Rapid Determination of α-Lactose in Whey Powders by Differential Scanning Calorimetry

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ABSTRACT

A new method for direct measurement of the amount of α- and β-lactose in whey powders by differential scanning calorimetry provides accurate results in only 2 to 3 h instead of 8 to 24 h required by the standard polarimetric method. Treatment of a whey powder with anhydrous methanol (2 h at room temperature) prior to a calorimetric analysis removes moisture, converts α-hydrate to α-anhydrous, and permits the detection of two melting transitions (α and β). Measurement of normalized peak height gives the relative amounts of α- and β-lactose in the original powder. Results are presented for whey powders with degrees of crystallinity ranging from 0 to 90% and for mixtures of α-hydrate and β-lactose as well as for lyophilized lactose. The technique measures total α-lactose content to within 5% of the value by polarimetric analysis.

INTRODUCTION

One of the most critical factors affecting the physical properties and market value of dried whey is the degree to which lactose has been crystallized as α-lactose monohydrate. The amorphous or glass form of lactose, resulting from rapid dehydration of whey, is highly hygroscopic and thus promotes excessive powder stickiness and caking.

The standard method for measuring the crystalline α-lactose content of whey powders and other dairy products is the polarimetric method of Sharp and Doob (12) which is based on the significant difference in specific optical rotation between α- and β-lactose. This method is accurate and reproducible yet suffers from two disadvantages. First, the presence of both crystalline forms in addition to lactose glass invalidates the procedure for calculating percentage crystalline α-lactose. One must assume that only two of the three possible lactose species (α, β, or glass) are initially present (4). Second, the attainment of mutarotational equilibrium requires at least 8 h (4, 7). Speeding the rate of mutarotation by raising the pH to 9 to 10, as with NH_3 (14), would require careful titration to the desired pH. Too little NH_3 would be ineffective because of the large buffer capacity of whey, and too much NH_3 could lead to the degradation of lactose and perhaps a change in specific optical rotation. Similarly, the possibility of thermal degradation of lactose argues against speeding mutarotation by raising the temperature of the sample. A new method, which would decrease analysis time without sacrificing accuracy is desirable.

Alternative methods have been proposed for the determination of crystalline lactose. For example, Anderson and Berlin (1) used thermogravimetric analysis to determine α-monohydrate content in sweet whey powders with reasonable accuracy, but acid whey powders were not amenable to their method. Susi and Ard (13) proposed a method based on far infrared absorption, but comparison with polarimetric analysis was not always favorable.

Results from the calorimetric study of the melting of α- and β-lactose by Berlin et al. (2) suggested that the large difference in melting point between the two anomers might permit differentiation between them in whey powder and thus provide a rapid and accurate method of analysis.

MATERIALS[2] AND METHODS

Crystalline α-lactose monohydrate was USP grade supplied by Foremost; crystalline β-lactose was Eastman Kodak white label. Further purification of each of these lactose samples

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[2] Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

was accomplished according to the method of Buma and van der Veen (5). Lactose glass was prepared by lyophilizing concentrated lactose solutions at mutarotational equilibrium (6).

Seven acid whey powders with varying amounts of crystalline lactose plus a sample of whey protein concentrate containing about 3% lactose were supplied by V. H. Holsinger.[3] Portions of several samples were exposed to ambient laboratory relative humidity to increase the content of crystalline α-lactose. One sample of high crystallinity was mixed with crystalline β-lactose to decrease the relative content of crystalline α-lactose. A final sample was prepared by grinding together weighed amounts of crystalline α- and β-lactose.

Polarimetry

Samples were analyzed for α-lactose content by the standard procedure of Sharp and Doob (12) with the latest values of specific optical rotation for α- and β-lactose (5). Optical rotation was measured with a Perkin-Elmer Model 141 automatic digital polarimeter.

Calorimetry

For calorimetric analysis, a duPont Model 990 Thermal Analyzer in the DSC mode was used. Standards (In, Sn) for calibrating temperature and enthalpy measurement were obtained from the Instrument Division of E. I. duPont de Nemours, Inc. Distilled, deionized water also was used for calibration.

Preliminary DSC experiments with 5- to 10-mg portions of whey powder or whey protein powder in standard aluminum sample pans indicated the need to pretreat the powders. Succeeding samples of lactose or whey powder were prepared for DSC analysis by mixing them with anydrous methanol at a vol/wt ratio of 20:1 (methanol/solid) and stirring for 2 to 3 h at room temperature in a tightly stoppered flask. Samples, which then were filtered and dried at room temperature under a stream of dry nitrogen, yielded final analytical results indistinguishable from these after more rigorous drying of 4 h in a 60 C vacuum oven. The DSC runs were at a programming rate of 10 C/min on dried samples (5 to 15 mg) contained in crimped aluminum pans. The reference material was an empty pan.

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RESULTS AND DISCUSSION

Figure 1 depicts two DSC thermograms obtained with an acid whey powder (75% of the lactose in the crystalline α-hydrate form) and a dried whey protein concentrate (3% total lactose, all amorphous), neither of which was subjected to methanol treatment. There is no evidence of fusion of lactose, but each thermogram has a broad change in baseline, indicative of a decrease in heat capacity, most probably the result of mass loss through decomposition at these elevated temperatures. The mass loss for several powders was in the range of 30 to 40% of the original sample mass.

Figure 2 shows thermograms of the same two powders after they were stirred in anhydrous methanol for 2.5 h at room temperature and dried under dry nitrogen. Methanol treatment such as this is sufficient to dehydrate the sample (1) and to convert α-lactose to a stable, anhydrous form (9). The thermogram for whey powder now shows two endothermic transitions attributable to the fusion of anhydrous α- and β-lactose. Comparison of these transitions with thermograms of purified and methanol-treated α- and β-lactose indicates that the lower temperature peak represents the fusion of α-lactose. Similar thermograms are observed with α- and β-glucose in samples containing both anomers (8). The fusion of lactose in whey protein concentrate powder is still not detectable be-

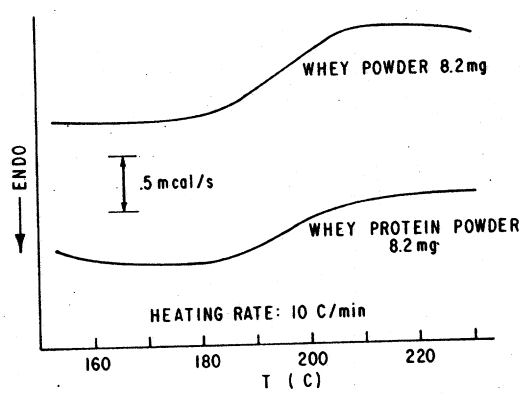

FIG. 1. DSC thermograms of whey powder and whey protein powder without treatment by methanol.

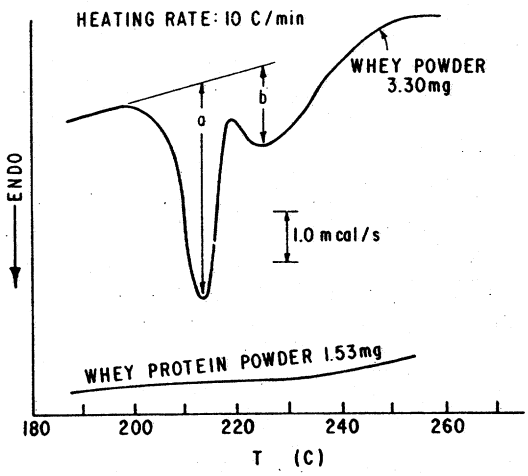

FIG. 2. DSC thermograms of whey powder and whey protein powder (Fig. 1) following dehydration in absolute methanol for 3 h at room temperature and subsequent removal of solvent. The thermogram for whey powder indicates the identification of peak heights, *a* and *b* for α- and β-lactose, respectively.

cause of the little lactose.

Comparison of Fig. 1 and 2 shows that for each powder the broad change in heat capacity is reduced in magnitude and shifted to a higher temperature following methanol treatment. Since the heat capacity change does not seem to require the presence of large quantities of lactose and is shifted to higher temperatures with reduced intensity upon dehydration, it may, in part, reflect protein degradation.

The appearance of an inflection on the trailing edge of the β-lactose fusion peak in Fig. 2 suggests that the onset of significant mass loss begins only after the temperature of maximum differential heat flow for β-lactose. This interpretation was verified by mass loss determinations after interrupting the temperature program at the midpoint of fusion of β-lactose. At this point in the thermal profile the sample was still light in color and lost less than 3% of its mass; samples heated beyond this temperature were charred and porous as a result of gas evolution.

The change in heat capacity following lactose fusion prohibits customary procedures for constructing baselines and measuring peak areas (3). Instead of the standard area measurements, a normalized-peak-height method was employed to calculate the relative amounts of α- and β-lactose. Figure 2 indicates the procedure for obtaining peak heights, labeled *a* and *b* for α- and β-lactose, respectively. The low temperature baseline was first extrapolated across the transition region, and the two peak heights were measured in relation to the extrapolated baseline. Each peak height was weighted according to the temperature-dependent calibration coefficient of the instrument as well as the difference in ΔH (fusion) between anomers.

The weighting factor, 1.30, was determined for the specific conditions and instrument of this study by recording isotherms of pure α-lactose and pure β-lactose, each treated with methanol according to the experimental protocol. The ratio of specific peak heights was 1.30 ± .08, which was identical to the value obtained for the ratio of fusion enthalpies. Thus, % α-lactose is calculated by the following equation:

$$\% \alpha = (100a/(a + b/1.30)$$

To calculate the % crystalline α-lactose, one needs the equilibrium ratio of β/α in lactose glass. All β-lactose is assumed to be in the amorphous state; the β/α ratio permits calculation of the correspoding amount of amorphous α. Previously, this β/α ratio was reported by Roetman and Buma (10) to be a function of drying temperature and to range between 1.3 and 1.6. More recently, however, Roetman and van Schaik (11) published data that indicate that the ratio is 1.25, independent of drying conditions.

The analytical results for several whey powders and mixtures of α- and β-lactose are listed in Table 1. The values are simply % total α, not % crystalline α-hydrate, which may be computed from the preferred β/α ratio. For example, if total α is 70% of all lactose, β = 30%, and if the β/α equilibrium ratio is chosen to be 1.25, then α (glass) is 30%/1.25, or 24%. Thus, crystalline α-lactose is (70%-24%) or 46% of all lactose on an anhydrous basis. The polarimetric values are means of duplicate determinations, with an average deviation of .64% α. The DSC analyses were performed in quadruplicate at the minimum; several samples were measured eight times. The average of mean deviations for these measurements was 1.18% α. Sample 11 was the same powder as sample 2, except for the addition of pure β-lactose before methanol treatment. Sample 7 was a humidified portion

TABLE 1. Comparison of DSC and polarimetric analyses.

Sample	% α-lactose	
	Polarimetry	DSC
Whey powder		
1	81.4	80.6
2	84.0	85.2
3	88.6	81.5
4	84.6	79.0
5	87.4	81.8
6	77.3	82.3
7	94.3	87.6
8	89.6	85.1
9	96.9	91.9
10	46.3	43.2
11	66.2	63.6
Lyophilized solution	54.6	58.5
α-hydrate/β mixture	60.5	59.5

of sample 6, and sample 9 was a humidified portion of sample 8.

The correlation between DSC and polarimetric analysis (Fig. 3) is close (r = .97), but the regression line crosses the ideal 45° line with an intercept at 7.0% α by DSC when α = 0% by polarimetry. The standard error of the intercept is 5.1 in units of % α. Overall, the average difference between the two methods is 4.00% α. The t value with 12° of freedom is 2.25, indicating a difference between methods at 95% confidence.

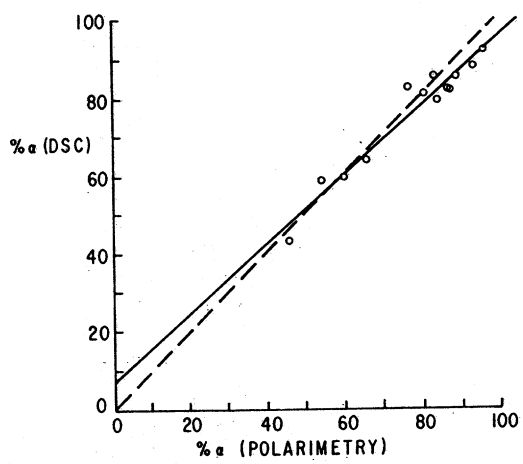

FIG. 3. Correlation of DSC and polarimetric analysis for total % α-lactose in whey powders and other samples from Table 1. The solid line is a linear least squares fit to the data. The dashed line is a 45° line of perfect correlation.

The range of greatest analytical interest and economic importance is 40 to 95% α, the range of greatest accuracy of the DSC method. Above 95% α the lactose is almost completely in the crystalline monohydrate form so small errors are not important. The region below 40% α, below the level of α-lactose in lactose glass, is also relatively unimportant because it rarely is encountered in the production of whey powders.

Neither the positions of the two fusion peaks nor the position of first deviation from the baseline varied with composition in a manner useful for quantitation of α- and β-lactose. α-Lactose fusion appeared at about the same temperature (within ± 3 C) for all samples except number 10, for which the peak was about 6 C lower than the mean. Fusion of β-lactose was scattered only slightly more widely. The lack of close correlation between melting temperature and composition might be explained by physical separation of α-lactose crystals from protein particles and amorphous lactose so that the α-lactose crystals melt independently. The melting points of α- and β-lactose in the amorphous phase would be affected mutually, however. Hence, the α-lactose in sample 10, which is almost entirely in the amorphous phase, would have more intimate contact with β-lactose and a lower melting point. The fusion peak of β-lactose in whey powders is lower than that of pure β-lactose by a fairly constant amount, because of the constant ratio of β/α in amorphous lactose. The fusion peak for α-lactose in treated whey powders is slightly broader than that for pure α-lactose since the former represents melting of α-lactose crystals at approximately normal temperature plus the melting of amorphous α-lactose at a lower temperature. The difference in melting points between these two is about 8 C (the difference between pure α-lactose and sample 10). Apparently this difference is insufficient to allow resolution of two distinct peaks. However, the width at half height was 5.0 C for pure α-lactose, 6.1 C for whey powder 5, and 8.5 C for whey powder 10. Such broadening may contribute to the error of the DSC method, particularly at lower α-lactose.

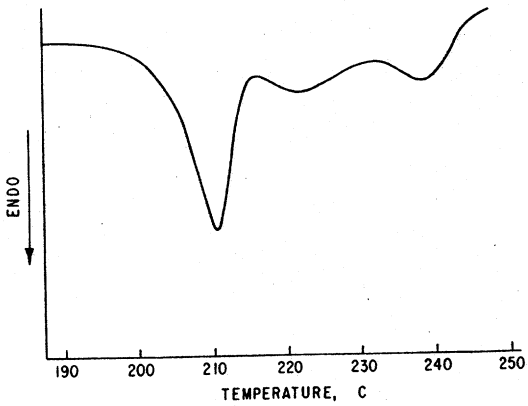

FIG. 4. DSC thermogram of sample 11 (whey powder + β-lactose) after methanol treatment, showing a resolution of two fusion peaks for β-lactose. The central peak has been assigned to β-lactose (amorphous), the high temperature peak to the crystalline β-lactose added before treatment with methanol.

In addition to speed, an advantage of the DSC method is the possibility of analyzing whey powders or lactose mixtures containing all three of the usual forms, amorphous, crystalline α-, and crystalline β-lactose. In contrast, the calculations of the Sharp and Doob method are based on the assumption that only one of the crystalline forms is present along with the amorphous lactose. An example of a mixture of all three forms is provided by sample 11, which was a mixture of sample 2 and pure crystalline β-lactose. The α content was calculated from the polarimetric analysis of sample 2 and the relative weights of sample 2 and added β-lactose. The DSC thermogram of sample 11 closely resembles that for sample 2 with the addition of a separate peak for pure β-lactose (Fig. 4). These two peaks for β-lactose probably result from physical separation of the pure β-lactose crystals as indicated by the temperature of the high-temperature peak, which is within 2 C of that for pure β-lactose. A value for *b* equal to the sum of the peak heights of the two β-lactose fusion peaks was used to calculate percent α in sample 11.

SUMMARY

A method has been presented for determination of the α-lactose content of whey powders with precision and accuracy comparable to the standard polarimetric technique. The advantages of this new method are a significant decrease in analysis time and an ability to detect the three usual forms of lactose instead of only two. With suitable calibration procedures this method can be used with either DSC or DTA instrumentation.

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