6.50	-
80°C 45 sec	12.7	3.32	144 m 30	6.56	-
	13.5	-	100 m 25	-	
	14.4	4.49	83 m	6.58	
	17.9		15 m 23	-	
	19.8	9.63	7 m 27	6.57	
	21.0	13.26	7 m 7	6.56	
	23.6	20.27	4 m	6.54	
	25.2	-	5 m 10	6.52	
	25.3	33.84	3 m 20	6.52	
	26.2	49.54	1 m 29	6.52	
	gel 30.2	253.93	-	6.55	
70°C 45 sec.	10.8	3.25	61 m 8	6.43	2.83
	11.4	3.31	54 m 59	6.44	3.20
	12.9	3.51	34 m 19	6.49	2.61
	13.3	3.62	16 m 42	6.44	3.70
	13.9	3.93	18 m 5	6.45	3.17
	14.2	4.03	23 m 14	6.48	3.13
	16.1	5.10	7 m 50	6.45	3.20
	19.2	8.34	3 m 48	6.47	4.24
	21.3	11.20	2 m 18	6.43	4.69
	26.8	30.16	0 m 55	6.44	7.70
	27.7	45.16	0 m 39 "	6.41	-
	28.1	46.54	-	6.40	-
	clumps 32.8	153.60	-	6.37	-
	gel 37.3	622.80	-	6.33	-
	gel 39.0	730.90	-	6.34	-
120°C 45 sec	18.3	17.22	16 m 10	6.71	
	18.9	22.45	14 m 59	6.72	
	19.6	26.18	14 m 59	6.69	
	20.1	37.86	12 m 16	6.73	
	22.7	162.49	6 m 53	6.69	

S E R I E S IV

Heat treatment	dry matter	viscosity	heat stability	pH	acidity (ml)
90°C 0 sec	13.7%	3.78 cP	-	6.61	2.62
	15.0	5.08	46 m 19 sec	6.64	2.72
	15.6	5.11	39 m 12	6.64	3.06
	15.8	5.61	31 m 51	6.62	2.64
	17.7	6.94	19 m 31	6.64	3.49
	21.5	22.94	4 m 24	6.65	4.30
	24.2	44.33	3 m 39	6.64	
	24.5	48.05	3 m 43	6.64	
	24.2	29.85	4 m 30	6.64	
	gel 31.0	411.60	-	6.61	-
	gel 33.2	545.74	-	6.59	-
80°C 0 sec	12.0	3.32	94 m 16	6.59	2.29
	13.8	4.61	63 m 11	6.65	2.76
	14.3	4.18	46 m 36	6.60	2.84
	16.7	6.43	16 m 8	6.59	3.37
	17.8	6.80	13 m 36	6.62	3.31
	18.8	8.37	5 m 58	6.63	3.82
	18.9	8.71	6 m 49	6.63	3.96
	19.9	13.99	3 m 25	6.63	3.95
	21.4	13.47	2 m 53	6.63	4.03
	24.7	26.64	1 m 6	6.56	2.53
	27.0	40.-	0 m 57	6.58	5.14
	27.3	51.64	-	6.58	5.84
	28.4	101.04	0 m 52	6.58	5.84
	gel 33.7	189.44	-	6.56	-
	gel 35.3	246.91	-	6.58	-
	gel 35.2	338.14	-	6.55	-
70°C 0 sec	11.5	3.09	62 m	6.61	2.28
	13.7	4.04	31 m 49	6.61	2.74
	14.5	4.25	22 m	6.62	2.81
	14.9	3.80	5 m 25	6.61	3.21
	14.3	4.31	21 m 4	6.61	2.79
	17.4	6.08	1 m 38	6.61	3.71
	20.3	8.14	1 m 1	6.59	4.32
	22.0	9.54	-	6.58	3.37
	23.0	10.90	0 m 51	6.58	5.06
	32.3	39.17	0 m 22	6.56	6.21
	36.1	67.29	-	6.54	-
	gel 36.7	115.69	-	6.52	-
	gel 37.2	186.54	-	6.52	-
	gel 41.2	320.21	-	6.51	-
	gel 44.2	458.70	-	6.49	-

Heat treatment	dry matter	viscosity	heat stability	pH	Acidity (ml)
100°C 0 sec	15.4%	5.90 cP	26 m 44	6.52	
	16.3	9.63	20 m 6	6.53	
	17.5	9.73	17 m 15.5	6.54	
	17.6	9.80	16 m 6.5	6.57	
	18.0	11.63	12 m 43.5	6.54	
	19.4	17.55	7 m 47.5	6.52	
	20.4	49.50	5 m 30.5	6.50	
	20.5	64.17	6 m 6	6.54	
	gel 20.7	50.62	7 m 33	6.53	
	gel 21.5	162.30	3 m 56	6.50	
	gel 21.8	131.64	-	6.51	
	gel 23.5	236.25	-	6.51	

Heat treatment	Dry matter	Viscosity	Heat treatment	Dry matter	Viscosity
Not heated	11.4	3.22	70° 0 sec (duplicate)	14.1	4.30
	13.1	3.57		21.9	9.75
	19.2	7.03		28.9	30.83
	19.3	7.28		31.4	46.53
	21.0	9.20		36.2	153.13
	21.0	15.50		40.2	362.04
	21.9	10.40			
	25.8	15.50	70° 45 sec (duplicate)	14.3	4.08
	28.9	31.00		18.9	7.15
	30.5	35.98		23.1	16.07
	30.7	53.72		25.7	25.62
	33.0	57.65		27.9	40.78
	36.6	205.15		30.1	69.75
	36.9	187.59		33.5	158.65
					Heat stability
Not heated	20.4	7.57	Not heated	13.7	29 m 8
	22.3	10.53		13.7	39 m 45
	26.9	25.73		15.6	8 m 41
	28.0	34.66		16.5	1 m 56
	29.5	56.80		17.5	2 m 54
	31.8	96.66		17.8	2 m 13
	32.3	92.13		19.2	1 m 40
				20.7	1 m 36
				21.3	1 m 7

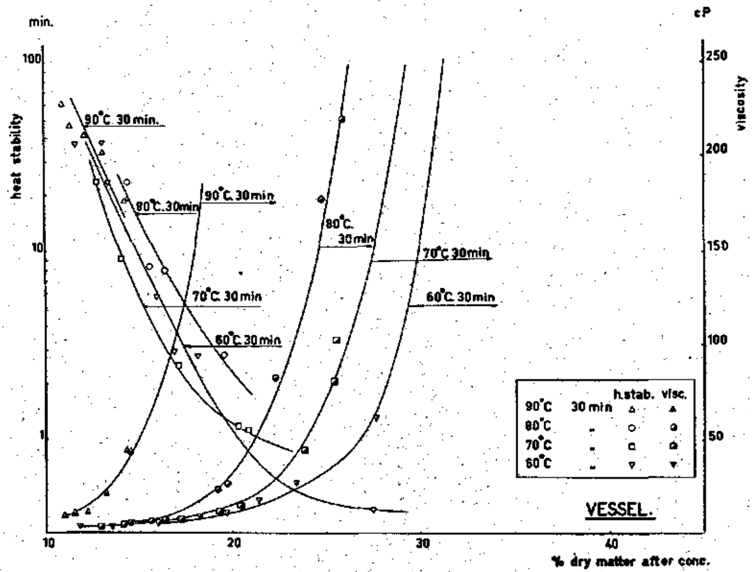


Fig 13. Series. 1.
Preheating with vessel, holding time 30 minutes.
Effect of temperature level on the viscosity and heat stability of evaporated soymilk.

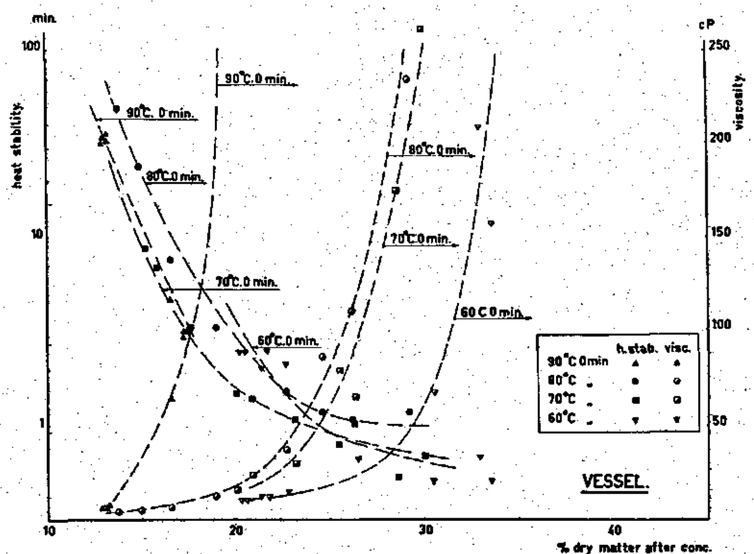


Fig 14. Series 2.
Preheating with vessel, holding time 0 minutes.
Effect of temperature level on the viscosity and heat stability of evaporated soymilk.

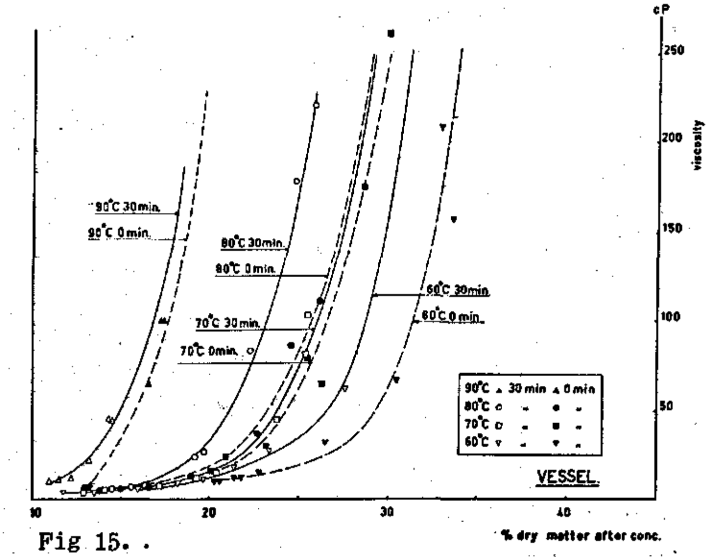


Fig 15.
Preheating with vessel.
Effect of holding time on the viscosity of evaporated soymilk.

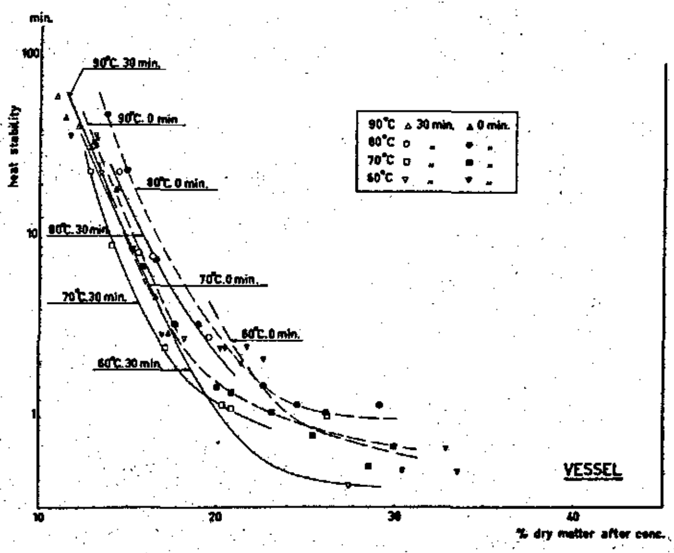


Fig 16.
Preheating with vessel.
Effect of holding time on the heat stability of evaporated soymilk.

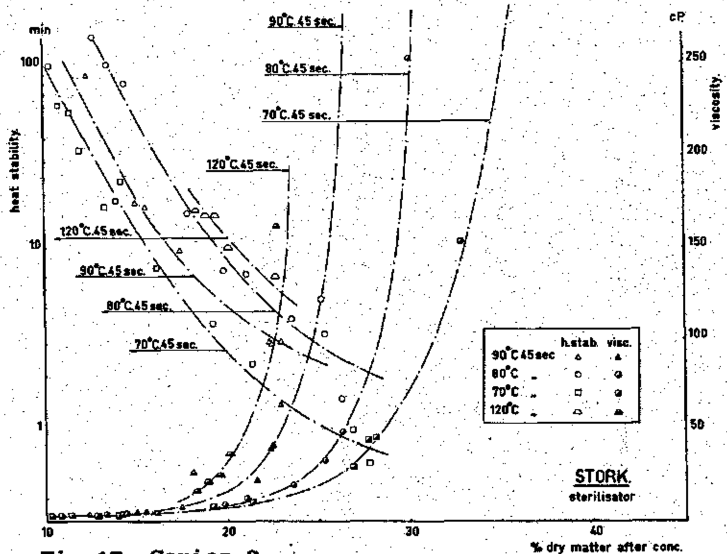


Fig 17. Series 3.
Preheating with flash sterilizator, holding time 45 seconds.
Effect of temperature level on the viscosity and heat stability of evaporated soymilk.

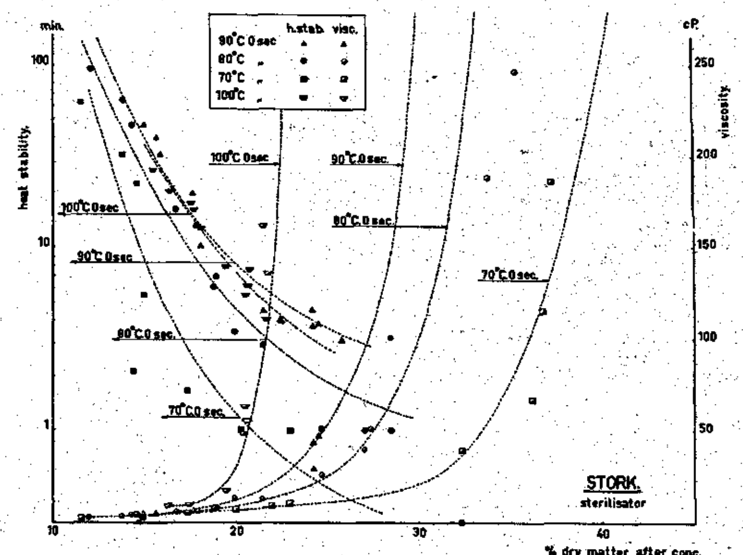


Fig 18. Series 4.
Preheating with flash sterilizator, holding time 0 seconds.
Effect of temperature level on the viscosity and heat stability of evaporated soymilk.

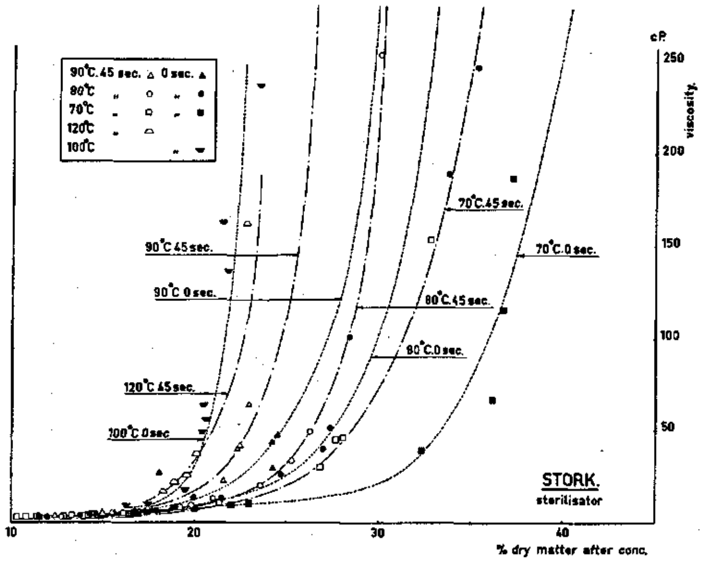


Fig 19.
Preheating with flash sterilizator.
Effect of holding time on the viscosity of evaporated soymilk.

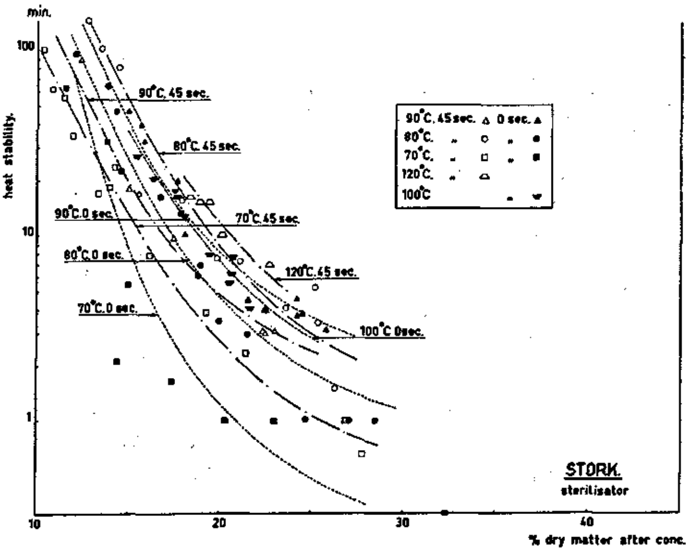


Fig 20.
Preheating with flash sterilizator.
Effect of holding time on the heat stability of evaporated soymilk.

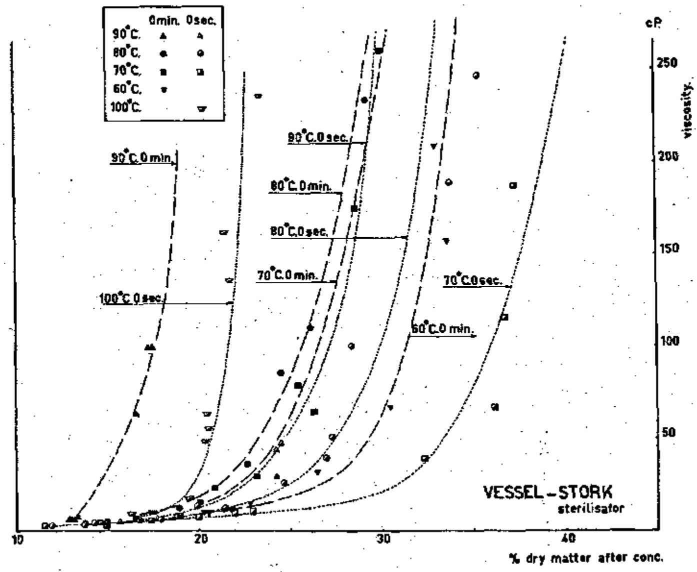


Fig 21.
Comparison of preheating with vessel and flash sterilizator.
Effect of temperature level on the viscosity of evaporated soymilk.

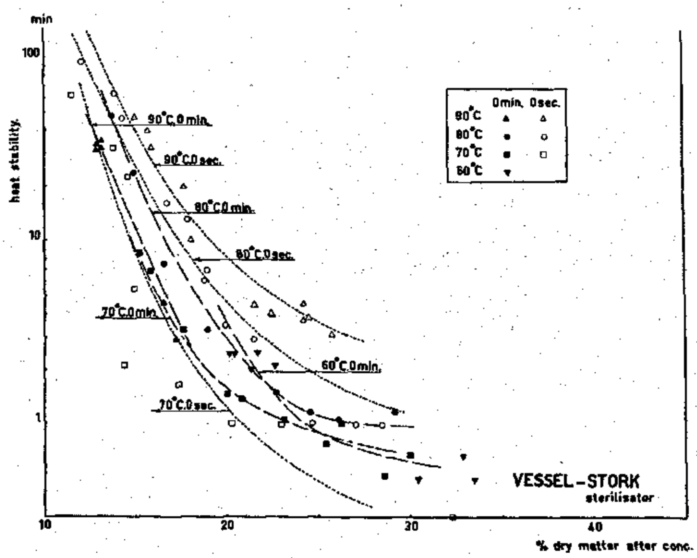


Fig 22.
Comparison of preheating with vessel and flash sterilizator.
Effect of temperature level on the heat stability of evaporated soymilk.

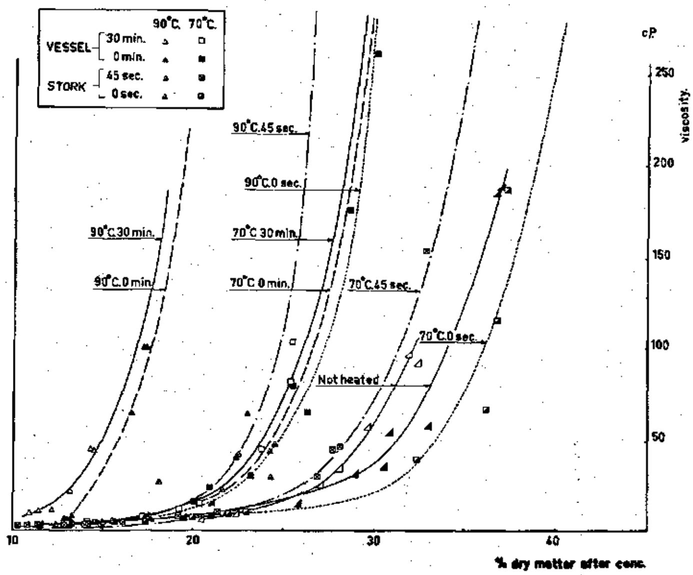


Fig 23.
Effect of the method of heating on the viscosity of evaporated soymilk.

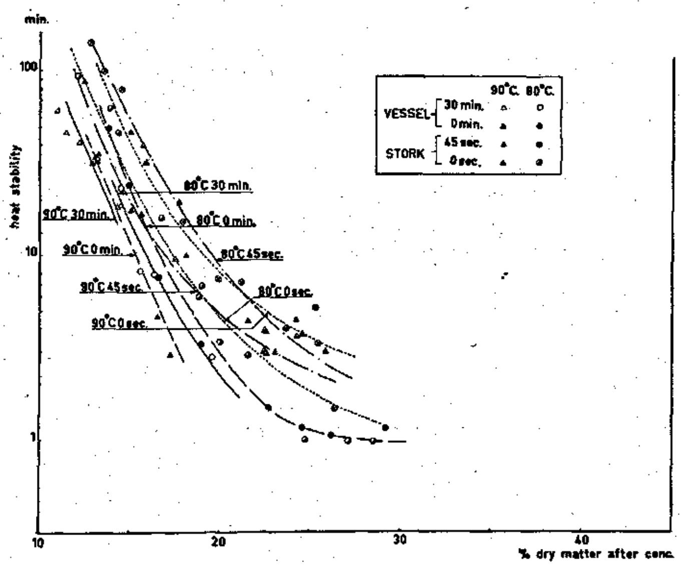


Fig 24.
Effect of the method of heating on the heat stability of evaporated soymilk.

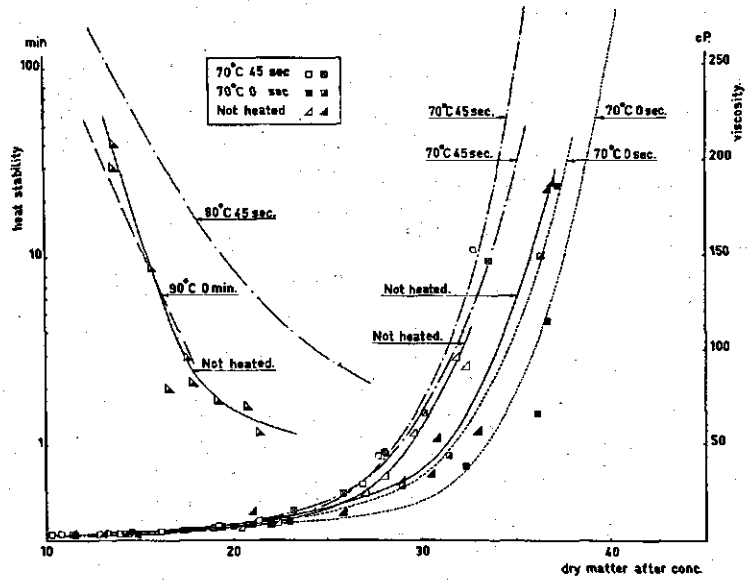


Fig 25. Duplication of experiments.

TABLE 5 Dry matter contents of evaporated soymilk corresponding with a viscosity of 100 cP.
Influence of preheating.

	Series 1 Batch	Series 2 Batch	Series 3 Continuous	Series 4 Continuous
Holding time / Heating-temperature	30 min	0 min	45 sec	0 sec
120°C	-	-	22.7 %	-
100°C	-	-	-	21.7 %
90°C	17 %	17.8 %	24.8 %	27.3 %
80°C	23.2 %	25.8 %	28.4 %	30 %
70°C	26.2 %	26.7 %	31.4 %	36 %
60°C	28.8 %	31.5 %	-	-
unheated	32.0 %	-	33.8 %	-

ments carried out with the vessel. The first graph, fig 13 shows the viscosity and heat stability curves when the milk is preheated with 30 minutes holding time, and the second one, fig 14, with 0 minutes holding time. The third graph, fig 15, compares the viscosity curves of 30 minutes holding time with those of 0 minutes. The same is done in fig 16 with the heat stability curves.

The next four graphs deal with the work done on preheating with the Stork sterilizator. The fifth graph, fig 17, shows the curves for viscosity and heat stability with a holding time of 45 seconds, and the sixth, fig 18, for a holding time of 0 seconds. The seventh graph, fig 19, compares the viscosity curves of 45 seconds holding time with those of 0 seconds, while the same comparison is made for the heat stability curves in fig 20.

A comparison of the viscosity curves of preheating zero minutes with the vessel and 0 seconds with the Stork sterilizator is made in fig 21, while in fig 22 the same comparison is made for the heat stability curves. The influence of the method of preheating on the viscosity curves of evaporated milk is shown in fig 23. For the heat stability curves this is done in fig 24.

In discussing the results we will pay attention to the dry matter content-reached after a certain heat treatment- corresponding with a viscosity of 100 cP. This viscosity is chosen as we may safely assume that then no gelling occurred yet. The heat stability will be discussed by considering the dry matter content reached by drawing a horizontal line at the 10 minute-leval.This value is chosen as it should be considered a minimum of sterilization.

When we take fig 15 (Preheating with vessel, effect of holding time and temperature level on the viscosity) and fig 19 (Preheating with flash sterilizator, effect of holding time and temperature level on the viscosity), we can draw an imaginary line at a viscosity level of 100 cP. At the points where this line crosses the viscosity curves the dry matter contents can be read by comparison with the horizontal axis. The values obtained in this way are shown in table 5.

If we compare the dry matter contents of series 1 reached after heating at different temperatures with the same holding time we can readily see that as the heating temperature increases the dry matter content decreases. This does not only apply to the points of the curves at the 100 cP level but also to most other points of the curves perhaps with the exception of those at the nearly horizontal part of the curves.

Considering the figures of series 2 we may say that the same holds for this series. As the temperature increases the dry matter contents diminishes. This means that if we preheat the milk with a vessel the viscosity barrier arises later if lower temperatures are used.

Comparison of the results of series 1 and series 2 shows that for each of the heating temperatures used the dry matter contents of series 2 are higher. This means that shortening the holding time causes the viscosity barrier to arise later. It may be seen that the influence of this factor is not so pronounced as that of the temperature level.

The results from series 3 show the same regularity as in the former two series. This is also the case with series 4. If we now compare the results of series 3 with the corresponding ones of series 4 we see that a higher dry matter content is reached if the holding time is shortened. Though the holding time has a definite influence, it is not very important. What counts in the first place is the influence of the temperature level.

We can measure the difference in effect of heating with the vessel and the continuous sterilizator by comparing series 2 and 4, which theoretically have the same holding time. Then we see (fig 21) that with the continuous sterilizator appreciably higher dry matter contents can be reached. This is especially the case with preheating at 70°C and also at 90°C. Evidently this may be ascribed to the much more rapid heating of the continuous sterilizator and presumably also to the rapid cooling.

Thus we can confirm that there actually exists such a thing as a viscosity barrier. When using the term "barrier" we especially have in mind the steep part of the viscosity curve, which in the proper sense of the word represents a barrier that cannot be overcome by evaporating.

The very regular sequence of the curves in fig 15 and fig 19 are prove of the systematic influence of temperature level and holding time of preheating on the viscosity of the evaporated product.

If we judge the results by quantitative standards we see that at about 20% or after 20%, as mentioned in the literature, there is a viscosity barrier. However we have found that also below 20% dry matter this barrier can arise if we choose the right preheating conditions (vessel, 90°C). We have also shown that the viscosity curves and thus the viscosity barrier can be moved to higher dry matter contents by lowering the heating temperature and reducing the holding time. In this respect the continuous flash sterilizator is more effective than the vessel. With the unheated milk we even reached a dry matter content of 32-34%. It is difficult to explain that flash heating at 70°C with no holding time gives a milk of even a higher dry matter content than the unheated, evaporated milk at 100 cP.

When we take fig 16 (Preheating with vessel, effect of holding time and temperature level on the heat stability) and fig 20 (Preheating with flash sterilisator, effect of holding time and temperature level on the heat stability) we can draw an imaginary line at the stability level of 10 minutes. At the points where this line crosses the stability curves the dry matter contents can be read by comparison with the X-axis. The values obtained in this way are shown in table 6.

TABLE 6 Dry matter contents of evaporated soymilk corresponding with a heat stability of 10 min.
Influence of preheating.

	Series 1 Batch	Series 2 Batch	Series 3 Continuous	Series 4 Continuous
Heating-temperature \ Holding time	30 min	0 min	45 sec	0 sec
120°C	-	-	20.6 %	-
100°C	-	-	-	18.8 %
90°C	15.0 %	15.3 %	17.0 %	19.1 %
80°C	15.9 %	16.7 %	19.6 %	17.4 %
70°C	14.0 %	14.8 %	15.8 %	14.8 %
60°C	14.9 %	-	-	-
unheated	15.5 %	-	-	-

On examining the figures of series 1 we see that heating at 80°C gives the highest dry matter content and thus we may say that this heat treatment gives the highest stability. For the three highest temperatures the sequence of increasing stability imparted to the evaporated milk is 70°, 90°, and 80°C.

Also in the second series heating at 80°C imparts the highest stability to the evaporated milk. It is interesting to note that also with this series the sequence in which the heat treatments increase the stability is the same as in the former series for the three highest temperatures.

Comparison of the figures of series 1 and 2 shows that for each of the heating temperatures the dry matter contents of series 2 are higher. This means that decreasing the holding time increases the stability.

Though the results of series 3 show the same order as in the former two series it should be noted that the determination of the heat stability of the heating experiment 90°C 45 sec was carried out about one day later as usual. Normally we may expect the order to be 70°, 80° and 90°C as in the following series. This experiment could not be repeated due to the fact that the original stock of soybeans was exhausted. It will be seen that heating at 120°C imparts an even higher stability.

In series 4 the order of heat treatments increasing the stability is 70°, 80°, and 90°C, if only the three lowest temperatures are considered. Clearly, heating at 90°C, has given the best results.

If we now compare the results of series 3 and 4 we see that in general the values of series 3 are higher. This means that -contrary to batch heating- lengthening of the holding time increases the stability, except for the heating experiment at 90°C, for 45 sec. As has been explained we may expect that this value would be higher if the stability determinations had been carried out in time.

A comparison of the stabilizing effect of batch (series 2) and continuous heating (series 4) will show (fig 22) that continuous heating imparts a higher stability to the evaporated product. The difference in stability seems to become greater with an increase in temperature.

Thus it appears that preheating has a systematic influence on the heat stability of the evaporated product. In comparison with the unheated milk the evaporated milks preheated with the vessel do not much improve their stability. The regular influence of preheating, however, is undeniable. The continuous sterilizator imparts a much more pronounced stabilizing effect to the milks.

The overall effect of preheating on the viscosity is depicted in fig 23. In order not to overcrowd the picture only the curves of 70°C and 90°C are drawn. As may be seen from this figure as well as from table 5 the curves shift to the right in the order series 1 - series 2 - series 3 - series 4. The same order applies to the 80°C-curves.

The overall effect of preheating on the heat stability is shown in fig 24, where only the curves of 90^{o} and 80^{o}C are drawn. The order in which the heat treatments increase the stability is series 1 - series 2 - series 4 - series 3. As has been pointed out before the order of the last two curves of 90^{o}C is reversed.

The experiments which have been duplicated, 70^{o}C 45 sec, 70^{o}C 0 sec and the unheated milk, are shown in fig 25. In accordance with the foregoing discussion we will judge their variation range at the 100 cP level. For the 70^{o}C 45 sec duplicates the difference is less than 1%, while for the 70^{o}C 0 sec curve this variation is about 1.5% of dry matter. The unheated milk gives a larger variation range, about 2%. From these duplicates one gets the impression that the more the milk is heated the less is the variation. Judging from these results the viscosity curves may be regarded as reproducible.

The foregoing discussion leads us to the following conclusions:

1. Raw soymilk can be concentrated to at least 30% without difficulties. However, this product can not be sterilized.

2. Heating the raw soymilk prior to evaporation gives rise to a viscosity barrier, which makes it impossible to concentrate the milk further as the evaporated milk cannot flow in the evaporator any more. The dry matter content at which this viscosity barrier arises depends upon the method of heating.

3. When *a steamjacketed vessel* is used to forewarm the raw milk, lowering the heating temperature from 90^{o}C to 60^{o}C as well as reducing the holding time from 30 to 0 minutes moves the viscosity barrier to higher dry matter contents.

4. When *the continuous, flash sterilizator is* used to forewarm the milk lowering the heating temperature from 120^{o} to 70^{o}C as well as reducing the holding time from 45 to 0 seconds moves the viscosity barrier to higher dry matter contents.

5. *In comparison* with the viscosity curves of the vessel, the curves of the continuous sterilizator for the corresponding heat treatments at 70^{o}, 80^{o}, and 90^{o}C are located at higher dry matter contents than those of the vessel.

6. Using a vessel, for a holding time of 30 minutes as well as 0 minutes, the heat stabilities of the evaporated milk are increased in the order 70^{o}, 90^{o}, and 80^{o}C. Reducing the holding time

from 30 to 0 minutes increases the stability.

7. Using a continuous sterilizator, for a holding time of 45 seconds as well as 0 seconds, the heat stabilities of the evaporated milks *tend* to be higher in the following order of heat treatments: 70^o, 80^o, 90^oC. Increasing the holding time from 0 to 45 seconds *tends* to increase the heat stability.

8. In comparison with the heat stability curves of the vessel those of the continuous sterilizator for the corresponding heat treatments at 70^o, 80^o, and 90^oC are situated at higher stabilities than those of the vessel.

9. In the range 70^o, 80^o, and 90^oC the overall effect of preheating is the shifting of the viscosity curves to the right, i.e. to higher dry matter contents, in the sequende.

   vessel 30 min-vessel 0 min-flash 45 sec-flash 0 sec.

After these conclusions we should consider again our starting point, where we assumed that -notwithstanding differences in structure between soy and animal milk- principles of preheating valid for animal milk might prove useful in application to soymilk.

As presumed, heating raw soymilk prior to evaporation does effect the viscosity of its evaporated product. With cowmilk, preheating may increase or decrease the viscosity of the concentrated product, whereas with soymilk the only effect of preheating is an increase in viscosity. Thus the influence is an unfavourable one.

Also the heat stability appears to be influenced by preheating. It may be said that in general preheating has a favourable effect on the stability.

The opposite effects of preheating on these two properties of the concentrated soymilk constitute a difficult problem that will be discussed in the last chapter of this work.

Addition of salts to the evaporated soymilk.

From the literature on animal milk it is known that the heat stability can be influenced by salts. With the purpose of increasing the heat stability of evaporated soymilk some experiments concerning this point were carried out to get an insight in the eventual role of salts in the sterilization process.

The experiments were carried out as follows: 0.75, 0.50, and 0.25 ml of 0.5n solutions of sodium citrate and potassiumphosphate were pipetted in separate flasks, adding 0.25, 0.50, and 0.75 ml water respectively. The same was done with 0.05n solutions. To each of these flasks, containing one ml of salt solutions, 50 ml of cold evaporated soymilk was added and thoroughly mixed. On these milks heat stability determinations were made.

In the following table some data on the salt influence are shown. In three cases the salts had no influence, while in the other three a definite effect could be observed. This ratio is not representative for the total number of samples investigated as these data were chosen on purpose so as to illustrate the differences in salt influence found. It will be easily understood that from these limited data no general conclusions can be drawn. They only indicate that research along these lines might be useful.

TABLE 7 INFLUENCE OF THE ADDITION OF SALTS ON THE HEAT STABILITY OF EVAPORATED SOYMILK

Heat treatment	120°C 45 sec	60°C 30 min	60°C 0 min	90°C 30 min	90°C 0 min	
Dry matter	18.9%	18.1%	20.8%	11.4%	12.8%	15.5%
Blancs	18 m 24 19 m 18	1 m 42 1 m 39	2 m 25 2 m 24	20 m 14 19 m 55	28 m 55 29 m 5	6 m 57 7 m 5
Na-citrate						
0.75 ml 0.5 n	20 m 50 48	1 m 25 24	2 m 34 34	48 m 0 50		
0.50 ml	22 m 50 21	1 m 46 37	2 m 36 37	38 m 0 43 m 30		
0.25 ml	21 m 34 19 m 40	1 m 33 36	2 m 24 25	32 m 30 33 m 0		
0.75 ml 0.05 n	20 m 28 18 m 50	1 m 26 10	2 m 26 28	22 m 30 19 m 53		
0.50 ml	20 m 38 19 m 58	1 m 39 33	2 m 26 28	17 m 30 15 m 30		
0.25 ml	18 m 37 19 m 58	1 m 39 33	2 m 26 26	21 m 18 50		
K-phosphate						
0.75 ml 0.5 n	24 m 0 22 m 4	1 m 41 36	2 m 14 22		60 m	16 m 0 15 m 49
0.50 ml	21 m 58 24 m 0	1 m 23 36	2 m 33 30		60 m	12 m 54 44
0.25 ml	23 m 2 22 m 5	1 m 48 44	2 m 24 26		60 m	11 m 41 55
0.75 ml 0.05 n	23 m 2	1 m 48	-			13 m 16 21
0.50 ml	20 m 6 22 m 10	1 m 48 47	-			10 m 2 10
0.25 ml	21 m 58 24 m 43	1 m 42 40	-			10 m 3 17
	no effect	no effect	no effect	effect	effect	effect

5.4 *Summary.*

The existing contradictory data on the evaporation of soymilk prompted the work done on this subject. Some authors state that soymilk can be evaporated without difficulty, while other sources conclusively prove the existence of a viscosity barrier at about 20% dry matter content. Therefore the behaviour of the viscosity and also the heat stability were studied.

As it is known that the viscosity and heat stability of evaporated animal milk are sensitive to heat, an attempt was made to influence these two properties of evaporated soymilk by application of heat prior to evaporation. Raw soymilk was subjected to four series of heat treatments. The heating experiments were carried out with a steamjacketed vessel, and a specially designed continuous, flash sterilizator. The experiments were designed in such a way that a possible effect of the coming-up time, temperature level, and holding time would manifest itself.

In our case the heating proved to be the factor that determines the position of the viscosity curves and thus also that of the viscosity barrier. In general it may be said that the less the heating the higher the dry matter content that can be reached by evaporation. This holds for the temperature level as well as for the coming-up time and the holding time. It is interesting to note that if the raw soymilk is evaporated without having been preheated even milks of more than 40% dry matter do not show any sign of gelling.

It seems that we now can bridge the two standpoints concerning the evaporation of soymilk, when taking into account the influence of heating on the viscosity. The raw soymilk can be concentrated to at least 30% dry matter content. If, however, the milk is heated prior to evaporation this heating will give rise to the existence of a viscosity barrier, the position of which depends upon the method of heating.

Also the heat stability of evaporated soymilk proves to be influenced by preheating as expected. This influence depends upon the coming-up time, temperature level, and holding time of the heat treatment.

6 SOYCURD

6.1 *Introduction.*

From soymilk a product called "toufu" is obtained. It is also called tofu (Japan), taohu (Java), and dauphu (Indochine). This product is obtained by precipitating the proteins of soymilk and pressing the precipitate. Due to the analogous method of manufacture of cheese from animal milk it is often designated as soycurd or beancurd. In the following we shall use the term soycurd or shorter "curd". As has been pointed out in the introduction of the chapter on the manufacture of soymilk the processing of soybeans to soycurd was already mentioned at the beginning of the Christian era.

Soycurd may be described as a product obtained from soymilk by precipitating the milkproteins followed by pressing the precipitate. Depending upon the composition of the milk and the method of manufacture the dry matter content of the curd is about 15 to 25%. Some analyses of the composition of the curd are given below.

	Java Prinsen Geerligs 1896	Indochina Bloch 1906	Indochina Monnier 1935	Japan Inouye 1894 frozen	Philippines Gibbs 1912 baked	osmosis
Water	76.15%	83.85%	81.40%	15.32%	73.0%	55.76%
Proteins	13.15	11.25	29.28	41.42	13.88	14.56
Fat	7.09	4.33	5.66	23.65	10.78	7.12
N-free extract	1.40	-	0.64	15.05	2.22	2.13
Ash	2.21	0.57	0.67	3.08	1.2	-

The figures from Inouye refer to frozen tofu, those of Gibbs to baked tofu and tofu kept in a salt solution.

From the soycurd a number of products is derived, which in comparison with the original curd contain less water. This decrease in water content is obtained in various ways: greater pressure, osmosis, drying, freezing, and baking. Also products are known in which micro-organisms play a role in the ripening process.

When soycurd is to be produced on a factory scale a number of technological prerequisites must be fulfilled. First the starting material, soymilk, must have a reasonably constant composition. In the experimental chapter on soymilk it has been shown that this will cause no difficulties. Second, the precipitating agent should give uniform yields when the precipitation conditions are kept constant. This means that some kind of standardization of the precipitating agent must be established. Third, the yield of precipitated proteins must be as high as possible.

As the first point was already studied, now attention was directed towards a standardization of the precipitating agent and to a study of the yield of precipitation.

6.2 *Literature.*

6.2.1 *Methods of manufacture.*

The literature on tofu is much more limited than that on soymilk. In the following some methods of manufacture are described.

In Java according to P R I N S E N - G E E R L I G S (1896) after the soaked beans are ground, the mass is boiled and filtered through a large cloth. The coagulation is effected by means of sour soymilk, and two hours later the half solid mass is pressed between two boards. Then it is cut into small cakes.

In Japan the proteins are precipitated with 2% of a mother solution, obtained from the salt manufacture out of seawater. The whey is filtered through a cloth and the precipitate is pressed slowly and then cut into pieces (I N O U Y E, 1894-97).

B L O C H (1906) described that in Indochina the milk is boiled and after 10-30 minutes it is transferred into another container to which the coagulating agent is added. The curd is placed between two sieves covered with cloth, and dependent upon the desired product more or less pressure is exerted. Then the cake is cut into pieces.

P I P E R and M O R S E (1923) using a 1% salt-solution of $MgCl_2$-added one part of salt solution to four parts of milk. Whole soyflour and defatted presscakes gave as good results as the soaked beans. From several varieties of soybeans the yield of soycurd was determined; the dry matter and crude protein content of this curd, however, were not mentioned.

I N O U Y E (1894-97) states that from the salt solution used the calcium- and magnesiumsalts are active but the sodiumchloride is not; on addition of calciumnitrate or magnesiumsulfate a precipitate is created,which is not the case with even large amounts of sodiumchloride or sodiumsulfate. An excess of magnesiumsulfate dissolves the precipitate again. Analysis of the salt solution showed the presence of 27.9% magnesiumchloride and 7.0% sodiumchloride. According to his calculations only about one fourth of the original amount of proteins in the beans was obtained in the soycurd.

The manufacturers, according to B L O C H (1906), used two solutions: an original solution and a solution for immediate use. This last one is a dilution of the original solution with four or five times the amount of water. The original solution is the residue of the salt manufacture from seawater. According to this worker 100 ml contain:

$MgCl_2$	29.21 %
$MgSO_4$	1.12
Na_2SO_4	6.24

None of both solutions contained animal or vegetable rennet. If the original solution is used the floccules are too large and too thick resulting in a curd different of appearance and consistency from the normal.

Furthermore BLOCH (1907) states that the chlorides and nitrates of calcium, barium, strontium, magnesium as well as magnesium sulfate have coagulating properties. This property is strongest with magnesiumsulfate: "Les Chinois, en employant le chlorure de magnésium à la coagulation du lait de soja bouillant, sont empiriquement tombé sur le coagulant d'élection de ce produit"

If the precipitation is carried out at a low temperature before cooking only with magnesiumchloride a precipitate is originated quickly and that only if large amounts are used. If the precipitation is carried out at low temperatures after cooking twice the amount of magnesiumchloride is necessary.

In Tonkin unpurified salt is used for precipitation (MONNIER, 1935). The solution contains 20 g of salt per liter. This worker too states that magnesium is one of the strongest precipitating salts. It was found that precipitation in the vicinity of the boiling point proceeds faster and can be carried out with smaller amounts of salts.

If the preparation of dâuphu is repeated every day, the salt-solution can be replaced by the "petit-lait aigre" or "eau-mère" from the preparation of the former day. This liquid has a pH 2.9 and has the following composition (g per liter)

dry matter	12.77 g	chlorides	0.24 g
nitrogen (total)	1.92	ash	2.62
oil	0.51	acidity	5.36
		glucides	traces

The liquid is strongly acid and thus it precipitates the proteins of the vegetable milk. The following acids were identified:

lactic acid	present
formic acid	traces
acetic acid	traces

MATAGRIN writes that according to Beltzer (1911) acetic acid gives a powdery curd only after long boiling; diluted sulfuric acid gives a clear precipitate after short boiling, which has a yellow-like colour; the action of hydrochloric acid lies between that of both foregoing acids.

6.2.2 Ripening of soy cheese.

The literature on this interesting subject is very limited.

An effort to initiate ripening is reported by INOUYE (1894-97), who mixed Swiss cheese with 10% sodiumchloride. The results, however, were poor.

KATAYAMA (1906) obtained symptoms of bacteriological ripening by mixing 450 g tofu, 60 g caseine, 60 g sodiumchloride and 20 g Swiss cheese. After storage at 15°C for five months the cheese appeared to have a compact consistency and formation of a crust had taken place. The taste is described as being agreeable. Notwithstanding the addition of 2 g milksugar no holes -due to gas formation- appeared. Increasing the amounts of milksugar did not give holes. The coarse surface, however, became smooth, while the taste of soybean had completely disappeared.

In Russia BELENKII and POPOVA (1933) fermented warm soymilk with milk Streptococci and cheese bacilli habituated to soymilk. After fermentation the product was allowed to stand at 33°C and the curd obtained was pressed.

According to the Institut International d'Agriculture (1936) the factory Caséo-sojaine, situated in the vicinity of Paris, succeeded in producing several sorts -soft, hard, ripened, not-ripened- of soycheese. In Germany experiments on giving soycheese the same taste as certain European cheeses were successful. This was accomplished by means of selected cultures of micro-organisms used for the preparation of European cheeses.

SMITH (1948) describes some sorts of cheeses in China as follows.

Chee-fan is a brownish soft cheese. It has an agreeable smell and taste. The curd cubes are inoculated with mold, salted, and placed in an appropriate storage house for about seven days for development of mold. The mold (Mucor) is grown on wheat flour. It exists in China in mold of wine and is white in colour. Also Aspergillus glaucus, blue in colour, apparently takes part in the cheese development. Next, the cubes are placed in an earthen crock or wide-mouthed bottle, and yellow wine and mold of wine are added. It is allowed to age in the wine for about one year.

Tsue-fan ("drunken cheese") is another type of cheese. The curd is boiled in water, cooled, and partly dried. It is molded and placed in yellow wine (rice wine) with wine mold added and aged for six months.

Hon-fan is a red cheese made in the same manner as tsue-fan except that soy sauce rather than wine is used in aging the cheese.

Significant variations in the processes, besides the micro-organisms, are the proportion of salt and the type of solution in which the cheese is aged. The cheese appears to vary somewhat with the locality in which it is produced, a variation due probably to the influence of climatic conditions on the activity of the fermenting organisms.

6.3 Experimental part.

6.3.1 Scope of work.

As may be seen from the discussion of the literature the details of making tofu differ locally. In general, however, it is possible to distinguish four different stages in the preparation of tofu and derivatives.

In the first stage, the protein is precipitated from the milk by the use of a precipitating agent. It is desirable that this agent has a constant composition. Besides the output of the precipitation should be as high as possible.

In the second stage, the curd is separated from the whey. In practice this is done by filtering through a cloth or a sieve, after which the whey is discarded. The curd has still a very loose structure.

In the third stage, the desired amount of water is removed from the curd by pressing or otherwise.

In the last stage the curd is inoculated with micro-organisms, thus initiating the ripening process.

Experimental work on the first stage, precipitation of proteins, is known from literature. This work is mostly qualitative in nature and only little attention was paid to the quantitative aspect of the precipitation process. Therefore a study was undertaken of the yield of protein precipitation, using various agents for coagulation. No researchwork was done on the ripening process; only the preparation of the fresh curd was investigated.

6.3.2 Design of experiments.

In actual practice only a limited number of agents are used to precipitate the proteins from soymilk. These agents are the mother liquid from seawater, sour milk, and spontaneous souring. It has been shown that the principal agents in the mother liquid causing precipitation are Mg, Ca, Cl, and SO_4 -ions.

Therefore we used three salts $MgCl_2$, $MgSO_4$, $CaCl_2$, and lactic acid as precipitating agents. Different concentrations and temperatures were used. The influence of the dry matter content of the milk was also studied.

The precipitation was carried out according to one uniform method, while also the separation of the precipitate was always done in the same way by means of a centrifuge.

Determinations of dry matter, crude protein, pH, and acidity were carried out on the original milk and on the whey. The amount of dry matter and crude protein in the curd is obtained by subtraction. All experiments as well as the determinations were done in duplicate. Thus the figures of table 5 are the averages of four determinations.

In total twelve precipitation series were designed.

In the first series $MgCl_2$ was used as a precipitating agent. From a 1 N solution 1,2,4, and 8 ml were added to 100 g of soymilk of 80°C. Roughly the salt concentrations were 0.01, 0.02, 0.04, and 0.08 N. In the second series the temperature varied from 40° to 90°C using two ml of the same salt solution.

The same kind of precipitation was carried out with $MgSO_4$ (series 3 and 4), and $CaCl_2$ (series 5 and 6).

To approach the effect of the mother liquid equal amounts of 1 N solutions of the former salts were mixed and varying amounts of the resulting liquid used for precipitation at 80°C (series 7).
In trying to raise the yield the same amounts and temperature were used, but the pH was lowered to 4.5 (series 8).

In series 9 the influence of the pH at 20°C was investigated, while in the next series the temperature was varied at a constant pH 4.5.

In the last two series another dry matter content of the milk, about 10 and 4%, was used for precipitation with $MgSO_4$ at 80°C.

6.3.3 Technique of experiments.

The precipitating agent was added to 100 g of soymilk, placed in a 325 ml centrifuge tube. The milk and the solution were well mixed with a glass stirrer. If high temperatures were used the tube was placed in a waterbath; when adjusting the pH of the milk the electrodes of the pH meter were placed in the milk while the solution was being added. Immediately after addition of the precipitating agent the content of the centrifuge tube was cooled to 20°C by means of ice water. Then the tube was put into a Martin Christ centrifuge and centrifuged for 5 minutes at about 3000 r.p.m. (diameter of centrifuge 40 cm).

The weight of the whey and curd was determined and the dry matter, crude protein content, pH, and acidity of the whey were determined as described for the manufacture of the milk. The indicator used for the titration of the whey was phenolphtaleine.

The dry matter and crude protein content of the original milk were also determined. By subtracting the dry matter and crude protein in the whey from those in the original milk the percentage of the dry matter and crude protein in the curd could be calculated.

6.3.4 *Results, discussion, and conclusions.*

Before starting the discussion some figures are given which may serve to illustrate the degree of accuracy of the determinations and also of the reproducibility of the experiments. Below are given the complete figures of series 2.

	grams	% dry matter	crude protein	pH	acidity	whey: % of orig. dry matter in whey	whey: % of orig. crude prot. in whey	curd: % of orig. dry matter in curd	curd: % of orig. crude prot. in curd
Whey 2.1.1	71.50	5.04	1.77	6.05	1.39	44.23	37.35	55.77	62.65
40 °C	[illegible]	5.02	[illegible]	6.02	1.35				
.1.2	71.55	4.19	1.57	6.03	1.37	38.19	31.37	61.81	68.63
		4.17	1.56	6.02	1.34				
	71.53	4.61	1.63	6.03	1.36	41.21	34.36	58.79	65.64
Whey 2.2.1	75.85	3.26	0.94	6.03	1.16	30.34	20.88	69.66	79.12
60 °C		3.25	0.94	6.01	1.15				
.2.2	69.10	3.04	0.92	6.03	1.11	26.69	17.65	73.31	82.35
		3.02	0.90	6.02	0.96				
	72.48	3.14	0.93	6.02	1.10	28.52	19.27	71.49	80.74
Whey 2.3.1	66.50	2.79	0.53	6.08	1.03	22.85	10.29	77.15	89.71
80 °C		2.79	0.53	6.08	1.02				
.3.2	67.20	2.72	0.55	6.03	1.04	22.37	10.36	76.63	89.64
		2.71	0.54	6.02	1.03				
	66.85	2.75	0.54	6.05	1.03	23.11	10.33	76.89	89.68
Whey 2.4.1	66.15	2.69	0.50	6.09	0.92	21.87	9.71	78.13	90.29
90 °C		2.68	0.50	6.08	0.89				
.4.2	65.10	2.75	0.55	6.03	0.99	22.86	10.08	77.14	89.92
		2.75	0.55	6.02	0.97				
	65.63	2.72	0.53	6.06	0.94	22.37	9.90	77.64	90.11

Starting milk dr.m. 8.15-8.13, cr.prot. 3.42-3.38. acidity 1.75-174, pH 6.36-6.33

dr.m. 7.84-7.81, cr.prot. 3.64-3.50, acidity 1.73-1.69, pH 6.40-6.39

The experimental results are summarized in table 8 and in fig 26 and 27.

First, the influence of *salts* (and temperature) will be discussed next the *pH* (and the temperature) and then *the combined action of salts and pH*. Last, the effect of *the dry matter content* is discussed.

On considering the results obtained it appears that the curves for the salts $MgCl_2$, $MgSO_4$, $CaCl_2$, and the combination of these three

TABLE 8.

Precipitation of proteins from soymilk.
Distribution of dry matter and crude protein in curd and whey.
Influence of salts, pH, temperature and dry matter content of milk.

Series	Precipitation conditions		whey weight (g)	whey dry matter (%)	whey crude protein (%)	whey pH	whey acidity	whey % of orig. dry matter in whey	whey % of orig. crude prot. in whey	curd % of orig. dry matter in curd	curd % of orig. crude prot. in curd
1	$MgCl_2$ in 80°C	1 ml	92.80	7.72	3.38	6.24	1.88	92.17	86.45	7.83	13.55
		2	66.35	2.80	0.49	6.10	0.99	23.44	8.77	76.57	91.24
		4	72.[illegible]	2.84	0.47	5.82	0.97	25.[illegible]7	9.04	74.24	90.97
		8	75.84	3.03	0.47	5.44	0.91	28.99	9.58	70.57	90.43
2	$MgCl_2$ in 2 ml	40°C	71.53	4.61	1.63	6.03	1.36	41.21	34.36	58.79	65.64
		60°	72.48	3.14	0.93	6.02	1.10	28.52	19.27	71.49	80.74
		80°	66.85	2.75	0.54	6.05	1.03	23.11	10.33	76.89	89.68
		90°	65.63	2.72	0.53	6.06	0.94	22.37	9.90	77.64	90.11
3	$MgSO_4$ in 80°C	1 ml	81.00	7.34	3.17	6.20	1.87	74.20	66.54	25.80	33.97
		2	66.55	2.81	0.58	6.04	1.01	23.38	9.97	76.62	90.03
		4	71.40	2.81	0.52	5.89	0.92	25.07	9.73	74.93	90.27
		8	75.40	3.02	0.51	5.78	0.90	28.50	10.11	71.50	89.90
4	$MgSO_4$ in 2 ml	40°C	72.90	4.11	1.58	6.11	1.40	36.48	28.68	63.52	71.33
		60°	71.90	3.22	0.98	6.05	1.22	28.37	17.76	71.64	82.24
		80°	65.08	2.84	0.62	6.07	1.07	22.69	10.01	77.31	89.99
		90°	61.15	2.88	0.59	6.04	1.06	21.53	9.01	78.48	91.00
5	$CaCl_2$ 1 n 80°C	1 ml	79.35	7.34	3.40	6.12	1.88	71.91	69.06	28.10	30.94
		2	65.05	2.66	0.55	5.91	0.91	21.22	8.89	78.78	91.11
		4	70.85	2.65	0.48	5.64	0.99	23.09	9.42	76.92	90.59
		8	76.25	2.80	0.49	5.47	1.06	26.15	9.66	73.85	90.34
6	$CaCl_2$ in 2 ml	40°C	65.65	3.70	1.30	5.86	1.18	29.87	21.87	70.14	78.14
		60°	72.65	2.94	0.83	5.86	1.07	26.42	15.60	73.59	84.40
		80°	67.00	2.65	0.54	5.87	0.91	21.80	9.21	78.21	90.80
		90°	64.33	2.69	0.51	5.85	0.93	21.31	8.44	78.75	91.56

7	Salt mixture 80°C	1 ml	86.70	7.77	3.61	6.26	1.89	82.30	80.88	17.71	19.13
		2	60.70	2.96	0.68	6.07	1.05	21.86	10.73	78.14	89.28
		4	71.38	2.75	0.52	5.78	0.90	24.00	9.56	76.01	90.44
		8	74.35	2.86	0.51	5.63	0.87	25.95	9.82	74.06	90.18
8	Salt mixture (pH 4.5)	1 ml	73.25	2.99	0.41	4.57	2.75	25.32	7.62	74.69	92.39
		2	74.25	3.04	0.43	4.56	2.73	26.13	8.17	73.88	91.83
		4	74.90	3.32	0.46	4.49	2.90	28.66	8.76	71.34	91.25
		8	77.53	3.10	0.49	4.43	2.85	27.44	9.78	72.26	90.23
9	Lactic acid 20°C	5.5	80.42	3.09	1.04	5.47	2.08	37.10	29.98	62.90	70.07
		4.5	82.19	2.96	0.56	4.54	3.17	36.29	14.89	63.71	85.11
		3.5	81.68	4.04	0.68	3.50	10.35	48.90	17.63	51.10	82.37
		3.0	75.31	5.84	1.57	2.98	22.18	65.95	38.23	34.06	61.78
10	Lactic acid (pH 4.5)	40°C	79.58	3.16	0.60	4.52	3.15	30.45	12.57	69.56	87.43
		60°C	82.10	3.02	0.54	4.56	3.09	30.03	11.65	69.98	88.35
		80°C	69.75	2.92	0.42	4.56	3.10	24.70	7.73	75.31	92.28
		90°C	63.33	3.02	0.47	4.61	2.96	23.07	5.99	76.94	94.02
11	Dry matter 10% $MgSO_4$ in 80°C	1	88.73	8.80	4.19	6.23	2.40	80.77	79.09	19.24	20.91
		2	58.75	4.17	1.20	6.11	1.34	24.68	15.06	75.33	84.95
		4	66.25	3.75	0.74	5.95	1.27	25.05	10.50	79.45	89.51
		8	69.88	3.90	0.73	5.80	1.21	27.47	10.71	72.54	89.29
12	Dry matter 4% $MgSO_4$ in 80°C	0.5	96.40	4.04	1.84	6.31	0.93	97.62	98.62	2.38	1.38
		1	75.63	1.56	0.38	6.17	0.59	29.58	16.02	70.43	83.98
		2	82.50	1.45	0.28	6.04	0.52	30.08	12.71	69.93	87.30
		4	84.23	1.58	0.23	5.93	0.53	33.33	11.05	66.67	88,95

Precipitation carried out with 100 gr of soymilk

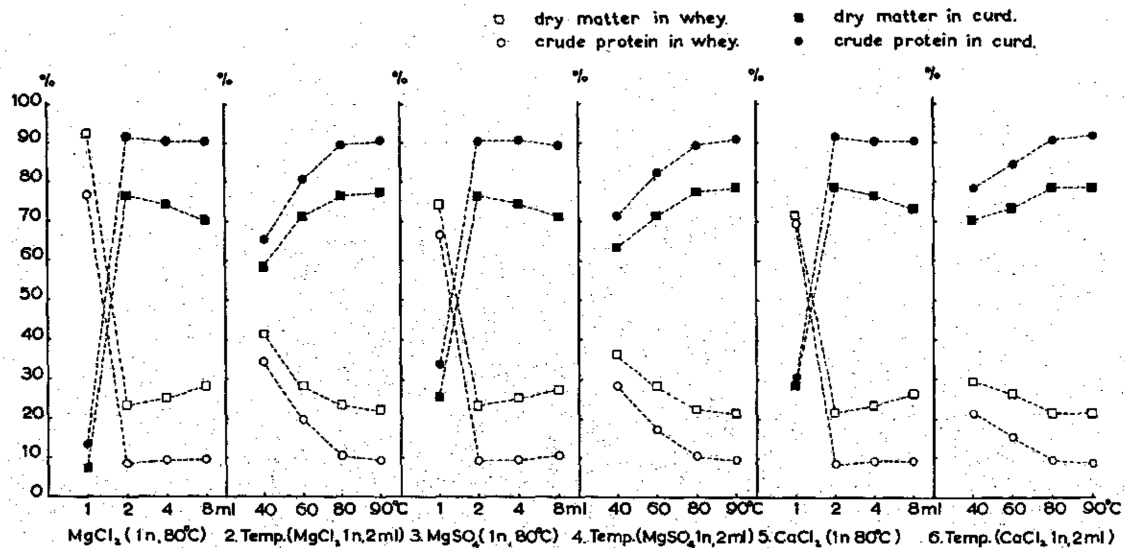


Fig 26.
Precipitation of proteins from soymilk.
Distribution of dry matter and proteins in curd and whey,
Influence of salts, concentration, and temperature.

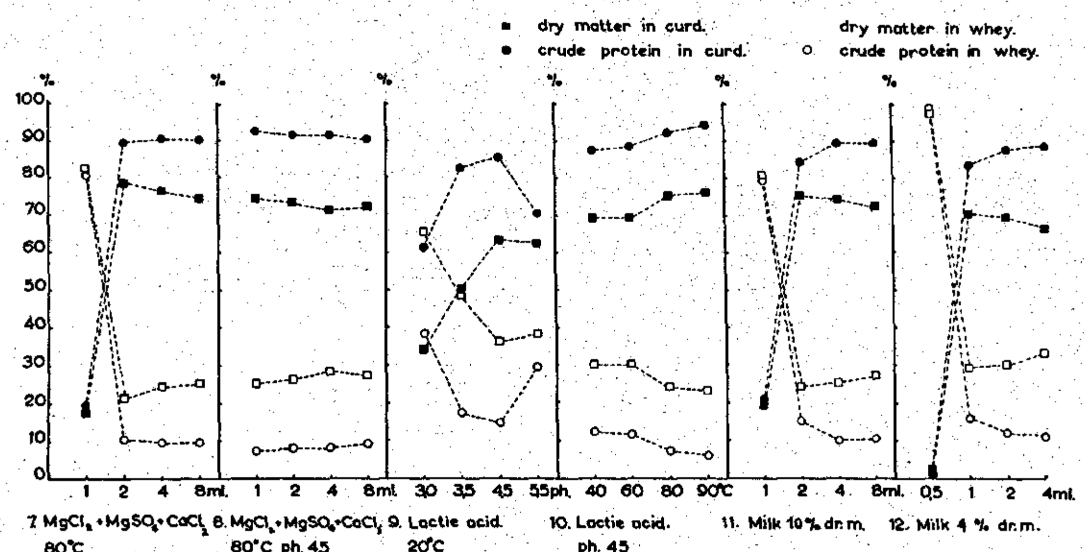


Fig 27.
Precipitation of proteins from soymilk.
Distribution of dry matter and proteins in curd and whey.
Influence of salt mixture, pH, concentration, temperature,
and dry matter content of milk.

salts (series 1, 3, 5, and 7) show a marked resemblance. These four series have in common that while addition of 1 ml of salt solution precipitates very little, 75 to 80% of the dry matter of the milk is obtained in the curd by doubling this amount. The yield of dry matter in the curd, however, does not rise if the quantity of the salt solution is increased.

In general, the same is true of the protein. An important difference, however, is that the protein yield figures are consistently higher than those for the dry matter. Evidently the proteins are better precipitated, as might have been expected. A rise in the amount of salt solution does not increase the yield. All four curves show about the same maximum yield which averages 90% of the original crude protein in the milk. On using 2 ml of salt solution the curd is solid (though soft) and the whey is light yellow transparant, whereas the curd has the appearance of a viscous liquid and the whey is milklike if 1 ml of salt solution is used.

Consequently the precipitating effect of each salt as well as that of the combination is quantitatively, practically the same. This implies that to obtain a high yield - *it is not necessary to use such a complicated solution as that applied by the population.* As on extraction the proteins are dispersed better than the other constituents of the dry matter of the bean, and the proteins are precipitated better than the other constituents of the milk, we may state that *the isolation of soyproteins by extraction followed by precipitation can give a high yield.*

When considering the influence of temperature on the action of the individual salts (series 2, 4, and 6) we find again a marked resemblance. The curves for the yield of dry matter rise regularly from about 60% to 80%, while the protein yield figures are higher than those for dry matter. Raising the temperature causes the differences to increase from about 7% to 13%.

The influence of the pH on the yield of precipitation at 20°C follows an unusual course (series 9). While on decreasing the pH the rise in yield of dry matter was smaller than expected, it appeared that the amount of dry matter precipitated decreased if the decrease in pH was continued. The maximum yield was about 55%.

In this case, too, there was a more marked reaction of the proteins to the precipitating agent so that an appreciably higher yield (85%) was reached. From the graph it can be clearly seen that if the pH decreases to 4.5 the proteins are precipitated in a larger degree than the other constituents of the dry matter.

If at pH 4.5 the temperature is raised from 40° to 90°C (series

10). it appears that the yield of the dry matter as well as that of the crude protein increase regularly. The difference in yield remains constant when the temperature is raised. The highest yield of proteins was 94.0%.

As mentioned before MONNIER (1935) states that the pH of the coagulating agent is 2.9. As this pH increases due to dilution this makes it understandable that an important part of the precipitating effect may be ascribed to the low pH as obtained with the empirical method used by the population of some East and South East Asiatic countries. Furthermore the precipitation is often carried out while the milk is boiling.

If simultaneously a low pH (4.5), high temperature (80°C) and salts are applied (series 8) a rather good reproduction of conditions of the empirical method-as applied by the population- is obtained. The curves for the yield of dry matter and crude protein remain almost horizontal, the protein yield figures being about 18-20% higher than those for the dry matter. The protein yield figures do not exceed 92.4%. Hence the empirical method may be considered very effective.

If milks with a higher or a lower dry matter content are used, 10% and 4% respectively, it appears that in principle the course of the curves is the same as that for 8% milk precipitated by a single salt. With the thin milk 1 ml of salt solution proved to be sufficient to obtain a solid curd, clear whey and a reasonable (85%) yield. As pointed out before, this amount of salt solution is not sufficient with the 8% milk, for which about 2 ml are needed. However, with the 10% milk it will be seen that these 2 ml are already insufficient. Apparently, as the dry matter content of the milk is increased more salt is needed to obtain efficient precipitation.

INOUYE (1894-1897) states that about one fourth of the total amount of proteins in the beans is obtained in the curd. LOSKA and MELNICK (1950) obtained 54% of the original proteins in the curd by adding 5 ml 0.2 n $MgCl_2$ solution to 25 ml of their milk. With another method they increased their yield to 74%, the curd being fluid, however. According to these workers the maximum yield for precipitation with $MgCl_2$ is between 0.01 and 0.03 n. This is in agreement with our experimental results.

It is interesting to note that on extraction of the soyproteins with $MgCl_2$-solutions a minimum yield is obtained at 0.025 n, according to SMITH, CIRCLE, and BROTHER (1938); at 0.010 n almost 30% more is extracted, whereas at 0.05 n only about 4% more is extracted. In general this is confirmed by our results.

From the foregoing the following conclusions can be drawn.

1. There are no appreciable differences between $MgCl_2$, $MgSO_4$, and $CaCl_2$, as regards their precipitating action on the proteins of soymilk. The combined action of these three salts does not show any essential difference either. About 90% of the crude protein can be precipitated by the use of a single salt.
2. Until up to about pH 4.5 more protein is precipitated if more lactic acid is used. At a lower pH less protein is precipitated. If the temperature is raised at least 90% of the original crude protein of the milk is obtained in the curd.
3. On lowering the dry matter content of the milk a less concentrated salt solution suffices, on raising the dry matter content a stronger salt solution must be used.

6.4 SUMMARY

Soycurd, a product derived from soymilk, is prepared in many ways. All these methods of manufacture have a number of stages in common: 1. The precipitation of the proteins, 2. the separation of the curd, 3. the lowering of the water content, 4. the ripening by means of micro-organisms. In this chapter the first stage, precipitation of the proteins, was studied.

The precipitating effect of a number of salts, $MgCl_2$, $MgSO_4$, $CaCl_2$, lactic acid, and of their combinations was studied at different concentrations and temperatures. The effects of each of these salts proved to be practically identical, while lactic acid produced a maximum yield at about pH 4.5 (20°C). When the dry matter content of the milk was changed, the course of the curves remained the same and proved only to be shifted in a horizontal direction.

7 SOY - YOGHURT

7.1 Introduction.

Preserving animal milk by means of microbiological souring has probably been practised from ancient times in many areas of Asia where nomadic tribes were living. As more than one sour milk product is known in the following we shall confine ourselves to souring by means of bacteria found in the so-called yoghurt.

Though the product may have been long known, according to LITTMANN (1934) it was not before the 17th century that statements on its use as human food became available. By way of Turkey the preparation of yoghurt seemed to have spread to the Balcan countries. In

Western Europe yoghurt became known not earlier than in the beginning of this century.

As has been shown in the chapter on heat preservation, soymilk in a raw condition is very subject to decay. Therefore it is useful to investigate whether it is possible to preserve soymilk by means of bacteriological transformations. Needless to say that the bacteria constituting yoghurt will have to accept soymilk as a substrate.

7.2 Literature.

With the exception of a French statement and a reference to a Russian investigation, hardly any literature is avialable on soy-yoghurt.

MATAGRIN (1939) states that vegetable milk is probably subjected to this kind of processing in those eastern countries where Bhudism prohibited the use of cow milk. According to this worker in Europe as well as in America there have been only few investigations concerning this point. He describes the preparation of soy-yoghurt and soykefir. However, it is not clear whether these methods of manufacture were developed after experimental work of the author or whether they were cited.

According to Matagrin in the preparation of *soy-yoghurt* the soymilk is concentrated to one half or one third, after which 2-3 ml "maya" (Bact. bulgaricum Barthel) or 15 ml of yoghurt is added per litre of milk. One ml of a streptococcus culture is recommended to avoid a peptone-taste. After mixing the temperature of the milk is maintained at 35-40°C until coagulation occurs. Then the product is cooled.

MILYUTINA (1948) produced *soykefir* by adding 4% Dispora caucasica to the soymilk. The beverage had an unpleasant taste if pure soymilk was used. Separation of the protein was prevented by the addition of about 0.05% of an aqueous agar[2] solution just before souring.

As may be seen the literature on this subject is very limited. However, we have got the impression that lactic acid bacteria do accept the soymilk as a substrate.

7.3 Experimental part.

7.3.1 Scope of work.

Judging from the scarce literature data available one might say that there is a reasonable chance that lactic acid bacteria will grow -and perhaps even grow well in soymilk.

Therefore *the first point* we have to confirm is, that if we take the two lactic acid bacteria present in yoghurt, the Thermobacterium bulgaricum and the Streptococcus thermophilus, and inoculate them then in soymilk either of them as well as both together do grow on this unknown substrate.

The second point -if the bacteria grow- is to study whether they grow sufficiently to have a preserving action on the milk. We could do this by counting the number of these two micro-organisms. But as we are more interested in their preserving action - as measured by their capacity of acid formation- we studied the acid formation by means of titration and the change in pH using an Electrofact pH-meter.

7.3.2 Design of experiments.

First the behaviour of each of the two bacteria, Thermobacterium bulgaricum and Streptococcus thermophilus, was determined and then their combined action was studied.

From tentative experiments it appeared that 45°C was a favourable temperature for the rodlike Thermobacterium. Therefore the influence of the inoculation percentage was studied at this temperature. Separate portions of soymilk were successively inoculated with 1, 2½, 5, and 10% of a pure culture of the Thermobacterium. By an inoculation percentage of 1% is meant that 10 ml of the pure culture was added to 1000 g of soymilk. The inoculated milk was poured into 50 ml bottles provided with tightfitting glass stops in such a way that no air was left in the bottles. The bottles were put in a waterbath. Then, using an inoculation temperature of 5%, the influence of the incubation temperatures 35°, 40°, 45°, 50°, and 55°C was investigated. After 1, 2, 4, 5, 6, and 7 hours and also after 24 hours the pH and the acidity were determined. For the acidity determination 10 ml were titrated with 0.1 n NaOH (indicator phenolphtaleine).

The same investigation was extended to the Streptococcus thermophilus. However, the temperature chosen for the study of the influence of the inoculation percentage was 40°C. The effect of the temperature was investigated in the range from 35°C to 50°C.

It appeared that for the Thermobacterium a temperature range from about 35° to 50°C can be used successfully, while for the Streptococcus this range was about from 35° to 45°C. Hence for the inoculation experiments with a mixture of both bacteria a temperature range from 35° to 45°C was chosen. In the mixture the ratio between the rods and the diplococci was 1 : 1. The sterilized milk was inoculated at a 1, 2½, and 5% level.

7.3.3 Technique of experiments.

The soymilk used in these experiments had a dry matter content of 8%. It was sterilized at 120°C for 10 minutes and then stored.

The inoculation material used was either a mixture of both bacteria or a pure culture of the bacteria isolated from the mixture. The original mixture was obtained from an institute where these mixtures are prepared.

The isolation of the bacteria was carried out as follows. One ml of a 10^{-5} dilution of the original mixture was mixed with a suitable substrate, according to BRIGGS, in Petridishes, and incubated at 37°C. After one or two days the colonies appeared on the agar. As the colonies of the rods and those of the cocci differ in shape a preliminary separation was obtained by inoculating a number of the colonies separately in tubes containing sterilized cow milk. After incubation at 37°C the milk coagulates, after which the contents were examined microscopically to ensure that the contents was made up of either rods or cocci. Then these pure cultures were inoculated in larger amounts of cow milk (100 ml). Every day two flasks of 100 ml cow milk were inoculated with 2½% of the Thermobacterium or the Streptococcus. Then the following day one flask was used for the inoculation experiments of the soymilk, while the contents of the other flask were used to inoculate another two flasks. The rods were incubated at 45°C, the cocci at 40°C, and then stored overnight in a refrigerator, so that the pH at incubation was about 4.5.

The mixture was inoculated in cow milk at a 2½% level for 2½ hours, cooled in water, and then stored in the refrigerator. The pH at incubation was about 4.5.

7.3.4 Results, discussion, and conclusions.

The results of the inoculation are shown in table 9 and represented graphically in fig 28, 29, and 30.

In general we may say that both bacteria accepted the soymilk as a substrate. It may also be said that -depending upon the experimental conditions- they grew well as was indicated by the acid formation and decrease in pH.

Fig 28 shows the influence of the inoculation percentage when the *Thermobacterium* was incubated at 45°C. The four curves for acidity and the four for the pH follow a regular course. When a low percentage (1%) was used the acidity increased slowly but at higher percentages the increase was much more pronounced. Then all four curves tended to become horizontal; and they did so sooner as the ino-

T A B L E 9. Inoculation of 8% soymilk with lactic acid bacteria.
Influence of inoculation percentage and temperature upon the acidity and pH.

			acidity (ml)							pH						
			1	2	4	5	6	7	24	1	2	4	5	6	7	24
Thermobacterium bulgaricum																
1.1	1%	45°C	1.46	1.70	2.79	3.25	3.55	3.76	4.03	6.47	6.26	5.49	5.18	5.09	4.99	5.03
.2	2½		1.65	1.76	2.80	3.66	4.13	4.19	4.48	6.32	6.17	5.43	5.14	4.98	4.91	4.88
.3	5		1.97	2.81	4.38	4.65	4.66	4.93	4.77	5.99	5.47	4.83	4.77	4.79	4.75	4.74
.4	10		2.56	3.70	5.31	5.55	5.63	5.66	5.66	5.65	5.08	4.62	4.65	4.65	4.69	4.68
2.1	35°C	5%	1.68	1.96	3.22	3.73	4.14	4.34	4.62	6.15	5.94	5.41	5.05	4.83	4.73	4.71
.2	40		1.79	2.29	4.24	4.24	4.42	4.23	4.82	6.10	5.63	4.71	4.67	4.68	4.66	4.70
.3	45		1.91	2.45	4.33	4.37	4.32	4.31	4.33	5.94	5.51	4.67	4.64	4.69	4.69	4.59
.4	50		2.01	2.60	4.30	4.63	4.69	4.65	4.70	6.09	5.46	4.81	4.69	4.64	4.66	4.72
.5	55		1.75	1.83	1.87	1.96	1.97	1.96	2.08	6.17	6.12	6.07	6.08	5.99	5.99	5.96
Streptococcus thermophilus.																
3.1	1%	40°C	1.51	1.55	1.57	2.01	2.87	3.39	5.53	6.33	6.33	6.29	5.93	5.49	5.04	4.41
.2	2%		1.56	1.57	1.97	2.67	3.54	4.25	5.71	6.28	6.31	5.94	5.52	5.06	4.71	4.33
.3	5		1.49	1.58	2.01	2.60	3.71	4.28	5.21	6.30	6.35	5.86	5.39	4.88	4.68	4.50
.4	10		1.82	1.90	3.11	3.92	4.66	5.07	6.37	6.12	6.01	5.20	4.87	4.61	4.52	4.26
4.1	35°C	5%	1.56	1.43	1.69	2.01	2.36	3.17	5.61	6.29	6.30	6.24	5.98	5.62	5.21	4.35
.2	40		1.51	1.56	1.87	2.49	3.87	4.34	6.09	6.38	6.24	5.97	5.43	4.89	4.67	4.22
.3	45		1.74	1.69	2.70	3.64	4.47	4.85	5.91	6.22	6.25	5.52	5.01	4.43	4.55	4.27
.4	50		1.66	1.69	1.83	1.82	1.79	2.00	3.72	6.20	6.15	5.99	6.00	6.02	5.91	5.12
Thb.bulg. + Strc.thermoph.																
5.1	1%	45°C	1.48	1.95	4.59	4.91	5.53	5.62	7.68	6.20	5.94	4.53	4.46	4.40	4.38	4.07
.2	2½		2.01	3.00	5.17	5.58	5.85	6.24	7.28	6.05	5.26	4.47	4.46	4.27	4.20	4.18
.3	5		2.40	3.87	5.22	5.97	6.26	6.38	7.07	5.18	4.92	4.43	4.32	4.27	4.21	4.24
6.1	1%	40°C	1.50	1.52	4.69	4.85	4.97	6.10	7.69	6.25	6.19	4.66	4.49	4.43	4.38	4.03
.2	2½		1.79	1.95	4.21	4.83	5.01	5.16	6.18	6.17	5.96	4.83	4.56	4.55	4.47	4.33
.3	5		2.12	3.40	4.95	5.88	6.04	6.23	6.71	5.79	5.10	4.54	4.28	4.24	4.19	4.16
7.1	1%	35°C	1.56	1.62	2.95	4.21	4.61	4.73	7.55	6.27	6.19	5.33	4.63	4.63	4.55	4.14
.2	2½		1.59	1.98	4.16	4.58	4.89	5.17	6.94	6.16	5.89	4.82	4.47	4.48	4.40	4.14
.3	5		2.07	2.44	4.52	5.03	5.54	5.70	6.41	5.88	5.62	4.63	4.53	4.40	4.34	4.21

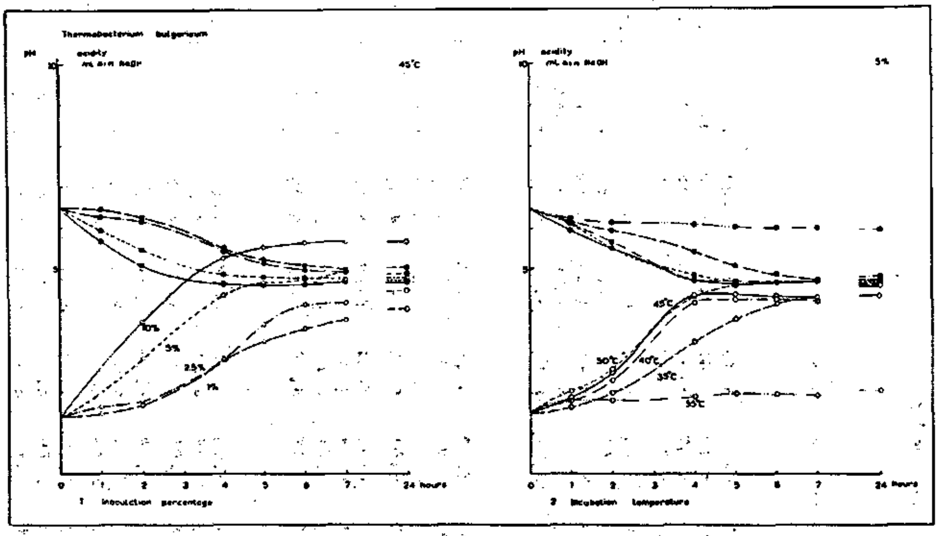


Fig 28.
Inoculation with Thermobacterium bulgaricum.
Influence of inoculation percentage and incubation temperature upon the acidity and pH.

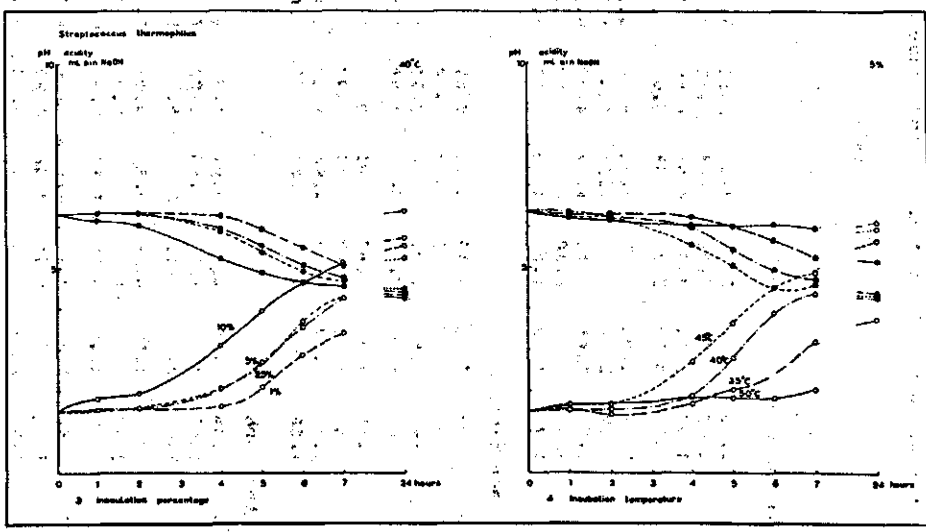


Fig 29.
Inoculation with Streptococcus thermophilus.
Influence of inoculation percentage and incubation temperature upon the acidity and pH.

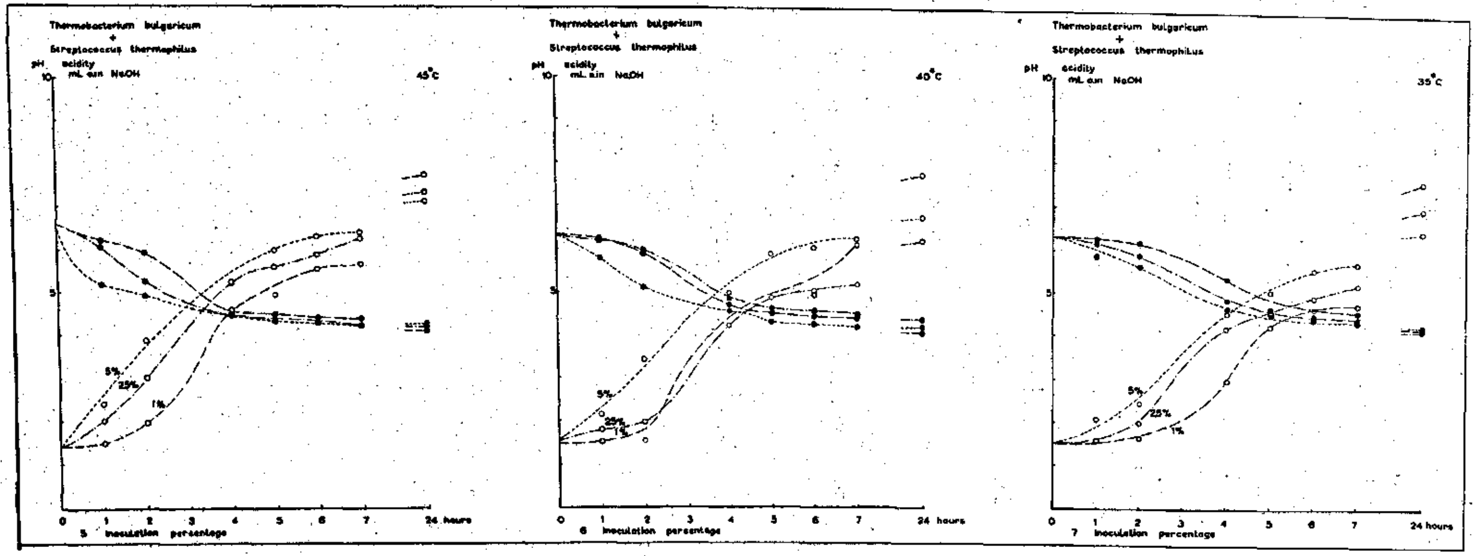


Fig 3D.
Inoculation with Thermobacterium bulgaricum and Streptococcus thermophilus.
Influence of inoculation percentage and incubation temperature upon the acidity and pH.

culation percentage was increased. The last part of the acidity curves is nearly horizontal. It may be said that there is little difference in acidity between 7 and 24 hours. It should be noted that the final acidity became higher as the inoculation percentage increased.

When the acidity increases the pH decreases. This may be seen from the same figure. In this sense the curves for the pH are the reverse of those for the acidity. It can also be seen that the final pH decreased as the inoculation percentage increased.

Under the experimental conditions the lowest pH and highest acidity reached were 4.6 and 5.7 respectively.

The effect of the temperature is shown in fig 28. Fifty five degrees proved to be too high for the Thermobacterium to form acid. On the other hand, 35°C is too low although after seven hours the acidity does not differ much from that obtained at temperatures between 35° and 55°. At first, however, the curves for 40°, 45°, and 50° clearly rose more steeply. There are hardly any differences between these three curves.

Judging from the rate of acid formation the *Streptococcus* also grew well in the milk (fig 29). However, it appeared that the acid formation was much slower than with the Thermobacterium. For instance, the acidity curve for 10% is about equal to that for 2½% for the Thermobacterium, up to about five hours. It is surprising to find here a further increase after seven hours, as with Thermobacterium neither the acidity nor the pH hardly changed after this period. The acidities thus obtained are appreciably higher than with the Thermobacterium.

In contrast to the Thermobacterium the acidity curves for the lower inoculation percentages did not rise until after three to four hours.

The influence of the temperature is very pronounced; 40° and 45°C proved to be favourable for the Streptococcus. Although the poor result at 35°C, however, was unexpected, the bad effect of 55°C upon the bacteria was not surprising. In this series, too, the acid production was very slow. After 24 hours, however, it surpassed that of the Thermobacterium.

The results of the inoculation of a mixture of both bacteria are shown in fig 30. It is clear that the course of the acidity curves show the characteristics of both curves for the individual bacteria. The first part of the curves is typical of the Thermobacterium, while the second part is influenced markedly by the Streptococcus.

Comparison of the first part of the 45°C acidity curves and those shown in fig 28 will reveal that -if the same inoculation percentages are compared- the acidity caused by the mixture is higher than that caused by the rod alone. The same is true of the second part of the 40°C curves if they are compared with fig 29. Thus it appears that, using the same inoculation percentage, incubation temperature and incubation time, a higher acidity is obtained with the mixture than with either of the two bacteria alone.

When an incubation temperature of 40°C was maintained the acidity generally decreased. An incubation temperature of 35°C is too low as may be seen from the slow rise of the acidity curves.

All the pH values reached after 24 hours lie between 4.5 and 4.0.

As may be seen from these experiments the efforts to convert soymilk into a sour product by means of lactic acid bacteria have given favourable results. We may expect that-due to the low pH- at least the preservability of the product is increased. Whether this product will come into use will, however, depend upon other factors such as the organoleptical properties and the nutritive quality. These factors as well as the preservability of the product were not investigated in this work.

From the foregoing the following conclusions can be drawn.

1. The lactic acid bacteria, Thermobacterium bulgaricum and Streptococcus thermophilus, did well in soymilk ($\pm$ 8% dry matter).
2. The acidity caused by the Thermobacterium bulgaricum reached a maximum of 5.7 ml 0.1 n NaOH (pH 4.7) after about five to six hours (inocul.perc. 10%, incub. temp. 45°C).
3. At first the Streptococcus thermophilus formed less acid than the Thermobacterium. However, after about seven hours -when the acid formation by the Thermobacterium had ceased the acidity increased further. At an inoculation percentage of 10% and an incubation temperature of 40°C after 24 hours the acidity was 6.4 ml (pH 4.3).
4. A combination of both bacteria also gave a regular acid formation. At an inoculation percentage of 2½% and an incubation temperature of 40°C an acidity of 4.8 ml (pH 4.6) was reached after five hours.

7.4 SUMMARY.

From literature it is known that preserving animal milk as sour milk by means of bacteriological changes is a long-established practice. In order to investigate whether lactic acid bacteria might prove useful in converting soymilk into sour milk a number of experiments were designed.

The lactic acid bacteria, Thermobacterium bulgaricum and Streptococcus thermophilus, were inoculated separately in sterilized soymilk (8%) at different inoculation percentages and incubation temperatures. The same was done with a combination of both.

Either of the bacteria as well as both together proved to be able to decrease the pH of the sour milk below pH 5.

8 GENERAL DISCUSSION.

The aim of this work has been to obtain information about the technological aspects of soymilk and some products derived from it: evaporated soymilk, soycurd, and soy-yoghurt. The preservation of soymilk by heat was also studied. In the preceding chapters the investigations of these subjects are described separately and the results are summarized. Here the practical consequences of these results will be briefly discussed.

In the chapter on the *manufacture of soymilk* we have demonstrated that if the same manufacturing conditions are maintained, a product of constant composition can be obtained. With respect to the manufacture on a technical scale we should pay due attention to the requirements of simplicity and speed.

What extraction ratio can be recommended for actual practice? In our opinion a ratio of about 10% should be chosen. By means of a single extraction milk of about 7.5% dry matter is obtained. There are no reasons for applying a lower ratio: in some cases higher ratios may be used, but then it will be more difficult to obtain satisfactory yields.

Now it might be questioned *whether it is desirable to dehull the beans*. From a technological point of view this is not necessary and even less desirable in view of the possible yield and the extent to which the suspension can be filtered. Therefore dehulling will only be applied if it is considered necessary from a nutritional point of view.

The manufacture of soymilk is a matter of considerable reduction in particle size of the raw material and of dispersing in water; both operations may be combined. The next question is whether *a wet or dry reduction in particle size* should be chosen. General experience as well as our own investigations have shown that a first reduction in particle size is best carried out dry, and a further reduction wet.

Therefore we would first recommend to ground the beans in a simple grinder, e.g. a hammermill, to a particle size of about 0.5 mm. Then this powder should be soaked for at least 4 to 6 hours, preferably at a low temperature, in water twice to three times the weight of the beans. A further reduction in particle size may be carried out with the ultimately desired amount of water and is thus combined with the extraction proper.

We have seen that the temperature has little influence on the extraction. Thus the grinding/extraction can be carried out at room-temperature. Furthermore the pH proves to have an important influence. If, however, the milk is destined for human consumption (either in a fresh, or concentrated form) it is not advisable to increase the pH considerably. Only if the milk is to be used for the manufacture of soycurd an increase in pH may be applied.

An important question is *which type of apparatus* is best suited for the grinding/extraction. In view of our experience we are of the opinion that it is best to use a modern grinder/mixer of which many types have appeared on the market during the last few years.

For our work we used an Ultra-Turrax with satisfactory results. A grinder/mixer has not only a high capacity for particle size reduction, but in addition it has a strong dispersing action. In a comparatively diluted suspension it is also effective, but it may be advisable to add little water to the soaked flour first and then after a short time of grinding add the rest of the water. With respect to other equipment (ball mill, runner mill, colloid mill) a grinder/mixer has the additional advantage of simplicity, low price, high capacity, and rapid action. The time of grinding/mixing is dependent upon the size of the batch, and the type of apparatus, and has to be determined empirically.

As stated before, *the separation of the milk* from the residue does not present great difficulties. For this operation we used a basket centrifuge with filter cloth. No doubt this apparatus is the most simple one. However, on using this apparatus the filter cake should be prevented from becoming too thick, as with increasing thickness the dry matter content of the milk becomes lower. The use of a clarifier is better justified as - by the right choice of diameter, number of revolutions, and holding time - it is possible to control accurately which particles will remain in the milk and which in the residue. However the disadvantage of an ordinary clarifier is the small capacity for solid substances. Therefore, if larger quantities of milk are to be produced, a more expensive continuous clarifier is necessary.

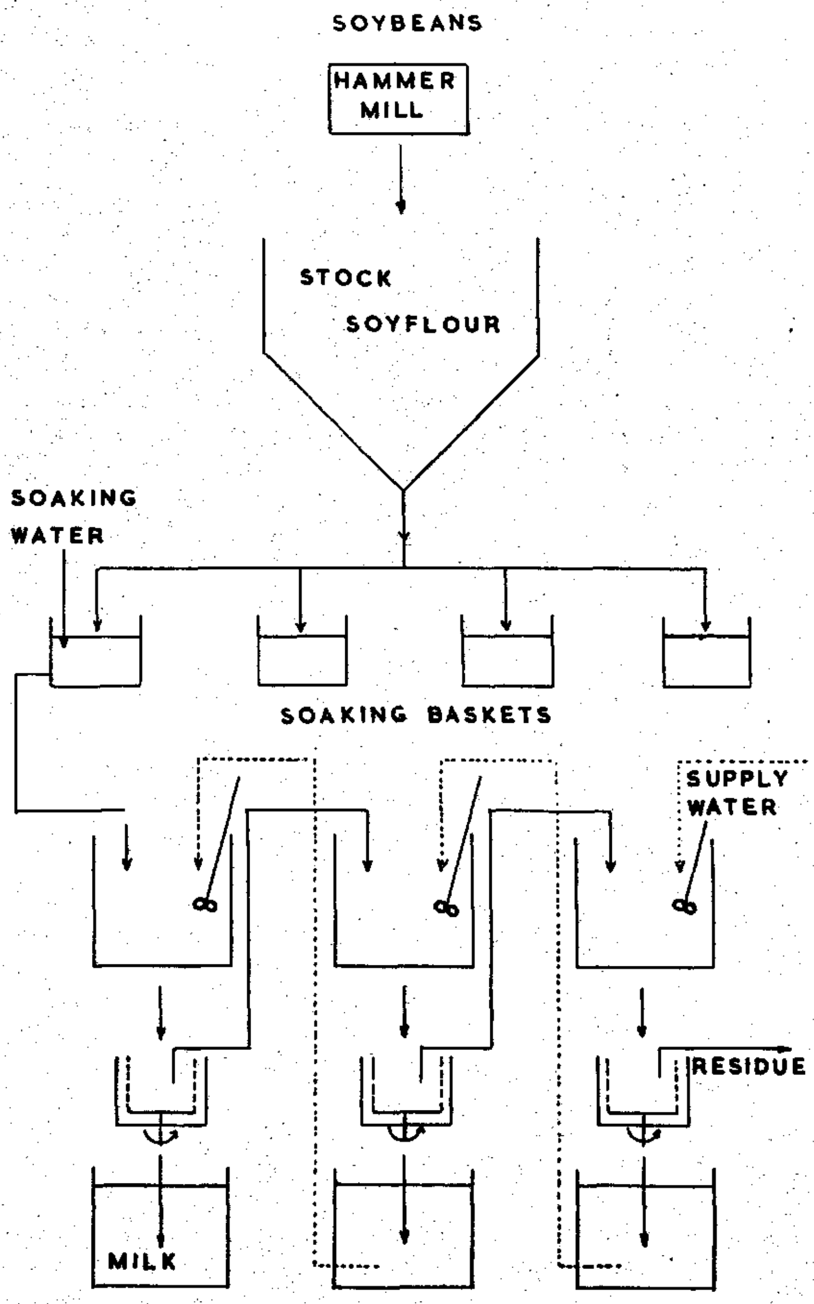


Fig 31. Flowsheet of the manufacture of soymilk.

If it is desired to increase the yield further we have seen that it is possible, to apply an extraction in several stages. No doubt a countercurrent extraction will then be used. At the second and third stage again a grinder/mixer may be used, so that a further reduction in particle size is effected, or a simple stirrer having only a dispersing action. Fig 31 shows a method of processing using an extraction in three stages.

As has been shown the manufacture of *raw evaporated milk* of about 30% dry matter content is quite possible. This product, however cannot be sterilized as its heat stability is too low. Although preheating appears to improve the stability, it still remains comparatively low. Moreover a result of preheating is that a viscosity barrier arises at a dry matter content depending upon the method of preheating.

This implies that it will be difficult to manufacture sterilized evaporated soymilk with more than 20% dry matter, as in that case the concentrated milk cannot be sterilized. By preheating at 70°C for 45 seconds a dry matter content of about 30% can be reached, while the micro organisms are partly destroyed.

If sugar is then used to preserve the concentrated milk in this way it is possible to manufacture *sweetened, condensed milk.*

As the same concentrated milk can be used for spray drying it will also be possible to manufacture *soymilk powder.*

If in actual practice an evaporator has to be chosen it should be remembered that for food products low temparatures and short holding times are preferable. For this purpose film evaporators are the only appropriate type. This type of evaporator has also the advantage that the holding time is rather uniform, which implies that the milk particles are subjected to a uniform heating.

If an evaporator with a long holding time is already present as part of the equipment of the factory it should be taken into account that the heating surface might damage the quality of the milk as regards its viscosity. In this case it is advisable to see to it that the temperature of the heating surface does not exceed 60°C. In a one effet evaporator by adaption of the vacuum a boiling temperature of about 30°C could be maintained. In this way detrimental heating in the evaporator is avoided. This method of manufacture, however, necessitates a separate heat treatment.

Foaming may be a complication in the execution of the process. According to our experience this foaming does not depend on the boiling temperature used, as it may occur both at low and high temperatures. It seems as if only the difference between the steam temperature and the boiling point is the determining factor, because when the steam temperature is raised above a certain level or when the vacuum is increased the milk starts foaming. These two observations give the impression that the foaming is caused by enlargement of the foam bubbles due to either a decrease of the pressure outside the bubbles or an increase of the pressure inside the bubbles because of a raise in temperature. With the film evaporator increasing the feed above a certain level also causes the milk to foam. In this case it seems to be the increase in the amount of foam bubbles that causes the milk to foam.

In the case of the film evaporator the foaming can be suppressed by increasing the speed of the rotor, while we have the impression that if the volume of the vapour separator is large enough no difficulties will occur. It might also be possible to combat foam by cooling of the passage between vapour separator and condensor, thus causing the foam bubbles to collapse.

Further research is necessary to investigate the possibility of further increasing the heat stability and of shifting the viscosity barrier to still higher dry matter contents or entirely dispense with it. We mention some lines along which future research may be developed.

1. Dry or wet heating the beans or meal.
2. Heating the concentrated milk after evaporation. This method is not unknown in experimental literature.
3. Addition of salts before or after evaporation. As has been shown in some cases we managed to increase the heat stability. As moreover the electrical charge of the proteins might be changed this could influence the viscosity.
4. Germination of the soybeans. As important changes take place during germination the tryptic inhibitor may disappear. Besides the amount of proteins diminishes and the milk may then acquire a higher heat stability.
5. Mixing with other vegetable proteins. The mixing may cause the stability to change.

Furthermore it should be noted that the work done was carried out with soybeans obtained from oil factories, and so presumably belonged to the commercial group of soybeans. Varieties of soybeans may be divided into three groups: commercial (grain), forage, and vegetable. The commercial varieties are used largely for processing into oil, meal, flour, and flakes. In the Orient varieties have been developed for use as green beans, bean curd, bean sprout, and numerous other food products; the term "vegetable varieties" is used to distinguish them from varieties grown for other purposes. This group is superior to commercial and forage types for vegetable milk, soy flour, bean curd etc. (USDA, 1952). It is possible that out of these vegetable varieties which number over one hundred, one or more will give no difficulties when processed to evaporated milk. In this connection it may be useful to consider the influence of the stage of ripeness on processing characteristics. Extensive and systematic research is needed to investigate this aspect of the problem.

In the production-distribution-consumption chain it will be some time before actual consumption of the milk takes place. Therefore pasteurization or sterilization will have to be applied. As has been shown pasteurization reduces the number of bacteria appreciably and the milk may be preserved for some days if pasteurization is combined with cold storage. The drawback, however, is that when the pasteurized milk acquires a higher temperature, bacteria will develop at a high rate. Even if the milk is kept at low temperatures the bacterial growth continues. Therefore, especially in warm climates sterilization should only be considered for application in practice. Sterilization may be carried out as usual with equipment already existing.

According to literature the mother liquid from seawater, spontaneous souring or sour soymilk are the means used to effect protein precipitation in soymilk. The objection against spontaneous souring is that the degree of souring is difficult to control, while furthermore protein decomposition may lead to a dangerous decay of the milk. Therefore the use of the mother liquid or its constituents is preferable. Since it has been shown in this work that it is not necessary to use all constituents of the mother liquid the question arises which of the constituents should be used. As far as the yield is concerned, the salts as well as lactic acid prove nearly equally effective.

The attractive point in the use of single salts is that if from a nutritional point of view it is desired to increase for instance the calcium content, this can be easily done by using a calcium-salt, e.g. calciumchloride.

From a technological standpoint, however, the use of acids (preferably lactic acid) has the advantage that a one-stage extraction already yields a very high percentage of the original protein if the pH is increased. The precipitation with acid yields nearly all the protein present in the milk. Another point in favour of the use of acid is that a low pH inhibits the growth of decay-bacteria.

The attractive point in the manufacture of soy-yoghurt is that the conversion of the soymilk can be effected in a simple way without the use of complicated equipment. Proper attention should be given to the incubation of a mixture of both lactic acid bacteria, Thermobacterium bulgaricum and Streptococcus thermophilus.

Since the nutritional qualities of this product are not yet known, this requires further investigation. Technologically, the important point is that the conversion of soymilk into a sour product does not present any difficulty.

SAMENVATTING

Gezien de grote tekorten aan dierlijk eiwit voor de voeding van de wereldbevolking en het feit, dat het in vele landen niet goed mogelijk zal zijn om op korte termijn deze tekorten op te heffen, is het noodzakelijk om te zien naar voedingsmiddelen met een hoog gehalte aan hoogwaardige, plantaardige eiwitten.

Het is bekend, dat de eiwitten van de sojaboon van hoogwaardige kwaliteit zijn. In enkele Aziatische landen is het gebruik van de soja als voedingsmiddel een gewoonte, die reeds van vele eeuwen voor het begin van de Christelijke jaartelling dateert. Voedingsmiddelen, zoals sojamelk en sojakaas, zijn derhalve geen nieuwe produkten. Technologische gegevens over de fabrikage van deze produkten zijn echter vrijwel niet aanwezig.

Het leek ons daarom van belang een onderzoek te verrichten betreffende de fabrikage van sojamelk en enkele hiervan afgeleide produkten teneinde het rendement en de kwaliteit te verbeteren en derhalve de kostprijs te verlagen. Tot de andere bestudeerde produkten behoren sojawrongel, sojayoghurt en ongesuikerde, gecondenseerde sojamelk. Ook de verduurzaming van de melk werd in het onderzoekprogramma opgenomen.

SOJAMELK

In de verschillende bereidingsmethoden van sojamelk, zoals die worden toegepast door de bevolking van enkele Oost-Aziatische landen, kunnen een aantal duidelijk te onderscheiden stadia aangewezen worden die als volgt aangeduid kunnen worden:

1) de voorbereiding;
2) de eigenlijke extractie;
3) de afscheiding van de melk;
4) de verbetering van de kwaliteit van de melk.

Aangezien de wijze waarop *de voorbewerking* plaats vindt in sterke mate de samenstelling van de melk bepaalt, werden diverse typen maalapparaten onderzocht. Hierbij werd zowel van de ongepelde, hele bonen als van de tot meel vermalen bonen uitgegaan.

Ook de *eigenlijke extractie* werd onderzocht teneinde een inzicht te krijgen in de factoren die gevarieerd zouden kunnen worden wanneer van een bepaald type apparaat wordt uitgegaan.

Over de afscheiding van de melk werd geen onderzoek verricht, aangezien zich hierbij geen problemen voordoen, althans niet bij de door ons gevolgde werkwijze. Een onderdeel van het laatste stadium, de houdbaarheid van de melk, werd in het onderzoek betrokken.

De volgende resultaten werden verkregen:

1. De proeven over de fabrikage van de melk zijn goed reproduceerbaar. Extractie van geweekt sojameel bij een extractieverhouding van 1 : 10 resulteerde in melk met ongeveer 7.7% droge stof, waarvan ongeveer de helft uit ruw eiwit bestond. Het rendement van de droge stof en het ruw eiwit bedroegen dan ongeveer 59.0, respectievelijk 68.0%. De eiwitten bleken derhalve beter dispergeerbaar te zijn dan het overige deel van de droge stof.

2. Extractie van geweekt sojameel gaf een hogere opbrengst dan extractie van de geweekte bonen. Zowel bij het meel als bij de hele bonen steeg de hoeveelheid melk bij betere vermaling, evenals het gehalte aan droge stof en ruw eiwit. Door een betere vermaling stegen derhalve ook de rendementen van de droge stof en het ruw eiwit.

3. De extractie verhouding heeft een grote invloed op de samenstelling van de melk. Bij een toenemende verhouding stijgen de gehaltes aan droge stof en ruw eiwit, terwijl van beide de rendementen dalen.

4. Ook de extractie pH is van grote invloed op het rendement. Het droge stof gehalte steeg bij toenemende pH echter niet in die mate als het gehalte aan ruw eiwit. Bij een pH van 9.5 bedroeg het rendement van ruw eiwit 80%.

5. De extractie temperatuur bleek in het gebied van 20° tot 50°C weinig invloed te hebben.

6. De extractie tijd had, nadat een zeker minimum overschreden was, eveneens weinig invloed op het rendement.

7. De rotatie snelheid van een Ika Ultraturrax had vrijwel geen effect op de extractie. De gehaltes aan en de rendementen van de droge stof en het ruw eiwit lagen echter wat hoger dan bij extractie met een Vibromixer.

8. Door het residu twee maal te wassen stegen de totale rendementen van de droge stof en het ruw eiwit aanzienlijk en naderden zij een grenswaarde, die - althans voor de droge stof - berekend kon worden. Het totale rendement van het ruw eiwit bedroeg 90%; geschat wordt, dat de grenswaarde bij de toegepaste werkwijze in de buurt ligt van 95%.

De consequenties voor de practische uitvoering worden in het hoofdstuk General Discussion besproken.

HOUDBAARHEID VAN SOJAMELK

Waar de sojamelk in hoofdzaak uit eiwitten, vetten en suikers bestaat vormt deze uiteraard een ideale voedingsbodem voor micro-organismen. In de literatuur zijn enkele aanwijzingen te vinden, die er op wijzen dat toepassing van een warmtebehandeling de houdbaarheid van de melk doet toenemen. Ook is het bekend, dat getracht is met behulp van chemicalien de houdbaarheid van de melk te verlengen. Aangezien het gebruik van chemische conserveermiddelen in voedingsmiddelen minder gewenst is, werd slechts getracht door middel van pasteurisatie en sterilisatie een betere bewaarbaarheid te verkrijgen.

De volgende resultaten werden verkregen:

1. Pasteurisatie van de rauwe sojamelk bij 60°C gedurende 30 min verlaagt het initiele bacterie-aantal aanzienlijk. De gepasteuriseerde melk blijft echter bij een temperatuur van 20 - 25°C nauwelijks een dag goed. Pasteurisatie in combinatie met bewaring bij lage temperatuur verhoogt de houdbaarheid tot ongeveer drie dagen.
2. Sterilisatie van 8% sojamelk bij 120°C gedurende 5 minuten blijkt voldoende te zijn om de melk gedurende tenminste een jaar houdbaar te maken.
3. Indien het droge stof gehalte van de melk boven de 12% stijgt dient de sterilisatie drastischer te worden uitgevoerd dan voor een 8% melk.

ONGESUIKERDE GECONDENSEERDE SOJAMELK

Om meerdere redenen kan het wenselijk zijn om de beschikking te hebben over gecondenseerde melk van tenminste 30% droge stof.
Een dergelijke verhoging van het droge stof gehalte zou met behulp van verdampers uitgevoerd kunnen worden. Het was echter bekend, dat zich bij het concentreren van sojamelk met behulp van verdampers moeilijkheden kunnen voordoen. In het kort kunnen deze moeilijkheden omschreven worden als het zeer sterk toenemen van de viscositeit van de sojamelk wanneer een droge stof gehalte van omstreeks 20% wordt overschreden, waardoor de hele melk in een puddingachtige massa overgaat. Door het optreden van dit verschijnsel is een limiet gesteld aan het concentreren door middel van indamping.

Getracht werd na te gaan of een warmtebehandeling van de rauwe sojamelk voor het indampen een belangrijke invloed zou kunnen hebben niet alleen op de viscositeit van het ingedampte product, maar tevens op de hitte-stabiliteit. Door middel van flash verhitting - met behulp van een speciaal ontworpen buizen warmtewisselaar - en langzame verhitting in een kookketel werd de invloed van verschillende temperatuurniveau's en verhittingstijden onderzocht. De experimentele resultaten gaven aanleiding tot het opstellen van de volgende conclusies:

1. Rauwe sojamelk kan zonder moeilijkheden tot tenminste 30% droge stof ingedampt worden.Het aldus verkregen product is echter niet steriliseerbaar.
2. Bij verhitting van de rauwe sojamelk voor het indampen treedt tijdens het concentreren een viscositeitsbarrière op, die het niet mogelijk maakt om de melk verder te concentreren. Het droge stof gehalte waarbij deze viscositeitsbarrière optreedt hangt af van de wijze van verhitting.
3. Wanneer een kookketel gebruikt wordt om de melk te verhitten, heeft het verlagen van de verhittingstemperatuur van 90^{o} tot 60^{o} C zowel als het verminderen van de verhittingsduur van 30 tot 0 minuten tot gevolg, dat de viscositeitsbarrière naar een hoger droge stof gehalte wordt verschoven.
4. Wanneer een continue,flash sterilisator gebruikt wordt om de melk te verhitten, heeft het verlagen van de verhittingstemperatuur van 120^{o} tot 70^{o}C zowel als het verminderen van de verhittingsduur van 45 tot 0 seconden tot gevolg, dat de viscositeitsbarrière naar een hoger droge stof gehalte wordt verplaatst.
5. In vergelijking met de viscositeitscurven van de melk behandeld in de kookketel zijn de curves voor de flash gesteriliseerde melk voor overeenkomstige temperatuurbehandelingen (70^{o}, 80^{o} en 90^{o}C) op een hoger droge stof niveau gelegen.
6. Bij gebruik van de kookketel geldt - zowel voor een verhittingsduur van 30 als van 0 minuten - dat de hitte stabiliteit in de volgorde 70^{o}, 90^{o} en 80^{o}C toeneemt. Wordt de verhittingsduur van 30 tot 0 sec verminderd, dan stijgt de hitte stabiliteit.
7. Bij gebruik van de flash sterilisator heeft - zowel voor een verhittingsduur van 45 als van 0 seconden - de hitte stabiliteit de neiging om toe te nemen in de volgorde 70^{o}, 80^{o} en 90^{o}C. Wordt de verhittingsduur verhoogt van 0 tot 45 seconden dan vertoont de hitte stabiliteit de tendens om toe te nemen.
8. In vergelijking met de hitte stabiliteits curven van de melk uit de kookketel zijn die voor de flash sterilisator, voor overeenkomstige warmte behandelingen op 70^{o}, 80^{o} en 90^{o}C, op een hoger niveau van hitte stabiliteit gelegen.

9. In het gebied 70°, 80° en 90°C is het overall effect van de voorverhitting het verschuiven van de viscositeits curven naar rechts, d.w.z. naar een hoger droge stof gehalte, in de volgorde ketel 30 min- ketel 0 min - flash 45 sec - flash 0 sec.

Beide eigenschappen - de viscositeit zowel als de hitte stabiliteit - blijken derhalve sterk door de warmtebehandeling beinvloed te worden. Bovendien blijkt, dat deze invloed aan een bepaalde wetmatigheid gebonden is. In het algemeen kan gezegd worden, dat naarmate de verhitting minder intensief is bij concentreren een hoger droge stof gehalte bereikt kan worden. Dit geldt zowel voor het temperatuursniveau als voor de coming-up time en de verhittingsduur.

SOJAWRONGEL

Evenals bij de bereiding van de melk kan ook in het fabrikageproces van sojakaas een aantal duidelijk verschillende stadia worden aangeduid: 1) precipitatie van eiwitten; 2) afscheiding van de wrongel; 3) vermindering van het watergehalte; 4) rijping van de kaas.

Voorwaarde voor een regelmatige produktie op grote schaal is, dat precipitatie onder bepaalde uniforme omstandigheden steeds een constante opbrengst zal geven. Tevens zal men een zo hoog mogelijke opbrengst verlangen.

Teneinde constante opbrengsten te verkrijgen is het nodig het precipiterende agens te standaardiseren. Hiertoe werd van een aantal zouten en van melkzuur afzonderlijk en in combinatie de precipiterende werking bij verschillende temperaturen en concentraties bestudeerd.

Uit de experimentele resultaten kunnen de volgende conclusies worden getrokken:

1. Bij het neerslaan van de eiwitten uit sojamelk met behulp van zoutoplossingen bij verschillende temperaturen en concentraties zijn voor $MgCl_2$, $MgSO_4$ en $CaCl_2$ geen noemenswaardige verschillen aan te wijzen. Ook de werking van een combinatie van deze drie zouten geeft geen essentiele verschillen te zien. Ongeveer 90% van de eiwitten is op deze wijze te precipiteren.

2. Wordt de precipitatie met behulp van melkzuur bewerkstelligd, dan wordt tot pH 4.5 meer eiwit neergeslagen naarmate de pH daalt. Echter blijkt, dat bij lagere pH minder eiwit wordt geprecipiteerd. In combinatie met een verhoging van de temperatuur is

het rendement van de precipitatie op te voeren tot tenminste 90%.

3. Bij een verlaging van het droge stof gehalte van de melk kan met een zwakkere zoutoplossing worden volstaan, bij verhoging van het droge stof gehalte dient een sterkere oplossing te worden gebruikt.

SOJAYOGHURT

Sojamelk, in hoofdzaak bestaande uit eiwitten, vetten en suikers, vormt een goede voedingsbodem voor diverse micro-organismen. De groei van deze organismen leidt tot een snel bederf van de melk. Nagegaan werd of het mogelijk is de twee melkzuurbacterien, Thermobacterium bulgaricum en Streptococcus thermophilus, in sojamelk te doen groeien om aldus een zuur melkproduct te verkrijgen. In een dergelijk zuur produkt zouden de rottingsbacterien weinig kans hebben om tot ontwikkeling te komen.

Hiertoe werden voor elk van de bacterien afzonderlijk en ook voor het mengsel van beide bacterien een aantal kweekproeven bij verschillende temperaturen en entpercentages opgezet.

Uit de proefresultaten werd het volgende geconcludeerd:

1. De melkzuurbacterien, Thermobacterium bulgaricum en Streptococcus thermophilus, groeien in 8% sojamelk goed.

2. De zuurtegraad teweeg gebracht door de Thermobacterium bereikte na ongeveer vijf tot zes uur, een maximum van 5.7 ml 0.1 n NaOH (pH 4.7) bij een inoculatiepercentage van 10% en een kweektemperatuur van 45°C.

3. In het begin vormt de Streptococcus minder zuur dan de Thermobacterium. Na zeven uur echter - wanneer de zuurvorming door de Thermobacterium reeds is opgehouden - blijft de zuurtegraad toenemen. Na 24 uur was de zuurtegraad 6.4 ml (pH 4.3) bij een 10% inoculatiepercentage en kweektemperatuur van 40°C.

4. Een combinatie van beide bacterien geeft een regelmatige zuurvorming. Na vijf uur werd een zuurtegraad van 4.8 ml (pH 4.6) bereikt bij een inoculatiepercentage van 2½% en een kweektemperatuur van 40°C.

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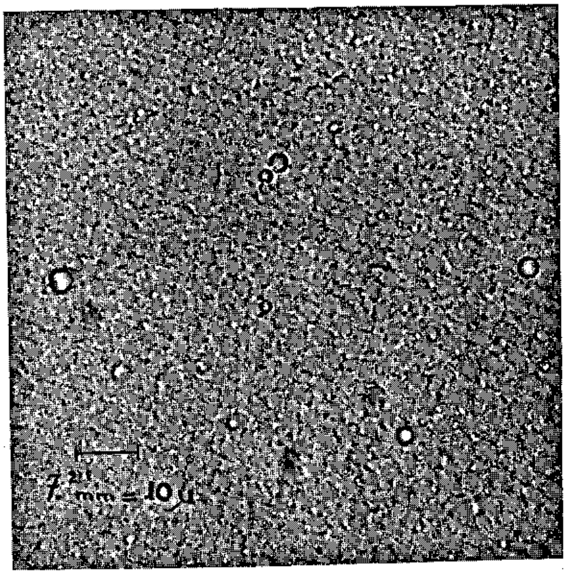
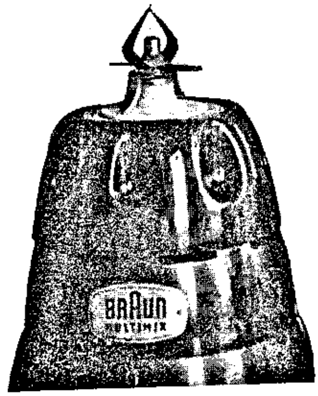
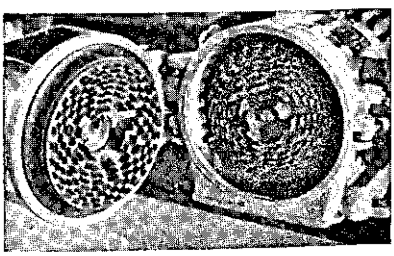


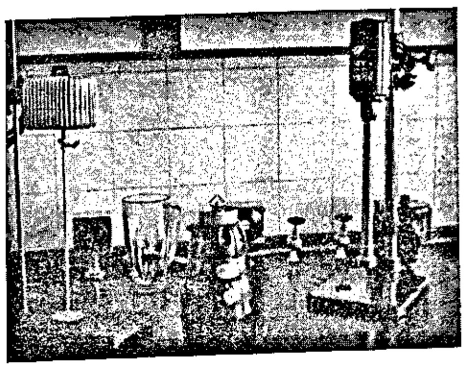
1. *Microphoto of soymilk (720 x)*



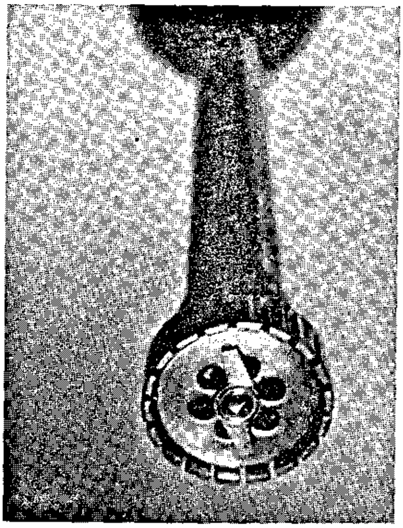
4. *Knives of Braun mixer*



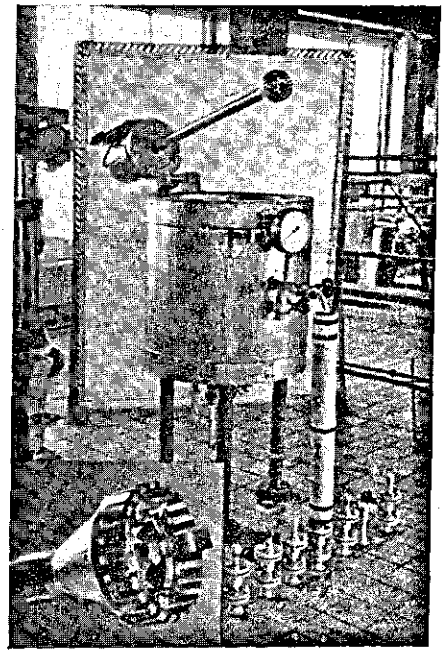
2. *Desintegrator*



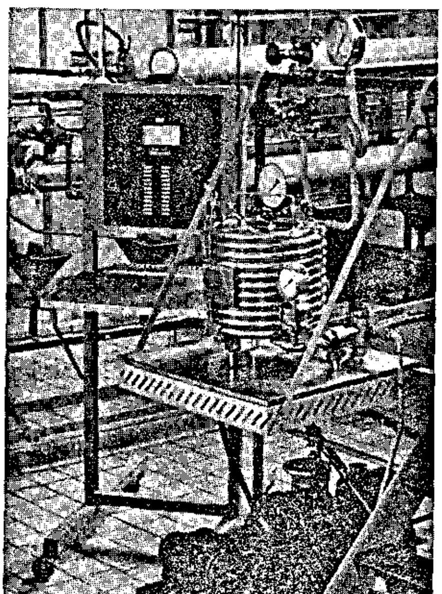
3. *Left: Vibromixer - Centre: Braunmixer*
Right: Ika Ultraturrax



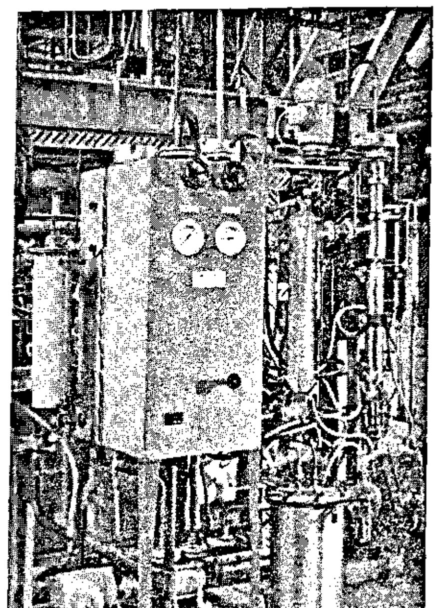
5. *Head of Ultraturrax*



6. *Steamjacketed vessel with mixer*



7. *Continuous flash sterilizator*



8. *Evaporation installation*
(Luwa film evaporator)

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13	10	MORSE,	(MORCE,
24	16	courses	sources
29	14	scare	scarce
36	5	4.1 Extraction ratio	4.1 Extraction pH
43	4	beans particles	bean particles
44	20	nog	not
44	37	beans particles	bean particles
48	36	is	=
49	14	implied	implies
51	6	an	and
63	11	en	an
66	6	BULL	BELL
66	28	applied	applies
67	26	possible	possibly
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42 Fig 3. Extraction of proteins from the soybean.
Distribution of dry matter and crude protein in milk and residue.
Influence of pretreatment.

42 Fig 4. Extraction of proteins from the soybean.
Distribution of dry matter and crude protein in milk and residue.
Influence of extraction conditions.

STELLINGEN

I

De conclusie van Huizenga, dat in 1939-'40 een gemiddeld inkomen van tien gulden per maand voor een arbeidersgezin (4-5 leden) op Java als een minimum, redelijk levensniveau dient te worden beschouwd, is niet gerechtvaardigd.

Huizenga, L. H., diss. Wag. (pp. 296), juni 1958

II

Vooroorlogse gegevens wijzen op een toestand van ondervoeding bij de bevolking op Java.

III

Het is mogelijk plantaardige melk zodanig te fabriceren, dat deze de rol van dierlijke melk als voedingsmiddel kan overnemen.

IV

Op economische gronden dient bij het streven de melkproductie in de tropische gebieden op te voeren meer profijt te worden getrokken van de productie van buffelmelk.

V

De opvattingen van DOS SANTOS en TROPA betreffende de rol van de veterinair bij de productie en verwerking van dierlijke melk zijn merendeels onjuist.

22 ième Session du Com. de l'Office
Int. des Epizooties, XLII: 537-546, 1954

VI

Voor het verhogen van het eiwitgehalte van de sojaboon ware het wenselijk reeds thans bij de cultuur aandacht te besteden aan de hiervoor in aanmerking komende maatregelen.

VII

Het gebruik van gips voor de bereiding van tofu dient te worden afgeraden.

VIII

Zowel voor hoog ontwikkelde als voor onderontwikkelde landen zijn de voorwaarden voor een juiste meningsvorming dezelfde. Deze voorwaarden zijn een objectieve en volledige voorlichting enerzijds en een critisch denkvermogen anderzijds.

Diss. Tan B. H.
Wageningen 1958