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Sha Wu, *China Agricultural University, China*
Dong Li, *China Agricultural University, China*
Shu-jun Li, *Chinese Academy of Agricultural Mechanization Sciences, China*
Bhesh Bhandari, *The University of Queensland, Australia*
Bao-ling Yang, *China Agricultural University, China*
Xiao Dong Chen, *Monash University, Australia*
Zhi-huai Mao, *China Agricultural University, China*

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Effects of Incubation Temperature, Starter Culture Level and Total Solids Content on the Rheological Properties of Yogurt

Sha Wu, Dong Li, Shu-jun Li, Bhesh Bhandari, Bao-ling Yang, Xiao Dong Chen, and Zhi-huai Mao

Abstract

The effects of incubation temperature (35-45oC), starter culture level (0.003-0.006%, w/v), and milk total solids content (12.32-15.68%, w/v) on the rheological properties of yogurt were investigated using central composite rotatable design (CCRD) and response surface methodology (RSM). Gelation time during milk fermentation and apparent viscosity of yogurt stored for 3 days were the rheological parameters studied. The results revealed that the gelation time and apparent viscosity of yogurt were significantly affected ($P < 0.1$) by incubation temperature, starter culture level, and total solids. Incubation temperature had the greatest influence on the gelation time. Higher temperature led to shorter gelation time, but resulted in lower apparent viscosity of yogurt. Apparent viscosity of yogurt was mainly affected by total solids. Higher apparent viscosities were also obtained at lower incubation temperature.

KEYWORDS: rheology, gelation, viscosity, yogurt, response surface methodology

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1. INTRODUCTION

Yogurt is the most popular fermented dairy product with high nutritional and health values. It is produced all over the world by acid coagulation of milk. Yogurt is a typical non-Newtonian fluid, exhibiting a shear-thinning, yield stress, viscoelasticity, and thixotropic (time-dependency) flow behavior (Afonso & Maia, 1999). The rheological properties of yogurt are indicative of gel formation and are important in the design of flow processes, quality control, processing and storage, and predicting the texture of yogurt. So, the rheological properties of yogurt during gelation have received much attention in the literature (Jumah et al., 2001; Lucey & Singh, 1997; Soukoulis et al., 2007). Rheological properties of yogurt depend on many factors, such as the type of starter culture (Gün & Işıklı, 2007; Vivian et al., 2007), incubation temperature (Haque et al., 2001), composition of milk (Gün & Işıklı, 2007; Gastaldi et al., 1997; Keogh & O'Kennedy, 1998), processing conditions (e.g., heat treatment, homogenization) (Labropoulos et al., 1984; Lucey, 2004), and storage time (Sodini et al., 2004).

Since yogurt is a viscoelastic material, dynamic rheological tests can provide information on behavior of gel formation during the fermentation process. Recently, rheometer has become an excellent and convenient tool in the study of milk gel structure. Rheometer working in dynamic mode provides the information of the storage modulus (G'), and the loss modulus (G''). G'-value is denoted as the elastic property and is a measurement for the solid nature of the mixture. On the contrary, G''-value, also known as the viscous modulus, is an indication for the fluid character of the food system (Steffe, 1996). Moreover, the complex modulus (G^*) is another useful parameter, which is defined as $G^* = (G'^2+G''^2)^{1/2}$. Using time sweep measurement, the gelation time is chosen as the time at which G^* is equal to 1 Pa, obtained from the curve of G^* against time (Tan et al., 2007).

One of the fundamental parameters, which characterize flow behavior of liquid and semi-liquid foods, is viscosity, which has been considered traditionally one of the main quality attributes to determine the overall quality and acceptability of yogurt. The viscosities and flow properties of stirred yogurt influenced by the composition have been studied by many researchers. Mohameed et al. (2004) found that a 5% increase in solids concentration doubled the apparent viscosity of labneh and the power law model fitted the apparent viscosity-shear rate experimental data satisfactorily. Domagała et al. (2006) found that the addition of 1 kg fat or 2 kg maltodextrin/100 kg yogurt caused an increase in apparent viscosity compared to the control yogurt. Lee & Lucey (2006) demonstrated that the use of higher milk preheating temperature and lower incubation temperature resulted in stirred yogurts with higher apparent viscosity, oral viscosity, and smoothness sensory attributes.

Response surface methodology (RSM) is an effective mathematical and statistical technique that can be used to evaluate the effect of some factors (independent variables) on one or more dependent variables (Ünal et al., 2003). It reduces the number of experimental trials required to evaluate multiple parameters and their interactions (Gunawan et al., 2005). RSM has successfully been applied to study and optimize the rheological properties (Ünal et al., 2003; Keogh & O'Kennedy, 1998; Lee & Lucey, 2004a) and kinetics of gelation (Kristo et al., 2003; Patrignani et al., 2007) of fermented milk.

Direct vat set (DVS) cultures are widely used in fermented milk products due to their high activity and can be added into the milk directly without sequential subculture. But the inoculum level is always decided empirically and few literatures are related to that. It is essential to provide information on process control conditions including starter culture level to improve the quality of yogurt. The aim of this study was to evaluate the combined effects of incubation temperature, starter culture level, and total solids content on the rheological properties of yogurt fermented with a direct vat set starter culture applying the response surface methodology.

2. MATERIALS AND METHODS

2.1 Materials

Starter culture used in this study was a freeze-dried commercial culture for Direct Vat Set (FD-DVS) YF-812 (Chr. Hansen, Denmark), which contained *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*. The culture was stored below -18^{o}C.

Pasteurized and homogenized milk was obtained from Beijing San Yuan Foods Co., Ltd.. To adjust the total solid content in the milk, low-heat skim milk powder (Inner Mongolia Yili Industrial Group Co., Ltd.) was used. This powder milk contained 1% of fat (w/w), 34% of protein (w/w), 4% of moisture (w/w), 52.5% of lactose (w/w) and 8.5% of ash (w/w). Standard granulated sugar was the product of Beijing Sugar Tobacco & Wine Co..

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2.2 Preparation of yogurt

The samples were prepared by mixing required amounts of pasteurized and homogenized milk, skim milk powder (SMP) and sugar (5%, w/v). The mixtures were heated to 40 ^{o}C and gently stirred to disperse the SMP and sugar. The amount of SMP used was variable depending on the total solids content of the milks (12.32-15.68%, w/v). The mixtures were further heat treated at 95 ^{o}C for 5 min in a thermostatically controlled water bath. After that, the mixtures were rapidly cooled down to ambient temperature and then inoculated with yogurt starter culture YF-812 at different levels (0.006-0.009%, w/v). Finally, inoculated samples were incubated at different incubation temperature (35-45 ^{o}C) until the pH-values decreased to 4.6. The time taken to reach pH 4.6 was considered as the indication of fermentation time as pH 4.6 is typically used as an endpoint for fermentation in the manufacturing of yogurt. After incubation, the coagulum was stirred manually for 20 s. All the stirred yogurt samples were kept in a refrigerator at 5 ^{o}C prior to the rheological tests.

2.3 Experimental design

The samples of yogurt were prepared according to a second order central composite rotatable design with three variables and five levels of each variable, resulting in 20 treatments. The independent variables of the design were incubation temperature, starter culture level, and total solids content. The uncoded and coded levels and experimental design are listed in ***Tables 1*** and ***Tables 2***.

Table 1. Level of independent variables used in second order central composite rotatable design

Independent variable	Coded levels of variables[a]					
	Symbol	-1.682	-1	0	+1	+1.682
Incubation temperature (^{o}C)	X_1	35	37	40	43	45
Starter culture level (%, w/v)	X_2	0.003	0.004	0.006	0.008	0.009
Total solids content (%, w/v)	X_3	12.32	13	14	15	15.68

[a] The passage from coded variable level to the origin level is given by the following equations: X_1= (incubation temperature -40)/3, X_2= (starter culture -0.006)/0.002, and X_3= (total solids -14)/1

The dependent variables (responses) were:

1. Gelation time (min): the time at which G^* was equal to 1 Pa obtained from the curve of G^* against time.

2. Apparent viscosity (Pa·s): the apparent viscosity of yogurt stored for 3 days at 5 °C at a shear rate of 10 s^{-1}.

Table 2. Second order central composite rotatable design matrix

Treatment	Coded variables			Uncoded variables		
	X_1	X_2	X_3	x_1	x_2	x_3
1	-1	-1	-1	37	0.004	13
2	-1	-1	1	37	0.004	15
3	-1	1	-1	37	0.008	13
4	-1	1	1	37	0.008	15
5	1	-1	-1	43	0.004	13
6	1	-1	1	43	0.004	15
7	1	1	-1	43	0.008	13
8	1	1	1	43	0.008	15
9	-1.682	0	0	35	0.006	14
10	1.682	0	0	45	0.006	14
11	0	-1.682	0	40	0.003	14
12	0	1.682	0	40	0.009	14
13	0	0	-1.682	40	0.006	12.32
14	0	0	1.682	40	0.006	15.68
15	0	0	0	40	0.006	14
16	0	0	0	40	0.006	14
17	0	0	0	40	0.006	14
18	0	0	0	40	0.006	14
19	0	0	0	40	0.006	14
20	0	0	0	40	0.006	14

The results were analyzed using RSM. This methodology allowed the modelling of the results using a second-order equation. The regression model between dependent variables (Y) and independent variables was:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j \quad (1)$$

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Where β_0 is the constant coefficient, β_i is the linear coefficient of variable i, β_{ii} is the quadratic coefficient of variable i, β_{ij} is the interaction coefficient of variables, and X_i and X_j are the coded values of independent variables.

The analysis of variance (ANOVA) was carried out based on the experimental data using the SAS statistical package (SAS Institute, Cary, NC). The statistical significance of the equation parameters for each response variable was also examined. Response surfaces and contour plots were developed using MATLAB software (Version 7.0.1, Mathworks, America), holding one variable constant in the second-order polynomial model. The results are means of two replicates.

2.4 Monitoring of pH-value of yogurt during fermentation

Inoculated milk in beaker was put into a thermostatically controlled water bath at incubation temperature. The pH-value of the milk during fermentation was measured continuously by immersing the glass electrode of the Orion 720Aplus Benchtop pH Meter (Thermo Orion Co., USA) in the milk. The pH meter was standardized over the range 6.86 to 4.01.

2.5 Dynamic time sweep

Measurements were carried out in a stress-controlled rheometer (AR-2000ex, TA Instruments Ltd., Crawley, UK), equipped with an aluminium parallel plates geometry (upper plate=40 mm diameter and 1mm gap). Strain sweeps (0.01-10%) were performed to determine the linear viscoelastic range at 0.1 Hz before dynamic time sweep. For characterization of the time-course of gel formation, the rheometer was pre-set at the appropriate incubation temperature. An aliquot of 1.26 mL inoculated milk was transferred to the rheometer plate, and silicon oil was used to cover the surface of the samples to prevent evaporation. To monitor the gelation process as the milk was fermented, the rheological measurements were performed at a fixed frequency of 0.1 Hz and a constant strain of 0.5% (linear viscoelastic region). Measurements were taken every 3 min until the pH-value of yogurt reached 4.6. The G^*, as a function of time, were calculated using the Rheology Advantage Data Analysis Software (version V5.4.7, TA Instruments, Crawley, UK). The G^* enabled to determine the gelation time.

2.6 Steady state flow

The flow behavior of yogurt samples was measured 3 days after preparation. A temperature of 5 ^{o}C, selected as representative of the usual consumption temperature of yogurt, was maintained during rheological tests. The yogurt sample was carefully loaded using a spoon. After lowering the upper plate, the sample was pre-sheared for 10 s at a shear rate of 10 s^{-1}, and then equilibrated for 20 min before measurement. The apparent viscosity of the sample as a function of shear rate was obtained by a logarithmic increasing (forward) shear rate from 1 to 100 s^{-1} and decreasing (backward) from 100 to 1 s^{-1}. The apparent viscosity of forward measurement at a shear rate of 10 s^{-1} was selected as one of the responses.

3. RESULTS AND DISCUSSION

3.1 Effects of incubation temperature, starter culture level, and total solids content on gelation time

A quadratic polynomial model as below (***Eq. (2)***) for gelation time was obtained using second-order design, after eliminating the statistically insignificant terms (P>0.1):

$$Y_1 = 204.98 - 41.37X_1 - 16.01X_2 + 4.78X_3 + 15.01X_1^2 + 8.24X_2^2 + 6.35X_2X_3 \quad (2)$$

Table 3 shows the ANOVA for gelation time of master model. The model F-value of 134.3766 and P<0.001 indicated that the quadratic polynomial model was significant at 99.9% confidence level. The model also showed statistically insignificant lack of fit. With a high coefficient of determination (R^2=0.992), the model was of good fitness of the experimental data in the response surface model of acceptability.

Eq. (2) shows that incubation temperature (X_1) and starter culture level (X_2) had both linear and quadratic effects on gelation time, whereas total solids content (X_3) only had a linear effect on gelation time. There were significant (P<0.01) interactions between starter culture level and total solids content for gelation time. Incubation temperature was the most significant (P<0.001) factor affecting the gelation time, as showed by the regression coefficient which has the highest absolute value among all other coefficients of the model. The linear effect was negative, which indicated an earlier coagulation of milk with increasing incubation temperature. It can be explained by an increase in the metabolic activity of the bacteria at high temperature. ***Fig. 1*** shows the *G** as a function of time for the tests 9 (35 ^{o}C) and 10 (45 ^{o}C), which just differ in the incubation temperature. Both profiles were typical for the gelation of milk and show a sigmoid shape with three stages corresponding to different viscoelastic behavior. In the first stage of fermentation, low values of *G** were measured without any significant changes. When *G** reached a value of 1 Pa, a sharp increase was measured, exhibiting rapid progress of the gelation process. In the last stage, *G** increased slowly and tended to plateau. ***Fig. 1*** displays the gelation time delayed from 175min to 313min when incubation temperature decreased from 45 ^{o}C to 35 ^{o}C. Lee & Lucey (2004b) reported that the gelation time was 132 min and 160 min for yogurt incubated at 45.7 ^{o}C and 40 ^{o}C, respectively and attributed the shorter gelation time of milk fermented at higher temperature to the higher rates of fermentation, aggregation of caseins, and thermal motion.

Table 3. ANOVA for gelation time of master model

Source	SS[a]	DF[a]	MS[a]	*F*-value	Prob>*F*
X_1	23368.05	1	23368.05	895.5849	0.0001****
X_2	3500.755	1	3500.755	134.1671	0.0001****
X_3	312.3159	1	312.3159	11.96957	0.0061***
$(X_1)^2$	3261.912	1	3261.912	125.0134	0.0001****
$(X_2)^2$	986.6257	1	986.6257	37.81261	0.0001****
$(X_3)^2$	1.811712	1	1.811712	0.069434	0.7975
$X_1 \times X_2$	10.125	1	10.125	0.388043	0.5473
$X_1 \times X_3$	66.125	1	66.125	2.534253	0.1425
$X_2 \times X_3$	322.58	1	322.58	12.36294	0.0056***
Model	31555.98	9	3506.22	134.3766	0.0001
Error	260.925	10	26.0925		
Lack of fit	190.6967	5	38.13934	2.715381	0.148497
Pure error	70.22833	5	14.04567		
Total	31816.91	19			

[a] SS: sum of squares; DF: degrees of freedom; MS: mean square.

Significant at: ****P<0.001, ***P<0.01, **P<0.05, *P<0.1

Fig. 2 shows the response surface and contour plot as functions of starter culture level and total solids content on the gelation time, as predicted from ***Eq. (2)***, while the incubation temperature was kept at 40 ^{o}C. The starter culture level exhibited an opposite effect on the gelation time depending on the total solids content. At the level of starter culture <0.005%, gelation time was found to decrease slowly with the increase of total solids content. However, at the level of starter culture >0.005%, gelation time increased rapidly. At the lowest level of total solids (12.32%), gelation time decreased from the maximum value (250 min) to the minimum value (180 min). At the highest level of total solids (15.68%), the gelation time first decreased to a certain level and then increased slowly due to the contribution of the interaction term.

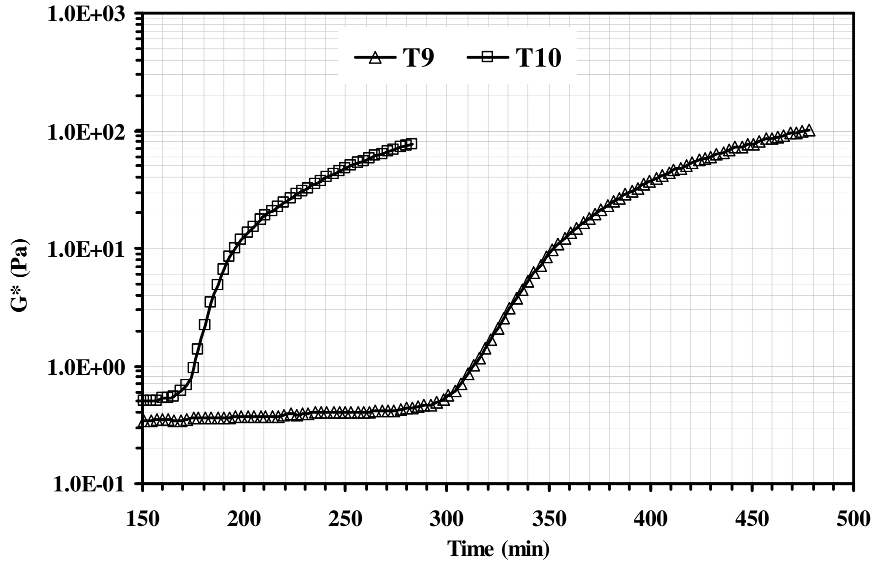

Fig. 1. Development of *G** as a function of time for tests of 9 and 10.

Starter culture level exerted a highly significant linear and quadratic effects (P<0.001) on gelation time. As would be expected, the higher the starter inoculum used, the higher the activity during gelation process. Lee & Lucey (2004b) described that the gelation time was 180 min and 119 min when inoculation rate increased from 0.5% to 4%, respectively.

Total solids content showed a positive effect on gelation time, the linear component of the regression being significant (P<0.01). Increasing total solids results in longer gelation time, which is attribute to the higher buffering capacity of milk solids, confirming the finding of Ozer et al. (1998), who observed that a higher total solids level provides better protection to bacteria from lowering of the pH-value.

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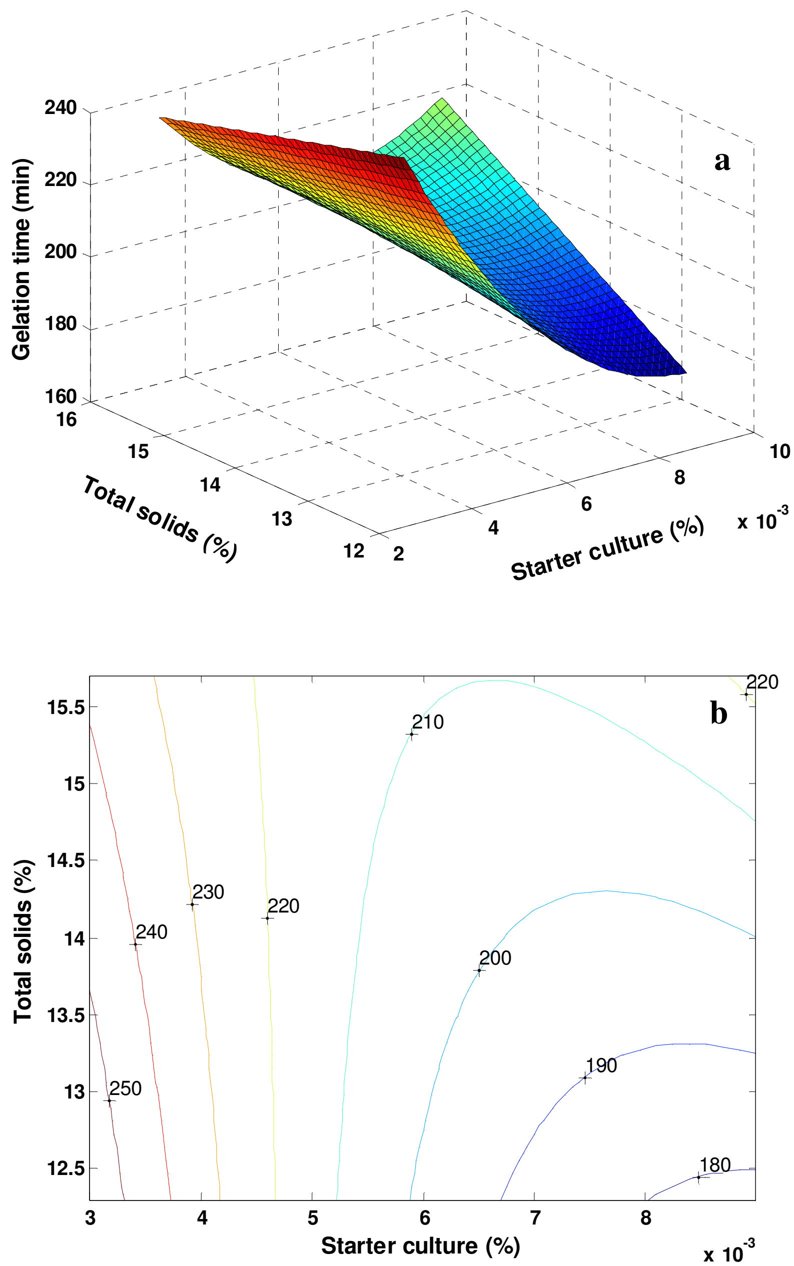


Fig. 2. Response surface (a) and contour plot (b) showing the effect of starter culture level and total solids content on the gelation time of yogurt.

3.2 Effects of incubation temperature, starter culture level, and total solids content on apparent viscosity

The quadratic polynomial model obtained for apparent viscosity of yogurt stored for 3 days, after eliminating the statistically insignificant terms (P>0.1), was:

$$Y_2 = 3.341 - 0.181X_1 + 0.360X_3 - 0.165X_1^2 - 0.178X_2^2 + 0.139X_3^2 \\ - 0.149X_1X_2 - 0.161X_1X_3 \quad (3)$$

Table 4. ANOVA for apparent viscosity of master model

Source	SS[a]	DF[a]	MS[a]	*F*-value	Prob>*F*
X_1	0.448	1	0.448	10.904	0.0080***
X_2	0.001	1	0.001	0.035	0.8547
X_3	1.765	1	1.765	42.985	0.0001****
$(X_1)^2$	0.391	1	0.391	9.523	0.0115**
$(X_2)^2$	0.458	1	0.458	11.162	0.0075***
$(X_3)^2$	0.278	1	0.278	6.761	0.0264**
$X_1 \times X_2$	0.178	1	0.178	4.325	0.0642*
$X_1 \times X_3$	0.207	1	0.207	5.034	0.0487**
$X_2 \times X_3$	0.037	1	0.037	0.894	0.3666
Model	3.831	9	0.426	10.367	0.0005
Error	0.411	10	0.041		
Lack of fit	0.334	5	0.067	4.370	0.0657
Pure error	0.076	5	0.015		
Total	4.242	19			

[a] SS: sum of squares; DF: degrees of freedom; MS: mean square.

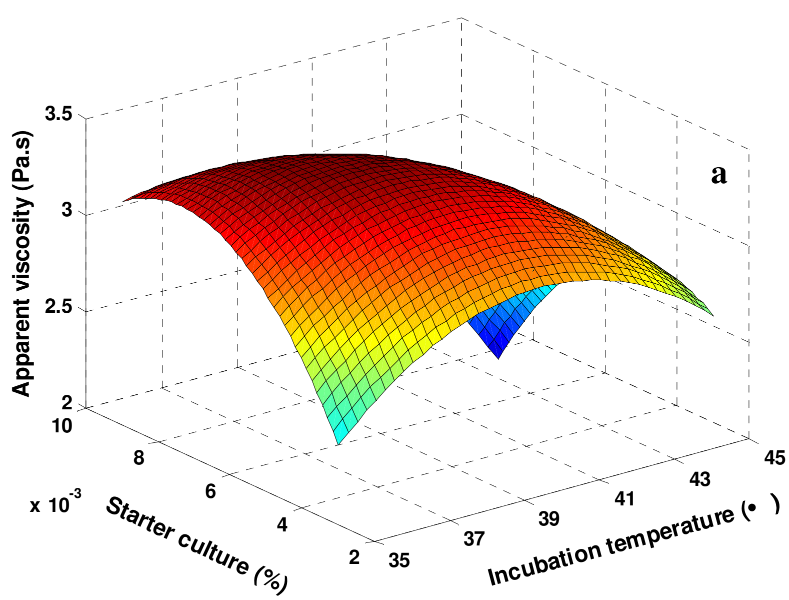
Significant at: ****P<0.001, ***P<0.01, **P<0.05, *P<0.1

Table 4 shows the ANOVA for apparent viscosity of master model. It was demonstrated that the lack of fit was not statistically significant. The statistical significance of the second-order polynomial, evaluated by the F-test, revealed that the regression was statistically significant at 99.9% confidence level, and the coefficient of determination (R^2= 0.903) showed a sufficient agreement between experimental and predicted values.

There was a strong and significant (P<0.01) influence of the quadratic factors of starter culture level (X_2^2) as well as linear factors of incubation temperature (X_1) and total solids content (X_3) on the apparent viscosity. The variable X_1 had a negative value for the linear term and quadratic term. The contrary occurred for variable X_3. The quadratic term of the variable X_2 and the interactions $X_1 \times X_2$ and $X_1 \times X_3$, affected negatively the apparent viscosity of the yogurt.

The three-dimensional response surface and contour plots presented in ***Fig. 3*** and ***Fig. 4*** are based on ***Eq. (3)***, illustrating the interactive effects of the independent variables on the apparent viscosity of yogurt, whereas the third factor was taken as a constant at its middle level.

Fig. 3 illustrates the effects of incubation temperature and starter culture level on the determination of apparent viscosity of yogurt. The response surface of apparent viscosity showed the maximum value (3.4 Pa·s) with incubation temperature locating between 37.5 ^{o}C and 38.5 ^{o}C combined with starter culture level locating between 0.006-0.007%. Higher incubation temperature and more starter culture level tended to result in lower apparent viscosity. The apparent viscosity was remarkably low at high values of incubation temperature and starter culture level.



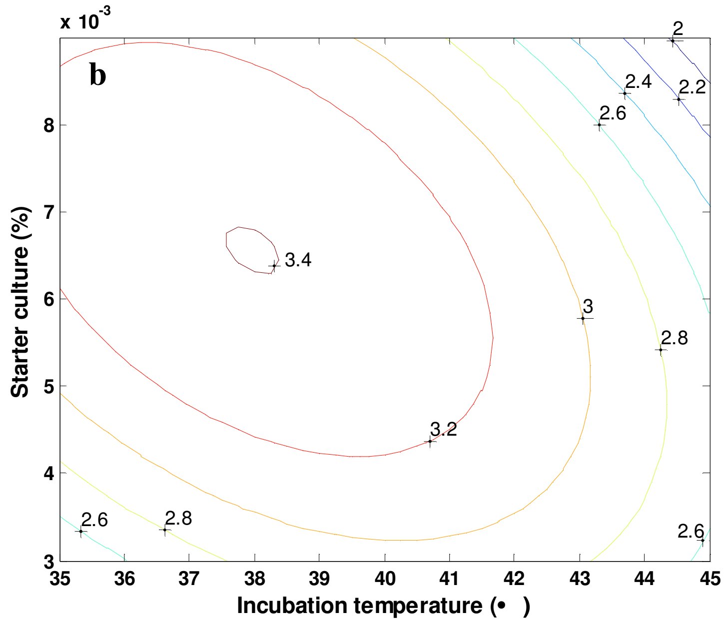
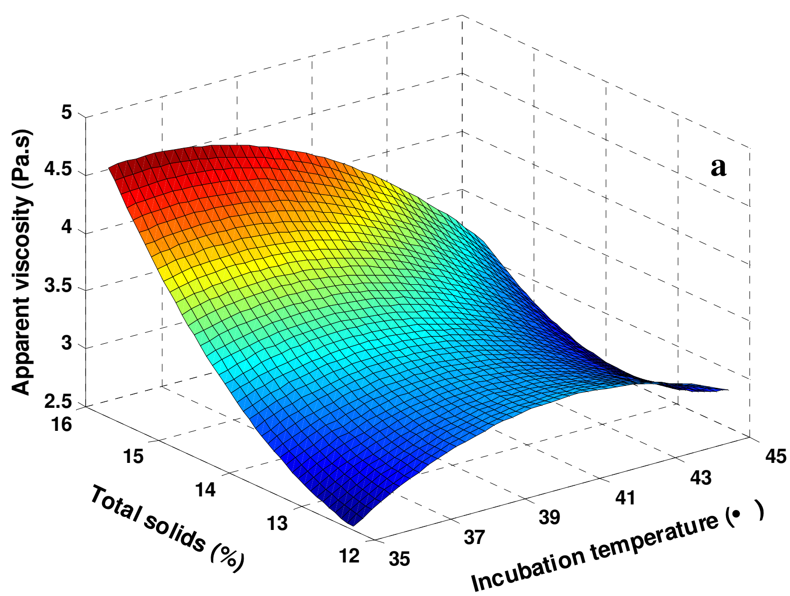


Fig. 3. Response surface (a) and contour plot (b) showing the effect of incubation temperature and starter culture level on the apparent viscosity of yogurt stored for 3 days at 5 °C.

Fig. 4 shows the effects of incubation temperature and total solids content on the determination of apparent viscosity of yogurt. Apparent viscosity of yogurt decreased with the increase of incubation temperature at total solids level>14%. At higher level of total solids content (15.68%), apparent viscosity value decreased from 4.6 Pa˙s to 3.2 Pa˙s when incubation temperature increased from 35 °C to 45 °C. At lower total solids levels, the apparent viscosity first increased with the increase of incubation temperature and then decreased. A higher apparent viscosity was found when the content of total solids was above 15.5%, and incubation temperature ranged from 35-39.5 °C.

The apparent viscosity of yogurt mostly depended on the total solids content, as manifested in highly significant linear (P<0.001) terms and the regression coefficient which has the highest absolute value among all other coefficients of the model. The positive regression coefficient of total solids content indicated that excessive total solids could improve the apparent viscosity of the yogurt. ***Fig. 5*** depicts the apparent viscosity as function of shear rate for the tests 13 (12.32% w/v solids) and 14 (15.68% w/v solids), which only differ in total solids level. Both flow curves had the shape of a hysteresis loop, exhibiting irreversible thixotropic behavior of yogurt. Shear thinning also observed in the rheograms. The apparent viscosity of stirred yogurt increased from 14.72 Pa·s to 22.72 Pa·s when the total solids content of the milk base were increased from 12.32% to 15.68% at the shear rate of 1 s^{-1}. Tamime & Robinson (1999) elevated total solids through fortifying milk solids-not-fat (MSNF) content of milk to achieve a more viscous, firm and consistent yogurt. According to the research of Barretto Penna et al. (2006), apparent viscosity at the shear rate of 100 s^{-1} of yogurt was increased from 1.3 to 3.0 Pa·s as the content of total solids in milk increased form 9.3% to 22.7%.

Incubation temperature is one of the main factors affecting the apparent viscosity, as showed in significant linear (P<0.01) and quadratic (P<0.05) terms. It can be seen from ***Fig. 3*** and ***Fig. 4*** that higher apparent viscosity values were obtained at lower incubation temperature. Since lower temperature resulted in a slow gelation, casein particles would have higher voluminosities and were probably more deformable than that at high incubation temperature, which allowed particles to aggregate with a larger number of protein-protein bonds between any two particles (Lucey et al., 1997). Thereby, a more rigid network with higher apparent viscosity value was obtained.



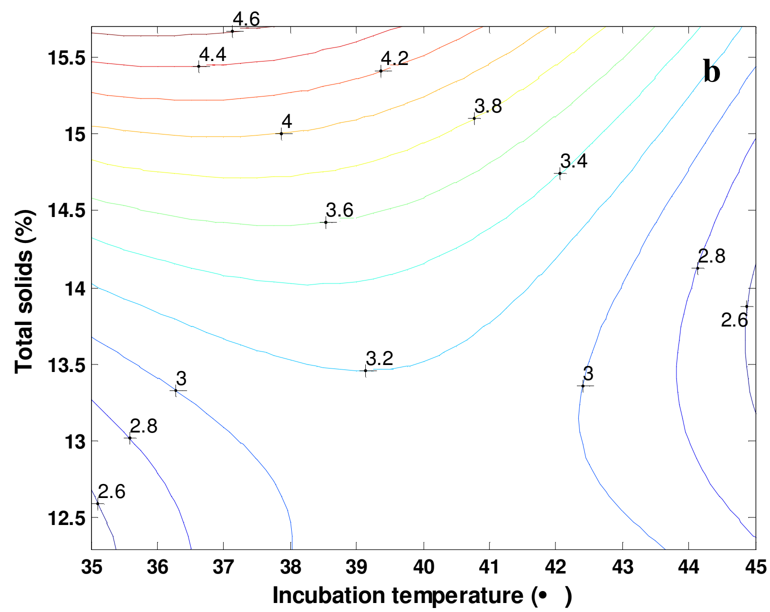


Fig. 4. Response surface (a) and contour plot (b) showing the effect of incubation temperature and total solids content on the apparent viscosity of yogurt stored for 3 days at 5 °C.

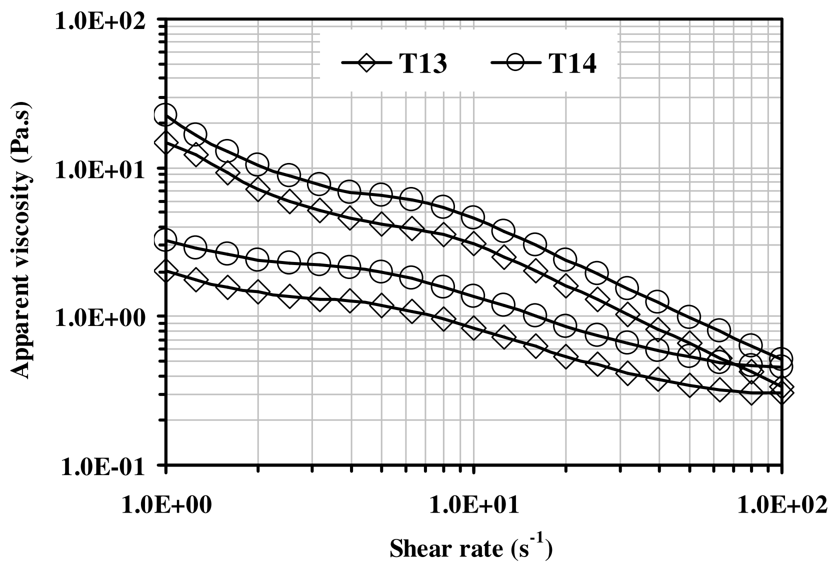


Fig. 5. Development of apparent viscosity as a function of shear rate for tests of 13 and 14.

The linear effect of starter culture level was insignificant (P>0.1), however, the quadratic term of which was found to be significant (P<0.01) and the effect was negative. Therefore, at high and low starter culture level, the apparent viscosity of yogurt was low.

4. CONCLUSIONS

Twenty different runs according to the central composite rotatable design were used to study the rheological properties of yogurt at different incubation temperature (X_1), starter culture level(X_2), and total solids content(X_3). Analysis of variance (F-test) showed that a second-order equation models were well adjusted to predict the experimental data. Lack-of-fit tests did not result in a significant F-value. R^2 were greater than 0.9 suggesting that the models were good fit. Incubation temperature had the greatest influence on the gelation time. Low temperature delayed the gelation time, but resulted in higher apparent viscosity. Apparent viscosity was strongly affected by the total solids content of milk base, with an increase in total solids content there was an increase in apparent viscosity. The surface response graphs show that the highest apparent viscosity was obtained when fermentation was carried out at temperatures ranging from 35 to 39.5 ^{o}C, in combination with total solids was up to 15.5% and medium starter culture level.

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