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POROSITY, SPECIFIC GRAVITY AND FAT DISPERSION IN BLUE CHEESES

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Abstract

Porosity was measured in Blue cheeses made from (i) homogenized butter oil-reconstituted nonfat dry milk (4% fat), (ii) homogenized butter oil-reconstituted nonfat dry milk (14% fat) standardized to 4% fat with reconstituted nonfat dry milk, (iii) homogenized raw cream (14% fat) standardized to 4% fat with raw skim milk, and (iv) homogenized pasteurized cream (14% fat) standardized to 4% fat with pasteurized skim milk. Cheeses made from (i) were the most porous and were significantly different from the other cheeses. In cheeses made from (i) and (ii), about 40% of the holes were less than $2 \mu\text{m}^2$ in area, while in cheeses made from (iii) and (iv), 50% of the holes were less than $2 \mu\text{m}^2$. Cheeses made from (iii) did not contain holes larger than $22 \mu\text{m}^2$, whereas cheeses made from (iv), (ii) and (i) contained 0.76, 1.29, and 5.27%, respectively, of holes larger than $22 \mu\text{m}^2$. Cheeses made from (i) had the lowest specific gravity and were significantly different from the others. Fat was well dispersed in cheese made from (i) with few small clusters. The other cheeses contained many large clusters of fat globules, and the fat distribution was less uniform than in cheeses made from (i).

Introduction

There is interest in making Blue cheese from butter oil and reconstituted nonfat dry milk in such countries as Egypt. A first attempt was made by Omar and Ashour (1982), who made Blue cheese from unhomogenized cream standardized to 3.8% fat with reconstituted nonfat dry milk and also from reconstituted dry whole milk. However, these cheeses were inferior to those made from fresh cow's milk.

In the manufacture of Blue cheese in the United States, it is common practice to separate whole milk into 12-14% fat cream and skim milk. The cream is homogenized and then recombined with the skim milk. A less common practice is to simply homogenize whole milk. The use of these homogenized products results in the production of an open bodied, porous cheese structure that favors mold growth and sporulation (Morris, 1981). The resulting cheese is superior to that made from unhomogenized milks.

It is postulated that homogenization and incorporation of air in the milk up to the time of adding rennet increases porosity and results in a less dense, more porous cheese than when unhomogenized milks are used (Morris, 1981). But information is lacking about porosity per se and density of Blue cheese as influenced by the homogenization treatment.

Overall curd structure may be visualized as a para-casein sponge in which fat globules, bacteria (Kimber et al., 1974), whey (Green et al., 1981) and gases (Kalab et al., 1982; Lowrie et al., 1982) are held. Whether the fat globules exist singly or in clusters, and the amount of entrapped air, undoubtedly influence the structure and thus the physical parameters of cheese, such as porosity, size distribution of holes in cheese, and density.

Cheese porosity is defined, theoretically, as the ratio of the volume of the holes (pores) in cheese to the total volume of cheese (Noel et al., 1987). Our interest in porosity was inspired by the work of Green et al. (1981). They measured coarseness of the protein matrix in curds and cheeses. This was done by printing micrographs of the matrix on transparent paper. For cheese samples, the micrographs were placed on a 28 x 19 line rectangular grid (Green et al., 1981). "The black parts of the photograph (casein) made the grid invisible, but the grid lines could be seen through the clear parts of the photograph (fat, whey, or holes). We simply counted the number of bits of grid line visible, ignoring their length. The frequency of phase change was (count x

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2) / total length of lines on the grid in mm. The coarseness was defined as the reciprocal of the frequency. Our prints were 72 x 48 mm and total length of lines on grid in mm" (Green, M.L. personal communication, 1987.).

To measure porosity accurately, all sections of cheese would need to be examined. Thus, in practice, the method for coarseness by Green et al. (1981), or similar approaches are feasible approximations to actual porosity.

Although pores have been noted in cheese structures and milk gels by Eino et al. (1976), Glaser et al. (1980), Green et al. (1978, 1981), Kalab and Emmons (1978), and by Kalab and Harwalkar (1973), porosity as such has not been measured.

Yiu (1985) studied fat distribution in commercial Blue cheese. She found large fat globules in the vicinity of the mold, while the fat globules away from the mold were smaller. It is not known whether or not the cheese was made from homogenized milks or homogenized creams.

Homogenization is essential to combine butter oil efficiently with reconstituted nonfat dry milk and to improve the quality of cheese over the use of un-homogenized mixtures. Comparability of resulting products with regular homogenized cream and milk in the manufacture of Blue cheese does not appear to have been investigated.

The literature on cheese density seems to be restricted primarily to work by Mayes and Radford (1983), who determined density in Cheddar cheese by determining the weight in air and the weight suspended in a liquid. They obtained values ranging from 1.073 to 1.078 g/mL at 20°C, and from 1.088 to 1.096 g/mL at 8°C.

In view of the aforementioned, the purpose of this work was to obtain information on porosity, density and fat distribution in Blue cheeses made from homogenized mixtures of butter oil and reconstituted nonfat dry milk.

Methods and Materials

Materials

The following materials were used (sources are indicated in parenthesis); butter oil (Level Valley Dairy Products, West Bend, WI); grade A low heat nonfat dry milk (NDM) (Mid-America Dairymen, Inc., St. Paul, MN); calf rennet (Pfizer, Inc., Milwaukee, WI); calcium chloride (Dairyland Food Laboratories, Inc., Waukesha, WI); Penicillium roqueforti powder (Dairyland Food Laboratories, Inc., Waukesha, WI); methylene blue and eosin (Hartman-Leddon Company, Philadelphia, PA); potassium hydroxide (Allied Chemical, General Chemical Division, Morristown, NJ); Sudan III dye (Sigma Chemical Company, St. Louis, MO); ethanol (Midwest Grain Products, Atchison, KS); acetone (Chemical MGF Corp., Gardena, CA); kerosene (Chemical Warehouse, University of Minnesota, Minneapolis, MN); monochlorobenzene and osmium tetroxide (Aldrich Chemical Company, Inc., Milwaukee, WI); formaldehyde (Hawkins Company, Minneapolis, MN); hematoxylin (E.M. Science, Cherry Hill, NJ); "hemo De" (a deparaffinizing mixture of terpene, mineral oil, and butylated hydroxy-anisol) (PMP, Medical Industries, Los Angeles, CA); sodium phosphate dibasic, hydrochloric acid and glacial acetic acid (Mallinckrodt, Inc., Paris, KY); monobasic sodium phosphate (Columbus Chemical Industries, Inc.,

Columbus, WI); glycerin, aluminum ammonium sulfate, sodium iodate and Permout Mounting Medium (Fisher Scientific Company, Fair Lawn, NJ); and paraffin (Surgipath, Grayslake, IL).

Cheesemaking
Butter oil, sufficient to produce 4% or 14% fat blends with reconstituted (9% solids) NDM were prepared. NDM was thoroughly dispersed in water heated to 43.5°C. The appropriate amount of butter oil was then added to the milk. The mixture was heated to 54.5°C with agitation until all the butter oil was melted. Both 4% fat in reconstituted nonfat dry milk (4% RNDM) and 14% fat in reconstituted nonfat dry milk (14% RNDM) were homogenized at 13790 + 3448 kPa (2000 + 500 psi) and 54.5°C. All products used in the manufacture of cheese were double stage homogenized at 13790 + 3448 kPa and 54.5°C in a Gaulin Model 125/ 83MF12A, 384L/H (Manton Gaulin Company, Inc., Everett, MA). After homogenization the 14% fat RNDM was standardized with reconstituted NDM to 4% fat (14-4% RNDM). Both mixtures were held overnight at 4-5°C. Two 454 kg portions of normal raw whole milk (3.8% fat) were used to make the control cheese. One portion was separated to get 14% fat cream and skim milk. The cream was homogenized, then recombined with the skim milk to make 4% fat milk (14-4% R). The other portion of normal raw milk was separated and both 14% fat cream and skim milk were pasteurized, then 14% fat pasteurized cream was homogenized and added back to the pasteurized skim milk to make 4% fat milk (14-4% P). Blue cheeses were made from 182 kg of reconstituted nonfat dry milks and 454 kg of 14-4% R and 14-4% P using the method described by Morris (1981), except that calcium chloride was added to 4% RNDM, 14-4% RNDM and the 14-4% P milks. Cheese-making trials were done in triplicate.

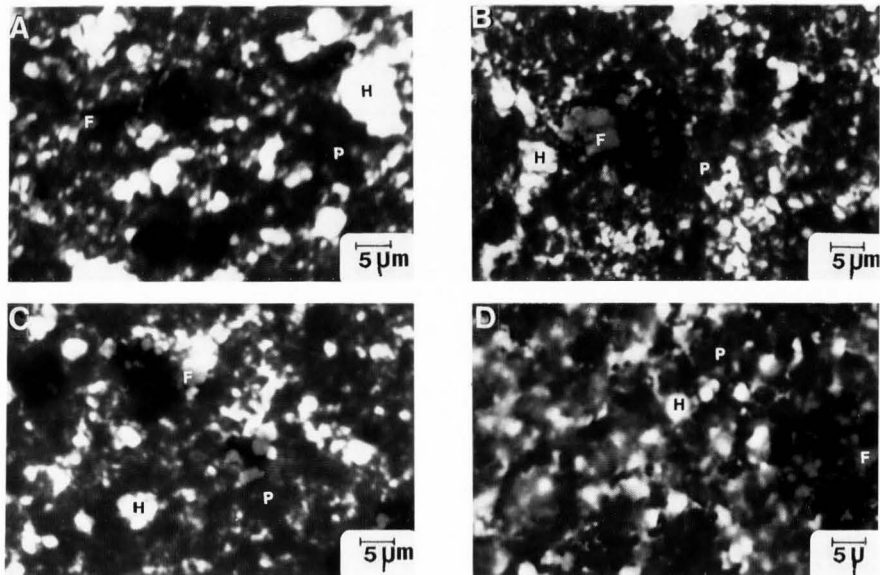
Table 1 describes the sample designations used in the rest of the paper.

Table 1. Sample designations.

| Designation | Blue cheese made from |
|-------------|--|
| 4% RNDM | Homogenized 4% fat in reconstituted nonfat dry milk |
| 14-4% RNDM | Homogenized 14% fat in reconstituted nonfat dry milk standardized to 4% fat with reconstituted nonfat dry milk |
| 14-4% P | Homogenized 14% fat pasteurized cream standardized to 4% fat with pasteurized skim milk |
| 14-4% R | Homogenized 14% fat raw cream standardized to 4% fat with raw skim milk |

Cheese porosity

Blue cheese samples were taken before salting, prepared and stained using the method of Hansson et al. (1966) with the following modification: sections were 4 μm thick prepared by using a cryogenic micrometer (Model CTD-International-Harris Cryostat



International Equipment Company, Needham Heights, MA). Two photomicrographs of each cheese were obtained using a camera (Nikon M-35S, 35 mm, Nikon, Tokyo, Japan) attached to a light microscope (Olympus BH, Olympus Optical Company Limited, Tokyo, Japan). A transparent micrograph was made for each photomicrograph obtained. Transparent

Table 2. The porosity of Blue cheeses. (Porosity = the total area of holes / the total area of the photograph.)

| Cheese samples | Porosity ^a | | Porosity ^b | SE |
|----------------|-----------------------|---------|-----------------------|--------|
| | Trial 1 | Trial 2 | | |
| 4% RNDM | 0.1259 | 0.0988 | 0.1124** | 0.0136 |
| 14-4% RNDM | 0.0716 | 0.0680 | 0.0698* | 0.0018 |
| 14-4% P | 0.0625 | 0.0598 | 0.0612* | 0.0014 |
| 14-4% R | 0.0530 | 0.0479 | 0.0514* | 0.0017 |

^a = mean of two photographs;

^b = mean of the two trials;

SE = standard error for the two trials;

* = not significantly different;

** = significantly different.

Figure 1. Light photomicrographs of sections of Blue cheeses:

A - 4% RNDM; B - 14-4% RNDM;

C - 14-4% P; and D - 14-4% R.

F = fat; P = protein matrix; H = holes

micrographs were used to measure the area of the holes (pores) using a Hipad digitizer (Houston Instrumental, Austin, TX) attached to an Apple II computer (Apple Computer, Inc., Cupertino, CA). The area of each hole on the micrograph was recorded in cm^2 and the total area of the photomicrograph was calculated in cm^2 . The ratio of the area of the holes to the area of photomicrograph was calculated and called porosity. Actual dimensions in μm^2 were used in the display of frequency distributions and in statistical calculations. This experiment was duplicated.

Specific gravity of cheese

Cheese samples were cut into 1 cm cubes. Four cubes were used for each type of cheese. The method described by Stoll (1966) to measure the specific gravity of curd using different solutions of kerosene and monochlorobenzene was used to measure specific gravity. The experiment was triplicated.

Distribution of fat in cheese

Cheese pieces (1 x 1 x 0.3 cm) were fixed in 10% buffered formalin solution (Hansson et al., 1966) and postfixed in 1.5% osmium tetroxide to stabilize the fat and stain it black. Routine Harris hematoxylin and eosin method (Luna, 1968) was used for

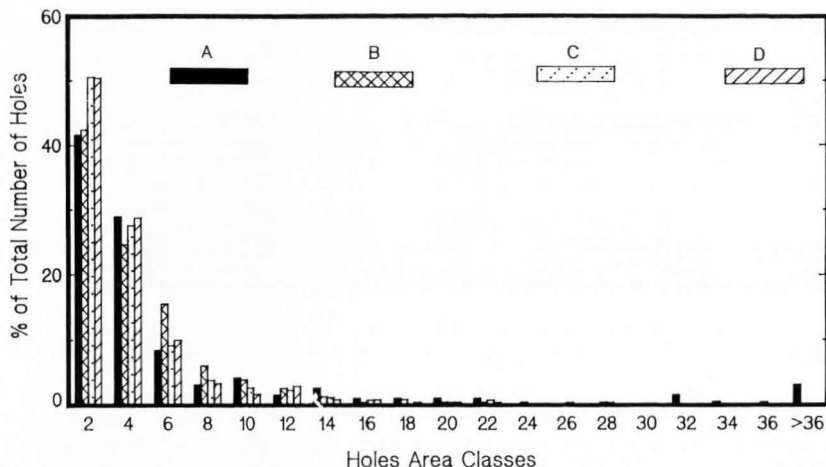


Figure 2. Size frequency distribution of holes (area in μm^2) in blue cheeses:
 A - 4% RNDM; B - 14-4% RNDM; C - 14-4% P; D - 14-4% R.
 The number (% of total number) is given per $\frac{1}{2} \mu\text{m}^2$ class widths.

staining and preparing the cheese on the slides except that the sections were not dipped in ammoniac or lithium and hemo De was substituted for xylene. The distribution of fat in cheese was studied using two from each of the four cheese types and photomicrographs were obtained using a Nikon camera attached to a light microscope. This experiment was duplicated. Therefore, four slides were evaluated for each type of cheese by counting the number of clusters in 8 fields for each slide.

Results and Discussion

Porosity of cheese was calculated by the ratio of total area of holes and the area of cheese observed. Holes as measured in this study contained whey or gases. Cheese made from 4% RNDM had the most porous structure and was significantly (P less than 0.05) different from other cheeses (Table 2 and Figure 1).

The frequency distribution of holes in the Blue cheeses is shown in Figure 2. About 50% of the total holes were less than $2 \mu\text{m}^2$ in Blue cheeses made from 14-4% R and 14-4% P, while cheeses made from 14-4% RNDM and 4% RNDM had only about 40% of the total holes less than $2 \mu\text{m}^2$. Cheese made from 14-4% R did not have any holes larger than $22 \mu\text{m}^2$, while cheeses made from 14-4% P, 14-4% RNDM and 4% RNDM had 0.76, 1.29 and 5.27% of the total holes larger than $22 \mu\text{m}^2$, respectively. Cheeses made from 4% RNDM were the only ones that had holes larger than $36 \mu\text{m}^2$ (3.16%).

Specific gravity of cheese

The mean specific gravities for each of the three trials along with the overall means are shown

in Table 3. Cheeses made from 4% RNDM had the lowest specific gravity and were significantly different (P less than 0.05) from the other cheeses.

Specific gravity values varied from 1.0339 to 1.0520 at 32°C . According to Mayes and Radford (1983), Cheddar cheese varies in density from 1.073 to 1.078 g/ml at 20°C and from 1.088 to 1.096 g/ml at 8°C . Thus, Blue cheese is much lighter than Cheddar cheese. Not surprising, the most porous cheese had the lowest specific gravity (cheese made from 4% RNDM) and the cheese that had the least porous structure had the highest specific gravity (cheese made from 14-4% R). This suggests that many of the holes contained gases and not whey.

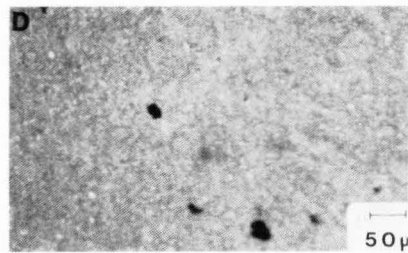
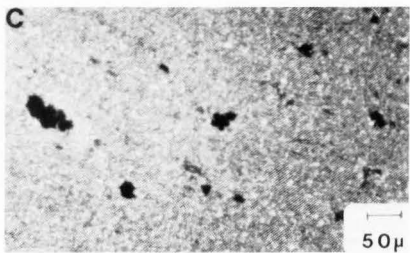
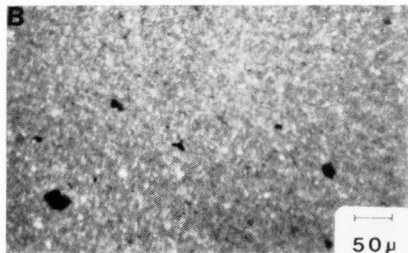
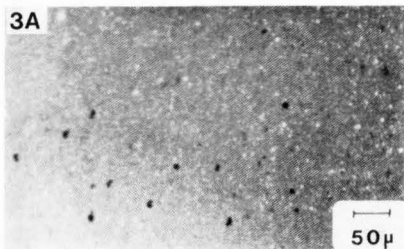
Distribution of fat in cheese

The distribution pattern of fat globules was studied in Blue cheeses made from the homogenized mixtures of 4% RNDM, 14-4% RNDM, 14-4% R and 14-4% P. The terminology to be used concerning the association of two or more fat globules is that of Mulder and Walstra (1974), whose plate 3 clearly shows the difference between flocs and homogenization clusters. The distribution of fat in Blue cheeses made from 14-4% RNDM, 14-4% R and 14-4% P was not uniform and existed mostly as clusters (Figs. 3B, C and D). The non-uniformity of distribution is similar to other cheeses (Hall and Creamer, 1972; Taranto et al., 1979). In contrast, fat in cheese

Figures 3 and 4 (on the opposite page). Light photomicrographs of sections of Blue cheeses showing: Figure 3: examples of overall distribution of fat; and Figure 4: examples of cluster formation.

A - 4% RNDM; B - 14-4% RNDM; C - 14-4% R; and D - 14-4% P. The black particles are fat.

Blue cheese porosity, density and fat dispersion



Bars = 50 μm (for all figures in Fig. 3).

Bars = 5 μm (for all figures in Fig. 4).

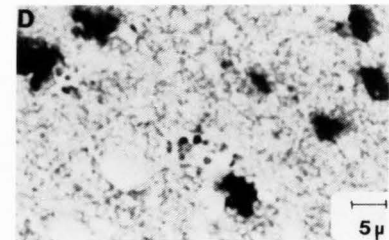
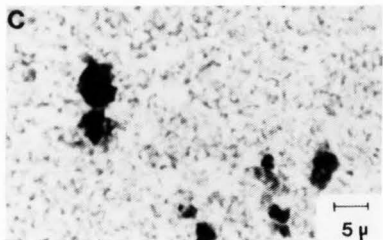
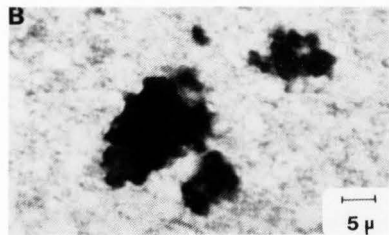
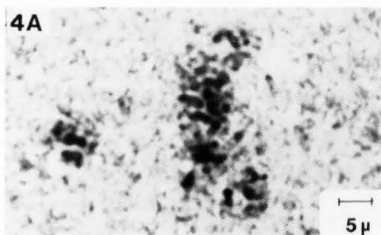


Table 3. The specific gravity of cheeses.

| Cheese samples: | Specific Gravity ^a | | | Specific Gravity ^b | SE |
|-----------------|-------------------------------|---------|---------|-------------------------------|--------|
| | Trial 1 | Trial 2 | Trial 3 | | |
| 4% RNDM | 1.0347 | 1.0358 | 1.0347 | 1.0351** | 0.0006 |
| 14-4% RNDM | 1.0449 | 1.0460 | 1.0430 | 1.0446* | 0.0012 |
| 14-4% P | 1.0453 | 1.0471 | 1.0453 | 1.0459* | 0.0006 |
| 14-4% R | 1.0483 | 1.0498 | 1.0471 | 1.0484* | 0.0006 |

^a = mean of four samples of cheese for trial; ^b = mean of the three trials; SE = standard error of the three trials; * = not significantly different; ** = significantly different.

Table 4. Fat distribution (clusters) in Blue cheeses.

| Cheese | Number of clusters ^a | | Number of clusters ^b | SE |
|----------|---------------------------------|---------|---------------------------------|------|
| | Trial 1 | Trial 2 | | |
| 4% RNDM | 2.0 | 2.0 | 2.0** | 0.00 |
| 14% RNDM | 5.0 | 5.0 | 5.0* | 0.00 |
| 14-4% P | 4.0 | 4.0 | 4.0* | 0.00 |
| 14-4% R | 5.0 | 4.0 | 5.0* | 0.5 |

^a = mean of the number of clusters in 16 fields for each trial; ^b = mean of the number of clusters in 32 fields in the two trials; SE = standard error of the two trials; * = not significantly different; ** = significantly different.

made from 4% RNDM was distributed more uniformly in the form of single globules (Fig. 3A) or as floccules (Fig. 4A), although a few clusters were present. Cheeses made from 14-4% RNDM, 14-4% R and 14-4% P had many clusters of fat entrapped in casein matrix (Figs. 4B, C and D). The number of clusters was significantly different from cheese made from 4% RNDM (P less than 0.05) as shown in Table 4. These results would be predicted from the work of Ogden et al. (1976), who found that homogenization of cream induced the clustering of fat globules due to sharing of fat globules by casein micelles. Clusters formed by homogenizing 14% fat mixtures were not dispersed by the second stage homogenization or by standardizing to a 4% fat mixture (unpublished data, Kebary, K.M.K.). Similarly, Knoop and Peters (1972) found in Camembert cheese made from partly homogenized milk that fat clusters were embedded in the casein matrix. As noted, cheese made from 4% RNDM had a few small clusters and floccules. In preliminary work on changes in the distribution pattern of fat globules in homogenized raw milk, homogenized pasteurized milk and in the homogenized 4% RNDM mixture left for 48h at 10°C, there was a slight increase in the number of floccules. This observation does not account for the relatively greater number of floccules observed in the cheese. Perhaps agitation of the milk, or other factors

during manufacture results in more aggregation of this type. Also, aggregation of fat globules was observed in Cheddar cheese (Taranto et al., 1979).

In conclusion, cheese made from 4% RNDM had the highest porosity and lowest specific gravity and was significantly different from cheeses made from 14-4% RNDM, 14-4% R and 14-4% P. Fat distribution was more uniform in cheese made from 4% RNDM than cheese made from 14-4% RNDM, 14-4% R and 14-4% P. Many clusters were noted in cheeses made from the 14-4% products.

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porosity decreases. It would appear to be possible to calculate the weight of air from the specific gravity if the composition of the cheese is also known. The formula is:

$$S = \frac{\%F + \%N + \%W + \%air}{(\%F / SF) + (\%N / SN) + (\%W / 1) + (\%air / S \text{ air})}$$

where S = specific gravity, F = fat, N = solids not fat and W = water. Using the S determined by experimentation, S of F = 0.93, S of N of 1.6007, S of air is 0.001205 (at 20°C and atmospheric pressure for dry air), and the cheese composition at the time the S was determined, the amount of air can be calculated by solving for % air. Unfortunately, we measured specific gravity of the cheeses before the cheeses were salted and before mold grew in them and determined cheese composition after salting, thus we do not have the needed information. We plan to do this experiment.

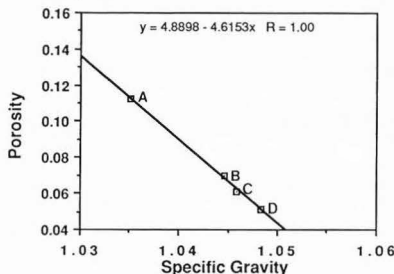


Figure 5. The relationship between porosity and specific gravity for the four cheeses used in these experiments:

A - 4% RNDM, B - 14-4% RNDM,
C - 14-4% P, and D - 14-4% R.

L.K. Creamer: Could porosity have been measured by weighing the cheese, weighing the cheese submerged in oil and then evacuating the cheese while submerged and weighing the degassed cheese?

Authors: Such an approach might be feasible if the pores contained only gases. But if some contain gas (air, carbon dioxide or ammonia) and others contain whey, the problem becomes more complicated. How well gases might be evacuated from the cheese matrix while immersed in oil would need to be explored.

L.K. Creamer: Why was porosity measured on only two sets of cheese?

M.L. Green: In Table 2, why only results of two trials when experiment was done in triplicate?

Authors: The overall project was concerned with the manufacture of Blue cheese from nonfat dry milk and butter oil. Three cheese-making trials were planned using the preparations indicated in this paper. Initially we planned on following acid development, sensory evaluation of the curds during manufacture, chemical composition of the cheeses, specific gravity

Discussion with Reviewers

M.L. Green: It should be possible to deduce the density of the contents of the holes from between-cheese comparisons of the proportions of holes and densities. This should help to identify hole contents. What does such an analysis reveal?

Authors: Your question led us to plot the means for porosity against the means for specific gravity to determine the regression equation and the correlation coefficient as shown in Figure 5. As would be expected, on average, as specific gravity increases,

of the cheeses, extent and distribution of mold growth, and chemical changes during ripening along with sensory evaluations. Trials were scheduled for the spring, summer, and winter. After the first trial we included porosity and fat distribution in the cheese to the parameters to be determined. To avoid possible complications of variations in salt content and mold growth in the cheeses, specific gravity, porosity and fat distribution were all determined on the cheeses just prior to dry salting. Cheeses from the first trial had been salted, punched, and mold had grown in them before we had decided to determine porosity and fat distribution. That is why specific gravity was run on the cheeses in the three trials and porosity and fat distribution were run only on the last two trials.

M.L. Green: In the absence of evidence that the fat globules are clustered in the milks at the start of cheesemaking, can the authors suggest why clustering should occur. Could the mode of aggregation of the casein have any influence?

Authors: We do have evidence that fat globules were clustered in the milks at the start of cheesemaking. This is a part of the unpublished work referred to in the text. The same is true in commercial manufacture as shown in Fig. 6. The creation of homogenization induced clusters is explained in the Ogden et al. (1976), and in the Mulder and Walstra (1974) references in the text. Since homogenization clusters are only redispersible with considerable energy, they are not broken up when the homogenized 14% fat mixtures are standardized to 4%. Ogden et al. (1976) also showed that the clusters stay intact after dilution. They also explain the effects of fat content, shareable surfactant (casein micelles) content, homogenization pressure, and surfactant particle size on the extent of clustering.

L.V. Ogden: Does the more open cheese that results from homogenization of 4% milk actually result in better mold growth and more acceptable product?

Authors: All of the cheeses made were very acceptable. Mold did grow more abundantly in the cheese made from the 4% fat containing butter oil-reconstituted nonfat dry milk blend than in the others. We did not use 4% homogenized normal milk but would expect similar results.

L.K. Creamer: On the basis of the present results would you recommend any particular manufacturing protocol?

Authors: For the manufacture of Blue cheese from mixtures of butter oil and reconstituted nonfat dry milk, i.e., 14-4% RNDM vs. 4% RNDM, based on porosity, specific gravity and mold growth, the 4% fat mixture would be preferred. But results from a consumer acceptance panel showed that there was no significant difference between the cheeses at four months of age, but at eight months the 14-4% RNDM cheeses were preferred. Since the cheeses were relatively comparable from a consumer viewpoint, the economics of homogenizing a lower volume of 14% fat product compared to homogenizing the 4% mixture would favor the manufacture from the 14-4% RNDM mixture using conventional methods.

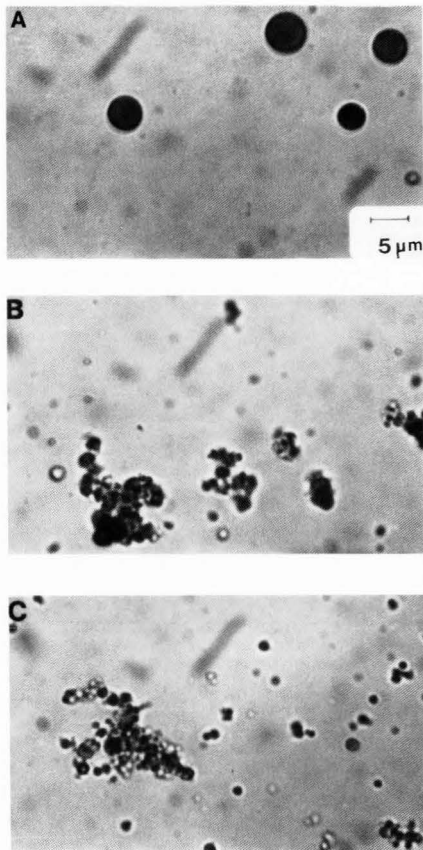


Figure 6. Effect of fat content on clustering and sizes of fat globules of normal milk (commercial samples) homogenized at 13790 + 3448 kPa. A - un-homogenized cream (14% fat); B - homogenized 14% fat cream; C - homogenized 14% cream diluted back to 3.4% fat with skim milk. The magnification is same in the three micrographs.