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Microstructure of Fat Free Plain Set-type Yogurt Containing Folic Acid

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Abstract

The objective of this study was to investigate the microstructure of folic acid-contained yogurt. Folic acids (25 and 50%) were added to milk preparation prior to pasteurization, then starter culture was added. The microstructure of yogurt containing folic acid was determined by the size of cluster of casein micelle using scanning electron microscopy and transmission electron microscopy. The cluster of casein micelle in yogurt containing folic acid were showed larger size than in control ($p<0.05$). In addition of 50% of folic acid, cluster of casein micelle per unit area was exhibited the highest number among tested yogurts. From these results, folic acid concentration of yogurt may be affected by mouth-feel of yogurt texture as well as the aggregation of casein micelle.

Key words : microstructure, fermentation, health, dairy, folic acid, yogurt

Introduction

The physico-chemical and sensory characteristics of folic acid fortified yogurts have been reported (Aryana, 2002; Boeneke and Aryana, 2003). During the initial stages in the manufacture of yogurt, the casein micelles in the fluid milk exist as suspended individual entities with net negative charges at the pH of the milk i.e 6.6~6.7 (Kosikowski, 1982). The lactic acid bacteria produce lactic acid which lowers the pH which in turn destablilzes the casein micelles (Kosikowski, 1982).

Casein micelles are comprised of submicelles that are held by bonds of calcium phosphate (Hojou et al., 1977). Calcium from the calcium caseinate colloidal suspension is gradually lowered to form calcium lactate a soluble salt. When the calcium and the pH lower to isoelectric point (pH 4.6~4.7) of the casein, the casein precipitates / coagulates by linking of the micelles in form of chains and clusters (Kalab et al., 1983) to a gel-like mass throughout the milk. It is in this protein matrix network that the liquid / whey phase is entrapped (Kalab et al., 1983).

Yogurts manufactured with high levels of folic acid resulted in a powdery mouth feel. This powdery mouth-feel may be due to increased localized protein aggregations. Addition of folic acid during the yogurt manufacture process might influence the sizes of the protein aggregates and their clusters. The objective of this work was to study the influence of folic acid on the microstructure of yogurt.

Materials and Methods

Yogurt Preparation

Plain set-type yogurts were manufactured with 0, 25, 50% of the 300 micrograms recommended daily allowance (RDA) for folic acid in a single cup of yogurt. Folic acid was added at 25% RDA at two stages i.e. before pasteurization viz. during the mix preparation and after pasteurization i.e. culture addition. Folic acid was also added at 50% of the RDA after pasteurization. Three replications were conducted.

Yogurt Fermentation

Fat-free plain set-type yogurt with no added sugar was manufactured according to Haque and Aryana (2002) in the Dairy Processing Plant, LSU Department of Dairy Science. Yogurt mixes were prepared in 3.785 L (one gallon) batches from

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fluid skim milk with 9% w/w nonfat solids. To increase total solids content in the mixes, 3% w/w nonfat dry milk and 0.5% w/w modified food starch (National starch, NJ, USA) were added. The mix was sweetened with 0.030% w/w aspartame (NutraSweet Co., Deerfield, IL, USA). Folic acid (Wright Nutrition Inc., Crowley, LA, USA) was incorporated at one quarter and one half of the recommended daily allowance of folic acid per 225 g cup of yogurt. Previously weighed ingredients were mixed into fluid nonfat milk and heated to 60℃ followed by homogenization with a two-stage homogenizer at a pressure of 3.43 MPa at the second stage and 13.73 MPa at the first stage. Homogenized mixes were batch pasteurized at 85℃ for 30 min and then rapidly cooled to 38℃ in an ice bath. Frozen culture concentrate of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (CH-3, yogurt culture, Chr. Hansen's Laboratory, Milwaukee, WI, USA) were weighed in an ethanol sterilized weigh boat which was air dried. After mixing, the inoculated yogurt mixes were filled into 250 g plastic cups and incubated at 38 + 2℃. Incubation was terminated at a pH of 4.5 which required approximately 6 h. The cups were chilled in an ice bath and then refrigerated at 5℃.

Scanning Electron Microscopy

Yogurt samples were cut into approximately 3 mm pieces and fixed in 1.4% v/v glutraldehyde in distilled water overnight in a refrigerator. Samples were equilibrated to room temperature and washed in 4 changes of room temperature distilled water over a 2 -minute period. Yogurt samples were dehydrated in a graded series of ethanol 35, 50% for 15 min each and in 70% ethanol overnight in a refrigerator. The following morning, samples were equilibrated to room temperature and dehydration was continued in 95% v/v ethanol for 15 min and 100% ethanol (3 changes over an hour). Yogurt samples were critical point dried using a critical point drying apparatus (Denton DCP-I, Denton Vacuum Inc, Moorestown, NJ, USA). Dried samples were mounted on double sided carbon sticky tape on aluminium stubs and sputter coated (Edwards S150 Sputter Coater, Edwards, UK) with gold palladium. Coated samples were stored in a desiccator. Yogurt samples were viewed using a Cambridge 260 Steroscan scanning electron microscope. Images were recorded on Polaroid type 55 P/N film (Polaroid Corp. Cambridge, MA, USA).

Determination of Cluster Size

Cluster size was determined on the positive prints by measuring using a ruler and converting to the respective micron scale.

Results and Discussion

The scanning electron microscope images of the yogurts are reported in Figs. 1 and 2. The control yogurt showed the network of casein micelles in chains and clusters (Fig. 1A). Similar observations were reported earlier (Kalab et al., 1983). Folic acid fortified yogurts viewed at a high magnification (Fig. 1. B, C, D) revealed that folic acid did not effect the size of the individual casein micelle that comprised the chains and the clusters.

At a lower magnification the casein micelle cluster size and number can be seen. Just as in the control (Fig. 2. A), the casein micelles were arranged in chains and clusters in the folic acid fortified yogurts as well (Fig. 2. B, C, D). The control yogurt had a smaller mean size of the casein micelle clusters compared to the folic acid fortified yogurts (Table 1). At high level i.e. 50% RDA, the size of the clusters were significantly larger compared to 25 and 0% of the RDA for folic acid. This may be because folic acid induced localized aggregation of the protein aggregates in yogurt. The stage of addition of folic acid, i.e before versus after pasteurization did not effect the size of the casein micelle clusters.

The number of the aggregate clusters did not increase with increased amount of folic acid (Table 2). Neither was the number of aggregate clusters impacted by the stage of addition of folic acid. The number of casein micelle clusters were higher ($p<0.05$) in the folic acid fortified yogurts compared to the control. Folic acid acidity induced a greater amount of protein aggregation in the product.

Astrid et al. (2003) reported formation of large disulfide-linked protein structures during acidification for the production of acid milk gels. Denatured whey proteins contributed to the protein network and the formation of additional disulfide bonds (Astrid et al., 2003). They further reported that sulfhydryl group disulfide bond interchange reactions took place at ambient temperatures and under acidic conditions. Microbial acidification for the preparation of dairy food gels has been reported earlier (Kosikowski, 1982).

Folic acid has appeared to have contributed to the micro-

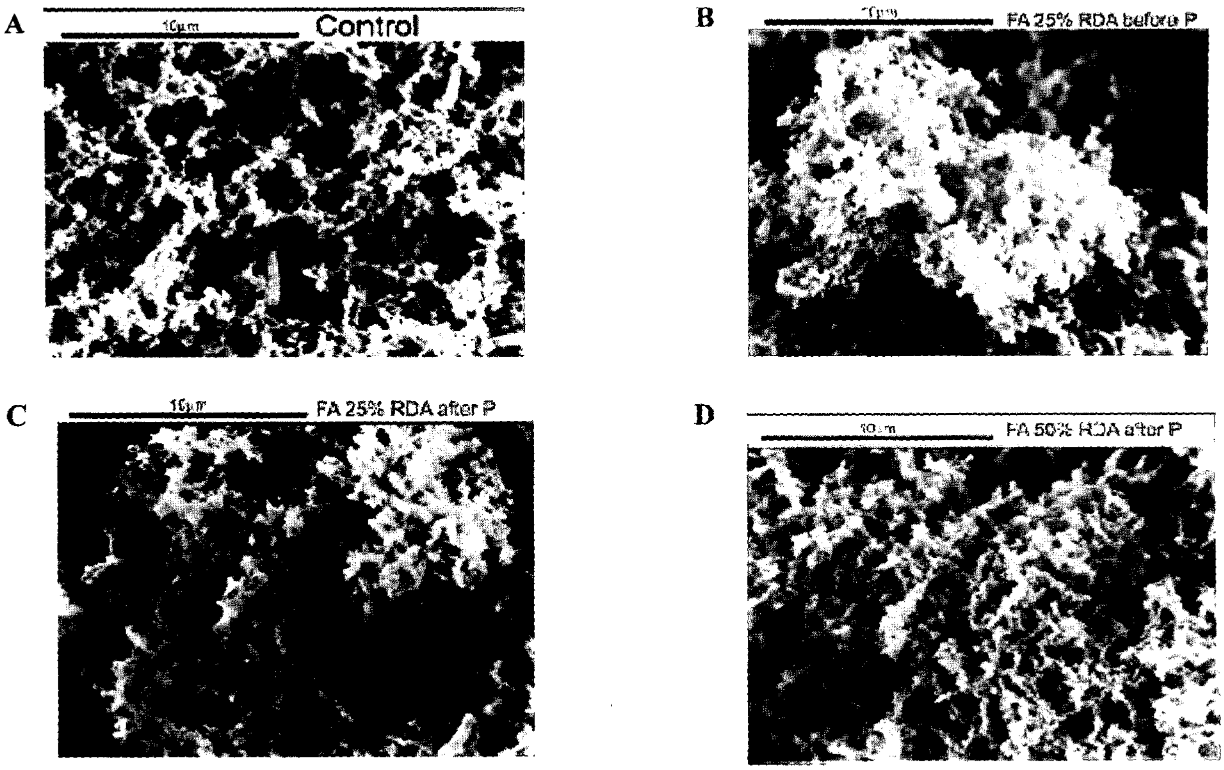

Fig. 1. **Scanning electron micrograph of yogurts at high magnification made using A) no folic acid / control, B) 25% RDA of folic acid before pasteurization, C) 25% RDA of folic acid after pasteurization, D) 50% RDA of folic acid after pasteurization.**

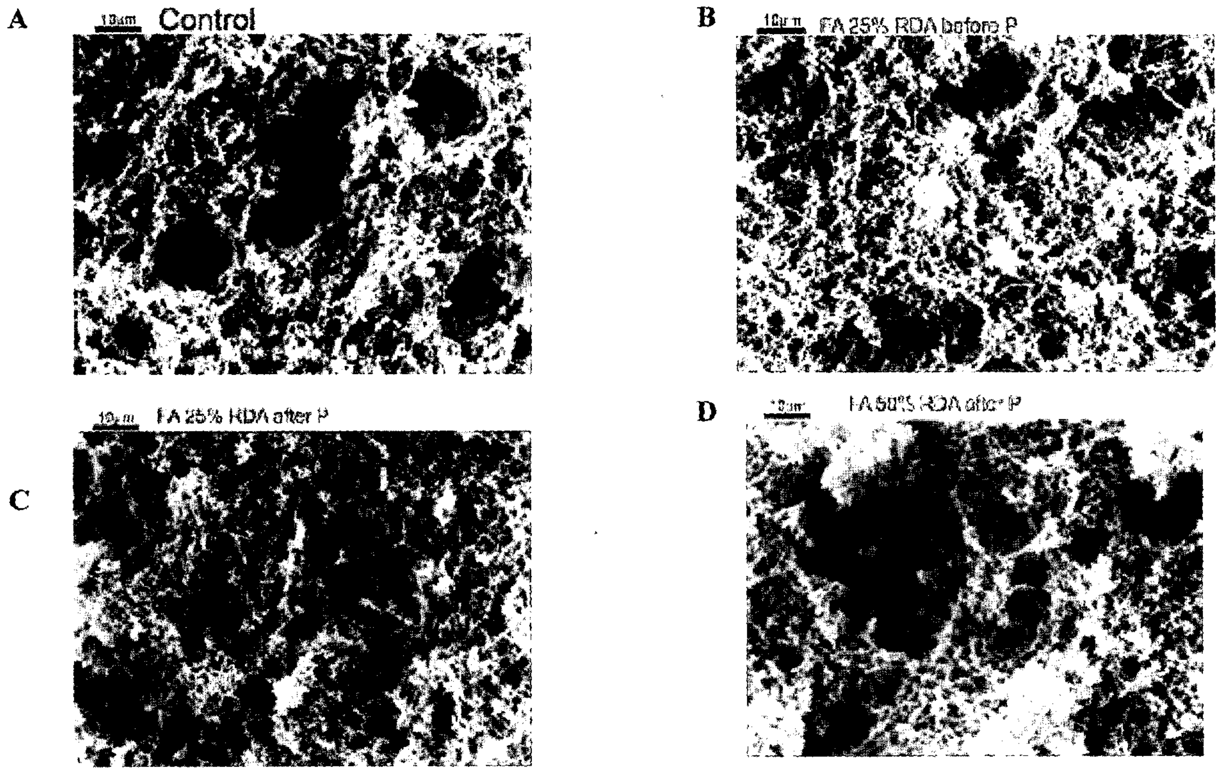

Fig. 2. **Scanning electron micrograph of yogurts at high magnification made using A) no folic acid / control, B) 25% RDA of folic acid before pasteurization, C) 25% RDA of folic acid after pasteurization, D) 50% RDA of folic acid after pasteurization.**

Table 1. Mean size of the casein micelle clusters

Treatment[1)]	Casein micelle cluster size (μm)
1	12.1±0.5[c]
2	13.2±0.7[b]
3	13.4±1.1[b]
4	14.8±0.9[a]

[a,b,c,d] Means with the same letter are not significantly different at $p<0.05$.

[1)] 1= Control, 2=folic acid incorporated at 25% RDA before pasteurization of mix (during mix preparation), 3= folic acid incorporated at 25% RDA after pasteurization of mix (during mix preparation), 4= folic acid incorporated at 50% RDA after pasteurization of mix (after culture addition).

Table 2. Mean number of the casein micelle clusters

Treatment	Number of casein micelle clusters per 100×100 μm^2 area of yogurt
1	4.3±0.7[b]
2	6.1±1.3[a]
3	6.5±1.1[a]
4	6.3±0.9[a]

[a,b,c,d] Means with the same letter are not significantly different at $p<0.05$.

1= Control, 2=folic acid incorporated at 25% RDA before pasteurization of mix (during mix preparation), 3= folic acid incorporated at 25% RDA after pasteurization of mix (during mix preparation), 4= folic acid incorporated at 50% RDA after pasteurization of mix (after culture addition).

bial acid produced to enhance the protein aggregation effects of the acid to result in larger cluster sizes and numbers.

In conclusion, folic acid addition increased the size of the casein micelle clusters and increased the number of clusters of casein micelles. This helps explain why yogurts contained with high levels of folic acid had a powdery mouthfeel.

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