FORTIFYING SOFT DRINKS WITH CHEESE WHEY PROTEIN

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□ THE SOFT DRINK INDUSTRY can serve as a model of successful merchandising. Although the tally is not yet complete, the soft drink industry expects to have overtaken coffee as the leading beverage next to water in the United States by the end of 1972. As a dubious measure of success, the empty containers from the ubiquitous soft drink are even considered to be environmental pollutants.

UNDER INCREASING PRESSURE

Because carbonated beverages consist primarily of water, sugar, flavoring, and carbon dioxide, many nutritionists consider them to be dietary pollutants, as well.

Actually, soft drinks provide only about 4.3% of the caloric requirement of the population of the United States and therefore should be of little national concern. However, their appeal to the young is strong and as more nutritious beverages such as milk and fruit juice are replaced in the diets of children and teenagers, nourishing materials such as calcium and protein are replaced by "empty" calories.

As a result, soft drink companies are under increasing pressure from consumer groups to improve the nutritional quality of their products (Nader, 1972). An obvious step toward better nutrition would be the fortification of soft drinks with valuable nutrients without detectable change in flavor or appearance.

Proteins isolated from cheese whey have unique functional properties which make them suitable for the fortification of carbonated beverages. Since acid cheese whey, a by-product of cottage cheese manufacture, is now being wasted and is producing serious pollution in some areas, a fortification program of this type could yield benefits to both soft drink consumers and cheese manufacturers.

FORTIFICATION INEXPENSIVE

Soft drinks consumed in the United States amount to an estimated 380 8-oz bottles per capita per annum, producing nearly $5 billion for the soft drink industry (Anonymous, 1971).

Statistics calculated from data compiled by the USDA's Statistical Reporting Service (1971) show the estimated amount of recoverable protein in the cottage cheese whey wasted annually in the United States to be about 43 million lb. Ultimately, this protein may sell for over $1.00/lb (Anonymous, 1972). If so, whey protein might represent a sizeable potential income to the protein processors.

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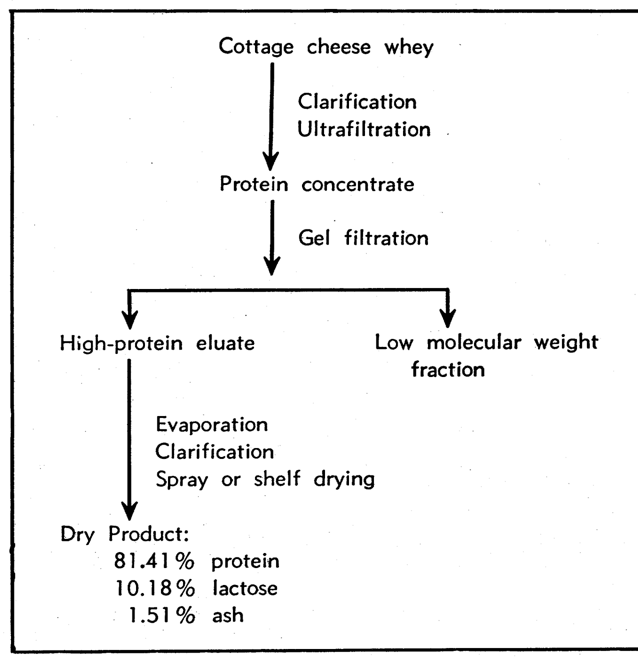

Fig. 1—**ISOLATION TECHNIQUE** *used by USDA's Dairy Products Laboratory to isolate undenatured proteins from cottage cheese whey*

When the volume of soft drinks manufactured in the United States is considered along with the potential amount of acid whey protein available and its projected price, calculations show that 12% of the total soft drink production could be fortified with 1% protein by weight at an added materials cost of about ¾ of a cent per 8-oz bottle. Fortification at this level would significantly increase the nutritive value of soft drinks.

PROTEINS ISOLATED & DRIED

The method we routinely use to isolate undenatured proteins from cottage cheese whey is schematically presented in Figure 1. Ultrafiltration and gel permeation, in sequence, remove lactose and salts from the whey protein. The resulting protein solution is condensed and dried using conventional techniques. Centrifugal clarifiers are used to remove insoluble material formed during the purification steps.

Protein loss entailed by this procedure is relatively high, but the quality of the dehydrated end product is excellent. Typical compositional data are shown at the bottom of the diagram. All studies were carried out with the dried product.

Whey proteins are an especially rich source of essential amino acids, particularly lysine. Table 1 shows the amino acid profile of the dehydrated product manufactured as described above. The amount of lysine shows that the processing steps required to produce the whey protein concentration do not damage the lysine to any great extent. Structurally, lysine is sensitive to heat damage in the presence of reducing sugars and is destroyed if overheated during product manufacture.

Table 1—**AMINO ACID PROFILE** *of a dehydrated high-protein isolate from acid cheese whey*[a]

Amino acid	g amino acid/100 g protein
Lysine	11.20
Histidine	2.05
Arginine	3.01
Aspartic acid	12.30
Threonine	6.18
Serine	4.76
Glutamic acid	20.41
Proline	5.18
Glycine	1.92
Alanine	6.11
Cystine[b]	2.51
Valine	6.42
Methionine	2.64
Isoleucine	6.92
Leucine	14.01
Tyrosine	3.50
Phenylalanine	3.82

[a] Amino acid analyses performed on acid hydrolysates on a Beckman Model 120-C Amino Acid Analyzer by the method of Spackman et al. (1958)
[b] Cystine was determined as cysteic acid after performic acid oxidation followed by acid hydrolysis according to the method of Moore (1963)

SAMPLES PREPARED & TASTED

Dehydrated whey protein concentrate was added to carbonated beverages using the formulations shown in Table 2. Since most of our experience is with dairy products, we used formulations and materials drawn from the National Soft Drink Association (1967). Solid carbon dioxide was used for carbonation.

Beverages fortified with 1% whey protein maintained excellent clarity and color during one year of storage at room temperature on shelves and in glass-fronted cabinets in the laboratory. When compared with a freshly made control, the color of the lime-flavored beverage had faded slightly, but the flavor remained unchanged after 203 days of storage. After one year, a slight stale whey flavor was noted in the fortified product.

Carefully prepared spray-dried protein concentrates also can be used to fortify the powders that are reconstituted with water to produce the popular "ade"-type beverages. Seven different flavors of these products were fortified with 0.5% and 1.0% whey protein.

Table 2—**COMPOSITION** *of protein-fortified carbonated soft drinks*

Ingredient	Composition %			
	Strawberry	Orange	Lemon	Lime
Sucrose	12.0	14.0	13.0	13.0
Flavoring	0.37	0.37	0.37	0.37
Citric acid	0.37	0.185	0.74	0.74
Protein	1.0	1.0	1.0	1.0
Water	86.26	84.44	84.89	84.89
Carbon dioxide volumes	2	1	1	1
pH before carbonation	2.50	2.66	2.35	2.46

The fortified beverages along with unfortified controls were then submitted, one flavor per panel, to 10-man taste panels of experienced dairy product judges selected for sensory acuity (Liming, 1966). The judges were asked to rate acceptance on the basis of a nine-point hedonic scale (Peryam and Pilgrim, 1957); the average scores for each flavor are shown in Table 3.

Table 3—**ORGANOLEPTIC EVALUATION** *of protein-fortified non-carbonated soft drinks*

Flavor	Hedonic ranking[a]		
	Control	0.5% Protein	1.0% Protein
Cherry	7.0	6.5	5.5
Grape	7.2	6.8	6.3
Tart lemon	5.7	5.2	5.5
Lemon-lime	7.2	6.5	6.2
Orange	6.2	5.9	6.0
Raspberry	6.3	6.7	6.4
Strawberry	6.5	5.9	6.0

[a] On 9-point hedonic scale; the higher the number, the better the flavor

The scores were then examined for significant differences by analysis of variance as described by Larmond (1970). At the 5% confidence level, only the scores of the cherry and lemon-lime flavored samples fortified with 1% whey protein deviated significantly from their controls.

Although these results indicate that fortification with 1% whey protein is not readily detectable in some instances, even by experienced judges, it may be difficult to produce soft drinks with protein levels approximating that of milk.

PROTEIN BEHAVIOR STUDIED

Very little information has been published about the solubility and stability of mixed whey proteins at the low values of pH that characterize most carbonated beverages. Guy et al. (1967) studied the denaturation of cottage cheese whey proteins by heat but did not investigate protein solutions of pH below 3.4.

While empirical studies indicate that soft drink fortification is possible, more fundamental data are needed to make commercialization feasible. We therefore studied the solubility and stability of whey proteins under acid conditions to provide some of the additional data required.

• **Protein Solubility.** Variation in the solubility of our whey protein concentrate with pH is shown in Figure 2. Also shown is the change in turbidity over the same pH range. The decrease in solubility is accompanied by a sharp increase in turbidity in the region of the proteins' isoelectric points. Somewhat unexpectedly, the protein showed complete solubility in the pH range 2–3.5 that is typical of carbonated beverages, with a clear solution resulting. This explains the clarity of the fortified beverages.

The data shown in Figure 3 indicate that beverage clarity could be maintained over a long period of time. The whey protein concentrates are most resistant to thermal denaturation at pH 3.5. As the pH range of most commercial carbonated beverages is near this region, these data illustrate that solubility changes on storage should be of little concern.

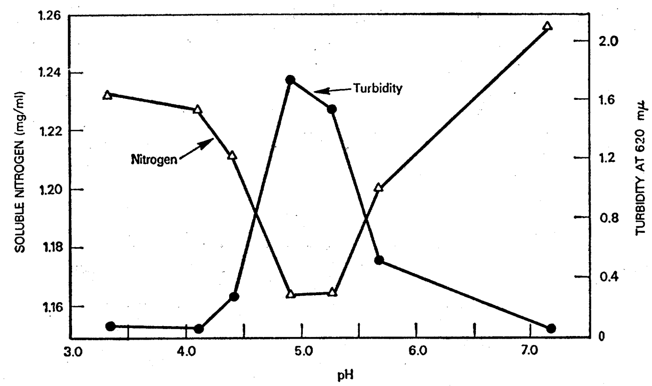

Fig. 2—**CHANGE IN SOLUBILITY** *and turbidity of a high-protein isolate from acid cheese whey with pH*

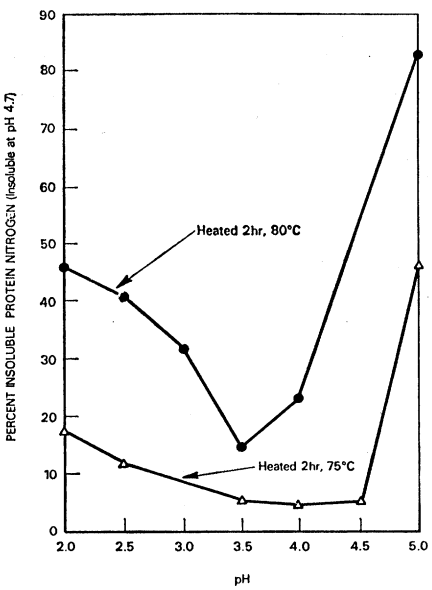

Fig. 3—**EFFECT OF ACID pH** *on the heat denaturation of a 1% whey protein solution*

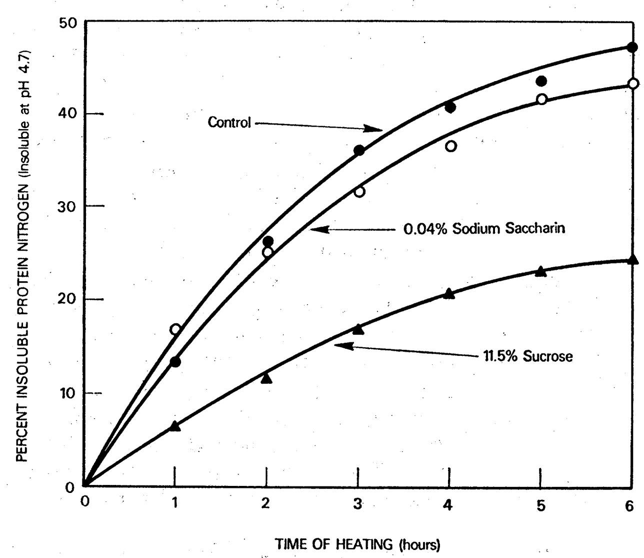

Fig. 4—**EFFECT OF ADDED SUCROSE** *and sodium saccharin on protein stability at 80°C and pH 3.3 when acidified with citric acid*

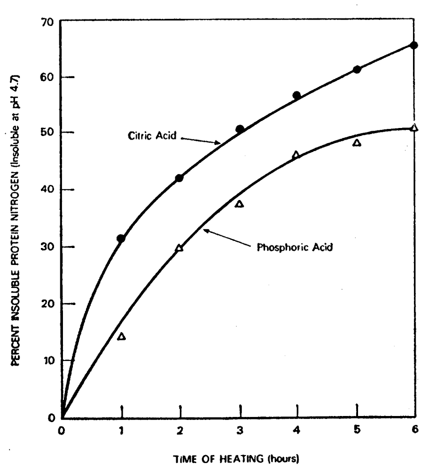

Fig. 5—**EFFECT OF TYPE OF ACID** *on protein stability at 80°C and pH 2.68*

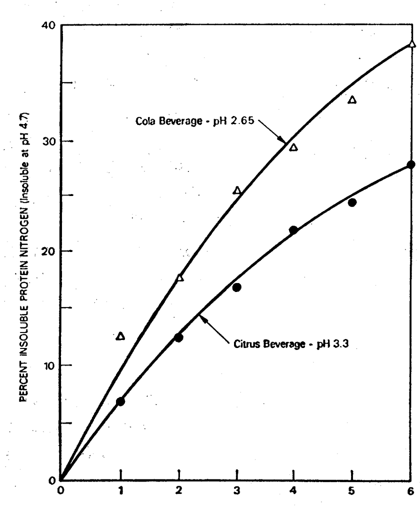

Fig. 6—**PROTEIN STABILITY** *in commercial soft drinks heated at 80°C*

Table 4—**CHANGES OCCURRING** *during room temperature storage for one year of a carbonated lime-flavored beverage containing 1% protein at pH 2.5*

Change	Percentage
Total protein insoluble at pH 4.7[a]	9.1
Total lysine lost	2.9
Sucrose inverted[b]	83.9

[a] All nitrogen determinations were carried out by the micro-Kjeldahl procedure of the AOAC (1970). Percent nitrogen was converted to percent protein by use of the factor of 6.38; non-protein nitrogen was determined on the supernatant after precipitation of the protein by use of 12% trichloroacetic acid

[b] The determination of percent invert sugar was carried out by a modification of the Folin-Wu procedure for blood glucose (Bausch and Lomb Optical Co., 1955)

Some of the chemical changes which had occurred in the fortified lime-flavored carbonated beverage at the time of tasting are shown in Table 4. Although 9% of the protein had become insolubilized, it precipitated only when the pH of the beverage was raised from 2.5 to 4.7. When bottles were opened for tasting, no sediment was apparent in the beverage. In spite of the presence of the reducing sugar formed by the inversion of sucrose, only 3% of the lysine had been destroyed in more than one year of storage.

• **Protein Stability.** Not only does this unusual resistance to thermal denaturation of the whey proteins at the acid pH of most soft drinks tend to favor fortification, but some of the soft drink ingredients themselves tend to stabilize the proteins that are added.

The effects of two popular sweeteners in soft drinks on the thermal stability of whey proteins at pH 3.3 are shown in Figure 4. After solutions containing 1% protein were heated for 6 hr at 80°C, the sucrose-containing solution had only half the amount of denatured protein found in the unsweetened control. Sodium saccharin, however, when added at the highest level permitted by FDA regulations (FDA, 1972), conferred very little protection against heat denaturation.

The type of acid used in the soft drinks also can influence the whey protein stability. Figure 5 demonstrates that the rate of denaturation of a 1% whey protein solution acidified with phosphoric acid to pH 2.68 is lower than that noted in solutions brought to the same pH with citric acid.

Figure 6 shows the stability of whey proteins in two popular commercial soft drinks when heated at the 1% level at 80°C. The whey proteins are less stable in the cola beverage of pH 2.65, which is lower than the point of greatest stability (pH 3.5). The pH of the citrus beverage is very close to the point where the proteins are most stable to heat denaturation. The presence of flavorings and colorings affects the protein stability only slightly in these particular products.

FEASIBLE, IF AVAILABLE

These data suggest that most soft drinks could be successfully fortified with cheese whey proteins. The success of such a project, however, depends upon whether cheese whey proteins can be concentrated undenatured at a reasonable price.

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