THE PHYSICAL AND CHEMICAL STABILITY OF SOYBEAN OIL FILLED MILK

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ABSTRACT

Filled milks were formulated from fresh skimmilk, vegetable oil and emulsifiers. The filled milks were pasteurized at 170 F (except in certain off-flavor studies), homogenized at 500/2500 psi and cooled to 36 F. Lightly hydrogenated salad oil, prepared from soybean oil, was quite acceptable when evaluated organoleptically at 24 hr. intervals over a period of approximately one week. Thiobarbituric acid and peroxide values revealed that very slight oxidation had occured during storage for approximately one week at 40 F. Four monoglyceride emulsifiers, with varying degrees of saturation, were used to stabilize the emulsion of soybean oil in skimmilk. Two of the more unsaturated monoglycerides tended to impart a bitter flavor to the milk when used at 0.1% (based on weight of product) and also were less efficient emulsifiers when compared to saturated monoglycerides. Development of a very undesirable sulfide-like odor and taste was revealed when extremely high pasteurization temperatures were used. The degree of off-flavor was directly proportional to the time and temperature of heating.

Two types of imitation milk, differing only in the type of protein, were prepared and evaluated. One series of imitation milks contained varying levels of calcium caseinate. Another group of imitation milks were formulated from combinations of skimmilk and soybean protein isolate. The isolate itself was then fractionated into two portions which were also used in combination with skimmilk proteins. The basic components of these two types of imitation milk consisted of proteins, corn sirup solids (low dextrose equivalent), dibasic potassium phosphate, monoglyceride, emulsifier, fat or oil and water.

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INTRODUCTION

Filled and imitation milk products are not only designed to fill the gap between demand and production of fluid milk in the future, but also to serve as economical substitutes for fluid milk at the present time. Milk substitutes are relatively new products to the consumer and appear to be readily accepted in States where their sale is legal.

A major portion of the filled milks now produced contain coconut fat as the replacement for milkfat. This substitution is often challenged since coconut is much more saturated than milkfat and contains smaller amounts of the essential fatty acids. Most imitation milks presently on the market also are nutritionally inferior to cow's milk, due mainly to the lower content of protein, calcium and phosphorus. The protein used in imitation milks has also created problems due to undesirable flavor characteristics of the isolated proteins.

The main purpose of this project was to evaluate a milkfat substitute, high in unsaturated fatty acids (including the essential fatty acids) which would be reasonably stable to oxidative attack when used in a filled milk product. Minor emphasis was placed on evaluating proteins now available for use in the formulation of imitation milk.

LITERATURE REVIEW

Filled milk was first introduced in the United States in 1916 (Siebert, 1967); however, Congress passed the Federal Filled Milk Act in 1923 to prohibit interstate shipment of this product (Doyle, 1967). Filled milk was labelled by this Act as an adulterated food article injurious to the public health, the sale of which constituted a fraud upon the public. At the same time many States also legislated against the production and sale of filled milk to protect the dairy industry (Australian Department Primary Industry, 1959) but this legislation has since been rescinded in a number of States (Anonymous, 1968 a).

The legal aspects pertaining to imitation milk may be summarized as follows (Food Processing, 1968):

a) Imitation milk is not covered by the Federal Filled Milk Act and may be shipped in interstate commerce; however, the filled milk laws of many States also prohibit the sale of imitation milk.

b) As of May 1968, twenty-nine States permitted sale of imitation products, fourteen allowed the sale of both filled and imitation milk; four States prohibited sale of both imitation and filled and four States had no laws covering milk substitutes.

Filled Milks

The Federal Filled Milk Act interprets filled milk as a product prepared by combining fats or oil other than milkfat with milk solids and the resulting product is in semblance of milk. According to Brink et. al. (1969) there are two types of filled milks presently on the market; one type contains fluid skim milk with or without milk solids and a vegetable fat in

semblance of milk. The other type is formulated from nonfat dry milk, vegetable oil, water and an additional source of protein such as soybean protein or sodium caseinate.

To date coconut oil has been the most common milkfat substitute used in filled milk formulations (Brink, 1968; Brink et al, 1969; Neu, 1967; Moczygemba, 1969; Rice, 1960; Moede, 1967; Saal, 1967 a; National Dairy Council, 1968; Rubini, 1969). Rice (1960) reported that hydrogenated soybean oil is also used in filled milks in the United States while olive oil and peanut oil have found corresponding usage in Spain and El Salvador. Other types of oil that have been used include palm kernel, corn and even specially treated whale oil (Australian Department Primary Industry, 1959). In order to prepare a diet low in saturated fats, Houk (1960) recommended using either corn, cottonseed or peanut oil with skimmilk to formulate filled milk. Cottonseed oil or combinations of cottonseed oil with corn and safflower oil are also utilized in filled milks (Brink, 1968). Klis (1968) reported that a selectively hydrogenated vegetable fat has been introduced by Proctor and Gamble as a milkfat substitute. Another hydrogenated vegetable fat claimed to be high in unsaturates has been marketed by Anderson Clayton and Co. (Anonymous, 1968 c).

Nutritional Value of Coconut Oil Filled Milk

There is no sound nutritional basis to justify replacing milkfat with coconut oil in the formulation of a filled milk (Brink, 1968; National Dairy Council, 1968). Use of coconut oil is based on its bland flavor, resistance to oxidation, desirable melting characteristics and price advantage over milkfat. (Brink, et al., 1969).

Coconut oil is a highly saturated fat and contains approximately 91 percent saturated fatty acids whereas milkfat contains 64 percent saturated fatty acids on an average. A maximum of two percent of the total fatty acids of coconut can be considered as "essential" while milkfat contains approximately three percent essential fatty acid (Bailey, 1964).

Keys (1967) found that saturated fatty acids with twelve to seventeen carbon atoms in the chain had a strong cholesterol-promoting action. The C_{12} fatty acid commonly called lauric acid, has the greatest hypercholesterolemic effect. Saturated fatty acids with fewer than twelve carbon atoms and those with eighteen or more in the chain were found to have little or no effect on serum cholesterol in man according to Keys. Lauric acid accounts for 45% of the fatty acids present in coconut fat (Bailey, 1964).

Hunter (1962) observed that Polynesians on a diet restricted in protein and calories still had much higher blood serum cholesterol levels than Polynesians on a more concentrated protein diet containing 1000 calories per day. Those on the low protein diet consumed 12 times as much coconut fat as those on the high protein diet and showed no signs of protein deficiency.

The necessity of essential fatty acid (linoleic and arachidonic) in the human diet has been well established as a requirement for growth and maintenance of dermal integrity (Holman, 1964; Hansen et al. 1963). According to Rice (1960), coconut fat increases blood cholesterol by reducing the effectiveness of linoleic acid. This author also indicates that lauric acid, fed in large amounts to humans, has deleterious effects, particularily when the diet is suboptimal. Moczygemba (citing Reiser, 1969)

states that coconut oil raises serum cholesterol levels more than butterfat or any other fat when fed to humans. Reports (Moczyemba citing Reiser, 1969) indicate that coconut oil retards calcium absorption, whereas milkfat promotes calcium absorption in the digestive tract of humans. Milkfat and unsaturated oils are well absorbed and appear to have little effect on calcium metabolism providing the calcium/phosphorous ratio of the diet is approximately one, (Lutwak, 1969). However, when the dietary fat is relatively poorly utilized as in the case of highly hydrogenated fats, both dietary calcium and phosphorous are excreted in greater concentrations. Rice (1960) found that coconut fat and milkfat have the same coefficient of digestibility but the absorption rate of milkfat is 71% opposed to 47.4% for coconut fat over a four hour period. In the same study it was noted that rats fed on ghee (a type of heated milkfat) had better growth rates than rats fed coconut oil. Brink (1968) reported that infants on a coconut diet had lower weight gains than those on evaporated milk. Moezygemba (citing Reiser, 1969), contends that coconut oil retards growth whereas milkfat promotes growth of man.

Brink et al. (1969) found that much of the coconut oil used in filled milk formulations is hydrogenated to increase stability of the fat. Keys (1967) indicated that hydrogenation is undesirable because the naturally occuring cis fatty acids are partially converted to the trans configuration which may not be utilized as well by the body.

Soybean Oil Filled Milk

Turpeinen et al. (1960) replaced whole milk by soybean oil filled milk and butter by a highly unsaturated margarine in the diets of patients to see what effect such replacements would have upon serum cholesterol levels.

Results showed there was a decrease of the mean serum cholesterol value which was statistically highly significant, though not large. Subjects with higher serum-cholesterol levels showed greater decreases, than patients with lower concentrations of serum cholesterol. Reduction of serum cholesterol may or may not reduce the incidence of atherosclerosis.

Thomasson et al. (1966) observed no abnormal changes in rats over a twelve week period when fed soybean oil hydrogenated by three different procedures. The utility of soybean oil in replacing milkfat in a filled milk has also been reported by Rakosky (1968). Jacobson et al. (1949), Murley et al. (1949) and Barker et al. (1952) found that freshly processed hydrogenated soybean oil was a suitable replacement for milkfat in the diet of young dairy calves. Calves fed crude soybean oil and refined soybean oil had lower weight gains than calves fed either milkfat or hydrogenated soybean oil.

The amount of α-tocopherol (Vitamin E) required by humans is directly related to the amount of unsaturated fatty acids in the diet (Herting and Drury, 1969; Holman, 1958). The ingestion of excessive amount of polyunsaturated fatty acids should therefore by accompanied by increased amounts of α-tocopherol in the diet.

Imitation Milk

Imitation milk differs from filled milk in that it contains no milk products as defined by the Federal Filled Milk Act. Sodium caseinate is a chemical and not a milk product, as defined by the Food and Drug Act (Anonymous, 1968 b). According to the National Diary Council (1968), the fat portion of most imitation milk consists of hydrogenated coconut oil with the protein portion consisting of caseinate and/or soybean protein.

Holland (1968) has developed two acceptable imitation milk formulations containing both sodium caseinate and soybean protein isolate.

Problems Associated With Imitation Milk

Flavor problems associated with sodium caseinate have limited the amount of protein that can be incorporated into a filled milk (National Dairy Council, 1968). Flavors characterized as "soapy," "gluey," "bitter" and "acrid" are often related with sodium caseinate (Cayen and Baker, 1963).

Analysis of imitation milks on the market (Brink et al. 1969; Koskowski, 1968: Coulter and Manning, 1968), revealed the protein level was suboptimal if this product was to be considered as a replacement for cow's milk. The protein portion of whole cow's milk is comprised of casein (82%) and whey protein (18%) with the latter having slightly higher nutritional value than casein. Formulation of imitation milk strictly from casein, to the same protein level as whole milk, would produce a product somewhat inferior to whole cow's milk (National Dairy Council, 1968).

Nicholas (1950) found that one of the major problems in preparing imitation milk is the incorporation of a protein of high biological value into a fluid system. Holland (1968) observed that it was not possible by his techniques to incorporate calcium and phosphorus in imitation milk to the same concentration as these minerals are found in cow's milk. Analysis of imitation milks by Brink (1968) and Kosikowski (1968) revealed that all imitation milks analyzed were low in minerals in comparison to cow's milk. Bronner (1969) observed there was not enough information indicating whether or not filled or synthetic milks can meet the nutritional needs for minerals as well as natural milks. Based upon present knowledge there is little reason to expect that increased use of milk substitues will lead to ill

effects on human health.

Role of Milk Substitutes

According to Floch, (1969); Brink et al., (1969); Smith, (1963); Glaser and Johnstone, (1952); Clein, (1951); Sternberg and Greenblatt, (1951), some humans, particularily infants, are allergic to the proteins present in cow's milk. Floch (1969) also points out that certain races cannot tolerate milk due to the presence of lactose. Such intolerance is thought to be due to a deficiency of lactase in the mucosa of the small intestine. In addition to problems associated with milk protein and lactose present in milk, Floch (1969) also states that bovine milk contains too high a concentration of saturated fatty acids and as a result milkfat has been implicated as a factor in atherosclerosis. The same author also states that milk proteins can induce ulcerative colitis in susceptible infants and adults. In order to combat the problems associated with bovine milk, substitutes have been developed from soybean protein and vegetable fat. Collins-Williams (1956) reported that "Powdered Sorbee," a soybean product designed for infants allergic to cow's milk, was a satisfactory substitute for cow's milk. Blumbert et al., (1963) reported that of the milk-free substitutes they studied, all were generally acceptable and grossly adequate.

Soybean Protein

World uses of soybean protein have been well documented (United States Department of Agriculture, 1967; Smith, 1963; Rakosky, 1968; Senti, 1963) but little information pertains specifically to the use of soybean protein in imitation milk formulations.

One of the major problems associated with soybean protein is the presence of a beany and bitter flavor. Attempts to remove the undesirable flavor with various solvents have been partially successful (Smith, 1961; Mustakas et al., 1961; Teeter et al., 1955; Elridge et al., 1963). Maga (1968) claimed that commercial soybean isolates are bland enough to be incorporated to a level of 3% (based on formula weight) in imitation milk, provided imitation milk flavor is also added. The threshold level for detection of soybean protein isolate in imitation milk was found to be 1% when no imitation milk flavor was added.

Brink et al., (1969) reported that properly processed soybean protein rates comparatively high in biological value but it is still lower than casein or whole milk proteins. Heat processing to inactivate growth inhibitors present in soybean protein also decreases the sulfur-containing amino acids, cystine and methionine. Harkins and Sarett (1967) reported a wide variation in protein quality of different soybean protein preparations. One product, with added methionine, proved to be nutritionally equivalent to casein. Holland (1968) indicated the major problem with soybean protein is the development of oxidative off-flavor caused by lipoxidase at the time of milling. This problem can be reduced to some extent by employing a processing procedure outlined by Wilkens et al., (1967).

Sasiki and Tsugo (1953) prepared synthetic milk from whey and soybeans by soaking the beans in water, grinding, adding whey to the ground soybeans, heating the mixture and removing the insoluble residue by press filtering. According to Nicholls (1950) the Chinese have used soybean emulsion and curd precipitated from this as a milk substitute. A soymilk factory has been operating in Hong Kong for several years.

Future of Imitation and Filled Milk

When imitation milk was first introduced on a national scale in the mid 1960's, it promised to be a great threat to the dairy industry in the United States (Saal, 1967 a; Saal, 1967 b; Norton, 1967; McKitrick, 1968; Anonymous, 1967; Anonymous, 1968 d). However, a very recent report by Quackenbush (1969) indicates that imitation milk has almost disappeared from the market, due mainly to poor quality and low nutritional value. Kosikowski (1969) indicates there is no great future for imitation milk unless the nutrient base is improved. Imitation milk could play a very important role in feeding tomorrow's population provided the nutritional aspect is given some consideration.

Filled milk appears to be well established in the United States as well as the Philippines and Mexico (Kosikowski, 1969). Most filled milk at the present time contains coconut fat as the milk replacement. This is a questionable substitute for milkfat, primarily because of the high content of saturated fatty acids. A more logical substitute for milkfat would be an oil high in unsaturated fatty acids.

EXPERIMENTAL PROCEDURE

Source of Materials[a]

Milk supply. The milk used in this study was obtained from the Michigan State University dairy department and separated into skimmilk plus cream in a Westfalia model LWA 205 separator.

Protein, corn sirup solids and emulsifiers. The calcium caseinate was obtained from Milk Proteins, Inc., Detroit, Michigan and the soybean protein isolate was provided by Central Soya, Chicago, Illinois. The 24 dextrose equivalent (24 DE) corn sirup solids were donated by American Maize-Products Co., Roby, Indiana. Distillation Products Industries (Rochester, New York) provided the four monoglyceride emulsifiers (Myvatex 8-20, Myverol 18-07, 18-30 and 18-85) used in this project.

Fats and oils. The types, common name and source of the various fats and oils used are listed on the following page.

[a] Throughout this thesis trade names of numerous fats, oils, proteins and emulsifiers are used to identify these products. Mention of certain products or equipment does not imply endorsement over others not cited.

Type	Brand Name	Source
Corn	Mazola	Supermarket
Cottonseed	Wesson	Supermarket
Olive	Sultana	Supermarket
Peanut	Planters	Supermarket
Safflower	Saff-O-lite	Supermarket
Coconut	Konut	Supermarket
Hydrogenated vegetable oil	Hydrol 92	Durkee Foods Chicago, Ill.
Hydrogenated vegetable oil	Kaola	Durkee Foods Chicago, Ill.
Hydrogenated vegetable oil	Durkex 100	Durkee Foods Chicago, Ill.
Hydrogenated vegetable oil	Durkex 500	Durkee Foods Chicago, Ill.
Hydrogenated vegetable oil	PG-25	Proctor and Gamble Cincinnati, Ohio

Formulation of Milk Products

Filled milk. Filled milks were formulated from skimmilk, commercial monoglyceride emulsifiers and vegetable fats and oils. The milkfat portion was replaced at concentrations of 0% (control), 25, 50, 75 and 100% with the various vegetable fat and oil products used.

Imitation milk. Two types of imitation milk was prepared. The first type was a completely synthetic milk formulated from calcium caseinate at concentrations of 1, 2, and 3% (based on formula weight) soybean oil, corn sirup solids (24 DE), emulsifiers, dibasic potassium phosphate (K_2HPO_4)

and water. A second type of imitation milk was prepared from corn sirup solids (24 DE), K_2HPO_4, soybean oil, emulsifier and water. Soybean protein isolate and Fractions I and II thereof were used in combination with skimmilk to constitute the protein fraction of this modified imitation milk. The three types of soybean protein were used at concentrations of 25 and 50 % (of the total protein content) with skimmilk constituting the remainder of the protein fraction.

Processing Procedure

General method. Each trial included a control sample of cow's milk containing the same percentage of fat (3.5%) as the imitation or filled milk. All samples of imitation and filled milks were forwarmed to 130 F, homogenized and pasteurized in that order. In certain studies on stability, some of the samples were first pasteurized and then homogenized. Prior to formulation, each monoglyceride emulsifier was dissolved (by heating if necessary) in the fat or oil.

Homogenization. All samples were homogenized in a two stage variable speed Manton-Gaulin homogenizer at pressures of 2000 and 500 psi on the first and second stages respectively. Continuous agitation on the intake side of the homogenizer, by means of a model F Lightnin stirrer, was necessary to insure that the oil portion was uniformly distributed in the aqueous phase during homogenization.

Pasteurization. All samples of imitation and filled milk were prepared in 10 pound lots and pasteurized by a batch process at 160 F in stainless steel beakers or by means of a Cherry-Burrell (HTST) heat exchanger (Spiratherm) equipped with an 8 second holding tube (Figure 1).

The milk was forced into the heating section of the Spiratherm by means of a size 10 Waukesha pump propelled with a Reeves variable speed drive. From the heating section the product was forced through the holding coil, into the cooling chamber which lowered the temperature to approximately 36 F at the point of exit. In order to obtain a holding time of 8 sec., the Reeves vari-drive was set at a pump speed of 6.5 on the 1 to 9 range. This speed maintained a back pressure of approximately 80 psi on the inlet side and prevented product from burning on in the heating section and holding coil during pasteurization. Thermocouples at the top and bottom of the holding coil were connected to a three point switch which was in turn connected to a pyrometer calibrated in degrees of F.

In order to take advantage of the sweet water cooling section of the HTST system the product had to be first homogenized, then pasteurized.

Flavor Studies

Sulfide off-flavor in soybean oil filled milk. In order to determine the critical time-temperature relationship involved in the formulation of sulfide-like odor and flavor, temperatures of 200, 205, 210 and 225 F were varied with individual holding times of 8, 4, and 0.3 sec. Filled milk pasteurized at these very high temperatures by means of the Spiratherm heat exchanger was evaluated organoleptically for sulfide-like off-flavor immediately after processing.

Development of oxidized flavor in filled milks. Filled milks were evaluated organoleptically at 0 time and at intervals not exceeding 48 hr. thereafter for a period of 6 to 8 days. A panel of experienced judges, working independently, was used for this purpose. The intensities of oxidized off-flavors were recorded as follows:

—— no oxidized flavor

± very slight oxidized flavor

+ slight oxidized flavor

++ moderate oxidized flavor

+++ pronounced oxidized flavor

Imitation milks were evaluated for oxidized flavor in addition to other off-flavors such as beany, watery, bitter, astringnet, etc.

Chemical Analysis

Iodine values. The iodine values of the various fats and oils were determined in triplicate by the official method outlined in the American Oil Chemists Society manual (1964).

Peroxide values. Peroxide values of the filled milks were determined in duplicate as soon as possible after formulation, then at daily intervals thereafter, for a period of one week. The method of Hills and Theil (1964) as modified by Stine et al, (1954) and Pont (1955) was employed for this determination with one further modification. A semi-automatic apparatus, as described below, was improvished for transferring aliquots of extracted fat from the Babcock cream test bottle to 10 ml volumetric peroxide flask (Figure 2).

1) Semi-automatic dispensing apparatus. This apparatus consists of a 500 ml separatory funnel connected to a three-way No. 2 stopcock by means of a 14/35 standard taper joint. A drying tube fitted with a standard taper (No. 27) joint was placed in the top of the benzene-methanol reservoir. A 0.5 ml Ostwald-Folin pipet was attached to the bottom of the three-way valve with a 12/5 ground glass joint and a piece of tygon tubing. The bottom portion of this three-way valve was inserted into a No. 7 rubber

stopper which was then fitted into a 500 ml aspirator flask. The remaining opening on the three-way valve was connected to a nitrogen line.

By turning the 2 three-way valves in the proper sequence, the lipid sample was drawn up into the 0.5 ml Ostwald-Folin pipet, then discharged with the benzene-methanol solvent and diluted to volume in the peroxide flask. Nitrogen (C P at 3 psi) was used to evaporate the residual solvent from the pipet in the preparation for the next sample.

2) Calculation of delivery weight. The lipid sample delivered by the apparatus described above was dispensed into a 50 ml beaker which was then placed in an oven at 212 F until a constant weight was maintained. The delivery weight was determined for both milkfat and soybean oil using the semi-automatic device and analytical transfer device. The semi-automatic dispensing apparatus was not used in the peroxide value determinations reported in the preliminary studies.

3) Reagents. All chemicals and solvents used in the peroxide determination were of ACS grade; water was deionized in a monobed and redistilled from glass.

a) Benzene-methanol solvent. A mixture of 7 volumes of thiophene-free benzene and 3 volumes of methanol was used. The benzene was redistilled and the methanol was dried by refluxing for 3 hr. with magnesium ribbon (5 g per litter) followed by distillation.

b) Ferrous chloride solution. (approximately 0.014 M)

Hydrated barium chloride dissolved in 50 ml of water was added slowly, stirring to a solution of 0.5 g of hydrated ferrous sulfate in 50 ml of water. To this, 2 ml of 10 N hydrochloric acid was added. The entire solution was then placed in a 100 ml graduated cylinder and the precipitated barium chloride allowed to settle for 16 hr. in a refrigerator at 40 F. The clear solution was decanted into a brown glass bottle and stored in a

refrigerator. All reagents were as free as possible of ferric ion.

c) Ammonium thiocyanate solution. Thirty g of ammonium thiocyanate was dissolved in water and made to a volume of 100 ml. The solution was transferred to a brown glass bottle and stored in a refrigerator at 40 F.

d) De-emulsification reagent. Fifty g of sodium citrate and 50 g of sodium salicylate were dissolved in 300 ml of water. To this was added 86 ml of redistilled n-butanol and enough water to make the volume up to 450 ml.

4) Procedure. Thirty ml of milk was added to a 9 g Babcock cream test bottle, followed by 15 ml of the de-emulsification reagent. After gentle agitation to insure thorough mixing of reagent and filled milk, the bottle was heated in a water bath maintained at 70 C (158 F) for 10 min. and then centrifuged 2 min. in a Babcock centrifuge. The fat was brought to the base of the neck by the careful addition of hot water (80 C) (176 F) and the bottle was again centrifuged 2 min. Hot water was again added to bring the fat up into the neck of the flask, and the bottle was centrifuged for 3 min. The fat was tempered by immersing the flask to the top of the fat line in water at 45 C (113 F) for 5 min. A final centrifuging (5 min.) was carried out. The fat was removed from the flask by means of the semi-automatic sampling device and placed in a 10 ml of volumetric flask. After addition of benzene-methanol solvent to the mark, the stoppered flask was inverted several times to dissolve the fat. Immediately after solution of the fat in the solvent, one drop of ferrous chloride, followed by one drop of ammonium thiocyanate reagent was added to the mixture in the flask. The flask was then shaken vigorously to disperse the reagents and placed in a water bath at 50 C (122 F) for exactly 2 min. for color development. The flask was placed in an ice water bath to lower the temperature to approximately 25 C (77 F). The contents of the flask were transferred to a cuvette.

Absorbancy was determined at 505 mu with a Coleman 20 spectrophotometer, adjusted to 100 percent transmittance with the benzene-methanol solvent.

A fat blank was run for both the filled milk and the cow's milk. A 0.5 ml sample of oil was handled in precisely the same manner except that no ferrous chloride reagent was added.

A reagent blank was made with each trial to check for possible deterioration of the reagents.

The peroxide values were expressed as the milliequivalents (meq) of oxygen absorbed per kg of fat. In making this calculation, the percent transmittance for the blanks must first be converted to ug of iron per 10 ml of solvent by means of the standard cuve (Stine et al., 1954). The net value for the unknown in terms of ug of iron per 10 ml of solvent is then calculated by substraction of the sum of the fat and reagent blanks. The peroxide value (as meq 0_2/kg fat) was calculated as follows:

$$\text{Peroxide value} = \frac{\text{Net ug Fe per 10 ml}}{\text{g of fat used} \times 55.84}$$

Thiobarbituric acid values. Thiobarbituric acid (TBA) values were determined in duplicate, by the procedure of King (1962), as soon as possible after product formulation and at 2, 4, 6, and 8 days intervals. The interfering pigments formed during the TBA reaction were removed by the method of Yu and Sinnhuber (1962). The TBA values of the soybean oil filled milk were then compared to the TBA values of cow's milk over the storage period of 8 days.

1) Reagents

a) 2-Thiobarbituric acid. The TBA (ACS) was recrystallized 3 times and 1.4 g of the recrystallized acid was added to 100 ml of 95% ethanol.

The TBA solution was freshly prepared for each trial.

b) Trichloracetic acid. One g of trichloracetic acid (ACS) was added per milliliter of deionized distilled water.

2) Procedure. One hundred ml of filled milk or cow's milk was added to 250 ml of erlenmeyer flask fitted with a standard taper glass stopper. After warming the milk to 30 C (86 F) 6 ml of trichloracetic acid solution was added, followed by 11.2 ml of 95% ethanol. After 5 minutes the contents of the erlenmeyer were filtered through No. 42 Whatman paper. To 50 ml of the clear filtrate, 5.6 ml of 2-thiobarbituric acid solution was added and the reaction carried out over a period of 1 hour in a water bath at 60 C (160 F). The reaction mixture was then cooled to 25 C (77 F) and passed over a standard cellulose column (1 x 12 cm). The interfering yellow pigments were eluted off the column with 28 ml of 0.1 N hydrochloric acid, followed by 20 ml of 0.1 N triethylamine to remove the red band of pigments which are associated with oxidized lipids. When the red band was approximately 1 cm from the bottom of the column the eluate was collected in a 10 ml volumetric cylinder and diluted to volume. The absorbancy of this solution was measured on a Beckman model DU-2 spectrophotometer at 532 mu. (CP Nitrogen at 4 psi was required to force the various solutions through the standard cellulose column used to separate the pigments).

Emulsion stability studies. The procedure as outlined in the Milk Industry Foundation Laboratory Manual (1959) was modified to determine the stability of the soybean oil in skimmilk emulsion formed by homogenization with the Myvatex 8-10 and Myverol 18-07 emulsifiers.

A representative sample of filled milk was placed in a 1000 ml graduated cylinder and stored under quiescent conditions for 48 hours at 40 F.

The top 100 ml of milk was siphoned off and the fat test of this portion was compared to the fat test of the remaining 900 ml. The percent difference between the fat test of the two portions has been termed homogenization efficiency and should not exceed 10% if an oil in skimmilk emulsion is stable. Each trial was duplicated.

Fractionation of soybean protein isolate. Smith and Wolf (1961) suggested using 85 to 90% isopropanol to remove the beany and bitter flavor from undenatured hexane-extracted flakes. This procedure was applied to soybean protein isolate.

Twelve hundred g of soybean protein isolate were added to 4000 ml of 90% isopropanol at 5 C (41 F) and centrifuged in 250 ml flasks at 2000 rpm for 5 minutes in an International Centrifuge (Model V) with a 16 inch head.

The less dense fraction of protein near the top of the flask, designated as Fraction I, was siphoned off and placed in a flat bottom stainless steel tray. The clear yellow middle layer of supernatant was likewise siphoned off but discarded. The protein on the bottom of the flask, designated as Fraction II, was removed and also placed in a stainless steel tray. Protein Fractions I and II were placed in a vacuum oven at 35 C (95 F) under 25 inches of vacuum for 8 hours to remove a major portion of the isopropanol. The Fractions were subsequently washed twice with distilled water to remove remaining traces of isopropanol.

Nitrogen analysis. The protein content of the soybean protein isolate, Fraction I and Fraction II, was determined by a micro Kjeldahl method described by Koler (1967). Nitrogen analyses were performed in duplicate and converted to percent protein by using a factor of 6.25.

EXPERIMENTAL RESULTS

Preliminary Studies with Filled Milks Prepared from Various Fats and Oils

Filled milks prepared from corn, cottonseed, olive, peanut and safflower oil as well as commercially hydrogenated fats including Kaola, PG-25, Durkex 100 and Durkex 500 were unacceptable in filled milk formulations, due to oxidized off-flavors initially present in the fat or oil (Table 1). Any filled milk (100% of milkfat replaced) which initially had a moderate oxidized flavor was considered unacceptable as a milkfat substitute. Of the twelve types of fats and oils listed on Table 1, only soybean, coconut, Hydrol 92 and Kaola were initially acceptable when used to totally replace milkfat. One of these milkfat substitutes, Kaola, (Table 2) was rejected after 6 days of storage at 40 F because of the intensity of oxidized flavor which had developed. Two other fats listed in Table 2, coconut and Hydrol 92, were acceptable for filled milk formulations with respect to oxidized flavor, but were too highly saturated to have any nutritional advantage over milkfat (Table 3). Soybean oil proved to be the only oil high in polyunsaturated fatty acids that was acceptable as a milkfat substitute (Table 2).

Studies with Soybean Oil Filled Milk

The results of gas-liquid chromatographic analysis of the methyl esters of the constituent fatty acids of soybean oil appear in Table 4. This oil was lightly hydrogenated and as a result the iodine value of 118 (Table 3) was somewhat lower than the average iodine value of 130 for a non-hydrogenated soybean oil. The flavor of this oil was essentially neutral and there was only a very slight increase in oxidized flavor over a period of 8 days (Table 2). These results were further substantiated by

thiobarbituric acid values (Figure 3). The TBA absorbancy of the extracted soybean oil increased from 0.034 to 0.055 over a period of 8 days while the absorbancy of the control sample (milkfat) changed from 0.040 to 0.064 over the same period of time. The peroxide values (Figure 4) of these same two products showed only slight fluctuations over a period of one week with an overall increase of 0.04 and 0.07 meq per kg of fat for the soybean oil filled milk and cows milk respectively.

The comparative accuracies of the analytical pipet and semi-automatic device for delivering aliquots of lipid into a peroxide flask by the semi-automatic device had higher mean delivery weights, smaller standard deviations with the 95% confidence limits being more narrow than samples delivered by the analytical transfer pipet.

The results of organoleptic evaluation of soybean oil filled milk containing various levels of monoglyceride emulsifiers are shown on Table 6. The Myverol 18-30 and 18-85 proved to be too bitter when used at the recommended level of 0.1% based on the formula weight. The Myverol 18-07 and Myvatex 8-20 appeared to contribute no undesirable off-flavor to the filled milk when used at a concentration of 0.1%. Properties of the monoglyceride emulsifiers used in this study appear in Table 7.

The stability of the soybean oil in skimmilk emulsion formed when Myverol 18-07 and Myvatex 8-20 emulsifiers are used, appears in Table 8. These results demonstrate that either emulsifier was acceptable when the samples were batch pasteurized and single homogenized. When HTST heat treatment was used to pasteurize the filled milks only the Myverol 18-07 proved acceptable when given a single homogenization. Use of the Myvatex 8-20 required double homogenization in order to insure stabilization of the oil emulsion. If the filled milk was first homogenized, then HTST

pasteurized, emulsion stability was reduced to the extent where double homogenization was necessary with either emulsifier.

The effect of varying time-temperature relationships during HTST pasteurization on the development of off-flavors in filled milk appears in Table 9. These data show that the off-flavor was evident at 205 F with an 8 sec. holding time and at 225 F with a 4 sec. hold. Reduction of the holding time to 0.3 sec. eliminated the off-flavors.

Studies with Imitation Milk

Flavor criticisms of imitation milk prepared with 1, 2, and 3% calcium caseinate appear in Table 10. The results revealed that use of calcium caseinate at concentrations of 1 and 2% (based on the formula weight) resulted in a product criticized as flat, watery and acid. When the protein content was increased to 3% calcium caseinate, flavors such as astringent, watery and bitter were predominant.

Treatment of soybean protein isolate with 90% isopropanol yielded two fractions. Fraction I was of low density and had a soft flaky texture whereas Fraction II was more dense. The texture of this fraction was coarse and mealy.

The three types of soybean protein were analyzed for protein content (Table 11). Fractions I and II contained approximately 90% protein which was about 3% more than the isolate from which they were fractionated. Imitation milks prepared from the three types of soybean protein were evaluated organoleptically immediately after formulation (Table 12). Fractionation of the soybean isolate with isopropanol did reduce the amount of beany flavor, particularly in Fraction I, but the milk product was still unsatisfactory. Fractions I and II appeared to disperse readily; however,

after 24 hr. of storage at 40 F a major portion of both protein fractions was found on the bottom of the storage flask. The isolate appeared to remain in solution, but the beany flavor was too intense to warrant consideration of this protein in formulation of imitation milk.

Table 1. Peroxide value and flavor score of filled milk prepared from various combinations of milkfat and vegetable fats or oils.

Fat or oil	Milkfat replaced (%)	Peroxide value (Meq/Kg fat)	Flavor[a] score
Corn	0	0.17	-
	25	0.24	+++
	50	0.73	+++
	75	0.87	+++
	100	1.16	+++
Cottonseed	0	0.55	±
	25	0.62	+
	50	1.10	+
	75	1.13	++
	100	1.78	++
Olive	0	0.21	-
	25	2.70	+++
	50	5.32	+++
	75	9.28	+++
	100	11.38	+++
Peanut	0	0.67	+
	25	1.95	++
	50	3.56	+++
	75	4.46	+++
	100	5.75	+++
Safflower	0	0.32	-
	25	1.55	++
	50	2.65	+++
	75	4.20	+++
	100	5.40	+++
Soybean	0	0.39	-
	25	0.08	±
	50	0.12	±
	75	0.18	±
	100	0.21	±
Coconut	0	0.37	-
	25	0.17	+
	50	0.28	++
	75	0.26	+
	100	0.21	+

Table 1 (Continued)

Fat or oil	Milkfat replaced (%)	Peroxide value (Meq/Kg fat)	Flavor[a] score
Hydrol 92[b]	0	0.26	+
	25	0.24	+
	50	0.14	+
	75	0.06	+
	100	0.13	+
Kaola[b]	0	0.42	-
	25	0.32	±
	50	0.39	-
	75	0.32	±
	100	0.29	-
Durkex 100[b]	0	0.42	±
	25	0.32	+
	50	0.39	++
	75	0.32	++
	100	0.29	++
Durkex 500[b]	0	0.33	-
	25	0.59	+
	50	0.82	+
	75	0.98	++
	100	1.15	++
PG-25[b]	0	0.15	-
	25	0.25	±
	50	0.30	+
	75	0.48	++
	100	0.59	+++

[a] - No oxidized flavor
± Very slight oxidized flavor
+ Slight oxidized flavor
++ Moderate oxidized flavor
+++ Pronounced oxidized flavor

[b] Trade name; composition of product not known.

Table 2. Peroxide value and flavor score of filled milk stored 6 to 8 days at 40 F

Fat or oil	Storage (days)	Peroxide value (Meq/Kg fat)	Flavor[a] score
Coconut	0	0.21	+
	1	0.09	+
	3	0.20	+
	6	0.06	+
Kaola[b]	0	0.29	-
	1	0.23	-
	3	0.47	+
	6	0.17	++
Hydrol 92[b]	0	0.13	+
	2	0.10	±
	4	0.02	+
	6	0.03	±
	8	0.09	±
Soybean	0	0.21	+
	2	0.21	-
	4	0.14	-
	6	0.15	±
	8	0.43	±

[a] - No oxidized flavor

± Very slight oxidized flavor

\+ Slight oxidized flavor

++ Moderate oxidized flavor

+++ Pronounced flavor

[b] Trade name; composition of product not known.

Table 3. Iodine value of fats and oils used in the preparation of filled milk

Fat or oil	Iodine value
Corn	119
Cottonseed	109
Olive	87
Peanut	97
Safflower	147
Soybean	118
Coconut	9
Hydrol 92[a]	4
Kaola[a]	46
Durkex 100[a]	97
Durkex 500[a]	83
PG-25[a]	99

[a] Trade name; composition of product not known.

Table 4. Fatty acid composition of lightly hydrogenated soybean oil

Composition of hydrogenated soybean oil[a]		
Fatty acid		Weight percent
Chain length	Name	
16:0	Palmitic	7.5
18:0	Stearic	1.9
18:1	Oleic	52.7
18:2	Linoleic	37.0
18:3	Linolenic	1.8

[a] Analysis by L. R. Dugan, Dept. of Food Science, Michigan State University.

Table 5. Comparative accuracy of an analytical transfer pipet and a semi-automatic device for delivering aliquots of soybean oil or milkfat into a peroxide flask.

Measurements	Transfer device studied			
	Semi-automatic		Analytical transfer	
	Soybean	Milkfat	Soybean	Milkfat
Number samples tested (n)	20	20	20	20
Mean delivery weight ($\bar{x}$)	0.4031g	0.4012g	0.3917g	0.3920g
Standard deviation (s)	0.0039g	0.0019g	0.0061g	0.0044g
95% Confidence limits	0.4031g ±0.0018g	0.4012g ±0.0009g	0.3917g ±0.0029g	0.3920g ±0.0021g

Table 6. Organoleptic evaluation of soybean oil filled milk containing various levels of monoglyceride emulsifiers

Chemical characteristics and quanity of emulsifiers used			Flavor noted in filled milk
Monoglyceride	Iodine value	Level (%)[a]	
Myverol 18-07	3	0.05	no flavor criticism
		0.10	no flavor criticism
		0.20	slightly bitter
Myvatex 8-20	22	0.05	no flavor criticism
		0.10	no flavor criticism
		0.20	slightly bitter
Myverol 18-30	40	0.05	no flavor criticism
		0.10	slightly bitter
		0.20	moderately bitter
Myverol 18-85	85	0.05	slightly bitter
		0.10	moderately bitter
		0.20	pronounced bitter

[a] Concentration of emulsifier based on weight of product.

Table 7. Gross composition of emulsifiers used in preparation of filled and imitation milks

Monoglyceride	Source	Minimum monoester content (%)	Other additives
Myverol 18-07	Hydrogenated cottonseed oil	90	0.02% citric acid
Myvatex 8-20	Edible fat and 20% hydrogenated vegetable oil	80	0.11% BHA, 0.0072% citric acid, 0.011% glycine and 0.011% H_3PO_4
Myverol 18-30	Edible tallow	90	0.02% BHA and 0.004% citric acid
Myverol 18-85	Refined cottonseed oil	90	0.02% glycine and 0.02% H_3PO_4

Table 8. Homogenization efficiencies of soybean oil filled milk prepared with Myverol 18-07 and Myvatex 8-20 monoglyceride emulsifiers

Method of processing			Homogenization efficiency[a]	
Type of pasteurization	Order of processing	Times homogenized	Monoglyceride	
			Myverol 18-07	Myvatex 8-20
Batch	Post. - Homo.	1	4.1	5.5
	Past. - Homo.	2	3.3	5.5
HTST[b]	Past. - Homo.	1	5.8	14.5
	Past. - Homo.	2	2.9	5.0
HTST[b]	Homo. - Past.	1	10.1	26.7
	Homo. - Past.	2	7.1	7.7

[a] Homogenization efficiency is the percent difference in the fat test of the top 100 ml and bottom 900 ml of product stored in a 100 ml graduated cylinder for 48 hours at 40 F under quiescent conditions (maximum of 10% allowed).

[b] Eight second holding time.

Table 9. Effect of time/temperature HTST pasteurization on off-flavor development in filled milk

Length of hold (seconds)	Off-flavors produced at various temperatures (F)			
	200	205	210	225
0.3	none	none	none	none
4.0	none	none	none	very slight sulfide
8.0	none	slight sulfide	moderate sulfide	very pronounced sulfide

Table 10. Organoleptic evaluation of fresh imitation milk prepared from three concentrations of calcium caseinate[a]

Calcium caseinate (%)[b]	Flavor criticism
1.0	Flat, watery, acid
2.0	Flat, watery, acid
3.0	Astringent, watery, bitter

[a] Imitation milk consisted of above concentrations of calcium caseinate in addition to 3.5% soybean oil, 0.05% K_2HPO_4, 8% corn sirup solids (24DE) and 0.1% Myvatex 8-20 emulsifier dispersed in an aqueous continuous phase.

[b] Percentage of calcium caseinate based on formula weight.

Table 11. Protein content of various types of soybean protein

Type of soybean protein	Protein content	
	Original (%)	Washed fraction (%)
Isolate	87.03	Not washed [a]
Fraction I	90.16	13.2[b]
Fraction II	89.79	12.6[b]

[a] Washing unnecessary since no isopropanol was present.

[b] This figure represents the amount of protein in solution after two washings with distilled water.

Table 12. Organoleptic evaluation of fresh modified imitation milk prepared from combinations of three types of soybean protein and milk protein[a]

Protein			
Type of soybean protein	Total protein[b]		Flavor criticisms
	Soybean protein (%)	Milk protein[c] (%)	
Isolate	25	75	Very beany, grainy, tart
Isolate	50	50	Very beany, grainy, tart
Fraction I	25	75	Slightly beany, grainy, tart
Fraction I	50	50	Slightly beany, grainy, tart
Fraction II	25	75	Moderately beany, grainy, tart
Fraction II	50	50	Moderately beany, grainy, tart

[a] Modified imitation milk consisted of above concentrations of soybean and milk protein in addition to 3.5% soybean oil, 0.05% K_2HPO_4, 8% corn sirup solids (24DE) and 0.1% Myvatex 8-20 emulsifier dispersed in an aqueous continuous phase.

[b] Total protein content of 3.2%.

[c] Milk protein added in the form of skimmilk.

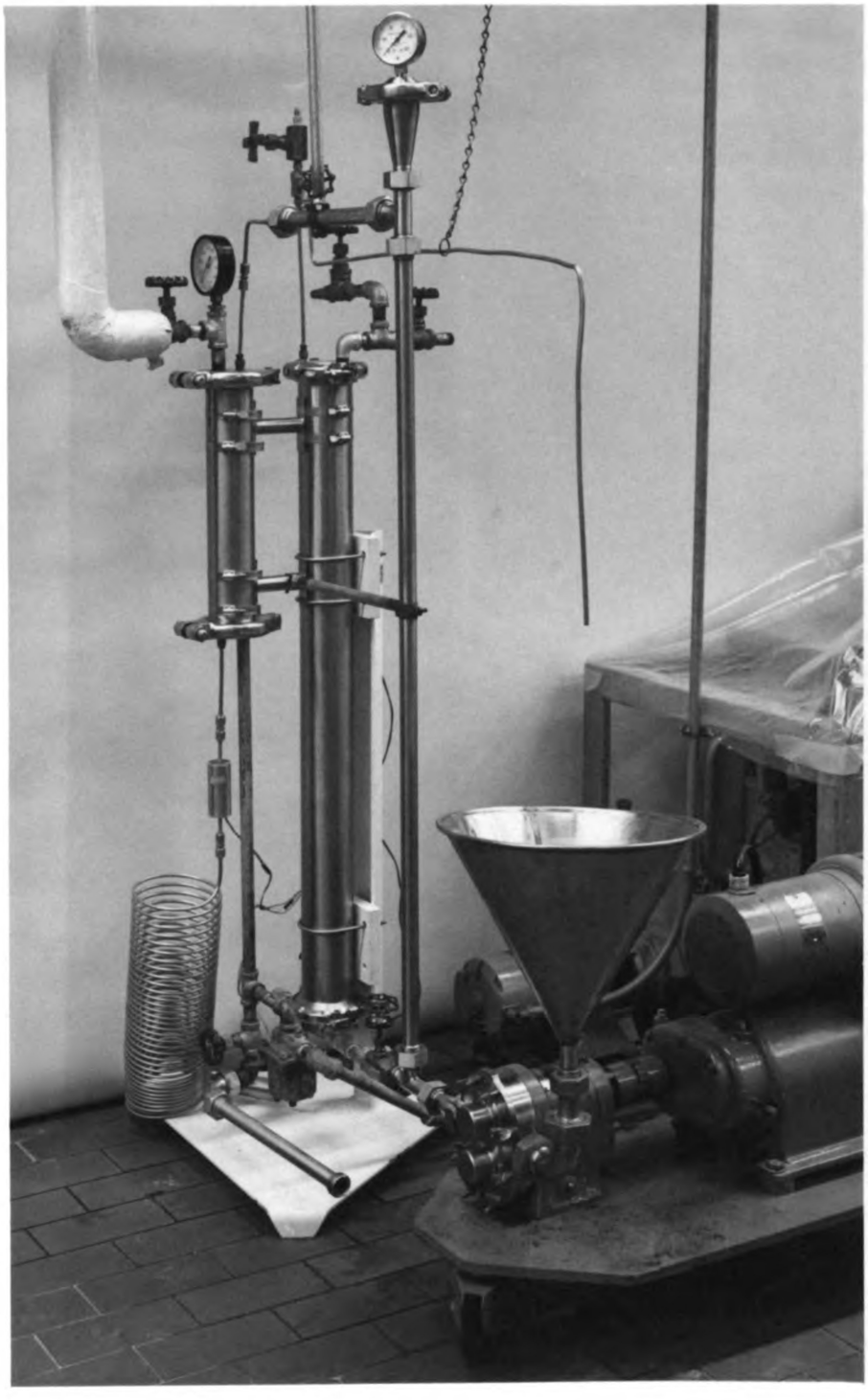

Figure 1. High temperature short-time (HTST) heat exchanger used to pasteurize milk.

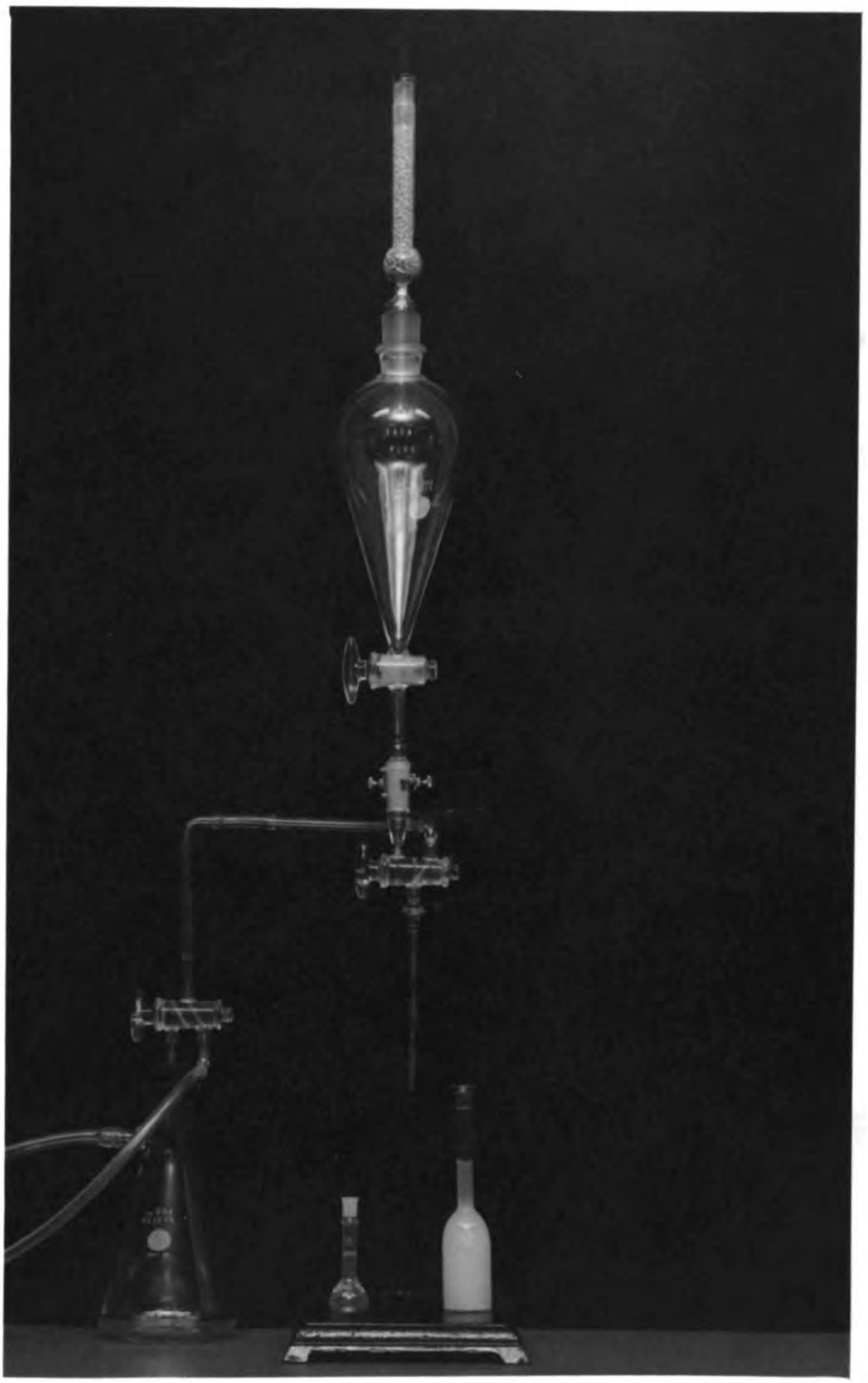

Figure 2. Semi-automatic peroxide apparatus designed for delivering aliquots of lipid from extraction bottle to peroxide flask.

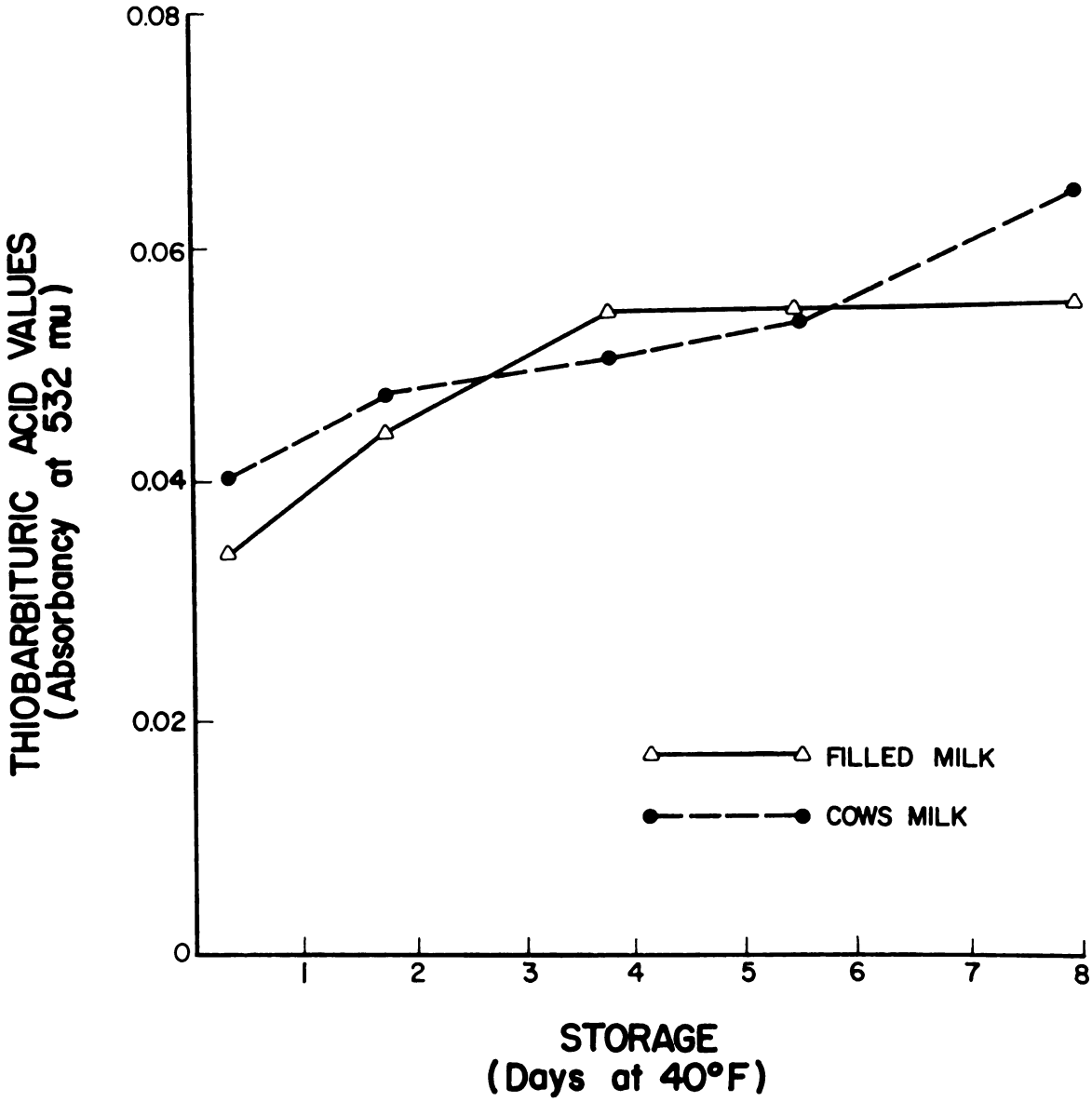

Figure 3. Thiobarbituric acid values of fat extracted from cow's milk and soybean oil filled milk during storage at 40 F for 8 days.

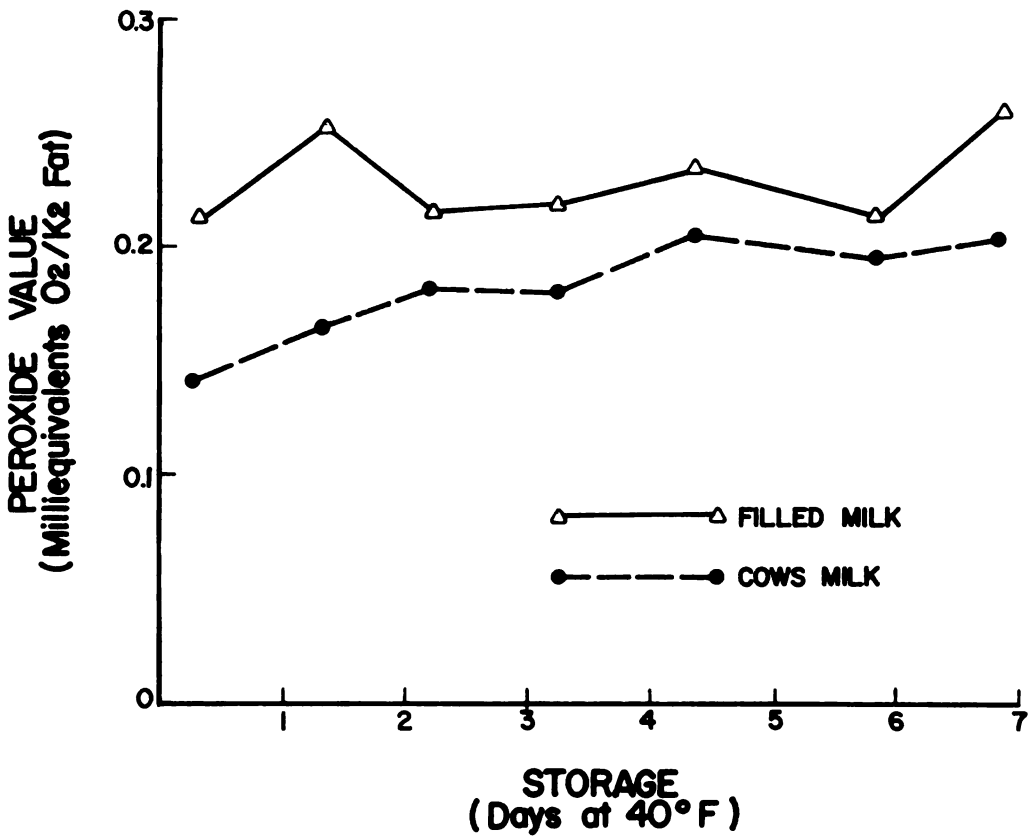

Figure 4. Peroxide values of fat extracted from cow's milk and soybean oil filled milk during storage at 40 F for 7 days.

DISCUSSION

Filled Milk Trials

The data in Table 1 indicate that the only acceptable highly unsaturated milkfat substitute, used in this research, was soybean oil. The oxidized flavor present in filled milks prepared with corn, cottonseed, olive, peanut and safflower oils respectively, indicates that undesirable autoxidation had occurred, probably after the oils had been refined. All of these oils were obtained in clear glass containers which allows the passage of visible light into their contents. Such light has the ability to catalyze the oxidation of unsaturated fatty acids.

Antioxidants which had been added by the oil seed processor to safflower oil did not effectively inhibit the development of oxidized flavor. Only the soybean and safflower oils of the group of oils evaluated contained antioxidants which had been added at the time of processing and refining.

The stability of the coconut fat and Hydrol 92 to oxidation is due to the very low content of unsaturated fatty acids. This is reflected in the iodine values (Table 3) of these fats.

The data presented in Table 1 do not substantiate earlier reports by Klis (1968) that PG-25 was an acceptable milkfat substitute. This fat proved to have a pronounced oxidized flavor when used to totally replace milkfat in the formulation of filled milk.

Kaola, a fat slightly more unsaturated than milkfat, proved to be an acceptable milkfat substitute when the product was first formulated (Table 1) but after 6 days of storage at 40 F, the filled milk had developed an objectionable oxidized flavor (Table 2). Kaola has been used in the preparation of frozen desserts as well as filled milk (Food

Processing, 1969); however, the filled milks prepared with the samples of Kaola available for this project were unacceptable.

The undesirable characteristic flavor of the corn and peanut oil also seriously limited their usefulness in this study.

The resistance of the soybean oil to oxidation may be attributed in part to the lower content of linolenic acid which is prone to rapid autoxidation. Light and selective hydrogenation of this oil results in the conversion of some of the naturally occuring cis isomers of the fatty acids to geometric isomers which are more resistant to oxidation. In addition, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) had been added after refining to help retard oxidation. The soybean oil was also packaged in amber glass bottles which may have also retarded oxidation of the oil. The resistance of the soybean oil to oxidation is reflected in both the TBA and peroxide values (Figures 1 and 2 respectively).

There is a close relationship between the degree of oxidized flavor and peroxide value, with a few apparent exceptions (Table 1). The presence of oxidized flavor in the original cow's milk, standardized to 3.5% milkfat, indicates that this off-flavor was apparently due to spontaneous oxidation of the cow's milk.

Use of the semi-automatic transfer device in the peroxide value determination (Figure 3) has several advantages: only one pipet is required which eliminates variation of delivery inherent in each pipet; rinsing of residual lipid from the pipet with solvent results in a higher and more uniform delivery weight; samples of extracted lipid can be transferred more quickly from the Babcock cream test bottle to the 10 ml volumetric flask when the semi-automatic device is used.

The Myverol 18-30 and 18-85 emulsifiers contributed some bitterness to the filled milk when used at 0.1% based on the formula weight. This bitterness may be a function of unsaturation and/or fat source. Miller (1960) indicated this flavor may be due to trace impurities of free fatty acids or fatty acid soaps in the monoglyceride.

The data in Table 8 reveal that Myverol 18-07 is a more efficient emulsifier than the Myvatex 8-20 when filled milk samples are HTST pasteurized and single homogenized. Such differences would indicate the hydrophilic to lipophilic balance (HLB) of the 18-07 monoglyceride emulsifiers is better suited to stabilizing the soybean oil in skimmilk emulsion than is the Myvatex 8-20. The HLB data were not available for the Myverol 18-07 and Myvatex 8-20 emulsifiers used in this study. When the soybean oil filled milk prepared with either Myverol 18-07 or Myvatex 8-20 monoglyceride emulsifiers was first homogenized, then pasteurized, the homogenization efficiency exceeded 10%. This indicates the heat treatment during pasteurization had detrimental affects upon the emulsion formed during homogenization.

The origin and characteristics of the sulfide-like off-odor and off-flavor produced at the various time-temperature relationships (Table 10), needs to be studied in greater detail. Senti (1963) states that little soybean oil goes into liquid cooking oils because of flavor and odor problems encountered at high temperatures in iron cooking utensils. The sulfide like off-odor and flavor in the soybean oil filled milk, pasteurized at high temperatures in the HTST heat exchanger, may be due to a lipid-protein interaction since the temperatures used result in serum protein denaturation and activation of sulfhydryl groups in these proteins.

Imitation Milk Trials

Imitation milk prepared with calcium caseinate as the protein source was not acceptable when the fresh product was evaluated organoleptically (Table 10). The flavor criticisms of flat and watery, made when 1 and 2% calcium caseinate were used, can be attributed to the low protein content. When the protein concentration is raised to 3% the product was still watery but the predominant astringent and bitter flavors are probably attributes of the protein and/or method of preparation of this chemical. Off-flavors may have also developed in storage following preparation of the calcium caseinate.

The soybean protein isolate, Fractions I and II, also failed to make an acceptable imitation milk when used in combination with skimmilk to constitute the protein portion of an imitation milk product. Fractionation of the isolate did reduce the beany flavor in the fractions recovered; however, in so doing the protein of the respective fractions was denatured to the extent where neither would solubilize sufficiently to stay in solution. Fractions I and II appeared to go into solution even more easily than the isolate from which they were fractionated but results showed that both fractions actually settle out during storage for 24 hours at 40 F. Fractions I and II may have been denatured by the isopropanol to the extent where the hydrophobic groups were buried deep in the molecule, with the hydrophilic groups projecting out from the surface. When the protein molecule is oriented in this manner, it would wet easily with water but never go into solution properly because of the internal hydrophobic nature of the molecule.

Treatment of soybean protein with alcohol is only one of the numerous methods that have been used to reduce the degree of beany and bitter flavor associated with soybean protein. This area is currently receiving a great deal of consideration by industry and it is quite conceivable that in the near future a very bland and acceptable milk protein substitute will be produced from soybeans.

SUMMARY

Filled milks were formulated from fresh skimmilk, twelve types of vegetable oils or fats and four types of monoglyceride emulsifiers. All milk samples were pasteurized at 170 F for 8 seconds (except in certain off-flavor studies), homogenized at 500/2500 psi and cooled at 36 F. Of the twelve fats and oils used, soybean oil was the only highly unsaturated oil which proved to be an acceptable milkfat substitute in filled milks stored at 40 F for eight days. A panel of four experienced judges was used for the purpose of evaluating the filled milk samples. Thiobarbituric acid and peroxide values were determined initially and during storage of soybean oil filled milk and cow's milk. There was only a very slight increase in the TBA and peroxide values of both the cow's milk and filled milk during these storage studies.

The more unsaturated monoglyceride emulsifiers (Myverol 18-30 and 18-85) tended to impart bitter flavors to the filled milk when used at 0.1% (based on weight of product). Myverol 18-07 and Myvatex 8-20 were acceptable when used at the recommended level of 0.1% based on the formula weight. Of these two emulsifiers, only the Myverol 18-07 had an acceptable homogenization efficiency of 10% or less when the soybean oil filled milk was HTST pasteurized at 170 F and single homogenized.

Development of a very undesirable sulfide-like odor and taste resulted when extremely high pasteurization temperatures were used. The off-flavor was evident at 205 F with an 8 sec. hold and at 225 F with a 4 sec. hold. Further reduction of the holding time to 0.3 sec. eliminated the off-flavor.

Imitation milks were formulated from low dextrose equivalent corn sirup solids, dibasic potassium phosphate, monoglyceride emulsifier and fat or oil dispersed in an aqueous continuous phase. The protein content

of these milks was varied between one to three percent, using fresh calcium caseinate. A modified type of imitation milk was also prepared using soybean protein in combination with skimmilk. Regardless of the protein used in preparation of the imitation milks, all had an objectionable off-flavor which could be attributed to the protein used in preparing them.

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