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Original research

Rheological properties of probiotic non-fat yogurt containing *Lactobacillus reuteri*: Effects of inulin addition, inoculum level and fermentation temperature

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A B S T R A C T

The simultaneous effects of inulin addition (0-4%w/w), probiotic inoculum level (1-3%v/v) and fermentation temperature (37-45°C) on the dynamic rheological properties of probiotic non-fat yogurt were studied using response surface methodology. Frequency sweep tests were performed to measure structure strength and type of structure. Linear viscoelastic range in term of strain (LVE), structure strength (G′ at LVE), yield stress (τ_y) and flow point (τ_f) of samples were measured doing strain sweep test. Results showed that Inulin concentration had the greatest influence on G' and τ_f, followed by fermentation temperature. A positive correlation between b value and level of syneresis was observed.

Keywords: Yogurt, Probiotic, Inulin, Rheological properties, Response surface methodology

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1. Introduction

Yogurt is the most popular fermented dairy product with high nutritional and health benefits. This positive aspect can be increased further by adding nutritional and pharmaceutical components such as probiotic bacteria and dietary fibers to the yogurt.

Probiotics are defined as live microbial supplement that beneficially affects the host by improving its intestinal microbial balance (Fuller, 1989). Probiotics provide numerous therapeutic benefits such as preventing intestinal infections, improving lactose intolerance, decreasing side-effects of antibiotics, improving the immune functions, preventing cancers and reducing blood cholesterol (Aryana & McGrew, 2007). Different strains of lactic acid bacteria are regarded as probiotics. *Lactobacillus reuteri* (previously *fermentum*) is one of the most documented probiotic strains which can colonize the intestine and vagina and decrease incidence of bacterial vaginosis and urinary tract infections (Hekmat et al., 2009). It is bile tolerant and survives passage through the human digestive tract (Gardiner et al., 2002; Reid et al., 2002).

Soluble dietary fibers also have beneficial effects on health, amongst which Inulin is a unique functional food ingredient because of its attractive technological and nutritional properties. The former include sugar and fat substitute, low caloric bulking agent, texture modifier and water-binding agent (Tungland & Meyer, 2002). Apart from its technological aspects, inulin provides several health benefits, namely acts as a prebiotic by promoting the selective growth of *Bifidobacterium* and *Lactobacillus* species in the human gastrointestinal system (Roberfroid et al., 1998), causes inhibition of harmful bacteria, enhances bioavailability of minerals (Tahiri et al., 2003), decreases blood cholesterol (Delzenne & Williams, 2002) and prevents colon cancer (Jenkins et al., 1999). Based on the combination of these benefits, the use of inulin in the food industry such as bread and other bakery products, table spreads, beverages, confectionery, frozen desserts, ice-cream, dressings and yogurt, especially low-fat yogurt, has grown recently.

Fat reduction in yogurt can cause defects such as lack of the sensory and textural quality (Guven et al., 2005). Since consumer concerns are associated to both nutritional and sensory aspects, textural and rheological properties of yogurt are essential characteristics for consumer acceptability. Therefore, the effect of inulin on sensory and rheological properties of yogurt has been studied by several researchers, and interestingly different results have been reported (Aryana & McGrew, 2007; Dello Staffolo et al., 2004; Guggisberg et al., 2009; Guven et al., 2005; Kip et al., 2006; Passephol et al., 2008). However, few reports were found on the effects of inulin addition on the viscoelastic attributes of probiotic yogurt.

Rheological properties of yogurt are also influenced by many other factors, for instance, composition of milk and its total solid content (Gun & Isykly, 2007; Kristo et al., 2003; Wu et al., 2009), the type of starter culture (Gun & Isykly 2007; Vivian et al. 2007), processing conditions such as heat treatment of milk (Labropoulos et al., 1984; Lee & Lucey, 2004a), fermentation temperature

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(Haque et al., 2001; Lee & Lucey, 2004a, b; Wu et al., 2009), inoculum level (Kristo et al., 2003; Lee & Lucey, 2004b; Wu et al., 2009) and storage time (Sodini et al., 2004). Incubation temperature mainly affects bacterial activity and thus the acidification process which can alter the taste and texture of the product (Radke-Mitchell & Sandine, 1986). Lee and Lucey (2006) reported that the use of lower fermentation temperature resulted in stirred yogurts with higher apparent viscosity. However, Haque et al. (2001) found that increasing incubation temperature gave a progressive increase in gel strength, viscosity and elastic modulus. On the other hand, the inoculum level is significant for a desired acidification rate and to guarantee a sufficient amount of final bacteria during the shelf-life of the product (Kristo et al., 2003). But the amount of inoculum is usually determined empirically (Wu et al., 2009). Therefore, it is important to provide information on process and incubation conditions including fermentation temperature and level of starter culture to develop the quality of yogurt.

Rheological measurements are extensively employed to predict and control the quality and structure of food systems. Since yogurt is a viscoelastic material, small and large deformation oscillatory testing give useful information on gel structure and behavior. The parameters measured in frequency sweep test are very sensitive to chemical composition and physical structure of gel (Cavallieri & da Cunha, 2008) and strain sweep data outside the LVE rang (large deformation) are more closely related to consumer perception (Guggisberg et al., 2009). Because of the thixotropic behavior of yogurt gel, the rheodestruction must be minimized. Therefore, vane measuring system was applied as a useful rheological method for yogurt.

The response surface methodology (RSM) is a group of statistical and mathematical techniques that can be used to design experiments, build models, study the effects of factors on dependent variables and search optimum conditions of factors for desirable responses (Myers & Montgomery, 2002). RSM has effectively been used to investigate and optimize the rheological properties of yogurt (Keogh & O'Kenndy, 1998; Lee & Lucey, 2004a; Unal et al., 2003).

Since there is no study on the interactive effects of inulin, incubation temperature and probiotic level on texture and rheological properties of non-fat yogurt, the objectives of this research were to model and evaluate, through RSM, the combined effects of long-chain inulin addition, fermentation temperature and inoculum level of probiotic on rheological properties of probiotic non-fat yogurt, containing *L. reuteri*; and to study the relationships between rheological parameters and level of syneresis.

2. Material and Methods

2.1. Culture preparation

L. reuteri (DSM 20016) was obtained from the DSMZ, Germany. Stock culture was stored in 40% glycerol at -20°C. The organism was subcultured three times before use in sterile de Man, Rogosa, Sharpe (MRS) broth (Merck, Germany) using 1% inoculum and incubated anaerobically for 18 h at 37°C.

2.2. Preparation of probiotic inoculum

To prepare *L. reuteri* DSM 20016 inoculum, pasteurized (85°C, 30 min) reconstituted skim milk (Pegah, Tehran, Iran, low heat, powder) containing 15.2% total solid including 1.2% yeast extract (Merck, Germany) was inoculated with 10% of overnight probiotic broth culture. After incubation for 18 h at 37°C, the resultant curdled milk was used to inoculate pasteurized reconstituted skim milk (14% total solid).

2.3. Yogurt manufacture

The determined amounts of skim milk powder were dissolved in distilled water, and supplemented with 0-4% (w/w) long-chain inulin (inulin HP with an average DP of 23, Orafti, Malvern, PA) to a final solid content of 14%. Milk samples were heat-treated at 85°C for 30 min in a thermostatically controlled water bath, cooled to 42°C and then inoculated with yogurt starter cultures. Standard yogurt starter (a 1:1 mixture of *S. thermophilus* and *L. delbreukii* sub-species *bulgaricus*) was used in a constant level of 2% (v/v) in all the tests and only the inoculum level of *L. reuteri* varied from 1% to 3% (v/v) according to the experimental design (Table 1). After a slow agitation to distribute the culture evenly, inoculated milk samples were incubated at different incubation temperature (37-45°C) until the pH value of 4.6 was reached. After incubation, samples were stored at 5°C before the rheological tests.

2.4. Rheological measurements

Immediately after inoculation, 37 mL of yogurt milk were transferred to the rheometer cup. Simultaneously similar sample was placed in the incubator (Digital incubator, Shimaz, Iran) and the pH was being monitored at the experimental conditions using a pH meter (Metrohm, 827 pH Lab, Swiss) with an electrode standardized at the experiment temperatures over the range from 7.0 to 4.0.

Rheological properties of yogurts were determined using a Physica MCR 301 Rheometer (Anton paar, GmbH, Graz, Austria) with the four-blade vane St14. the vane was introduced into the cup vertically using the dimensions suggested by Steffe (1996). The sample surface was covered with a solvent trap to avoid evaporation. Temperature was regulated by a viscotherm VT2 circulating bath and a controlled peltier system (Anton paar) and fixed at the experimental temperature with an accuracy of ± 0.01 °C. For the strain sweep tests the deformation was elevated from 0.01 to1000% at a constant frequency of 1Hz to determine: 1) Limit value of linear viscoelastic range in terms of strain (LVE or γ_L). 2) Structure strength or firmness in terms of elastic and complex modulus (G′ and G* respectively) at LVE. 3) Viscous or loss modulus (G″) which is an indication for the fluid character of the mixture. 4) Resistance to mechanical force or yield stress (τ_y) which could be calculated from limiting value of LVE range in term of shear stress. 5) Cross over (G′=G″), which occurs when the LVE is completely left and G′ can cross G″. 6) Flow point (τ_f), the stress in which the structure is ruptured to such an extent causing the material to flow (G′=G″). 7) Damping factor (tan δ) or the ratio of loss modulus to elastic modulus. For the frequency sweep, a constant strain of 1% was adjusted and the frequency lowered from 16 to 0.01 Hz. Power low model ($G'=A\ \omega^b$) was fitted on experimental data and regression coefficients (A) and (b) were determined as a measure of structure strength and type of structure respectively. Low values of (b) show a gel structure whereas higher values indicate a gel like structure (Knudsen et al., 2006; Mezger, 2006).

2.5. *Determination of syneresis*

The level of spontaneous syneresis in undisturbed set yogurt was determined according to Amatayakul et al. (2006). A cup of set yogurt (4°C) weighed and kept at an angle of about 45° to collect the separated whey on the side of the cup. A needle attached to a syringe was used to remove the whey from the surface of the sample, and the cup of yogurt was weighed again. The test was performed within 10 s to avoid further leakage of whey from the gel. The syneresis was expressed as the percentage weight of the whey over the initial weight of the yogurt sample.

2.6. *Experimental design and statistical analysis*

The Experimental design was a face-central composite plan with three variables and three levels for each factor, resulting in 20 treatments. The independent variables were inulin content, probiotic inoculum level and fermentation temperature. The coded and actual levels and experimental design are shown in Table 1 and 2. The quadratic polynomial model was fitted to each response, according to the following equation:

Table 1. Levels of independent variables used in face-central composite design.

Independent variable	Coded levels of variables			
	Symbol	-1	0	+1
Inulin content (%w/w)	X_1	0	2	4
Probiotic inoculum level (%v/v)	X_2	1	2	3
Fermentation temperature (°C)	X_3	37	41	45

Table 2. Second order central composite design matrix.

Run	Coded variables			Uncoded variables		
	X_1	X_2	X_3	x_1	x_2	x_3
1	-1	-1	-1	0	1	37
2	1	-1	-1	4	1	37
3	-1	1	-1	0	3	37
4	1	1	-1	4	3	37
5	-1	-1	1	0	1	45
6	1	-1	1	4	1	45
7	-1	1	1	0	3	45
8	1	1	1	4	3	45
9	-1	0	0	0	2	41
10	1	0	0	4	2	41
11	0	-1	0	2	1	41
12	0	1	0	2	3	41
13	0	0	-1	2	2	37
14	0	0	1	2	2	45
15	0	0	0	2	2	41
16	0	0	0	2	2	41
17	0	0	0	2	2	41
18	0	0	0	2	2	41
19	0	0	0	2	2	41
20	0	0	0	2	2	41

$$Y = B_0 + \sum_{i=1}^{3} B_i X_i + \sum_{i=1}^{3} B_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} B_{ij} X_i X_j \quad (1)$$

where Y is the response, β_0, β_i, β_{ii} and β_{ij} are regression coefficients for intercept, linear, quadratic and interaction effects, respectively and X_i and X_j are the coded values of independent variables. The quality of fit of the model and its statistical significance were expressed by determination coefficient (R^2) and F-value, respectively. The significance of the regression coefficients was determined by t-value (at an alpha level of 0.05). The analysis was carried out using coded units. Minitab 14 (Minitab Inc., State College, PA, USA) was employed for response surface analysis and drawing of plots. In addition, Microsoft Excel 7 software (Microsoft, Redmond, U.S.A.) was used to plot the curves.

Table 3. Effect of inulin content on rheological properties of yogurt samples inoculated with 2% v/v probiotic and incubated at 41°C.

Rheological parameters	Inulin content (%w/w)		
	0	2	4
G' at LVE (Pa)	2790.71	1753.15	1309.99
G″ at LVE (Pa)	684.62	451.25	345.54
G* at LVE (Pa)	2873.45	1810.31	1354.81
Tanδ at LVE	0.245	0.257	0.263
G', G″ at Cross over (Pa)	204.72	147.73	105.51
γ_L (%)	16.7	18	17.1
τ_y (Pa)	406	275	193
τ_f (Pa)	1048.21	775.66	568.01
A(Pa.s^b)	2041.91	1268.02	943.84
b	0.159	0.166	0.168

Table 4. Effect of probiotic inoculum level on rheological properties of yogurt samples containing 2% w/w inulin and incubated at 41°C.

Rheological parameters	Probiotic inoculum level (%v/v)		
	1	2	3
G' at LVE (Pa)	1719.03	1753.15	1409.24
G″ at LVE (Pa)	426.14	451.25	378.39
G* at LVE (Pa)	1771.06	1810.31	1459.16
Tanδ at LVE	0.247	0.257	0.268
G', G″ at Cross over (Pa)	154.81	147.73	141.98
γ_L (%)	19	18	16
τy (Pa)	286	275	198
τ_f (Pa)	789.79	775.66	687.88
A(Pa.s^b)	1286.11	1268.02	957.67
b	0.161	0.166	0.165

Table 5. Effect of fermentation temperature on rheological properties of yogurt samples containing 2% w/w inulin and inoculated with 2% v/v probiotic.

Rheological parameters	Fermentation temperature (°C)		
	37	41	45
G' at LVE (Pa)	1452.38	1753.15	2031.23
G″ at LVE (Pa)	343.48	451.25	521.86
G* at LVE (Pa)	1492.45	1810.31	2097.21
Tanδ at LVE	0.236	0.257	0.256
G', G″ at Cross over (Pa)	144.57	147.73	170.53
γ_L (%)	19.5	18	18.5
τ_y (Pa)	245	275	328
τ_f (Pa)	762.03	775.66	906.78
A(Pa.s^b)	1093.51	1268.02	1467.62
b	0.152	0.166	0.169

3. Results and Discussion

3.1. *Rheological properties*

The effect of inulin addition, probiotic inoculum level and fermentation temperature on different rheological parameters obtained from strain sweep and frequency sweep tests are presented in Table 3-5. A considerable decrease (about two times) in the G', G″, G*, cross over (G′=G″), τ_y, τ_f and A values of yogurt samples was observed with increasing inulin concentration from 0 to 4%, whereas damping factor and b value slightly increased (Table 3). The least values of G', G″, G*, cross over (G′=G″), τ_y, τ_f and A were obtained at probiotic inoculum level of 3 % (v/v). On the other hand, yogurts inoculated with 2% (v/v) probiotic showed the maximum values of G', G″ and G* (Table 4). Results showed that by increasing fermentation temperature in yogurt manufacture, G', G″, G*, cross over (G′=G″), τ_y, τ_f and A values could be increased (Table 5).

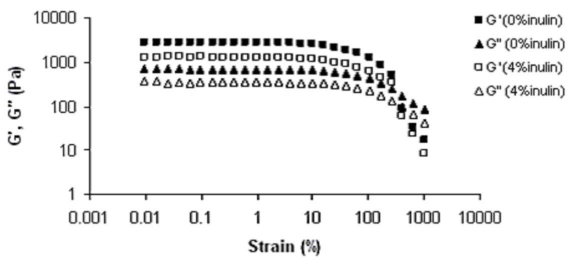

Fig. 1. Effect of inulin content on G′ and G″ of yogurts as a function of strain. Samples inoculated with 2% (v/v) probiotic and incubated at 41°C.

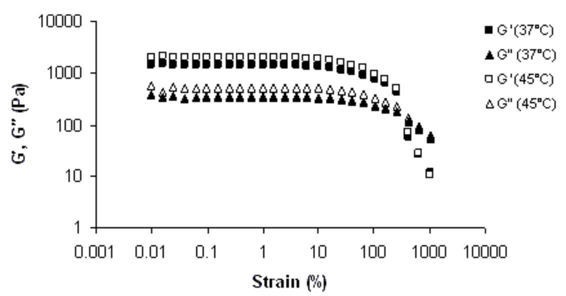

Fig. 2. Effect of fermentation temperature on G′ and G″ of yogurts as a function of strain. Samples containing 2% w/w inulin and inoculated with 2% v/v probiotic.

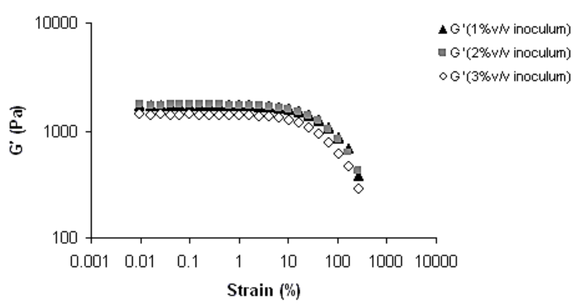

Fig. 3. Effect of probiotic inoculum level on G′ of yogurts as a function of strain. Samples containing 2% w/w inulin and incubated at 41°C.

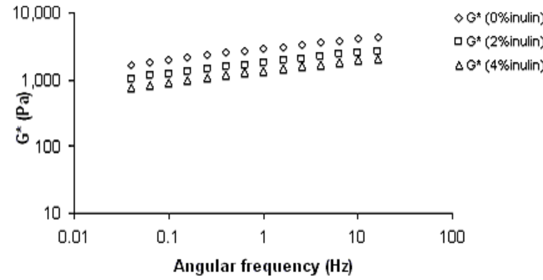

Fig. 4. Variation of G* as a function of frequency for yogurts containing different levels of inulin. Samples inoculated with 2% v/v probiotic and incubated at 41°C.

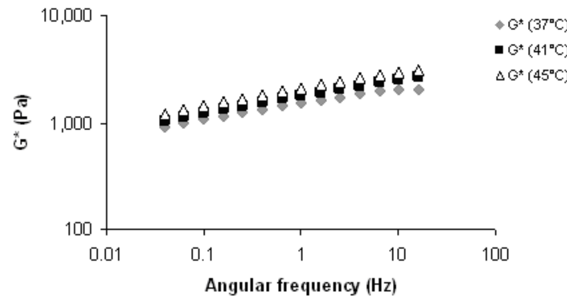

Fig. 5. Variation of G* as a function of frequency for yogurts incubated at different temperatures. Samples containing 2% w/w inulin and inoculated with 2% v/v probiotic.

The effects of inulin content and incubation temperature on the G′ and G″ of yogurts as a function of strain are shown in Fig. 1 and 2. It can be seen that G′ values of all yogurt samples were greater than G″ over the tested strain range, which is predictable for solid viscoelastic systems. The length of the LVE indicates that G′ and G″ values are independent of the oscillation strain (reversible elastic). Higher inulin content and lower fermentation temperature led to decrease in G′ at LVE which means less elastic behavior and firmness. Fig. 3 shows that samples with medium probiotic inoculum level (2% v/v) had a higher G′ value which means higher structure strength. However, the effect of inoculum level on G′ was less than that of inulin content and fermentation temperature.

Frequency sweep tests were used to study time-dependent behavior. The slope values of log-log plots of G′ versus ω indicate the frequency dependence of the elastic modulus of tested samples. Fig. 4 and 5 show the variation of G* as a function of frequency for samples which just differ in the inulin content and fermentation temperature, respectively. The log G* values of yogurt samples with different inulin content increased linearly as log frequency increased (Fig. 4). The similar trends were observed for yogurts incubated at different temperatures (Fig. 5).

3.2. *Correlation between rheological properties and synersis level*

The correlation coefficients between the small and large deformation oscillatory rheological properties of yogurt gels prepared at different conditions are shown in Table 6. The correlation matrix showed strong positive correlations among G′, G″, G*, cross over (G′=G″), τ_y, τ_f and A value with $0.914 \leq r \leq 1$ (p

< 0.01). These parameters except τ_y ($p > 0.05$) negatively correlated with γ_L ($-0.515 \le r \le -0.665$), which indicate that higher firmness (higher G′) is accompanied by shorter linear viscoelastic range and also higher yield stress in term of flow point. The G' value also had negative correlations with tanδ and b value, although these correlation coefficients were not statistically significant ($p > 0.05$). Paseephol et al. (2008) also found significant positive correlations between G′, G″ and complex viscosity (η*); these parameters were negatively correlated with tanδ. Furthermore, they reported that viscoelastic parameters (G′, G″ and η*) from dynamic test were positively correlated with the firmness value from penetration test. Lee and Lucey (2004b) found a significant positive correlation between G′ and yield stress. Results indicated that according to the b value by increasing in tanδ (at LVE) the structure will be changed from gel to gel like.

Syneresis (whey separation) is an important physical defect in yogurt which is related to an unstable network (Lee & Lucey, 2004a). In the present study among viscoelastic parameters only "b" value significantly correlated with syneresis and the correlation was positive, indicating that less whey separation occurred in gel structures rather than gel like structures. Ozcan-Yilsay et al. (2007) reported that weak yogurt gels exhibited high levels of syneresis.

Table 6. Pearson's correlation coefficients between dependent variables.

	$G'_{(LVE)}$	$G''_{(LVE)}$	$G^*_{(LVE)}$	$\tan\delta_{(LVE)}$	(G′=G″)	γ_L	τ_y	τ_f	a	b	syn
$G'_{(LVE)}$	-										
$G''_{(LVE)}$	0.994**	-									
$G^*_{(LVE)}$	1.000**	0.994**	-								
$\tan\delta_{(LVE)}$	-0.424	0.322	-0.419	-							
(G′=G″)	0.955**	0.935**	0.955**	-0.516*	-						
γ_L	-0.642**	-0.665**	-0.643**	0.007	-0.559*	-					
τ_y	0.927**	0.914**	0.927**	-0.481*	0.916**	-0.328	-				
τ_f	0.952**	0.930**	0.951**	0.533*-	0.992**	-0.515*	0.926**	-			
a	0.998**	0.985**	0.998**	-0.472*	0.959**	-0.625**	0.929**	0.959**	-		
b	-0.262	-0.159	-0.257	0.906**	-0.376	-0.046	-0.319	-0.378	-0.300	-	
syn	0.353	0.422	0.357	0.412	0.281	-0.258	0.372	0.298	0.325	0.509*	-

* ,** Significant at the 0.05 and 0.01 level, respectively.

Table 7. Pearson's correlation coefficients between dependent variables.

Source of variation	DF	Sum of squares	Mean square	F-value	p-value
Regression	9	4762130	529126	45.86	0.000
Linear	3	4436282	1478761	128.16	0.000
Square	3	257026	85675	7.43	0.007
Interaction	3	68823	22941	1.99	0.180
Lack of fit	5	71128	14226	1.61	0.308
Pure error	5	44260	8852		
Total	19	4877517			
Factors	**DF**	**Coefficient estimate**	**Standard error**	**t-value**	**P-value**
Intercept	1	1820.69	36.93	49.304	0.000
X_1	1	-631.87	33.97	-18.602	0.000
X_2	1	-69.09	33.97	-2.034	0.069
X_3	1	198.97	33.97	5.858	0.000
X_1^2	1	243.66	64.78	3.762	0.004
X_2^2	1	-242.56	64.78	-3.745	0.004
X_3^2	1	-64.88	64.78	-1.002	0.340
$X_1 X_2$	1	31.18	37.98	0.821	0.431
$X_1 X_3$	1	-86.11	37.98	-2.267	0.047
$X_2 X_3$	1	-14.71	37.98	-0.387	0.707

R^2=97.6% and adjusted R^2=95.5%

3.3. RSM models for G′ (at LVE) and flow point (τf)

Table 7 and 8 show the ANOVA for the quadratic model for G′ (at LVE) and flow point, respectively. The results revealed that the second-order regressions to produce the quadratic models were significant ($p < 0.001$). The lack-of-fit tests which determine the fitness of the models, were insignificant ($p > 0.05$). Determination coefficients (R^2) were high indicating that the models accurately predict the degree of G′ (at LVE) and τ_f in the experimental region (Myers & Montgomery, 2002). The second-order polynomial models for G′ (at LVE) and τ_f are expressed as Equations 2 and 3, respectively.

$$Y = 1820.69 - 631.87X_1 - 69.09X_2 + 198.97X_3 + 243.66X_1^2 - 242.56X_2^2 - 64.88X_3^2 + 31.18X_1X_2 - 86.11X_1X_3 - 14.71X_2X_3 \quad (2)$$

$$Y = 778.486 - 195.684X_1 - 33.115X_2 + 49.883X_3 + 25.387X_1^2 - 44.218X_2^2 + 51.352X_3^2 + 5.475X_1X_2 - 25.625X_1X_3 - 16.650X_2X_3 \quad (3)$$

Table 7 shows that inulin content (X_1) had both significant linear and quadratic effects on G′ (at LVE), whereas fermentation temperature (X_3) only had a linear effect. The linear effect of starter culture level was insignificant ($p > 0.05$), however, the quadratic term was found to be significant. There was also a significant (p <

0.05) interaction between inulin content and fermentation temperature for G′ at LVE. The full-term model (Equ. 3) was considered for the flow point, although it seemed that only the linear terms of variables significantly influenced it (Table 8).

Table 8. Analysis of variance and estimated regression coefficients for flow point (τ_f).

Source of variation	DF	Sum of squares	Mean square	F-value	P-value
Regression	9	440128	48903	24.49	0.000
Linear	3	418771	139590	69.90	0.000
Square	3	13646	4549	2.28	0.142
Interaction	3	7711	2570	1.29	0.332
Lack of fit	5	11386	2277	1.33	0.382
Pure error	5	8584	1717		
Total	19	460099			

Factors	DF	Coefficient estimate	Standard error	t-value	P-value
Intercept	1	778.486	15.36	50.673	0.000
X_1	1	-195.684	14.13	-13.847	0.000
X_2	1	-33.115	14.13	-2.343	0.041
X_3	1	49.883	14.13	3.530	0.005
X_1^2	1	25.387	26.95	0.942	0.368
X_2^2	1	-44.218	26.95	-1.641	0.132
X_3^2	1	51.352	26.95	1.906	0.086
$X_1 X_2$	1	5.475	15.80	0.347	0.736
$X_1 X_3$	1	-25.625	15.80	-1.622	0.136
$X_2 X_3$	1	-16.650	15.80	-1.054	0.317

R^2=95.7% and adjusted R^2=91.8%

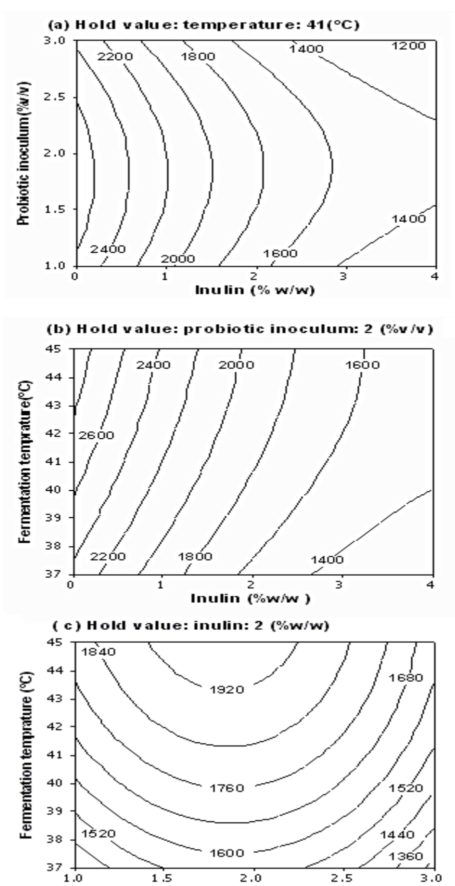

Fig. 6. Contour plots for G′ (at LVE). (a) Interaction of inulin content and probiotic inoculum level, (b) interaction of inulin and fermentation temperature and (c) interaction of incubation temperature and probiotic inoculum level.

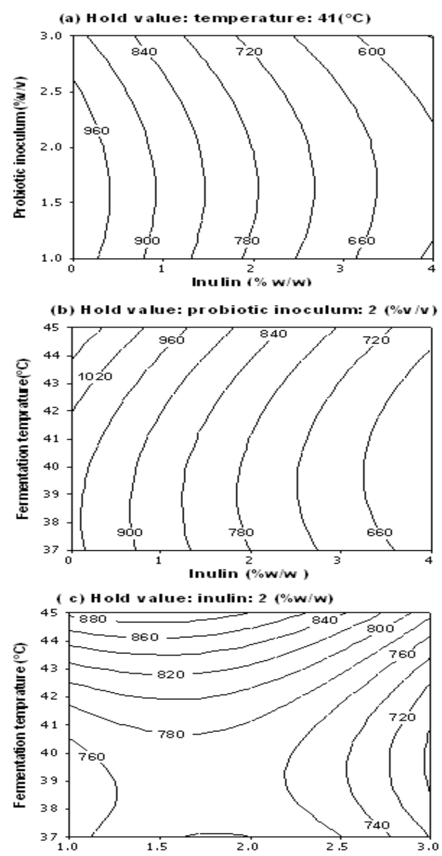

Fig. 7. Contour plots for flow point (τ_f). (a) Interaction of inulin content and probiotic inoculum level, (b) interaction of inulin and fermentation temperature and (c) interaction of incubation temperature and probiotic inoculum level.

The effect of inulin content was the strongest factor in determination of G′ (at LVE) and τ_f, as obviously demonstrated by the highest absolute value of regression coefficient among all other coefficients of the model. Fig. 6 and 7 show contour plots of the models fitted to the experimental data for G′ (at LVE) and τ_f, respectively. Fig. 6(a) demonstrates the interactive effects of inulin content and probiotic inoculum level on G′ (at LVE). It shows that inulin content did a strong negative effect; higher inulin content resulted in lower G′ value. However, inoculum level only showed a little effect on G'. The similar effect of inulin content was observed on τ_f (Fig. 7(a)). These results are in agreement with the findings of Passephol et al. (2008); they reported that inulin-containing yogurts compared with control had lower values of firmness, yield stress, apparent viscosity, storage modulus, loss modulus, and complex viscosity. The firmness of yogurt generally depends on the number and strength of bonds between casein particles (Lucey et al., 1997). Higher total solids and specially protein content would cause an increase in the number of casein-casein bonds in the gel network and thereby more compact and much firmer structure (Tamime, 2006). According to the research of Kristo et al. (2003), high total solids resulted in large increase in G′. In the present study, yogurt milks were standardized to 14% total solids. Therefore, samples containing inulin had less net protein content. Moreover, the inulin molecules would not support the structure because of their inability to form a cohesive network with the protein matrix. In contrast they could act as inactive fillers or destructive fillers, and hinder the protein matrix formation, thus reducing the degree of cross-linkage and firmness of yogurt gels (Passephol et al., 2008). Similarly, the addition of native whey proteins to yogurt milk caused a decrease in yogurt gel strength. (Guggisberg et al., 2007). However, Guggisberg et al. (2009) reported that higher firmness and yield stress values were obtained in yogurts containing higher inulin levels. But instead of standardizing the total solids, the protein content of milk was standardized in their study, thus increasing inulin content led to increase in total solids values.

Fig. 6(b) and 7(b) illustrate the effects of inulin content and fermentation temperature on G′ (at LVE) and τ_f, respectively. In these graphs G′ (at LVE) and τ_f values increased with decrease in inulin content and increase in fermentation temperature. However, the effect of inulin content was much stronger.

The interaction between fermentation temperature and probiotic inoculum level is shown in Fig. 6(c) and 7(c). It can be seen that yogurts formed at higher temperatures had higher G' and τ_f values compared with those formed at lower temperatures. This result concurs with the conclusions of Haque et al. (2001) who reported that increasing incubation temperature gave a progressive increase in gel strength, viscosity and storage modulus (G′) of the yogurt samples. Other researchers have also observed an increase in viscosity with increasing fermentation temperature (Schellhaas & Morris, 1985; Skirver et al., 1993). In contrast, the negative effect of incubation temperature on gel strength has been reported in some literatures (Kristo et al., 2003; Lee & Lucey, 2004b; Wu et al., 2009). The integrity of casein micelles in milk is related to the local balance between electrostatic repulsion and hydrophobic interaction (Horn, 1998; Lucey, 2002). During fermentation, electrostatic repulsion decreases due to the production of acids by starter bacteria, therefore assisting the casein micelles to aggregate into chains and clusters through hydrophobic bonds. Further pH reduction to isoelectric point causes extensive casein–casein attractions (Tamime, 2006). According to Horn (1998) the positive force to agglomeration of micelles is attributed to hydrophobic interactions; and because they are of entropic origin, enhance progressively as temperature is increased ($\Delta G=\Delta H-T\Delta S$). Consequently, increasing incubation temperature would develop hydrophobic association and thereby the firmness and rigidity of yogurt gels.

Fig. 6(c) and 7(c) show that at a constant fermentation temperature, with the increase of inoculum level G′ (at LVE) and τ_f were initially found to increase slightly and then decreased. Therefore, at low and high levels of starter culture, the G′ (at LVE) and τ_f values of yogurt were low; however, middle level of probiotic inoculum led to higher firmness. These results confirm the findings of Wu et al. (2009), who observed that the highest apparent viscosity was obtained at intermediate starter culture rate. Lee and Lucey (2004b) also reported that medium to high amounts of inoculum give yogurts with less textural defects and lower syneresis; they concluded that lower yield stress in yogurt samples inoculated with low starter culture levels (e.g. 0.5%) can be due to large pores and weaker associations between casein particles. Inoculation rate, as well as fermentation temperature, directly influences the rate of acidification (Lee & Lucey, 2004b), thus it is feasible that some of the differences between samples' properties result from the different kinetics of gel formation.

3.4. *Elaboration of G′ (at LVE) and τf*

The optimal values of independent variables can be obtained by solving regression equations. Response optimizer of RSM package was employed to determine the values of factors in the regression equations (Equ. 2 and 3) to achieve the maximum values of G' and τ_f. The optimum values of the variables were 0% w/w, 1.7715% v/v and 45ºC for inulin content, probiotic inoculum level and fermentation temperature, respectively. The models predict that the maximum values of G' and τ_f which can be obtained using the above optimal conditions are 2930.0285 Pa. and 1136.7306 Pa., respectively. The values indicate that lower inulin concentration, middle levels of probiotic inoculum and higher fermentation temperature result in higher values of G' and τ_f and consequently higher firmness.

4. Conclusion

This study was performed to determine how inulin content, probiotic inoculum level and fermentation temperature affect rheological properties of probiotic non-fat set yogurt containing *L. reuteri*. The results indicated that the quadratic models were well adjusted to predict the experimental data. Inulin addition made a softening effect on yogurt; with increasing inulin content the G′, G", G*, $\tau_{y,}$ τ_f and A value decreased. In contrast, incubation temperature had a positive influence on structure strength. Results showed that the highest G' and τ_f values were obtained by a combination of low inulin concentration (0% w/w), high fermentation temperature (45ºC) and middle level of probiotic inoculum (1.7715% v/v). Determination the correlations between rheological parameters and level of syneresis showed a significant positive correlation between b value and level of spontaneous whey separation. Finally, it should be noted that probiotic, yogurt and inulin itself are well known for their health benefits, and together they can provide an interesting functional food with high consumer acceptability and commercial applications.

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