

MILK SOLIDS DETERMINATION

Rapid Determination of Nonfat Dry Milk Solids in Cereal Bars by Paper Chromatography

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A paper chromatographic method for screening cereal bars for nonfat dry milk solids content reduces the 2 days required for the classical procedures to 2 hours. The method combines clean-up with migration of lactose in a water extract of cereal bars containing other contaminants. The mobile solvent is 85 ml. of acetone made up with water to 100 ml. Partitioning and clean-up are aided by impregnating the paper with 1 ml. of heavy petroleum oil made up with petroleum ether to 100 ml., after spotting and drying. Identification of spots on the developed chromatogram is restricted to lactose and dextrose, using Soxhlet's modification of Fehling's solution as a chromogenic reagent and heating for 5 minutes at 100° C. in an oven. Spots are yellow against a blue background; semiquantitative estimates may be made of lactose or nonfat dry milk solids content. A ball-point pen line along both margins of the chromatographic paper automatically and permanently records the advancing solvent front.

THE CLASSICAL PROCEDURE (5, 7) for the determination of nonfat dry milk solids in cereal bars is based on extraction, precipitation, fermentation, and chemical estimation of the amount of lactose present in the cereal bars. A factor is then used to convert lactose to nonfat dry milk solids. Not only does this method require considerable analytical time, but quantitative recovery of the nonfat dry milk solids in a sample of cereal bars is subject to question. Grimbleby (4) predicted that the binding of lactose by protein could be a significant source of variation in lactose determination, as different methods of protein precipitation would liberate varying amounts of bound lactose. He based his conclusions, in part, on the data of Francis and Smith (3), which indicated that results for lactose determined on sera prepared by coagulating milk with various clearing agents showed a variation up to 10%, according to the agent used. Dutra, Jennings, and Tarassuk (2) showed the extent of lactose binding to protein in milk, and found that increases in temperature and time of heating increased the amount of lactose-protein complexes. Another source of error lies in the indirect determination of the per cent of nonfat dry milk solids

in a product by using a factor to convert lactose to nonfat dry milk solids. At least three conversion factors—1.9, 2, and 2.06—are available for calculations (6-8). The military specification for cereal bars (7) attempts to obviate the error introduced with conversion factors by specifying an end item requirement in terms of 6.8% of lactose by test rather than the 14% of nonfat dry milk solids required in the formula for the bar.

To reduce the number of procedural steps and to shorten the over-all time of analysis, a rapid paper chromatographic method was developed capable of semiquantitative screening of cereal bars for nonfat dry milk solids content by direct comparison with the component nonfat dry milk solids as a standard. While this paper is limited to cereal bars, the method has also been applied to screening cocoa beverage powder, tomato soup, and ice cream and cake mixes for nonfat dry milk solids content, and to vanilla tablets for lactose content. Approximately 2 hours total time, or 30 minutes actual working time, is required for an analysis.

Method

Apparatus. Chromatographic apparatus as specified by Mitchell (9).

Oven regulated at 100° C.

Pipet graduated at 4, 5, and 6 μ l.

Reagents. Immobile solvent—1 ml.

of heavy petroleum oil made up to 100 ml. with petroleum ether.

Mobile solvent—85 ml. of undistilled, reagent grade acetone made up to 100 ml. with water.

Chromogenic reagent—Soxhlet's modification of Fehling's solution (7).

Nonfat dry milk solids—use component of sample being tested.

Procedure. Add 10.0 grams of composite sample passing No. 10 sieve to a 300-ml. Erlenmeyer flask. Add 100 ml. of water. Shake at intervals over a 10-minute period to dissolve lactose. Filter through rapid filter paper to remove coarse particles. Use the first milliliter or so of filtrate. Filtration of the entire mixture is unnecessary.

Spot a 5- μ l. drop of filtrate on the chromatographic paper at the starting line, 1 inch from bottom and a minimum of 1 inch from side. Spot 5-, 4-, and 6- μ l. drops of standard containing equivalent amount of lactose. For example, if the unknown is thought to contain 14% of nonfat dry milk solids, weigh 1.40 grams of nonfat dry milk solids into an Erlenmeyer flask and add 100 ml. of water. Treat in the same manner as the unknown and use the filtrate for spotting. Repeat spots, alternating unknown with standard at intervals of 1 inch until the paper is filled. Draw a line with a ball-point pen $\frac{1}{4}$ inch from the edge of the chromatographic paper and perpendicular to the starting line to mark the solvent front (line auto-

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Table I. Collaborative Study of Per Cent of Nonfat Dry Milk Solids in Cereal Bars

Method	Sample A ^a	Sample B ^a	Sample C ^a
Standard	14.0	13.0	15.0
LABORATORY I			
Classical	14.0	17.7	17.9
Chromatographic	14.0	14.0	14.0
LABORATORY II			
Classical	^b	^b	^b
Chromatographic	14.0	13.3	14.0
LABORATORY III			
Classical	16.5	13.8	16.5
Chromatographic	15.4	14.0	16.8

^a Per cent nonfat dry milk solids by classical method equals per cent lactose \times 2.06.

^b Not reported.

matically marks advancing solvent front and forms permanent record). Dry the spotted paper at 100° C. for 15 minutes. Dip in immobile solvent, covering spots. Immediately withdraw paper and dry at room temperature in the hood for 5 minutes. Develop with mobile solvent to 5 inches above the starting line. Dry at room temperature in the hood for a few minutes. Spray the paper while it is still in the hood with chromogenic reagent lightly on one side, then on the other side to incipient dripping. Immediately suspend paper in 100° C. oven for 5 minutes.

Estimation of Nonfat Dry Milk Solids. Compare the area and intensity of the yellow color of 5- μ l. spots of standard and unknown at the same R_f value. Estimate concentration of nonfat dry milk solids in the unknown to nearest 0.1%.

Results and Discussion

None of the other contaminants in cereal bars—fats, proteins, starch, sucrose, dextrose, and salt—had any noticeable effect on the migration of lactose. Lactose exhibited the same R_f value whether present with contaminants extracted from cereal bars, from nonfat dry milk solids, or when chemically pure.

As expected, none of the contaminants in cereal bars reacted with the chromogenic reagent with the exception of dextrose, which has a different R_f value. The fact that dextrose reacts may be of informational value, since the formula-

tion (7) for cereal bars requires the use of sucrose rather than dextrose, or mixtures of sucrose and dextrose. The yellow spots against a blue background remained visible for several weeks. The spots varied in intensity, proportional to the concentration of lactose present in a curve of standards, ranging from 1 to 24% lactose. The latter concentration is more than triple the expected lactose concentration in cereal bars, and begins to present tailing problems in migration when 5- μ l. spots are used.

To facilitate the visual comparison between standard and unknown, like volumes of solutions are compared. Good results have been obtained using 5 μ l. each of standard and unknown, with additional spots of 4 and 6 μ l. of standard as limits. These limits aid greatly in comparing the 5- μ l. spots. At the concentration recommended for the standard, aliquots of 4, 5, and 6 μ l. give spots representing 11.2, 14.0, and 16.8% of nonfat dry milk solids, respectively. While this appears to be a wide range, the differences in the spots obtained were such that analysts could estimate nonfat dry milk solids to 0.1% (Table I). These data were obtained from a collaborative study by three laboratories of three unknowns, set up in an experimental design intended to give the maximum amount of information from the minimum amount of data. These unknowns were prepared from a composite sample of cereal bars submitted by a contractor to meet the 14.0% nonfat dry milk solids formulation (7). The cereal bars were assumed to con-

tain this amount of nonfat dry milk solids and represented the unknown of this concentration to be analyzed. A portion of this material was used to prepare the 13.0 and 15.0% unknowns by adding either sucrose or nonfat dry milk solids to give the desired percentage composition on the basis of total weight. Data in Table I indicate that the experimental method produces data statistically comparable to the classical procedure. Laboratory II had difficulty with the classical procedure and failed to report test results; hence, these were excluded from the statistical analysis. The F and "t" tests indicate that the chromatographic method is slightly more accurate than the classical method, in that the former has more values within the two sigma control limits of the formulation.

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