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UTILIZATION OF WHEY PROTEINS

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The ever increasing imbalance between world food protein supply and demand has caused the expenditure of greater effort in the evaluation of alternative protein sources. These efforts have focused primarily on the incorporation of vegetable or single-cell proteins into traditional protein foods. While much progress has resulted from these efforts, a source of highly nutritious animal protein, cheese whey, remains largely underutilized. The 30 billion pounds of whey produced annually by the cheese industry in the United States (U.S. Dept. of Agriculture 1974) contain nearly 200 million pounds of protein, of which little more than half are currently utilized in feed and food formulations. It is imperative that this valuable protein be recovered and returned to the food supply.

It is not only important that the quantity of protein in the diet be increased, but also that the quality be raised significantly. The traditional food utilized for these purposes has been nonfat dried milk; however, potential demands for milk powder can easily exceed supplies as was recently experienced in the United States. It is therefore logical to look at the composition of the 6.5% total solids contained in fluid whey and to compare it with the composition of nonfat dried milk; these data are presented in Table I (Hargrove et al. 1974). Although the fat, ash and moisture contents of the powders are comparable, whey has only one-third the protein content of milk powder and contains significantly elevated levels of lactose. The removal of the caseins, the major milk proteins, means that whey protein is composed chiefly of β-lactoglobulin and α-lactalbumin, two proteins with unique functional characteristics. Despite these differences, dried whey can serve as a nonfat milk replacer in a wide variety of food applications.

Table I

Composition of Dried Sweet Whey and Nonfat Milk

Nutrient	Approximate Content	
	Whey	Nonfat Milk
Protein	12.9	35.9
Fat	1.1	0.8
Ash	8.0	8.0
Lactose	71.2	52.3
Lactic acid	2.3	-
Water	4.5	3.0

*Agricultural Research Service, U.S. Department of Agriculture.

Whey proteins are of particular importance not only because of their functional properties, but because they possess an excellent balance of essential amino acids (Wingerd 1971; Wingerd et al. 1970). A comparison of the essential amino acid profile of whey protein with the Food and Agricultural Organization (FAO) of United Nations standard profile is presented in Table II (Lambert 1972). The FAO nutritional profile is required for optimal growth in humans and animals. From the data presented, it can be seen that whey protein exceeds the standard requirements in each of the essential amino acids. As expected, the protein efficiency ratio (PER) is quite high, ranging between 3.0 and 3.4 depending on processing conditions. Whey protein fortification has therefore been recognized as an excellent means of raising the PER of a variety of cereals. The increased nutritive value of cereal proteins fortified with 3.5% whey protein is shown in Fig. 1 (Hutton 1974). The ability of whey proteins to raise the PER of soy protein is also recognized (Hutton 1974).

Table II

Comparison of Essential Amino Acid Content of Whey Protein, Casein and the FAO Standard

Amino Acid	Grams per 100 g. Protein		
	FAO Standard	Whey Protein	Casein
Methionine	4.2	4.3	3.4
Leucine	9.0	15.5	16.4
Lysine	4.2	8.2	8.2
Phenylalanine	2.8	4.0	5.5
Threonine	2.8	5.5	4.5
Valine	4.2	5.5	7.3
Tyrosine	2.8	3.7	6.2
Tryptophan	1.4	2.5	1.4

The most readily available form of whey, of course, is in the liquid state. However, since whey is over 93% water, utilization of the 1% protein and other nutrients contained therein is dependent on water removal. This is accomplished by precondensing the whey to increase the total solids level prior to spray or roller drying.

The dried whey solids, as shown earlier, contain over 70% lactose; therefore, although much dried whey is utilized in food applications, protein fortification requires use of whey fractions or concentrates with substantially higher protein contents.

Technology is currently available for the recovery of whey proteins in a variety of product forms ranging from spray dried whey (10% protein) to dried whey protein concentrates containing over 80% protein. Fractionation methods used to separate the whey proteins from low molecular weight components, principally lactose and salts, range from simple precipitation procedures to the use of sophisticated gel permeation, ultrafiltration and electrodialysis techniques. The whey proteins recovered in these processes vary in functional properties which are primarily related to the degree of denaturation incurred in processing.

This can vary between 5 and 100 percent denaturation. Representative applications for whey protein concentrates of varying functionality will be presented later.

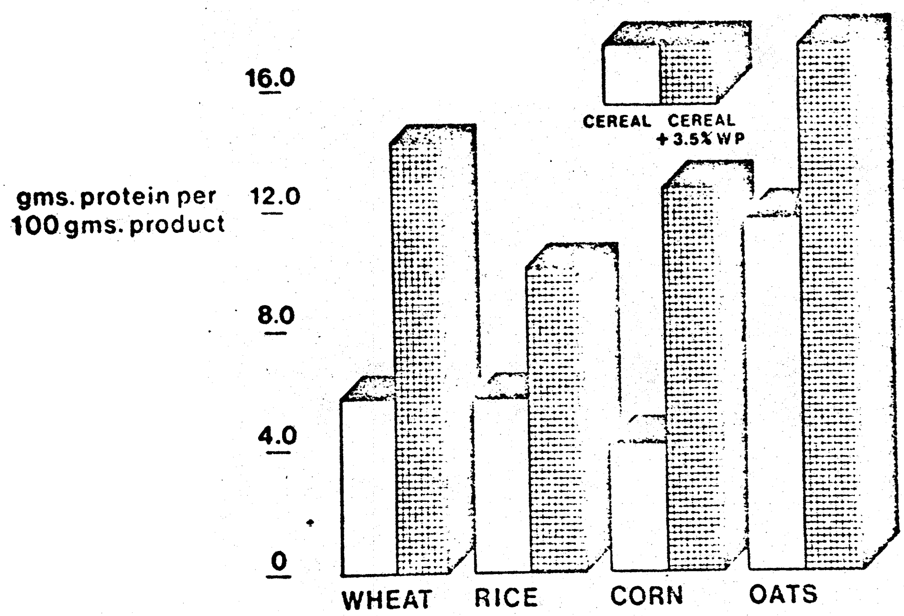

Fig. 1. Effect on Balanced Protein (FAO) of Supplementing Cereals with 3.5% Whey Protein

Probably the simplest technique for whey protein isolation is that of heat coagulation. Although this process is an old one, it has recently been reinvestigated at our Center by Sinnamon (1974). When whey is held at a high enough temperature for a sufficient period of time, whey proteins will coagulate, with the amount of denaturation dependent on the time-temperature-pH relationship. At best, up to 80% of the crude protein can be precipitated. Temperatures and times that have been used in the past ranged from 185°-212° F with holding times of 15 minutes or more. Sinnamon (1974) studied the relationships between pH, temperature and holding times. These results are shown in Fig. 2 and 3. Figure 2 illustrates that at 250° F, a maximum of 60% of the protein is coagulated in the pH range 5.5-6.5, with optimum coagulation predicted to occur at pH 6.0. The effect of holding times is shown in Fig. 3, from which can be seen that the amount of protein coagulable is independent of holding times in the range of 4-16 minutes. Precise control of conditions is not required; the whole process is quite simple. The composition of typical products is shown in Table III. Reduction of the ash content by solubilization of the calcium salts can be obtained by adjusting the whey slurry to pH 4.6 prior to isolation of the protein. This simple technique can be applied to acid whey to yield concentrates containing 60-85% protein. The protein obtained in this manner is completely denatured, a functional property required for specified food uses to be discussed later.

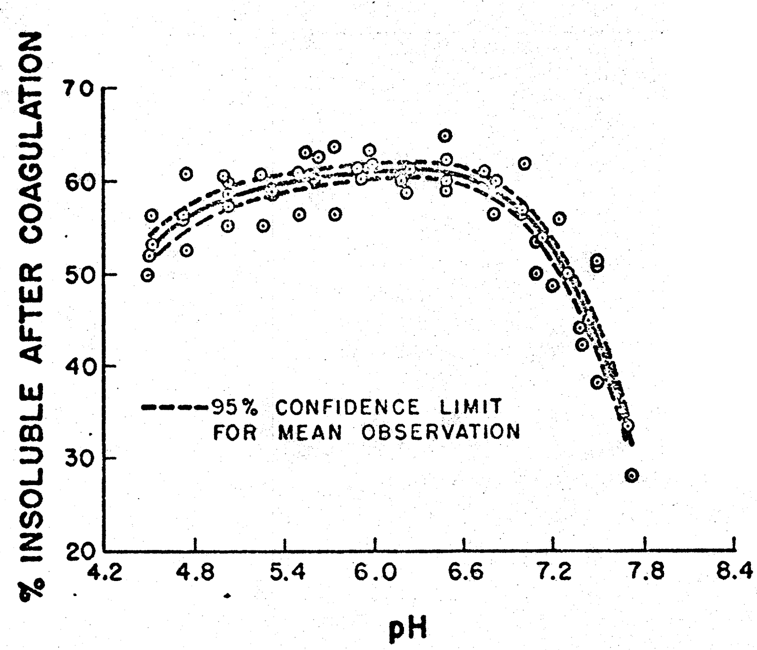

Fig. 2. Effect of pH on the Amount of Protein Precipitated After Holding at 250° F for 15 Minutes.

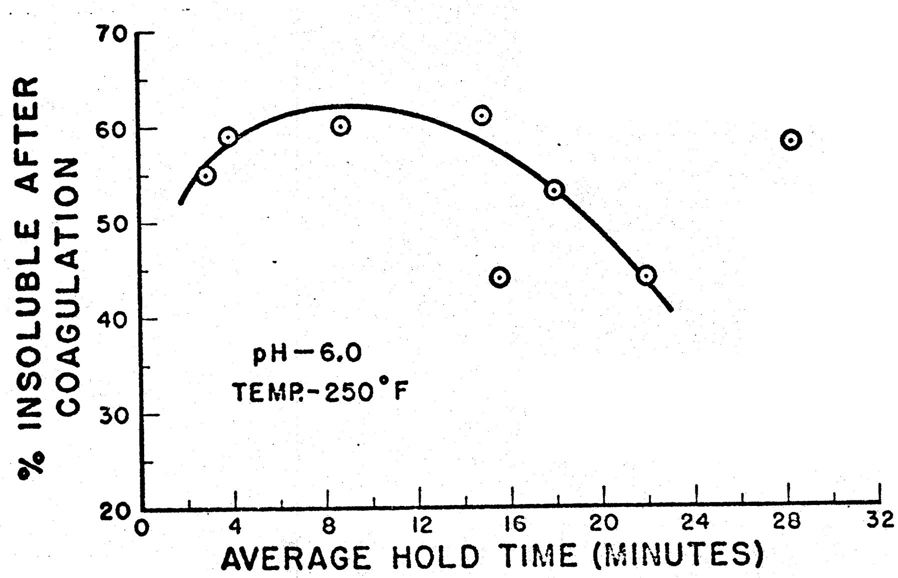

Fig. 3. Effect of Holding Time in the Amount of Protein Precipitated from Cottage Cheese Whey at 250° F and pH 6.0

Table III

Composition of Heat Coagulated Whey Protein Fractions

Type	Coag pH	% Protein (MFB)	% Ash (MFB)	% Lactose (MFB)
Conventional - 95° C	6.0	65-70	20-25	10
High temperature - 120° C	6.0	65-70	20-25	10
High temperature + ash resolubilization*	6.0	>85	<5	10

*pH adjusted to 4.6 prior to separation of proteins.

Another process developed in our laboratories is that of Holsinger et al. (1975), which combines aspects of three basic methods. These consist of polyphosphate precipitation, ultrafiltraion and gel permeation. Although each of these methods can be used to prepare whey concentrates, none yields fractions of the desired protein level (80-90%) when used singly. Accordingly, the protein in acid whey was preconcentrated either by polyphosphate precipitation or by ultrafiltration and then subjected to gel permeation. A flow diagram of the selective precipitation - gel permeation technique is shown in Fig. 4. Briefly, acid whey is forewarmed to 110° F and acidified to pH 2.5 with hydrochloric acid; precipitation is then accomplished by the addition of 0.5% sodium hexametaphosphate. The precipitate is collected by centrifugation. A tenfold concentration of whey protein is obtained in this manner. The precipitate is redissolved at pH 6.8 and applied to a G-25 Sephadex column. The protein elution diagram from the gel column is shown in Fig. 5. The high protein eluate was condensed and dried using conventional dairy plant equipment.

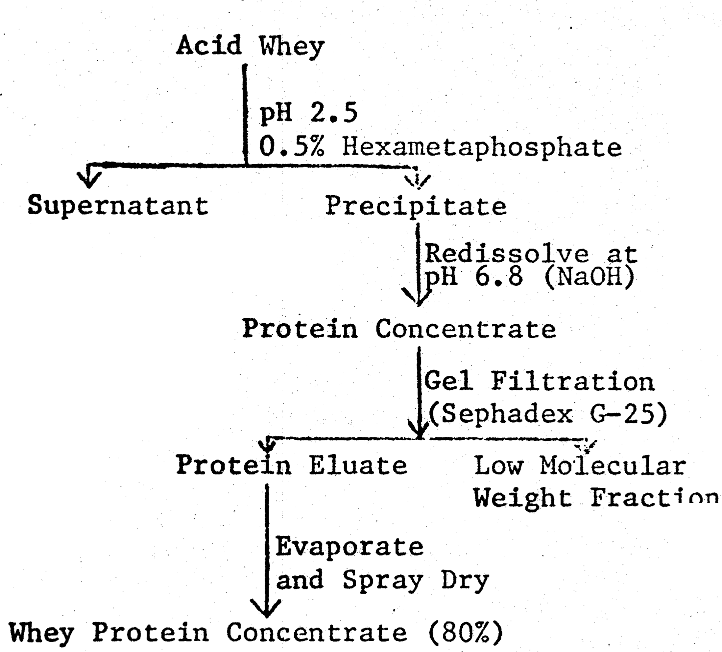

Fig. 4. Flow Diagram for Preparation of Whey Protein Concentrate by Phosphate Precipitation and Gel Filtration

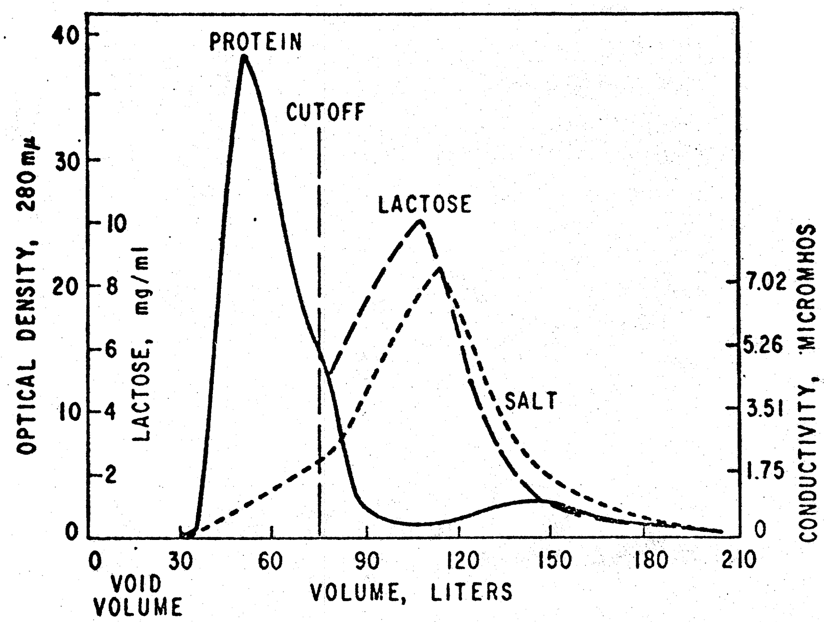

Fig. 5. Elution Diagram of a Whey Protein Concentrate from Sephadex G-25 Column

Preconcentration of whey proteins by ultrafiltration requires thorough whey clarification to remove fat and residual casein fines which, if not removed, would result in clogging of the U.F. membrane. This process removed 80% of the whey solids and 90% of the water. A 30-liter charge of the retentate was applied to the gel column and eluted as in Fig. 5.

The compositions of the protein isolates thus obtained are shown in Table IV. It can be seen that preconcentration with polyphosphate results in a high ash (14%) product and reflects the ability of the whey proteins to bind polyphosphate. The amount of protein insoluble at the isoelectric region of pH 5 is substantially higher in the polyphosphate derived protein. This is attributed to differences in pH at which the concentrates were applied to gel column (polyphosphate at 6.8 and ultrafiltration at 4.7). Previous work (Guy et al. 1967) has demonstrated that minimum protein insolubilization occurs at pH 3.4 and increases with increasing pH. This denaturation can be reduced or eliminated by keeping processing temperatures low. Irrespective of the degree of denaturation produced in the various fractionation procedures, these concentrates possess desired functionalities matching specific food requirements. Examples of applications of both dried whey and whey concentrates follow.

In 1973 and 1974, shortages of nonfat dry milk necessitated reductions in quantities available for distribution in international preschool feeding programs. The idea of a beverage that would combine the nutritional benefits of whey and soy served as a starting point for the development of a beverage powder to replace nonfat dry milk in U.S. Food-for-Peace (Title II) programs. Through the cooperative efforts of many government agencies, the dairy industry, nutrition researchers, and private relief organizations, a whey-soy beverage has been developed and is currently in procurement for distribution abroad (Holsinger et al. 1973;

Holsinger et al. 1974). A whey-soy product developed earlier in our laboratories (Guy et al. 1969) served as a base for further nutritional modifications. These modifications included elevation of the protein content to 20%, increased caloric density through incorporation of soybean oil, addition of high molecular weight corn syrup solids and incorporation of vitamin and mineral premixes. The ingredient formulation is shown in Table V and the nutrient composition in Table VI. When reconstituted to a level of 15% total solids, the beverage has the nutritional equivalent of milk. Acceptability studies in six countries indicated that between 50 and 72% of the children would accept the product (Rodier et al. 1973). This product is currently being purchased by the U.S.D.A. in amounts of 8-10 million pounds per month, with a projected annual purchase of 120 million pounds.

Table IV

Composition of Whey Protein Concentrates

Component	Phosphate-Gel	UF-Gel
Protein	78.7	90.0
Lactose	2.9	7.6
Fat	-	0.5
Ash	13.7	0.3
Water	1.9	3.0
Insolubles, pH 5.1	34.3	3.5

Table V

Ingredients for Formulation of Whey-Soy Drink Mix

Ingredients	Pounds for 2,000 Pounds of Final Food	
	Using only Full-Fat Soy Flour	Using only Defatted Soy Flour
Sweet-type whey solids	826	826
Soy flour, full-fat or defatted	730	594
Soybean oil, refined, deodorized, partially hydrogenated, stabilized	244	380
Corn syrup solids	182.5	182.5
Mineral premix	15.17	15.17
Vitamin premix	2.33	2.33

Table VI

Composition of Whey-Soy Drink Mix

Protein	20.5
Carbohydrate	50.8
Fat	19.5
Ash	6.0
Moisture	2.6
Fiber	0.6
Calories/100 g.	460

Whey protein concentrates produced by the simple process of heat coagulation are fully denatured, yet this very denaturation is the prime functional property. A popular food that is low in protein nutrition is pasta, which has a PER value of less than 1.0. Pasta can therefore become an excellent vehicle for increasing protein consumption. A successful protein-fortified pasta product should have a protein content of approximately 20%, a PER 95% that of casein, it should be manufactured without modification of the conventional pasta process, and should retain consumer acceptability. Sufficient amounts of whey protein concentrates were added to durum flours to raise the protein content from 13 to 20% and the products evaluated for nutritional and organoleptic qualities. No difficulties were encountered preparing enriched pasta products from protein concentrates containing primarily denatured (insoluble) proteins; however, departure from normal manufacturing processes were required to prepare pasta fortified with undenatured (soluble) proteins. The protein concentrates used and the resultant PER's obtained are shown in Table VII. Texture profiles of control and fortified pastas indicated differences which could not be eliminated by variations in cooking time. Taste tests using a nine point hedonic scale to determine overall flavor-texture differences indicated that the enriched pasta was still an acceptable product. Other foods, such as cereals, also present opportunities for protein fortification using heat-coagulated whey protein concentrates.

Table VII

PER Values of Whey Protein Fractions and Macaroni Products

Dietary Source of Protein	Corrected PER
Casein control	2.50
Plain macaroni	0.70
Heat-coagulated insoluble protein (75%)*	3.05
Macaroni with heat-coagulated protein	2.41**
Industrial experimental soluble protein (70%)	3.05
Macaroni with industrial protein (70%)	2.31
Macaroni with soluble protein (50%)	2.06

*Percentages in parenthesis indicate protein content of concentrates.

**Not significantly different from casein ($p = 0.05$).

Whey protein concentrates containing little denatured protein have functional properties which include good solubility and absorptive capacity, and high surface activity. This type of whey protein concentrate, prepared by the ultrafiltration-gel permeation process, meets the requirements for use in the fortification of highly acid carbonated beverages (Holsinger et al. 1973). As soft drinks replace nutritious beverages such as milk and fruit juices in the diet, "empty" calories replace quality nutrients. One obvious step toward better nutrition is to fortify soft drinks with nutrients without changes in flavor or appearance. Protein fortification at the 1% level with full solubility at the pH of soft drinks (2.5-3.5) requires the use of undenatured protein. Thus, technology is available to prepare and fortify soft drinks and improve the protein intake of our young people.

The fractionation processes discussed are representative elements of a number of commercial separation processes. Similarly, dried whey powder and concentrates ranging from 35-90% protein are currently available from commercial sources. While these products are finding markets in a variety of food uses, further utilization is needed to eliminate waste of valuable nutrients and reduce environmental pollution. Although the whey proteins have good functional properties similar to other proteins, e.g., soy and cottonseed, their outstanding property is the ability to raise the protein quality of a wide variety of food products. It is hoped that in the future, nutritionists and food technologists will reexamine the qualities of whey and whey protein and develop formulation for their incorporation.

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