Error in the Analysis of Hydroxymethylfurfural in Processed Milk

2810

E. S. DELLA MONICA, J. C. CRAIG, JR., and M. J. CALHOUN
Eastern Utilization Research and Development Division, USDA
Philadelphia, Pennsylvania

Abstract

The Keeney-Bassette method for determining hydroxymethylfurfural (HMF) content of whole milk powder was studied. The variables examined were: solids of milk, age of reagents, and method of sample preparation. Results indicate that total HMF color is a function of the solids content of milk with high solids yielding more color. A seasonal variation in HMF color, as well as a daily variation within each season, also was observed. This source of error was eliminated by establishing a blank value for each new lot of milk processed.

A second type of time-oriented variation in HMF color formation was observed. For a given powder the HMF value gradually increased as the oxalic acid aged.

It is concluded that the method is satisfactory for measuring HMF and HMF precursors in processed milk, provided the solids content of the milk is kept constant, a raw milk blank is established for each milk, and oxalic acid is prepared fresh daily.

There is ample evidence that the presence of hydroxymethylfurfural (HMF) and its precursors in food is an indication of the heat exposure of the product (5-9). Hodge (3) has indicated that the formation of HMF is one of a number of pathways, whereby the nonenzymatic browning reaction proceeds. Keeney and Bassette (4) developed a method designed to detect early stages of the browning reaction in heated milk. This was done by forcing the initial compounds of the browning reaction, via an acidic reaction, to follow the HMF reaction pathway. The HMF thus produced was measured colorimetrically.

Craig et al. (2) found the determination of HMF (4) to be a very sensitive indicator of heat exposure and suitable for estimating the flavor stability of stored whole milk powder. In that study very low concentrations of HMF (1-5 μmoles per liter) were encountered. At these low levels, the experimental error in analyzing powders within any day ranged from ± 10 to $\pm 80\%$ of the determined value.

In subsequent studies the Keeney-Bassette Method B, as modified by Craig and associates, was used routinely. During this work a time-dependent bias was observed in this determination. As a result the following investigation was undertaken in an effort to establish and reduce this source of variation. The intended result of this study was the reduction of both the time-dependent bias and the within-day error described in our earlier study (2). Variables such as the solids content of the milk sample, the age of the reagents, and the method of sample preparation were studied.

Experimental Procedure

Whole milk powders, produced by the process of Aceto and associates (1), were used in this study. The powders were canned under nitrogen and stored at 0 degree F. Headspace gas was analyzed for oxygen content and the sample discarded if O_2 level exceeded 0.1% O_2 as measured by a Beckman Model E-2 Oxygen Analyzer.[1] Milk concentrate (45% total solids) used in this study was also obtained from the above process.

Hydroxymethylfurfural was determined by the Method B of Keeney and Bassette (4), employing the modifications outlined by Craig et al. (2). The milk powder was prepared for analysis by either one of two methods. The first method was to reconstitute a quantity of milk powder to a fluid whole milk containing 12% total solids. A 10-ml aliquot of this solution was analyzed. The alternate method was to weigh 1.2 g of milk powder directly into the reaction test tube, reconstitute with 8.8 ml H_2O, and analyze. Both a reagent and milk blank were determined. Results are reported either as optical density values at a wavelength of 443 mμ or as μmoles HMF per liter of milk.

[1] Received for publication August 8, 1967.

[1] Mention of companies and commercial products anywhere in the paper does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

All color measurements were made with a Beckman Model B Spectrophotometer using 1-cm pyrex cells.

Results and Discussion

Effect of solids content. The effect of solids content on the HMF value for a given milk sample is shown in Figure 1. Although an increase in HMF color was expected, a curved line was unexpected. Statistical evaluation of the data indicated a significant ($P = 0.05$) relationship between color produced and solids content. It was also observed that the wavelength of maximum absorption remained unchanged. The slight linear increase in color observed with higher whey solids levels was not sufficient to explain the nonlinear increase in HMF color. In an attempt to explain the nonlinear HMF color-solids relation, both raw whole and raw skimmilk were employed. These, representing zero HMF milks, were concentrated to approximately 25% solids by lyophilization (no heat applied). The concentrates then were reconstituted to various solids levels and analyzed for HMF color. Results obtained with raw whole milk show a nonlinear relationship identical to that obtained with heated milk. Similar results were obtained with raw skimmilk. Thus, it appears that the total HMF color produced is a function of the solids content of milk when analyzed. For this reason a milk blank should be established using raw or low heat milk of known solids content. All subsequent milk samples, both concentrated and dried, should be adjusted to this solids level before sampling and analyzing.

Seasonal and daily variation. Keeney and Bassette originally assumed a constant number (0.055) as the optical density value for the whole milk blank. However, an examination of the apparent HMF color values for raw, pasteurized, and concentrated milks accumulated over an 18-month period indicated variation with time. The variation appeared to be seasonal in character. Illustrated in Figure 2 are the data for pasteurized whole milk, showing clearly the magnitude of the day-to-day variation in optical density (milk blank) and, with this, the potential for a large error inherent in using a constant milk blank value.

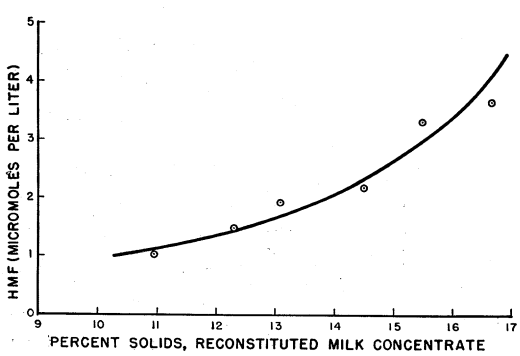

FIG. 1. Hydroxymethylfurfural (per liter milk, 12% solids) versus per cent solids of reconstituted whole milk concentrate analyzed.

To test the suspected periodicity of these data a Fourier synthesis was performed employing an 1130 IBM Computer. The curved line plotted in Figure 2 is the result of this calculation and shows the nature of the variation. An analysis of variance indicated a highly significant (99% level) correlation between optical density and time (season). It was established that the variation was not due to analytical error. Thus, the practice of determining a daily milk blank for each lot of milk received was established.

This blank value applies to all samples prepared from a particular lot of milk. This practice has been followed throughout the following studies.

Source of variation. During this investigation a second type of time-oriented variation of color values of whole milk powders was observed. It appeared as a steady but gradual increase in HMF color for a number of analyses, then a sharp drop in HMF values followed by another increase. Statistical analysis of a series of data revealed that the variation was independent of the sample. Consequently, a series of experiments was initiated to find the source of this variation with time. To do this a large batch of screened, whole milk powder was thoroughly blended, canned under nitrogen ($<0.1\%$ O_2), and held at 0 degree F, as a constant sample to be studied. There were approximately 200 g powder per can. The following sources of variation were considered for study: (1) method of sampling, i.e., direct weighing of the powder versus reconstitution of the powder and sampling, (2) the trichloroacetic acid used, (3) the oxalic acid used.

While the three studies were conducted simultaneously, each was independent and could be analyzed separately.

Direct weighing versus reconstitution. In this experiment the standard powder was analyzed a number of times in the following manner: Powder was reconstituted to fluid milk (12% total solids), sampled and then analyzed, or was weighed directly into individual reaction tubes, reconstituted, and analyzed. The reagents used in this series of experiments were not freshly prepared and will be designated as

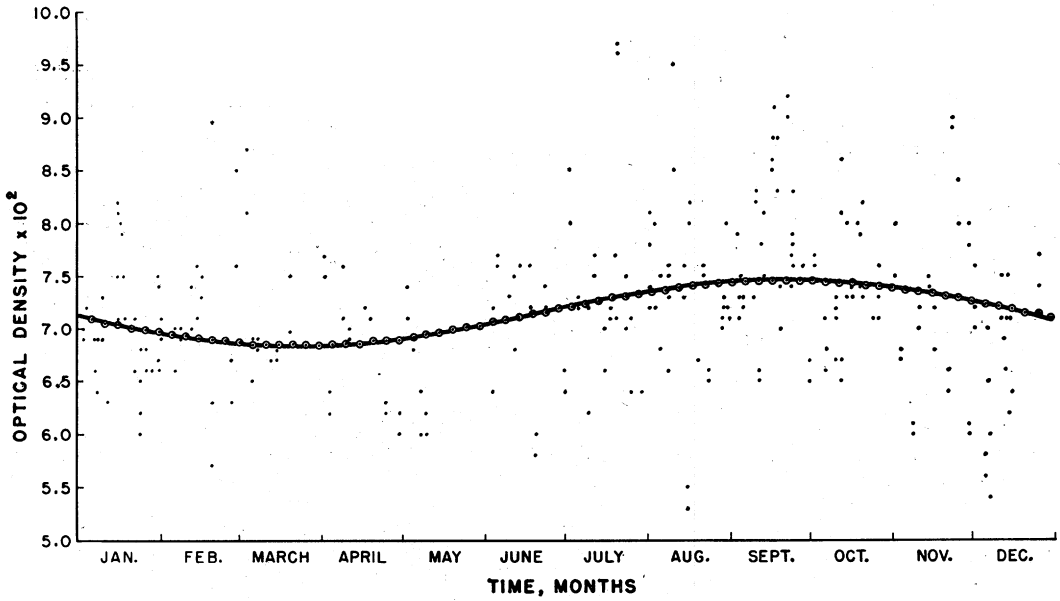

FIG. 2. Effect of time on HMF color of pasteurized market milk. ⊙——⊙ calculated line, • experimental values.

regular solutions throughout this study. The data were analyzed statistically and the results shown in Table 1. These results indicate no significant difference between the methods of sampling. The data also show, however, that optical density was varying with time regardless of the method of sampling. These findings suggest that either of the reagents may be the factor responsible for the time-oriented variation in color formation.

Age of trichloroacetic acid. Of the reagents used, the first to be tested was the trichloroacetic acid (TCA). In this series of experiments a slightly aged (four to five days old) TCA solution was compared to a freshly prepared solution. The results, shown in Table 2, indicate a questionable difference between the two TCA solutions. Once again, however,

TABLE 1
Direct weighing versus reconstitution

Analysis of variance (three-way ANOVA)				
Source	SS	dF	ms	F
Total	10.990	23		
Replications (error)[a]	7.298	18	0.405	
(R) (method of sampling)	0.920	1	0.920	2.506
(D) (days)	2.716	2	1.358	3.700*
(R × D) (interaction)	0.056	2	0.028	<1.

[a] Within-groups error (within each unit day or basic set).

TABLE 2
Aged versus fresh trichloroacetic acid

Analysis of variance (three-way ANOVA)				
Source	SS	dF	ms	F
Total	16.234	23		
(D) (days)	12.364	2	6.182	39.38*
(R) (reagents)	0.667	1	0.667	4.248
Replicates (error)	2.842	18	0.157	
(R × D) (interaction)	0.361	2	0.181	1.152

a significant relation with time was observed (Table 2). These results suggest that the TCA solution was not the factor causing the day-to-day variation.

Age of oxalic acid. This study was identcial to that employed for testing TCA solutions, in that the standard milk powder, stored at 0 degree F, was analyzed. In these experiments a regular TCA solution was used. Initially, an oxalic acid solution was prepared and used for 22 days. Results indicated a gradual but regular increase in the amount of color produced by the reaction (similar to that shown in the left half of Figure 3). This suggests that aging oxalic acid is responsible for the increase in color.

The above experiment was repeated over a 30-day period. A gradual increase in color again was observed (Figure 3). At this point a fresh oxalic acid solution was prepared and

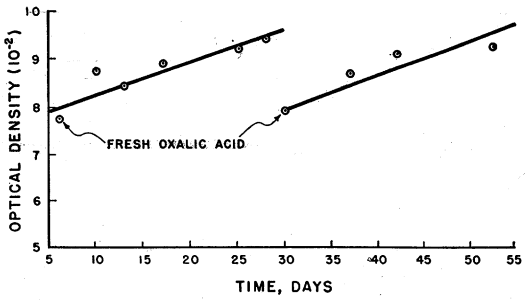

FIG. 3. Apparent HMF content of a standard milk powder versus age of oxalic acid.

utilized for the determination of HMF of the standard powder. On this day the HMF color reverted back to the original level of color obtained 30 days previously. Analyses of the standard powder were continued for an additional 22 days, using the second preparation of oxalic acid. As this acid solution aged, the color once again began to rise (Figure 3), indicating that oxalic acid was the most likely factor responsible for the increase.

As a final check the standard powder was analyzed using an oxalic acid solution prepared fresh daily along with an aged solution. Results showed that the optical density with the fresh oxalic acid remained essentially constant, while the values with the aged oxalic acid showed irregularities in optical density.

Conclusion

The determination of the quantity of HMF formed during processing can be a reliable objective analysis for measuring incidence of heat exposure of milk provided: the solids content of the milk to be analyzed is kept constant; a raw or low heat milk blank is established for a particular lot of milk; and the oxalic acid is prepared fresh daily.

References

(1) Aceto, N. C., Sinnamon, H. I., Schoppet, E. F., and Eskew, R. K. 1962. Continuous Vacuum Drying of Whole Milk Foam. J. Dairy Sci. 45: 501.

(2) Craig, J. C., Jr., Aceto, N. C., and Della Monica, E. S. 1961. Occurrence of 5-Hydroxymethylfurfural in Vacuum Foam-Dried Whole Milk and Its Relation to Processing and Storage. J. Dairy Sci., 44: 1827.

(3) Hodge, J. E. 1953. Chemistry of Browning Reactions in Model Systems. J. Agr. Food Chem., 1: 928.

(4) Keeney, M., and Bassette, R. 1959. Detection of Intermediate Compounds in the Early Stages of Browning Reaction in Milk Products. J. Dairy Sci., 42: 945.

(5) Luh, B. S., and Kamber, P. J. 1963. Chemical and Color Changes in Canned Apple Sauce. Food Technol., 17: 105.

(6) Luh, B. S., Leonard, S., and Marsh, G. L. 1958. Objective Criteria for Storage Changes in Tomato Paste. Food Technol., 12: 347.

(7) Potter, F. E., and Patton, S. 1956. Evidence of Maltol and Hydroxy-Methylfurfural in Evaporated Milk as Shown by Paper Chromatography. J. Dairy Sci., 39: 978.

(8) White, J. W., Jr., Kushnir, I., and Subers, M. H. 1964. Effect of Storage and Processing Temperatures on Honey Quality. Food Technol., 18: 555.

(9) Zubeckis, E. 1966. Hydroxymethylfurfural in Canadian Apple Juice. Can. J. Plant Sci., 46: 125.