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Original Article

Comparative Effects of Iranian Native and Commercial Probiotic Incorporation on Rheological Behavior and Syneresis Characteristics of Yogurts

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ABSTRACT

Background and Objectives: The aim of the present study was to investigate effects of incorporating Iranian native and commercial probiotic strains (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium lactis*) on the rheological and syneresis properties of yogurts.

Materials and Methods: Samples were heated (90 °C, 15 min) after reconstituting in milk (13% SNF). After cooling down, the starter culture and probiotics were incorporated. Incubation was carried out at 42 °C until the pH decreased to 4.5. Syneresis was assessed based on Amatayakul *et al*. method. Rheological characteristics were assessed using dynamic oscillation (strain sweep and frequency sweep) and rotation assays.

Results: Results revealed significant improvement in characteristics of the probiotic yogurts, compared to the control sample. Based on the results of rheological assessments, yogurts showed viscoelastic behaviors. In general, yogurts containing the native strain of *L. acidophilus* provided further desirable rheological and syneresis characteristics.

Conclusions: Therefore, it is strongly recommended to use native Iranian *L. acidophilus* in probiotic yogurt production.

Keywords: *Bifidobacterium*, *Lactobacillus*, Yogurt, Rheological characteristics

Introduction

There are direct relationships between food and health, popularizing functional food consumption world (1, 2, 3). According to Food and Agriculture Organization/World Health Organization (FAO/WHO), probiotics are live microorganisms with beneficial health effects as administered in sufficient amounts. In fact, viability is a critical factor for considering a microorganism as a probiotic. However, it has been reported that dead cells and cell metabolites can include health benefits (4). These effects, known as core benefits, include regulation of intestinal transit, normalization of perturbed microbiota, turnover of enterocytes, competitive exclusion of pathogens, colonization resistance with neurological, immunological and endocrinological effects as well as production of short-chain fatty acids and various bioactive compounds (5). Proteolytic activities of probiotics and prebiotics can affect sensory and rheological characteristics of the food products. Therefore, consumption of fermented dairy products (containing living bacteria) such as probiotic yogurts shows beneficial effects on human health. Yogurt is a unique carrier for probiotic bacteria. It includes nice texture and organoleptic characteristics, high nutritional values and excellent adaptability. *Lactobacillus* spp. and *Bifidobacterium* spp. are known as the most common commercial probiotics used in yogurt production (6, 7). Various factors affecting probiotics in yogurts, including food matrices, production procedures and storage conditions (oxygen extent, pH, temperature, inoculation rate, time and packaging materials) (8). Changes in yogurt characteristics such as viscosity can occur as a result of alteration in protein proportions and ion contents even as total protein and fat are adjusted to a fixed extent as well as changes in process and storage conditions (9).

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Mousavi *et al*. (2019) reported that survival of *Lactobacillus acidophilus* and texture and organoleptic characteristics of the flaxseed enriched probiotic yogurts are affected by the proportion of flaxseed and storage time. Furthermore, addition of flaxseed to probiotic yogurts caused a higher growth rate of *Lactobacillus acidophilus* (up to 8.82 CFU/ml), compared to the control sample (6.87 CFU/ml). Flaxseed enhanced the yogurt viscosity, hardness, cohesiveness, gumminess and water holding capacity (WHC). However, decreases in yogurt syneresis and adhesiveness were seen (10). Sarwar et *al.* (2019) assessed physicochemical and microbiological characteristics of the produced synbiotic yogurt by probiotic yeast of *Saccharomyces boulardii* in combination with inulin (1–2% w/v). Microrheological analysis showed that the values of G_0 and G" slightly decreased by the addition of inulin. Physiochemical parameters such as pH, acidity and protein content were in the normal range (similar to the control), while the fat content of the synbiotic yogurt significantly decreased. Addition of 1% inulin in yogurt formulation decreased the yogurt syneresis and preserved the viability of *S. boulardii* after storage for 28 days. Inulin improved the texture (dense, compressed and homogeneous structure), organoleptic characteristics and the survival of *S. boulardii* with a viable count of higher than 6.0 log CFU/g in the yogurt, as generally needed for probiotics. There is no evidence assessing rheological characteristics and syneresis of the probiotic yogurts using various Iranian native probiotics. Therefore, the aim of this study was to assess effects of Iranian native probiotic strains in comparison with commercial strains on the rheological and syneresis characteristics of the yogurt.

Materials and Methods

Probiotic strains and starter culture: Commercial lyophilized starter culture (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) as well as commercial probiotic strains (*Lactobacillus acidophilus* LA-5, *Lactobacillus casei* 431 and *Bifidobacterium lactis* BB-12) were supplied by Chr. Hansen A/S, Horsholm, Denmark. Iranian native probiotic strains were purchased from TakGene, Tehran, Iran. Yogurt cultures and probiotic strains were stored at -18 °C according to the manufacturer's instructions.

Production of set yogurts: In this study, various probiotic yogurt treatments were used, including yogurt bacteria (Y-C) as control, yogurt bacteria and native strain of *L. acidophilus* (YLA_n), yogurt bacteria and native strain of *L. casei* (YLC_n), yogurt bacteria and native strain of *B. lactis* (YBL_n), yogurt bacteria and commercial strain of *L. acidophilus* (YLA_c), yogurt bacteria and commercial strain of *L. casei* (YLC_c), and yogurt bacteria and commercial strain of *B. lactis* (YBL_c). All samples were prepared using potable water and reconstituted skim milk powder (Pegah Dairy Industry, Tehran, Iran). Samples were heated (90 °C, 15 min) after reconstituting in milk (13% SNF). After cooling down (44 °C ±1), starter cultures and probiotics were incorporated. Then, incubation was carried out at 42 °C until pH decreased to 4.5 ±0.01 from 6.41 ±0.03. Samples were cooled down and stored at 5 °C for 28 days.

Rheological assessment: Rheological behaviors of the prepared samples were studied using dynamic oscillation and rotation shear assays and rheometer (MCR301, Anton-Paar, GmbH, Graz, Austria) vane geometry. All experiments were carried out at 20 °C ±1. The strain sweep assay was carried out (strain range of 0.01–1000% at fixed 1 Hz) to investigate limiting values of the linear viscoelastic range (LVE), structural strength (*G*' at LVE), cross over ($G' = G''$) and resistance to mechanical force or yield stress (Tau *y* as an indicator of structural strength) (12). In frequency sweep assays, the frequency ramp ranged 0.01–100 Hz. Rheological parameters, including elastic modulus (G'), viscous modulus (G''), damping factor ($\tan\delta = G''/G'$), complex modulus (G^*) and "*a*" and "*b*" factors were estimated (13,14). Rotation shear assays were carried out in two intervals as described by Donmez et *al.* (2017) and Norouzbeigi et *al.* (2020) with modifications as follows: the first interval for 10 s at a steady shear rate of 200 rps to achieve a better homogenization and create a similar beginning status for all treatments and the second interval at a shear rate of 0.5–300 rps to achieve apparent viscosity (15,16). Data were properly fitted with the power-law model and assessed using Rheoplus Software v3.21 (Anton Paar, Graz, Austria).

Syneresis assessment: Level of the spontaneous syneresis in undisturbed set yogurt was assessed according to Amatayakul *et al*. (2006). First, the cup of set yogurt was weighed at 4 °C (W_1). Then, yogurt was stored at an angle of nearly 45° to collect the separated whey on the side of the cup. A needle was used to remove the separated whey. Weight of the yogurt cup was recorded (W_2). To prevent further leakage of whey from the gel, the entire assay was carried out within 10 s. The syneresis was expressed using Eq. 1 (17):

$$\text{Syneresis (\%)} = W_2 / W_1 \times 100 \qquad (1)$$

Statistical analysis: All experiments were carried out in triplicate. Results were statistically analyzed using completely randomized design at a confidence level of 0.95. Curve Expert v.6, SPSS v.24 and Excel 2013 were used to carry out statistical and regression analyses.

Results

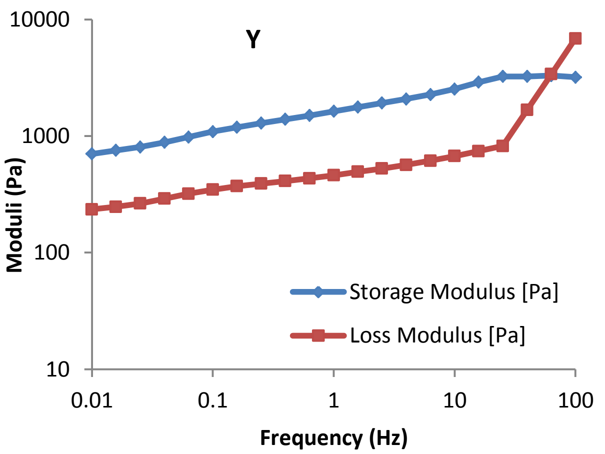
Rheological characterization

Frequency scan assay: Figure 1 (a–g), showed changes in the storage modulus of the various samples at a frequency of 0.01–100 Hz. Results of the frequency scanning assay are represented in Table 1. It was detected that the elastic modulus of all treatments was higher than the viscous modulus. Storage module (elastic component) and drop modulus (viscous component) showed similar frequency dependences. Furthermore, samples showed various elasticities and poor viscoelastic gel characteristics (Fig. 1H); as Hassan et *al*. (2003) reported (18). Similar rheological behaviors were observed in all samples. However, significant differences were seen between the elastic components of the Iranian and commercial probiotic strains. Generally, YLan and YLac samples showed the highest and the lowest elastic components, respectively. Increases in elastic components of all samples were seen at higher frequencies; as shown by Blisto *et al*., 2017, and Costa *et al*., 2019 (21, 22). Figure 1i represents elastic and viscous components of the samples at various frequencies. Therefore, addition of probiotic bacteria changed the firmness of yogurt. Compared to the control, YLan sample included the highest elasticity and viscosity, while YLac sample showed the lowest values.

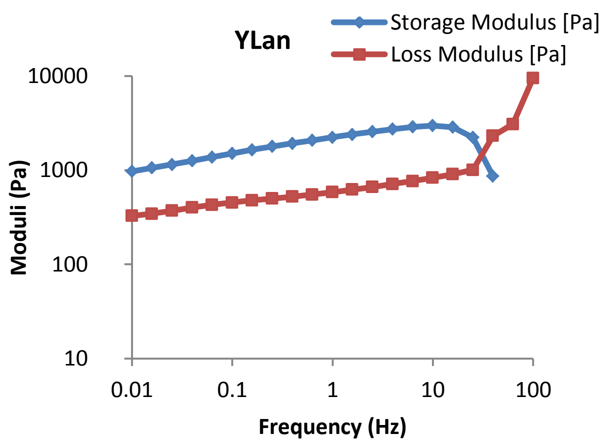
Table 1. Textural attributes of various yogurt samples

Treatments	a	b	R^2	tanδ
Y-C	1658^{e}	0.193^{a}	0.98	0.310^{a}
YLA_n	2205^{a}	0.167^{d}	0.99	0.294^{d}
YLA_c	1294^{g}	0.164^{d}	0.99	0.293^{d}
YLC_n	1938^{b}	0.171^{c}	0.99	0.300^{c}
YLC_c	1542^{f}	0.168^{cd}	1.00	0.301^{c}
YBL_n	1781^{d}	0.180^{b}	0.99	0.304^{b}
YBL_c	1871^{c}	0.179^{b}	0.98	0.301^{c}

*Means in a column with different small letters represent significant differences ($P < 0.05$).



(a)



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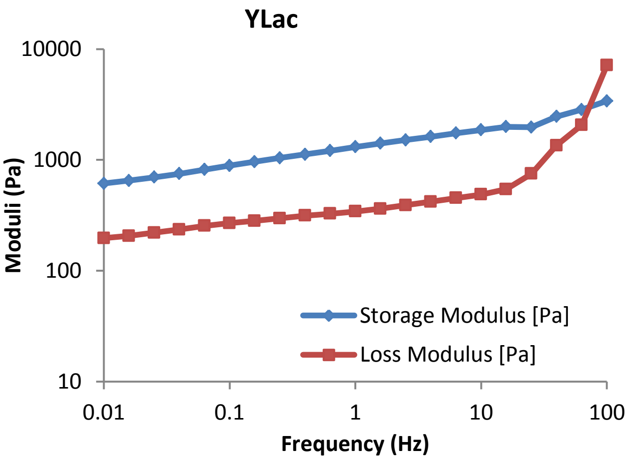

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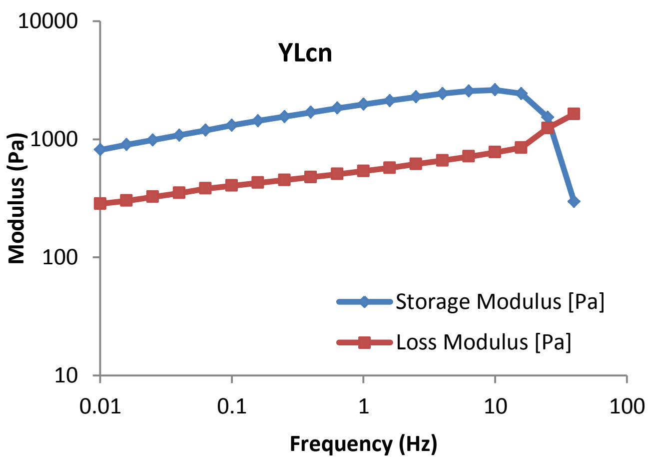

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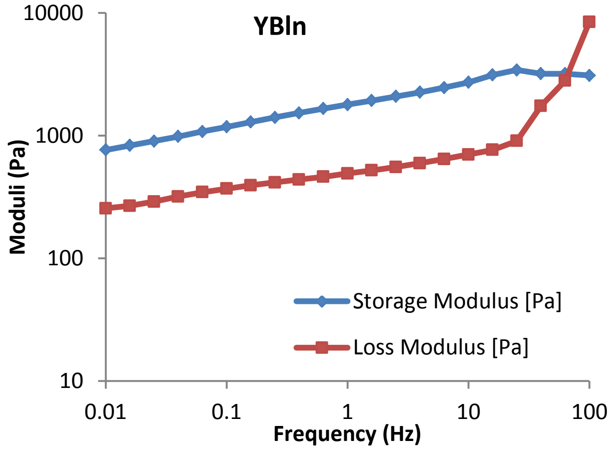

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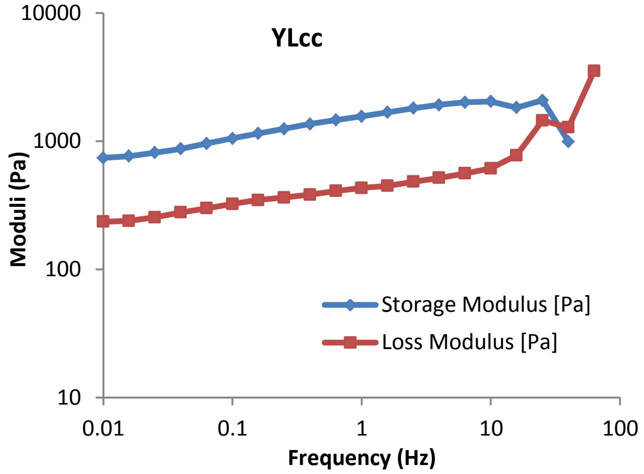

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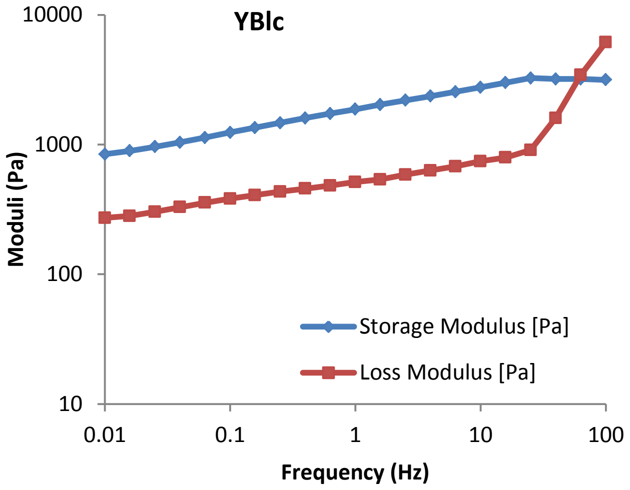

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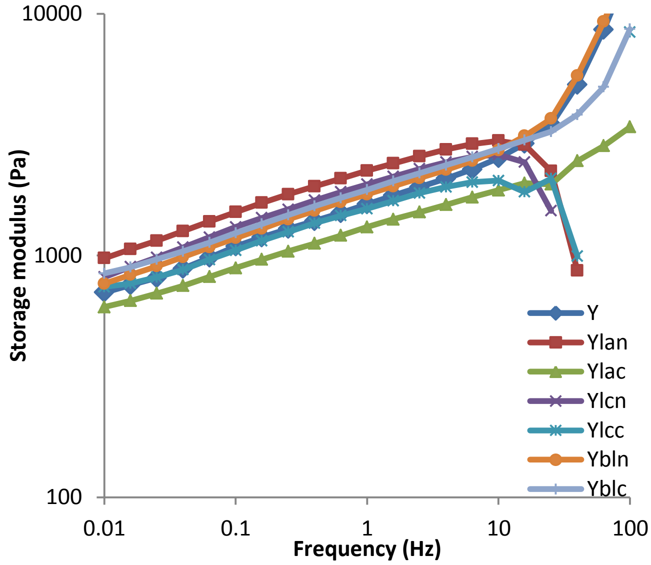

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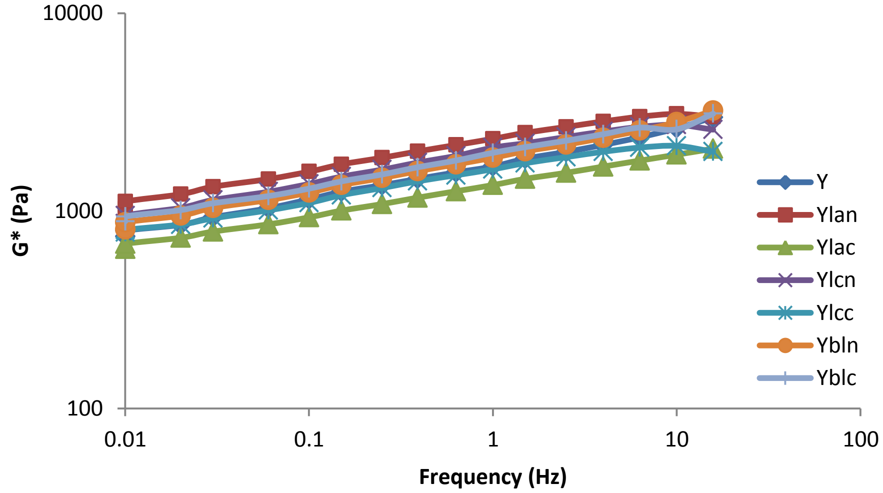

(i)

Figure 1. Storage and loss moduli of various yogurt samples Treatments: Y–C, yogurt bacteria; YLA_n, yogurt bacteria and native *Lactobacillus acidophilus*; YLA_c, yogurt bacteria and commercial *Lactobacillus acidophilus*; YLC_n, yogurt bacteria and native Iranian *Lactobacillus casei*; YLC_c, yogurt bacteria and commercial *Lactobacillus casei*; YBL_n, yogurt bacteria and native Iranian *Bifidobacterium lactis*; and YBL_c, yogurt bacteria and commercial *Bifidobacterium lactis*

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As shown in Table 1, tanδ, "*a*" and "*b*" values of the samples were significantly various ($p < 0.05$). The Y-C included the highest "*b*" value while YBL_n and YBL_c showed the lowest value. The YLa_n and YLa_c included the highest and the lowest "a" values, respectively. Based on the viscoelastic materials, higher "b" values show the viscoelastic behavior of the gel-like structures and a further sensitivity to mechanical stresses. In power-law, the higher "*a*" (y-intercept) values refer to stronger gel structures. The YLan and YLac samples included the highest and the lowest "*a*", respectively. The higher tanδ refers to the lower strength of the gel structure. Ratio of the viscous components was higher than elasticity. YLa_c and Yla_n included the highest and the lowest values of tanδ, respectively.

Strain scan assay results: Intersection of the elastic and viscous components is considered as the elastic boundary (Table 2). The sample structure breaks and begins to flow; as Meyer *et al.* (2011) reported. Values of the elastic and viscous components were similar. Intersection of the elastic component and the viscosity (over cross) were significantly various in various samples ($p < 0.05$). Moreover, YLCn and YLA_c included the highest and the lowest elastic ranges, respectively. Based on the results from the strain scan assay (Table 2), the YLa_c sample included the lowest structural strength (elastic modulus in the range linear viscoelasticity). Considering G*, "*a*", "*b*" and tanδ, the YLA_n sample showed the highest structural strength. Table 2 shows significant differences between the stress levels of the samples corresponding to the end of the linear region ($p<0.05$). Higher levels of this parameter demonstrate higher structural stabilities and stronger gel structures. Stress is the minimum necessary force to flow the sample (24). In fact, YLAn included the highest limit stress representing that a further mechanical force was needed to follow (12). Apparent viscosity of the samples (at a constant stress point) was shown in Fig. 2. Samples Y and YLan showed the lowest and the highest apparent viscosities, respectively. These results revealed effects of the probiotic bacteria on the yogurt texture. Figure 2 represents significant improvements in apparent viscosity of the probiotic yogurt, compared to the control (25).

Table 2. Rheological characteristics of the control and prepared yogurt samples

Treatments	Storage modulus (Pa)	Cross over (Pa)	Tau (y) (Pa)
Y-C	1661.03^{e}	124.00^{b}	6.20^{d}
YLA_n	2187.94^{a}	121.00^{c}	8.34^{a}
YLA_c	1284.84^{g}	106.00^{d}	4.90^{f}
YLC_n	1988.76^{b}	170.66^{a}	7.39^{b}
YLC_c	1550.66^{f}	121.07^{c}	5.91^{e}
YBL_n	1776.95^{d}	121.60^{c}	6.63^{c}
YBL_c	1859.77^{c}	121.68^{bc}	6.21^{d}

*Values with different letters in each column are significantly different ($p< 0.05$).

Syneresis characteristics: Table 3 illustrates syneresis assessment results of the samples. Significant differences were seen in the syneresis levels of various yogurts ($p < 0.05$). in general, YLA_n and YLA_c showed the lowest and the highest syneresess, respectively. Levels of YLA_n, YLC_n and YBL_c samples were statistically similar ($p > 0.05$). However, syneresis of YLA_c was 62.34% higher than that of YLA_n.

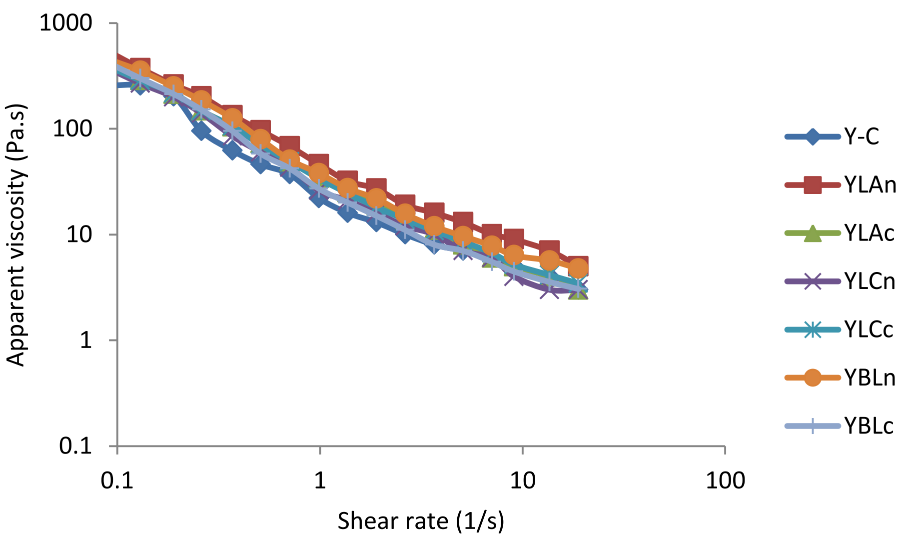

Figure 2. Apparent viscosity of various yogurt samples

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Discussion

Rheological characterization

Frequency scan assay: Results were similar to reported results by Hasan *et al*. in 2003 and KrivoroTova et *al*. in 2017 (18, 19). Use of higher frequencies caused increases in elasticity and viscosity of the sample components. Elastic and viscous components reached the intersection point; therefore, all samples showed the flow point. In viscoelastic region, all samples showed linear behaviors (such as solid). Before the flow point, the elastic component was higher than the elastic component. After the linear viscoelastic range, the viscosity component was higher and the samples showed quasi-liquid behaviors. These results were similar to those by Zargaran et al. (2013). As Costa et al. (2019) described, the higher complex modulus caused further firmness in the treated yogurt rather than the control.

Strain scan assay results: Costa *et al*. (2019) reported that limit stresses generally existed despite an interactive structure or cross-linking and probiotic bacteria seemed to amplify these linkages (22). Decreases in apparent viscosity were possibly linked to the effects of the bacterial enzymes on the casein micelle matrix (Ariana and McGrew, 2007). Type of the fiber and storage time are factors affecting apparent viscosity of the samples (26). Ariana and McGraw (2007) reported insignificant differences between the apparent viscosity of yogurt samples containing inulin and oligofructosis. Yogurt is a pseudoplastic product (Costa et *al*., 2019), meaning that increases in shear stress lower its viscosity. In the current study, YLan and Y showed the highest and the lowest apparent viscosities, respectively. This demonstrated the effects of probiotics on strengthening the gel network and improving the viscosity (22).

Syneresis characteristics: In this study, consistencies were reported between the results of the syneresis and the rheological characteristics of the yogurt samples. Gel structure of the samples with higher elastic modulus and yield stress was further compact. It brought higher serum maintenance and lower syneresis (27,9,28). Compared to the probiotic samples, incorporating inactivated probiotic cells into yogurts caused lower syneresis and higher WHC (12). Brennan and Tudorica (2008) reported that yogurt syneresis was affected by the milk fat content. They also stated that the fat globules decreased the casein aggregation, improved the three-dimensional (3D) network and produced further compact structures (29). Amatayakul *et al*. (2006) reported that the yogurt syneresis decreased by increases in total solids and use of extracellular polymeric substance-producing starter cultures (17). These dissimilar syneresis results could be attributed to use of various total solid levels (13%) in this study (30,31). Total solids extended and starter culture types affected whey separation of the yogurt. Increases in total solid contents promoted the gel network stability and density. Moreover, water was delimited rather tightly, causing higher firmness (21). Fortifying milk protein network with skim milk powder, whey protein isolate and sodium caseinate is effective in decreasing or preventing yogurt syneresis (29). Donmez *et al*. (2017) investigated that green tea and green coffee powders (rich in polyphenols) improved set yogurt whey separation and its rheological characteristics (16). Parvarei et *al*. (2021) compared addition of probiotics and paraprobiotics to the greatest mean pH drop rate, mean acidity increase rate, mean redox potential increase rate, final acidity and final redox potential in yogurts containing inactivated *L. acidophilus* (added before fermentation). Samples containing *L. acidophilus* and *B. lactis* included the highest acetaldehyde contents. After 28 days, samples containing paraprobiotics (*L. acidophilus*) included the highest lactic acid levels. The *L. acidophilus* showed greater effects on acetaldehyde generation in yogurts after 28 days of storage. Addition of paraprobiotics increased viability of the starter cultures. Moreover, incorporation of inactivated probiotic cells into yogurts decreased the syneresis and increased the WHC (32).

Conclusion

Results of this study revealed that the probiotic strains significantly affected syneresis and rheological characteristics of the yogurts. Native and commercial probiotic strains were effective in strengthening the casein network and yogurt structure as well as decreasing the syneresis. Therefore, use of the Iranian native *L. acidophilus* strain is recommended in probiotic yogurt production as it provides better rheological and syneresis characteristics after fermentation. Further studies are needed to assess effects of other Iranian native probiotic strains on rheological and syneresis characteristics of the yogurts during fermentation and storage.

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