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Original Article

Characterization of yogurts made with milk solids nonfat by rheological behavior and nuclear magnetic resonance spectroscopy

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ABSTRACT

The effect of adding milk solids nonfat (MSNF) on the physical properties and microstructure of yogurts was investigated. The physical properties of fat free yogurt, fat free with MSNF yogurt, whole fat yogurt, and whole fat with MSNF yogurt were analyzed using shear viscosity, viscoelasticity, and texture analysis. The two yogurts with MSNF had higher consistency coefficient (*K*), storage modulus (*G*′), yield stress, and hardness. To gain insight into the multiphase system, nuclear magnetic resonance (NMR) and brightfield microscope images were acquired. The addition of MSNF significantly modified NMR relaxation time; T_1 values were reduced significantly. Brightfield microscope images showed that the size of the protein network of the two yogurts with MSNF added was greater than that of the two yogurts without MSNF added. The microstructural information supported the physical information. The results showed that the increase in MSNF contributed positively to strengthening the physical/mechanical properties of yogurt.

1. Introduction

Yogurt, a cultured dairy product, is milk fermented by lactic acid bacteria, mainly *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. Because of the existence of a large number of live bacteria, yogurt has therapeutic effects, such as digestion enhancement, enterogastric peristalsis boosting, appetite enhancement, anticarcinogenic activity, and reduction of serum cholesterol [1]. Yogurt also contains many bioavailable proteins, minerals, and vitamins [2]. Therefore, yogurt has become a popular favorite among consumers around the world. In China, yogurt production has risen dramatically with an annual growth rate of more than 10% in 2009–2013 [3].

Yogurt is a complex gel that is mainly composed of denatured protein and milk fat globule. The fat content of yogurt directly influences the final strength of the gel network structure [4]. When the fat content of yogurt decreases, a more

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fragile gel network structure of yogurt forms, and this also leads to less desirable rheological properties, texture characteristics, taste, and flavor [5].

In order to produce good-quality fat free, low fat, and reduced fat yogurt, it is a common practice to add stabilizers to yogurt, such as pectin, gelatin, and κ-carrageenan [6,7]. The addition of stabilizers in yogurt has a negative influence on consumers' acceptance, because more natural yogurt products are preferred [8]. Under the premise of not using stabilizers, it is a challenge to manufacture fat free, low fat, and reduced fat yogurt with the desired gel network structure, creamy aroma, mouthfeel, and little whey-off during storage [9].

Increasing the protein content of yogurt offers an alternative way to strengthen the gel network structure [10]. Denatured proteins act as fillers or binders within a casein matrix [5]. The main components of denatured protein are caseins that are mainly composed of four kinds of monomeric protein: α_{s1}-casein, -α_{s2}-casein, β-casein, and κ-casein [11]. The content of these four kinds of monomeric proteins directly influence the final strength of the gel network structure [8]. Ozlem and Nursel [12] assessed the gel network and water holding capacity of yogurt with the increasing protein content. In the research of Denin-Djurdjević et al [13] and Fetahagić et al [14], the viscosity of yogurt with added dry matter was investigated. In 2006 and 2008, Isleten and Karagul-Yuceer [15,16] compared the physical and sensory attributes of the fat free yogurts made from reconstituted skim-milk powder with the fat free yogurts fortified with whey protein isolate, sodium caseinate. Peng et al [17] monitored the pH, storage modulus, loss tangent, yield stress, and permeability values of yogurts with milk protein isolate and micellar casein as the protein reinforcer during fermentation.

To analyze the effect of milk solids nonfat (MSNF) on the physical behavior of yogurts, the physical/mechanical properties fat free yogurt, fat free with MSNF yogurt, whole fat yogurt, and whole fat with MSNF yogurt were compared through rheological measurements in this work. The structural characterization of the four types of yogurts was performed using nuclear magnetic resonance (NMR) relaxometry and brightfield microscope images.

2. Materials and methods

2.1. Materials

Pasteurized, homogenized whole fat, and fat free milk fortified with Vitamins A and D were purchased from a local supermarket in Davis, CA, USA. Instant nonfat dry milk solid (total fat 0%, total carbohydrate 52.2%, protein 34.8%) was also purchased from a local supermarket.

Yogurt Bulgarian starter culture (skim milk and/or lactose, lactic culture, ascorbic acid) was purchased from New England Cheese Making Supply Company (South Deerfield, MA, USA).

2.2. Yogurt preparation

The MSNF (39.4 g) and a portion of the whole fat milk (1 L) or a portion of the fat free milk (1 L) were mixed thoroughly at room temperature. The yogurt was prepared using two different procedures to address the constraints of the characterization methods. Yogurt was prepared in a 2-L batch using Yogotherm Yogurt Incubator (New England Cheese Making Supply Co.; www.cheesemaking.com) and also prepared in reusable glass jars using the Automatic Yogurt Maker (New England Cheese Making Supply Co.; www.cheesemaking.com). For the 2-L batch, the ingredients (1 L fat free milk, mixture of 1 L fat free milk and 39.4 g MSNF, 1 L whole fat milk, mixture of 1 L whole fat milk and 39.4 g MSNF) were heated to 88°C, maintained between 85°C and 90°C for 30 minutes, and cooled to 43°C. The starter culture was added to the cooled milk and incubated for 6 hours. After the incubation was complete, the yogurt was stored at 4°C for 15 hours.

The procedure was the same for the yogurt made in the Automatic Yogurt Maker except that the yogurt was incubated in glass jars with screw-on plastic caps.

Samples prepared for the rheological property measurements and the yield point determinations were incubated in the Yogotherm Yogurt Maker. The other samples were incubated in the automatic yogurt makers.

2.3. Property measurement

2.3.1. Kinematic viscosity of milk samples

The kinematic viscosity of the four milk samples was determined using the Cannon Fenske-Routine Viscometer (Cole-Parmer North America, Vernon Hills, IL, USA, Size 200; Cole-Parmer, www.coleparmer.com). The values are given as the average and 1 standard deviation of four measurements.

2.3.2. Moisture content of yogurt samples

An HR83 Halogen Moisture Analyzer from Mettler Toledo LLC (Columbus, OH, USA) was used for moisture content determination. Measurements were performed in triplicate.

2.3.3. Shear viscosity of yogurt samples

The shear viscosity tests were performed using a rotational rheometer (CVO 50, Bohlin Rheometer; Malvern Instruments Ltd, Malvern, UK) with a cone and plate measuring system at 25 ± 0.1°C. The shear viscosity tests were performed under the controlled rate mode. The shear rate range was 0.1–100/s. For each experimental run, the yogurt sample was stirred for 30 seconds with a tablespoon and recovered 10 minutes in the container prior to the shear viscosity tests. Measurements were performed in triplicate.

2.3.4. Viscoelasticity: dynamic testing

The dynamic viscoelastic properties of the yogurt samples were measured on the CVO 50 rheometer with the cone and plate measuring system at 25 ± 0.1°C. The amplitude sweep tests were performed at 0.1 Hz, 1 Hz, and 10 Hz to identify the linear viscoelastic range for the yogurt samples. The amplitude sweep tests were performed under the controlled stress mode in the range of 0.03–50 Pa. The frequency sweep tests were performed in the frequency range of 0.1–10 Hz under constant stress. Measurements were performed in triplicate.

2.3.5. Viscoelasticity: transient testing

The stress relaxation test was performed on a TA-XT2i Texture Analyser (Texture Technologies Corp., Scarsdale, NY, USA) using a TA-25 probe (an aluminum cylinder with 50.8 mm diameter, 2026.83 mm^2 in contact area). The test mode was compression with a pretest speed of 10.00 mm/s, test speed of 0.50 mm/s, and post-test speed of 10.00 mm/s. The target value was 10% strain with a hold time of 60 seconds. The trigger force was 4.5*g*. Measurements were performed in triplicate.

2.3.6. *Large deformation testing*

The yogurt consistency and flowability were determined via a back extrusion test. The back extrusion test was performed on a CT3 Texture Analyzer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). The back extrusion test used a TA3/100 flat cylinder probe (25.4 mm in diameter, 35 mm in length; Brookfield Engineering Laboratories). Yogurt samples were tested in the Automatic Yogurt Maker jars (67 mm in diameter, 83 mm in length), 75% full without stirring. The samples were tested immediately after removal from storage at 4°C. For the back extrusion test, the test type was compression with a pretest speed of 2 mm/s, a test speed of 0.5 mm/s, and a post-test speed of 0.5 mm/s. The target distance was 25 mm, and the trigger force was 4.5*g*. Measurements were performed in triplicate.

The differences in the extrusion circle were small in this research. To clarify the differences, statistical analysis was performed on the hardness, hardness work done, adhesive force, and adhesiveness data. Hardness is the force necessary to attain a given deformation. It is the peak force of the compression cycle. Hardness work done is defined as the energy required to drive the probe to hardness value (area under peak). Adhesive force is the force necessary to break the probe from the sample. It is the height of the negative peak. Adhesiveness is the work necessary to break the probe from the sample area (area of the negative peak).

2.3.7. *NMR relaxometry*

The spin–lattice relaxation T_1 and T_2 were acquired on a 1-Telsa permanent magnet NMR spectrometer (Aspect Imaging, Shoham, Israel). All yogurt samples were tested in a plastic cylinder (27.5 mm in diameter, 37.6 mm in length) 80% full at 25 ± 0.1°C.

A saturation recovery sequence was used for T_1 relaxation time measurement. A total of 40 delay time points were used ranging from 1.057 milliseconds to 6.282 seconds. For T_2 relaxation time measurement, a Carr–Purcell–Meiboom–Gill sequence was used with an echo time of 0.5 milliseconds and 3600 echoes. Measurements were performed in triplicate.

2.3.8. *Brightfield microscope images*

Brightfield microscope images of the four yogurt samples were acquired using an Olympus IX71 microscope (Olympus Inc., Center Valley, PA, USA) at 60× magnification. For imaging, the yogurt samples were diluted 10 times and then mounted onto the slide. The images were captured within the same field of view.

2.3.9. *Statistical analysis*

The mean and standard deviation of data over three replicates were calculated. A one-way analysis of variance method was used for statistical analysis of data to determine the influence of formulation. A *p* value ≤ 0.05 was regarded as statistically significant.

3. Results and discussion

3.1. *Shear viscosity of yogurt samples*

The kinematic viscosity values for the fat free milk, fat free milk with MSNF, whole fat milk, and whole fat milk with MSNF were 1.719 ± 0.016 mm^2/s, 2.108 ± 0.022 mm^2/s, 1.936 ± 0.006 mm^2/s, and 2.391 ± 0.010 mm^2/s at 22°C, respectively. The addition of MSNF only slightly increased the kinematic viscosity.

Shear stress versus shear rate relationships for fat free yogurt, fat free with MSNF yogurt, whole fat yogurt, and whole fat with MSNF yogurt are shown in Figure 1. The typical shape of the curves indicated the presence of a yield stress and a shear thinning behavior [18]. The existence of yield stress is generally linked to the existence of an interactive or cross-linked structure [19]. It can be used to characterize the firmness of the yogurt [20]. As can be observed in Figure 1, the yield stress of the whole fat with MSNF yogurt was the highest, and the lowest was that of the fat free yogurt. This indicated that the whole fat with MSNF yogurt was the firmest among the four yogurts, and the added dry milk solids played a more important role than the fat of milk. The shear thinning behavior arises from the alignment of the biopolymer molecules with the field of shear and the weak physical interactions of the biopolymer–biopolymer interactions [5]. As shown in Figure 1, the two yogurts with MSNF added showed higher values of shear stress compared with the other two without MSNF. The fat free yogurt without MSNF showed the lowest value of shear stress. The rheological behavior of yogurt is influenced by a three-dimensional network formed by protein [21]. The enhanced milk protein content facilitated the yogurts to form strong protein–protein bonds [22].

The power law equation (Eq. 1) [23] was used to describe the rheological behavior of the fat free yogurt, fat free with MSNF yogurt, whole fat yogurt, and whole fat with MSNF yogurt over the shear rate range of 0.1–100/s. The power law equation was also applied for yogurt in other research studies [24].

$$\sigma = K(\dot{\gamma})^n, \tag{1}$$

where σ is the shear stress (Pa), K is the consistency coefficient (Pa s), γ is the shear rate (/s), and n is the flow behavior index (dimensionless).

The results for the power law constants (n and K values) are shown in Table 1. The four curves showed good fit with the power law equation, with a minimum R^2 of 0.988. The four yogurt samples were pseudoplastic fluids as evidenced by the flow behavior index $(n) < 1$. The same observation was obtained by Cruz et al [21]. Compared with the two yogurts without MSNF, the two yogurts with MSNF added had a higher consistency coefficient (K). It indicated a comparatively thicker structure of the two yogurts with MSNF added. Fetahagic et al [14] and Gun and Isikli [12] also reported that the viscosity of the yogurt with dry milk solids was relatively high.

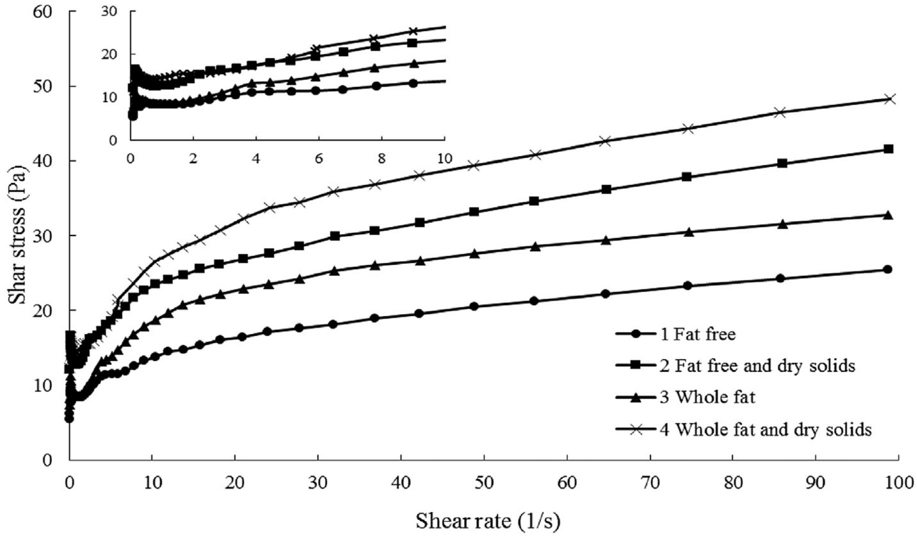

Figure 1 – Shear stress versus shear rate relationships for fat free yogurt, fat free with milk solids nonfat (MSNF) yogurt, whole fat yogurt, and whole fat with MSNF yogurt.

Table 1 – Flow parameters of fat free yogurt, fat free with milk solids nonfat (MSNF) yogurt, whole fat yogurt, and whole fat yogurt and whole fat with MSNF yogurt.

	K (Pa s)	*n*	Minimum R^2
Fat free yogurt	10.39 ± 1.21	0.16 ± 0.02	0.994
Fat free with MSNF yogurt	14.95 ± 1.94	0.18 ± 0.03	0.991
Whole fat yogurt	11.05 ± 0.90	0.22 ± 0.01	0.988
Whole fat with MSNF yogurt	16.21 ± 1.38	0.19 ± 0.03	0.990

3.2. *Viscoelasticity: dynamic testing*

Viscoelastic properties characterize the extent and strength of internal structures present in yogurt samples [25]. Figure 2 shows the dependence of storage modulus (G') and loss modulus (G'') on shear stress at 0.1 Hz in the range of 0.03–50 Pa. As can be observed from Figure 2, the yogurt sample showed a weak gel behavior as evidenced by $G' > G''$ over the range of 0.05–10 Pa. Sendra et al [25] and Cruz et al [26] obtained similar results for the viscoelastic properties of yogurt. A linear viscoelastic range between 0.1 Pa and 5 Pa was observed from Figure 2. The stress of 1 Pa was chosen for the frequency sweep tests.

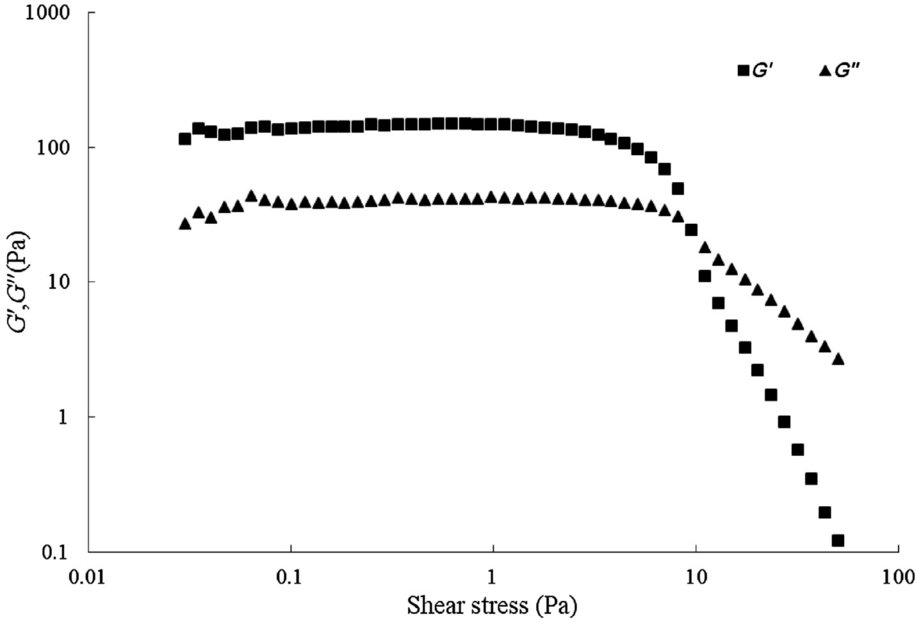

Figure 2 – Amplitude sweep results for the fat free yogurt.

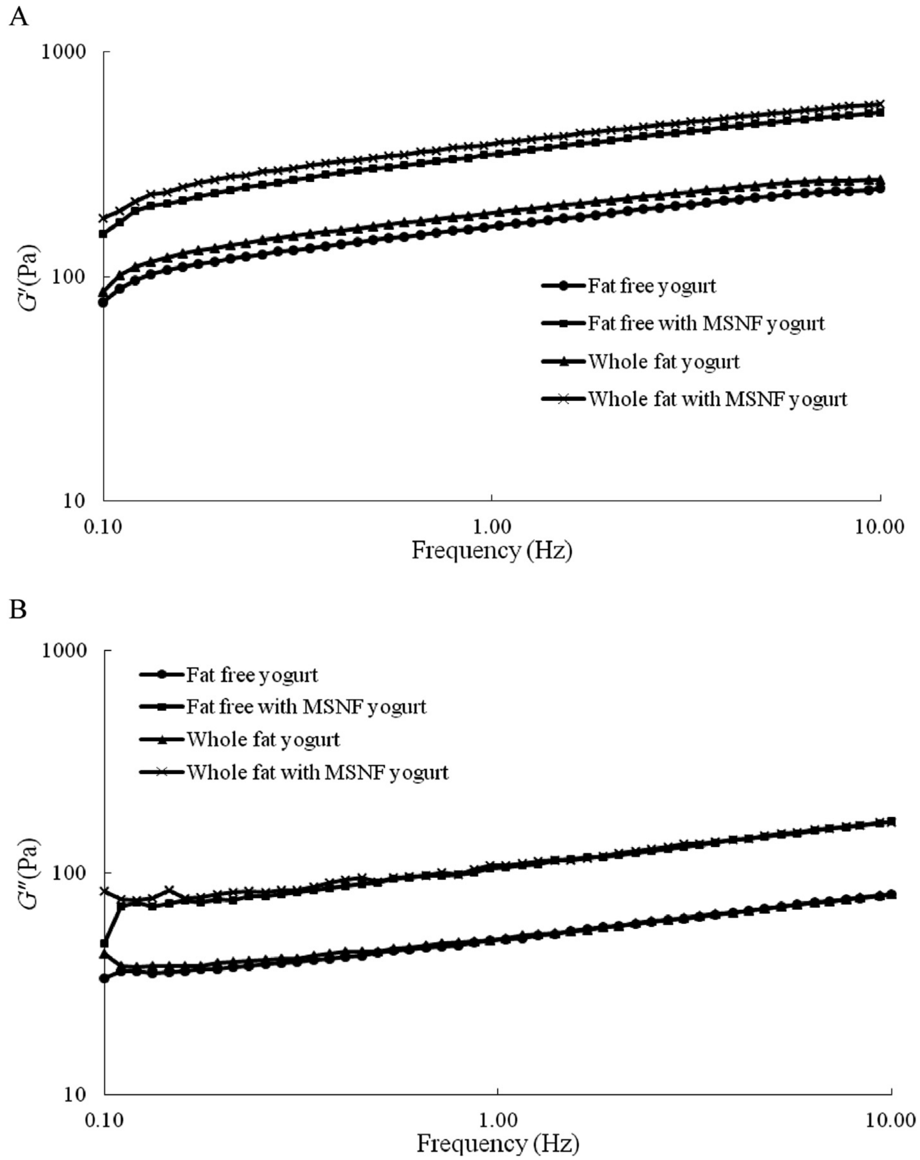

Figure 3 – Dependence of G′ and G″ on frequency for the fat free yogurt, fat free with milk solids nonfat (MSNF yogurt), whole fat yogurt, and whole fat with MSNF yogurt.

The dependence of *G*′ and *G*″ on frequency is shown in Figures 3A and 3B, respectively. For all four types of yogurt, the values of *G*′ and *G*″ increased with the increase in frequency. The same trend was also observed by Ramírez-Sucre et al [27]. Figure 3A shows that *G*′ values increased with the added MSNF and the increase in fat content. *G*′ is related to the stiffness of the network and reflects the solid-like properties [24,28]. The result from Figure 3A indicated that the yogurt with MSNF added had a firmer body. It was consistent with the results obtained in the viscometry analysis. Figure 3B illustrates that the *G*″ values of the whole fat with MSNF yogurt and the fat free with MSNF yogurt were the same, whereas the *G*″ values of the fat free yogurt and the whole fat yogurt were the same. *G*″ is related to the viscous component of the yogurt and reflects the liquid-like properties [29]. The results indicated that the viscous behavior of the yogurts with the same protein content were almost the same.

3.3. *Large deformation testing*

Texture is a very important characteristic of yogurts [30]. It is closely linked with the yogurt's inner structure, which finally determines the overall quality of the yogurt [31]. Because back extrusion is a simple, rapid, and low-cost method, it is often used in the texture measurement of fluid and semisolid foods [32]. Figure 4 shows the typical curves acquired in back extrusion tests using the TA3/100 flat cylinder probe for fat free yogurt, fat free with MSNF yogurt, whole fat yogurt, and whole fat with MSNF yogurt. As shown in Figure 4, the peak values for yogurts with MSNF added were much greater than those without MSNF added. In the compression circle, the difference in the peak value brought by the increase in protein content was greater than that brought by the increase in fat content. It indicated that proteins played a more important role in the texture of

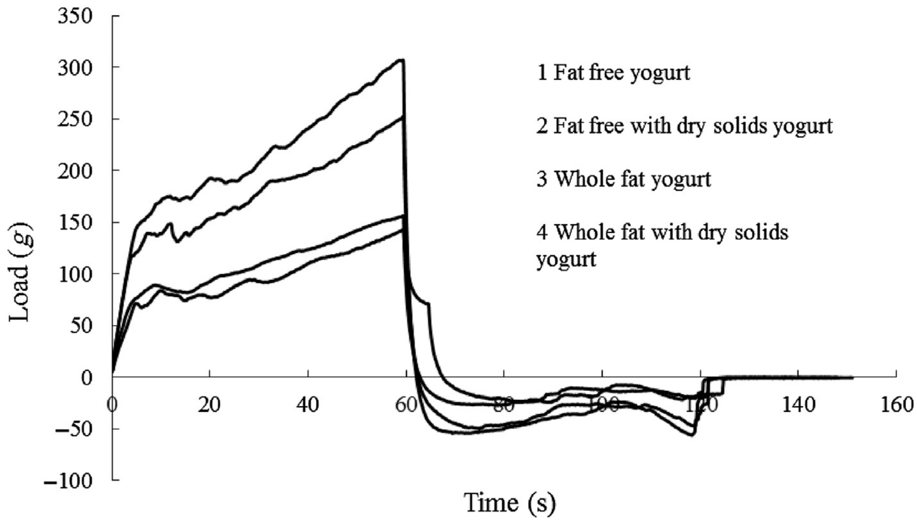

Figure 4 – **Back extrusion tests for the fat free yogurt, fat free with milk solids nonfat (MSNF) yogurt, whole fat yogurt, and whole fat with MSNF yogurt. (A) Cylinder probe. (B) Mesh probe.**

Table 2 – Statistical analysis results for back extrusion tests using TA3/100 flat cylinder probe.)

Sample name	Hardness (g)	Hardness work done (mJ)	Adhesive force (g)	Adhesiveness (mJ)
Fat free yogurt	145.67 ± 3.47^{a}	28.23 ± 0.72^{a}	26.92 ± 4.68^{a}	4.82 ± 1.52^{a}
Fat free with MSNF yogurt	252.25 ± 21.75^{b}	51.22 ± 4.78^{b}	58.00 ± 10.97^{b}	10.32 ± 2.40^{b}
Whole fat yogurt	156.67 ± 3.30^{a}	31.62 ± 1.16^{a}	32.5 ± 6.87^{a}	5.15 ± 2.49^{a}
Whole fat with MSNF yogurt	307.67 ± 38.51^{c}	60.75 ± 4.95^{c}	58.83 ± 6.75^{b}	11.47 ± 1.69^{b}

$^{a-c}$ Means with different letters in the same column are significantly different ($p < 0.05$).
MSNF = milk solids nonfat.

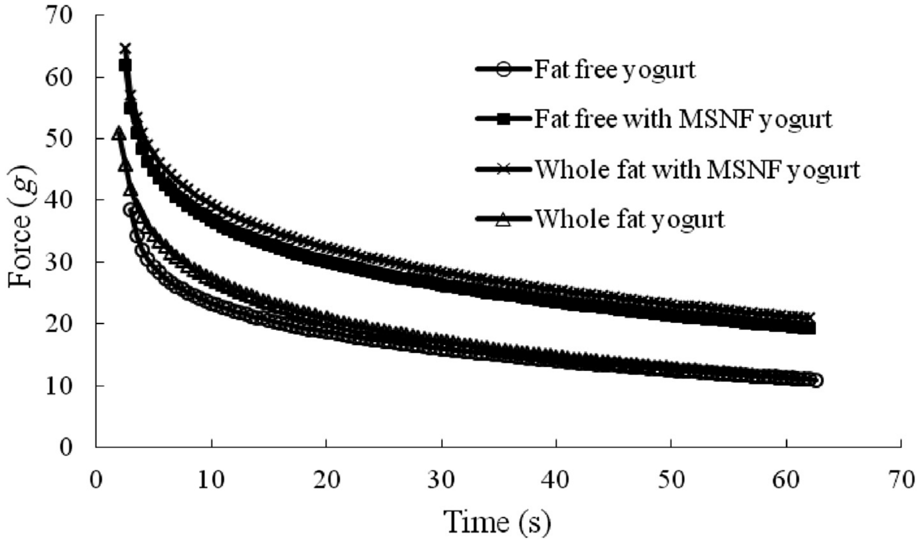

Figure 5 – **Relaxation curves for the fat free yogurt, fat free with milk solids nonfat (MSNF) yogurt, whole fat yogurt, and whole fat with MSNF yogurt.**

yogurt. Texture of yogurt is based on the strings of casein micelles interacting physically with each other [33]. In the extrusion circle of Figure 4, the differences in the four yogurt samples are small. In the research of Vercet et al [33] the differences in the extrusion circle were also smaller than those in the compression circle.

The statistical results for the hardness, hardness work done, adhesive force, and adhesiveness acquired in the back extrusion tests are given in Table 2. The whole fat with MSNF yogurt had the highest value of hardness, hardness work done, adhesive force, and adhesiveness. The fat free with MSNF yogurt ranked second to the whole fat with MSNF

Table 3 – Statistical analysis of the Maxwell parameters for fat free yogurt, fat free with milk solids nonfat (MSNF) yogurt, whole fat yogurt, and whole fat with MSNF yogurt.

	F_e (g)	F_0 (g)	λ_{rel} (s)	Minimum R^2
Fat free yogurt	15.10 ± 3.45	39.27 ± 6.26	14.78 ± 1.40	0.965
Fat free with MSNF yogurt	20.71 ± 4.91	49.07 ± 7.43	14.23 ± 0.25	0.962
Whole fat yogurt	13.23 ± 2.84	43.78 ± 5.36	14.28 ± 0.89	0.967
Whole fat with MSNF yogurt	23.80 ± 1.30	56.63 ± 2.51	14.77 ± 0.41	0.965

yogurt. Meanwhile, the fat free yogurt had the lowest value of hardness, hardness work done, adhesive force, and adhesiveness. The analysis of variance results for back extrusion tests are also shown in Table 2. The adhesive force and adhesiveness for the two yogurts with MSNF added were significantly different from those without MSNF added.

3.4. *Viscoelasticity: transient testing*

The fat free yogurt, fat free with MSNF yogurt, whole fat yogurt, and whole fat with MSNF yogurt were subjected to a stress relaxation test to observe the integrity of the structure [34]. As demonstrated in Figure 5, all four curves show exponential relaxation. The two yogurts with MSNF added (whole fat with MSNF yogurt and fat free with MSNF yogurt) showed a similar trend, whereas the other two yogurts without MSNF added showed a similar trend. The two yogurts with MSNF added showed a greater resistance. The observation was in accordance with that reported by Bhattacharya [35]. The Maxwell model given in Eq. (2) [23] was used for the description of stress relaxation data of the four yogurt samples.

Table 4 – Relaxation time (T_1 and T_2) of the fat free yogurt, fat free with milk solids nonfat (MSNF) yogurt, whole fat yogurt, and whole fat with MSNF yogurt.

	Moisture content (%)	T_1 (ms)	T_2 (ms)
Fat free yogurt	86.58 ± 0.05	1273.6 ± 14.9	190.7 ± 0.3
Fat free with MSNF yogurt	82.80 ± 0.24	1004.3 ± 9.9	139.7 ± 0.5
Whole fat yogurt	84.84 ± 0.12	1095.3 ± 7.3	175.2 ± 0.4
Whole fat with MSNF yogurt	80.81 ± 0.24	936.3 ± 6.5	133.0 ± 0.4

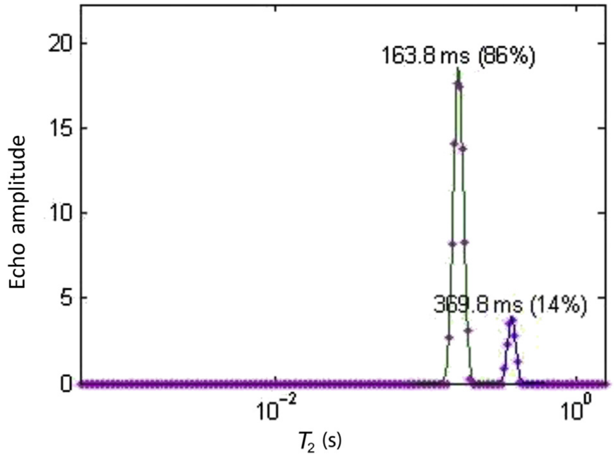

Figure 6 – T_2 relaxation spectrum of fat free yogurt.

$$F = F_e + (F_0 - F_e)\exp\left(\frac{-t}{\lambda_{rel}}\right), \tag{2}$$

where F is the decaying force (g), F_e is the residual force (g), F_0 is the initial force (g), and λ_{rel} is relaxation time (seconds).

Table 3 gives the mean ± 1 standard deviation for F_e, F_0, and λ_{rel}. As can be observed, the four curves were well fit with the Maxwell model, as evidenced by R^2 values > 0.962. The whole fat with MSNF yogurt was the hardest, as evidenced by the highest F_0 and F_e value. The fat free with MSNF yogurt was next to the whole fat with MSNF yogurt. The results were consistent with those obtained in the texture analysis.

3.5. *NMR relaxometry*

Relaxation times T_1 and T_2 of the fat free yogurt, fat free with MSNF yogurt, whole fat yogurt, and whole fat with MSNF yogurt were determined to gain insight into the multiphase system. Table 4 shows the T_1 and T_2 values for the four yogurt samples. The addition of nonfat dry milk solids significantly modified the behavior of the NMR signals. The T_1 values were reduced significantly from 1273.6 milliseconds to 1004.3 milliseconds (fat free to fat free with dry milk solids) and from 1095.3 milliseconds to 936.3 milliseconds (whole fat to whole fat with dry milk solids), respectively. It indicated that the water molecules were in fast exchange between water associated with proteins and free water. The moisture content (Table 4) also showed that the two yogurts with MSNF added had lower water content. Because proteins have relaxation times shorter than 100 microseconds [36], the T_1 values for the two yogurts with MSNF added were reduced. The research of Lucas et al [37] demonstrated the effect of protein on the behavior of the NMR signals of ice cream. They reported that water relaxation was sensitive to the protein structure.

Figure 6 illustrates the T_2 relaxation spectrum of fat free yogurt. The presence of two peaks was observed. The first peak had a higher proportion in all four yogurts (higher than 75%; the other three spectra are not shown). It was strongly associated with the water in the multiphase system [34]. The relaxation time for the second peak varied from 294.6 milliseconds to 600.8 milliseconds (the other three spectra are not shown). It is mainly associated with water entrapped in the protein network [34].

3.6. *Brightfield microscope images*

Figure 7 shows the brightfield microscope images of the fat free yogurt, fat free with MSNF yogurt, whole fat yogurt, and whole fat with MSNF yogurt. As can be observed, the protein network was the fundamental structure of yogurt [12]. When reaching the isoelectric point of the proteins, an aggregated

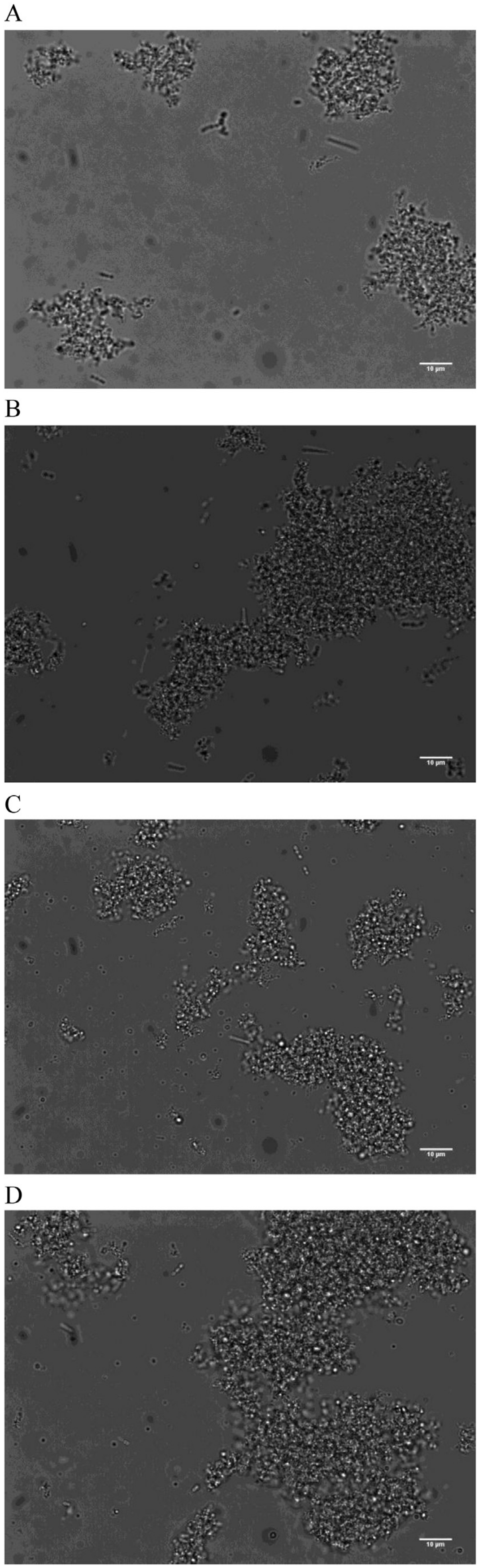

Figure 7 – Brightfield microscope images. (A) Fat free yogurt. (B) Fat free with milk solids nonfat (MSNF) yogurt. (C) Whole fat yogurt. (D) Whole fat with MSNF yogurt.

network enclosing fat globules and serum was established between the proteins [25]. Figure 7 illustrates that the size of the protein network of the two yogurts with MSNF added was greater than that of the two yogurts without MSNF added. The microscope results supported the results of viscometry analysis, back extrusion, and stress relaxation testing.

4. Conclusion

The effect of adding MSNF on the physical behavior of yogurt was studied in this research. MSNF addition contributed positively to the shear viscosity, viscoelasticity, and texture of the yogurts. The microstructural information acquired via NMR relaxometry and brightfield microscope supported the perceptions of texture. Flavor metabolites analysis will be conducted to gain a better understanding of how MSNF influences physical behavior.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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