Asian Journal of Chemistry

# Effect of Power Ultrasound and Microstructure Change of Casein Micelle in Yoghurt

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In present studies, the changes in micro structural of yoghurt casein micelles during application power ultrasound has been investigated. The gel structure of yoghurt contributes strongly to its characteristic texture which is an important organoleptic property perceived by the consumer and strongly dependent on the distribution of porosity in network of case gel. The experimental results demonstrated that the microstructure of ultrasound treated milk yoghurt for 0, 5, 10, 15, 20, 25 and 30 min at 20 KHz frequency, had more interconnected chains of regularly-shaped casein micelles, with reduced particle size as well as more spherical in shape, exhibiting a smooth more regular surface and presenting more uniform size distribution and more homogenous porosity. This work offer the power ultrasound improved the gel texture and useful in manufacturing process of yoghurt.

Key Words: Ultrasound, Microstructure change, Casein micelle, Yoghurt.

### **INTRODUCTION**

Casein micelles in milk are aqueous protein colloidal particles and are essential for the production of fluctuated and gelled products such as yoghurt, cheese and ice-cream. The structural properties and the stability of yoghurt are quite complicated and a number of factors influence the results, related to chemical compositions and processing conditions<sup>1</sup>. Casein micelle is a polymerization model that envisages two cross-linking routes for assembly of the micelle. They are cross-linked by individual caseins through hydrophobic regions of caseins and bridged involving colloidal calcium phosphate<sup>2</sup>.

The formation and integrity of the micelle is controlled by a balance between attractive and repulsive faces in casein micelles<sup>2,3</sup>. Whey separation and several rheological changes have been implicated to excessive rearrangement of particles making up the gel network before and during gel formation<sup>2</sup>. Yoghurt is formed during the slow lactic fermentation of milk lactose by the thermophilic lactic acid

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bacteria. The rigidity of yoghurt gels is an important texture attribute and is effective in preventing whey separation. The power ultrasound proved to be useful in the homogenization of casein micelles and disintegrated them into smaller (diameter) particles and decrease in milk turbidity and increase milk viscosity<sup>3-6</sup>.

Furthermore, intense cavitations considerably reduced particle size micelles and induced more uniform size distribution of casein micelle in yoghurt. This study is devoted to investigate milk treatment effect on the microstructure of yoghurt gels and to understand the yoghurt microstructure to establish relationship between ultrasound treatment and another influence factors.

### **EXPERIMENTAL**

**Yoghurt preparation:** Skim milk (0.0-0.2 % fat and 9.17-9.20 % total solids with skim milk powder increased the total solids content to 14 % is heated at 85 °C for 0.5 h. The milk was cooled in a water batch to 43 °C for the yoghurt preparation and was treated with ultrasound for 0, 5, 10, 15, 20, 25, 30 min at 20 KHz frequency, three replication were carried out for each test. The processed milk was inoculated (0.2 % v/v) with two freeze dried yoghurt starter cultures, a mixture of *Staphylococcus thermophilus* and *Lactobacillus bulgaricus*. The fermentation was carried out at 43 °C and fermentation process was monitored by continuous recording of pH values to measure the acidification rates, during fermentation until the pH value reached  $4.6 \pm 0.1$ . The yoghurt was cooled in an ice bath and then stored at 4 °C for 15-16 h.

**Ultrasonic equipment:** Ultrasonic piezoelectric source use (Dr. Hielscher, Germany) irradiated at 20 KHz, sonotrude immersed 2 cm below the free surface of milk.

**Microstructure analysis:** Yoghurt sample were kept over night in 2 % glutaraldehyde and 2 % paraformaldehyde and 0.05 M phosphate buffer at 4 °C for fixation. These samples were rinsed three times with 0.05 M phosphate buffer for 10 min, each, following samples were post fixed in 2 % osmium tetroxide for 1.5 h, rinsed twice with 0.05 M phosphate buffer for 10 min, each and dehydrated with increasing concentrations of ethanol each (30, 50, 70 and 95 %, 3 time at 100 %) and dried then with carbon-coated in vacuum using. Yoghurt was examined with a scanning electron microscopy (SEM) (1450 Vp model, Germany).

#### **RESULTS AND DISCUSSION**

The scanning electron micrographs of yoghurt gel made with ultrasonic waves are presented in Figs. 1-7 depending on different time of ultrasound.

Treatment led to differences in organization of the gel network. Ultrasoundtreated milk yoghurt microstructure (Figs. 2-7) is composed of chains casein micelles, forming an regular network enclosing the void spaces, that the pores are very large compared the yoghurt made without treating of ultrasound, (Fig. 1), micelles are less inter connected the protein network appears in dark gray and void spaces in white, Vol. 21, No. 2 (2009)

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Fig. 1. Scanning electron microscopy micrograph of yoghurt fermented untreated (0 min)



Fig. 2. Scanning electron microscopy micrograph of yoghurt fermented untreated (5 min)



Fig. 3. Scanning electron microscopy micrograph of yoghurt fermented untreated (10 min)

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Fig. 4. Scanning electron microscopy micrograph of yoghurt fermented untreated (15 min)



Fig. 5. Scanning electron microscopy micrograph of yoghurt fermented untreated (20 min)



Fig. 6. Scanning electron microscopy micrograph of yoghurt fermented untreated (25 min)

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Fig. 7. Scanning electron microscopy micrograph of yoghurt fermented untreated (30 min)

the fine microstructure of yoghurt from ultrasound milk can be attributed to the decrease of micelle size after treating ultrasound<sup>6-9</sup>.

As clear in figures ultrasound increased the number and strength of bonds between casein and another proteins such as whey proteins. The application of ultrasound leads to decrease in the mean hydrodynamic diameter of casein particles, with decrease in milk turbidity and lightness and probably an increase in viscosity of the milk<sup>8</sup>.

The presence of small particles explain the decrease in the apparent lightness in microstructure study. It is also observed in ultrasound treated milk held at 4 °C, that the micelles were fragmented, forming small particles. They are more spherical in shape, more regular surface and present more uniform size distribution. The use of ultrasound to introduce denaturizing aggregation and gel formation of milk proteins, has been studied by many researchers<sup>6-9</sup>.

The behaviour of protein under ultrasound implies that hydrophobic interactions and ionic effects are liable to disruption by ultrasound, while the formation of hydrogen bond is favoured by ultrasound. These bonds contribute to protein conformation and structural interactions in solution. Any changes associated with them resulted in modifications to the overall structure. The ultrasound treatment of milk induced a partial and irreversible dissociations of casein micelles and arrange of interaction may form a closer casein micelle structure<sup>10</sup>.

### Conclusion

The results showed that the use of ultrasound to treat milk before fermentation affected the micro-structure of yoghurt. The microstructure of ultrasound treated milk yoghurt shows more regular surface and more uniform size distribution which resulted in improved gel texture and viscosity. Thus, ultrasound treatment before fermentation is proved to be a better process for uniform consistent microstructure with less physical defects<sup>8-10</sup>.

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(*Received*: 26 June 2008; *Accepted*: 15 October 2008) AJC-6951