Seasonal Variation of Phospholipids and Their Influence on the Foaming Characteristics of Concentrated Whole Milk

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Abstract

Qualitative observations of continuous vacuum foam drying of whole milk indicated that dryer performance was influenced by seasonal variations in milk concentrate foaming activity. An investigation showed that the phospholipids content of whole milk varies significantly with season and that this is directly related to a similar variation in the static rate of foam subsidence of whole milk concentrate. These seasonal variations are characterized by high phospholipids and rate of foam subsidence levels in the winter, and low levels in the summer. Seasonal variation in phospholipids is most pronounced in the lipid phase.

The development of a process for the vacuum drying of concentrated whole milk foams (1, 9) has involved an extensive research program. Since the foaming characteristics of whole milk concentrates profoundly affect many aspects of this process, part of the research program has included studies of factors affecting these characteristics. Recent investigations have led to the development of a quantitative static foam test to study the effects of temperature and viscosity on milk concentrate foams (6), and these correlations have proven useful in understanding certain aspects of the drying problem. However, pilot plant observations indicated that other factors intrinsic to the milk itself were also affecting dryer performance through foaming activity. Specifically, qualitative observations indicated that during winter months, milk concentrate foams subsided quickly to thin, easily dried films whereas, in the summer, foams were much more stable, and persisted as thick films difficult to dry. Consequently, a study was initiated to determine what milk constituents might be causing this variation.

A screening of some naturally occurring constituents likely to affect surface activity was carried out, employing the static foam test (6). These constituents included calcium, citrate and phosphate ions, the proteose-peptone fraction, and the phospholipids. Addition of the ions did not influence the foaming characteristic of whole milk even when added at levels several times greater than their reported seasonal differences (7). On the other hand, phospholipids and proteose-peptone did have a marked influence even at levels lowers than occur naturally.

Of these two constituents, phospholipids were of particular interest, because the initial foam tests indicated that an increase in phospholipids content destabilized the milk foams and, therefore, might serve as a convenient additive to decrease the stability of summer foams. On the other hand, proteose-peptone, as indicated from the same preliminary foam testing, had an opposite effect from phospholipids and would consequently have to be removed from summer milk to improve the drying operation. Preliminary work by Craig (3) indicates that the concentration of surface active agents can be altered by a foam fractionation technique. This was not considered a convenient processing operation, as compared with a simple phospholipids addition. In light of this, a year's seasonal investigation was undertaken which included observation of whole milk phospholipids content in conjunction with foam studies.

Experimental Procedure

Experiments were performed on batches of homogenized-pasteurized[1] whole milk diluted with enough pasteurized[1] skimmilk to adjust the milk fat content of the resultant blend to 3.25% on a fluid basis (12% total solids). The milks were obtained from a large pooled commercial source. Approximately every 2 wk a sample was analyzed for phospholipids and its foaming characteristics determined. Other samples were analyzed for phospholipids only.

Phospholipids analysis. Phospholipids content of whole milk and its centrifugally isolated cream fraction were determined following the general scheme outlined by Greenbank and Pallansch (5). The cream was isolated in a Spinco

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[1] Pasteurized at 162 F for 16.5 sec.

Model L Ultracentrifuge [2] operating for 1.5 hr at 22,600 × g. The extracted lipids were ashed and phosphorus content determined by the Fiske and Subbarow method (4), using $FeSO_4$ as the reducing agent (10). Optical density measurements were made in a Beckman Model B Spectrophotometer at a wave length of 650 mμ. The milligrams of phospholipids per gram of fat was obtained by multiplying the milligrams of phosphorus per gram of fat by the factor 25 (5). Phospholipids content of the skimmilk fraction was obtained as the difference between whole milk and the cream phospholipids content.

Determination of milk concentrate foam characteristics. Foam determinations were carried out on milk concentrates, employing the procedure and apparatus described by Holden et al. (6). This procedure, referred to by Bikerman (2) and others as a static foam test, measures independently two foam attributes,[3] foaming ability expressed as initial foam height (Hi) in inches, and foam stability expressed as rate of foam subsidence (R_{fs}) in inches/minute.

Since viscosity plays an important role in determining the foaming characteristics of whole milk concentrates, the viscosity of a 50-ml aliquot of each concentrate sample foamed was determined in a Saybolt Furol tip viscosimeter at 21.1 C as Saybolt Furol Seconds (SFS).

Results

Phospholipids content data collected in the course of these experiments included analysis of 44 separate whole milk samples. Twenty-seven of these samples were also analyzed for milk concentrate foam characteristics. Collection dates of these samples were evenly distributed throughout the period from November 15, 1962, to November 16, 1963.

Seasonal variation on phospholipids content. Phospholipids concentrations determined throughout the experimental period included three data sets, i.e., total phospholipids content of the whole milk and phospholipids content of the cream fraction from which phospholipids content of the skim fraction was calculated. A preliminary statistical analysis was performed on each data set, to test for seasonal variation. This analysis consisted of a rather arbitrary breakdown of the 1962-1963 period into four equal seasons determined by the equinoxes, and then a season-to-season cross-comparison in all possible combinations for each set of data by means of analysis of variance. These analyses indicated distinct seasonal differences.

Since seasonal variation in naturally occurring compounds such as phospholipids might be expected to be cyclic and essentially continuous, further data evaluation was performed to test this hypothesis. This evaluation was carried out by subjecting the three sets of data to a mutliple regression analysis, employing a cyclic mathematical model as follows:

$$y = a_0 + a_1 \sin\left(\frac{2\pi}{365}t\right) + a_2 \cos\left(\frac{2\pi}{365}t\right)$$

where:

- y is the daily phospholipids content of either the whole milk (y_{pw}) or the cream fraction (y_{pc}) or the skim fraction (y_{ps}) in milligrams phosphorus per gram of fat.
- t is the time in days of the phospholipids analysis (Day 1 is November 15, 1962).
- a_1 and a_2 are coefficients obtained from the regression.
- a_0 is the regression intercept (equal to the yearly mean phospholipids content, $\bar{y}_{pw}$, $\bar{y}_{pc}$, or $\bar{y}_{ps}$ for this particular function).

Figures 1, 2, and 3 show results of the regression analysis performed on the data sets, plotted along with the raw data as phospholipids concentration against time, in days. Table 1 includes a summary of the regression analysis for the three data sets and includes the regression coefficients for each set, the F ratio obtained from an analysis of variance associated with each set, and the maximum and minimum phospholipids levels predicted from the regression line for each set of data. The F ratios reported in Table 1 indicate a probability

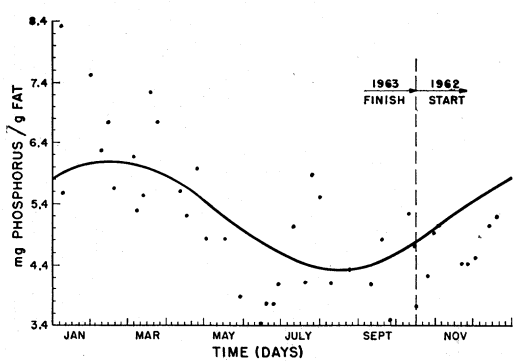

FIG. 1. Seasonal variation in the total phospholipids concentration of whole milk.

[2] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

[3] These two attributes are defined as independent, in that they are measured independently. This does not preclude a functional relationship between the two attributes, just as temperature and pressure for a gas are measured independently but are functionally related.

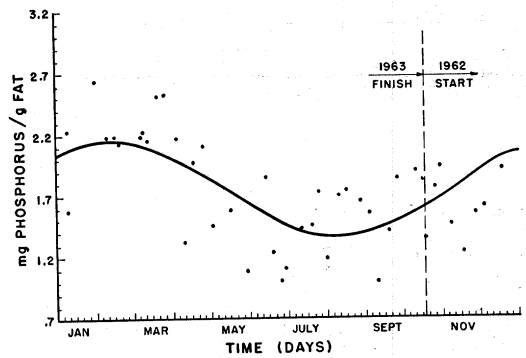

FIG. 2. Seasonal variation in the phospholipids concentration of the cream fraction of whole milk.

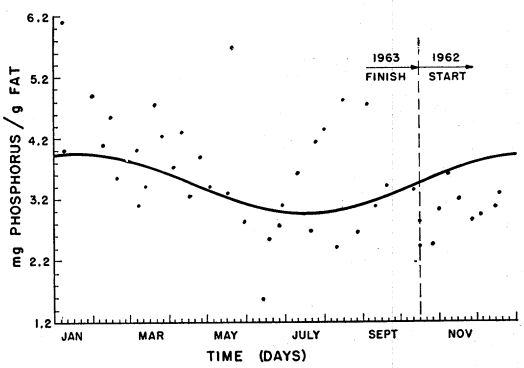

FIG. 3. Seasonal variation in the phospholipids concentration of the skim fraction of whole milk.

of better than 95% that the regressions fit the data for each data set.

Examination of Figures 1, 2, 3, and Table 1 illustrates the seasonal variation in phospholipids levels. These variations are characterized by high levels in the winter and low levels in the summer.

The skim fraction phospholipids content, although varying significantly with season, does not seem to be as strongly affected by season as the phospholipids in the cream fraction. This is suggested by the relatively low F ratio for the skim fraction regression. Also, since skim phospholipids were determined by difference, they are subject to the random errors of the two other determinations. Another indication of the smaller seasonal variation in the skim fraction phospholipids was shown by a statistical comparison ("t" test) of the maximum, minimum, and yearly mean values. For the total and cream fraction phospholipids, maximum values were significantly higher than the yearly mean values which, in turn, were significantly higher than the minimum values. In contrast, for the skim fraction, a significant difference exists between maximum and minimum values, but none between maximum and mean, or minimum and mean. It appears, then, that the seasonal variation observed in whole milk phospholipids concentration is due primarily to variations in phospholipids level taking place in the lipid phase of the milk, with

TABLE 1

Summary of regression analysis for phospholipids concentration as function of time based on the model:

$$y = a_0 + a_1 \sin\left(\frac{2\pi}{365}t\right) + a_2 \cos\left(\frac{2\pi}{365}t\right)$$

Fraction	a_0 Mean value for period 10/16/62 to 10/16/63 (mg P / g fat)	a_1 (mg P / g fat /day)	a_2 (mg P / g fat /day)	F Ratio	Maximum predicted value (mg P / g fat)	Minimum predicted value (mg P / g fat)
Total phospholipids in whole milk, y_{pw}	5.20	0.7633	−0.4602	9.9	6.09 (2/15/63)	4.28 (8/17/63)
Phospholipids in cream, y_{pc}	1.74	0.3542	−0.1720	11.9	2.15 (2/9/63)	1.35 (8/12/63)
Phospholipids in skim, y_{ps}	3.45	0.4868	−0.0195	3.6	3.94 (1/12/63)	2.97 (7/13/63)

little seasonal variation in the aqueous phase.

Seasonal variation in foaming characteristics. The first step in evaluating the foam characteristic data was the correction of the raw data to a standard viscosity of 25 SFS. This step was necessary because it was impossible to produce milk concentrate batches of constant viscosity on a day-to-day basis and, as has been shown previously (6), viscosity markedly effects the foaming attributes under study. Consequently, each of the two foaming attributes observed, rate of foam subsidence and initial foam height, were correlated with viscosity by means of linear regressions. The following correction relationships were obtained from these correlations and applied to the means of the raw data points.

$$R_{fsc} = R_{fso} + 0.047\ (\eta_o - 25)$$
$$H_{ic} = H_{io} + 0.132\ (\eta_o - 25)$$

where:

- R_{fsc} and H_{ic} are the rates of foam subsidence and initial foam heights corrected to 25 SFS in inches/minute and inches, respectively.
- R_{fso} and H_{io} are the observed rates of foam subsidence and initial foam height in inches/minute and inches, respectively.
- η_o is the observed viscosity in SFS.
- 0.047 and 0.132 are correction factors obtained from linear regressions of R_{fso} and H_{io} on η_o.

Because of the narrow range of viscosities encountered in these experiments (15-35 SFS) a linear relationship could be employed without loss of accuracy, even though a curvilinear relationship exists over a broad viscosity range (6).

Statistical analyses for seasonal variation in R_{fsc} and H_{ic} were performed next. These analyses were carried out in exactly the same manner as that for phospholipids content, described

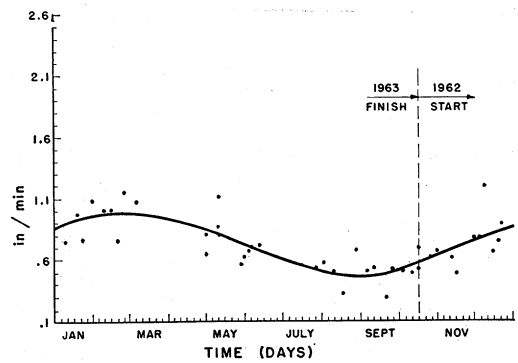

FIG. 4. Seasonal variation in foam stability as measured by the static rate of foam subsidence of whole milk concentrate.

previously. The analysis indicated distinct seasonal variation in R_{fsc} but none whatever for H_{ic}. Figure 4 and Table 2 show the details of the R_{fsc}-seasonal relationship, characterized by high rate of foam subsidence in the winter and low rate of foam subsidence in the summer. The F ratio in Table 2 indicates a high-fit probability of the cyclic regression model. The predicted maximum R_{fsc} value is significantly higher than the yearly mean value which, in turn, is significantly higher than the minimum value.

Relationship between rate of foam subsidence and phospholipids concentration. In the light of the similarity between the phospholipids concentration-time relationships and the rate of foam subsidence-time relationship, a correlation of phospholipids concentration and rate of foam subsidence was indicated. To test this hypothesis, a multiple regression model was employed which produced the following relationship significant at greater than the 95% level:

$$R_{fso} = 1.012 - 0.0147\,\eta_o + 0.299\,Y_{pc}$$
$$R_{fso} = 1.074 - 0.0164\,\eta_o + 0.199\,Y_{pw}$$

TABLE 2

Summary of regression analysis for rate of foam subsidence, corrected to a viscosity of 25 SFS, as a function of time based on the model:

$$R_{fsc} = a_0 + a_1 \sin\left(\frac{2\pi}{365}t\right) + a_2 \cos\left(\frac{2\pi}{365}t\right)$$

a_0 Mean value for period 10/16/62 to 10/16/63	a_1	a_2	F ratio	Maximum predicted value	Minimum predicted value
(in./min)	*(in./min/day)*			*(in./min)*	
0.734	0.1838	−0.1667	25.8	0.990 (2/26/63)	0.465 (8/28/63)

where:

R_{fso} is observed rate of foam subsidence (uncorrected for viscosity) in inches/minute.

η_o is viscosity in SFS.

Y_{pc} and Y_{pw} are phospholipids concentrations in milligrams phospholipids/gram fat for the cream fraction and whole milk, respectively.

R_{fs} did not correlate significantly with skim fraction phospholipids content.

It should be noted that the above relationships were determined for observed R_{fs} rather than the viscosity-corrected R_{fs}. Therefore, they include viscosity as a variable, along with phospholipids concentration. This treatment is statistically more accurate than correlating phospholipids concentration with the viscosity-corrected data. The positive sign of the coefficient of the phospholipids concentration indicates that an increase in phospholipids level leads to an increase in rate of foam subsidence.

Although initial foam height did not correlate with season, it was still tested for a possible correlation with all three sets of phospholipids data. No significant (at $P = 0.05$) relationship was observed.

Discussion

The seasonal variation of phospholipids content reported in this paper has not been recorded. However, indications of variations are found in the Literature. Nickerson in 1960 (7) studied more than 23 constituents in milk from various areas in California. He showed that the total phosphorus content of milk varied with season and the total phosphorus content, which presumably included phospholipid phosphorus, was similar to the seasonal variability of phospholipids reported herein, i.e., high in the winter and low in the summer. However, it should be pointed out that a seasonal variation in total phosphorus content would not necessarily be indicative of a seasonal variation in phospholipids, in which phosphorus is but a small fraction of the total.

The role of phospholipids in determining the foaming characteristics of milk has been reported by others. Richardson and El-Rafey (8), in studying the effects of milk fat in the foaming of milk, concluded that the nature of the interface material between the fat globule and the aqueous medium has a profound influence on foaming. They showed that the addition of varying amounts of soybean phospholipids to a 10% milk fat-skim milk emulsion decreased foam stability as phospholipids concentration increased.

This work should not be construed as excluding other naturally occurring constituents of the milk system, not studied in our investigation, as contributing to seasonal variation in foam behavior. For example, it has been suggested that the mono- and di- glycerides in milk fat could affect milk concentrate foam behavior.

An investigation into the factors that lead to the seasonal variations of phospholipids and, hence, foaming activity of cows milk is beyond the scope of this work. It is suggested, however, that changes in diet experienced by dairy herds throughout the year may be responsible for these variations. The usual annual cycle of fresh grass to silage and back might well be investigated from the standpoint of a possible influence on phospholipids, to shed some light on this problem.

Acknowledgment

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