The effect of high-temperature heat treatment and homogenization on the microstructure of set yogurt curd networks

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SUPPLEMENTARY FILE

SUPPLEMENTARY MATERIALS AND METHODS

Set yogurt preparation

Culture LB81, which includes *Lactobacillus delbrueckii subsp bulgaricus* 2038 and *Streptococcus thermophiles* 1131, was used in this study. This starter culture has been used in the commercial production of Meiji Bulgaria Yogurt LB81 and other products in Japan. The culture LB81 stored at −80°C was inoculated (0.15%, w/w) into heated 10% (wt/wt) skim milk (95°C, 10 min) and then incubated at 37°C to reach pH 4.6.

Supplementary Figure S1 illustrates the procedure used for making the set yogurt. The yogurt mixture was obtained by mixing raw milk, skim milk powder, and water; it contained 3.0% (wt/wt) fat, 9.5% (wt/wt) SNF (solids-not-fat), and 3.6% (w/w) protein. The raw milk and skim milk powder were supplied by Meiji Co. (Tokyo) facilities. The yogurt mixture was pre-warmed at 75°C. Prior to the heat treatment described below, each mixture was homogenized (model H20, Sanwa Engineering, Hyogo, Japan) at 10 (first stage) + 5 MPa (second stage) or 35+5 MPa. The yogurt mixtures were then pasteurized at 95°C for 5 min or at 130°C for 2 sec. Heat treatment at 95°C for 5 min was conducted as vat heat treatment. The heat treatment at 130°C for 2 sec was conducted with an indirect plate exchange system (Powerpoint International, Saitama, Japan).

In this study, we defined the control condition as heat treatment at 95°C for 5 min and homogenization at 10 and 5 MPa. After heat treatment, the yogurt mix were cooled to 40°C and then inoculated with 3% (w/w) of the yogurt bulk starter culture. After the yogurt mix and yogurt bulk starter culture were mixed, 80 g of the mixture was placed in 100-mL polystyrene cups. The yogurt mix was fermented until the pH reached 4.6. After fermentation, the yogurts were stored at 5°C. Fermentation took approx. 3 hr to complete.

Particle size distribution

We determined the distribution of the sizes of the fat particles of the fat-containing yogurt mixture by a laser diffraction scattering method with a laser diffraction particle size analyzer (SALD2200, Shimadzu, Kyoto, Japan) as follows. Fat-containing yogurt mixture was added into the circulating cell of the apparatus containing deionized water. Triplicate measurements were taken at 25°C, and the surface-weighted mean diameter ($d_{3,2}$) and the volume-weighted mean diameter ($d_{4,3}$) were recorded.

We determined the distribution of the sizes of the casein micelles by using the non-fat yogurt mixture and a laser diffraction scattering method with a laser diffraction particle size analyzer (LS230, Beckman Coulter, Brea, CA, USA). Non-fat yogurt mixture was added into the circulating cell of the apparatus containing deionized water. Triplicate measurements were taken at 25°C, and the volume-weighted mean diameter ($d_{3,2}$) was recorded.

Assuming that the distribution of fat globules follows a normal distribution, we calculated the volume of fat globules smaller than 'x' μm (F_x: Suppl. Fig. S2) contained in 100 g of fat-containing yogurt mixture by using the mean value and standard deviation of the fat globules. The lower cumulative distribution of the fat globules of 'x' μm (F_x) was estimated with the use of the standard normal distribution table. (F_x) was approximated with the following equation:

$$(F_x) = [\text{fat}\ (\%) / \text{s.g.}_{\text{fat}}] \times P(x, \mu, \sigma)$$

where (F_x) is the volume of the fat globules smaller than 'x' μm contained in 100 g of yogurt mixture, s.g._{fat} is the specific gravity of the milk fat, and P(x, μ, σ) is the lower cumulative distribution.

Physical characterization of the yogurt

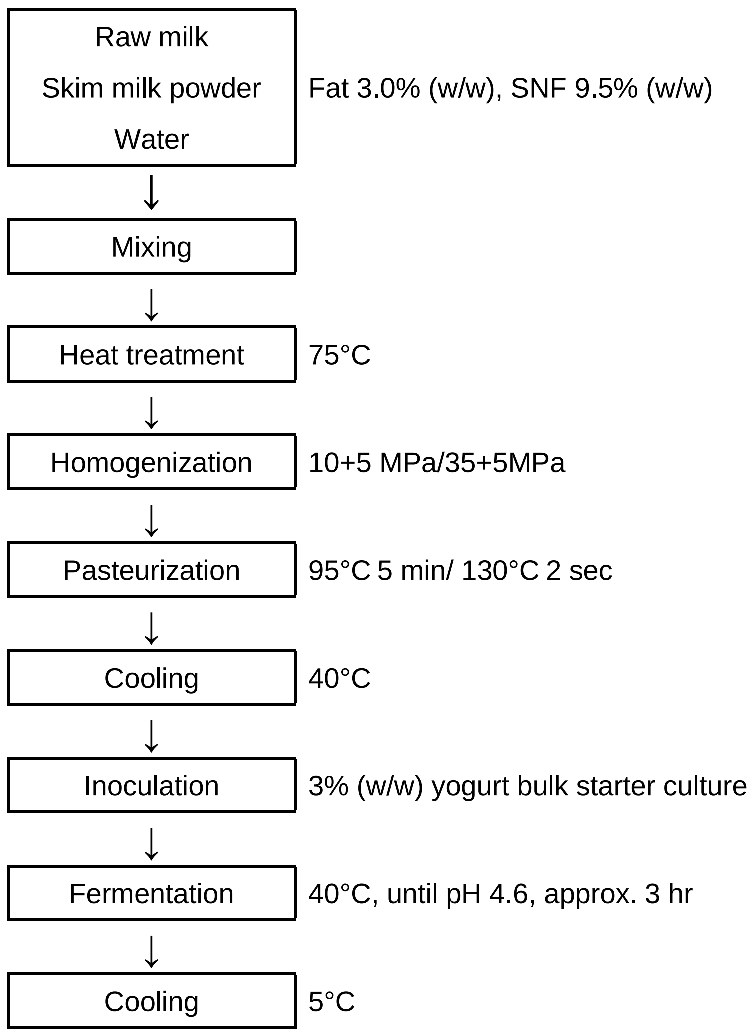
The physical characteristics of the yogurt were the curd firmness, penetration angle, and the degree of syneresis. The curd firmness of yogurt represents the yogurt's resistance to breakage. The curd firmness and the penetration angle of the yogurt were measured by a CurdmeterMAX ME-500 (Asuka Kiki, Tokyo, Japan). The pressure was applied by a yogurt knife. A load of 2.5 g was applied for 1 sec, and the positive area on the chart when the surface ruptured was used as the curd hardness (Ichimura et al. 2022). We used the penetration angle of the elastic surface curve as an index of the smoothness of the texture (Horiuchi et al., 2009). A greater penetration angle represents a rougher tissue.

The yogurt samples were taken out of the refrigerator at 5°C just before the measurements of the curd firmness and penetration angle, thus simulating the temperature during transportation. The degree of syneresis is an indicator of syneresis to the surface. The degree of syneresis was determined using the centrifugation method: yogurt samples (40 g) in 50-mL tubes were centrifuged at 1,182*g* for 10 min at 25°C. The number of g of supernatant fluid separated per 100 g of yogurt was defined as the degree of syneresis.

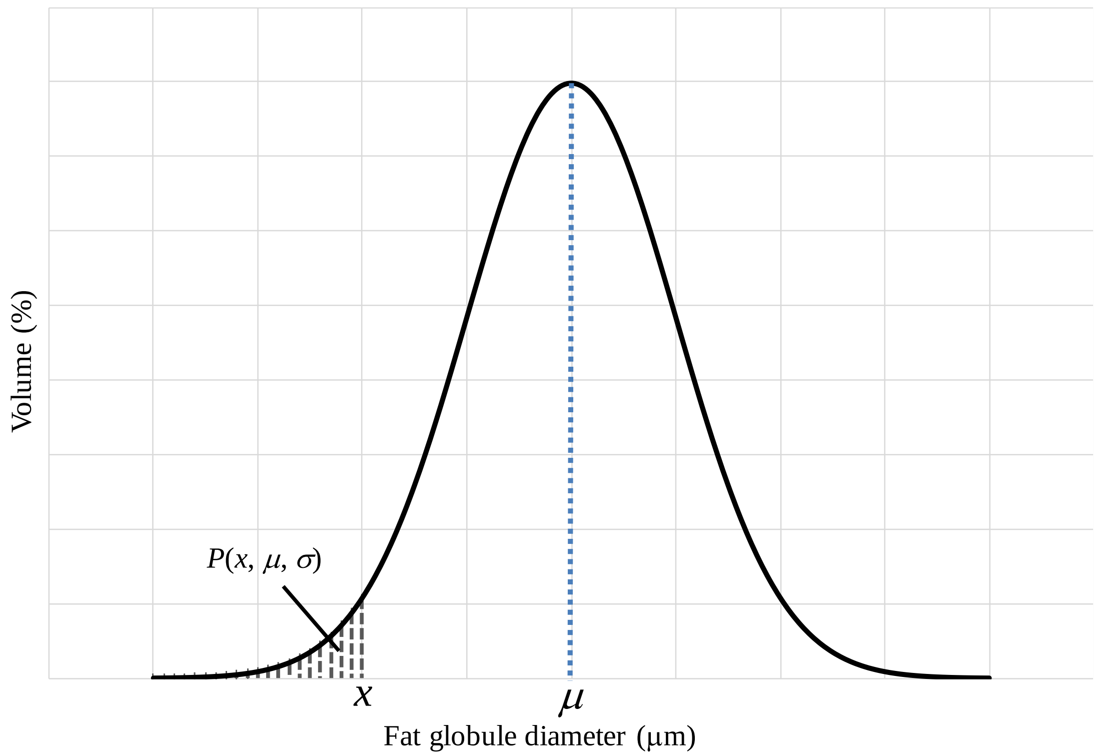
REFERENCES

Horiuchi H, Inoue N, Liu E, Fukui M, Sasaki Y and Sasaki T 2009 A method for manufacturing superior set yogurt under reduced oxygen conditions. *Journal of Dairy Science* **92**, 4112-4121.

Ichimura T, Osada T, Yonekura K and Horiuchi H 2022 A new method for producing superior set yogurt, focusing on heat treatment and homogenization. IDF Factsheet 001/2018-02. Heat Treatment of Milk – Overview. *Journal of Dairy Science* **105**, 2978-2987.



Suppl. Fig. S1. Schematic flow diagram of set yogurt preparation. SNF: solids-not-fat.



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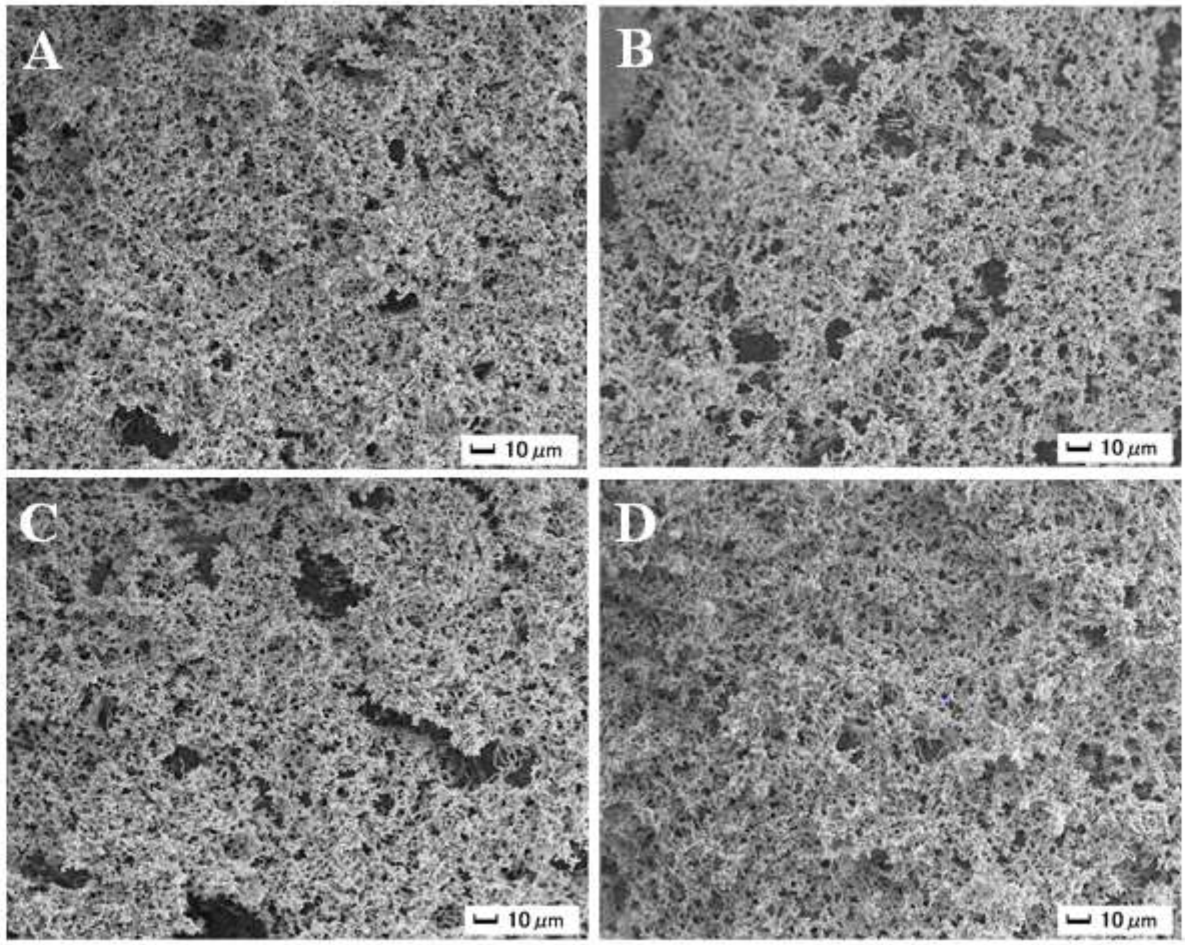
83 **Suppl. Fig. S2.** The particle size distribution: (P(x, μ, σ)), i.e., the lower cumulative

84 distribution of the fat globules of x μm, (μ) mean diameter, and (σ) standard deviation.

The microstructure of set yogurt



Suppl. Fig. S3. Scanning electron micrograph of yogurts with different conditions of homogenization and heat-treatment temperature. **A:** Heat treatment at 95°C for 5 min and homogenization at 10+5 MPa. **B:** Heat treatment at 130°C for 2 sec and homogenization at 10+5 MPa. **C:** Heat treatment at 95°C for 5 min and homogenization at 35+5 MPa. **D:** Heat treatment at 130°C for 2 sec and homogenization at 35+5 MPa.