

WHEY-MILK: A POTENTIAL MILK SUBSTITUTE FROM DIRECT-
ACID-SET COTTAGE CHEESE WHEY AND MILK

by

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INTRODUCTION

Growth of the cheese industry with its contribution to environmental problems, and high prices for alternative protein sources have focused attention on whey supply and utilization. Liquid whey is a valuable food source containing about 6.35--6.50% high quality solids. However, large amounts are still underutilized. An estimated 29.5 billion pounds of liquid whey were produced in 1975, with 25.3 billion pounds of sweet whey from "hard" cheese and 4.2 billion pounds of acid whey from cottage and cream cheeses. This was equivalent to total of about 2.0 billion pounds of whey solids (13, 46).

Increased amounts of liquid whey are dried, condensed, or used to produce lactose. In 1975, 766 million pounds of dry whey were produced, about 64 percent for human food and the rest for animal feed (46).

Municipalities insist that a cheese plant effluent entering their sewerage systems must be below 200 ppm of biological oxygen demand (BOD). But, in practice, the effluent of plant food wastes is about 3,500 ppm of BOD, while BOD of whey is 32,000 ppm or higher, requiring rotary vane oxidation treatment to reduce it to the acceptable level (37). It is very costly for cheese plants to reduce BOD of whey, whether through municipal sewerage systems or their own treatment system.

Cottage cheese plants are generally too small to produce enough whey for economical drying. Also acid whey does not withstand heat during drying as well as sweet whey, and dry acid

whey has relatively less utility value for food products (46). The cottage cheese industry usually uses pasteurized skim milk which is separated from grade "A" raw milk. In fact, cottage cheese usually is manufactured in plants that process both fluid milk and cottage cheese.

This experiment was designed to develop a new milk-like product subsequently referred to as "whey-milk" using whey, cream and raw milk which are readily available in dairy plants. If this product is found acceptable to consumers dairy plants could save a considerable amount of money in investment of whey disposal equipment or from paying penalties for dumping whey into the streams. Moreover, the dairy plants might sell such whey-milk at relatively low price.

The objectives of this research were to develop whey-milk that could be manufactured in a dairy plant; to study the acceptability of this product; and to determine its composition and shelf-life. In this study, whey was obtained from cottage cheese made by the direct-acid-set method, which is commonly adopted in the dairy industry (38). The acid whey was neutralized with potassium hydroxide, then standardized with raw milk and cream before being pasteurized by the low temperature long time (LTLT) method in the laboratory. The whey-milks were evaluated by either hedonic or triangle taste tests. Whey-milk also was manufactured and packaged in the Dairy Processing Plant of KSU evaluated by consumers by a paired comparison test.

REVIEW OF LITERATURE

Whey Availability and Markets

As estimated by the Whey Products Institute (WPI), total production of fluid whey for 1974 was 30.5 billion pounds, 26.4 billion pounds of sweet-type and 4.1 billion pounds of acid-type; and for 1975 was 29.5 billion pounds, 25.3 billion pounds of sweet-type and 4.2 billion pounds of acid-type. Further, the 30.5 billion pounds of fluid whey in 1974 and 29.5 pounds in 1975 are both equivalent to approximately 2.0 billion pounds of whey solids (13).

Utilization of dry and condensed whey for human food and animal feed, lactose, and whey solids in wet blends in 1975 amounted to 1,153 million pounds and accounted for 58% of the available solids. This represented the greatest amount used for drying and other processing in a five-year period and amounted to an increase of 285.8 million pounds of solids in 1975 compared with 1970. For 1975, the whey solids reported as "used for dry whey" included those in dry "whole" whey and modified whey products such as partially delactosed, partially demineralized, and partially delactosed/demineralized whey. Statistics on these products were collected and reported for the first time in 1975. Total production of dry whey (exclusive of modified whey products) in 1975 was 594.6 million pounds and accounted for only 28.7% of the available whey solids. Modified dry whey products by 1975 reached an annual production of about 171 million pounds. Together with whey solids in wet blends they accounted for much of the

increased utilization in recent years (56).

The results of a survey conducted by the Whey Products Institute indicated bakeries and dairies were the most prevalent users of dry whey in 1975, accounting for 62% of the dry sweet whey and 75% of the dry acid whey. For dry sweet whey, 85% of the total sales going directly to dairies was for frozen products.

The most common uses of modified sweet whey were in infant foods, dairy products, and prepared dry mixes. Modified acid whey products were heavily used in bakeries (46).

Problems and Treatment of Whey Pollution

Approximately 54% of the nutrients from milk are found in cheddar cheese whey, and about 73% of the non-fat milk-nutrients show up in cottage cheese whey (37). These nutrients, if unused, place costly burdens on sewerage systems. For example, the biological oxygen demand (BOD, 5-day) of fluid whey is 32,000 ppm. Fluid whey still is being dumped into available ditches, streams, rivers and even the oceans throughout the world. It is a practice not to be condoned because it is detrimental to the environment. In the absence of effective pollution laws and centralized whey driers, the problem will continue and worsen (37).

Major cheesemaking countries are hoping to convert whey into a valuable national asset, but there is a considerable difference between its disposal and utilization. Alternate disposal methods are to spray whey into a lagoon until the organic components are oxidized and to spray it over land as a natural fertilizer. Both methods dispose of considerable amount of whey, but ultimately

they become self-limiting (37).

Frank Groves (29) reported in the Whey Products Conference in 1972 that the most common methods of whey disposal in 1962 were: returning whey free to farmers, dumping it as wastes or sewage, selling it to processors, and paying processors to collect it. The change in whey disposal and utilization has been the result of rigid enforcement of whey disposal regulations, increased costs of labor and equipment, high cost operating return routes. The small cheese factory is still in a disadvantageous position in whey disposal because its volume is too small to justify installing disposal or processing equipment.

Composition of Sweet Whey and Acid Whey

Whey is the separated watery portion of milk usually obtained by acid, heat or rennet coagulation. It is opaque and greenish with total solids of 6.1 to 6.5% and a BOD of 32,000 ppm or higher (37). Whey contains approximately 50% of the original milk solids, i.e. most of the lactose, 20 to 24% of the protein and almost all of the vitamins (64).

Basically, there are two major types of whey; sweet whey from cheddar, Swiss and other rennet cheeses, and acid whey from cottage cheese and similar cheeses and acid casein. The typical composition of cheddar and cottage cheese wheys is shown below (16,50):

	Cheddar cheese whey	Cottage cheese whey
Protein	0.62%	0.70%
Non-protein nitrogen	0.19%	--

	Cheddar cheese whey	Cottage cheese whey
Fat	0.04%	--
Lactose	4.60%	4.50%
Ash	0.56%	0.60%
Total solids	6.10%	6.50%

Direct-acid-set cheese making utilizes phosphoric or hydrochloric acid acidulants which change the whey character. The acidified whey powder does not contain lactic acid, but contains higher phosphorus or chloride ions (48).

The proteins of whey derived from cow's milk have been described by Larson and Rolleri (44) as containing lactalbumin 68.1% (which contains α -lactalbumin 19.7%, β -lactoglobulin 43.7%, and serum albumin 4.7%), proteose-peptone 18.9% and immunoglobulin 13.0%.

Webb et al. (66a) indicated that sweet fluid whey contains about 5% lactose, but differs according to the amount fermented during the setting of cheese. There is usually 0.85% protein and 0.75% ash in acid whey, and 1.10% protein and 0.50% ash in sweet whey. The difference in ash is mainly due to the content of calcium phosphate, which is present in acid whey in appreciable proportions, but not in sweet whey. The latter contains a small amount of precipitable protein which accounts for the greater amount of protein, and makes isolating the lactose more difficult.

Nutritional Properties of Whey Protein

Whey proteins have a relatively high concentration of lysine which is deficient in wheat and rice protein. Whey proteins also

contain adequate tryptophan, the limiting amino acid in corn (25). It has been reported that the addition of only 4% whey protein to corn-meal can more than double its protein efficiency ratio (PER) (68).

The lysine content of whey protein is a approximately 10%, however, lysine is sensitive to heat in the presence of reducing sugars. This lowers the level of available lysine. Different methods of preparation of spray dried whey protein concentrates affect the availability of lysine ranging from 60 to 94% for electro dialysis and ultrafiltration, respectively (32, 52). Morr et al. (52) found an inverse relationship between the amount of available lysine and the residual lactose (reducing sugar).

A recent study undertaken by Erdmen et al. (23) demonstrated the nutritive value of fractionated-whey protein, bacterial cells and ammonium lactate recovered from L. bulgaricus continuous fermentation of cottage cheese whey. Based on feeding trials with rats, the recovered protein fraction had a PER of $4.1 \pm .15$ which was higher than the PER of $3.34 \pm .26$ for casein.

Various whey products were studied by Forsum and Hambraeus (26) for the degree of protein denaturation and protein nutritional qualities. The biological value and true digestibility of whey proteins were high in all products although individual whey proteins showed varying degrees of denaturation. Furthermore, all whey products contained a surplus of available lysine as compared to the content of the Food and Agriculture Organization (FAO) reference amino acids pattern.

The proteins in cottage cheese whey were comparatively stable

to heat; 91 C for 30 min was required to denature 80% of the protein in whey, whereas 81 C for 30 min denatured the same amount of proteins in skim milk (30).

McDonough et al. (49) reported that all essential amino acids were in excess in whey protein concentrate when compared with reference protein of the World Health Organization (WHO). Feeding trials of rats indicated the bioavailability of the dried concentrates was higher than that of both casein and skim milk powder. Addition of dried whey concentrate to nonfat dry milk as a 40% blend raised the PER of nonfat dry milk from 2.51 to 2.83.

Problems of Acid Whey Treatment

Acid whey is more difficult to dry. The higher the acidity of the whey, the more acute are the problems. Defects such as improper crystallization, corrosion of drier parts, and development of brown burnt particles occur particularly when whey is neutralized (40). More acid in the whey requires higher drying temperatures. According to Young (69), the easiest drying is achieved with heat denatured whey protein and simultaneous heat shocking to give satisfactory crystallization. New concepts have been developed by DeLaval, NIRO, and Pillsbury for drying acid whey. Essentially, a two-stage drying effect is created in which continued crystallization of lactose to a crystalline alpha-hydrate from occurs in the first stage (wet powder) to achieve nonhygroscopicity of the final powder with 4 to 5% water. If crystalline alpha-hydrate crystals are not formed, the lactose glass of the whey

powder absorbs water easily making the powder sticky (37).

A study made by Mavropoulou and Kosikowski (48) indicated that free amino acids and soluble peptides are present at higher significant level in acid whey powder than in sweet whey powder. Such nonprotein nitrogen compounds are derived from milk, casein, soluble protein, and microbial cells and they migrate into the fluid whey, exerting a potentially important influence on functionality and flavor.

Studies of flavored drinks made with cottage cheese whey by Demott et al. (17, 18) indicated that development of a suitable drink from cottage cheese whey has been hampered somewhat by the carry-over of culture flavor into the drink. However, by the use of whey from the direct-acid-set method, this objection was avoided, yet it had approximately the same composition.

Previous Studies of Whey Beverages

Using cheese whey as a beverage, especially for therapeutic uses, can be traced back to the ancient Greeks. Hippocrates, in 460 B.C., prescribed whey for an assortment of human ailments. In the middle ages, whey was recommended by many doctors for varied diseases, and, by the mid-19th century, whey cures reached a high point with the establishment of over 400 whey houses in West European countries (63, 64). As late as 1942 in Spas in Central Europe, dyspepsia, uremia, arthritis, gout, liver diseases, anemia and even tuberculosis were treated with ingestion of up to 1.5 kg of whey per day (58).

A. Beverages resembling soft drink

The cheapest, most efficient method of preparing a whey-based beverage is to drain whey from the cheese vat, pasteurize, deodorize if desired, flavor appropriately, and package for later consumption (33, 34). One of the best known commercial whey beverages is Rivella (1) which was originally developed in Switzerland and is now manufactured in Holland, Australia and Germany. The product is based on lactic fermentation of clarified whey followed by addition of sugar, fruit flavors and carbonation.

The utilization of whey in combination with other materials to manufacture a fruit flavored beverage was described by Guy, et al. (31). Soya flavor was combined with either sweet or cottage cheese whey and mixed with sugar, citric acid, stabilizer and flavoring, the mixture was homogenized, concentrated under vacuum and vacuum shelf-dried. The product had an acceptable citrus flavor and a representative sample of the beverage had a pH of 4.4 and contained 16.5% solids.

Kosikowski (38, 39) described an acceptable beverage made by incorporating up to 6% acid whey powder into reconstituted frozen orange juice. The blend contained 2.5 times the amount of protein of orange juice alone. The acid whey powder at 6% imparted a slight salty taste, but when the content was reduced to 4%, tasters rated the flavor of the product excellent.

Besserezhnov (7) prepared a yoghurt flavored beverage with freshly pasteurized sweet whey inoculated with a 10% culture of Lactobacillus bulgaricus, L. acidophilus, L. helveticus, L. casei and Streptococcus thermophilus. After 24 h incubation,

the product was cooled and packaged.

Engel (22) patented a method of making a whey product which consisted of preparing a yeast culture of whey, sugar and yeast. The method indicated the products were kept at different temperature for different periods of time. O-way, a product developed at Michigan State University (11), was conceived as a breakfast meal incorporation either sweet or acid whey and orange juice. One volume of fresh orange concentrate was mixed with four volumes of dedorized whey and packaged. It contained .7 to 1.0% protein.

Researchers at University of Arizona (53) combined 25 to 40% whey with grapefruit juice and 7 to 20% other fruit juices and tested these drinks by sending them into homes as a commercially sterile canned product. A peach-grapefruit-whey combination received an average score of 5.9 on hedonic scale of 1 to 7. A second series of beverages, using Vinifera grape juice, whey, and 3% passion fruit juice also had good acceptance in preliminary studies. In another study, an orange flavored drink (54) containing 33% cottage cheese whey was rated 6.3 by 51 tasters whereas a nonwhey drink rated 4.7. Acceptable drinks containing 80 to 90% whey and flavored with 10% natural strawberry puree or 20% natural peach puree receive acceptable scores by panels.

Whey Kwas, a Polish fermented beverage (42, 65), was made from fresh sweet whey. After separation and deproteinization, whey was cooled to 42 C and inoculated with 5% thermophilic starter. After 2-hr incubation, it was treated with yeast and

caramel coloring and packaged in bottles. After 40 h at 8 C, the Kwas was ready for consumption. It had a slightly lactic taste, a clear greenish-yellow color and an acidity not over 45 SH.

Holsinger and associates (35) fortified soft drinks with whey protein manufactured by ultrafiltration, followed by gel permeation, vacuum evaporation, and spray or vacuum shelf-drying. They demonstrated that carbonated beverages could be fortified with up to 1% whey protein without detectable change in flavor or appearance, provided the whey proteins were isolated in undenatured form.

Demott (18) recently developed an orange and lemon-lime-flavored drink which was formulated using cottage cheese whey made by the direct-acid-set method. Two levels of sweetness were used in each flavored drink. A group of 21 panelists scored the drinks on a 6-point hedonic scale. The panel preferred the orange-flavored drink over the lemon-lime ($p < .5$), describing the orange-flavored drink as "like moderately" and lemon as "like slightly". Also a tomato-flavored beverage was described by Demott et al. (19). Cottage cheese whey made by the short-set culture method was used to make tomato-drink by adding 6% dried tomato-spice flavoring material, and the product evaluated by 10 panelists to have a pleasing taste.

B. Milk-like beverages

Downhan (20) patented a process for making products claimed to resemble human milk. Sweet whey was homogenized with the following ingredients: cream, butterfat, milk sugar, sugar, skim

milk, sodium citrate. The finished beverages were reported to be useful for infant and invalid feeding.

Researchers at Michigan State University (11) prepared a product called Way-Mil, an imitation milk formulated from whey, selected vegetable hydrocolloids, and, in some applications, skim milk. The beverage contained 2.4% fat and 1 to 1.5% protein. The fat-protein dispersion was claimed to be physically stable for 3 to 4 weeks.

Edmonson et al. (21) and an anon (2) developed a sterile milk-like beverage from sweet whey and cream. This product, condensed to 35% total solids and flavored with chocolate, was sterilized then homogenized and canned aseptically. When reconstituted to 17.5% total solids. This product scored 6.5 on 9-point hedonic scale compared to 6.9 for commercial chocolate milks.

Bodmershof (8) prepared a sparkling beverage from a mixture of 40% sour milk, 50% whey, and 10% fruit juice. This concoction was bottled under 7 N/m^2 of carbon dioxide and was claimed to keep for several months.

A soy-whey milk made from soybeans and cottage cheese whey was developed at the University of Illinois (4). The beany flavor was destroyed by boiling the whole soybean before it was broken for incorporation into the whey. The product had a flavor resembling that of egg nog.

A whey-soy drink, developed under a joint project sponsored by USDA and AID, was described by Pallansch (57). The product was formulated as follows: sweet whey solids 41.7%, full fat soy flour 36.9%, soybean oil 12.3% and corn syrup solids 9.1%. Acceptability studies were carried out in six developing countries representing

a variety of cultures. Only one of these countries, Sierra Leone, found this product to be unacceptable.

Cerna (12) described an uperised aseptically packaged infant milk product produced in Finland and other European countries. The infant milk product with 13.6% total solids was formulated from 74% demineralized whey and 24.7% cream, with added lactalbumin, maltose, sunflower oil, barley flour and vitamins and iron.

Direct-Acid-Set Cottage Cheese

Historically, cottage cheese was made in farm-houses and home in Central Europe. Colonial America adopted the cheesemaking practices in its cottages, so the name of "cottage cheese" was derived. There are two types of cottage cheese, i.e. cultured cottage and direct-acid-set cottage cheese. The direct-acid-set method shortens the processing period, and has become popular, recently.

Deane and Hammond (15) originally applied acid anhydrides as mesolactide and D-glucono-delta-lactone (GDL) to skim milk. Although results were acceptable, the method was expensive and subject to long delay from the hydrolysis of the anhydride.

In 1965, little (45) patented a method for making cheese curd from milk comprising: (a) refrigerating milk to a temperature above freezing to about 60 F wherein coagulation will not occur within a pH range of 4.00 to 4.95. (b) setting milk while it is at said temperature by acidifying to a pH from 4.00 to 4.95 and adding proteolytic enzymes in amounts to effect casein coagulation while maintaining said milk at temperature to about 60 F. Another method was patented for Corbin Jr. and Somerset (14) in 1969. In the

patented procedure, cheese curd is manufactured by acidifying cold milk with free acid, heating to 80 F, and adding acidogen and proteolytic enzyme, allowing cheese curd to form, cutting curd after about 1 h and cooking.

Ernstrom (24) described an efficient apparatus known as a continuous curd former was developed to carry out the process whereby the acid-producing function of a culture starter was replaced by the direct addition of acid to cold skim milk in such a way that a definite curd structure was developed. In the continuous process, treatment of the milk with rennet appeared to increase the firmness of the final product. Direct acid, continuous cottage cheese process was described recently by Born and Muck (9). The commercial size process can be divided into four operations: acidification, curd formation, cooking and curd conditioning.

White and Ray (67) indicated that the direct-acid-set method needed only 33% cutting time that was needed in the conventional method. In addition, the direct acidification method required cooking time only 40% of that used in the conventional method.

Sharma et al. (61) reported that for cottage cheese made from milk with 3.1 ± 0.1% protein, yields averaged 14.6 and 13.8% for the direct-set and culture methods, respectively.

Sensory Evaluation

Taste testing in recent years has become highly sophisticated for determining flavor differences and preferences. The proper tests employed on a laboratory scale can provide food processors with invaluable information about consumer acceptance of food prod-

ucts. the tests supplemented with consumer panels can establish a reliable basis from which processors may plan marketing strategy(5).

The methods indicated by Larmond (43) are difference tests and preference tests. In difference tests the members of the panel merely are asked if a difference exists between two or more samples. Preference or acceptance tests determine representative population preferences. The total scores from trained panels can be used to predict preferences obtained by panels of 100 to 160 untrained persons.

The Manual on Sensory Testing Methods (3) emphasized that sensory testing requires special controls of various kinds. If they are not employed, results may be biased or sensitivity may be reduced. Most of these controls depend directly upon the physical setting in which the tests are conducted. Major ones include control of irrelevant odor stimulation, elimination of psychological distraction, provision of a generally comfortable work environment. In running a sensory evaluation, such conditions as samples and their preparation, panelists, testing conditions and questionnaires should be well-studied beforehand in order to eliminate all influential factors that may contribute biases.

The consumer test is used to measure acceptance of a product. Although the fate of a food product depends on consumer acceptance, formal studies of consumer preference are recent. Consumer studies are completely separated from laboratory panels which do not attempt to predict consumer reaction (43).

An investigation conducted by Brant and Arnold (10) showed that evaluation methods reported most frequently include: triangle

test, used by 66% of the 56 responding companies; hedonic scale scoring 57% and paired comparison 55%. Multiple comparison, general preference, ranking, and degree of preference methods were used by approximately one third of the firms. Most employed more than one type of test procedure. Interpretation of sensory data is performed via manual calculation and computers. Twenty-two firms used both manual and computer calculation, 20 employed manual means only, and 3 used computers only. Most food companies actively applied statistical methods to interpret their sensory data.

Sidel and Stone (62) emphasized that accuracy of information provided by the sensory study will depend upon selection of an appropriate experimental design and appropriate analysis of the data. Experimental design refers to all the primary components of a sensory experiment. These components are: product objective, test objective, testing environment, samples, judges, response forms, serving procedure, and data analysis. The experimental design should be selected well in advance of the sensory test and should be chosen to at least provide: (a) an unbiased estimate of the effect to be measured, (b) a valid estimate of the variability of the estimated effect, (c) an opportunity to use a simple mathematical model in the analysis of data and for testing a specific hypothesis concerning the true effects and (d) efficiency in terms of cost per unit of information.

To design an experiment, Prell (59) also pointed out one should state the objectives clearly. The objectives should be classified as major and minor, since certain experimental designs give greater precision for some treatment comparisons than for others. Another

should state the measurement to be made, sample variables and level of variables, replications, inherent variability, and physical limitations. The efforts made to reduce the experimental error should be described.

EXPERIMENTAL

Experiment 1. A "Whey-Milk" Made in the Laboratory

Introduction

Fresh pasteurized skim milk (71.6 C/ 16 sec) was obtained from the KUS Dairy Plant and used to make cottage cheese by the direct-acid-set method. A laboratory process was designed to simulate the plant process. Instead of collecting curd, cottage cheese whey was collected as a starting material to make whey-milk. The acid whey first was neutralized to pH 6.6 to 6.7 with either potassium hydroxide or sodium hydroxide. The neutralized whey the was centrifuged to eliminate any residual curd particles.

In Experiment 1, a total of 15 vats of cottage cheese were made under controlled conditions. The whey-milk was made by combining pasteurized whey, whole milk, and cream to meet a compositional standard. The finished product was evaluated by a group of trained panelists to determine its acceptability.

Objectives

The main objectives of this experiment were to:

1. determine the possibility of making an acceptable milk-like product by blending neutralized acid whey and milk.
2. compare the organoleptic properties of the whey-milk and regular milk.
3. predict consumer acceptance of the whey-milk through a consumer taste panel.

4. study the composition and shelf-life of the whey-milk.

Materials and Methods

Laboratory process for making cottage cheese whey. The experimental cottage cheese whey was collected by the direct-acid-set methods. The cheese was made in an 8-1 stainless steel tank which was placed in a temperature controlled water bath. The direct-acid-set method developed by Vitex Laboratories (27) was modified to facilitate the manufacture of cottage cheese in the experimental vat. Refrigerated skim milk was acidified to pH of 4.95 to 5.00 with a special food grade acidulant, Vitex 750TM, which was diluted with an equal volume of distilled water. Acidified milk then was heated to 26.5 C. \pm .5 C, pH measured and a specified amount of D-glucono-delta-lactone (GDL) added according to the pH value of the heated-acidified milk (see Table 9 in the Appendix). The GDL, dissolved in water, (1 kg/1.389 l of water) was added to the milk after the milk temperature reached 32 C. After this, Vitex coagulator (51.6 ml/378 l of milk) was diluted with a least 5 times its volume of cold distilled water and added to the milk. The milk in the vat was allowed to set and coagulate for 60 \pm 5 min. The curd was cut at a pH 4.6 to 4.7 using horizontal and vertical 1-cm cheese knives and further acidified with Vitex 750TM (88.5 ml/378 l of milk) to prevent matting. The curd was stirred constantly during cooking up to 54 C and held for 15 min. The whey was drained into -----
TM--Vitex 750, a starting-acid for cheese, contains phosphoric acid, lactic acid, diacetyl, artificial flavors.

a clean flask through a sieve and refrigerated until used to make our whey-milk.

Whey treatment. The collected-whey was held at 4 ± 1 C and its pH measured. Usually, the pH was 4.3 to 4.4 after acidification of the cut curd with Vitex 750TM. The whey then was neutralized to pH 6.6 to 6.7 with a 50% aqueous potassium hydroxide solution (8 g/l of whey). This liquid potassium hydroxide solution was added slowly to the whey while stirring vigorously. The mixture then was clarified in a centrifuge at 1,000 to 1,200 r.p.m. for 5 min and the clear whey decanted.

Formulation of whey milks of different compositions. The whey-milk consisted of neutralized whey, cream and KSU milk of 3.25% fat content. The finished product contained 3.25% fat. Whey and cream were heated separately up to 63 C in a water bath then transferred to a temperature-controlled bath with an automatic shaker and held separately at 63 ± 1 C for 30 min to pasteurize (28, 41). In this experiment, whey was added to KSU milk so as to contain 30%, 40% and 50% whey. In order to find whether additional lactose would affect the sweetness of finished product, 0.5%, 1.0%, 1.5% of additional lactose was added to the whey-milk containing 40% whey. Milk and cream added to standardize the whey-milk to 3.25% fat.

Analytical methods

A. Microbiological tests. The Standard Plate Count (SPC) method described by Housler (36a) was used to determine the viable

bacterial populations of whey-milk during storage. Coliform counts were made using Difco dehydrated violet red bile (VRB) agar, prepared according to Difco's directions 24-48 h prior to use. Procedures used for enumerating coliform colonies were those recommended in Standard Methods (36b).

B. Chemical analyses. Fat content of both the KSU milk and whey-milk were determined by the Babcock method as described in A.O.A.C. (6a). Protein concentrations were analyzed by a dye-binding method (36c) using protein analyzer--Model L. Part No. L-1,000 (Udy Analyzer Company). The method is based upon the fact that proteins have an affinity for certain dyes (i.e. Orange G) and form insoluble complexes with them. In this procedure milk was added to a dye solution and mixed thoroughly. The insoluble dye-milk complex was removed by filtration and the clear solution of uncomplexed dye remaining was determined spectrophotometrically and related to absorbed dye and protein concentration.

Total solids were determined by the Mojonnier gravimetric method (51) and ash by the A.O.A.C. method (6b). About 5 g prepared samples were weighed in platinum dishes, evaporated to dryness on a steam bath and ignited in a furnace at 550 C until the ash was carbon free. The residual ash was weighed and calculated as percentage ash. Solutions of ash obtained from the experimental milks were used to determine calcium either by atomic absorption or atomic emission and potassium and phosphorus by the atomic emission method only (6c).

The colorimetric phenol-sulfuric acid method of Marier and Boulet (47) was used to determine lactose in our experimental milk.

In this method, 2.0 ml of a test solution, or a standard lactose monohydrate solution or water was pipetted into a colorimeter tube, followed by 0.10 ml of 89% w/v phenol. Six milliliters concentrated sulfuric acid was added slowly, letting the acid run down the side of the tube, the mixture swirled to obtain good mixture, and allowed to stand for 10 min at room temperature. The lactose contents of milk samples were calculated based on the absorbance value of standard lactose solution at 490 μ m.

Sensory evaluation. Preparation of samples, testing conditions and design of the questionnaires were in accordance with recommended procedures (3, 5, 43, 55). In Experiment 1, a group of 11-13 trained panelists were invited to judge the experimental milk products. A set of three-digit random numbers were assigned to each sample so that the panelists could receive samples coded differently. Each time, no more than six samples were presented for the panelists to evaluate. Since a panelist's eating habits might affect test results, the taste panels usually were scheduled at 10:00 A.M. or 3:00 P.M. Deodorized distilled water at room temperature was available for the panelists to rinse their mouths between each sample. Other conditions such as temperature of sample (15.5 C), order of presentation and type of containers also were carefully controlled.

The following procedures for judging milk were suggested by Nelson and Trout (55).

1. remove the lid and smell the contents of the container at once to detect any odor that might be present.

2. take a generous sip and roll the milk around in the mouth, during which time the mouth was closed and the breath slowly passed out through the nose.
3. expectorate the sample.
4. inhale slowly through the mouth.
5. rinse cups or glasses with water before new samples are poured into them and to rinse mouth with water between each samples.

These procedures were followed for all whey-milk organoleptic evaluations other than the consumer survey.

A. Hedonic test. A hedonic test system was used to determine both difference and preference. The score card used by panelists is shown in Fig. 1 in the Appendix. The panelists were presented about four samples and asked to rate the flavor for each sample on the nine-point scale. The points on the scale were associated with descriptive terms, such as "dislike extremely", "dislike moderately", "neither like nor dislike", "like moderately", etc. Numerical values then were assigned to each descriptive term, starting with one, for "dislike extremely" and ranging up to nine for "like extremely".

Results from the hedonic test were analyzed by an analysis of variance. The F-ratio was interpreted by reference to standard tables in which values indicate significance at 5% and 1% levels. F-ratio is obtained by the following equation:

$F = \frac{MSs}{MSe}$, Where: MSs = Sum of square of samples/degree of freedom of sample.

MSe = Sum of square of error/degree of freedom of sample.

A significant difference among the samples was established by comparing the F-ratio with table values.

B. Triangle test. In the triangle test, each panelist received three coded samples, two of them identical and one different. The panel members were asked to taste the samples and indicate on a form (see Fig. 2 in the Appendix) which were paired samples and which were the odd ones. If a panelist could not detect a flavor difference between the three samples, he was instructed to make a guess. Each panelist therefore had one opportunity in three of correctly identifying the odd sample by chance alone.

The classical t-Test was used to analyze this data. The t-value was obtained from the following equation:

$$t = \frac{\text{observed proportion} - \text{theoretical proportion}}{SE_{\text{per}}}$$

$$\text{Where } SE_{\text{per}} = \left(\frac{p \times q}{N} \right)^{\frac{1}{2}} = \left(\frac{.333 \times .667}{\text{No. of panelists}} \right)^{\frac{1}{2}}$$

p = probability of successful selection

q = probability of unsuccessful selection

T-value was interpreted by reference to t-table in which the values are shown to indicate the significance at 5% and 1% levels.

Experiment 2. Whey-Milk Made in the KSU Processing Plant from Commercial Whey

Introduction and Objectives

Cottage cheese whey was provided by the Meadow Gold Plant, Beatrice Foods, in Topeka. After the acid whey was neutralized and clarified to eliminate curd sediment, whey-milk was formulated

using whey, cream, raw milk and dry skim milk, and then pasteurized and homogenized. The final product was standardized to contain 3.25% fat and 40% whey. In this experiment, a total of three batches of whey-milk were processed at the KSU Dairy Plant. One $\frac{1}{2}$ gallon of experimental product and one $\frac{1}{2}$ gallon of KSU homogenized milk were sold for the price of one to customers with the provision that the customers evaluate the milks.

The experiment was designed to establish, if whey-milk could be prepared on a pilot or semi-commercial scale using conventional dairy processing equipment. And to determine the milk's acceptability by subjecting it to evaluation by consumers.

Materials and Methods

Preparation and processing of whey into whey-milk. Cottage cheese whey was neutralized to pH 6.7 with potassium hydroxide. The neutralized whey then was clarified to obtain clear whey. Whey-milk was standardized to contain 40% whey and 3.25% fat with cream, raw milk, and whey. In one trial additional 0.5% NFDM was added. After thorough mixing, the raw blend was pasteurized at 71.6 C for 15 seconds and homogenized at 2,000 lbs/in (28, 41). The finished product was packaged in $\frac{1}{2}$ gallon Pure-pak cartons.

Analytical methods. The microbiological tests and chemical analyses were the same as described in Experiment 1.

Consumer evaluation. In this experiment, a paired-comparison test was employed to evaluate the experimental product. Each consumer was presented with coded samples, i.e. the regular KSU milk

and experimental whey-milk, and asked to identify the regular milk. In addition, each consumer also was asked to indicate a preference for one of the two samples.

A t-Test was employed to analyze the percentage of correct responses in the paired comparison test in this experiment. However, p and q values of theoretical proportion were all 0.50.

RESULTS AND DISCUSSIONS

Experiment 1. Whey-Milk Made in the Laboratory

Preference Test

Part of cottage cheese whey used in the experiment first was neutralized with calcium hydroxide (5.25 g Ca(OH)/1 of whey) to adjust the pH from 4.4 to 5.8 and further with potassium hydroxide (1.99 g/1 of whey) from 5.8 to 6.7. A second aliquot of whey was neutralized with potassium hydroxide only. Cream was added to both neutralized wheys to increase the fat content to 2.0% and 3.25%. From these treated wheys, four whey-milk samples were formulated. These four plus KSU milk were tasted and assigned hedonic scores by five judges. An analysis of variance showed that there was no difference ($P < .01$) between the samples or panelists scores. In other words, the four whey-milk samples were as good as the regular KSU milk. The results are shown in Table 1.

Whey-milk made from wheys neutralized with calcium and potassium hydroxide were similar in flavor. However, since calcium hydroxide yielded more precipitate we decided to neutralize with potassium hydroxide.

Difference Test

The triangle test was adopted to differentiate the whey-milk with various whey concentrations from control milk. Whey-milk containing 30%, 40% and 50% whey arranged separately by random with control milk for the panelists to select the odd sample out

of the three in each group. This test was conducted twice using 11-13 panelists. Results are shown in Tables 2 and 3. From statistical tables (3), I found that milk containing 50% whey could be differentiated ($p < .05$) while that containing 40% whey or less did not show any significant difference.

Another triangle test also was used to differentiate whey-milk samples containing 40% whey with addition of 0.0%, 0.5%, 1.0% and 1.5% lactose from KSU milk. These results presented in Table 4 show that whey-milk with 1.0% additional lactose or higher was significantly different ($p < .01$) while addition of 0.5% lactose or less will not affect the taste.

Experiment 2. Whey-Milk Made in the KSU Processing Plant from Commercial Whey

Difference Test

Since panelists in Experiment 1 could not distinguish the whey-milk containing 40% whey from the regular milk by the triangle taste test, Experiment 2 was consumer evaluation. The finished product was distributed through the University Dairy Sales room evaluated by customers in their homes. A questionnaire was included with the samples (Fig. 3 in Appendix). Consumers were instructed to complete the questionnaire and return it in the self addressed-stamped envelope.

The modified paired comparison test was adopted to evaluate the product. The results from Trial 1 in Table 5 showed that 74 out of 104 tasters were able to identify the correct coded

Table 1. Hedonic scores and analysis of variance of KSU milk vs. whey-milks made from cottage cheese whey.

Panelists	Samples 1, 2					Total
	A	B	C	D	E	
Scores ³					
1	8	6	7	8	6	35
2	6	6	6	5	6	29
3	7	7	8	7	3	32
4	4	4	4	7	7	26
5	<u>4</u>	<u>5</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>27</u>
Total	29	28	30	33	29	149
Ave score	5.8	5.6	6.0	6.6	5.8	

- All whey-milk contained 50% whey and 50% KSU milk
- A= Whey-milk of 3.25% fat, whey neutralized with KOH + Ca(OH)₂.
 B= Whey-milk of 3.25% fat, whey neutralized with KOH.
 C= Whey-milk of 2.00% fat, whey neutralized with KOH + Ca(OH)₂.
 D= Whey-milk of 2.00% fat, whey neutralized with KOH.
 E= KSU milk of 3.25% fat.
- Scoring range: 9 for "like extremely" --- 1 for "dislike extremely".

Table 2. Triangle test results comparing whey-milk containing 30%, 40% and 50% whey and regular KSU milk (Trial 1.).

<u>Judgement</u>	Sample group 1,2		C*
	A	B	
Correct	5	5	8
Wrong	6	6	3
<u>Degree of difference</u>			
Slight	4	4	6
Moderate	1	1	1
Much	0	0	1
Extreme	0	0	0
<u>Acceptability</u>			
KSU milk	4	2	5
Whey-milk	1	3	3

1. all samples contain 3.25% fat.
 2. A = KSU milk vs. whey-milk containing 30% whey.
B = KSU milk vs. whey-milk containing 40% whey.
C = KUS milk vs. whey-milk containing 50% whey.
 3. Degree of difference and acceptability were recorded only for those making correct judgements (43).
- * Significance at ($P < .05$).

Table 3. Triangle test results comparing whey-milk containing 30%, 40% and 50% whey and regular KSU milk (Trial 2.)

<u>Judgement</u>	Sample group 1,2		C*
	A	B	
Correct	6	6	7
Wrong	6	6	5
<u>Degree of difference</u>			
Slight	5	4	1
Moderate	1	1	6
Much	0	1	0
Extreme	0	0	0
<u>Acceptability</u>			
KSU milk	4	3	5
Whey-milk	2	3	2

- all samples contained 3.25% fat.
 - A = KSU milk vs. whey-milk containing 30% whey.
B = KSU milk vs. whey-milk containing 40% whey.
C = KSU milk vs. whey-milk containing 50% whey.
 - Degree of difference and acceptability were recorded only for those making correct judgements (43).
- * Significance at (P<.05).

Table 4. Triangle test results comparing whey-milk containing 40% whey with 0.0%, 0.5%, 1.0%, and 1.5% lactose added vs. regular KSU milk.

<u>Judgement</u>	Sample group 1, 2		C**	D**
	A	B		
Correct	7	8	9	11
Wrong	6	5	4	2
<u>Degree of difference</u>				
Slight	3	7	5	7
Moderate	1	1	2	3
Much	3	0	2	1
Extreme	0	0	0	0
<u>Acceptability</u>				
KSU milk	4	4	3	7
Whey-milk	2	3	6	4

1. All samples contained 3.25% fat.

2. A = KSU milk vs. whey-milk without added lactose.

B = KSU milk vs. whey-milk with 0.5% added lactose.

C = KSU milk vs. whey-milk with 1.0% added lactose.

D = KSU milk vs. whey-milk with 1.5% added lactose.

3. Degree of difference and acceptability were recorded only for those making correct judgements (43).

** Significance at (P<.01).

Table 5. Consumer evaluation of whey-milk by the paired comparison tests.

Samples selected as milk by consumers¹

Trial	Whey-milk ²	Milk	Total
1	30	74*	104
2 ³	42	55	97

Preference

Trial	Whey-milk ²	No preference	Milk	Total
1	17	35	60	112
2 ³	31	36	45	112

1. See Fig. 3 in Appendix.
 2. Whey-milk containing 40% whey.
 3. Fortified with 0.5% NFDM.
- * Significance at (P<.05).

sample. This meant that the tasters were able to differentiate between whey-milk and regular milk. As shown in Table 7, the total solids and fat contents of whey-milk made in Trial 1 were lower those of Trial 2. It appears that the whey-milk was diluted with water in pipelines and processing equipment. As a result, it tasted flat compared with the regular milk. An additional 0.5% NFDM was added to the whey-milk in Trial 2 to overcome the criticism of "flat" attributed to whey-milk in Trial 1.

The results of Trial 2 presented in Table 5 show that 55 of 97 tasters selected the correct sample. These indicated that consumers could not differentiate these two samples ($P < .01$).

Preference Test

Since whey-milk would have a definite economic advantage over regular milk, we compared those who preferred the whey-milk as well as those who did not discriminate against it (no preference) to those who preferred milk (60). By combining the 35 no preference responses with 17 that preferred whey-milk in Trial 1, 52 of the 112 consumers either would accept or prefer the whey-milk. In Trial 2 in which an additional .5% nonfat dry milk solids was added over half, 67 of 112, preferred or would not discriminate against (no preference) whey-milk (see Table 5). In Trial 2 almost half selected the whey-milk as regular milk.

In our paired comparison testing, family members appeared to respond less independently than individual tasters. Interesting, in Trial 2, 27 of 31 respondents indicated a preference for what they had incorrectly identified as milk. And 38 of the 45 correctly

identifying the milk indicated a preference for regular milk. The same pattern was observed in Trial 1. This suggests that we have a "built in" bias in favor of "the natural milk." That which we identify as milk we are likely to prefer.

Composition and Quality of Whey-Milk

Table 6 and 7 list the composition of regular milk, human milk (Table 6), and whey-milk (50% whey-Table 6, 40% whey-Table 7). Generally, whey-milk contains lower total solids and protein than cow's milk. However, by adding the proper amount of lactose, whey-milk would be similar in composition to human milk.

The pH, total viable bacteria and coliform counts of both whey-milk and the regular milk were examined periodically during storage. Table 8 shows that these counts and pH values were stable. The total bacteria counts of whey-milk were lower than those of regular milk. The bacteria counts of neutralized whey also were comparatively low.

Table 6. Composition of human milk, laboratory-prepared whey-milk and KSU milk.

Component	Human milk ¹	Whey-milk ²	KSU milk
	--%--	--%--	--%--
Total solids	12.57	11.48	12.35
Fat	3.67	3.24	3.25
Protein	1.63	2.31	3.47
Lactose	6.98	5.07	4.92
Ash	0.21	0.86	0.71

Remarks: 1. Fundamental of Dairy Chemistry (66b)

2. Whey-milk containing 50% whey.

Table 7. Composition of KSU milk and plant processed whey-milk.

Component	Whey-milk ¹	Whey-milk ¹	KSU milk
	(Trial 1.)	(Trial 2.) ²	
	--%--	--%--	--%--
Total solids	10.83	11.50	12.42
Fat	2.65	3.15	3.30
Protein	2.32	2.43	3.45
Lactose	4.96	5.01	4.90
Ash	0.90	0.91	0.77
Ca (mg%)	73.3	64.3	72.4
P (mg%)	128.0	143.0	103.0
K (mg%)	101.0	114.0	61.8

Remarks: 1. Whey-milk formulated with 40% whey content.
2. 0.5% nonfat dry milk solids added.

Table 8. PH, SPC and coliform counts of KSU milk and whey-milk during storage.

Days in storage	Whey-milk			KSU milk		
	SPC/ml	Coliform/ml	pH	SPC/ml	Coliform/ml	pH
(Experiment 1.)						
0	200	0	6.7	430	0	6.7
5	160	0	6.6	200	0	6.7
10	100	0	6.7	170	0	6.7
15	170	0	6.6	250	0	6.6
(Experiment 2.)						
0	140	0	6.8	260	0	6.8
5	180	0	6.7	300	0	6.7
10	210	0	6.7	340	0	6.7
15	200	0	6.7	360	0	6.7

Remarks: Grade "A" pasteurized milk:

1. Not more than 20,000 SPC/ml.
2. Not more than 10 coliform/ml.

CONCLUSIONS

Base on the results obtained in this study, the following conclusions may be drawn:

1. A milk-like product containing 40% whey or less with milk solids and fat adjust to approximately that of cow's milk was similar in flavor to a commercial milk.

2. There was no difference in flavor ($P < .05$) between whey-milk containing 40% whey prepared in the laboratory (milk solids and fat adjusted to near that of cow's milk) and a commercial milk when evaluated by experienced judges using a triangle taste test.

3. There was no difference in flavor ($P < .05$) between whey-milk containing 40% whey and 0.5% added NFDM prepared in the University processing plant (using commercial whey and the University milk and cream) and a commercial milk when evaluated by consumers using a paired comparison test.

4. Whey-milk prepared in the University processing plant was found to be as acceptable as commercial milk.

5. Whey-milk, containing 40% whey, with 0.50% added lactose or less could not be distinguished from a commercial milk by using a triangle taste test.

6. Under the controlled processing and storage conditions of this study, the microbial quality and shelf-life of whey-milk were comparable to the regular milk.

7. Instead of expensive disposal of cottage cheese whey, it appears practical to utilize whey in a whey-milk that, from an

ingredient-cost standpoint, could be sold at lower price than commercial milk.

APPEDIX

Table 9. Amount of D-glucono-delta-lactone (GDL) required to acidulate 10 l of heated-acidified skim milk.

pH of heated-acidified milk	g of GDL/10 l skim milk
5.00--5.05	39.01
5.05--5.15	42.01
5.15--5.20	48.01
5.20--5.25	54.02
5.25--5.30	60.02
5.30--5.40	72.02

Fig. 1

HEDONIC SCALE SCORING

DATE _____

TASTER _____

PRODUCT _____

Taste test these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for this attitude.

Degree of preference	CODES				
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					
	REASONS				

Remarks:

Numerical Values: Like extremely = 9.

Dislike extremely = 1.

Fig. 2

TRIANGLE TEST
DIFFERENCE ANALYSIS

DATE _____

TASTER _____

PRODUCT _____

Instructions: Here are three samples for evaluation. Two of these samples are duplicates. Separate the odd sample for difference only.

(1)

(2)

Sample

Check odd sample

(3) Indicate the degree of difference between the duplicate samples and the odd sample.

Slight _____ Much _____

Moderate _____ Extreme _____

(4) Acceptability:

Odd sample more acceptable _____

Duplicate sample more acceptable _____

(5) Comments:

Date _____

Taster (family) _____

Evaluate these two milks.

1. Place a check (✓) in the space that corresponds to the code letter that you believe identifies the regular milk.

G _____ or M _____

2. Which one do you prefer?

G _____ M _____ no preference _____

3. Comments or suggestions:

PAIRED COMPARISON TEST

Date _____

Taster (family) _____

Evaluate these two milks.

1. Place a check (✓) in the space that corresponds to the code letter that you believe identifies the regular milk.

G _____ or M _____

2. Which one do you prefer?

G _____ M _____ no preference _____

3. Comments or suggestions:

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WHEY-MILK: A POTENTIAL MILK SUBSTITUTE FROM DIRECT-
ACID-SET COTTAGE CHEESE WHEY AND MILK

by

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AN ABSTRACT OF A THESIS

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If cottage cheese whey is not utilized, it may contribute seriously to environmental pollution. This experiment was designed to use whey in a milk-like product called "whey-milk".

Cottage cheese whey was neutralized with potassium hydroxide to pH 6.6 to 6.7 and a variety of whey-milks containing 30%, 40% and 50% of this neutralized-clarified whey were standardized with milk and cream to yield a product with 3.25% fat and 11.5% total solids. These milks were evaluated for flavor by trained panelists using a triangle taste test. Fat, protein, lactose and mineral contents were determined. The pH and total bacteria counts of the whey-milk during storage were similar to those of a commercial milk.

Whey-milk containing 40% whey or less was found to be statistically identical to regular milk by a triangle taste test, whereas whey concentrations of 50% or more were detectable by the taste panel. Addition of 0.5% lactose to whey-milk (40% whey) was not detectable organoleptically by the triangle taste test, however, 1.0% was detectable. Subsequently a whey-milk containing 40% neutralized whey, 0.5% NFDM and 3.25% milk fat was made in the University Dairy Plant and evaluated by consumers by a paired comparison test. This whey-milk was found to be no different ($P < .05$) from the regular milk.

Cottage cheese whey contains high quality proteins. These proteins represent approximately 0.7% of the whey. Unfortunately, this whey is considered a waste product in many dairy plants. However, this whey could be utilized to produce whey-milk, thus providing dairy processors a profitable and marketable product.