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CASEIN AND WHEY PROTEIN DETERMINATION

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Present ice cream standards specify that the nonfat milk-solids content of ice cream depends on fat content. Thus, as the fat increases above 10 percent, the nonfat milk-solids can be decreased until the total milk-solids content reaches a minimum of 20 percent (4).

The protein component of the milk-solids-non-fat fraction contains two broad categories of milk proteins: caseins and whey proteins. Quantitatively determining these two types of proteins in processed dairy products, such as ice cream, is a problem both for manufacturers and regulatory agencies.

A total nitrogen determination would not permit their differentiation. Nor could the nitrogen content of either casein or whey proteins be reliably determined separately after their separation, since some whey proteins complex with casein, via sulfhydryl-disulfide bonds, when subjected to heat treatment (2,3,6). Thus, to determine the casein or whey protein content of ice cream, the complex must be broken or some other unique property of the proteins must be utilized.

In seeking to develop a routine method of assaying casein and whey proteins in ice cream, we made several attempts to break the complex they had formed. None of the attempts were successful. We then considered several other schemes of milk protein analysis and decided on the one that appeared to be the simplest and most direct.

The method we selected is based on the finding that among milk proteins, casein accounts for nearly all protein-bound phosphorus (5). We reasoned that if casein could be isolated, whether alone or as a complex with whey protein, the phosphorus content of the iso-

TABLE 1
Percentage Of Casein In Processed Dairy Products

Product	%Casein*
Coffee creamer	2.98
Nonfat dry milk	26.56
Vanilla ice cream	3.51
Chocolate ice cream	2.15
Strawberry ice cream	2.07
Vanilla ice milk	2.98

* Data from Douglas *et al.* (1982).

late could be used to calculate the amount of casein actually present.

To accomplish this, we precipitated all protein with 12 percent trichloroacetic acid (TCA), the only effective one of the precipitating agents tested. The precipitate, which was free of fat and all inorganic material, was redissolved, and aliquots of the solution were analyzed for nitrogen and phosphorus. Total protein content and casein content were calculated, and the total protein minus the casein gave the whey protein content.

Whether the composition of an ice cream complies with federal regulations depends not only on its milk protein content but also on its fat content. Therefore, a method for measuring both contents with a single sample would be ideal.

The fat-free aqueous residue from the Mojonnier fat test (1) was found to be an excellent starting sample for protein determination. Thus we worked out a combined procedure for fat and casein-phosphorus determination in ice cream. Our procedure can be broken down into five basic steps: 1) fat extraction, 2) protein precipitation,

3) total protein determination, 4) phosphorus determination, and 5) calculation of casein.

Fat Extraction

- Weigh well-mixed, thawed ice cream samples (5 to 6 g) into Mojonnier fat extraction flasks and add 0.12-0.13 g of sodium chloride.
- Perform standard Mojonnier fat extractions.

Protein Precipitation

- Transfer the aqueous residues from the fat extractions to 120-ml beakers, warm them in a water bath to 41 degrees C, and bubble nitrogen through the samples for 2 hours to remove alcohol. A manifold can be prepared from glass tubing, Y connectors, rubber tubing, and Pasteur pipettes to accommodate any desired number of samples. Once started, this operation



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can proceed unattended.

- Quantitatively transfer these samples to 100-ml volumetric flasks and make up to volume with deionized water.

- Pipette 10-ml aliquots into 120-ml beakers and add 10 ml of H₂O and 40 ml of 18 percent TCA to each to make a final TCA concentration of 12 percent.

- Mix the samples 5 to 10 minutes.

Total Protein Determination

- Filter the precipitated samples under suction with a glass fiber-type filter disc, smooth side in and folded in quarters for conical filter funnels, or smooth side up for Buchner funnels.

- Wash the precipitate from the beakers and stirring bars onto the filter with 12 percent TCA solution from a squeeze bottle. Wash beakers, stirring

bars, and precipitate several times to remove all water-soluble and inorganic phosphorus esters.

- Remove TCA from the precipitate, beakers, and stirrers by washing them with ethyl ether. Separate the washings from the precipitate by filtration with a very slight suction. Filtration of the ether washings tends to be difficult. It is best started by holding the hose from the vacuum source against the flasks intermittently until flow commences. Thereafter, it usually proceeds without suction. Vigorous suction will puncture the filter, but the flow cannot be started without suction.

- Insert the stems of the funnels into 100-ml volumetric flasks, and dissolve the precipitate with warmed (50 degree C) 0.1 N sodium hydroxide solution, allowing the solutions to drain into the flask. Wash the beakers and stirring utensils with warm alkali and pour the washings into the funnels. Sodium hydroxide solution is best dispensed from a squeeze bottle.

- Cool the protein solution and make it up to 100 ml volume with sodium hydroxide.

- Pipette 25 ml aliquots and analyze them for nitrogen; then, calculate total protein. Samples should be taken immediately and acidified, because under alkaline conditions some nitrogen may be lost on standing.

Phosphorus Determination

- Any of the many available procedures for phosphorus determination may be chosen. We used the colorimetric procedure of Meun & Smith (7).

- Calculate the phosphorus concentration after subtracting the reagent blank, which is treated the same as the sample.

Calculation of Casein

- Using the phosphorus values, calculate the casein content of each sample. The percentage of phosphorus in casein is somewhat variable, with literature values ranging from 0.79 to 0.86 percent. We used a value of 0.85 percent and the following formula to find the percentage of casein:

$$\frac{\text{mg Phosphorus} \times 100}{0.0085} \times \frac{\text{g of sample}}{1000} \times \frac{\text{ml of sample used in phosphorus determination}^*}{10^6}$$

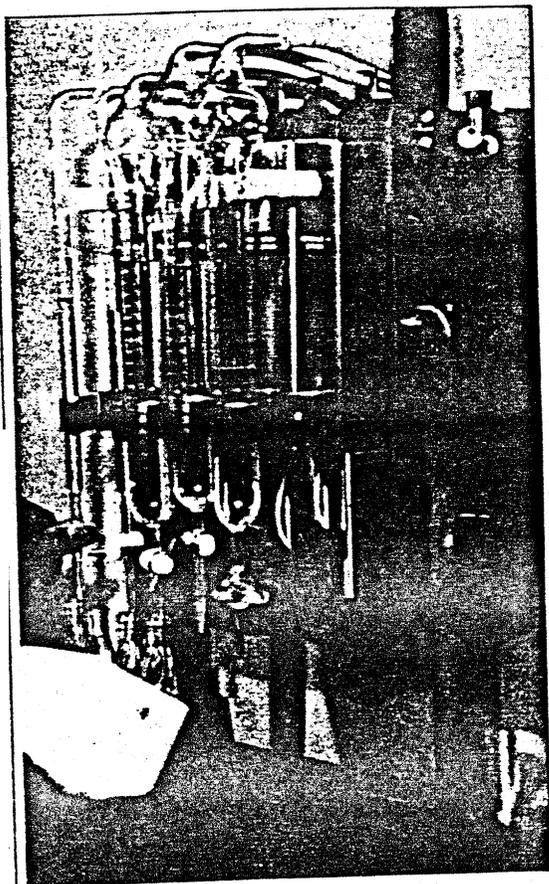
(* In the Meun & Smith procedure this value is the number of milliliters used from the redissolved precipitate for phosphorus determinations.)

Table 1 shows the percentage of casein in various processed dairy products determined by computing the nitrogen-to-phosphorus ratio after analysis of the precipitated proteins. Assuming 0.85 mg phosphorus/g casein, casein comprises 80 percent of the total protein, and protein comprises 36 percent of the milk-solids-non-fat, the values given in the table can be calculated.

The Eastern Regional Research Center's Food Science Laboratory has successfully demonstrated a procedure for determining casein and whey protein in ice cream by measuring its phosphorus-to-nitrogen ratio. This procedure can also be used to demonstrate whether or not ice cream has been adulterated with other proteins. □

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Removal of fat by Mojonnier extraction.