University of Tennessee, Knoxville

TRACE: Tennessee Research and Creative Exchange

Doctoral Dissertations

Graduate School

12-1978

Utilization of heat precipitated whey protein

Firyal B. Al-Dabbagh

Follow this and additional works at: https://trace.tennessee.edu/utk_graddiss

Recommended Citation

Al-Dabbagh, Firyal B., "Utilization of heat precipitated whey protein. " PhD diss., University of Tennessee, 1978.
https://trace.tennessee.edu/utk_graddiss/7883

This Dissertation is brought to you for free and open access by the Graduate School at TRACE: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Doctoral Dissertations by an authorized administrator of TRACE: Tennessee Research and Creative Exchange. For more information, please contact trace@utk.edu.

To the Graduate Council:

I am submitting herewith a dissertation written by Firyal B. Al-Dabbagh entitled "Utilization of heat precipitated whey protein." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Hugh O. Jaynes, Major Professor

We have read this dissertation and recommend its acceptance:

A. M. Campbell, J. L. Collins, B. J. Demott

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a dissertation written by Firyal B. Al-Dabbagh entitled "Utilization of Heat Precipitated Whey Protein." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Hugh O. Jaynes, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

D

Ag-VetMed
Thesis
786
.A383
cop. 2

UTILIZATION OF HEAT PRECIPITATED WHEY PROTEIN

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Firyal B. Al-Dabbagh

December 1978

1371121

ACKNOWLEDGMENTS

The author wishes to express her genuine appreciation and gratitude to Dr. H. O. Jaynes, major professor, for his advice, guidance, and valuable encouragement throughout the graduate program.

Sincere appreciation and thanks is extended to Dr. A. M. Campbell, Dr. J. L. Collins, and Dr. B. J. Demott who participated in reviewing the manuscript and serving on the Graduate Committee.

The author also acknowledges Dr. J. T. Miles, Head of the Department of Food Technology and Science, for his encouragement and participation as a graduate committee member.

A special word of thanks is due to Miss Angie Cabrera, for her help in the laboratory work, and Mrs. Mary Mills for her assistance in typing the dissertation.

Finally, I wish to express to my husband, Nabeel, my sincere appreciation and thanks for his constant encouragement, genuine interest, and enthusiasm during my study, and for my sweet children, Aseel, Khaleel, and Muhammed, for their patience and understanding throughout my graduate study at the University of Tennessee.

ABSTRACT

The purpose of this study was to coagulate whey protein by heat denaturation, recover it, and use it in food products. Fresh liquid whey at pH 4.5 was heated at 90°C for 20 minutes. Heated whey was cooled to room temperature (about 25°C) and centrifuged 15 minutes at 750xg. About 42 percent of the protein was recovered.

The mean particle diameter of the sedimented material was 29.5μ. Electrophoresis indicated that blood serum albumin and β-lactoglobulin were essentially precipitated while some α-lactalbumin remained in suspension.

The sediment was collected and used as a basic matrix in sandwich spread and as a partial replacement of NFDM in ice cream. The products showed desirable quality when examined by sensory panels. Up to 30 percent of the NFDM in ice cream could be replaced.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

CHAPTER I

INTRODUCTION

When casein is removed from skim milk, the remaining aqueous phase is designated as whey or milk serum. A further refinement in terminology reflects the method by which the casein is removed from skim milk. If precipitated by acid at pH 4.6, the aqueous phase is "acid whey." If removed by the action of rennin-like enzymes, as in the production of cheddar cheese, the aqueous phase is "rennin" or "sweet whey."

Acid whey, although practically free of casein components, contains more calcium, whereas sweet whey, in addition to the usual complement of whey protein, has a higher content of free amino acids and casein fragments resulting from enzymatic cleavage.

Annual whey production in the world is estimated to be 74 million tons or 162.8 billion pounds, 95 percent of which is cheese whey. The remaining 5 percent results from the manufacture of casein and heat or acid coagulated milk products in various parts of the world. In the United States approximately 29.5 billion pounds of liquid whey are produced each year. This whey contains more than one billion pounds of lactose and 170 million pounds of protein as well as large quantities of water soluble vitamins and minerals.

About 56 percent of the whey solids are currently utilized for human and animal food, and the rest is disposed of by spraying onto fields or by dumping in sewage systems. Quite apart from the environmental

problems created by these methods of whey disposal, is an economic and value loss.

The Chemical Oxygen Demand (COD) of whey has been noted as ranging from 60,000 to above 70,000 ppm. depending upon the specific cheese making process. Every 1,000 gallons/day of raw whey accepted by a city sewage system is equal to the waste of 1,800 people. For the oxidation of 1,000 lb. of raw whey discharged into a stream about 4,500,000 gallons of unpolluted water are required.

During the last few years interest has been increasing in whey and whey protein. This has been due largely to the fact that disposal of whey through sewage systems has been prohibited in many industrialized countries. This situation has led the dairy industry to consider new ways of utilizing whey and its components. Of special interest are whey proteins because of their high nutritive value.

Whey, particularly in modified form or as separated components, can be used in many food products as well as in animal feed. The work reported herein was undertaken as a part of a whey utilization system that could be used by dairy plants producing cottage cheese. Protein in cottage cheese whey was coagulated by heat and recovered by centrifugation. The recovered concentrate was then used as an ingredient in ice cream and as a base for several sandwich spreads.

CHAPTER II

REVIEW OF LITERATURE

Whey, the yellowish-green liquid portion of milk remaining after coagulation of casein during manufacturing of cheese or casein, contains about half of the milk solids, most of the lactose, about one-fifth of the protein, and most of the vitamins and minerals. Five to ten pounds of whey are produced from each one pound of cheese manufactured. The growth of the cheese industry, increased environmental concerns, and periodic high prices for alternative protein sources have focused attention on supply and utilization of whey.

Whey Production

An estimated 29.5 billion pounds of liquid whey were produced in 1975, with 23.9 billion pounds of sweet whey coming from hard cheese and 5.6 billion pounds of acid whey coming from cottage and cream cheese (78). This was equivalent to a total of about 2.0 billion pounds of whey solids. The supply of whey is growing. From 1970 through 1975, the statistics show that whey production increased over 20 percent. If this trend continues, whey production would reach 36 billion pounds by 1980 (128).

About 56-60 percent of the whey solids are currently utilized in human and animal feeds and the rest is disposed of by spraying onto fields or by dumping in sewage systems. 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 of this was used for human food and the rest for animal feed (78).

In the USDA economic report (78), it was mentioned that in 1975 for the first time the total whey data were divided into dry whey, partially delactosed, partially demineralized, and partially delactosed-demineralized. Therefore, the 1975 dry whey production numbers are not strictly comparable to earlier years. Condensed whey production reported for 1975 was 87.8 million pounds (solid content). This includes only whey that was condensed as a final marketable product and does not include the quantity used at the plant or shipped to another plant for further processing into dry whey or modified whey products. Lactose production was 136 million pounds for that year. The total whey solids equivalent of the dry whey products, condensed whey, lactose, and wet blend was 1,153 million pounds, 58 percent of total whey solids available.

Whey Pollution

Until the 20th Century, nobody learned much about what happened to whey. Then came the realization that whey, discharged in large amounts into rivers and streams, was a fatal pollutant; it killed fish. When dumped into the fields near a cheese plant, it gave off a rank, repulsive, skunk-like odor. Whey pollution became so extensive that the Environmental Protection Agency (EPA) issued strict regulations forcing cheese manufacturers to discontinue dumping whey into sewer systems by January 26, 1976 (129).

The Chemical Oxygen Demand (COD) of whey has been noted as ranging from 60,000 to above 70,000 ppm depending upon the specific cheese making process (3). Every 1,000 gallons/day of raw whey accepted

by a city sewage system is equal to the waste of 1,800 people. For the oxidation of raw whey discharged into a stream 4,500,000 gallons of unpolluted water are required (84). So dairy plants may have to build treatment facilities--aeration and/or ponds--at great cost. This adds to the cost of manufacturing cheese.

Composition and Nutritional Value of Whey

Quite apart from the environmental problems created by these methods of whey disposal, there is also an economic and value loss. Whey contains an excellent array of high quality nutrients, such as albumin, lactose, vitamins and minerals. However in its utilization as food or feed, whey is not necessarily the product of choice for supplying energy and nutrients. Feeding liquid whey to hogs dates back to ancient Rome (97).

Prior to World War II, the major outlet on the farm for whey in both Europe and the United States was as feed for swine (37). This was logical since up to that time most dairy farmers also raised swine. Since that time, both dairy and swine farming have become more specialized, and frequently many miles apart, making it no longer realistic to feed whey to swine because of the costs of transportation. Thus there was a decline in feeding liquid whey to swine. Since whey can no longer be discharged indiscriminately into rivers and streams and since there is no economical way of getting it to hogs and other animals and since new legislation regarding its disposal has added to costs of cheese manufacture, cheese makers have redoubled their efforts to find new uses for whey.

Many authors have discussed the nutritional value of whey. One practical way of looking at the nutritional value of whey is to compare it with dried skim milk (NFDM), which is already being used in large amounts to upgrade the protein of the diet.

Table 1 shows the approximate content of the major nutrients in both NFDM and dried whey (45). The total protein content of whey is much lower than that of NFDM (about one-third). Not only is the protein level lower, but the types of the proteins are different, since casein of milk is removed during cheese making. The proteins remaining in whey are chiefly lactalbumin and lactoglobulin.

Fat content and water content are similar for the two products. The food calories available from the two products are essentially the same, since the total carbohydrates plus protein level add up to 86-87 percent.

The other major difference in nutritive content of dried whey is the percentage of lactose. This high lactose content can create serious problems for human feeding.

Utilization of Whey

Long before whey was utilized in human food, it was fed as fluid to swine (97). Ruminants can consume up to 30 percent of their dry-matter intake as liquid whey without impaired performance while swine may have diarrhea when more than 20 percent of their dry matter is liquid whey (37).

The compositions of whey and several whey products were discussed by Schingoe (107). The protein content of dried whole whey is comparable to that in barley, oats and wheat. Whey protein is one of the

Table 1. Nutrients in Dried Whey and Non-fat Dry Milk

Nutrient	Approximate Content	
	Whey* (percent)	Non-fat Dry Milk** (percent)
Protein	12.9	35.8
Fat	1.1	0.7
Ash	8.0	7.9
Water	4.5	4.0
Lactose	73.5	51.0

*350 calories/100 gm.

**359 calories/100 gm.

highest quality naturally occurring proteins having a Protein Efficiency Ratio (PER) of 3.0-3.2 compared to casein at 2.5 (28, 39). Whey is a source of energy primarily because of its high lactose content. The energy value of dried whole whey is comparable to the energy value of shelled corn and slightly higher than that of most other feed grains. Whey is also a relatively good source of calcium, phosphorous, sulfur, and water soluble vitamins. About 40 percent of the calcium and 43 percent of the phosphorous of the original milk normally remain in whey. Sodium chloride accounts for most of the remaining ash in whey (97).

Some problems have been encountered with whey feeding. Keeping the whey fresh to avoid problems of palatability and possible excessive urination at high intakes is one. Sweet whey may be more palatable than acid whey (47, 121).

Welch and Nilson (121) observed teeth erosion when whey was fed for long times and mixed with molasses before feeding. Lynch et al. (79) observed cases of bloat initially in experiments in which acid whey was fed to Holstein steers but prevented bloat by feeding hay at 0.4 percent body weight during the remainder of the experiment.

Acid resistant tanks must be used for storage of whey to prevent corrosion caused by acids produced during whey's fermentation. Flies and other sanitation problems also may be experienced, especially during warm weather.

Condensed whey has been fed to cattle either untreated (122) or fermented and ammoniated (62). Fermented ammoniated condensed whey (FACW) is made by allowing whey to ferment producing lactic acid, neutralizing the acid by bubbling anhydrous ammonia through the whey,

and then condensing to 55-65 percent solids (61, 62). The final product contained 7-10 percent nitrogen, had a pH of 5.5-6.3 and exhibited handling properties comparable to urea-molasses based liquid protein supplements. Condensed whey was unpalatable to cows when fed alone but was consumed readily when mixed with equal amount of molasses. No palatability problems were encountered when condensed whey with added urea and molasses was fed with silage (122).

Weight gains and conversion of feed dry matter to weight gains by calves and steers fed FAWC were the same as with those fed soybean meal (87) and slightly higher than for those fed urea-molasses-based liquid protein supplements (55, 56, 87). Digestibilities of FACW, urea and soybean meal rations were the same for sheep (87).

Dried whey is the most suitable form to be used in food or feed. Most of the commercial dried whey is made so that the lactose is in the large crystalline form. This stabilizes the produc and prevents it from picking up excessive quantitites of moisture and caking (45).

Dried whey has been fed to nonruminants for many years with favorable results. Including dried whey in the ration increased weight gains and feed efficiencies for poultry (1, 13), swine (25), horses (59), and rats (127).

Increased protein digestibility and nitrogen retention (11), increased fat digestibility, as well as improved mineral absorption and retention (59), have been observed commonly when dried whey or lactose are fed to nonruminants.

The problem in whey feeding is lactose intolerance. Rats can tolerate up to 35 percent lactose (68). However they showed signs

of lactose intolerance such as diarrhea with diets containing 20-30 percent lactose (98).

Swine are more tolerant to lactose than are poultry and rats and easily can consume 15-20 percent dried whey rations without difficulty (12). Becker et al. (1957) found that 40 percent dried whey (30 percent lactose) rations caused some diarrhea in finishing pigs, while Ekstrom et al. (34), observed no harmful effects from feeding up to 40 percent dried whey.

Dried whey has been fed to ruminants too. The main problem concerning researchers in feeding dried whey to cows is milk fat depression. Huber et al. (63), demonstrated that the percent fat in milk was maintained at pretreatment levels when as little as 10 percent partially delactosed whey was incorporated into the concentrate ration. Incorporating 20, 30 and 60 percent dried whey or partially delactosed whey into the concentrate ration had no significant additional effect on fat percentage (63). Studies by Rosser et al. (102) and by many others, indicated that the minerals and lactose were the components in whey most responsible for maintaining milk fat percentage.

There is probably no subject in the dairy industries today arousing greater interest than that of whey utilization. Many authors have discussed the use of whey in various food products. Many others have suggested that whey could be used in the formulation of nutritious soft drinks or high-protein beverages and also might be used as an additive in soups and fruit juices (6, 67, 119).

Using cheese whey as a beverage in human nutrition, especially for therapeutic purposes, 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 various diseases, and by the mid-19th Century, whey cures reached a high point with establishment of over 400 whey houses in Western Europe (114). As late as the 1940's in Central Europe, dyspepsia, uremia, arthritis, gout, and liver diseases were treated with the ingestion of up to 1500 g of whey per day (60). Available literature indicates that whey beverages have been studied extensively in Germany and Eastern Europe (46, 73, 81, 86, 93).

Preparing beverages from whole liquid whey is the cheapest and most efficient method. It is based on draining the whey from the cheese vat, pasteurizing, deodorizing, if desired, flavoring appropriately, and packaging for later consumption (60).

The whey flavor, particularly that of acid whey, is most compatible with citrus flavors, particularly orange. Several experimental citrus-flavored beverages have been developed, for which good consumer acceptability was claimed. Both cheddar and cottage cheese wheys have been used.

O-way, a product developed at Michigan State University (21), was visualized as a breakfast meal incorporating either sweet or acid whey and orange juice. One volume of fresh orange juice concentrate was mixed with four volumes of deodorized whey and packaged. The product contained 0.7 to 1.0 percent protein. The authors suggested that the beverage could be carbonated and sold as a nutritious soft drink.

Researchers at the University of Arizona (91) combined 25-40 percent whey with grapefruit juice and tested these drinks by sending them

into homes as commercially sterile canned products. A peach-grapefruit-whey combination received an average flavor score of 5.9 on a hedonic scale of 1-7. A second series of beverages, using Uinifera grape juice, whey and 3 percent passion fruit juice also had good acceptance in preliminary studies.

Nelson and Brown (92) prepared an orange flavored drink containing 33 percent cottage cheese whey. The product was rated 6.3 by 51 tasters whereas a nonwhey drink rated 4.7. Acceptable drinks containing 80 to 90 percent whey and flavored with 10 percent natural strawberry puree or 20 percent natural peach puree received acceptable scores by panels.

A drink based on cheddar cheese whey has been formulated at Mississippi State University (74). This product was prepared by mixing whey, sugar, orange concentrate, citric acid and other ingredients to produce a pH of 3.8 and total solids content of 16.5 percent in the finished beverage for which a shelf-life of at least 14 days at 5, 10 or 32°C was claimed. A total of 956 consumers of all ages sampled the beverage, and 76.5 percent of the respondents rated the drinks acceptable. Personal interviews with 46 families showed that 90 percent would purchase the product for $0.38 per liter.

A new beverage based on whey, called Freshi, was developed by the Verbands-Molkeri, a dairy co-op in Berne, Switzerland (72, 73). This product contained about 50 percent purified whey plus sugar, water, and natural orange flavoring with lemon and grapefruit flavors added for topnotes. The whey mixture was heated at 90°C and packaged aseptically. Since the product was highly acid, the low sterilization temperature

was possible, and shelf-life was claimed to be about six months without refrigeration.

Kosikowski (69) has shown that an acceptable beverage could be made by incorporating up to 6 percent acid whey powder in reconstituted frozen orange juice. The blend contained 2.5 times the protein of orange juice alone. Acid whey powder at 6 percent showed a slightly salty taste; when the content was reduced to 4 percent, tasters rated the flavor of the product excellent. The orange juice concentrate could be thawed to a thick slurry, the proper amount of acid whey powder mixed in and the mixture recanned and frozen at -25°C. After one month of storage, the reconstituted beverage retained the quality of the freshly reconstituted blend.

Whittier and Webb (124) described the preparation of prune juice by extracting dried prunes with clarified whey. Fresh sweet whey was allowed to sour to pH 4.8; the whey was then further acidified to pH 4.5 with citric acid and lemon juice, boiled, and then the clarified liquid filtered. Dried prunes were then extracted with two parts whey by either cooking or leaching the prunes in the whey. The final extract (pH 3.8-4.0) was canned and sterilized at 100°C for 30 minutes. The use of whey in this manner helped reduce hydrogen swells which after two or three months often cause corrosion of cans of water-extracted prune juice.

Laessing (71) prepared a nonalcoholic beverage concentrate to be diluted with water. The mixture was composed of freeze-concentrated fermented whey containing 5 percent lactic acid plus 1.3 times its weight in sucrose. After holding at 100°C to produce invert sugar and reduce

microbial contamination, the concentrate was ready for use. Laessing suggested adding fruit juices or flavorings.

A frozen whey concentrate has been developed (116) which, when diluted one to three with gingerale, had possibilities as a party punch base. The product, in which at least half the liquid was fresh sweet whey, was similar to frozen orange juice concentrate and was claimed to have no whey flavor.

Besserezhnov (14) has described a simple process for preparing a yoghurt flavored beverage. Freshly pasteurized sweet whey was inoculated with a 10 percent culture consisting of Lactobacillus bulgaricus; L. acidophilus, L. helveticus, L. casei and Streptococcus thermophilus. After 24 hours incubation, the product was cooled and packaged.

Non-alcoholic beverages from deproteinized whey have been the subject of considerable research in several countries. The whey may have been fermented either before or after protein removal and the finished beverage may or may not be sparkling. This type of beverage represents a more nutritive version of the carbonated soft drinks and still beverages so familiar to the American consumer especially.

The most popular method of deproteinization is by heating whey which is usually acidified, at about 90°C for different times. The coagulated whey proteins are then removed by filtration or centrifugation, and the clear supernatant is processed further to produce the desired effect.

Tannic acid, herbal leaf extracts containing large amounts of tannins, or natural fruit juice containing tannin are also efficient

protein precipitants (60), particularly in conjunction with heat. The latter two means add desirable flavors to the whey base. Several beverages have been developed by similar procedures. Treatments with proteolytic enzymes help protein coagulation and increase the soluble nitrogen content of whey, thus adding to the nutritive aspects of the beverages (60).

A patent issued to Mauroy (85) described a process in which the whey was condensed to two-thirds of its original volume and neuteralized to pH 7 before clarification by heat. The filtrate was used as a soft drink base.

Dordević and Koehler (31) described the manufacture of a carbonated fruit flavored clear beverage. They recommended that the whey be deodorized after protein removal and filtered and de-aerated after flavoring to reduce oxidation in the finished product.

The beverage was then pasteurized and bottled on a regular bottling line equipped with machines for impregnating with carbon dioxide under a pressure of one to two N/M^2. The beverage was stable for at least 30 days. The final pH was 3.7; the product contained 4.4 percent lactose, 14.8 percent total sugar and 188 mg calcium/100 g. If an opaque beverage was desired, the filtering step after flavoring was eliminated.

Rzewuska-Rutte (104) gave an excellent description of the utilization of whey in the soft drink industry in Poland. Experiments showed whey deproteinization necessary to produce a beverage of good keeping quality. Deproteinization with heat at pH 7 removed 63 percent of the protein. Mixing with natural fruit juice deproteinized the whey

further so one liter of the final product contained 3 to 4 g of soluble protein. The best soft drinks contained 95 percent whey. Musts and concentrates of several types of fruits were used. Taste panel results showed that musts of cherry, strawberry, and blackberry were not suited to the whey flavor. Citrus and peppermint flavors were preferred, and beverages from sweet whey were superior to those from acid whey.

Murray (90) has patented a process for deproteinizing whey with citric and tannic acid. Heated whey was acidified with 10 to 15 g/l of citric acid followed by addition of 0.7-1.0 g/l tannic acid. After filtering the whey may be flavored and bottled. Murray also described the clarification of whey by filtration through fine particles (80 percent pass 70μ mesh) of diatomaceous earth in a conventional pressure filtering apparatus.

Maeno (80) and Romanskaya and Kalmysh (101) outlined processes whereby whey was incubated with a proteolytic enzyme for several hours. After the precipitate was removed, the filtrate was sterilized with ultrasonic waves in Maeno's process. In the Romanskaya and Kalmysh (101) process, the filtered whey was condensed to one-fourth or one-eighth the initial volume, flavored and bottled.

Fermented beverages have been made from whey. Whey is incubated with yeasts and sucrose for various times to develop carbonation. Although in this step traces of alcohol may be produced, the beverages are considered to be nonalcoholic by their developers.

Rivella, a sparkling, crystal clear infusion of alpine herbs, first appeared in Switzerland in 1952 (2, 114). Rivella was prepared by fermenting deproteinized whey with lactic acid bacteria, filtering,

condensing to 7:1 concentrate, adding sugar and flavoring, refiltering, diluting and carbonating, after which the product was bottled and pasteurized. The finished beverage contained 9.7 percent TS, 0.125 percent total nitrogen and pH was about 3.7. Twenty to thirty million liters are sold annually.

Blazek et al. (15) patented a method for the manufacture of a dietetic whey beverage. The whey was first inoculated with 2 to 5 percent culture of lactose fermenting organisms such as S. lactis, S. diacetilactis, Saccharomyces fragilis, or Torulopsis sphaecica, either singly or in combination, and incubated at 15-25°C to pH 4.4-4.6. After addition of ethanol, the mixture was boiled to coagulate the protein and filtered. The pH of the filtrate was adjusted to 5.0 and flavorings such as sultanas or apples and citric acid and vitamins were added. The beverage was then diluted, reclarified, pasteurized, the pH readjusted to 5.0, carbonated, and bottled. A later patent of Blazek et al. described the addition of 2 percent cation exchanger in the hydrogen cycle after the initial fermentation to reduce the pH of the whey to 3.8-4.0. The whey was then clarified, filtered, decolorized with carbon and used as a beverage base.

During World War II, Schulz and Drache (109) developed an acid whey concentrate called Lactrone for beverage use. It contained 17 percent total solids, 10 percent lactic acid, 2 percent protein and peptone, 2 percent reducing sugars and 3 percent ash. The whey was fermented first with Kefir culture. Schulz and Drache (109) recommended Kefir fermentation followed by vacuum evaporation as being the best for removing the whey taste. After the alcohol was distilled off, the

resulting stable product was concentrated. Vitamin C, when added to this product, was stable and enhanced the flavor of the diluted beverage.

Alcoholic beverages can also be manufactured from whey with proper techniques. A good beverage should be transparent, clear, and preferably sparkling. Deproteinization of whey would be especially important in the production of such beverages.

The shortages of raw materials arising from World War II accelerated research toward development of good quality alcoholic beverages derived from whey, and some success was achieved, particularly in the manufacture of whey beers. Whey wines and other low-alcoholic beverages have also been produced.

Roeder (100) patented a process for a beverage base in which the whey was concentrated to 25-33 percent of the original volume, deproteinized, cooked with 0.1-0.2 percent hops, and decolorized with carbon. The product at this stage had no whey taste or smell. It was then fermented to 0.75-1.5 percent alcohol, after which sugar and fruit juice or other flavorings could be added.

Whey has many properties which make it suitable for the manufacture of beer-like beverages. Because whey contains material similar to the colloids of beer wort, it has a great capacity for binding carbonic acid. Whey, like beer wort, has a high salt content. Some constitutents in whey, after prolonged heating under pressure, develop caramel-like flavors which are similar to the taste and odor of cured malt. Lactose is only slightly sweet so it does not alter the taste of the finished beverage. Various types of beer-like whey beverages

(Whey-beer) have been manufactured in Germany.

Roeder (99) proved that whey has a greater buffering capacity than beer wort, and therefore, the pH of the whey is crucial to the proper development of acidity in the mash. In the normal mash process, if the acidity of the whey is incorrect, the mash does not develop the acidity necessary to precipitate the protein upon cooking with hops. Tannic acid present in the hops helps precipitate the protein. Roeder also showed that up to 30 percent of the malt could be replaced by deproteinized whey by cooking at pH 4.5-5.5 with the hops. Then the filtrate was blended with the malt wort. Fermentation with bottom-fermenting beer yeasts followed. Because these cannot ferment lactose, enough malt constituents with fermentable carbohydrates had to be used so that the intended contents of carbon-dioxide and alcohol were guaranteed.

A whey beer could be produced from malt with up to 50 percent whey addition. Starch and sugar syrup were added, and the mixture was cooked with hops and fermented with top fermenting beer yeasts. The beer was refermented with sugar before decantation and pasteurization (60).

Another type of whey beer was produced by substituting other starch carriers, such as potatoes or maize, for the malt. The starch was broken down by diastase during the mash process into normal hydrolysis products of dextrin and maltose (60).

The growth of so-called pop wines has been spectacular since 1967, and in 1971, accounted for 231 million liters, about 25 percent of the wine produced in the United States. These products, aimed at

the younger consumer, have relatively low alcoholic content and are mostly fruit flavored. Wines based on whey would seem to fit in with the natural beverage trend and some promising products have been developed (60).

Engel (35, 36) has patented a process for producing a sherry-like alcoholic beverage from whey. Fresh whey and sucrose in the proportions of 2.5:1 up to 9:1 were fermented with 1.2-9.9 percent baker's yeast. This mixture was allowed to age for 3-5 months. For the first few days it was held at 17.8°C and gradually cooled. After 10 days, a black crust formed on the surface, this was removed, and an oily film which subsequently formed was also removed. The liquid was then siphoned from the tank and stored in the dark for 10 to 54 days to permit flavor development. After chilling to -33°C for four days to fix the flavor, it was aged at 10°C for several days, at which time it was ready for use as a flavoring agent or a beverage.

Zadra (131) patented a process for making a carbonated alcoholic beverage from whey. After protein removal, the whey was cooled to 35°C and lactose and 1 percent powdered almonds were added. After 4 hours incubation, the liquid was decanted, cooled to 4-6°C, treated with beer yeast, fermented, decanted, stored at 0-2°C under pressure for 1 to 2 weeks, filtered, and bottled. By hydrolyzing the lactose to its monosaccharides, glucose and galactose, no added sucrose was needed for fermentation. Zadra has also developed a noncarbonated coffee flavored alcoholic beverage from whey (132).

Yoo and Mattick (130) have studied the production of alcohol by Saccharamyces fragilis fermentation of both sweet and acid whey. They

found that ethyl alcohol production from whey was maximum with a lactose concentration of 12 percent. Lactose in acid whey fermented more rapidly and produced more alcohol than sweet whey. They produced an acceptable whey wine containing 10 percent alcohol when 16 percent sucrose was added to a 10 percent acid whey solution.

High protein beverages using whey offer attractive possibilities, not only from the standpoint of the protein processors, but also because such beverages have a great potential for popular acceptance. These beverages fall into two groups: those that may be considered milk-like or extended milk and those that resemble soft drinks.

Bodmershof (16), prepared a sparkling beverage from a mixture of 40 percent sour milk, 50 percent whey, and 10 percent fruit juice. This was bottled under 7 N/M^2 of carbon dioxide and was claimed to keep for several months.

Downham (32) patented a process for making a product claimed to resemble human milk. Sweet whey was homogenized with some of the following ingredients: cream, butterfat, milk sugar, sugar, skim milk, and sodium citrate. The finished beverages were reported to be useful for infant and invalid feeding.

Researchers at Michigan State University (21) have prepared a product called WayMil, an imitation milk formulated from whey, selected vegetable oils, vegetable hydrocolloids, and in some applications, skim milk. The beverage contained 2.4 percent fat and 1-1.5 percent protein. The fat protein dispersion was claimed to be physically stable for three to four weeks.

Edmondson *et al.* (4, 33) have developed a sterile milk-like beverage from sweet whey and cream. This product, condensed to

CRANESE CREST

35 percent total solids and flavored with chocolate, was sterilized by a standard high temperature short time (HTST) procedure, then homogenized and canned aseptically. When reconstituted to 17.5 percent TS, this product scored 6.5 on a nine point hedonic scale compared to 6.9 for commercial chocolate milks.

A recent study by Vajdi and Pereira (117) described the use of whey as a milk substitute in the production of strawberry, lemon and chocolate beverages. The pH of liquid whey was adjusted to 6.7 with 0.1N KOH. Strawberry drink was prepared by mixing 2.59 Kg of 35 percent cream, 2.27 Kg of sugar, 2.72 Kg of skim milk powder, stabilizer and flavoring to 39 Kg of liquid whey. The mixture was stirred vigorously, heated at 82°C for two minutes, and homogenized at 35.2 and 105.52 Kg/cm^2. After homogenization, the product was cooled to 10°C and bottled. Lemon drink was prepared by a similar method by mixing of 2.59 Kg of 35 percent cream, 2.27 Kg of sugar, flavor and stabilizer to 40.8 Kg of whey concentrate. Chocolate drink was prepared by mixing 2.27 Kg of sugar, 0.91 Kg of chocolate, 3.63 Kg of whey powder, and stabilizer to 38.6 Kg of liquid whey. These products had low production costs and good flavor. Shelf life studies showed no change in state or flavor during one month of storage under refrigeration. In addition, taste panels found no significant difference between commercial chocolate milk and the chocolate whey drink.

Demott et al. (29) prepared a tomato-flavored beverage from cottage cheese whey. Dried tomato spice flavoring material (5.4 or 6 percent) was added to cottage cheese whey taken from the vat at draining time. The mixture was allowed to remain at 4°C overnight.

The tomato flavored whey drink and two commercial brands of tomato juice were tasted informally in the laboratory by ten untrained panelists. The samples were tested for difference and overall acceptability. The panelists could distinguish a difference between tomato juice and the tomato-flavored whey, but they found the latter to have a pleasing flavor and good texture.

In the past, the soybean has offered a cheap nutritious source of protein for increasing the protein content of whey. Soy protein is low in lysine, as well as in the sulfur-containing amino acids. Whey proteins, on the other hand, are particularly rich in lysine and also contain significant amounts of methionine and cystine. Therefore, a soy-whey beverage with a protein content equivalent to or greater than that of milk would appear to be a desirable addition to the available food supply.

A soy-whey milk made from soy beans and cottage cheese whey has been developed at the University of Illinois (7). The beany flavor was destroyed by boiling the whole bean before it was broken for incorporation into the whey. The product had a flavor resembling that of eggnog after proper flavoring.

A high-protein beverage powder, from soy and whey, readily reconstitutable in water, would be easier and cheaper to transport and store than a fluid product. This would be particularly useful for shipment to developing countries where protein is in short supply.

Loewenstein and Paulraj (76) have shown that a powder made by coprecipitating and drying defatted soy flour and whey protein to a final blend of three parts soy to one part whey protein was clearly

superior to soy protein alone in promoting growth in rats.

Saski and Tsugo (106) described the manufacture of synthetic milk powder from whey and soybean by extraction of the soybeans with hot whey. Their research led Guy and his associates (52) to develop a process for spraydrying a soy-whey mixture which yielded a powder containing 67 percent sweet whey solids and 33 percent full fat soy flour. A powder containing 55 percent sweet whey solids, 28 percent soy flour and 17 percent corn oil could also be manufactured by this process. The formulation of this product was flexible and permitted the easy addition of flavorings and carbohydrates to produce a powder readily dispersible in water to yield a beverage containing 2.7 percent protein. Citrus and cherry vanilla flavors were highly acceptable to a trained taste panel (4, 51).

Kraft (70) patented a process for making a spray dried food ingredient powder which contained four parts comminuted sesame seed to three parts whey solids. Sesame is high in methionine, the limiting amino acid in milk protein. The powder manufactured by this process could be readily dry blended with sugar and cocoa to make a nutritious beverage mix which, when reconstituted with water, produced a drink containing 15-20 percent TS.

Kraft Company and four other United States Corporations such as Foremost, Land O'Lakes and Borden, with adequate research facilities, have developed a method for drying whey under hygienic conditions, which permits its use in products for human consumption. At a recent press conference (105), officials of the Kraft Industrial Food Division said "research in applications and uses for whey in cooking, baking and

frying has been going on for several years and one reason for this continued interest has been that dried whey can be made and sold for less than non-fat dry milk."

Whey is used to replace part of the non-fat dry milk normally used in preparing commercial foods. Whey has been used as a tenderizer; it helps to produce a better crust for pie dough and soft textured baked goods. It gives a more pleasing color to almost any product than is the case if non-fat dry milk is used alone.

Bread with whey as an ingredient was said to have been better colored and smoother-textured. Sweet rolls and coffee cakes with whey in the dough have been more evenly browned and definitely tastier (105). Ice creams, fudges, caramels, syrups, toppings, coatings and fillings were all said by Kraft officials to yield better products as a result of the addition of whey. Whey, they claimed, improved the blending of candies and produced a smoother appearance in these products.

Many other products have been discussed by Kraft Company (105) and, as a proof, Kraft officials pointed to a number of large bakeries which use whey in their products. Some of the largest biscuit companies now list whey among the ingredients on packages of cookies and crackers.

One of the newest whey-base products Kraft has introduced is Compound Coating Classic, a whey blend used to replace milk solids in the coatings used by ice cream novelty makers, candy makers and bakers. The coatings are used in cookies, graham crackers, ice cream bars and other confections. They are called compound coatings to distinguish them from true chocolate coatings, which are legally defined by federal regulations.

Concentration and drying of whey are expensive because of the low concentration (6-7 percent) of solids in the fluid whey. Also, the high proportion of lactose and salt in the solids limits the utilization of dried whey in food or feed. Thus, an economical process that would separate proteins from liquid whey, and at the same time remove at least part of the lactose and salts, would greatly increase the feasibility of processing whey for food uses.

Proteins of Whey

The proteins of whey represent about 20 percent of total milk proteins. The two principle components, β-lactoglobulin and α-lactalbumin, both products of mammary synthesis, account for 70-80 percent of the protein content (123). Blood serum albumin, immunoglobulins and the proteose-peptones account for the remaining proteins of whey. Additionally, numerous enzyme proteins and proteins with specific metabolic functions, e.g., lactoferrin, have been identified and in many instances isolated from whey.

Table 2 shows composition and some characteristics of whey protein.

The primary structure of β-lactoglobulin was established by Frank and Braunitzer in 1967 (42), revealing 162 residues and a calculated molecular weight of 18,362. Monomeric β-lactoglobulins are further characterized by the presence of one free sulfhydryl group and two disulfide groups. Whitney (123) deduced the positions of the two disulfide bonds. One is always found between the residues CyS_{66} and CyS_{160}; the other is present in equal distribution between CyS_{106}

Table 2. Distribution and Characteristics of Milk Proteins

Component	Approximate Concentration Percent of Skim Milk Protein	g/l	Approximate Molecular Weight	Groups/Molecule -S-S	SH
Casein	78-85	27.2			
α_s Casein	45-55				
α_{s1} - Casein		13.6	23,500	0	0
β - Casein	25-35	8.2	24,000	0	0
K - Casein	8-15	4.1	19,000		
γ - Casein	3-7	1.4	43,800	0	0
Whey Proteins	12-25	6.8			
β - Lactoglobulin	7-12	3.6	18,300	2	1
α - Lactalbumin	2-5	1.7	14,200	4	0
Immunoglobulins	1.5-2.5	0.6	1,730,000	present and variable	
Serum albumin	0.7-1.3	0.4	69,000	17	1
Proteose-Peptone	2.0-4.0	0.7	4,000-40,000	0	0

Source: Brunner (20).

and CyS_{119} or between CyS_{106} and CyS_{121}. This is an unusual situation in protein structure and could impart a unique binding property to β-lactoglobulin.

The primary structure of α-lactalbumin was established by Brew et al. in 1970 (18), yielding 123 amino acid residues with a calculated molecular weight of 14,176. This protein has some apparent similarity with hen's egg lysozyme. α-Lactalbumin exerts a significant biological function by participating as a "modifier" protein in the lactose synthetase system which is responsible for the synthesis of lactose (19).

Bovine serum albumin has been crystallized from milk and was shown to be compositionally and physically similar to bovine blood serum albumin (96). Although the entire amino acid sequence has not been elucidated, present evidence suggests that there exists some degree of microheterogeneity. Two features of its structure include a free sulfhydryl group at position 34 in its N- terminal peptide and 17 intramolecular disulfide bonds.

The term immunoglobulins describes a family of high molecular weight proteins that share common physical and chemical characteristics and antigenic determinants (24). Immunoglobulins occur in serum and other body fluids. In cow's colostrum they serve to transfer passive immunity to the calf, protecting it against disease until its own immune defenses are activated. All immunoglobulins are monumers or polymers of a four-chain molecule consisting of two short chain polypeptides and two long chain polypeptides which are cross linked by disulfide bonds.

The proteose-peptone fraction of whey proteins could be defined as that portion of the milk protein system not precipitated by heating

to 95°C for 20 minutes and subsequent acidification to pH 4.7, but precipitated by 12 percent (W/V) trichloroacetic acid (103). Compositional and physical characteristics of the major components indicate that they are low to intermediate molecular weight phosphoglycoproteins (4,000-40,000). Their amino acid profiles are characterized by low concentration of aromatic residues and relatively high concentrations of glutamic and aspartic acids. Low concentrations of methionine account for all the sulfur containing amino acids.

Whey contains several unique proteins which are present in lower concentrations than the components already identified. These "minor" proteins are lactoferrin, lactollin, serum transferrin and M-1 glycoproteins.

Nutritional Value of Whey Protein

The amino acid profile of whey indicates that this by-product was shown to have value as a supplement or partial substitute for other proteins in food products (26, 28, 39). A World Health Organization (WHO) group discussed methods for evaluating proteins and illustrated the use of a reference protein for calculating the chemical score as follows:

> The content of each essential amino acid in a food protein is expressed as a percentage of the content of the same amino acid in the same quantity of a protein selected as a standard. The amino acid showing the lowest percentage is called the limiting amino acid, and this percentage is the chemical score. (128)

This WHO group also considered factors involved in the amino acid needs of different individuals and cautioned against use of the amino acid profile as the only means of evaluating proteins.

The WHO group published a provisional pattern of essential amino acids. The ideal protein should contain essential amino acid in these proportions, but not necessarily in the same amount per gram of protein. Tryptophan concentration of a particular protein sometimes has been used as unity, and all the other amino acids in the protein calculated on this basis.

The greatest differences in the amino acid profiles of casein, whey protein and the provisional FAO pattern were shown (26) to be in the amount of tyrosine, which is low in whey protein, and in the amount of sulfur-containing amino acids, which are low in casein as shown in Table 3.

Lactalbumin, the major protein component of whey, was found to be slightly superior to casein in a dietary study on rats (28). For example, rats fed a diet containing 20 percent casein as the sole protein source showed approximately the same weight gain as a similar group fed a diet containing 12 percent lactalbumin as the sole protein source.

Nutritional evaluation of whey protein concentrates and whey protein fractions was performed by Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) assays as well as by calculation of chemical scores (39). Weanling male rats (21 days old) were fed experimental diets containing 10 percent protein for four weeks. Food intakes and weight gains were recorded and PER values calculated after each week. Another set of 28 day old weanling male rats were fed experimental diets containing 9 percent protein for 10 days, after which the nitrogen content of the rats was determined by carcass analysis to measure the NPU.

Table 3. The Essential Amino Acids of Casein and Whey Protein

Amino Acid	Percent in Casein	Percent in WPC	FAO Pattern
Isoleucine	6.1	5.6	4.2
Leucine	9.2	8.2	4.0
Lysine	8.2	7.4	4.2
Phenylalanine	5.0	2.9	2.8
Tyrosine	6.3	1.7	2.8
Sulfur Containing	3.1	4.4	4.2
Threonine	4.9	5.7	2.8
Threonine	4.9	5.7	2.8
Tryptophan	1.7	1.8	1.4
Valine	7.2	5.6	4.2
Total	51.7	43.3	31.4

Data from Demott (28).

For calculating the chemical score Forsum (39) used egg and human milk reference amino acid patterns, as well as the FAO Provisional pattern. Forsum (39) showed that amino acid contents of the concentrates and of all the fraction were almost adequate to cover the needs of the human infant and more than adequate to cover those of young children.

Protein Recovery

There has been considerable effort over the past 20 years to devise and implement processes for recovering whey protein concentrate (WPC) such as complex formation and precipitation procedures, reverse osmosis (RO)(84), gel filtration (GF), ion exchange (IE)(26), electrodialysis (ED) and ultrafiltration (UF) (27), which retain the proteins in a native and functional state.

Although the technology for these processes has been fairly well developed, they have not been as universally adopted by the industry as had been projected. Delaney (26) studied the composition, properties and uses of WPC prepared by different processes. Wide compositional differences were evident between products prepared by individual processes (e.g. protein contents ranging from 30-70 percent for WPC prepared by UF). Even wider differences occurred in the composition of WPC prepared by different processes. Delaney also showed that the amino acid pattern in WPC does differ significantly from that in whey powder. However the overall amino acid profile of WPC was excellent.

Undenatured whey protein concentrates are valuable to food processors because of their functional properties. Such properties have

been delineated by Morr (89) as: dispersibility/solubility, viscosity/stabilization, elasticity/cohesion/adhesion/dough properties, emulsification, foam expansion and stability, water sorption and gelation/fiber formation.

The performance of WPC in food products has been studied by many workers (82, 83).

DeVilbiss et al. (30), Guy et al. (53), and Volpe and Zabik (118) studied the performance of WPC in bread and cake baking.

Foremost Food Company, Division of Foremost-McKesson, Inc. (38), had announced the development of whey concentrate providing protein in levels to 35 percent and called Fortein. Its principle nutritional benefit is its balanced profile of all essential amino acids. The product has been used in a wide variety of food products including cereals, instant breakfast, candy, baked goods, desserts and toppings, baby foods, frozen ice and sherbet bars, and beverages.

WPC were shown to possess a wide range of functional or food technological properties. Some examples of practical applications of these properties include the fortification of acid fruit drinks and carbonated beverages with WPC (60). The addition of 2 percent WPC to cake flour increases volume, produced a more moist and tender crumb and increases tolerance to variations in formulation and baking conditions (125). WPC has been found particularly suitable as a replacement for skim milk in a number of baking applications--such as prepared cake, biscuit and flour mixes. WPC has also been used as a base material for the manufacture of coffee whiteners, whipped toppings, imitation sour cream, and instant breakfast food (77, 108). WPC is beginning to find

application as an egg white extender and whipping agent in the preparation of low fat-whipped toppings and meringue type confections (17).

As mentioned before, undenatured WPC are valuable to food processors because of their "functional" properties. Heat coagulated whey protein on the other hand, though nutritionally equal to undenatured whey protein and superior to most others in animal feeding has found limited use in the manufacture of foods due to its insolubility and gritty characteristic (126).

Functional protein alone, however, cannot fit all the needs of the food industry. In food formulation where protein sources are required primarily for nutritional reasons, functionality may be neither necessary nor desirable. Schoppet et al. (108), for example, showed that whereas it is fairly easy to produce a highly nutritious 20 percent protein pasta by conventional means using an insoluble heat coagulated whey protein concentrate, it is quite difficult by conventional means to produce the same product using an undenatured or soluble whey protein concentrate. Similarly, the work of Smith et al. (110) indicates that among non-meat protein additives, insoluble protein produces a high protein hot dog (over 30 percent protein) with greater emulsion stability than its soluble form.

Denaturation of whey protein appears when whey proteins are subjected to temperatures greater than 60°C. They change from their globular structure to a more random open conformation. This denaturation can be followed by the Harland and Ashworth (54) procedure, based upon their loss of solubility at pH 4.6. However, the rate of denaturation of the individual proteins varies. Larson and Rolleri (75)

followed the denaturation of the individual whey proteins by free-boundary electrophorisis. From their results, it may be seen that the whey proteins can be arranged in the following order according to the ease of denaturation: immunoglobulins>serum albumin> β-lactoglobulin> α-lactalbumin. Many bonds are involved in the denaturation such as disulfide, hydrophobic, hydrogen and ionic bonds.

Heat coagulation of whey protein is simple. If whey is held at sufficiently high temperature for a sufficient period of time, some of the protein will denature and coagulate. Conventionally, heat coagulation has been carried out at 85-100°C requiring a holding time of 15 or more minutes (43, 44, 50, 54, 75, 88, 94, 95, 103, 115). Figure 1 describes the process.

Buchheim and Jelen (22) studied the microstructure of heat coagulated whey protein curd. They used light microscopy and freeze-fracturing election microscopy to study the particle size and micro-structure of the heated protein precipitated from electrodialysed, ultra-filtered and pH-adjusted wheys. Loose structure and small particles were observed after heating untreated wheys at pH 4.5. All pretreatments reducing mineral content resulted in less fragile coagulum with larger particle size. Heating UF whey at pH 6.5 produced very large, firm particles; however, a significant amount of protein did not precipitate under these conditions. All samples heated pH 4.5 showed more compact and well define microstructure when compared to electron micrographs of sample heated at pH 6.5.

Panzer et al. (95) described a process to obtain a low moisture, free-flowing protein powder of high nutritional value through high

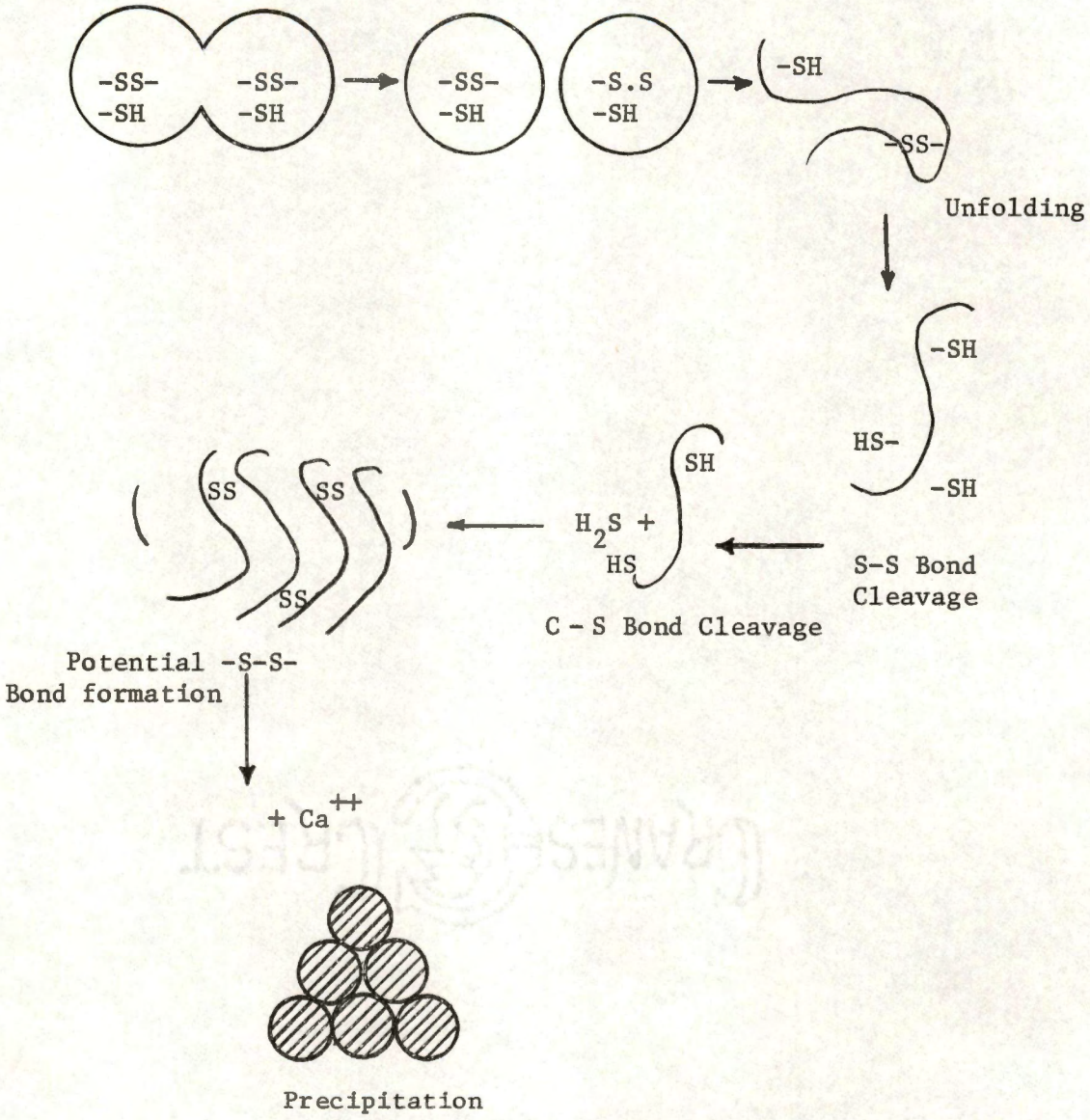


Figure 1. The Effect of Heat on β-Lactoglobulin.

temperature (120°C) heat coagulation of cottage cheese whey protein in a constant stirred reaction vessel using steam injection heating. Dried protein concentrates prepared by the suggested process contain 65-95 percent protein. Panzer et al. (95) claimed that all the coagulable protein can be removed in 8 minutes of holding time in pH range of 5.5-6.5. They also found that increasing coagulation temperature to above that of conventional methods (85-100°C) significantly decreases processing time without adversely affecting product quality.

A potential of heat-precipated WPC as a meat extender was demonstrated by both objective and subjective evaluation (65). However, it appeared that with deep-fried meat balls made from plain ground beef the WPC could be used only in small amounts without negatively affecting quality attributes such as appearance, texture or mouthfeel.

Fresh pork sausage extended with 20 percent WPC was rated slightly higher than the same sausage without the WPC (64).

Jelen and McIntyre (66) have evaluated the sensory properties of beef, pork and turkey meat loaves and beef patties containing heat-coagulated WPC by hedonic scale taste panel procedures. Beef products showed significant linear regression between decreasing hedonic scores and increasing percent WPC when served under normal light, but not under red light. Pork and turkey loaves containing 0, 5 or 10 percent WPC did not differ significantly. They also found that products containing 20 percent WPC were significantly inferior. Poor appearance of beef products containing WPC may have influenced their low rating. Ground pork and ground turkey appeared more compatible with the WPC than ground beef.

Whey protein concentrate was used in pasta (macaroni) to increase its PER value (112). A durum granular flour with different levels of WPC were used to prepare the pasta with up to 20 percent protein. Various tests were applied to the enriched and unenriched product to evaluate the produce. The authors concluded from the observations on processing and nutritional and organoleptic testing that pasta can be successfully fortified with whey proteins; that the addition of insoluble whey proteins can provide a real improvement in the nutritional quality of the pasta; that the product can be easily adapted to commercial practice; and that consumers will find such an enriched product acceptable.

Demott et al. (29) described a simple process for preparing heat denatured WPC. The precipitates were blended with xanthan gum and onion-flavoring to produce a chip dip.

CHAPTER III

MATERIALS AND METHODS

Source of Whey

Cottage cheese whey prepared by rennet coagulation was obtained from a local dairy plant and stored at 4°C until used.

Heat Denaturation of Whey Proteins

Nine combinations of time, flocculant and pH at 90°C were used to determine optimum precipitation. These were 10, 20, or 30 minutes. The three pH levels were: 4.5, 5.75, and 7.0.

The pH of 200 ml batches of whey was adjusted to the desired value with 2N NaOH. The samples were then divided into three groups. The first group was heated at 90°C in a water bath for 10 minutes, the second group for 20 minutes, and the third group for 30 minutes.

Addition of Flocculant

To 200 ml of whey, 10 or 20 ppm of alum ($Al_2 (SO_4)_3$) were added. The pH's of the sample were adjusted to 4.5, 5.75 or 7.0. The samples then were heated at 90°C for 10, 20 or 30 minutes. Two replicates of each treatment were performed.

Recovery of Denatured Protein

The heated whey was cooled to room temperature (about 25°C). Then the samples were centrifuged 15 minutes at 750 x g in an International #2 centrifuge. The sediment was collected and stored at 0°C until used.

Analysis of Recovered Material

The wet weight of the sediment was determined by weighing the centrifuge bottle empty, then weighing it after centrifuging the sample and decanting the supernatent.

Protein in the original whey, the sediment and the supernate were analyzed by the Kjeldhl method. A sample of 1-2 gm for sediment and 15 gm for supernate and original whey, and a conversion factor of 6.38 x %N were employed.

The ash content of the recovered material was determined by using the AOAC method (8). Samples were dry-ashed at 550°C for 4 hours and ash was determined as follows:

$$\text{Percent ash} = \frac{\text{weight of dry sample (gm) X 100}}{\text{weight of wet sample (gm)}}$$

For total solids, a 2.0 gm sample of the recovered material was weighed into a tared pan. The sample was dried to a constant weight in a vacuum oven at 60°C and 380 torr (about 16 hours). The weight of the dried sample was recorded. Total solids were calculated.

The lactose content of the recovered sediment was calculated by difference. The protein and ash content were subtracted from the total solids in the sediment.

Electrophoresis of Protein

The method of Weber and Osborn (120) was used to determine the distribution of protein by gel electrophoresis. This was applied to sediment, supernate, and original whey. Following Weber and Osborn (120) a gel was prepared. Acylamide A (13.5 ml) and 15 ml gel buffer were

mixed together, deareated for 2 minutes and then 1.5 ml ammonium persulfate, freshly prepared, and 0.045 ml TEMED (N, N, N, N-tetramethyl ethylenediamine) were added. The gel tubes were filled with a syringe and left 15-20 minutes until gel formation was complete.

The protein samples were prepared by placing 9 parts of 0.01M sodium phosphate (pH 7.0) containing 1 percent SDS (sodiumdodecyl sulfate) and 1 percent of 2-mercaptoethanol in test tubes in a 100°C bath. One part of the protein solution was added to a tube. The tube was capped and incubated for 2 minutes. The sample was then cooled to room temperature. The final protein concentration was about 1.0 mg/ml and volumes as small as 30-60 µl were used.

Samples for electrophoresis were prepared by placing the following combination on a square piece of parafilm:

Five µl tracking dye solution (0.05 percent bromophenol blue in 0.01 M phosphate pH 7.0), 3-5 crystals of sucrose, 5 µl of 2-mercaptoethanol, and 30-60 µl of protein solution. They were mixed well and placed on the top of the gel tube in the electrophoresis apparatus using a micropipet. The current was adjusted so that each tube received not more than 8 amp.

After 6 hours the dye band reached the bottom of the gel tubes. At that time the gel tubes were removed and the center of the dye band in each tube was marked with a needle dipped in water-insoluble India ink. The gel tubes were then placed in capped test tubes containing a staining solution consisting of:

Cromassie Brilliant Blue R 250	1.25 gm
Methanol	227 ml
Glacial acetic acid	46 ml
Water	to 500 ml

The gel tubes remained 12 hours in the staining solution, then were removed, and washed with distilled water and placed in a destaining solution which was prepared as follows:

Methanol	50 ml
Glacial acetic acid	75 ml
Water	to 1 liter

This process removed excess dye from the gels and rendered the protein bands visible. After destaining, the distance from the top of the gel to the India mark in the dye band was measured. The distances from the top of the gel to the center of each protein band on the gel were also measured and by using the following formula the mobility of each protein was calculated.

$$R_m = \frac{x}{y}$$

where R_m = relative mobility

x = distance from the top to the center of each band

y = distance from the top of the India ink mark

Particle Size - Photomicrography

Slides of sediment suspension (0.1 ml/100 ml H_2O) were prepared and photographs were taken under different magnifications using a phase contrast microscope with a 35 mm camera attachment. The magnification best suited to the purpose was 40X.

Applications

Sandwich spread. Sandwich spreads using the whey protein precipitate as a basic matrix were prepared using the following ingredients:

Ingredients	Weight
Whey protein concentrate	50
Mayonnaise	20
Pickle relish	10
Catsup	2.5
Sugar	0.03
Salt	0.03
Xanthan gum	0.20

Tuna, ham, or cheese in bits were added as variations.

Proximate analysis was performed on the basic sandwich spreads. Protein, ash, and total solids were carried out as described earlier for the whey materials.

Fat in sandwich spread was determined by the following ether extraction method. A 33 x 80 Whatman extraction thimble with a small pad of glass wool was weighed empty and then filled two-thirds with sample to be extracted. The difference between the two weights gave the weight of the sample. The filled thimble was inserted into Soxhlet extraction tube. A 250 ml round flat-bottom flask with ground glass joint was weighed and 100 ml of 1:1 mixture petroleum ether and hexane was added, then the flask was attached to the bottom of the Soxhelt tube with a water cooled condenser attached above. Fifty ml

of ether solvent was added by a funnel through the top of the condenser to cover the sample. The sample was extracted by heating on a hot plate for 6 hours. Ether was evaporated under a hood overnight and the last trace was removed in a vacuum oven at 60°C. The flask with the fat was re-weighed and fat percent was calculated according to the following formula:

$$\text{percent fat} = \frac{\text{weight of fat (gm)}}{\text{weight of sample (gm)}} \times 100$$

Overall acceptability, flavor and consistency of sandwich spreads prepared with WPC as a matrix and with other flavoring ingredients were assessed by a consumer panel. The panel involved 40 untrained persons. Four samples were presented to the panelists in small paper cups under cool-white fluorescent lighting in the sensory evaluation room.

Score cards using a seven-point scale, shown in Figure 2, were used by the panelists to score flavor and consistency. A five-point scale was used for overall acceptability, as shown in Figure 2. All data from sensory panels were analyzed by analysis of variance and, where appropriate, means separations were measured by Duncan's Multiple Range Test (113).

Use in Ice Cream

Small batches of vanilla ice cream were prepared using the wet concentrate WPC as a partial replacement for nonfat dry milk (NFDM). Levels of WPC replaced 0, 10, 20, or 30 percent of the NFIM in ice cream mixes.

The method of Sommer (111) was used to calculate the ice cream formula shown in Table 4.

Date _____ Name _____

You are given four samples of sandwich spread. Please evaluate each sample for all quality attributes listed. Check (✓) the term that best describes each characteristic of the sample.

Quality Attributes	Sample Codes			
Flavor	_____	_____	_____	_____
Very desirable	____	____	____	____
Desirable	____	____	____	____
Slightly desirable	____	____	____	____
Neither desirable or undesirable	____	____	____	____
Slightly undesirable	____	____	____	____
Undesirable	____	____	____	____
Very undesirable	____	____	____	____
Consistency				
Very desirable	____	____	____	____
Desirable	____	____	____	____
Slightly desirable	____	____	____	____
Neither desirable or undesirable	____	____	____	____
Slightly undesirable	____	____	____	____
Undesirable	____	____	____	____
Very undesirable	____	____	____	____
Overall Acceptability*				
Very good	____	____	____	____
Good	____	____	____	____
Fair	____	____	____	____
Poor	____	____	____	____
Very poor	____	____	____	____

*Consider all the characteristics by which you would usually evaluate this food.

Comments:

Please answer the following questions after you have evaluated the samples.

1. Are there any changes that you would like to suggest for the samples? If so, please specify.

2. Would you be willing to serve this product in your home?
   Yes _____ No _____

Thank you very much for participating in this test.

Figure 2. Score Card for Sandwich Spread

Table 4. Composition of Ice Cream Mixes

NFDM Replacement by WPC (percent)	Ingredients[a]					
	Cream	NFDM	WPC	Sugar	Stab.	Water
			lb.			
0	8.81	2.97	0	4.5	.045	13.67
10	8.81	2.68	1.92	4.5	.045	12.05
20	8.81	2.38	3.84	4.5	.045	10.43
30	8.81	2.08	5.76	4.5	.045	8.81

[a]Cream = 45.5 percent fat.
WPC = 14.70 percent solids.

Four batches of mix containing 30 pounds each were prepared as follows: The proper amounts of sugar, NFDM, WPC, stabilizer and water for each bath were weighed and mixed together in 5 gallon stainless steel milk cans. The cream for each batch was weighed and set aside.

The mixes were then pasteurized by placing the cans in water bath (75°C). When the temperature of the mixes reached 60°C, the pre-weighed amount of cream for each batch was added.

The temperature of the water bath was maintained at 73°C by injecting steam into the water. The mixes were kept at 72°C for 30 minutes to complete pasteurization. They were stirred by hand. Ice cream mixes were then neutralized to pH 6.7-6.9, with 25 percent NaOH, and then homogenized at 2500 psi and cooled in an ice bath and kept at 4°C until the next day when they were frozen. A soft serve ice cream freezer was used for freezing the mixes. The four ice cream batches were held at -20°C for hardening.

Samples of the ice cream were presented to an expert panel of six people. Judges examined the samples and scored the flavor and body and texture using the score card shown in Figure 3. Data from these sensory panels were analyzed by analysis of variance.

CONTEST

DATE_____ A.D.S.A. ICE CREAM SCORE CARD D.I.S.A._____CONTESTANT No._____

PERFECT SCORE	CRITICISMS	SAMPLE NO 1	2	3	4	5	6	7	8	9	10	TOTAL GRADES
FLAVOR 45	CONTESTANT SCORE ➧											
	GRADE SCORE											
	GRADE CRITICISM											
	COOKED											
	LACKS FLAVORING											
	TOO HIGH FLAVOR											
NO CRITICISM 10	UNNATURAL FLAVOR											
	HIGH ACID											
	LACKS FINE FLAVOR											
	LACKS FRESHNESS											
	METALLIC											
	OLD INGREDIENT											
NORMAL RANGE 1–10	OXIDIZED											
	RANCID											
	SALTY											
	STORAGE											
	LACKS SWEETNESS											
	SYRUP FLAVOR											
	TOO SWEET											
BODY AND TEXTURE 30	CONTESTANT SCORE ➧											
	GRADE SCORE											
	GRADE CRITICISM											
NO CRITICISM 5	COARSE/ICY											
	CRUMBLY											
	FLUFFY											
NORMAL RANGE 1–5	GUMMY											
	SANDY											
	SOGGY											
	WEAK											
COLOR 5	ALLOWED PERFECT IN CONTEST ➧	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
MELTING QUALITY 5	ALLOWED PERFECT IN CONTEST	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
BACTERIA 15	ALLOWED PERFECT IN CONTEST	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	
TOTAL 100	TOTAL SCORE OF EACH SAMPLE ➧											
	TOTAL GRADE PER SAMPLE											FINAL GRADE

TEAM RANK	Code	Grade
	1	
	2	
	3	
	TOTAL	
	RANK	

RANK

F649
3/77-2M

Figure 3. Score Card for Ice Cream.

CHAPTER IV

RESULTS AND DISCUSSION

The effect of heat on whey proteins has been studied extensively. A combination of time, pH and flocculant to give most practical conditions for protein recovery was involved. According to Guy et al. (50) and many others heating at 90°C was required for complete denaturation of whey protein.

Table 5 shows the analysis of variance of the factors affecting WPC recovery. Heat, pH, flocculant, and two interactions were significant. No interactions involving replication were significant, so all such interactions were pooled into the "b" error term, excepting heat x replication. The latter interaction was used as a split plot error term ("error a") to give a more strenuous test for the effect of heat which was of prime importance.

Alum in two concentrations, 10 ppm and 20 ppm, was used as flocculant to increase the recovery of whey proteins. The percentages of recovered protein with and without flocculant $Al_2(SO_4)_3$ are shown in Table 6. The addition of 10 ppm alum is shown to be superior to either 0 or 20 ppm, but when tested, it gave a distinctive taste of alum. Based on this fact we decided to drop the use of flocculant.

Heating of whey under three different pH levels was also investigated. The mean values of protein recovery in Table 7 show heating at pH 7.0 and 5.75 superior to pH 4.5. But the low total solids in the precipitate as shown in Table 8 made it inconvenient to use the

Table 5. Analysis of Variance of Effects of Heating Time, Flocculant and pH on Recovery of Whey Protein

Source	df	Mean Square[a]
Heat	2	67.103**
Rep	1	0.008
Heat x Rep (error a)	2	0.4039
pH	2	49.294**
Flocculant	2	21.587*
pH x Heat	4	106.142**
Floc x Heat	4	6.744
Floc x pH	4	4.521
Floc x pH x Heat	8	10.579*
Error (b)	24	4.031

[a]Significant variation: * <0.05, ** <0.01.

Table 6. The Effect of Alum on Protein Recovery from Heated Whey

Flocculant (alum)	Recovered Protein[a]
ppm	Percent
0	41.55b
10	43.65a
20	42.04b

[a]Means followed by same letter are not significantly different at $P \leq 0.05$. n = 18.

Table 7. The Effect of pH on Protein Recovery from Heated Whey

pH	Recovered Protein[a]
	Percent
7.00	43.81a
5.75	42.85a
4.50	40.59b

[a]Means followed by same letter are not significantly different at $P \leq 0.01$. n = 18.

Table 8. Total Solids of Recovered Sediment from Heating at 90°C for Three Times

Time	Total Solids[a]		
	$pH_{4.5}$	$pH_{5.75}$	$pH_{7.0}$
Minutes		Percent	
10	13.67	14.12	11.61
20	16.01	19.85	13.05
30	15.57	18.00	12.51

[a]Means of duplicates.

precipitate on a practical basis in ice cream mix. Adjustment from pH 4.5 to 5.75 made a difference of only 2.26 percent recovery. This small increase in protein recovery does not practically justify the adjustment of the whey to pH 5.75 before heating. On the other hand pH 4.5 is the optimum for fungal lactose which could be applied industrially.

Whey was heated at 90°C for three periods of time, 10, 20 and 30 minutes to determine the optimum time for maximum protein recovery. From data shown in Table 9, 20 or 30 minutes heating was superior to 10 minutes. Since there is no significant difference in heating 20 or 30 minutes, 20 minutes heating time was considered to be more economical in industry.

The combinations of heat and pH were the most important factors since the addition of alum left an undesirable taste in the precipitate. Table 10 shows the interaction of heat and pH on protein recovery with no flocculant added. The condition of pH 7.0 and 20 minutes was superior to any others, but because of the factors mentioned before, pH 7.0 was not desirable. The treatment that was used, pH 4.5 and 20 minutes heating, was not significantly different from any of the other good combinations.

Protein Distribution by Electrophoresis

Figure 4 shows the electrophoretic patterns of the different fractions of whey involved in the study along with a control gel containing blood serum albumin (BSA), β-lactoglobulin, and α-lactalbumin, the principle proteins in whey. Referring to Figure 4, it is evident that the actual migration of the proteins was not uniform among the

Table 9. The Effect of Heating Time on Protein Recovery from Heated Whey

Heating Time	Recovered Protein[a]
(90°C)	Percent
10 minutes	40.21b
20 minutes	43.78a
30 minutes	43.26a

[a]Means followed by same letter are not significantly different at $P \leq 0.01$. n = 18.

Table 10. Effect of Interaction of Heat and pH on Protein Recovery from Heated Whey with No Flocculant

pH	Time of Heating	Protein[a]
		(Percent)
7.00	10	36.81cd
7.00	20	46.99a
7.00	30	43.86ab
4.50	10	35.53d
4.50	20	41.26b
4.50	30	40.32bc
5.75	10	43.41ab
5.75	20	41.88b
5.75	30	43.93ab

[a]Means followed by same letter are not significantly different at $P \leq 0.05$. $n = 2$.

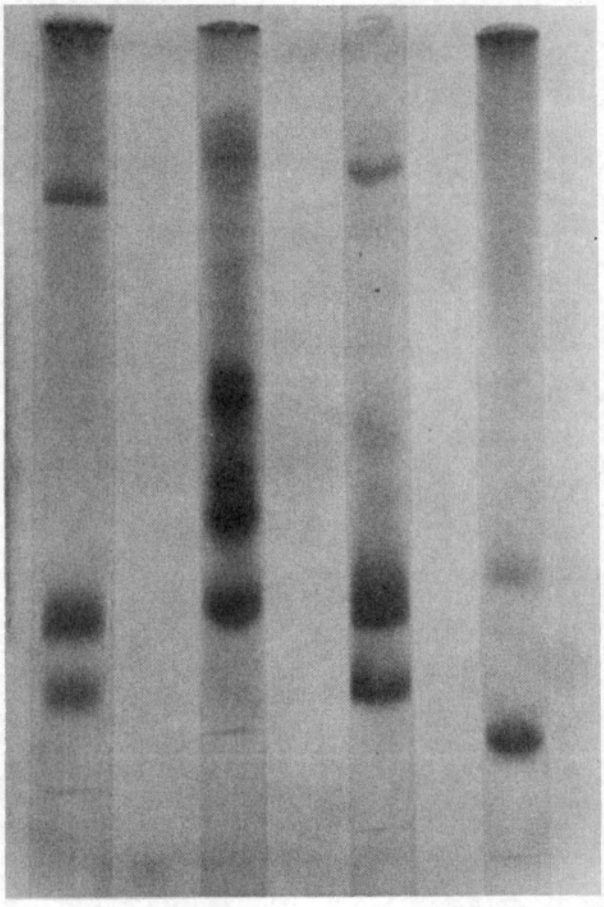


Figure 4. Electrophoretic Patterns of Whey Proteins in; A - Reference Proteins (BSA, β-Lactoglobulin, α-Lactalbumin); B - Unheated Whey; C - Heat Precipitated Whey Protein, D - Heated Whey Supernate.

four gel tubes. Therefore relative mobilities were calculated and are shown in Table 11. From the figure and Table 11 the three whey protein fractions and residual casein (R_m = 0.5-0.69) were evident in the unheated whey and the sedimented, heat-coagulated protein. A small amount of β-lactoglobulin and a relatively large proportion of the α-lactalbumin remained suspended in the supernate after heating. This is in agreement with literature that reports α-lactalbumin as having the greatest resistance to heat. There were no bands from the BSA or casein in the supernate, indicating their precipitation by the heating process. The precipitation conditions used for the sample reported were pH 4.5, 20 minutes heating at 90°C.

Particle Size

Figure 5 shows a typical photograph of WPC particles suspended in water (0.1 ml/100ml H_2O). The diameters of groups of particles in different size classes were measured and plotted against number of particles. Figure 6 shows the particle size distribution in heat precipitated whey protein.

The average diameter was calculated by the following formula:

$$dav = \frac{\Sigma nidi}{\Sigma ni} = 29.5\mu$$

where dav = average diameter

$\Sigma nidi$ = summation of the diameter times the number of particles that have the same diameter.

Σni = summation of particles.

Table 11. Relative Mobilities of Whey Protein Fractions Separated by Zone Electrophoresis

Control Component		Unheated Whey	Heat Prec. Protein	Supernate
BSA - 0.21		0.20	0.19	--
β-lactoglobulin	.79	0.72	0.74	0.70
α-lactalbumin	0.89	0.82	0.85	0.87
Other 1	--	0.50	0.50	--
2	--	0.69	--	--

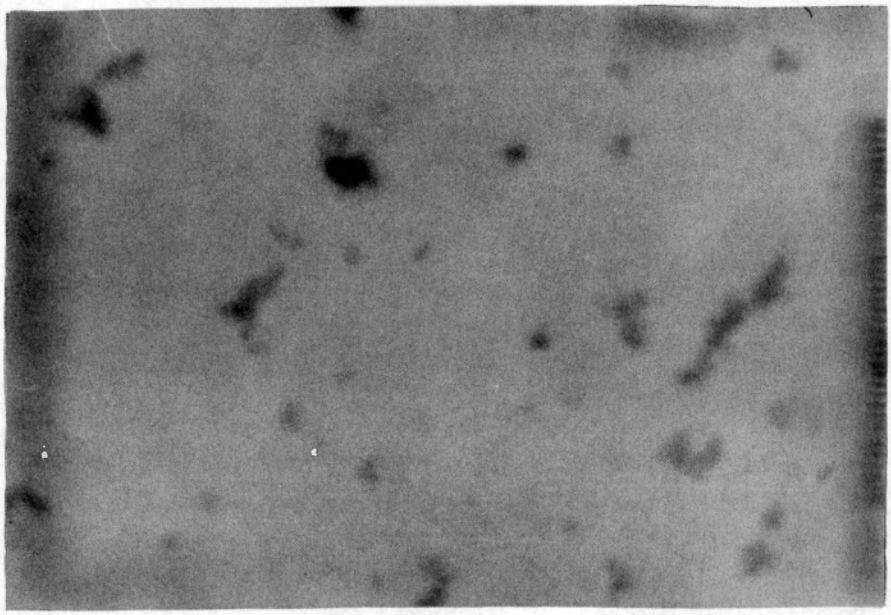


Figure 5. Water Suspension of Heat Precipitated Whey Protein (.1 ml/100 ml H_2O).

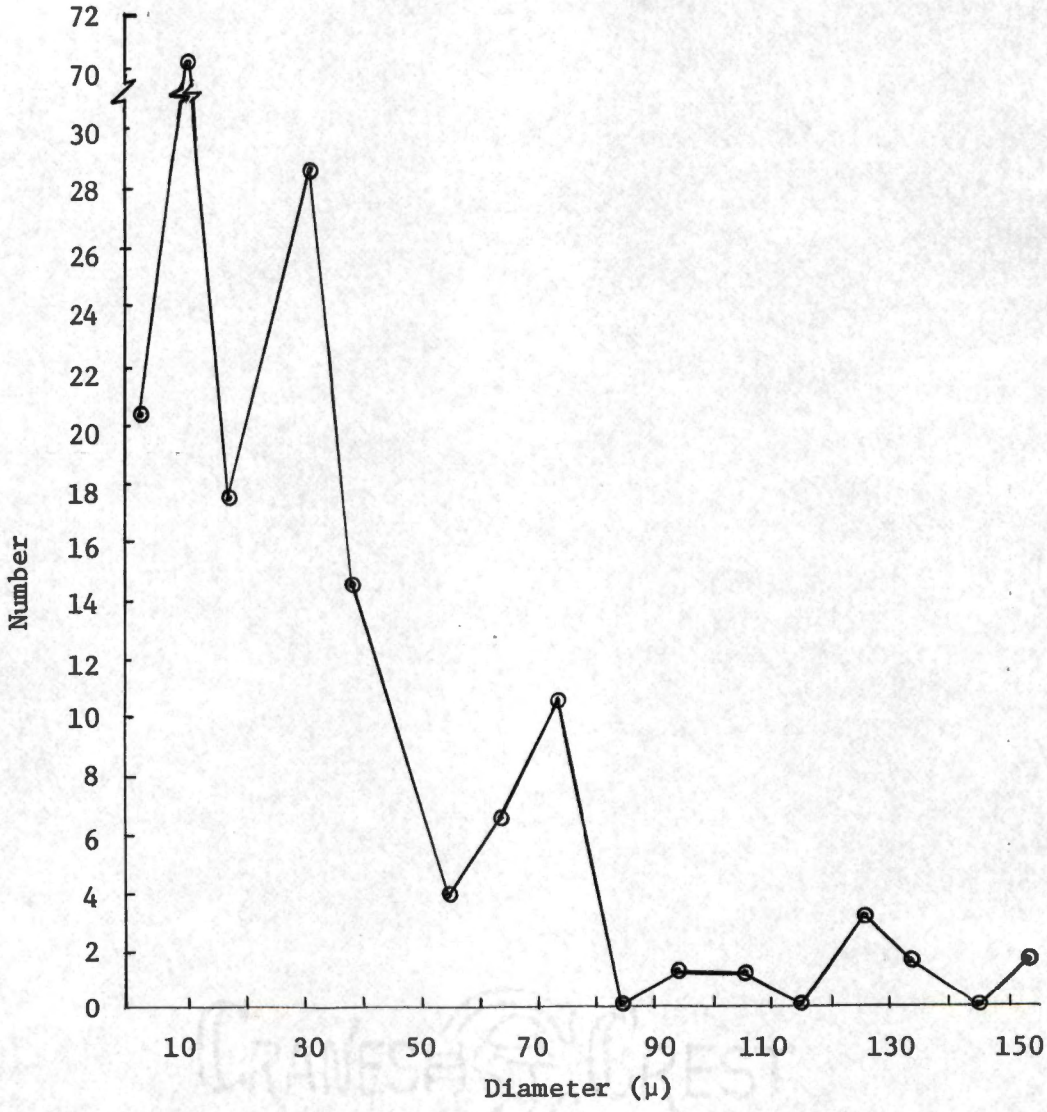


Figure 6. Distribution of Particles in Heat Precipitated Whey Protein.

Applications

The WPC was used in two food products, a sandwich spread and as a replacement for non-fat dry milk in vanilla ice cream.

Sandwich Spread

Four variations of a sandwich spread using the WPC as a basic matrix were prepared and evaluated by sensory panels. The variations were (a) basic spread, (b) basis spread plus 20 percent (weight) ham bits, (3) basic spread plus 20 percent cheddar cheese bits, (d) basic spread plus 20 percent shredded canned tuna. The proximate composition of the basic spread is shown in Table 12. The composition of the spreads with added ingredients would vary as the composition of the added materials varied.

Overall acceptability, flavor and consistency of sandwich spreads prepared with WPC as a matrix and with other flavoring ingredients were assessed by a consumer taste panel with 40 members.

Table 13 shows the mean sensory scores for sandwich spread when served to the panelists without crackers. Plain sandwich spread was significantly different in flavor, consistency and overall acceptability from the sandwich spreads that had flavoring ingredients such as ham, cheese or tuna added.

The analysis of variance in Table 14 shows that there was significant differences for the treatments and panelists in all three attributes. A second set of sandwich spreads were tested the same as the first set but were served on saltine crackers. These were more acceptable to the panelists. The data are shown in Table 15 and analyzed in Table 16.

Table 12. Composition of Basic Sandwich Spread with No Added Ingredients

Component	Percent[a]
Protein	4.86
Fat	48.36
Ash	1.60
Moisture	46.06

[a]All data are means of two replicates.

Table 13. Mean Sensory Scores for Sandwich Spreads Without Crackers

Spread	Mean Scores[a]		
	Flavor	Consistency	Overall Acceptability
Plain	3.86a	4.03a	3.18a
Spread with Ham	2.40b	2.80b	2.18b
Spread with Cheese	2.68b	2.80b	2.28b
Spread with Tuna	2.68b	3.10b	2.28b

[a]Sensory response scale 1-7 (very desirable - very undesirable). Means in columns followed by the same letter are not different at $P < 0.01$.

Table 14. Analysis of Variance of Sandwich Spreads Served Without Crackers

	d.f.	Mean Square[a]
I. Flavor		
Treatment	3	16.72**
Panelists	39	3.50**
Error	117	1.59
II. Consistency		
Treatment	3	17.69**
Panelists	39	4.37**
Error	117	0.89
III. Overall Acceptability		
Treatment	3	8.80**
Panelists	39	1.84**
Error	117	0.66

[a]Significant variation - * $\leq$ 0.05, ** $\leq$ 0.01.

Table 15. Mean Sensory Scores for Sandwich Spreads Served with Crackers

Spread	Mean Scores[a,b]		
	Flavor	Consistency	Overall Acceptability
Plain	2.39a	2.51a	2.15a
Spread with Ham	2.81	2.54a	2.56a
Spread with Cheese	2.42a	2.27a	2.17a
Spread with Tuna	2.02a	1.93b	1.78b

[a]Sensory response scale 1 - 7 (very desirable - very undesirable).

[b]Means in columns followed by the same letter are not different at $P \leq 0.01$.

Table 16. Analysis of Variance of Sandwich Spreads Served with Crackers

	d.f.	Mean Square[a]
I. Flavor		
Treatment	3	4.17**
Panelists	39	2.09**
Error	117	0.78
II. Consistency		
Treatment	3	3.29**
Panelists	39	2.03**
Error	117	0.67
III. Overall Acceptability		
Treatment	3	4.17**
Panelists	39	1.11**
Error	117	0.45

[a]Significant variation - * $\leq$ 0.05, ** $\leq$ 0.01.

Ice Cream

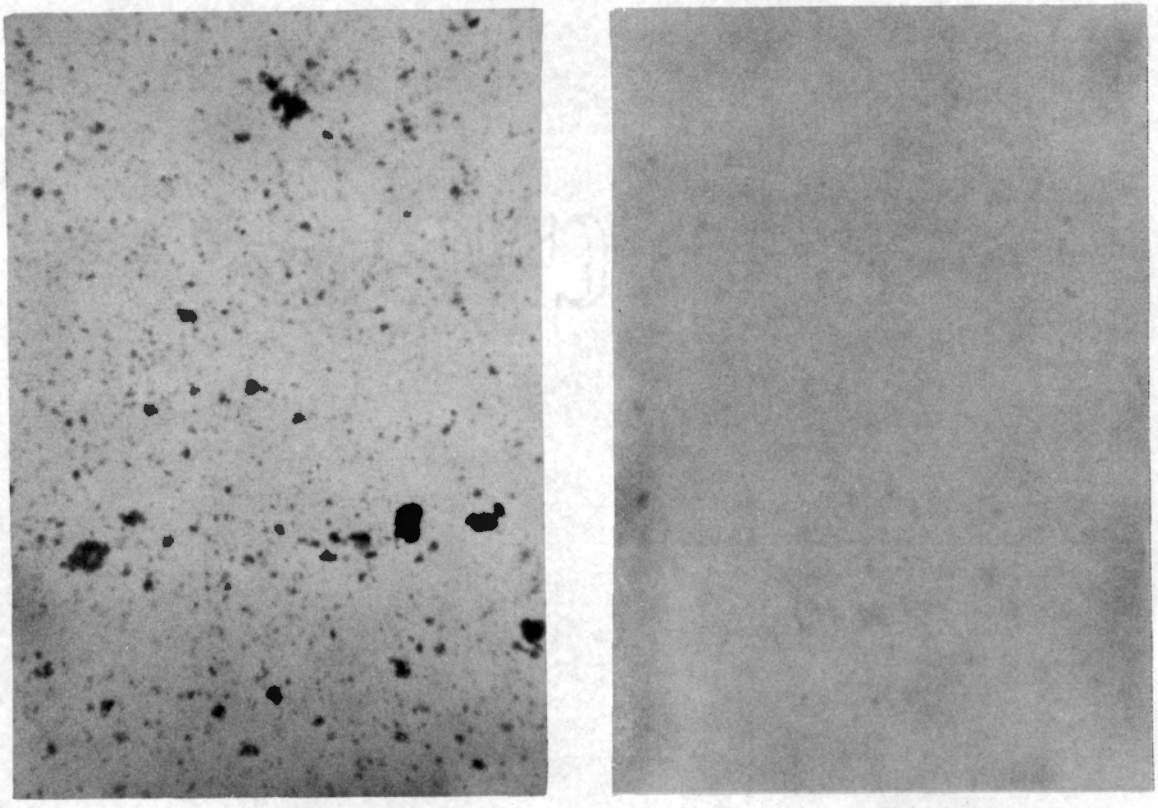
Four small batches of vanilla ice cream were prepared with the following combinations of WPC and NFDM: A - 100 percent NFDM, B - 90 percent NFDM + 10 percent WPC, C - 80 percent NFDM + 20 percent WPC, D - 70 percent NFDM + 30 percent WPC. The mixtures were weighed, pasteurized, neutralized to pH 6.7-6.9 with 25 percent NaOH and then homogenized and kept at 4°C until the next day when they were frozen.

A soft-serve ice cream freezer was used for freezing the mix, and then the ice cream was held at -20°C for hardening. Samples of the first batch of the ice cream were presented to an expert panel of six people. The ice cream had mealy texture and off flavor.

Figure 7 shows a photomicrograph of the two batches of ice cream mix. The first batch was very lumpy due to the large particle size and had high consistency; churning was evident.

The problem with body and texture of the first batch of ice cream was due to the technical operation of the freezer. This was controlled by decreasing the freezing time which gave an ice cream with good body and texture and with a drawing temperature - 5°C. Also some adjustment was made in the calculation of the ice cream mixes which could have had some effect on the ice cream body and texture.

A second batch of ice cream was presented to the same panelists. The ice cream had very smooth body and texture and had good flavor, even the one that had the highest percent of WPC. The data are shown in Table 17 and analyzed in Table 18. Table 17 shows the mean sensory score for the second ice cream batch. The flavor of the control batch was significantly higher than the other batch. On the flavor scale



Batch 1 Batch 2

Figure 7. Photomicrographs (40X) of Two Batches of Ice Cream Mix Showing Churned Fat in Batch 1.

Table 17. Mean Sensory Scores for the Second Batch of Vanilla Ice Cream

Attribute	Mean Score[a]
Flavor[b]	
0 WPC (control)	8.8a
10 percent WPC	8.1b
20 percent WPC	7.6b
30 percent WPC	8.1b
Body and Texture[b]	
0 WPC (control)	4.1a
10 percent WPC	3.6a
20 percent WPC	3.6a
30 percent WPC	4.0a

[a]Means followed by the same letter are not significantly different at $P \leq 0.05$. $n = 6$.

[b]Perfect scores: Flavor-10, body and texture - 5.

Table 18. Analysis of Variance of Sensory Scores on the Second Batch of Vanilla Ice Cream

Source	d.f.	Mean Square[a]
I. Body and texture		
Treatment	3	0.37
Panelist	5	0.27
Error	15	0.16
II. Flavor		
Treatment	3	1.78**
Panelist	5	0.87*
Error	15	0.28

[a]Significant variation - * $\leq$ 0.05, ** $\leq$ 0.01.

a perfect score was 10 points. The range of scores on the batches containing WPC was 7.6-8.1 with none of the three significantly different. A score of 8 would be considered acceptable. There was no significant difference in body and texture of any of the four batches. This leads to the conclusion that normal homogenization was adequate to disperse the particulate PWC in the mix in particles below the size which would be felt in the mouth. Table 18 shows the analysis of variance of sensory scores on the second batch.

From these results it would appear that up to one-third of the NFDM could be replaced by heat-precipitated WPC without seriously affecting the quality of vanilla ice cream.

CHAPTER V

SUMMARY AND CONCLUSIONS

In this study, whey protein was coagulated by heating at high temperature, 90°C. Based on the results the following conclusions were made:

1. Twenty minutes heating at 90°C was as good as 30 minutes heating for protein recovery, and was more economical.
2. Heating at pH 7 was superior to either pH 5.75 or pH 4.5 but because of the low solids at higher pH, pH 4.5 was sufficient and more practical to use industrially.
3. A flocculant such as aluminum sulfate could increase protein recovery but left an undesirable taste.
4. The basic proteins in whey were coagulated and precipitated by heat although small amounts of β-lactlogobulin and α-lactalbumin stayed in whey supernate after centrifugation.
5. WPC had a wide range of particle size when suspended in water.
6. Heat precipitated whey protein was successfully used in two food products, sandwich spread and vanilla ice cream.

Sandwich spreads prepared with WPC, plain or with added ingredients such as ham, cheese or tuna, were desirable and acceptable by sensory evaluation.

The wet WPC was successfully used in vanilla ice cream as partial replacement of non-fat dry milk. Homogenization, when properly applied, removed any mealiness contributed to the ice cream by particles of WPC.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Al-Ubaidi, Y. Y., and Bird, H. R. 1964. Assay for the unidentified growth factor in dried whey. Poult. Sci. 43:1484.

2. Anon. 1960. "Rivella" - a new form of whey utilization. Dairy Ind. 25(2):113.

3. Anon. 1967. Waste prevention and disposal. In "Manual for Milk Plant Operators" 3rd Ed. pp. 194-221. Milk Industry Foundation, Washington, D.C.

4. Anon. 1968. Dairy drinks made from cheese whey. Amer. Dairy Rev. 30(8):52.

5. Anon. 1969. Potential competition for synthetics in orange juice acid whey powder blend. Quick Frozen Foods 31(66):87.

6. Anon. 1969. New dairy product trends in Soviet Union. Milk Ind. 64(5):31.

7. Anon. 1972. New soy-whey milk tastes like egg nog. Mod. Dairy 51(2):8.

8. AOAC. 1975. "Official Methods of Analysis." 12th ed. Assoc. Offic. Analytical Chemists, Washington, D.C.

9. Arbuckle, W. S. 1972. "Ice Cream." The Avi Publishing Co., Inc. Westport, CT, Chap. 5, p. 59.

10. Baldwin, A. E. 1868. Improved process of treating milk to obtain useful products. U. S. Patent 78,640.

11. Balloun, S. L., and Khajerarn, J. K. 1974. The effect of whey and yeast on digestibility of nutrients in feather meal. Poult. Sci. 53:1084.

12. Becker, D. E., Terrill, S. W., Jensen, A. H., and Hanson, L. J. 1957. High levels of dried whey powder in the diet of swine. J. An. Sci. 16:404.

13. Berry, E. P., Carrick, C. W., Roberts, R. E., and Hague, S. M. 1943. Whey solubles as a source of growth factors in chick rations. Poult. Sci. 22:252.

14. Besserezhnov, A. S. 1968. Method for the preparation of a whey beverage. USSR patent 210,645. Dairy Sci. Abst. (1969).

15. Blazek, Z., Sulc, J. and Boeswart, J. 1966. Processing acid whey for food purposes. Czech. Patent 118,405, Chem. Abstr. 68:2124W (1968).

16. Bodmershof, W. 1959. Method for the preparation of sparkling milk drink of good keeping quality. Australian patent 205,328. Dairy Sci. Abstr. (1960).

17. Borgwardt, J., Petzold, Hand, Bordwardt, J. 1976. German Democratic Republic patent 122,326. Dairy Sci. Abstr. 1977.

18. Brew, K., Castellino, F. J., Vanaman, T. C., and Hill, R. L. 1970. The complete amino-acid sequence of bovine α-lactalbumin. J. Biol. Chem. 245:4570-4582.

19. Brodbeck, U., Penton, W. L., Tanahashi, N. and Ebner, K. E. 1967. The isolation and identification of the B protein of lactose synthetase as α-lactalbumin. J. Biol. Chem. 242:1391-1397.

20. Brunner, J. R. 1977. Milk protein. In "Food Proteins." Whitaker and Tannenbaun ed. The AVI Publishing Co., Inc. Westport, CT, Chap. 7, p. 175.

21. Brunner, J. R., Finley, J. W., and Blakely, L. 1969. Whey forms base for new dairy drinks. Amer. Dairy Rev. 31(6):60.

22. Buchheim, W. and Jelen, P. 1976. Microstructure of heat-coagulated whey protein curd. Milchwissenschaft 31(10):589.

23. Bunce, G. E. and King, K. W. 1966. Dietary protein level and amino acid retention in the young rat. Proceedings of the seventh international congress of nutrition in Hamburg, 5:134. Pergamon Press, New York.

24. Butler, J. E. 1969. Bovine immunoglobulins: A review. J. Dairy Sci. 52:1895-1909.

25. Cheeke, P. R., and Stangel, D. E. 1973. Lactose and whey utilization by rats and swine. J. An. Sci. 37:1142.

26. Delaney, R. M. M. 1976. Composition, properties and uses of whey protein concentrates. J. Soc. Dairy Tech. 20:91.

27. Delaney, R. A. M. and Donnelly, J. K. 1974. "Reverse Osmosis and Membranes-Theory-Technology-Engineering." Sourirajan, Marcel Dekker, ed. New York.

28. Demott, B. J. 1972. Nutritional value of casein and whey protein. Food Product Develop 6(10):86.

29. Demott, B. J., Helms, A. B., and Sanders, O. G. 1977. Tomato-flavored beverage and onion-flavored chip dip made from cottage cheese whey. J. of Food Protection, 49(8):540-542.

30. DeVilbiss, E. D., Holsinger, V. H., Posati, L. D. and Pallansch, M. J. 1974. Properties of whey protein concentrate foams. Food Technol. 28(3):40.

31. Dordević, L. and Koehler, V. 1966. Refreshing beverages from whey. Dairy Sci. Abst. (1967).

32. Downham, W. S. 1914. Whey emulsion. U. S. Patent 1,085,380.

33. Edmondson, L. F., Avants, J. K., and Douglas, F. W., Jr. 1968. Utilization of whey in sterilized milk product. J. Dairy Sci. 51:931.

34. Ekstrom, K. E., Benevenga, N. J., and Grummer, R. H. 1975. Effects of various dietary levels of dried whey on the performance of growing pigs. J. Nutr. 105:846.

35. Engel, E. R. 1948. Fermenting whey. U. S. Patent 2,449,064; Chem. Abstr. 42:9072h.

36. Engel, E. R. 1952. Improvements in or relating to process of producing an alcoholic beverage and a solid residue from whey. British patent 669,894; Dairy Sci. Abstr. 1952.

37. Fife, C. L., and Nilson, K. M. 1969. Production, disposal, and use of whey in Vermont. Bull. 558, Univ. VT., Burlington.

38. Foremost Foods Company. 1971. Am. Dairy Rev. 33(2):18.

39. Forsum, E. 1974. Nutritional evaluation of whey protein concentrates and their fractions. J. Dairy Sci. 57:665.

40. Forsum, E., Hambraeus, L., and Siddiqi, I. H. 1974. Large-scale fractionation of whey protein concentrates. J. Dairy Sci. 57:659.

41. Fox, P. F., and Morrissey, P. A. 1977. Reviews of the progress of Dairy Sci. the heat stability of milk. J. Dairy Res. 44:627.

42. Frank, G., and Braunitzer, G. 1967. The primary structure of β-lactoglobulins. Physiol. Chem. 358:1691-1692.

43. Genvrain, S. A. 1970. Improvements in the process for extracting protein from whey. British patent 1,190,448.

44. Genvrain, S. A. 1971. Process for the continuous extraction of soluble proteins from lactoserum. British patent 1,243,988.

45. Gillies, M. T. 1974. "Whey Processing and Utilization." Economic and Technical Aspects, NDC Corporation. Park Ridge, New Jersey.

46. Glazachev, V. V. 1960. Production of cultured dairy products. Dairy Sci. Abstr. 24:2715 (1962).

47. Gordon, C. H., Lynch, G. P., and McDonough, F. E. 1972. Feeding liquid whey to dairy animals. P. 78 in Proc. Whey Prod. Conf. ERRL. Publ. No. 3779.

48. Gordon, W. G., and Kalant, E. B. 1974. Proteins of milk in "Fundamentals of Dairy Chemistry." Webb, Johnson and Alford ed. The AVI Publishing Company, Inc., Westport, CT, p. 87.

50. Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1967. Denaturation of cottage cheese whey protein by heat. J. Dairy Sci. 50:828.

51. Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1968. Citrus flavored beverages made from cheese wheys and soy flours. J. Dairy Sci. 51:932.

52. Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1969. Spray-dried cheese whey-soy flour mixture. J. Dairy Sci. 52:432.

53. Guy, E. J., Vettel, H. E., and Pallansch, M. J. 1974. Effect of cheese whey protein concentrate on the baking quality and rheological characteristics of sponge doughs made from hard red spring wheat flour. Cereal Sci. Today 19:551.

54. Harland, H. A., and Ashworth, U. S. 1945. The preparation and effect of heat treatment on the whey protein of milk. J. Dairy Sci. 28:879.

55. Henderson, H. E., Crickenberger, R. G., and Reddy, C. A. 1974. Effect of fermented ammoniated condensed whey on dry matter consumption. J. Dairy Sci. 57:635.

56. Henderson, H. E., Reddy, C. A., and Crickenberger, R. G. 1974. Fermented ammoniated condensed whey (FACW) as a protein source. J. An. Sci. 39:240.

57. Hidalgo, J. and Gamper, E. 1977. Solubility and heat-stability of whey protein concentrate. J. Dairy Sci. 60:1515.

58. Hillier, R. M. 1976. The quantitative measurements of whey proteins using polyacrylamide gel elecrophoresis. J. Dairy Res. 43:259.

59. Hintz, H. F., Schryer, H. F., and Lowe, J. E. 1971. Comparison of a blend of milk products on linseed meal as protein supplements for young growing horses. J. An. Sci. 33:1274.

60. Holsinger, V. H., Posatl, L. P. and DeVilbiss, E. D. 1974. Whey beverages: A review. J. Dairy Sci. 57:849.

61. Huber, J. T., Boman, R. L., and Henderson, H. E. 1974. Value of fermented-ammoniated whey as a nitrogen supplement for lactating cows. J. Dairy Sci. 57:635.

62. Huber, J. T., Boman, R. L., Henderson, H. E. and Kulasek, G. W. 1975. Fermented-ammoniated whey as a nitrogen supplement of lactating cows. J. Dairy Sci. 58:748.

63. Huber, J. T., Polan, C. E., and Rosser, R. A. 1967. Effect of whey on milk composition and rumen volatile fatty acids in restricted-roughage rations. J. Dairy Sci. 50:687.

64. Jelen, P. 1974. New uses for the heat-acid whey fraction products. Proceedings 3rd whey products conference, Chicago, USDA, Philadelphia, PA.

65. Jelen, P. 1975. Use of coagulated lactalbumin from cheese whey in ground meats. J. Food Sci. 40:1072.

66. Jelen, P. and McIntyre, D. 1977. Sensory evaluation of meat products containing coagulated cheese whey lactalbumin. J. Food Sci. 42:281.

67. Keay, J. 1971. Whey powder. Food Mfg. 46(11):36.

68. Koehler, A. E., and Allen, S. E. 1934. The nutritive value of lactose in man. J. Nutr. 8:377.

69. Kosikowski, F. V. 1969. Nutritional beverages from acid whey powder. J. Dairy Sci. 51:1299.

70. Kraft, J. H. 1972. Food composition prepared from whey and comminuted sesame: U. S. Patent 3,669,678.

71. Laessing, H. 1916. Non-alcoholic concentrated beverage and process for its manufacture. U. S. Patent 1,119,440.

72. Lang, F. and Lang, A. 1967. New yogurt flavors and UHT produce in Switzerland. Milk Ind. 61(3):26.

73. Lang, F. and Lang, A. 1969. More advances in the manufacture of new milk-based food product. Milk Ind. 64(6):64.

74. Laonipon, R. and Cardwell, J. T. 1971. Development of a nutritious drink made from fresh whey. J. Dairy Sci. 54:450.

75. Larson, B. L. and Rolleri, G. D. 1955. Heat denaturation of the specific serum protein in milk. J. Dairy Sci. 38:351.

76. Loewenstein, M., and Paulraj, V. K. 1972. Preparation and growth producing evaluation of a concentrated coprecipitate of soy cheese whey protein. Food Prod. Develop. 5(8):56.

77. Loewenstein, M., Reddy, M. B., White, C. A., Speck, S. J. and Lunsford, T. A. 1975. Using cottage cheese whey fractions or their derivatives in ice cream. Food Prod. Develop. 11:91.

78. Lough, H. W. 1976. Whey supply and utilization. USDA Economic Research Service DS-361, pp. 35-41.

79. Lynch, G. P., McDonough, F. E., Rough, D. K., Smith, D. F. and Gordon, C. H. 1975. Growth and carcass evaluation of Holstein steers fed liquid acid whey. J. Dairy Sci. 58:1688.

80. Maeno, M. 1958. Beverage from whey. Japanese patent 3787. Chem. Abstr. 53:5586h (1959).

81. Mann, E. J. 1972. Whey beverages. Dairy Ind. 37(3):153.

82. Mann, E. J. 1974. Whey Utilization - Part I. Dairy Industries. 39(8):303.

83. Mann, E. J. 1974. Whey Utilization - Part 2. Dairy Industries. 39(2):343.

84. Marshall, P. G., Dunkley, W. L., and Lowe, E. 1968. Fractionation and concentration of whey by reverse osmosis. Food Technol. 22:969.

85. Mauroy, M. 1960. Soft drinks prepared from whey. French patent 1,224,651; Chem. Abstr. 55:19065b (1961).

86. McCormick, R. D. 1973. Younger consumer is target for beverage marketers. Food Prod. Develop. 7(3):17.

87. McCullough, M. E., Neville, W. E., Jr., and Monson, W. J. 1972. Ammoniated whey as ingredient in complete livestock rations. Feedstuffs 17(50):27.

88. Modler, H. W. and Emmons, D. B. 1976. Properties of whey protein concentrate prepared by heating under acidic conditions. J. Dairy Sci. 60:177.

89. Morr, C. V. 1976. Whey protein concentrate: an update. Food Technol. 14:18.

90. Murray, M. 1968. Liquid food product and process for preparing same. U. S. patent 3,419,398.

91. Nelson, F. E. and Brown, W. C. 1969. Whey utilization in fruit juice drinks. J. Dairy Sci. 52:900.

92. Nelson, F. E. and Brown, W. C. 1971. Whey as a component of fruit flavored drinks. J. Dairy Sci. 54:758.

93. Nielson, V. H. 1970. Pollution problems facing cottage cheese makers. Amer. Dairy Rev. 32(8):30.

94. Nielson, M. A. and Coulter, S. T. 1973. Comparison of some functional properties of undenatured and denatured cottage whey protein. J. Dairy Sci. 56:76.

95. Panzer, C. C., Schoppet, E. F., Sinnamon, H. I., and Aceto, N. C. 1976. Continuous coagulation of cottage cheese whey. J. Food Sci. 41:1293.

96. Polis, B. D., Shmukler, H. W., and Custer, J. H. 1950. Isolation of a crystalline albumin from milk. J. Biol. Chem. 187, 349-354.

97. Price, W. V., Bohstedt, G., and Rupel, J. W. 1944. Whey for livestock. Univ. WI. Circular 340.

98. Riggs, L. K. and Beaty, A. 1947. Some unique properties of lactose as a dietary carbohydrate. J. Dairy Sci. 39:939.

99. Roeder, G. 1939. Improved process for manufacturing fermented beverages. British patent 500,912; Dairy Sci. Abstr. 1:230.

100. Roeder, G. 1959. Method for the utilization of whey for human nutrition. German patent 1,046,459; Dairy Sci. Abstr. 22:2473 (1960).

101. Romanskaya, N. N. and Kalmysh, U. S. 1971. Beverage from whey. USSR patent 322,173; Chem. Abstr. 76:71306j (1972).

102. Rosser, R. A., Polan, G. E., Chandler, P. T. and Bibb, T. L. 1971. Effects of whey components and methionine analog on bovine milk fat production. J. Dairy Sci. 54:1807.

103. Rowland, S. J. 1938. The precipitation of the proteins in milk. 1. casein, 2. total proteins, 3. globulin, 4. albumin, and proteose-peptone. J. Dairy Res. 9:30.

104. Rzewuska-Rutte, J. 1967. Utilization of whey in the soft-drink industry. Chem. Abstr. 67:81288h.

105. Saal, H. 1976. Kraft seeks improved uses for whey and finds new applications. American Dairy Rev. 9:14.

106. Saski, R., and Tsugo, T. 1953. The manufacture of synthetic milk powder from whey and soybean. II. The manufacture and nutritive value of synthetic milk powder. Proc 13th Int. Dairy Congr. 4:606.

107. Schingo, D. J. 1976. Whey utilization in animal feeding. A summary and evaluation. J. Dairy Sci. 59:556.

108. Schoppet, E. F., Sinnamon, H. I., Talley, F. B., Panzer, C. C. and Aceto, N. C. 1976. Enrichment of pasta with cottage cheese whey proteins. J. Food Sci. 41:1297.

109. Schulz, M. E. and Drache, K. D. 1947. Fruit whey beverage. Milchwissenchaft 2:276.

110. Smith, G. C., Hyunil, J., Carpenter, Z. L., Mattil, K. F., and Carter, C. M. 1973. Efficiency of protein additives as emulsion stabilizers in frankfurters. J. Food Sci. 38:849.

111. Sommer, H. H. 1951. "Theory and Practice of Ice Cream Making." Madison, Wisconsin.

112. Stauffer Chemical Company. 1971. U. S. Patent no. 3,269,843.

113. Steel, R. G. D., and Torrie, J. H. 1960. Principles and procedures of statistics. McGraw Hill Publishing Company, New York, N.Y.

114. Susli, H. 1956. New type of whey utilization: A lactomineral table beverage. Proc. 14th Int. Dairy Congr. 1(pt.2):477.

115. Towned, R. and Gyuricsek, D. M. 1974. Heat denaturation of whey and model protein systems. J. Dairy Sci. 57:1152.

116. True, L. C., and Ousley, T. J. 1970. Dairy waste pollutant or profit? Mississippi Farm Res. 33(11):1.

117. Vajdi, M. and Pereira, R. R. and 1973. The feasibility of whey utilization for the production of various drinks. Mod. Dairy 52(3):14.

118. Volpe, T. and Zabik, M. E. 1975. A whey protein contributing to loaf volume depression. Cereal Chem. 52:188.

119. Webb, B. H. 1972. Recycling whey for profitable uses. Amer. Dairy Rev. 34(6):32A.

120. Weber, K. and Osborn, M. 1975. Protein and sodium dodecyl sulfate: Molecular weight determination on polyacryl amide gels and related procedures. In "Proteins." Neur and Hill ed. Academic Press, New York, N.Y., p. 179.

121. Welch, J. G. and Nilson, K. M. 1973. Feeding liquid whey to dairy cattle. J. Dairy Sci. 56:681.

122. Welch, J. G., Nilson, K. M., and Smith, A. M. 1974. Acceptability of whey concentrate mixture for dairy cows. J. Dairy Sci. 57:634.

123. Whitney, R. McL. 1977. Milk proteins in "Food Colloids." Graham, ed. The AVI Publishing Co., Inc., Westport, CT. Chap. 2, p. 66.

124. Whittier, E. O. and Webb, B. H. 1950. "By-products from milk." Reinhold Publishing Corp., New York.

125. Wingerd, W. H. 1971. Lactalbumin as a food ingredient. Dairy Sci. 54:1234.

126. Wingerd, W. H., Saperstein, S., and Lutwak, L. 1970. Bland, soluble, whey protein concentrate, has excellent nutritional properties. Food Technol. 24:758.

127. Womack, M., and Vaughan, D. A. 1972. Whey and whey products as cereal supplements. J. Dairy Sci. 55:1081.

128. World Health Organization. 1965. Protein requirements. Technical Reports Series No. 301. World Health Organization, Geneva.

129. World Health Organization. 1976. Health laws and regulation. Canada International Digest of Health Legislation 27(2):267-293. Geneva, Switzerland. Dairy Sci. Abstr. 39(9):583. 1977.

130. Yoo, B. W., and Mattrich, J. F. 1969. Utilization of acid and sweet cheese whey in wine production. J. Dairy Sci. 52:900.

131. Zadra, L. 1954. Carbonated alcoholic beverage from milk whey. Italian patent 502,920; Chem. Abstr. 51:7646i (1957).

132. Zadra, L. 1955. Alcoholic beverage from whey. Italian patent 535,705; Chem. Abstr. 53:1628i (1959).

VITA

Firyal Basheer Al-Dabbagh was born July 1, 1948, in Baghdad, Iraq. She attended Al-Hareery High School and graduated in June, 1964. She attended the University of Mosul and was graduated with the degree of Bachelor of Science in Chemistry in June, 1968.

In September, 1974, she entered the Graduate School at the University of Tennessee, Knoxville, to study for the degree of Master of Science in Food Technology and Science. She received the Master of Science with a major in Food Technology and Science in December, 1976.

From December, 1976, to the present she has been continuing her education toward the degree of Doctor of Philosophy in Animal Science at the University of Tennessee.

Mrs. Al-Dabbagh has been a member of the Institute of Food Technologists since February, 1975.