DIRECT-ACID-SET COTTAGE CHEESE WHEY AS A BASE FOR A SHELF-STABLE ATHLETIC-TYPE DRINK

by

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INTRODUCTION

Whey disposal is a problem for most cheese processing plants. Municipal sewage plants impose high surcharges when they accept it, because of its high BOD (biochemical oxygen demand). The equipment needed for processing it for animal or human consumption is costly and not readily available to small cheese producers.

The cost of disposing of or processing the whey could be eliminated by using the whey in beverage formulations. This research centered on formulating a beverage using direct-acid-set (DAS) cottage cheese whey as the major ingredient that would be similar in composition and flavor to electrolyte-carbohydrate containing athletic-type drinks. The cost of making such a drink should be comparable to that of products currently on the market. The equipment needed, such as batch pasteurization tanks and desludging clarifier, already exists in cottage cheese processing plants.

The objectives of this research were to:

1. develop a process for formulating an athletic-type drink from whey,
2. test the process for making a shelf-stable product,
3. compare its organoleptic properties to an existing commercial product, and determine the consumer acceptance of the drink,
4. compare its composition to that of an existing product,
5. analyse the beverage for sugar and test it for flavor stability and shelf life.

Acid whey for this research was donated by Beatrice Foods Company (Meadow Gold), Topeka, Kansas, or Steffen Dairy Foods Company, Wichita, Kansas. Prior to its use, the pH of the whey was adjusted to 5.2 with potassium hydroxide. It was heated to 90°C and held at that temperature for 10 min to coagulate proteins. The majority of the proteins were removed by centrifugation or filtration. Sufficient calcium hydroxide was added to obtain a pH of 5.6 to 5.7. More potassium hydroxide was added to reach the optimum pH of 6.5 for enzymatic hydrolysis of lactose. A sufficient amount of ß-galactosidase was added to hydrolyse more than 80% of the lactose to glucose and

galactose. Two parts of the processed whey were added to one part water; this reduced the salty character, and citric acid was added to lower the pH to 3.7±0.05. The desired sweetness was obtained by adding sucrose and glucose. Orange flavoring (Norda Natural and Artificial Emulsion # EP-10,806; and Blanke Baer Orange Extract) was added to give the drink a desirable flavor. The product was heated to 88°C, bottled, capped and held 5 min at that temperature to control microorganisms.

REVIEW OF THE LITERATURE

I. Whey Utilization and Disposal

Utilization of cheese whey is still a challange in the United States. Cheese production has been increasing steadily to the point where, in 1981, the Whey Products Institute estimated that the total whey production in the United States reached a record high of 42 billion pounds, including 3.9 billion pounds of acid whey and 38.1 billion pounds of sweet whey. Of the total whey produced, only 54.6% was further processed. This whey was processed into concentrated whey; dry whey for human and animal consumption; modified dry whey products such as reduced lactose whey, reduced minerals whey, and whey protein concentrate; whey solids in wet blends; and whey solids utilized for lactose (Anonymous, 1982b).

Due to water pollution legislation in the 1960's cheese manufacturing plants have had to find means of disposing of whey without causing pollution to natural water systems. Disposing of it through a sewage system is costly, and a waste of the nutrients present in whey. Cottage cheese whey contains 73% of the nutrients of the skim milk used for making the cheese. If the whey is disposed of through the municipal systems, it is costly to the dairy plant, and a waste of the available nutrients (Kosikowski, 1978). Many municipal sewage systems are not equipped to remove the oxygen-consuming pollutants that are in whey, and refuse to accept dairy wastes (Gillies, 1974). The biochemical oxygen demand (BOD) of whey is 32,000 ppm or higher. This is caused by protein and lactose in the whey (Harper et al., 1971; Kosikowski, 1978). Sewage treatment plants often insist that cheese-plant discharges have a BOD of 200 ppm or less to avoid payment of a surcharge. Equipment used to treat the wastes (decrease the BOD), or to condense or dry the whey, is very expensive (Kosikowski, 1978). Eighty-five percent of the unprocessed whey comes from plants with the approximate daily production of 9,000 kg (19800 pounds) of whey or less. Milk processing plants that produce cottage cheese are normally among the small

whey producers. In these plants it may not be economically feasible to purchase the equipment to further process whey, so they are more inclined to dump the whey in municipal sewers, and pay the surcharge (Jelen and LeMaguer, 1976).

Demott (1976) stated that drying acid whey is a problem because of the low pH of 4.6. It does not withstand heating and other processes; it lumps together, and clogs the dryer. Kosikowski (1978) stated that the more acidic the whey, the higher is the drying temperature required. Furthermore, the high acidity is corrosive to the equipment used for drying (Kosikowski, 1978). During drying of acid whey, oversized lactose crystals are formed, and adhere to the interior of the dryer making cleanup difficult (Gillies, 1974). Currently, research is being conducted to utilize whey for producing wine, gasohol, and beverages (Delaney, 1981; Kosikowski, 1979).

II. Composition of Whey

The Federal Food and Drug Administration defines wheys as follows: "the liquid substance obtained by separating the coagulum from milk, cream or skim milk in cheese making. Whey obtained from procedures in which a significant amount of lactose is converted to lactic acid, or from the curd formation by direct acidification of the milk, is known as acid whey. Whey obtained from procedures in which there is insignificant conversion of lactose to lactic acid is known as sweet whey" (Anonymous, 1981b).

The compositions of sweet, acid, and direct-acid-set (DAS) wheys are found in Table 1. Lactose is slightly less in acid whey than in sweet whey. This can be attributed to more lactose fermentation during cottage cheese manufacturing. The total protein of acid whey is less than sweet whey, whereas the nonprotein nitrogen level is a little greater. There is generally more ash in acid whey than in sweet whey, and less fat. This is due to the fact that most acid whey comes from cheese made from skim milk (Glass and Hedrick, 1977). The fat and water contents of dried whey

Table 1. Composition of sweet, acid, and direct-acid-set (DAS) fluid wheys.

	Sweet[a]	Acid[a]	DAS[b]
Fat	0.3%	0.08%	0.24%
Protein	0.9%	0.9%	0.70%
Lactose	4.9%	4.4%	c
Ash	0.6%	0.8%	0.88%
Total solids	6.3%	6.1%	7.25%

[a] From Harper (1972)

[b] From Blackburn (1980)

[c] Not available

are similar to non-fat dry milk (NFDM), but the protein content is about 1/3 of that in NFDM. The types of protein differ also. Casein is the major protein in NFDM, whereas lactalbumin and lactoglobulin are the chief proteins in whey. The ash content is similar to that of NFDM, but the mineral composition between the two differs considerably (Gillies, 1974).

A. Lactose

Lactose is the major component of whey solids, comprising of about 70% of the total solids. It is the characteristic sugar found in milk, other than trace amounts of glucose and galactose, and the only sugar present in milk of most animals. It is a disaccharide that yields D-glucose and D-galactose upon hydrolysis. The systematic nomenclature is 4-0-ß-D-galactopyranosyl-D-glucopyranose. It occurs in both the alpha and beta forms. Lactose is normally found in dairy products in either of two crystalline forms--alpha-lactose monohydrate and anhydrous beta-lactose, or amorphous mixture of alpha- and beta-lactose. The solubility of lactose at 25°C is 24.8 g/ 100g water (Nickerson, 1974). The relative sweetness of lactose is low compared to other sugars (See Table 2). Hydrolyzing the lactose to glucose and galactose results in sweeter products. As the temperature increases the difference in the relative sweetness between these sugars decreases. The difference in sweetness is greater at lower temperatures (Tsuzkike and Yamazaki, 1953).

B. Proteins

Casein is the major milk protein. It coagulates during cheese making and forms the cheese curd. The non-casein proteins (whey proteins) remain in the whey during cheese making. These constitute 14 to 24% of the total milk protein. The major whey protein in milk is ß-lactoglobulin (57.2% of the total whey protein). It has an isoelectric point of 5.3. Alpha-lactalbumin constitutes 20.9% of the total whey protein. It has an isoelectric point of 4.2 to 4.5. Other proteins present in the whey

Table 2. Relative sweetness of some sugars compared to sucrose.

Sugar	Relative sweetness[a]
Sucrose	100
Lactose	39
Glucose	69
Galactose	63
Fructose	114

[a] Cited from Aurand and Woods (1973)

are immunoglobulins which comprise 15.1% of the total whey protein; euglobulins, about 6.6%; pseudoglobulins, about 5.3%. There is 21.1% of the total whey protein that is identified as the proteose-peptone fraction (Gordon and Kalan, 1974). Table 3 shows the percentages of the four major protein fractions in the protein portion of whey.

C. Minerals

The mineral content of acid whey is usually higher than that of sweet whey (See Tables 1 and 4). The amounts of calcium, phosphorous, zinc, and copper are greater in acid whey than in sweet whey. The potassium and sodium contents are similar between the two types of whey. Factors that can influence the mineral content in whey are: type of cheese, geographic area, stage of lactation, source of milk (breed or animal), care during storage, and processing and specific operations (Glass and Hedrick, 1977).

D. Vitamins

Whey is a good source of vitamin B_{12}, riboflavin, pantothenic acid, biotin, and choline (See Table 5). Glass and Hedrick (1977) reported that for acid and sweet wheys there was no significant difference for thiamin, vitamins B_6 and B_{12}, tocopherol, pantothenic acid, folacin, and choline. However, acid whey generally has less riboflavin, biotin, niacin, vitamins A and C than sweet whey. There was considerable variation among the samples analyzed. They speculated that the difference could be due to the cheese processing treatment and the drying procedure. Bacterial growth prior to drying can cause variations in levels of some vitamins. The content of unstable vitamins can decrease during storage, particularly vitamins A, B_6, and biotin (Glass and Hedrick, 1977).

Table 3. Major protein fractions in whey[a].

Protein	% of whey protein
Beta-lactoglobulin	50.0
Alpha-lactalbumin	18.4
Immunogolbulins	13.2
Bovine serum albumin	5.3

[a] Calculated from the data of Gordon and Kalan (1974)

Table 4. Mineral composition of sweet and acid fluid wheys[a].

Mineral	Sweet	Acid
	mg/100ml	
Calcium	61.46	168.28
Phosphorus	76.72	111.16
Sodium	90.09	76.09
Potassium	129.85	134.05
Magnesium	12.46	15.66
Zinc	0.147	0.567
Iron	0.063	0.091

[a] Calculated from the data on dry whey (Glass and Hedrick, 1977)

Table 5. Vitamins in sweet and acid fluid wheys.

Vitamin (units/ 100g)	Sweet	Acid
Vitamin A (IU)	9.52	7.49
Vitamin C (mg)	0.10	0.02
Vitamin B_6 (mg)	0.04	0.04
Vitamin B_{12} (µg)	0.17	0.175
Tocopherol (mg)	0.004	0.005
Thiamin (mg)	0.035	0.034
Riboflavin (mg)	0.15	0.13
Pantothenic Acid (mg)	0.8	0.8
Biotin (µg)	3.01	2.45
Niacin (mg)	0.09	0.08
Folacin (mg)	0.001	0.002
Choline (mg)	7.28	7.07

[a] Calculated from the data on dry whey (Glass and Hedrick, 1977)

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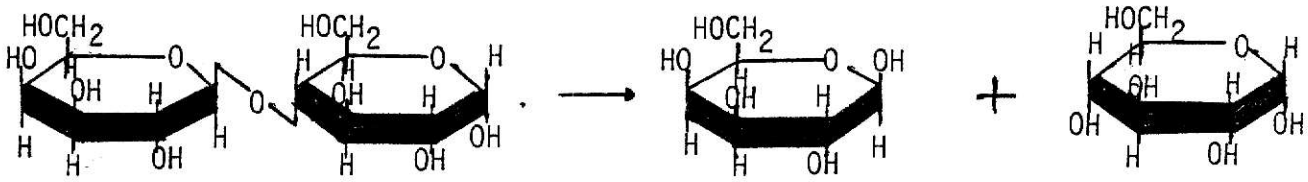
III. Hydrolysis of Lactose

A. Beta-Galactosidase

By enzymatic hydrolysis, the lactose molecule is split into glucose and galactose as shown below.



LACTOSE β-D-GALACTOSE α-D-GLUCOSE

Beta-galactosidase is the trivial name for the enzyme that catalyzes hydrolysis of lactose. The name "lactase" that traditionally has been used by authors because of its convenience is, however, an obsolete term (Richmond et al., 1981). Beta-galactosidase is widely distributed in nature, and can be found in plants (especially almonds, peaches, apricots, and apples), animal organs (such as the intestines), yeast, bacteria, and fungi. The yeasts in which ß-galactosidase is commonly found are Kluyveromyces lactis, K. fragilis, and Candida pseudotropicalis. It also is found in certain bacteria such as Escherichia coli, Bacillus megaterium, Streptococcus lactis, S. thermophilus, Lactobacillus bulgaricus, and L. helveticus, as well as in fungi such as certain species of Aspergillus and Mucor (Shukla, 1975). Beta-galactosidase from different sources varies considerably in many of its extrinsic properties, such as optimum temperature and pH, although the specificity of the enzyme remains essentially the same (Richmond et al., 1981).

The commercial ß-galactosidase (Maxilact L 2000 from G.B. Fermentation Industries) is a purified liquid ß-galactosidase preparation from the dairy yeast

Kluyveromyces (Saccharomyces) lactis. Its optimal pH range is 6.4 to 6.7 at 30°C, and the optimal temperature is 35 to 40°C. However, it is active at temperatures down to 4°C, and pH of 6.3 to 6.8 (Anonymous, 1979).

Bouvy (1975) recommended that the lactose be as dilute as possible before hydrolysis. Galactose is a competitive inhibitor of the ß-galactosidase, and the transgalactosidase activity is more pronounced when lactose is more concentrated (Bouvy, 1975). The enzyme is more active in the presence of potassium than sodium. Potassium hydroxide is recommended over sodium hydroxide for adjusting the pH of the whey. Low concentrations of manganese are essential in maintaining the active structure of the enzyme. Magnesium and cobalt can partly replace manganese in this function. Heavy metals, such as lead, strongly inhibit the enzyme activity (Bouvy, 1975).

B. Formation of Oligosaccharides

During hydrolysis of the glycosidic linkage of lactose, some monosaccharide units may be transferred to active acceptors such as monosaccharides, polysaccharides, or alcohols (Wierzbicki and Kosikowski, 1973). These molecules become the acceptor of the ß-D-galactose moiety instead of water. This transfer reaction is called transgalactosidation. Because a small amount of galactose is transferred to other sugar molecules during hydrolysis, slightly more free glucose than galactose is present after the reaction (Bouvy, 1975).

Burvall et al. (1979) suggested that trisaccharides could be formed by a galactose molecule linking to a lactose molecule. Tetrasaccharides and longer polysaccharides could be formed by a similar mechanism. The authors also suggested that disaccharides other than lactose could be formed; however, they could not be detected by separation techniques used in their study.

The number of types of oligosaccharides formed during lactose hydrolysis with Aspergillus niger galactosidase varied from three to eleven (Pazur et al., 1958).

Wierzbicki and Kosikowski (1973) reported that five different oligosaccharides resulted from the activity of ß-galactosidase of Aspergillus niger. These appeared at low concentrations of 1 to 2% of the total lactose present before hydrolysis. At the lactose concentration of more than 4%, the oligosaccharides appeared in larger amounts (Wierzbicki and Kosikowski, 1973). Burvall et al. (1979) measured oligosaccharide formation in a 5% lactose in a buffer (0.05M potassium phosphate buffer at pH 6.8) solution which was inoculated with Saccharomyces lactis ß-galactosidase (Maxilact). They found that the maximum oligosaccharide formation was about 5% of the total sugar concentration.

Burvall et al. (1979) found that the medium where the hydrolysis takes place, whether it be milk, whey or a buffer solution, affects the oligosaccharide formation. The pH of the medium also can determine the degree of oligosaccharide formation. Smaller amounts of oligosaccharides were produced from lactose in milk than in a buffer solution that contained lactose (Burvall et al., 1979). They suggested that these oligosaccharides can cause intestinal discomfort in the form of flatulence.

C. Depression of Freezing Point

Hydrolysis of lactose is accompanied by a corresponding decrease in the freezing point of the solution. Complete hydrolysis of 50g/ kg of lactose in solution shows a reduction of the freezing point of 0.273°C. This physical property can be used to measure the amount of hydrolysis that occurs. The method is simple, and the reproducibility and accuracy are good (Nijpels et al., 1980).

Acidification of the solution can result in a further decrease of the freezing point, and so the pH must remain constant during hydrolysis (Nijpels et al., 1980). The freezing point is depressed approximately 0.0066°C by the addition of 0.1g ß-galactosidase/ 100ml substrate. During lactose hydrolysis oligosaccharide formation is likely to cause the freezing point depression to be less than it would be if there

were only simple breakdown of lactose into glucose and galactose (Baer et al., 1980).

D. Lactose Intolerance

The enzyme ß-D-galactosidase is deficient in many non-caucasian adults. The insufficienty of ß-galactosidase production in the digestive tract is known as lactose intolerance. It causes abdominal cramps, gaseous distention, and in some cases diarrhea when a large amount of lactose is consumed (Nickerson, 1974). Almost all Japanese and African blacks are lactose intolerant. Sixty-four percent of U.S. blacks and 6% of U.S. whites lack the enzyme (Anonymous, 1979).

IV. Removal of Proteins

A number of procedures have been employed to clarify whey for subsequent uses. Some of these methods are as follows:

1. A chelated protein complex may be precipitated out of the whey by added ferric salts (Block and Bolling, 1955).

2. Polymeric phosphates are used to precipitate the protein (Gordon, 1945).

3. Anionic polyelectrolyte 1-carbo-methoxy-2-carboxy-3-acetoxybutylene copolymer at 50 ppm (based on liquid whey) added to hot whey at a pH of 4.0 to 5.2, and agitated for 2 min, precipitates the protein (Rogers and Palmer, 1966).

4. Gel filtration (Steiner, 1968) has been used in a commercial plant in Sweden to clarify whey.

5. Ultrafiltration (Kosikowski, 1978) separates whey components under pressure across a thin, semi-permeable membrane film. Much of the water, lactose, soluble salts, lactic acid, and nonprotein nitrogen are transferred to the film's outer surface, leaving behind on the inner side, protein and insoluble salts in suspension.

Another method of protein removal from sweet whey by heat was published by Burkey and Walter (1947). The procedure is as follows:

1. Place the whey in the vat in which it is to be heated, determine the pH, and adjust to 6.3 to 6.5. The proteins are most stable to heat at this pH.

2. Heat the whey to at least 94°C (200°F), or as near to boiling as possible, and stir constantly while it is being heated.

3. As soon as the whey is heated, add the acid, or a calcium chloride solution, to the hot whey with constant stirring until precipitation is complete. At this point discontinue stirring, and allow the precipitated protein to stand for a few min to permit it to accumulate.

4. The precipitated protein may be recovered from the whey with a Swiss-cheese dipping cloth, or by draining off the whey, depending on the size and kind of vat and disposition that is to be made of the recovered protein.

The amount of precipitating agent added should be sufficient to produce visibly complete precipitation. It is necessary to add enough acid to lower the pH of the hot whey to between 4.8 and 5.3 for complete precipitation. Calcium chloride solution is then added to adjust the pH to between 5.5 and 5.8. When an insufficient amount of precipitating agent is added, the precipitation will be incomplete. The addition of an excessive amount of precipitating agent is likely to prevent proper flocculation and the protein, instead of collecting into a mass of curd, will remain in small particles that are difficult to recover.

Aside from the precipitating agents, the primary factor in the removal of proteins from cheese whey is heat. The heating process is the most expensive part of the procedure. The whey must be heated to a temperature of at least 90.5°C (195°F) to provide complete protein precipitation. The protein curd may be removed from the hot whey either by means of dipping cloth or by draining the whey off through a vat drain fitted with a fine mesh whey strainer. Approximately 3.5 pounds of whey protein

curd can be obtained from 100 pounds of cheese whey (Burkey and Walter, 1947). Recovery of the protein can be made through a desludging clarifyer. The separation can be commenced immediately without allowing the mass to stand unagitated (Van der Merwe and Downes, 1981).

V. Direct-Acid-Set Cottage Cheese Whey

Acid whey is the principal byproduct of cottage cheese manufacturing. There are two types of cottage cheese i.e. cultured and direct-acid-set (DAS) cottage cheese. Cultured cottage cheese is produced commercially by the addition of selected strains of lactic acid-producing bacteria into pasteurized skim milk. After a period of time the bacteria produce enough acid to reduce the pH and form a soft coagulum. It is cut and cooked to produce curds and acid whey. This cultured method is time consuming, and occasionally subject to failure due to slow growth of starter organisms. The starter culture is propagated in special tanks to guard against bacteriophage and contamination that cause failure of the process. Microbial contamination can cause off flavors (Kosikowski, 1978).

An alternate method is the DAS method. The patented process developed by Corbin (1971) utilizes an acid mixture, coagulator, and acidogen for direct acidification. A mixture of citric acid, lactic acid, and phosphoric acid (Vitex 750) are added to 5°C milk to obtain a pH of 4.9 to 5.0. The milk is then heated to 32°C followed by the addition of D-glucono-delta-lactone (GDL or Vitex 850) and rennet (Vitex cottage cheese coagulator). After an hour the curd is cut, and additional Vitex 750 is then added to obtain a pH of 4.4 to 4.5. The curd is then cooked, drained, and washed as in the conventional method (Corbin, 1971).

The cooking and cutting times for cottage cheese made by the DAS method are 40 and 33% shorter, respectively, than with the short set culture method (White and Ray, 1977). Sharma et al. (1977) reported a 6% yield increase in DAS cottage cheese

over the cultured method. Extended shelf life, improved consistency, and a 1.0 to 1.5% yield increase were reported by Gerson (1970). Since DAS cottage cheese whey is not cultured, it does not have that "whey taint" that is in cultured cottage cheese whey. The DAS whey flavor blends into drinks or beverages better than cultured cottage cheese whey (Demott, 1975).

VI. Beverages Formulated with Whey

A familiar whey beverage in Europe is Rivella (Anonymous, 1960). It was originally developed in Switzerland, and it has been marketed in Holland, Germany, and Australia. It is fermented, clarified whey that is sweetened, flavored, and carbonated.

A Polish scientist developed a soft drink utilizing deproteinized whey (Rzewuska-Rutte, 1967). Deproteinization of whey was carried out at 90°C and pH 7.0, and 63% of the protein was precipitated. Citrus and mint flavors resulted in more acceptable flavored soft drinks than did apples, cherries, strawberries, blackberries, and raspberries. He found that the best soft drinks were prepared with 95% whey.

Kosikowski (1968) incorporated up to 6% whey powder into reconstituted frozen orange juice. It contained 2.5 times more protein than plain orange juice, and was reported to be an acceptable product. He reported that the addition of 6% added whey powder imparted a salty flavor. At 4% added whey powder, however, a taste panel rated the flavor as excellent.

O-whey is a breakfast meal formulated from either neutralized acid whey or sweet whey and orange juice (Brunner et al., 1969). Orange juice is added to deodorized whey at a ratio of one volume concentrate to four volumes whey. The whey was deodorized by vacuum treatment and it can be packaged as a liquid, condensed, or dried product.

Nelson and Brown (1971) reported that an orange juice flavored drink prepared with 33% unmodified cottage cheese whey was rated 6.3 on a 7-point hedonic scale by 51 tasters, whereas the non-whey drink was rated 4.7. They found that whey contributed significantly to the color and flavor stability of the orange drink. Drinks using strawberry or peach puree and modified whey were also rated good to excellent by their expert panel members.

Demott and Park (1972) developed an orange drink that consisted of 65% acid whey, 0.2% citric acid, 28% added water, 5% sucrose, 2% orange concentrate, and 0.0006% saccharin. Thirteen out of 15 students trained in product evaluation prefered this drink to a conventional orange drink formulated by the university dairy processing plant.

Demott (1975) also used DAS cottage cheese whey to formulate an orange flavored drink, and a lemon-lime flavored drink. They consisted of whey, orange or lemon-lime concentrate, sugar, and saccharin. A 21 member panel preferred the orange flavored drink to the lemon-lime flavored drink.

Lactofruit is a soft drink developed by the Battelle Research Center in Geneva, Switzerland (Anonymous, 1978). It is obtained from deproteinated and 50% lactose-hydrolysed whey. Protein is removed by ultrafiltration. Lactose hydrolysis is carried out by enzymatic electrocatalysis. The enzyme is fixed to a tridimensional electrode. The influence of the electrical field developed between the ß-galactosidase from Aspergillus niger, electrode and the counter electrode makes it possible to control such parameters as pH and the immediate environment of the enzyme without adding anything to the whey. The composition of the liquid base is approximately 12.5g/ l of galactose, 25.0g/ l of lactose, 4 to 5g/ l of mineral salts, and 2g/ l of nitrogenous materials.

VII. Milk-Like Drinks Formulated with Whey

Way-Mil is an imitation milk formulated with neutralized acid whey or sweet whey, and 2 to 3% vegetable oil (Brunner et al., 1969). The product is bland and can be flavored with chocolate or fruit flavor.

Chen et al. (1979) formulated an imitation milk that contained four parts neutralized DAS whey, six parts whole milk, and 0.5% non-fat dry milk solids. Twenty percent of the people who sampled the product could not tell it from milk, and 26% favored the imitation milk.

Blackburn and Bassette (1982) prepared a chocolate flavored dairy drink. It consisted of four parts neutralized DAS whey and six parts whole milk. Chocolate at 1.44% and sugar at 4.5% were added for flavoring and 0.2% nonfat dry milk was added for both flavor and body. From a sensory evaluation of this product 22 of the 37-member consumer panel preferred the whey drink to a conventional chocolate drink, and seven panelists expressed no preference.

VIII. Athletic Drinks

A great amount of body fluid can be lost by evaporation of perspiration while exercising heavily (Francis, 1979). If this fluid is not replaced, one can suffer from heat exhaustion or stroke. Along with perspiration, a number of body electrolytes are lost. Principally these are sodium, potassium, and chloride. The body fluid can be replaced by water or by a commercially available athletic drink. Athletic drinks can help replace electrolytes and supply extra energy during prolonged exercise (Anonymous, 1980a). The most commonly available athletic drink on the market is Gatorade^{R}, produced by Stokely-Van Camp (Indianapolis, Indiana). It consists of water, glucose, fructose, electrolytes (namely sodium, potassium and chloride), citric acid, and flavorings. It has a pH of 3.5 (Coyle et al., 1978; Francis, 1979). The compositions of Gatorade^{R} and other commercial drinks are listed in Table 6.

Table 6. Electrolyte composition and pH of athletic-type drinks[a].

	Gatorade[b]	Breaktime[c]	Body Punch[d]
Sodium (meq/l)	22.7	22.4	9.85
Potassium (meq/l)	2.5	9.8	4.97
Chloride (meq/l)	13.5	14.8	8.46
Carbohydrate (g/l)	4.6	1.1	2.5
pH	3.5	3.46	3.00

[a] From Coyle et al. (1978)

[b] Manufactured by Stokely-Van Camp

[c] Manufactured by Johnson and Johnson

[d] Manufactured by Starting Line Sports

The American Dietetic Association has set guidelines for electrolyte drinks (Anonymous, 1980a). They should contain no more than 10meq of sodium per liter, 5meq of potassium per liter, and 2.5% glucose. Not more than 20 to 30meq of sodium chloride per liter, and 50 to 60g of glucose per hour are well tolerated by the digestive system (Anonymous, 1980a).

Coyle et al. (1978) compared gastric emptying rates for commercial athletic drinks to those of water. Breaktime®, Body Punch®, and water contributed 35, 37, and 39% more fluid, respectively, to the system in 15 min than did Gatorade®. Breaktime®, Body Punch®, and water were not found to be significantly different in their mean volume of fluid emptied. It has been shown that carbohydrates delay gastric emptying in proprotion to their concentration (Hunt, 1960). Since Gatorade® has a higher sugar concentration than the other two drinks, it may account for the slow emptying rates. It also has been found that the greater the electrolyte concentration of the original drink, the greater is the body's gain of that electrolyte in 15 min (Coyle et al., 1978).

IX. Sensory Methods

Discriminatory tests are used to determine whether a difference exists between samples (Larmond, 1977). The triangle test is the most popular discriminatory sensory test. Sixty-six percent of the 56 companies surveyed by Brandt and Arnold (1977) used it. This test has had wide use in the food industry. Food companies are concerned with the effects of variables such as formulation, processing, raw materials, and packaging materials on the quality of their products (Brandt and Arnold, 1977). During a triangle test a panelist receives three coded samples. Two are actually the same sample, and one is different. The panelist is asked to identify the odd sample. The test can be used to determine if ingredient substitution or some other change in manufacturing results in a detectable difference in the product. Results are based on the probability that if

there is no detectable difference, the odd sample will be selected by chance one third of the time (Larmond, 1977).

Preference tests are used to determine the acceptability of a product. The hedonic scale is commonly used in preference tests. It is used by 57% of the 56 food companies surveyed by Brandt and Arnold (1977). "Hedonic" is defined as "having to do with pleasure." The panelist expresses his or her degree of liking or disliking. Nine- and 7-point hedonic scales are commonly used (Larmond, 1977). Hedonic scoring is well suited for consumer testing (Brandt and Arnold, 1977). The paired comparison test was used by 55% of the food companies surveyed by Brandt and Arnold (1977). For the preference test the panelist is given two coded samples, and is asked which he/she prefers (Larmond, 1977). The hedonic scale is used slightly more than the preference test (Brandt and Arnold, 1977).

Multiple comparison tests set no limits statistically on the number of samples that may be evaluated at one setting, nor on the kind of information that may be obtained. Results from multiple comparison tests may indicate not only whether there is a difference, but also a measure of the difference, as well as whether there is an improvement or a deterioration. The panelist is presented two or more samples simultaneously or singly in random order. The panelist may be required to evaluate each sample on the basis of a particular scale (Kramer and Twigg, 1970).

Scoring can be used to evaluate the intensity of some specific characteristic. The panelist records his judgement on a graduated scale. The intervals on the scale can be labeled with numbers or descriptive terms. The inclusion of standards will help minimize panel variability. Variability is a marked disadvantage when scoring is used in storage stability studies (Larmond, 1977).

It is common practice to apply statistical methods to interpret sensory data. Fifty-six percent of the 56 companies surveyed by Brandt and Arnold (1977) used the Student's t test, and 47.5% used analysis of variance. Chi-square, Kramer's rank test,

Duncan's multiple range test, and the F-ratio are used to a lesser extent (Brandt and Arnold, 1977).

X. Heat Processing Methods

A heat preservation method commonly known as "hot filling" is recommended for fruit juices such as apricot, grapefruit, orange, and pineapple. The juice is normally heated to 88°C (190°F) in a plate type heat exchanger, filled into cans or bottles (making sure that the closing temperature is at least 80°C or 175°F), sealed, held for three minutes, and then rapidly cooled. For glass containers a cooling temperature of about 55°C (130°F) must be used first to prevent thermal shock breakage. The final cooling water temperature should be 15.5 to 26.6°C (60 to80°F) (Pflug and Esselen, 1967).

MATERIALS AND METHODS

I. Beverage Made in the Laboratory

Commercial direct-acid-set (DAS) cottage cheese whey was provided by the Beatrice Foods Company (Meadow Gold) in Topeka, Kansas, and Steffen Dairy Food Company in Wichita, Kansas. The whey, cooled to 5 to 7°C, was transported to Kansas State University by car in one gallon plastic containers. Upon arrival it was placed in the K.S.U. Dairy Processing Plant milk cooler and stored there until it was used. The flavor was clean and fresh, and the pH ranged from 4.2 to 4.5.

A. Preparation of the Beverage Base from Whey with Various Neutralizing Agents

1. Potassium hydroxide

DAS cottage cheese whey was neutralized to pH 6.5 with saturated potassium hydroxide (1.0g KOH/ 0.9ml water), and heated to 37°C. The whey lactose was hydrolyzed with ß-galactosidase by incubating it at 37°C for 4 hr. After incubation,the pH was reduced to 5.2 with citric acid, and the product was heated with stirring to 90°C, held at that temperature for 10 min with stirring, and filtered to remove the protein.

2. Magnesium oxide

DAS cottage cheese whey was neutralized with laboratory grade magnesium oxide at 0.265g/ 100ml acid whey as described by Blackburn (1980). The mixture was agitated for 15 min with a magnetic stirrer and refrigerated for 12 to 16 hr for the sediment to precipitate. Then the supernatant was decanted, and the pH was adjusted to 6.5 to 6.6 with more DAS cottage cheese whey if necessary. The lactose hydrolysis was accomplished as described above. After the pH was reduced to 4.5 with citric acid, the product was heated to 90°C, and held for 10 min to coagulate the proteins that then were filtered out.

3. Calcium hydroxide

Calcium hydroxide was tried as the neutralizing agent at a level of 0.15g/ 100ml of whey. The whey was agitated with a magnetic stirrer for 15 min. A pH of about 5.4 to 5.5 was reached. Then, saturated potassium hydroxide was added to raise the pH to 6.5. Lactose hydrolysis was carried out as described above. The pH was adjusted back to 5.2 with citric acid, the whey was heated to 90°C ,and held for 10 min to coagulate the protein which was then filtered out.

4. Combination of potassium hydroxide and either calcium hydroxide or magnesium oxide

To decrease the salty taste a combination of neutralizing agents was investigated. Saturated potassium hydroxide was added to DAS cottage cheese whey to pH 5.2. The whey was heated to 90°C and held for 10 min with continuous stirring. The whey was filtered, and then 0.1 to 0.2g of calcium hydroxide at 0.01g increments was added per 100ml of filtrate. The mixture was agitated for 15 min to dissolve the calcium hydroxide. At all levels of added calcium hydroxide, the pH was raised to 5.7. The pH then was adjusted to 6.5 with saturated potassium hydroxide, and cloudiness was removed by filtration. Then hydrolysis of the lactose was commenced.

Magnesium oxide was tried in the place of calcium hydroxide with the above procedure. It was added to the acid whey at concentrations of 0.05 to 0.10g/ 100ml resulting in a pH 5.7 to 5.9. Saturated potassium hydroxide was used to further increase the pH to 6.5 for the lactose hydrolysis.

5. Neutralizing agents selected for formulation

Calcium hydroxide was used for final formulation in the amount of 0.1 to 0.15g per 100ml of deproteinated whey. Potassium hydroxide was used to increase the pH to 5.2 for protein removal and for final pH adjustment to 6.5.

B. Formulation of the Beverage

1. Acids

Five acids were compared to determine which one blended favorably with the whey. Those tried were tartaric acid, fumaric acid, phosphoric acid, malic acid, and citric acid. A combination of citric and malic acid was also tested. The pH of the deproteinated, lactose-hydrolysed whey was adjusted to 3.5 with one of the said acids. Sucrose was added at a level of 2% when comparing the acids. A five member expert panel evaluated the acidified whey samples and expressed their opinions on the flavor the acids imparted to the whey.

2. Sugars

A level of 2% added sucrose was used during prototype formulations. Glucose was used along with sucrose in the final formulation to make the sweet flavor linger in the mouth. For the final product, levels of 4% w/v sucrose and 1% w/v glucose were used to make it comparable in sweetness to Gatorade^{R}

3. Flavorings

Most of the sensory work was done with the beverage flavored with Norda's atural and Artificial Orange Emulsion (#EP-10,806), East Hanover, New Jersey. It imparted an orange color as well as flavor. A flavor level of 0.3%v/v was used when work was being done with the whey base. At this level the orange color was darker than orange drinks on the market. A level of 0.2%v/v was closer to the color of Gatorade^{R}, and was used for the final formulation. In addition to the Norda flavoing Blanke Baer's Orange Extract, Fenton, Missouri, was added at a level of 0.3%v/v, in addition to Norda's, to improve the orange flavor.

4. General procedure for preparing the beverage

A 4-liter pyrex beaker with a magnetic stir bar was used as a vat, and a Corning PC-351 hot plate-stirrer was used for stirring and heating. The temperature of the whey was adjusted to 25°C and saturated potassium hydroxide was added to obtain

a pH of 5.2. Under continuous stirring the fluid was heated and held at 90°C for 10 min. The coagulated protein was removed by filtration using Whatman #4 filter paper. The filtrate was allowed to cool to 25°C. Calcium hydroxide was added to the filtered whey at 0.1 to 0.15g/ 100ml of filtered whey, and agitated by a magnetic stir bar for 15 min. This gave a pH of 5.6 to 5.7. The pH then was adjusted up to 6.4 to 6.5 with saturated potassium hydroxide solution. The liquid was filtered again with Whatman #4 filter paper to remove cloudiness caused by calcium hydroxide and some protein precipitation at that pH. The finished product was formulated by adding one part distilled water to two parts whey, adjusting the pH down to 3.7 with saturated citric acid; adding 1%w/v glucose and 4%w/v sucrose; and adding sufficient orange flavor (as described in the previous section) to make an acceptable orange flavored athletic drink. The flow diagram of this process is illustrated in Figure 1. The formula of the finished product is found in Table 7.

C. Sensory Methods

Triangle tests as described by Larmond (1977) were utilized to determine if panelists could pick up differences between two different methods of processing whey for the beverage. The test was used extensively for comparing neutralizing agents. See Appendix A.1 for an example of the form used. Panelists were food science and non-food science students who were familiar with taste testing. The panelists were given the option of making comments. These comments were used to make decisions on changing the processing methods and formulations.

Three to five persons trained in taste testing were used as an expert panel in developing the formulations. The panelists tasted the prototype beverages and then discussed improvements that could be made.

Preference tests were used to determine if the whey drink was preferred to Gatorade^{R} An example of the form used is found in Appendix A.2. The panelists all had had various taste panel experiences. Each panelist was given two coded samples,

Figure 1. Flow diagram of laboratory processed whey beverage.

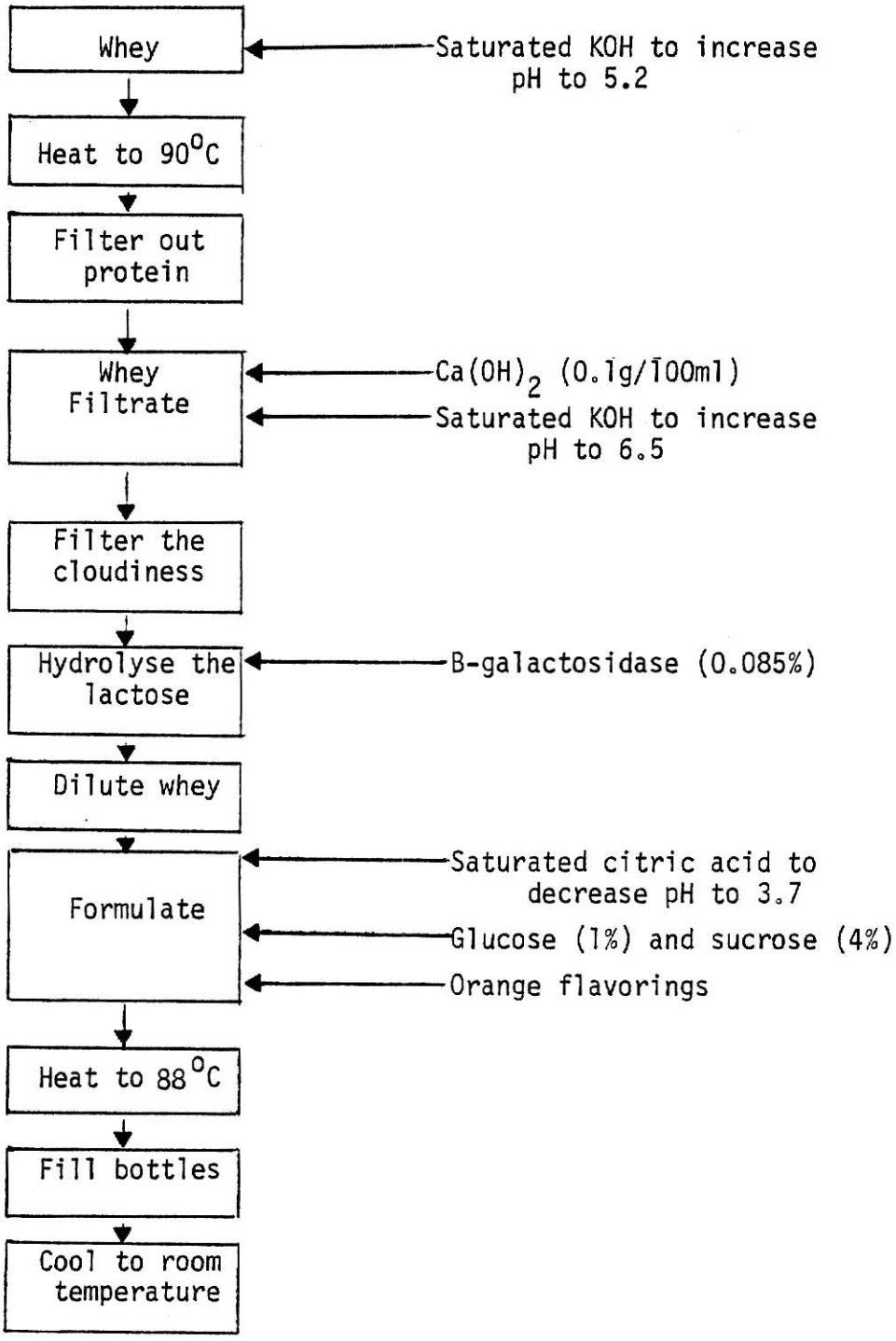


Table 7. Formula for whey-based athletic-type drink.

Ingredient	% Composition	Amt./32 fl. oz.
Whey	62.5	592.0ml
Water	31.3	296.5ml
Sucrose	3.8	36.0g
Glucose	0.9	8.5g
Saturated citric acid[a]	0.7	6.6ml
Flavoring[b]	0.5	4.7ml
Saturated potassium hydroxide[c]	0.3	2.8ml
Calcium hydroxide	d	d
Total	100.0	32 fl. oz. (947.1ml)

[a] 146g citric acid/ 100ml water

[b] 0.2% Norda Natural and Artifical Orange Emulsion and 0.3% Blanke Baer Orange Extract

[c] 1.0g potassium hydroxide/ 1.0g water

[d] 0.1 to 0.15g/ 100ml whey base

had had various taste panel experiences. Each panelist was given two coded samples, and asked which he preferred.

D. Heat Processing Method Used For Controlling Microorganisms

The heat processing method used was similar in practice to that described by Pflug and Esselen (1967). The beverage was heated to 88°C in a beaker on a hot plate and poured into the bottles. The bottles were sealed, inverted and held for 5 min, and then turned right side up and cooled to room temperature in a cold water bath.

E. Microbiological and Analytical Procedures

1. Microbiological Analysis

Standard plate counts were made using plate count agar, and yeast and mold counts were made using potato dextrose agar (A.O.A.C., 1980). Three bottles of hot filled beverage were stored in an incubator at 37°C for 7 days, and then duplicate 1ml samples from each bottle were plated for each test to determine the presence of microorganisms.

2. Chemical Analysis

Test samples were analyzed by the K.S.U. Department of Animal Sciences and Industry Nutrition Laboratory for protein, calcium, phosphorous, sodium, potassium, chloride, and magnesium. Protein was analyzed by the Kjeldahl procedure (A.O.A.C., 1980). A dry sample was ignited in a muffle furnace to produce carbon-free ash (A.O.A.C., 1980). Ash solutions were prepared to be run through a Jarrell-Ash Atomic Absorption Instrument to determine calcium, magnesium, sodium, and potassium. Phosphorous was determined by the spectrophotometric molybdovanadate method (A.O.A.C., 1980). The chloride was determined by a titrimetric kit from Sigma Chemical Company, St. Louis, Missouri. Titration was done with a standardized mercuric nitrate solution using diphenylcarbazone to visually indicate the endpoint (Anonymous, 1982a). The percent of lactose hydrolysis was determined by the cryoscope method (Nijpels et al., 1980), using Fiske Milk Cryoscope Model J-61 (Fiske Associates,

Inc., Uxbridge, Massachusetts). The freezing point of the whey is measured before and after lactose hydrolysis. The difference between the two measurements was divided by 0.273 and multiplied by 100 to get the percent hydrolysis.

II. Pilot Plant Processed Beverage from Whey

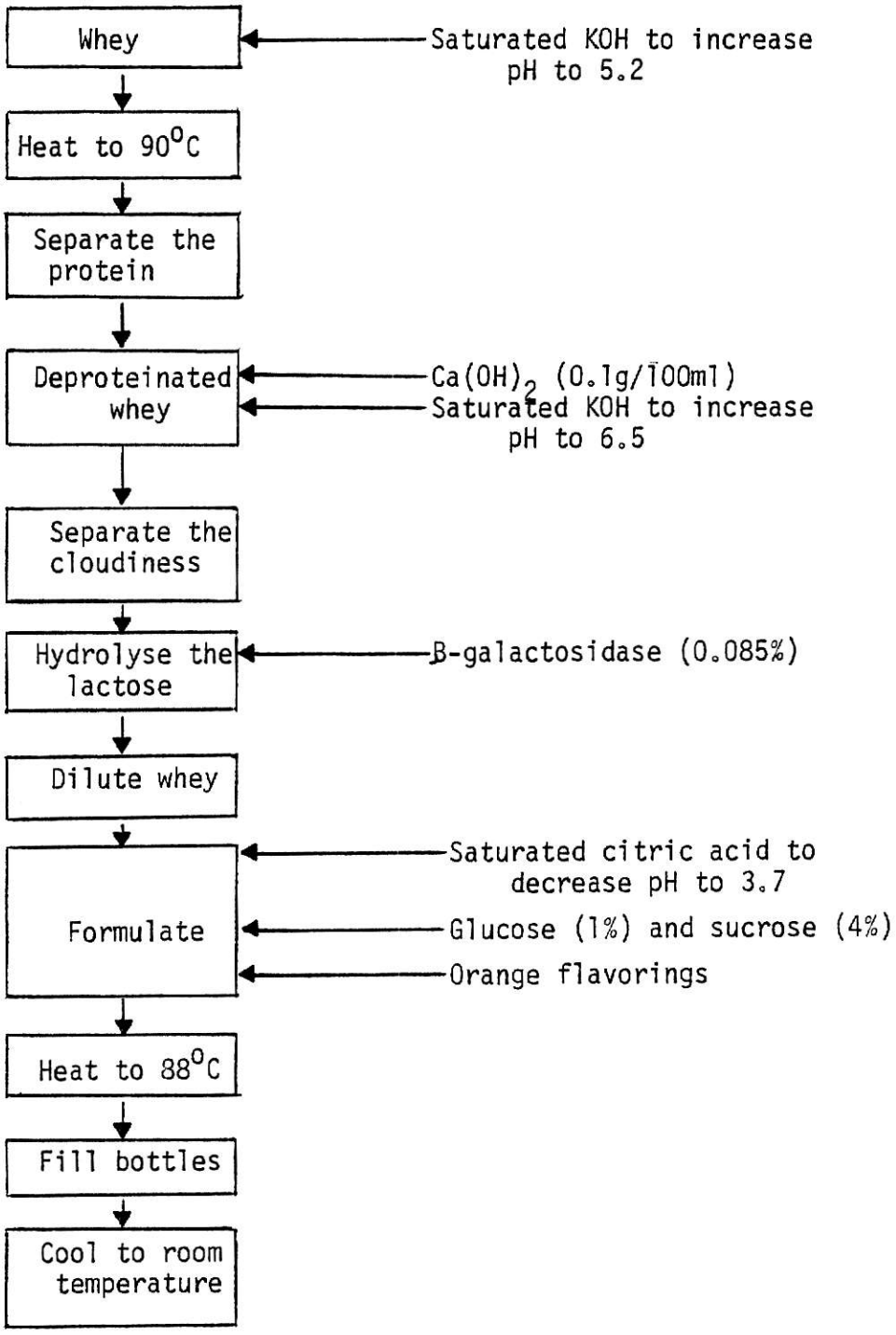
A. Preparation of Beverage from Whey

Fresh whey at pH 4.2 was obtained from Beatrice Foods Company (Meadow Gold), Topeka, Kansas. It was stored at 2 to 3°C before and after transporting to Manhattan, Kansas. Processing of the whey was done in the K.S.U. Dairy Processing Plant.

Twenty gallons of whey were placed in a steam jacketed vat, and neutralized with saturated potassium hydroxide to a pH of 5.2. It was heated to 90°C (195°F) and held for 10 min and then run through a dairy cream separator to get a clear liquid. It was run through the separator twice for best results. Calcium hydroxide at a level of 0.12g/ 100ml whey (90.96g in total) was added and then agitated for 15 min. A pH of 5.6 was reached. Saturated potassium hydroxide was then added to obtain a pH of 6.45. The whey was run through the separator again. Beta-galactosidase (Maxilact^{R}) was added at a level of 0.085% (48.25ml) to 15 gallons of deproteinated whey. The deproteinated whey was hydrolyzed in stainless steel milk cans at 5°C for 18 hr.

Five gallons of water was added to ten gallons of the deproteinated, lactose-hydrolyzed whey and saturated citric acid was added to lower the pH to 3.74. One percent (w/v) glucose, 3%w/v sucrose, 0.2%v/v Norda Natural and Artificial Orange Emulsion, and 0.3%v/v Blanke Baer Orange Extract were added. The mixture was heated to 88°C and poured into 8 Fl. Oz. Standard N Round bottles, finish 28-405 (Riekes Container Co., Lenexa, Kansas). The bottles were capped with metal closures, inverted, and held for 5 min, and then cooled in a walk-in cooler. Seven cases of the 8 Fl. Oz. bottles of beverage were produced. A flow diagram of the process is found in Figure 2.

Figure 2. Flow diagram of plant processed whey beverage.



To monitor and adjust the pH, 200ml samples were taken from the vat, and the amount of neutralizer or acidulant necessary to adjust to the desired pH was measured. From this measurement the amount needed for the whole vat was calculated. The percent hydrolysis was determined by the cryoscope as described in the laboratory preparation section. When the sugars were added the Brix reading was monitored with a refractometer (American Optical Corporation, Buffalo, New York) so that 8.5° Brix would be the final reading. This was to prevent the error of adding too much sugar.

A filler was not available for filling and capping the bottles so this step was done by hand. Four liters of the beverage were heated at one time, and poured into bottles. The bottles were capped manually and placed upside down in the case. After 5 min they were turned upright, and placed in the walk-in cooler to cool down.

B. Consumer Evaluation

Black and white gummed labels were put on bottles (See Appendix A. 3). Seventy-five labeled bottles were singly placed in brown bags along with an evaluation sheet, instructions, and a self-addressed stamped envelope for returning the evaluation sheet. The test samples were distributed to customers of the K.S.U. Dairy Sales Counter. The consumer was instructed to cool the sample, and drink it from the bottle, or pour into a glass but use no ice.

A 7-point hedonic scale (See Appendix A. 4) was used for the consumer acceptance test (Larmond, 1977). An average value was determined from total responses as well as the percentage of responses for each category.

C. Shelf Life Study

A multiple comparison test (Larmond, 1977) was conducted to evaluate the stored samples using eight panel members trained in food product evaluation (Larmond, 1977). The reference sample was stored at 5°C for the duration of the test. Test samples were stored at 21, 32, and 37°C to accelerate changes in shelf life. Panelists

tasted all three samples as well as the reference every 2 weeks to detect changes in sweetness, orange flavor, overall flavor, and color. Reference scores were set by having the panelists evaluate the 0 day samples for the said characteristics. An average of the scores was calculated and used for the reference score for the duration of the experiment. Each characteristic was scored by comparing it to the reference sample. (See Appendix A. 5).

D. Microbiological and HPLC Procedures

1. Microbiological Analysis for Product Sterility

Seven bottles (8 fl. oz.) were chosen randomly from the seven cases of the product processed in the pilot plant. They were incubated at 37°C for 15 days, and checked for visual changes after 7 and 15 days. On day 15, each was sampled aseptically and 1ml plated in each of the four different agars:

1. Standard plate count agar for aerobic viable cell counts,
2. Orange serum agar for acid tolerant bacterial counts,
3. Anaerobic agar in an anaerobic jar for anaerobic bacterial counts,
4. Potato dextrose agar (pH adjusted to 3.5 with 10% tartaric acid; 0.1% tetracycline and 0.1% chlorohexanimide added to prevent bacterial growth) for yeast and mold counts.

Duplicates were made of each analysis. The plate count agar was incubated at 32°C for 48 hr; the orange serum agar and the anaerobic agar were incubated at 37°C for 48 hr. Potato dextrose agar was incubated at room temperature for 5 days (A.O.A.C., 1980; Leininger, 1976).

2. Separation and Quantitation of Carbohydrates by High Performance Liquid Chromatography

Stability of sugars during storage was analyzed by high performance liquid chromatography (HPLC). Samples of the beverage stored at 5, 21, and 37°C for 4 and 8 weeks were diluted to one part beverage to four parts water. The diluted samples

were run through Sep-Pak C_{18} Cartridges (Waters Associates, Inc., Milford, Massachussetts), as described (Anonymous, 1981a), to remove lipid, colored materials, and residual proteins.

A standard carbohydrate solution was prepared from analytical grade reagents. All sugars involved were dissolved in water (w/v) at a concentration near that estimated in the diluted test samples. The standard solution contained 0.05% lactose, 0.34% galactose, 0.55% glucose, 0.15% fructose, and 0.54% sucrose. Prior to injections the standard was run through a Sep-Pak as described in the sample preparation.

The HPLC system consisted of a Beckman Model #100A solvent delivery system, an Altex #210 septumless injector, an Altex refractive index detector Model #156 with Fisher Recordall Series 5000 chart recorder. The precolumn was a AX-GU Ion Exchange LiChrosorb-Anion Column (Brownlee Labs Inc., Santa Clara, California) which removes unwanted anions. The analytical column was a μ-Spherogel Carbohydrate 7.5 column. It was held at 80°C by a water jacket on the column and a Fisher Model #80 circulating water bath. The eluent was deionized, boiled, and degassed water held at a temperature of 62°C. The flow rate was 0.6ml/ minute. A Hamilton 25 μl syringe was used to inject a sample into a 20 μl sample loop.

RESULTS AND DISCUSSION

I. Beverage Made in the Laboratory

A. Preparation of Beverage Base from Whey

The first attempt at deproteinating the whey involved neutralizing it with potassium hydroxide to a pH of 6.5 for lactose hydrolysis, and readjusting the pH with an acid to 5.2 for protein coagulation by heat. In this preliminary study it was found that after using potassium hydroxide to increase the pH, a pH of 5.2 was best for heat-coagulating the whey proteins. A sparkling clear supernatant resulted with this treatment after filtering. At that pH, no further protein precipitation was seen upon heating a second time. In fact there was no difference in the degree of turbidity in any of the wheys after the second heating. Table 8 shows the visual measure of clarity of whey after removal of proteins by filtration at various pH's. The preliminary studies also showed that heating to 90°C at pH 5.2 for 10 minutes was sufficient to coagulate proteins in whey. Table 9 shows the percent of proteins removed from whey after heating at various temperatures. At 90°C, 57% of the proteins was removed. No further removal of protein was observed at higher temperatures. Therefore, 90°C was selected as the best temperature for economic reasons as well as convenience. At that temperature 10 min was the time required for complete protein coagulation. Less than 10 min resulted in a cloudy appearance.

Magnesium oxide when used as a neutralizing agent produced the clearest liquid upon heating at pH 4.5. No precipitate was observed after reheating the filtered whey. However, it was found that the amount of protein removed was less with magnesium oxide than with potassium hydroxide. With magnesium oxide, only 50% of the protein was removed whereas 57% protein was eliminated when potassium hydroxide was used. It was also difficult to adjust the pH with magnesium oxide because of its insolubility in water. When magnesium oxide was used to neutralize the whey a large amount of magnesium remained in solution. Twenty-three times more

Table 8. Visual measure of clarity of whey after protein removal at various pH's when the pH was increased with potassium hydroxide.

pH	Before heating	After 1st heating	After 2nd heating
4.0	Turbid	Turbid	Turbid
4.2	Turbid	Turbid	Turbid
4.4	Turbid	Clear	Clear
4.6	Turbid	Slightly turbid	Slightly turbid
4.8	Turbid	Very slightly turbid	Very slightly turbid
5.0	Turbid	Clear	Clear
5.2	Turbid	Clear	Clear
5.4	Turbid	Slightly turbid	Slightly turbid
5.6	Turbid	Slightly turbid	Slightly turbid
6.0	Turbid	Turbid	Turbid

Table 9. Heat treatment and its effect on removal of proteins from whey.

Temperature (°C)	% Protein removed
85	52.25
90	57.0
95	57.0
98.5	57.0

magnesium was found in this whey filtrate than in the original whey. As a result 200ml of this deproteinated, lactose-hydrolyzed whey would meet the U.S.R.D.A. for magnesium of 300mg to 350mg per day (See Table 10) (Anonymous, 1980b)

A salty taste was a problem when potassium hydroxide was used as the neutralizing agent; so other chemicals were tried. Calcium hydroxide was tested as a neutralizing agent. An expert panel of four members preferred the calcium hydroxide as a neutralizing agent to potassium hydroxide, but they still found a salty taste.

To minimize the salty taste, the process was rearranged. The pH was adjusted to 5.2 using potassium hydroxide and protein was removed, and then the pH was further adjusted to 6.5 for lactose hydrolysis. The amount of potassium hydroxide required was the same as when the pH was initially adjusted to 6.5. The total amount of potassium hydroxide required was 0.4% in both cases. Since rearrangement did not help reduce the salty character, calcium hydroxide was incorporated into this process. The pH was adjusted to 5.2 with potassium hydroxide, and the protein was removed in the usual way. Then, 0.1 to 0.15g calcium hydroxide per 100ml whey was added to the deproteinated whey, and it was agitated for 15 minutes. This increased the pH to 5.6 to 5.7. The pH was further raised to 6.5 with potassium hydroxide and it was filtered or centrifuged again. This method reduced the amount of potassium hydroxide used by 25% (from 0.4 to 0.3% potassium hydroxide). Less citric acid was required to lower the pH to 4.0 with this method. With no calcium hydroxide 0.7% citric acid was needed, and with calcium hydroxide only 0.46% citric acid was needed.

This same method was tried using magnesium oxide. The same amounts of potassium hydroxide and citric acid were required, but only 0.05g magnesium oxide per 100ml of whey was needed to reach a pH of 5.7. Analysis of calcium, potassium, and magnesium for the two previous methods mentioned shows that the amount of magnesium is greatly increased over that in direct-acid-set (DAS) cottage cheese whey, when magnesium oxide is used to neutralize it (See Table 11). When calcium

Table 10. Mineral and protein composition of unprocessed and processed[a] direct-acid-set wheys.

	MgO process	KOH process	Unprocessed whey
	in meq/l		
Calcium	0.27	0.15	0.27
Phosphorus	0.67	0.51	0.60
Magnesium	1.05	0.04	0.05
Sodium	0.27	0.34	0.18
Chlorine	0.35	0.31	0.31
Potassium	0.13	0.31	0.23
Protein	0.477%	0.374%	0.946%

[a] Neutralized with the indicated neutralizer to pH 6.5

Table 11. Mineral content of unprocessed and deproteinated, lactose-hydrolysed processed[a] wheys.

	MgO	$Ca(OH)_2$	Unprocessed whey
	in meq/l		
Calcium	0.063	0.081	0.26
Potassium	0.37	0.35	0.25
Magnesium	0.14	0.05	0.071

[a] The pH was adjusted to 5.2 with potassium hydroxide. Then, the protein was removed and the indicated neutralizer was added to increase the pH to 5.6.

hydroxide is used the calcium concentration is decreased from that of DAS cottage cheese whey. Also the cost for laboratory grade magnesium oxide is $81.25/ 500g, whereas the cost of laboratory grade calcium hydroxide is only $15.75/ 500g, or five times less (from the VWR, 1982 catalog). In addition, solubility of calcium hydroxide is much greater than that of magnesium oxide. Calcium hydroxide dissolves at 0.185g/ 100ml water at 0°C and 0.077g/ 100ml at 100°C, while magnesium oxide dissolves 0.00062g/ 100ml in cold water and 0.0086g/ 100ml at 30°C (Windholz, 1976).

B. Formulation of the Beverage

According to an expert panel of four, citric acid and malic acid blended well with the whey flavor. Malic acid, however, did not blend as well as citric acid with the orange flavor. Citric acid blended better than a combination of citric and malic acid. Therefore citric acid was used as the acidulant in the final formulation. The final pH of 3.7 accentuated the orange flavor better than a lower or higher pH. For the final product levels of 4%w/v sucrose and 1%w/v glucose were used to make the sweetness comparable to Gatorade^{R}. The sucrose alone resulted in a strong sweet taste at first, and then it dropped off sharply. A level of 0.2%v/v Norda Natural and Artificial Orange Emulsion was closer to the color of Gatorade^{R}, and was used in the final product. Blanke Baer Orange Extract was added at a level of 0.3%v/v to increase the orange flavor.

C. Sensory Analysis during Formulation

Using a triangle test 18 panelists compared two formulations of whey, one processed with calcium hydroxide and the other with magnesium oxide as neutralizing agents in conjunction with potassium hydroxide (See Appendix A.1). Five persons were able to distinguish a difference, 13 could not. Therefore, there was no statistically significant difference between the two products. Those who did detect a difference considered the difference to be moderate (See Appendix Table 16). Many panelists complained about a salty taste. Kosikowski (1968) reported that adding 4% whey

powder to reconstituted fruit juices resulted in an excellent flavor, and 6% added whey powder gave an acceptable, but salty taste. With this information in mind, one part water was added to two parts whey to give about 4% whey solids. It was compared to Gatorade^{R} in a preference (paired comparison) test (See Appendix A. 2). Twenty-eight panelists participated in the preference test. The results were as follows: 64% (18 members) preferred the whey-based athletic-type drink to Gatorade^{R}, 36% (10 members) preferred Gatorade^{R}. In a two sample difference test one sample must be selected 20 times out of 28 judgements to be significantly different at a 5% level. The difference was not statistically significant, but the majority preferred the whey based athletic drink.

D. Microbiological and Analytical Evaluation

1. Microbiological analysis

The heat processing method used appeared to preserve the beverage in 8 Fl. Oz. bottles. There was no microbial growth detected in any of the three samples analysed. Microbial tests conducted were total viable cell count, yeast and mold counts, and anaerobic counts from the samples stored in an incubator at 37°C for 8 days.

2. Chemical Analysis

The results of the mineral and protein analysis, and caloric content of the final product are shown in Table 12. The sodium and chloride contents are slightly lower than those of Gatorade^{R}. The sodium level of this beverage is closer to that recommended by the Americian Dietetic Association of 10meq per liter than Gatorade^{R}. The potassium levels of both products are similar to that recommended by the American Dietetic Association of 5 meq per liter. The formulated product is lower in chloride content than Gatorage^{R}. However, it comes closer to complying with the American Dietetic Association recommendations for athletic drinks than does Gatorade^{R}. Magnesium and calcium are not added to Gatorade^{R}, and the phosphorous content was not available for the commercial drink; so comparison could not be made

Table 12. Comparison of minerals, protein, and caloric content of whey-based athletic-type drink and Gatorade®.

	Whey based athletic drink	Gatorade[a]
	in meq/l	
Calcium	2.7	b
Phosphorus	21.7	b
Sodium	14.52	22.7
Potassium	2.56	2.5
Chloride	2.31	13.5
Magnesium	2.2	b
Calories	25 cal/100ml	21 cal/100ml
Protein	0.17%	b

[a] From Coyle et al. (1978)

[b] Not available

for these three minerals. There is a 0.17% level of protein in the whey product which improves the mouth feel without the addition of hydrocolloids.

II. Plant Processed Athletic Drink Made from Whey

A. Preparation of Beverage in the University Dairy Processing Plant

The amount of saturated potassium hydroxide necessary to make the pH adjustments for large batches was calculated by measuring the amount needed to adjust 200ml of whey to the desired pH. This method worked well and was necessary for avoiding over-adjustedment of pH.

When separating the protein it was found that running it through the separator once resulted in a relatively clear whey, but it had a dull appearance. Running it through a second time resulted in a sparkling clear whey. The pH decreased 0.03 units between the first and the second run.

In the laboratory the addition of 0.1g calcium hydroxide per 100ml of deproteinated whey was sufficient to increase the pH to 5.6. In the plant with a larger batch it took at least 0.12g/ 100ml to reach the same pH. Ninety-four percent of the lactose was hydrolysed into glucose and galactose in 18 hr at 5°C when the ß-galactosidase (0.085%) was added to the deproteinated whey. The whey was diluted with one part water to two parts whey. Then the pH was lowered to 3.74 with saturated citric acid.

B. Consumer Evaluation

Sixty-nine out of 76 consumers responded to the consumer acceptance evaluation. The average response on the 7-point hedonic scale was 4.26, which fell between "like slightly" and "neither like nor dislike". Forty-seven point eight percent (47.8%) reported some degree of liking, 14.5% reported that they neither like nor dislike the beverage, and 37.7% reported some degree of disliking. The response with the most number of votes was "like moderately" with 27% of the votes (See Table 13).

Table 13. Results of consumer acceptance evaluation on plant prepared samples.

Rating Scale	# of responses	% of total
7-like very much	4	5.7
6-like moderately	19	27.5
5-like slightly	10	14.5
4-neither like nor dislike	10	14.5
3-dislike slightly	15	21.7
2-dislike moderately	6	8.7
1-dislike very much	5	7.2

The ratings obtained may have been affected by a number of factors other than quality of the test sample. These are characteristics of the test subjects or of the test situation, transitory attitudes or expectations of the subjects (Anonymous, 1968). It was not determined if the panelists used athletic-type drinks regularly. If they don't use them regularly, they may rate this beverage low because of the bland flavor. They were given instructions on sample preparation, but since the panelists tasted the product in their homes we had little control over the conditions for tasting. If they failed to cool it down properly that could affect the flavor, and consequently the quality value assigned.

C. Shelf Life Study

The results of the shelf life study are depicted in graph form in Figures 3 through 6. The numerical results can be found in Appendix Table 17. All four categories (sweetness, orange flavor, overall flavor, and color) showed little change during 10 weeks of storage at any of the storage temperatures. The panelists detected little difference between the beverages stored for 10 weeks at 5 and 37°C. The subjectiveness of the test could account for some fluctuations in the results.

The rate of a reaction is about doubled, for many reactions, by 10°C rise in temperature (Brady and Humiston, 1975). This is known as the Q_{10} theory. The 32°C stored beverage showed no flavor change over the 21°C stored beverage during 10 weeks. If the reaction rate of off-flavor development in this beverage follows the Q_{10} theory then the beverage stored at 21°C would show no flavor change for more than 20 weeks. If we apply the same principle to the beverage stored at 37°C over the 21°C stored beverage, one can project the shelf life of the product to be more than six months at 21°C. Because of the time limit on this study, testing the theory on this product is limited. However, according to the results obtained thus far, the product appeared to be stable at room temperature for at least six months.

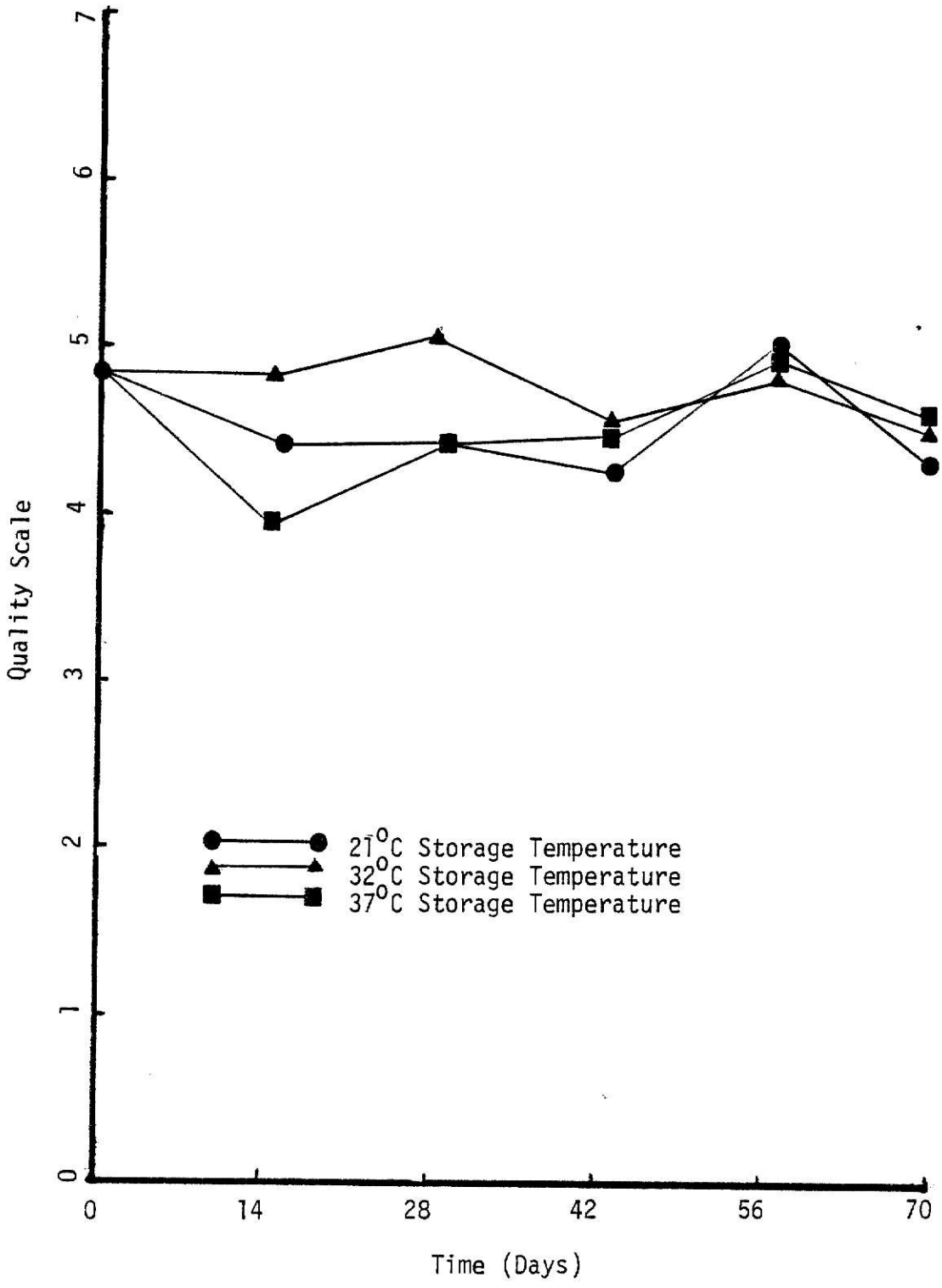


Figure 3. Shelf life study of sweetness. Each data point indicates the average of the scores of eight panelists.

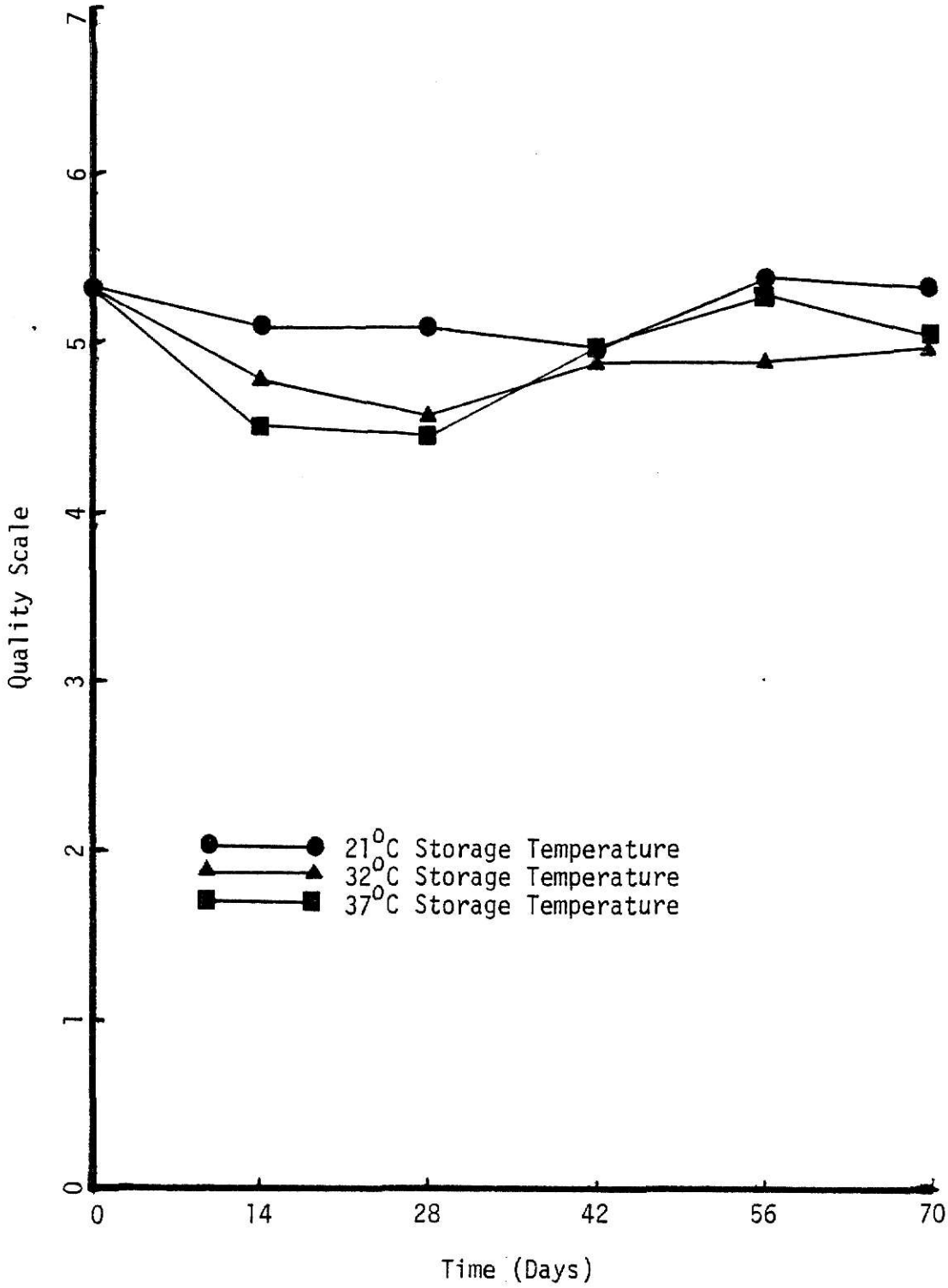


Figure 4. Shelf life study of orange flavor. Each data point indicates the average of the scores of eight panelists.

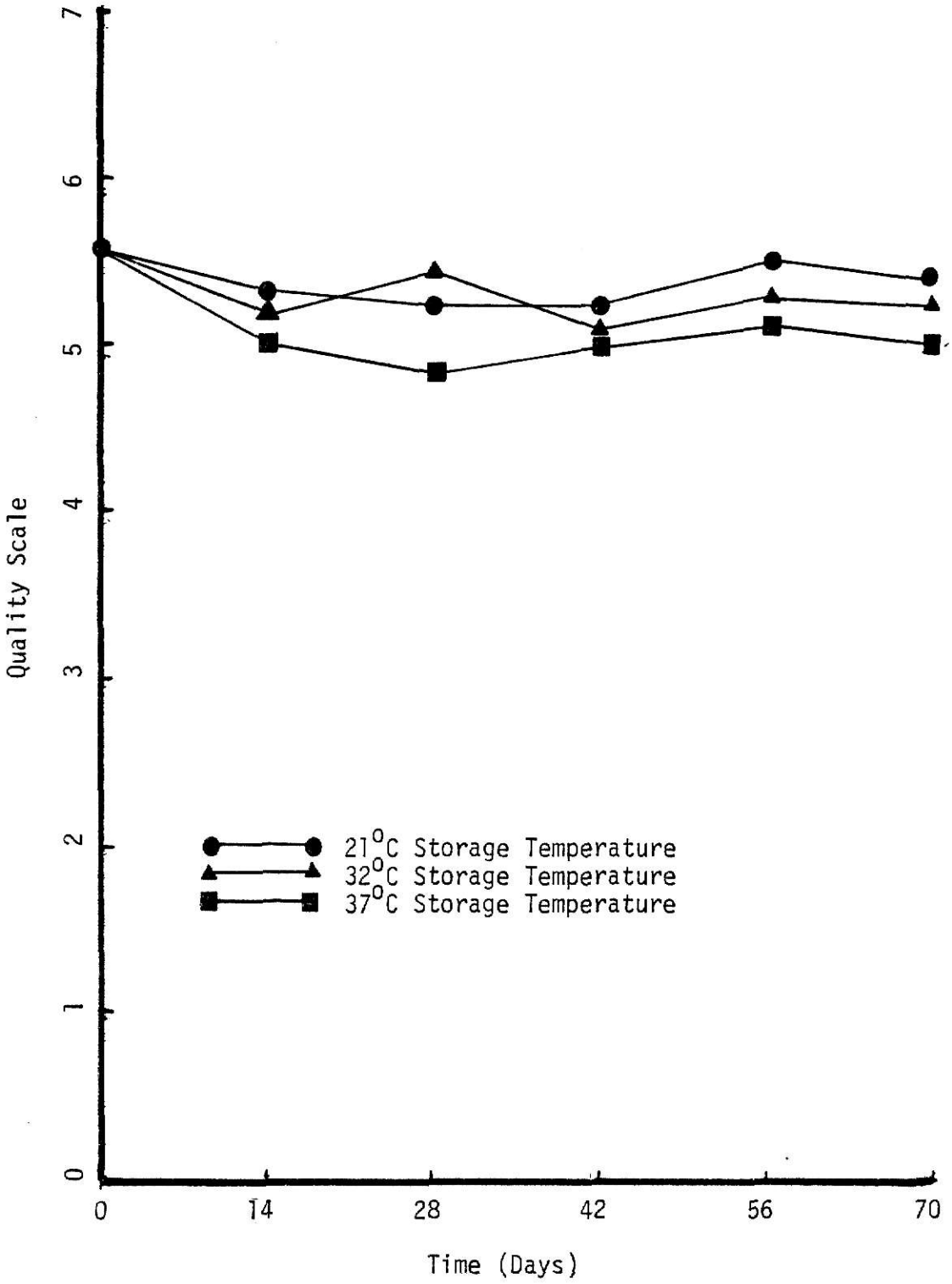


Figure 5. Shelf life study of overall flavor. Each data point indicates the average of the scores of eight panelists.

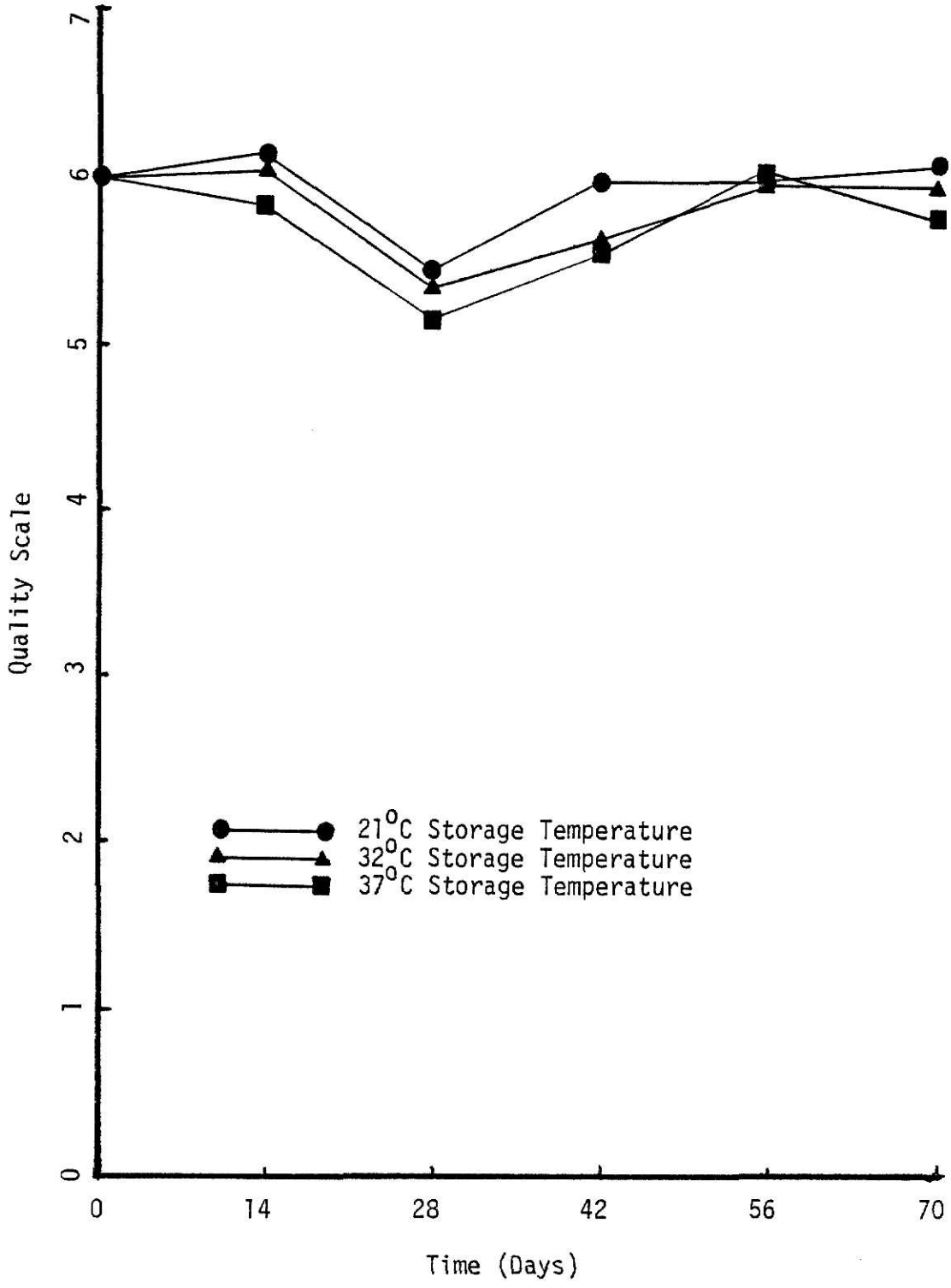


Figure 6. Shelf life study of color. Each data point indicates the average of the scores of eight panelists.

D. Microbiological and HPLC Analysis

1. Microbiological Analysis

There was no microbial growth detected from the beverage samples stored for 15 days at 37°C. All four microbiological tests conducted (viable cell count, acid tolerant bacterial count, anaerobic bacterial count, and yeast and mold counts) showed no growth of microorganisms in 7 replicates. There was no visible change in the color or appearance observed. These results indicated that the heat treatment applied was adequate to control all microorganisms. Therefore, this product appeared to be "commercially sterile". Commercial sterility means that it has the degree of sterility at which all pathogenic and toxin forming microorganisms have been destroyed as well as other more resistant types which, if present, could not grow in the product and produce spoilage under normal storage conditions (Tanner, 1944).

2. Separation and Quantitation of Carbohydrates by High Performance Liquid Chromatography

The separation of sugars by high performance liquid chromatography is shown by the chromatograms in Figures 7 and 8. Upon observance of the relative peak heights, one can see that the beverage stored at different temperatures for 4 and 8 weeks has different concentrations of the five sugars. No interference from the whey base used was observed on the peaks. The peak heights of the stored samples were compared to those in the standard solution; and the percentages in Table 14 were calculated.

The sucrose peak (Peak #1) is from the sucrose that was added for sweetness. The lactose shoulder (Peak #2) is the residual lactose remaining after being hydrolyzed by the ß-galactosidase. The glucose peak (Peak #3) originated from a sum of glucose addded for sweetness and glucose that resulted from lactose hydrolysis and from acid hydrolysis of the sucrose. Galactose (Peak #4) is present as a product of the lactose hydrolysis. The amount of sucrose in the sample decreased as the storage temperature increased. Accordingly, the amount of fructose (Peak #5) and glucose increased. This is

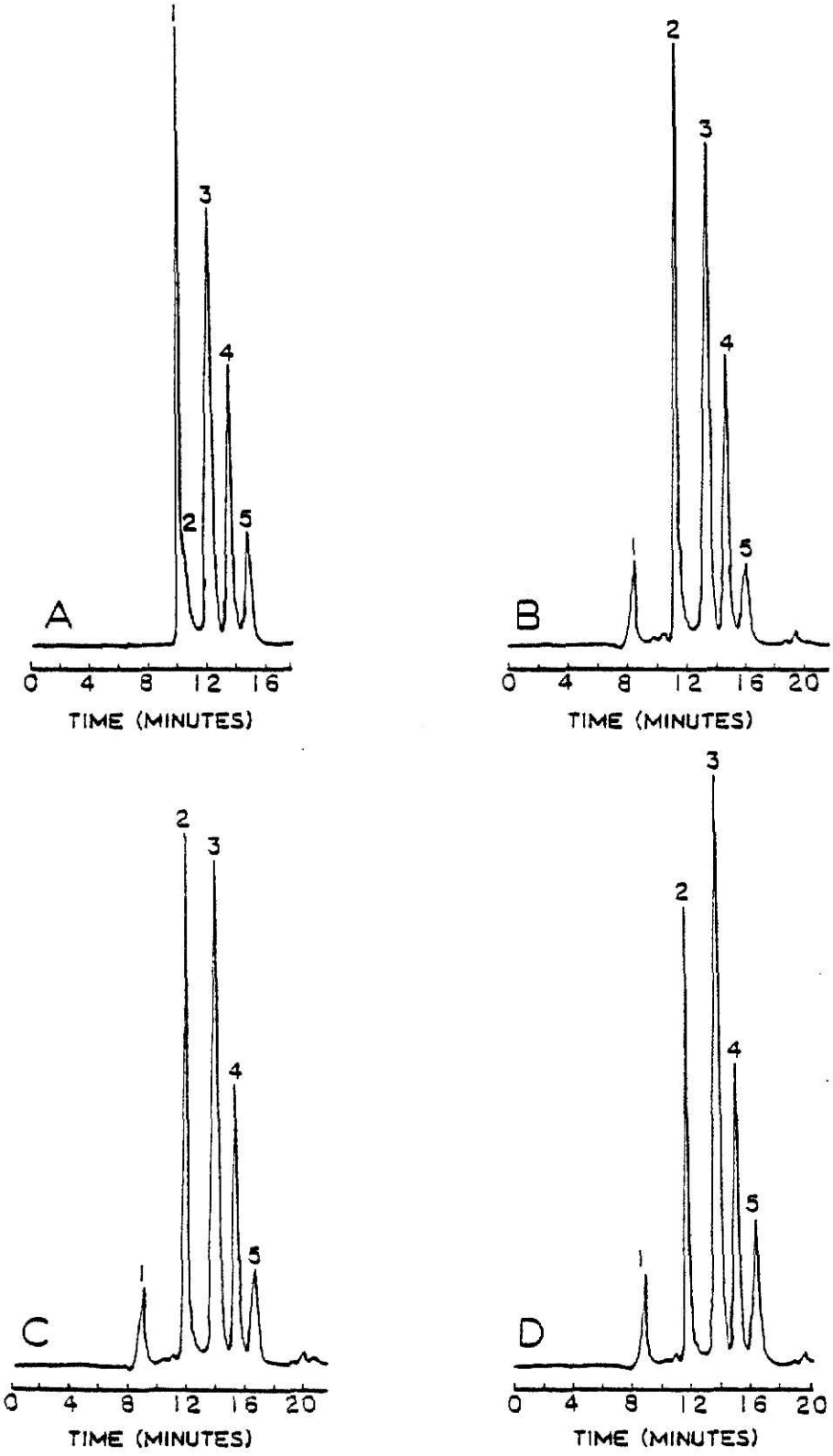


Figure 7. High performance liquid chromatograms of standard solution and product stored for four weeks: 1)sucrose, 2)lactose, 3)glucose, 4)galactose, and 5)fructose. μ-Spherogel Carbohydrate Column (80°C); solvent, H_2O; flow rate, 0.6ml/min; injection volume, 20μl; RI detector, 16x.

A) Standard solution
B) Stored at 5°C
C) Stored at 21°C
D) Stored at 37°C

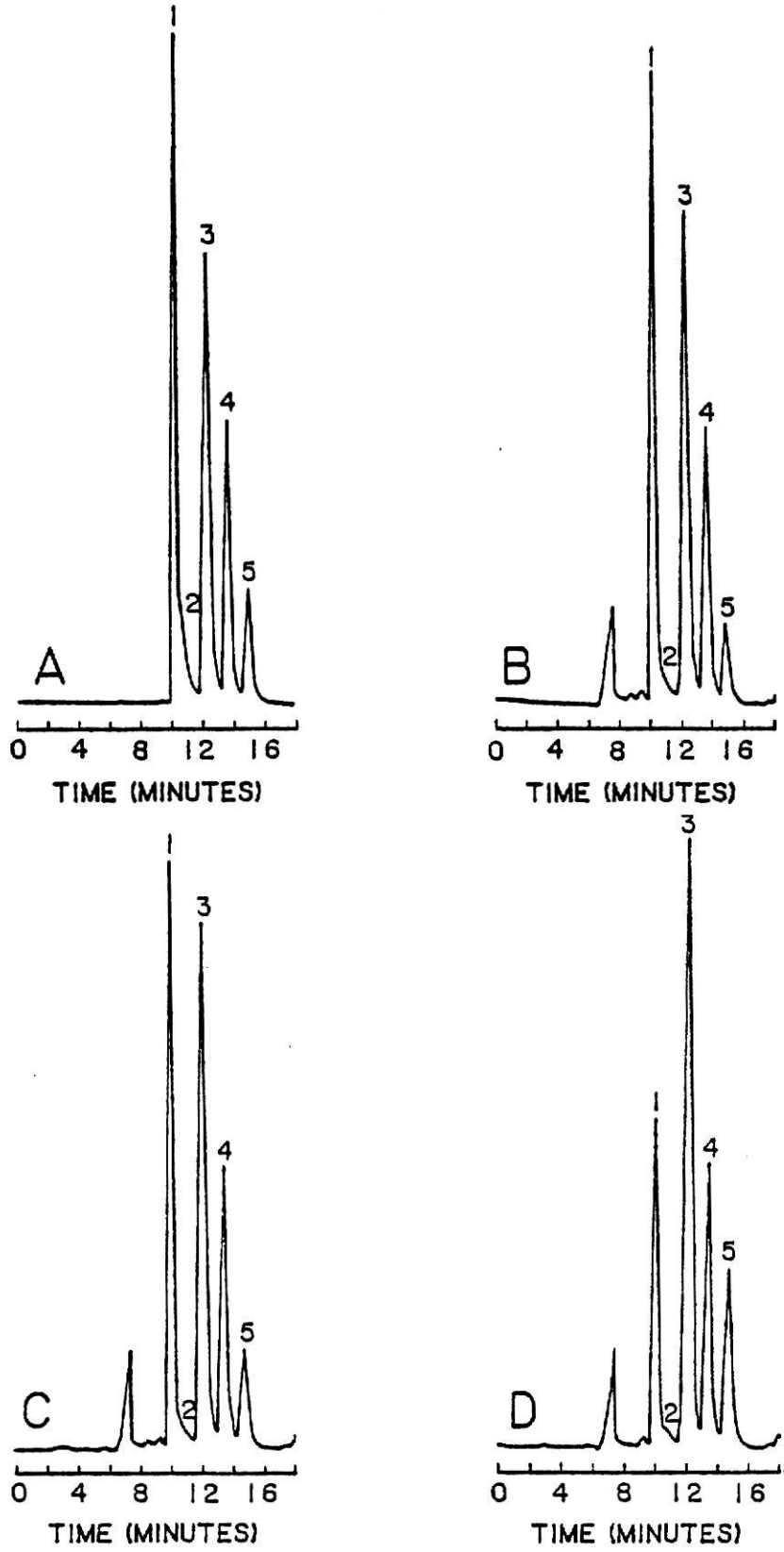


Figure 8. High performance liquid chromatograms of standard solution and product stored for eight weeks: 1)sucrose, 2)lactose, 3)glucose, 4)galactose and 5)fructose. μ-Spherogel Carbohydrate Column (80°C); solvent, H_2O; flow rate, 0.6ml/min; injection volume, 20μl; RI detector, 16x.

A) Standard solution
B) Stored at 5°C
C) Stored at 21°C
D) Stored at 37°C

Table 14. Sugar levels in the beverage samples stored at three different temperatures.

Carbohydrates	5°C	21°C	37°C
	% Concentration at storage temperature (4 weeks)		
Galactose	1.70	1.60	1.75
Glucose	3.05	3.05	3.60
Fructose	0.50	0.60	0.95
Lactose	0.05	0.04	0.04
Sucrose	2.50	2.20	1.90
Total	7.80	7.49	8.24
	% Concentration at storage temperature (8 weeks)		
Galactose	1.67	1.73	1.74
Glucose	3.07	3.29	3.90
Fructose	0.54	0.68	1.29
Lactose	0.06	0.06	0.04
Sucrose	2.49	2.37	1.35
Total	7.83	8.13	8.32

due to acid hydrolysis of the sucrose by the acidic conditions present in the beverage (Hodge and Osman, 1976). At eight weeks one can see that further hydrolysis of the sucrose has taken place in the 37°C sample, whereas the 5 and 21°C storage samples have remained relatively unchanged in sucrose content. In addition, acid hydrolysis of oligosaccharides formed during lactose hydrolysis by ß-galactosidase can be a cause for further increase in the galactose and glucose level.

Fructose is a sweeter tasting sugar than sucrose, but glucose is less sweet than sucrose. Fructose is 14 relative sweetness units sweeter than sucrose, and glucose is 31 relative sweetness units less sweet than sucrose. The difference in the three sugars during storage should result in an overall decrease in sweetness. However, the change in the sugars present in the 37°C sample was not detected by the panel, in terms of sweetness, during the shelf-life study. The panelists observed little change in the sweetness.

E. Cost of Formulation

The whey based athletic-type drink ingredient cost per 32 Fl. Oz. was $0.14. This does not include the savings from sewage plant surcharges on whey. Refer to Table 16 for a cost breakdown. The retail price of a leading commercial athletic drink is at least six times higher than the ingredient cost of this whey based athletic beverage.

Table 15. Ingredient cost of producing a 32 Fl. Oz. quantity of the whey-based athletic-type drink.

	Unit cost[a]	Cost
Whey	d	d
Water	e	e
Potassium hydroxide	1.69/lb.	0.009
Calcium hydroxide	3.47/lb.	0.003
Maxilact L2000	14.52/lb.	0.017
Sucrose	0.33/lb.	0.028
Glucose	0.24/lb.	0.005
Citric acid	5.73/lb.	0.055
Orange flavor and color[b]	12.50/gal	0.006
Orange extract[c]	19.20/gal	0.014
Total cost per 32 Fl. Oz. quantity		$0.14

[a] Based on wholesale price as of March 1, 1983

[b] Norda, East Hanover, New Jersey

[c] Blanke Baer, Fenton, Missouri

[d] Savings on surcharge by sewage plant not included

[e] Negligible

CONCLUSIONS

Based on the data obtained in this study, the following conclusions have been drawn:

1. Direct-acid-set cottage cheese whey can be used as a beverage base by deproteinating the whey with proper pH adjustments and heat.
2. An athletic-type drink can be formulated from the deproteinated, lactose hydrolysed whey.
3. Of the persons who compared the formulated beverage to Gatorade^{R}, a greater number preferred the whey based beverage than did the number that preferred Gatorade^{R}.
4. In a consumer evaluation more persons liked the beverage than did not like it.
5. Under controlled processing conditions this whey-based athletic-type drink can be made free from microorganisms.
6. The beverage is stable in organoleptic quality under normal storage conditions.
7. The beverage is a good source of the electrolytes that are needed during heavy physical exercise.
8. The ingredient cost for a 32. fl. oz. quantity is $0.14, which is reasonably inexpensive.
9. This beverage can be processed in a milk processing plant with available equipment; such as a steam jacketed kettle and a cream separator.

APPENDIX

A. 1. Questionnaire for triangle test.

QUESTIONNAIRE FOR
TRIANGLE TEST

NAME_____ DATE_____

PRODUCT_____

Two of these three samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample.

Code	Check odd sample
_____	_____
_____	_____
_____	_____

2. Indicate the degree of difference between the duplicate samples and the odd sample.

Slight _____
Moderate _____
Much _____
Extreme _____

3. Acceptability:

Odd sample more acceptable _____
Duplicates more acceptable _____

4. Comments:

A. 2. Questionnaire for preference test.

NAME_____ DATE_____

PRODUCT_____

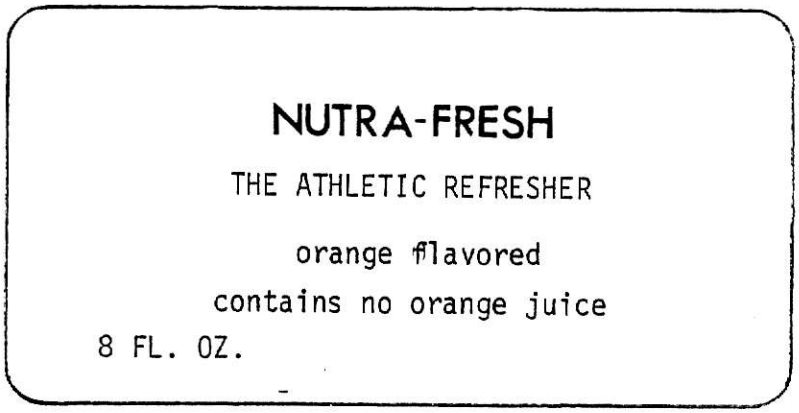
Taste the two coded samples in the following order:

_____ _____

Which of these two samples do you prefer? _____

Comments:

A. 3. Lable that was placed on bottle for consumer acceptance test.



A. 4. Questionnaire for consumer evaluation.

ORANGE FLAVORED ATHLETIC DRINK

Taster's Name_____

You received a paper bag that contains a bottle of orange flavored athletic drink. Taste this sample and check how much you like or dislike it.

INSTRUCTIONS:

-Cool the bottle of sample overnight in the refrigerator.

-Drink the sample right from the bottle when evaluating, or pour it into a glass. Please do not put ice into the sample when taste testing.

-Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample.

LIKE VERY MUCH	_____
LIKE MODERATELY	_____
LIKE SLIGHTLY	_____
NEITHER LIKE NOR DISLIKE	_____
DISLIKE SLIGHTLY	_____
DISLIKE MODERATELY	_____
DISLIKE VERY MUCH	_____

-Place this sheet in the envelope supplied, and drop it into the mail.

Thank you for your cooperation.

Sincerely,

Karen L. Crippen

A. 5. Questionnaire for multiple comparison test.

MULTIPLE COMPARISON TEST

Product: _____ Name _____

Date _____

Directions: Please evaluate each sample for the quality factor listed below, using the appropriate scale.

Quality Factor and Scale

	Reference (R)	Sample Code		
A. Sweetness				
7 Extremely 6 5 Moderately 4 3 Slightly 2 1 None	4.8			
B. Orange Flavor				
7 Extremely 6 5 Moderately 4 3 Slightly 2 1 None	5.3			
C. Overall Flavor				
7 Extremely 6 5 Moderately 4 3 Slightly 2 1 None	5.6			
D. Color				
7 Extremely 6 5 Moderately 4 3 Slightly 2 1 None	6.0			

Table 16. Detectable difference in the triangle test.

Extent of difference	# of Persons	x	Score	=	Subtotal
Slight	2		1		2
Moderate	2		2		4
Much	1		3		3
Extreme	0		4		0
Total					9
Average					1.8[a]

[a] 1.8 = moderate difference in flavor

Table 17. Results of multiple comparison test for shelf life study. Each value indicates the average of the scores of eight panelists.

	Temperature		
Day	21°C	32°C	37°C
Sweetness:			
0	4.8	4.8	4.8
14	4.5	4.9	4.0
28	4.5	5.1	4.5
42	4.3	4.6	4.5
56	5.1	4.9	5.0
70	4.3	4.5	4.6
Orange Flavor:			
0	5.3	5.3	5.3
14	5.1	4.8	4.5
28	5.1	4.6	4.5
42	5.0	4.9	5.0
56	5.4	4.9	5.0
70	5.3	5.0	5.0

Table 17. Continued.

Day	Temperature 21°C	32°C	37°C
Overall Flavor:			
0	5.6	5.6	5.6
14	5.3	5.2	5.0
28	5.3	5.5	4.8
42	5.2	5.1	5.1
56	5.5	5.3	5.1
70	5.4	5.2	5.0
Color:			
0	6.0	6.0	6.0
14	6.1	6.0	5.8
28	5.4	5.3	5.1
42	5.9	5.6	5.5
56	5.9	5.9	6.0
70	6.0	5.9	5.7

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DIRECT-ACID-SET COTTAGE CHEESE WHEY AS A BASE FOR A SHELF-STABLE ATHLETIC-TYPE DRINK

by

KAREN L. CRIPPEN

B.S. Kansas State University, 1981

AN ABSTRACT OF A MASTER'S THESIS

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Manhattan, Kansas

1983

An athletic beverage was developed from direct-acid-set cottage cheese whey. First, the pH of the whey was adjusted to 5.2 with a saturated potassium hydroxide solution. The whey was heated with stirring to 90°C and held for 10 min to coagulate the protein. It was filtered or centrifuged to remove the precipitated proteins. Calcium hydroxide was added to increase the pH to 5.6 and potassium hydroxide was added to further bring the pH up to 6.5. It was filtered or centrifuged again to remove the cloudiness caused by the addition of calcium hydroxide and additional protein precipitation. Beta-galactosidase was added to hydrolyse the lactose into glucose and galactose at 5°C for 18 hr. Then, one part water was mixed with two parts whey before citric acid was added to lower the pH to 3.7. Sucrose,glucose, orange flavor and color were added to make an acceptable orange flavored beverage.

The levels of electrolytes, such as sodium and potassium in this product were similar to those in commercially available athletic drinks.

In-house sensory analysis indicated that more people preferred the whey-based drink to a leading commercial athletic drink. According to results of a product evaluation by consumers at the K.S.U. Dairy Sales Counter, 48% of those who tried it liked it to some degree, 14% neither liked nor disliked it, and 38% disliked it to some degree.

During storage the added sucrose was hydrolysed into glucose and fructose to some extent, however, the taste panel did not detect any change in sweetness.

When the sterilization process "hot filling" was used on this beverage it was found to be commercially sterile. The stability of the product during storage was good and estimated to be longer than 6 months.

The whey-based athletic-type drink ingredient cost per 32 Fl. Oz. was $0.14. The retail price of a leading commercial athletic-type drink is at least six times higher than the ingredient cost of this whey-based athletic-type drink.