Quantitative Recovery of Carboxymethylcellulose from Milk

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The food stabilizer carboxymethylcellulose (CMC) may be recovered from a tryptic digest of milk by solvent precipitation of the fraction soluble in 12.5% trichloroacetic acid. Free boundary electrophoresis in phosphate buffer, pH 7, revealed the isolated CMC was identical to the original material and was free of protein. The isolation is quantitative, and the amounts may be measured spectrophotometrically. The precision of the determination performed on 10 grams of sample was ±1.7 mg. of CMC (standard deviation). The mean recovery from skim milk, whole milk, and ice cream mix containing 0.05 to 0.4% CMC was 92.7 ± 3.4%. The method cannot be applied directly to chocolate milk because of the interference by chocolate constituents. However, if the results are corrected for this interference, recovered amounts are highly correlated with the expected values.

Polysaccharides with strong hydrophilic properties are accepted in the food industry as stabilizing agents. Interest in the mechanism of stabilization of fluid milk products required the development of a suitable test for determining the distribution of these additives in different milk fractions. A method for the quantitative analysis of sulfated polysaccharides, such as carrageenan, has been reported (Hansen and Whitney, 1960), but the absence of readily identifiable groups in carboxymethylcellulose (CMC) has so far precluded the determination of this substance in milk. Qualitative methods for the detection of common food stabilizers have been reported (Bundesen and Martinek, 1954).

A procedure is presented in this paper for the quantitative determination of CMC in a number of dairy products, including milk and ice cream. The method is based upon the complete isolation of CMC from the milk system followed by a colorimetric determination of the recovered material.

EXPERIMENTAL

Materials. Sodium carboxymethylcellulose (7 HP, Hercules Powder Co., Wilmington, Del.) has a designated substitution range of 0.65 to 0.85. Trypsin solution (2% w./v.) was prepared in water from Difco certified enzyme (1 to 250, Difco Laboratories, Inc., Detroit, Mich.). Dioctyl sodium sulfosuccinate (Complemix-100, American Cyanamid Co., Pearl River, N.Y.), a 0.4% (w./v.) solution was prepared by dissolving the reagent in water at 80° to 90° C. Trichloroacetic acid (25% w./v.) was prepared from the analytical reagent. Absolute ethanol and ethyl acetate were technical grades.

Sample Preparation. Sodium carboxymethylcellulose was dispersed in the samples at room temperature by sprinkling the powder onto the surface of the liquid under rapid agitation. The samples were then homogenized several times with a hand-operated laboratory homogenizer to facilitate complete dispersion.

Electrophoresis. Samples for electrophoresis were dialyzed against sodium phosphate buffer (pH 7.0, ionic strength 0.1) for 24 hours. Electrophoresis was performed at 0° to 2° C. in a Perkin-Elmer Model 38-A instrument at a field strength of 0.4 volt per cm.

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Isolation Procedure. Ten grams of samples are weighed into a 50-ml. centrifuge tube. For hydrolysis of the protein, the pH is adjusted to 8.5 with 4 drops of 1*N* sodium hydroxide, 1 ml. of trypsin solution is added, and the sample is incubated at 40° C. for 3 hours. If the sample contains much fat, such as in ice cream mix, it is necessary to remove the fat at this step by Mojonnier extraction (Milk Industry Foundation, 1959). To ensure a complete dispersion of CMC, 10 ml. of dioctyl sodium sulfosuccinate solution (DSS) is added to the tryptic digest after completion of hydrolysis. The hydrolyzed protein is precipitated by the addition of 20 ml. of trichloroacetic acid solution (TCA). The mixture is centrifuged at 4000 to 5000 G for 30 minutes, and the supernatant is collected in a 250-ml. glass centrifuge bottle. To recover residual CMC, the precipitate is dispersed in 5 ml. of DSS and neutralized with 30% sodium hydroxide (indicator paper) and the protein is again precipitated with an equal volume of TCA. Separation of CMC from the combined supernatants is achieved by the addition of 1 volume of absolute ethanol and 2.5 volumes of ethyl acetate. The mixture is allowed to stand at room temperature for 30 minutes with intermittent shaking during which time a complete precipitation of CMC occurs. The supernatant is carefully decanted after centrifugation at 4000 to 5000 G for 15 minutes. To remove traces of protein and lactose, the CMC precipitate is again dispersed in DSS and sodium hydroxide to neutral or slightly alkaline pH followed by treatment with TCA. The purified CMC is finally obtained by solvent precipitation of the supernatant as before.

Colorimetric Analysis. The isolated CMC is hydrolyzed in the centrifuge bottle with 0.1*N* sulfuric acid for 2 hours at 95° to 100° C. The partially hydrolyzed solution is then cooled and diluted with 0.1*N* sulfuric acid to yield a CMC concentration of 0.025 to 0.200 mg. per ml. The microcolorimetric method by Dubois *et al.* (1956) and modified by Marier and Boulet (1959) is used to determine the quantity of CMC in the solution. For this method, 2 ml. of sample is layered over 6 ml. of concentrated sulfuric acid, 0.1 ml. of 80% phenol is added, and the contents are quickly mixed. The color is allowed to develop in the warm mixture of 10 minutes before cooling it to room temperature. The absorbance is then measured at 490 mμ and compared with a standard curve prepared from CMC hydrolyzed in 0.1*N* sulfuric acid.

RESULTS AND DISCUSSION

Ionic polysaccharides and milk proteins undergo complex interactions and cannot readily be separated without prior degradation of the proteins. In preliminary trials, several proteolytic enzymes, including trypsin, pronase, and a combination of trypsin and pepsin, were used to hydrolyze the milk protein. The trypsin treatment, under the conditions indicated in the procedure, yielded satisfactory results. Attempts to remove the hydrolyzed protein from the CMC by exhaustive dialysis or by treatment with ion exchange resins were not successful, presumably because of an interference by glycopeptides. However, CMC was soluble in TCA and a complete separation of CMC from the proteinaceous material could be achieved at the concentration of 12.5% TCA followed by centrifugation. The dispersion of CMC in TCA was materially enhanced by the use of DSS, a wetting agent for food gums (Whelan and Klis, 1966). The supernatant obtained by centrifugation contains, in addition to CMC, soluble constituents from the milk, including lactose, salts, and peptides. CMC could be precipitated from this system by decreasing the polarity of the solution which was accomplished by the addition of a mixture of absolute ethanol and ethyl acetate in the ratio of 1 to 2.5. However, it was not possible to obtain a clear separation of the CMC from the tryptic digest of ice cream mix because of an association of fat with the hydrocolloid (Harper *et al.*, 1967). This difficulty could be eliminated by performing a fat extraction of the sample immediately after the hydrolysis.

The characteristics of the isolated material were determined by free boundary electrophoresis. The electrophoretic patterns of the recovered material as compared with the original stabilizer are shown in Figure 1. The patterns are homogeneous with no apparent interference of protein, and the mobilities are nearly identical. Therefore, the recovered CMC appears to be pure and has not been significantly affected by treatments during the isolation.

Particular attention was given in this study to methods for the determination of the amount of isolated material. Attempts to weigh the quantity of the recovered CMC after drying it under standard conditions failed because of a lack of reproducibility in the results. The anthrone method (Medvedeva and Konyushko, 1966) was generally unsatisfactory because of difficulties in obtaining a reproducible absorption maximum. The phenol–sulfuric acid method (Dubois *et al.*, 1956; Marier and Boulet, 1959) was used instead for the quantitative determination. Although CMC is capable of developing color directly in the reaction mixture without prior acid hydrolysis, the nonhydrolyzed CMC has a tendency to agglomerate and adhere to the glass walls, causing inconsistency in pipetting samples. It was impossible to improve the dispersion characteristics of nonhydrolyzed CMC at this step by the use of DSS because this reagent interferes with the colorimetric determination.

The developed method was applied to different milk products containing from 0.05 to 0.4% CMC. The CMC was recovered from 10 grams of each sample by the isolation procedure, and following the acid hydrolysis, the volumes were adjusted to 200 ml. Spectrophotometric analysis was performed on duplicate 2-ml. portions of these solutions. Average absorbance values are plotted in Figure 2 and show a close agreement between the standard curve and the isolated quantities of CMC from skim milk, whole milk, and ice cream mix.

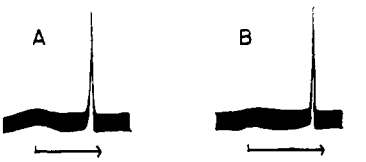

Figure 1. Ascending electrophoretic patterns of CMC

Concentration 0.5%, sodium phosphate buffer, pH 7.0, ionic strength 0.1, temperature 0 to 2° C., field strength 0.4 volt/cm.
A. Original CMC, mobility: −18.9 sq. cm. volt^{-1} sec.$^{-1}$
B. Isolated CMC, mobility: −18.6 sq. cm. volt^{-1} sec.$^{-1}$

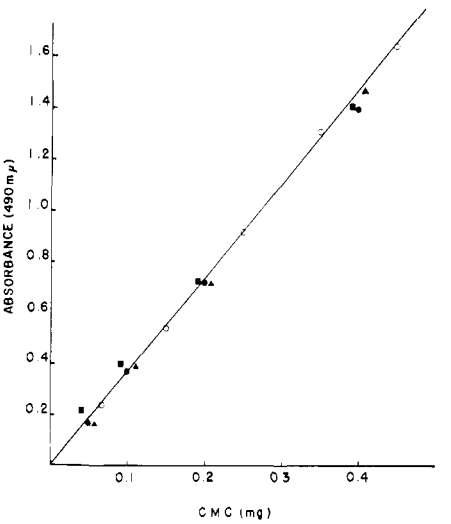

Figure 2. Spectrophotometric determination of CMC

● Skim milk ▲ Whole milk ■ Ice cream mix
○ Standard curve for CMC ($\hat{Y}$ = 3.68 X −0.009)

The absorbance data were converted to CMC concentration in terms of milligrams per 10 grams of sample by comparison with the standard curve and the values are presented in Table I. Examination of the data revealed that the standard deviation for the spectrophotometric determination of CMC in the 2-ml. portion taken for analysis was ±0.012 mg., corresponding to an analytical error of ±1.2 mg. for the original, undiluted sample. The combined experimental error for the isolation of CMC and for the final spectrophotometric analysis was ±1.7 mg. These two values are not significantly different, suggesting that the proposed isolation procedure is reproducible.

For all three products the calculated correlation coefficients indicated an excellent linear relationship between the recovered and expected amounts. The accuracy of the method was assessed by the slope of the regression equation for the combined data, $\hat{Y} = 0.927\,X + 1.42$, which shows a

Table I. Recovery of CMC from Different Milk Systems

CMC Added	CMC Found,[a] Mg./10 Ml. Whole milk	Skim Milk I	Skim Milk II	Ice Cream Mix I	Ice Cream Mix II	Average
5	4.1	4.5	5.5	6.6	6.9	5.8 ± 1.5
	4.1	5.0	5.8	6.4	8.7	
10	9.4	9.5	10.5	11.6	13.1	10.9 ± 1.1
	10.4	10.9	10.4	11.3	12.1	
20	18.2	19.3	21.9	20.2	21.2	20.1 ± 0.9
	19.6	20.2	19.5	20.0	21.1	
40	40.6	38.3	39.3	38.9	39.9	38.4 ± 1.3
	37.6	38.0	33.4	38.4	39.6	

[a] The standard deviation from analyses of duplicate series (8 degrees of freedom) was ±1.7 mg./10 ml. The standard deviation from duplicate analyses on single hydrolyzates (20 degrees of freedom) was ±1.2 mg./ml.

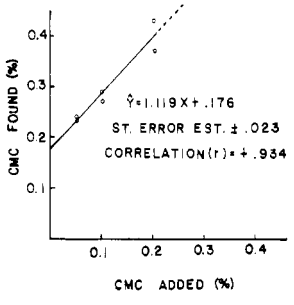

Figure 3. Recovery of CMC from chocolate milk

mean recovery of CMC of 92.7%, with an uncertainty of ±3.4% as determined by the 95% confidence interval for this estimate. The positive intercept of the regression equation of 1.42 mg. per 10 grams of sample suggests some contribution by the milk constituents, possibly by native polysaccharides present in milk proteins. This interference is comparatively small and is important only for measurements of the lowest levels of CMC. Therefore, if a greater accuracy is required, the data may be corrected by solving for X in the regression equation or basing the standard curve on a suitable milk system.

The method does not measure CMC specifically if other hydrocolloids are present, since most polysaccharides would be isolated by this procedure as well. This effect is illustrated in Figure 3 by the analysis for CMC in chocolate milk, containing approximately 5% of chocolate. Evidently, the high content of pectin or starch in the chocolate interferes with the results. However, when the quantity of these other hydrocolloids is known, recovery values are highly correlated with the expected values.

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