Effect of Sweeteners on the Microstructure of Yogurt

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The objective was to determine the effect of a peptide sweetener, Aspartame, compared to a carbohydrate sweetener, sucrose, on the microstructure of yogurt. Microstructure was determined by transmission and scanning electron microscopy. Without the sweeteners, casein micelles, that make up the yogurt matrix, were observed in single longitudinal polymers. When Aspartame was used, casein micelles formed double longitudinal polymers. In comparison sugar caused casein micelles to form clusters. Data show that type of sweetener impacts state of association of casein micelles and thus effects microstructure.

Keywords: cultured, dairy, fermentation, structure, sweetener

Principal component analysis of yogurt revealed high consumer acceptability for sweetness characteristics and lower consumer acceptability for samples with plain yogurt character (Barnes *et al.*, 1991). Hence consumers want the sweetness in yogurt but health reasons dictate decreased intake of calories and reduction of sugars. To decrease calories in yogurt, artificial sweeteners have been incorporated (Nabros & Gelandi, 1986; Keating & White, 1990). Farooq and Haque (1992) reported that nonfat yogurts with Aspartame, a popular peptide based sweetener, had half the calories per serving compared to regular yogurt containing 3.25% fat and 4% sucrose. Mozaffar and Haque (1992) and Haque (1993) have shown that amphipathic peptides (like Aspartame) effect protein-protein association, which is a primary requisite during gel formation. On the other hand sugars such as sucrose are traditionally used by enzymologists to stabilize enzyme proteins (Choi & Kosikowski, 1985). Such hydrophilic solutes impact water structuring and hence influence protein-protein association (Kalab & Harwalkar, 1972). This in turn may impact the amount of entrapped water or pore dimensions. The size of pores of the casein micelle network is a major factor affecting susceptibility of yogurt to syneresis (Harwalkar & Kalab, 1986). Various factors such as heating of milk, total solids content, pH, lactic acid bacteria and thickening agents have been reported to affect the microstructure of yogurt (Kalab *et al.*, 1983). It would be interesting to see the effect of a peptide sweetener, Aspartame, compared to a carbohydrate sweetener, sucrose, on yogurt gel network formation.

Yogurt was made as described earlier by Farooq and Haque (1992) with slight modification. Yogurt mixes were prepared in 1 l batches. Fluid skim milk with 9% milk solids not fat (MSNF) was obtained from the Mississippi State University Dairy Plant. Other ingredients added on weight per volume basis were 0.5% modified food starch, 3% non fat dry milk (NDM), 0.02% Aspartame (NutraSweet Co., Deerfield, IL), and 3% maltodextrin or 3% sugar. The overall total solids (TS) content was 15.5% (Table 1). Every effort was taken to keep TS constant to avoid its

impact on microstructure (Kalab *et al.*, 1983; Harwalkar & Kalab, 1986). Ingredients were thoroughly mixed in fluid skim milk heated to 60°C and homogenized at 35.15 and 140.62 kg/cm². This was followed by batch pasteurization at 85°C for 30 min and rapid cooling to 38°C. Thawed culture concentrate (# 380) comprising of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Chr. Hansen=s Lab. Milwaukee, WI), were then added to inoculate the base mixes that were then poured into 250 g cups and incubated at 38°C. After approximately 10 h, or when the pH reached 4.4, the incubation was terminated by placing cups in an ice bath followed by refrigeration at 5°C.

Yogurt samples for scanning electron microscopy were taken from the center of the cup. Yogurt was cut with the help of a sharp blade into 1 mm³ pieces. Sample processing was according to Aryana and Haque (2001). Samples were fixed in 1.4% glutaraldehyde (Sigma, St. Louis, MO) overnight in a refrigerator. These were then washed in six changes of water over a two min period. Samples were dehydrated in a graded series of ethanol (35, 50, 70, and 95%; 15 min per change). Dehydration was continued in 100% ethanol (Electron Microscopy Sciences, Fort Washington, PA), (three changes over one hour) followed by critical point drying (Polaron critical point dryer, Ted Pella, Inc., Redding, CA) and mounted with double sided carbon sticky tape (Electron Microscopy Sciences) on aluminum stubs (Electron Microscopy Sciences). This was followed by sputter coating (Polaron sputter coater, Watford, UK). Samples were viewed Cambridge S 300 SEM (Leo, Electron Microscopy Inc. Thornwood, NY).

Sample processing for transmission electron microscopy was also according to Aryana and Haque (2001). Yogurt was cut with the help of blade into 1 mm³ pieces. Samples were then fixed in 1% glutaraldehyde (Sigma) in 0.1 M potassium phosphate buffer (Fisher Scientific, Fair Lawn, NJ) (pH 7.2) overnight in a refrigerator, rinsed three times (10 min each) in buffer (0.1 M potassium phosphate buffer pH 7.2) and post fixed in 1% osmium tetroxide (Fisher Scientific) for 30 min. The fixed specimens were rinsed three times in distilled water, stained with 1% aqueous uranyl acetate (Fisher Scientific) for 30 min and rinsed three times over a 15 min period in distilled water. Dehydration was in 35, 50, 70, 95% ethanol for 15 min, and finally in 100% ethanol

(Electron Microscopy Sciences) for an hour (three changes). The dehydrated samples were placed in propylene oxide (Electron Microscopy Sciences) (two changes over an hour). Infiltration was done. These were then placed overnight in an oven at 70°C for 15 h. Thin sections were cut using a Reichert-Jung ultramicrotome (Cambridge Instruments Inc. Buffalo, NY) and collected on formvar (Electron Microscopy Sciences) coated copper grids (Ernest F. Fullam, Inc. Schenectady, NY) and stained with uranyl acetate (Fisher Scientific) and Reynolds lead citrate (J.T.

Baker Chemical Co. Phillipsburg, NJ) (Reynold, 1963). Sections on the grid were washed in distilled water, air dried and in a JEOL 100 CX II TEM (JEOL, Peabody, MA).

The experiment was conducted using a completely randomized design. Three replications were conducted.

Transmission electron microscopic (TEM) observations revealed that in absence of any sweetener, the yogurt matrix was predominantly casein micelles arranged in longitudinal polymers (Fig. 1A). Yogurt made with Aspartame caused casein micelles to arranged themselves in double longitudinal polymers (arrows) (Fig. 1B) indicating an increased tendency to polymerize. Aspartame is a methyl ester of L-aspartic acid and L-phenylalanine (Israelow, 1983; Vetsch, 1985). Thus it is a highly soap-like molecule with distinctly separate hydrophobic and hydrophilic zones. Such molecules influence hydrophobic interactions that play an important role during thermal gelation (Tanford, 1980; Busk, 1984).

Yogurt made with sugar showed a matrix in which casein micelles associated in form of clusters (arrows) in some spots

Table 1. Ingredients for yogurt (% by weight).

Ingredients	Yogurt with sugar	Yogurt with aspartame	Yogurt without sweetener
Fluid skim milk solids	9	9	9
Non fat dry milk	3	3	3
Starch	0.5	0.5	0.5
Maltodextrin	0	2.98	3
Sugar	3	0	0
Aspartame	0	0.02	0

Fig. 1. Transmission electron micrograph of A) yogurt made from skim milk in absence of sweetener. Casein micelles arranged in longitudinal polymers. Bar represents 2 µm. B) yogurt in presence of aspartame. Casein micelles seen in double longitudinal polymers. Bar represents 2 µm. C) yogurt in presence of sugar. Casein micelles in clusters in some spots. Bar represents 2 µm. D) yogurt made from whole milk in absence of sweetener. Fat globules (f) seen associated with casein micelles.

Fig. 2. Scanning electron micrograph of A) yogurt made with aspartame. Casein micelles predominantly in double longitudinal polymers (arrows). B) yogurt made with sugar. Casein micelles seen in clusters in some spots.

(Fig. 1C) causing the matrix to form large void spaces implying greater entrapment of water.

The difference between yogurts made from skim milk and whole milk was the relative absence of fat globules in skim milk (Fig. 1A) compared to whole milk (Fig. 1D). These fat globules (f) were associated with the casein micelles. Aguilera and Kinsella (1991) reported compatibility of fat globules and casein micelles in protein matrix of filled gels and in whey protein isolate gel matrix (Yost & Kinsella, 1992). Tamime *et al.* (1984) reported that yogurts made with anhydrous milk fat had shorter casein micelle chains compared to yogurts made with protein based fat substitute Simplesse 100.

Number of rows of casein micelles were higher in yogurt with sweeteners compared to control. Scanning electron microscopic (SEM) observations confirmed the TEM observations. Casein micelles were predominantly in double longitudinal polymers (arrows, Fig. 2A) when Aspartame was used. Sugar caused casein micelles to cluster (Fig. 2B).

Kalab and Emmons (1975), when studying the effects of

thickening agents on the microstructure of yogurt, reported that starch and carrageenan contributed a considerable change to the microstructure. They observed yogurt with 0.4% carrageenan gave clusters of casein micelles connected with thin fibers. The present data show that use of Aspartame, even at the small usage level of 0.2% (w/v) compared to 3% w/v usage level for sucrose, caused significant changes in the microstructure of yogurt gels.

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