

# The Interaction Effect of Mixing Starter Cultures on Homemade Natural Yogurt's pH and Viscosity

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## Abstract

Dairy yogurts are common food products consumed by people all over the world. Due to the simple process, many people have made their own natural yogurt at home. The fermentation due to the starter culture causes the textural properties of dairy yogurt. However, the literature is surprisingly scarce on the topic of starter culture interactions in the development of textural properties of dairy yogurt. This study investigated the interaction effect of three common starter cultures, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, on the viscosity of homemade yogurt. Using Design Expert software, a 10-run mixture model experiment was designed to examine the textural properties developed by single or multiple inoculation of these starter cultures. All yogurt formulations reached the isoelectric point of milk and had pHs in the range 3.97 to 4.32. Yogurt formulations with *L. acidophilus* and *S. thermophilus* resulted in viscosities which were similar to commercial yogurt viscosity (1.77 Pa·s), while *L. bulgaricus* resulted in yogurt with a lower viscosity. Based on the mixture model, *L. acidophilus* had most influence on the yogurt viscosity, followed by *S. thermophilus* and *L. bulgaricus*. In conclusion, *L. acidophilus* can be used as a single starter culture or combined with other starter cultures to develop high viscosity homemade yogurt. A Combination of *S. thermophilus* and *L. acidophilus* can also be used to develop high viscosity yogurts. However, *L. bulgaricus* should not be inoculated alone or become a dominant ratio in multiple starter culture inoculation as it will decrease the overall homemade yogurt viscosity.

**Keywords:** Viscosity; Yogurts; Starter culture; *Lactobacillus acidophilus*; *Lactobacillus bulgaricus*; *Streptococcus thermophilus*

## 1 Introduction

Yogurt is one of the basic fermented foods consumed worldwide. It is produced by fermenting bacteria in milk at appropriate temperatures (incubating temperature) and time. Yogurt's development is said to originate from the nomads in the Middle East (Tamime & Robin-

son, 1999). The fermenting bacteria are now marketed as starter culture products (Mazzoli, Bosco, Mizrahi, Bayer, & Pessione, 2014). Each starter culture will coagulate the substrate where it is inoculated. The coagulation is due to accumulation of lactic acid that converts casein into lumps of micelles (Lee & Lucey, 2010). *Lactobacillus acidophilus*, *Lactobacillus bulgari-*

*cus* and *Streptococcus thermophilus* are some of the most utilized starter cultures in the production of dairy products (Delorme, 2008). Both *L. acidophilus* and *L. bulgaricus* possess proteolytic capability, that is the capable of hydrolyzing food proteins to peptides and amino acids to contribute to texture, taste and aroma of fermented products (McSweeney & Sousa, 2000). The key role of *S. thermophilus* is to breakdown lactose to lactic acid, and therefore reduce pH to preserve the product. *S. thermophilus* also produces secondary metabolites, such as exopolysaccharides and flavoured compounds (Broadbent, McMahon, Welker, Oberg, & Moineau, 2003). These probiotic starter cultures are now available for public use and sold in specialised shops for food ingredients. Thus, the general public can easily produce homemade natural yogurt for personal consumption (Fisberg & Machado, 2015). However, homemade natural yogurts are usually made without preservatives, unlike most commercial yogurts (O'Rell & Chandan, 2013; Smith, 2015). The literature regarding homemade natural yogurt is surprisingly scarce. Thus, this study aims to contribute towards the body of literature by investigating the interaction effect of mixing starter cultures on homemade natural yogurt's pH and viscosity.

## 2 Materials and Methods

### 2.1 Yogurt Preparation

Three common yogurt starter cultures were used: *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. All three starter cultures were obtained from Custom Probiotics (Custom Probiotics Inc.<sup>TM</sup>, California) in powder form. Pasteurized milk fresh from a local dairy farm in Sabak Bernam, Selangor, under the Farm Fresh Milk brand was obtained. 100 ml of the dairy milk was poured into respective cups, before it was inoculated with approximately 1 g of starter cultures. Table 1 shows the mixture design that was used to make the 10 yogurt formulations, where each yogurt contains a different ratio of the 3 selected probiotic strains. The inoculation volume was based on the weight of the single starter culture powder or

cumulative of multiple starter culture powders. For example, formulation 7 required one third of all three starter cultures. Therefore, 0.33 g of all three starter cultures was inoculated into the dairy milk. After inoculation with starter cultures, the milk samples were homogenized using an orbital shaker (MS Major Science, MS-NOR-30, USA) for 15 minutes at 100 rpm.

The mixture design was a simplex Centroid design, and it was developed on Design-Expert<sup>®</sup> Version 6.9 (State-Ease, Inc. Minneapolis, 2003). All homemade yogurts were incubated at 37 °C for 24 hours via a Trio Yogurt Maker (Trio Kaden (M) Sdn Bhd, TYM-7, Malaysia). All yogurt samples were analyzed within a maximum of 3 days to ensure freshness.

### 2.2 Yogurt pH Measurement

Yogurt pH measurements were made using a benchtop pH meter (Mettler Toledo Inc., Parkway, USA). The pH-meter was calibrated before it was used for yogurt measurement. The yogurt samples were measured immediately after the incubation period (Sadler & Murphy, 2010).

### 2.3 Yogurt Viscosity Measurement

A rotational viscometer (Brookfield, model DV II, USA) was used to measure the viscosity of the dairy yogurts. Approximately 50 ml of yogurt in a 50-ml beaker was used as a sample during measurement. The viscosity measurements were made at room temperature (27 °C) using a Brookfield LV spindle no. 4 at 10 r.p.m..

## 3 Results and Discussion

### 3.1 pH of Yogurt

Table 2 shows the pH values of the 10 yogurt formulations which lie within the range 3.97 to 4.32. This pH range is consistent with the 4.0 to 4.5 pH values reported by Gahruie, Eskandari, Mesbahi, and Hanifpour (2015) for commercial yogurts.

Single inoculation *L. bulgaricus* produced the

Table 1: Mixture experiment design of multiple species inoculation

Sample: Dairy milk Inoculation volume : 1 g				
Run	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus bulgaricus</i>	<i>Streptococcus thermophilus</i>	
1	1	0	0	
2	0	1	0	
3	0	0	1	
4	$\frac{1}{2}$	$\frac{1}{2}$	0	
5	$\frac{1}{2}$	0	$\frac{1}{2}$	
6	0	$\frac{1}{2}$	$\frac{1}{2}$	
7	1/3	1/3	1/3	
8	2/3	1/6	1/6	
9	1/6	2/3	1/6	
10	1/6	1/6	2/3	

lowest yogurt pH of the ten formulations (Table 2). Dual starter inoculation containing *L. bulgaricus* (formulations 4 and 6) also had low pH values when compared to the other yogurt formulations. This is probably one of the reasons why the dairy industry utilises multiple inoculations when producing commercial yogurt (Leroy & De Vuyst, 2004).

### 3.2 Viscosity of Yogurt

Table 3 shows the viscosity of the 10 yogurt formulations which were within the range 2.307 Pa·s to 1.058 Pa·s. According to Lee and Lucey (2010), gelation occurs when pH is just above the dairy milk isoelectric point (pH 5.2) and the observed viscosities are indicative of gelation in all 10 formulations. Normally, commercial yogurt contains multiple starter cultures and has an average viscosity of 1.77 Pa·s (Ares et al., 2007). However, not all commercial yogurts declare which starter cultures are used. Several yogurt formulations are near or exceed the commercial yogurt viscosity (formulations 3, 5, 6 and 10) (Table 3).

Yogurt formulations with *L. acidophilus* have the highest viscosity (formulations 1 and 8), followed by those with *S. thermophilus*. When *L. acidophilus* and *S. thermophilus* are combined (formulation 5), the yogurt viscosity increases above that of *S. thermophilus* alone. When *L. acidophilus* and *L. bulgaricus* were combined (for-

mulation 4), the resulting yogurt had a higher viscosity than the yogurt with a single *L. bulgaricus* inoculation.

*L. acidophilus* seems to have high viscosity capability. This was shown even in multiple starter culture yogurt formulations, for example, in formulation 8 where *L. acidophilus* was the dominant starter culture ratio. Conversely, *L. bulgaricus* seems to decrease yogurt viscosity whenever it is used to inoculate. Yogurt formulations with dominant *L. bulgaricus* starter culture ratio have lower viscosities than yogurt formulations without *L. bulgaricus* inoculations (formulation 6 and 9).

### 3.3 Model System

Table 4 shows coefficient estimates, regression coefficient ( $R^2$ ), model significance and lack of fit results for dairy yogurt viscosity. The values indicate the significance of equations or models that were developed from the design. If the probability of the F value obtained is less than 0.05, it means that model or equation can be developed confidently ( $p < 0.05$ ). According to Henika (1982),  $R^2$  values of more than 0.75 can be used for prediction purposes. However, the closer the  $R^2$  is to unity, the better the empirical model fits the actual data. The lack-of-fit test is a measure of the failure of the model to represent the data in the experimental domain at which points were not included in the regression, or in other words,

Table 2: pH of ten dairy yogurt formulations

Formulation	Ratio Strain			pH
	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus bulgaricus</i>	<i>Streptococcus thermophilus</i>	Natural Yogurt
1	1.00	-	-	4.31±0.02 <sup>a</sup>
2	-	1.00	-	3.97±0.03 <sup>d</sup>
3	-	-	1.00	4.24±0.01 <sup>b</sup>
4	0.50	0.50	-	4.04±0.03 <sup>c</sup>
5	0.50	-	0.50	4.29±0.01 <sup>ab</sup>
6	-	0.50	0.50	4.09±0.01 <sup>c</sup>
7	0.33	0.33	0.33	4.31±0.03 <sup>a</sup>
8	0.67	0.16	0.16	4.30±0.02 <sup>ab</sup>
9	0.16	0.67	0.16	4.31±0.03 <sup>a</sup>
10	0.16	0.16	0.67	4.32±0.02 <sup>a</sup>

a-e Different letters indicate significant difference (p ≤ 0.05)

Table 3: Viscosity of ten dairy yogurt formulations

Formulation	Ratio Strain			Viscosity (Pa.s)
	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus bulgaricus</i>	<i>Streptococcus thermophilus</i>	Dairy Yogurt
1	1.00	-	-	2.1597±0.31 <sup>a</sup>
2	-	1.00	-	1.2410±0.00 <sup>de</sup>
3	-	-	1.00	1.7660±0.07 <sup>abcd</sup>
4	0.50	0.50	-	1.9250±0.05 <sup>abc</sup>
5	0.50	-	0.50	1.8970±0.17 <sup>abc</sup>
6	-	0.50	0.50	1.5940±0.15 <sup>bcd</sup>
7	0.33	0.33	0.33	1.5050±0.08 <sup>cde</sup>
8	0.67	0.16	0.16	2.3077±0.21 <sup>a</sup>
9	0.16	0.67	0.16	1.0579±0.14 <sup>e</sup>
10	0.16	0.16	0.67	1.6840±0.28 <sup>bcd</sup>

a-e Different letters indicate significant difference (p ≤ 0.05)

variations in the models cannot be accounted for by random error.

The F-value obtained was less than 0.05 (0.0109). The  $R^2$  for yogurt was 0.6334. The yogurts also displayed statistically insignificant lack-of-fit, thus indicating that the experimental data was satisfactorily accepted for model fitting even though the  $R^2$  of the dairy yogurt is less than 0.75, it can be used with confidence (Henika, 1982; Yusnita, Aida, Maskat, & Aminah, 2007). According to the Design Expert software, there were interactions between binary and tertiary components. However, these interactions were not significant and were removed in order to develop a more robust model using the significant single components.

The canonical Scheffe's equations for the dairy yogurt viscosities were obtained from the software. The models were analyzed as follows. The positive (+) sign in the equation meant that the response values increased with an increase of the variables, whether it is linear (A, B, C), binary (AB, AC, BC) or ternary combination (AC (A-C)). There is an inverse meaning when a negative (-) sign is shown on the variables.

The canonical Scheffe's equation for dairy yogurt viscosity is shown in equation 1:

$$\text{Viscosity} = 0.222 \cdot A + 0.12236 \cdot B + 0.15282 \cdot C \quad (1)$$

where A is *L. acidophilus*, B is *L. bulgaricus* and C is *S. thermophilus*.

In equation 1, the single inoculation of *L. acidophilus* had the highest influence on the homemade dairy yogurt viscosity followed by *S. thermophilus* and *L. bulgaricus*. *L. acidophilus* has the highest mathematical weigh (+0.222). This is because *L. acidophilus* has the ability to convert 23 % of lactose into lactic acid. The accumulating lactic acid then contributes to the viscosity of the yogurt (Anjum et al., 2014). Formulations 1 and 8 of the homemade dairy yogurts validate this statement.

The second highest was *S. thermophilus* (+0.15282). *S. thermophilus* has the ability to breakdown lactose into glucose and galactose. *S. thermophilus* then proceeds to digest glucose monomers for growth (Broadbent et al., 2003; Drouault, Anba, & Corthier, 2002). Almost all formulations that contain *S. thermophilus*

had high viscosity, especially when paired with *L. acidophilus* (formulation 8 and 10). This is because *L. acidophilus* also benefited from the breakdown of lactose, since it utilizes galactose in its growth (Noh & Gilliland, 1993).

*L. bulgaricus* had the lowest influence in dairy yogurt viscosity (+0.12236). Although *L. bulgaricus* was able to breakdown peptides into free amino acids that can contribute to viscosity and sensory quality (McSweeney & Sousa, 2000), it relies on other starter cultures for its growth. *L. bulgaricus* requires galactose or glucose for its metabolism but it cannot breakdown the lactose in order to feed itself (Nguyen et al., 2012; Zourari, Accolas, & Desmazeaud, 1992). This is an example of why dairy yogurt requires an emulsifier in its formulation (O'Rell & Chandan, 2013; Smith, 2015).

Table 4: Coefficient estimates, model significance, regression coefficient and lack-of-fit values for Dairy yogurt viscosity

Coefficients	Ratio Strain in Yogurt
	Dairy Yogurt
A	+0.22222
B	+0.12236
C	+0.15282
Model (Prob> F)	0.0109
Regression Coefficient ( $R^2$ )	0.6334
Lack of fit	0.7218

## 4 Conclusions

There are significant differences in the texture of homemade dairy yogurts inoculated with different ratios of starter culture. All homemade yogurt formulations reached the isoelectric point of milk and had pHs in the range 3.97 to 4.32. The most viscous homemade yogurt (2.3 Pa·s) came from a multiple inoculation formulation with *L. acidophilus* as the dominant ratio. When results are fitted into a Scheffe's canonical special quadratic equation for three components, the model shows that *L. acidophilus* brings the most positive influence to increase homemade yogurt viscosity. This is followed by *S. thermophilus* and *L. bulgaricus*. In conclusion, *L. acidophilus* can be used as a single starter culture or combined

with other starter cultures to develop high viscosity homemade natural yogurts. A combination of *S. thermophilus* and *L. acidophilus* can also be used to develop high viscosity homemade yogurts. However, *L. bulgaricus* should not be inoculated alone or become the dominant ratio in a multiple starter culture inoculation since it will decrease the overall viscosity of homemade yogurt.

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