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Research Article

Selection and study on technological parameters of symbiotic starter cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for production of Vietnamese yogurt

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Abstract

In this paper ten symbiotic starter cultures for yogurt production were examined for their coagulation time, titratable acidity, pH at the moment of coagulation. Their maximum rate of acidification was also determined by model of fermentation kinetics. Three starter cultures were selected for production of Vietnamese yogurt.

With the selected starter culture, yogurt from natural milk and reconstituted whole milk was obtained. Their coagulation time, acidity, maximum rate of acidification and rate of acidification during storage of product were studied.

As a result of this study and mathematical modeling, we concluded that maximum rate of acidification at moment of coagulation and during storage was affected by the type of milk used in yogurt production.

Keywords: mathematical model, kinetics, maximum rate of acidification, coagulation time, Vietnamese yogurt

Abbreviations:

BLIS - Bacteriocin-Like Inhibitory Substances

NDF - non-dimensional form

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Introduction

The benefits of traditional Bulgarian yogurt on human health have been investigated since many years. The yogurt has immunostimulatory effect due to components in the cell wall of the lactic acid bacteria such as peptidoglycan, teichoic acids, lipoproteins, exopolysaccharides (Takahashi et al. 1993; Akira et al. 2006) and bioactive substances produced from the growth of microorganisms in starter culture (Sanders 1999; Lourens-Hattingh and Viljoen 2001; Zubillaga et al. 2001).

Yogurt also has antimicrobial activity due to high concentration of active microorganisms *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These lactic acid bacteria maintain the balance between the bacterial species in the gastrointestinal tract by producing short-chain acids (mainly lactic acid), bacteriocins, (Bhatia et al., 1989), BLIS (Bacteriocin-Like Inhibitory Substances) and other metabolites which inhibit enteropathogens (Davidson et al. 2000; Fang et al. 2001). Consuming yogurt is beneficial in lowering the high level of total serum cholesterol, which is considered a factor for cardiovascular disease (Akalın et al. 1997; Anderson and Gilliland 1999). This effect can be explained by high proteolytic activity of *Lb. bulgaricus*, which synthesizes low molecular weight bioactive peptides such as α -lactotensin (His- Ile- Arg- Leu) from β -Lactoglobulin (Yoshikawa et al. 2000). Furthermore, the high hydrolysis activity of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in yogurt can improve lactose tolerance in human (McBean 1999).

Vietnamese dairy industry is developing rapidly in the recent years, although the first industrial dairy products were produced in 1990 (Khoi and Dung 2014). However, there has been still limited research about the Vietnamese yogurt and its production. The Vietnamese yogurt has specific organoleptic characteristics such as sweet taste and viscous consistency. The cause for the sweet taste of Vietnamese yogurt can come from the low acidity of the yogurt and addition of a certain amount of sugar during their production. The starter cultures used to produce Vietnamese yogurt mainly include strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

In Vietnam, a large percentage of milk powder was used to produce yogurt and dairy products due to the shortage of natural cow's milk. Natural cow's milk obtained in Vietnam provides only 30% of domestic demand and the remaining 70% is imported from different countries such as: New Zealand, America, Singapore, and Netherlands.

Having reviewed the cited literature, the aim of the present paper was to study on technological parameters of symbiotic starter cultures and the effect of different type of milk using for Vietnamese yogurt production.

Materials and Methods

Combinations starter cultures.

Table 1. Combinations starter cultures of different type of lactic acid bacteria and ratio

Nº	Types of lactic acid bacteria	Ratio
1A	<i>Lb. bulgaricus</i> LBBG2 + <i>Str. thermophilus</i> STBG1	1:10
1B	<i>Lb. bulgaricus</i> LBBG2 + <i>Str. thermophilus</i> STBG2	1:20
2A	<i>Lb. bulgaricus</i> LBNV1 + <i>Str. thermophilus</i> STVN1	1:3
2B	<i>Lb. bulgaricus</i> LBNV1 + <i>Str. thermophilus</i> STVN4	1:6
3A	<i>Lb. bulgaricus</i> LBBG4 + <i>Str. thermophilus</i> STVN1	1:3
3B	<i>Lb. bulgaricus</i> LBBG4 + <i>Str. thermophilus</i> STVN4	1:12
4	<i>Lb. bulgaricus</i> LBNV1 + <i>Str.</i> <i>thermophilus</i> STBG2	1:1:10
5A	<i>Lb. bulgaricus</i> LBBG4 + <i>Str. thermophilus</i> STBG1	1:3
5B	<i>Lb. bulgaricus</i> LBBG4 + <i>Str. thermophilus</i> STBG2	1:10
6	<i>Lb. bulgaricus</i> LBNV3 + <i>Str.</i> <i>thermophilus</i> STBG2	1:1:12

Preparation of the Vietnamese yogurt. Cow's milk (natural or reconstituted whole milk) was pasteurized at $95 \pm 1^\circ\text{C}$ for 15 min, cooled to $44 \pm 1^\circ\text{C}$ and divided into different batches. After packing in polystyrene containers, the milk is

inoculated with 2% starter culture from the selected combinations. The samples are thermostatically incubated at temperature of $44 \pm 1^\circ\text{C}$ until coagulation of milk. After that, the yoghurt samples were cooled to $20 \pm 1^\circ\text{C}$ for 20 min, then cooled and stored at $4 - 6^\circ\text{C}$.

Determination of titratable acidity. Titratable acidity was determined according to ISO 6091:2010.

Modeling the kinetics of the fermentation process. Models 1 and 2 were used for the kinetics of the fermentation process, as the pH and titratable acidity were presented in a non-dimensional form (Tishin and Fedorov 2016; Bouguettoucha et al. 2011; Gordeev et al. 2017). Identification of the model parameters was performed by the method of least squares.

Model 1:

$$\frac{dpH_b}{d\tau} = -V_{max} \cdot pH_b \Rightarrow pH_b = pH_{b0} \cdot e^{-V_{max}t}$$

Model 2:

$$K_T = 1 + (q_b \cdot \tau)^n$$

Where:

V_{max} is maximum rate of acidification, 1/h;

pH_b – pH in non-dimensional form;

pH_{b0} – initial value of pH in non-dimensional form;

K_T – titratable acidity in non-dimensional form;

q_p – rate of post-acidification, 1/h;

n – exponent indicator or the change in rate of post-acidification over time (characterized for intensity of post-acidification);

τ – cultivation time, 1/h.

Results and Discussion

Ten combinations of symbiotic starter cultures with selected strains of *Lactobacillus bulgaricus*, characterized by antimicrobial activity were obtained. The obtained starter cultures were studied for coagulation time, pH and titratable acidity at moment of coagulation, maximum rate of acidification to select starter cultures for production of Vietnamese yogurt with desired quality. The

results of these studies were presented in Figure 1 and Table 2.

According to the presented data in the Figure 1 and Table 2, combinations starter cultures 1A, 1B and 4 had the shortest coagulation time (2.45 h). The titratable acidity of these combinations was determined in short range from 75 ± 0.4 to $80 \pm 0.4^\circ\text{T}$.

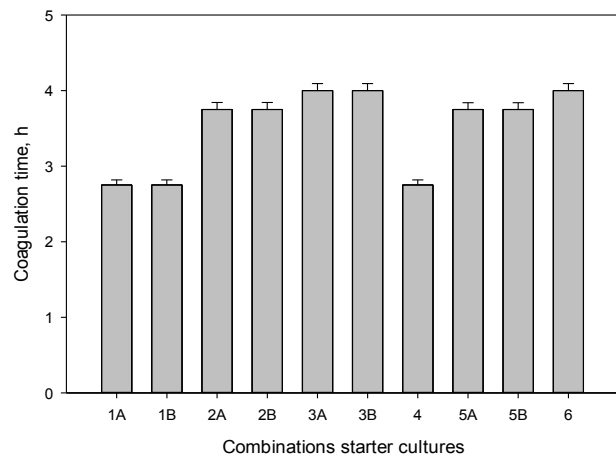


Figure 1. Coagulation time with different combinations of starter cultures

A similar trend is observed for the values of pH at the moment of coagulation (from 5.00 ± 0.02 to 5.05 ± 0.03). For combinations starter cultures 2A, 2B, 5A and 5B, coagulation time were observed with one hour longer (3.45 h). At the moment of coagulation, combinations starter cultures 2A, 2B, 5A and 5B had lower titratable acidity (from 60 ± 0.3 to $66 \pm 0.3^\circ\text{T}$) than combinations starter cultures 1A, 1B and 4; respectively higher pH (pH is varied in the range of 5.28 ± 0.04 to 5.35 ± 0.03). The longest coagulation time of the milk (4 h) was observed in combinations starter cultures 3A, 3B and 6.

Titratable acidity and pH of combinations 3A, 3B and 6 were close to those obtained in combinations starter cultures 2A, 2B, 5A and 5B which are ranged from 61 ± 0.3 to $68 \pm 0.3^\circ\text{T}$ and the pH - from 5.25 ± 0.04 to 5.35 ± 0.02 .

As shown in Table 2, we also observed that the combinations starter cultures 2B, 3B and 6 had significantly weaker acidification activity.

Titratable acidity of those combinations after 24h are lowest, which is respectively $90 \pm 0.4^\circ\text{T}$; $88 \pm 0.4^\circ\text{T}$ and $76 \pm 0.4^\circ\text{T}$.

Table 2. Titratable acidity and pH at the moment of coagulation and after 24h storage.

Combination	At moment of coagulation		After 24 h	
	Titratable acidity, °T	pH ^a	Titratable acidity ^a , °T	pH ^a
1A	80 ± 0.4	5.00 ± 0.02	100 ± 0.5	4.78 ± 0.02
1B	77 ± 0.4	5.00 ± 0.03	95 ± 0.5	4.82 ± 0.03
2A	66 ± 0.3	5.28 ± 0.04	93 ± 0.5	4.84 ± 0.02
2B	61 ± 0.3	5.35 ± 0.03	90 ± 0.4	4.93 ± 0.04
3A	68 ± 0.3	5.25 ± 0.04	91 ± 0.4	4.88 ± 0.03
3B	61 ± 0.3	5.35 ± 0.02	88 ± 0.4	5.03 ± 0.05
4	75 ± 0.4	5.05 ± 0.03	96 ± 0.5	4.82 ± 0.03
5A	66 ± 0.3	5.28 ± 0.03	95 ± 0.5	4.83 ± 0.02
5B	60 ± 0.3	5.35 ± 0.04	89 ± 0.4	4.95 ± 0.03
6	62±0.4	5,28±0.02	76±0.4	5.11±0.05

^a Data represents the mean values and standard deviation (n = 3).

The differences in coagulation time, titratable acidity and pH at the moment of coagulation were explained by the composition and the ratio between the lactic acid bacteria, as well as in the specific acidification ability of the various strains.

To determine the effect of the acidification ability of the starter cultures, the models of kinetics of pH reduction (acidification of milk) were studied and the maximum rate of acidification was determined. The data are presented in Table 3.

According to Table 3, combinations 1A, 1B and 4 were characterized with the highest values of maximum rate of acidification (0.109, 0.105 and 0.111 1/h). This is the main reason for their shortest coagulation time. In the other tested combinations, the maximum rate of acidification was significantly lower and varied within the range of 0.048 to 0.064 1/h.

From the obtained results, combinations starter cultures 2B, 3B and 6 were selected for further experiments as the most suitable combinations to produce Vietnamese yogurt according to the specific organoleptic characteristics – with lower acidity, sweet and slightly expressed lactic acid taste.

Table 3. The rate of acidification of different combination starter cultures

Combination	V _m	R ²
1A	0.109	0.9984
1B	0.105	0.9986
2A	0.064	0.9990
2B	0.059	0.9991
3A	0.053	0.9991
3B	0.048	0.9992
4	0.111	0.9984
5A	0.063	0.9990
5B	0.058	0.9992
6	0.052	0.9991

Yogurt products were obtained from natural cow's milk and reconstituted whole milk using the selected starter cultures. The effect of the type of milk on coagulation time, titratable acidity, pH,

maximum rate of acidification, and rate of post-acidification during the storage of yogurt was studied.

Figure 2 shows the comparative results between the experimental data and the data obtained by modeling of pH kinetics during the formation of yogurt using natural cow's milk using the selected starter cultures. The kinetic parameters for the models were presented in Table 4.

As shown in Figure 2, model's data were corresponded to the experimental data with very high accuracy and could be used to predict the dynamics of lactic acid fermentation. According to data in Table 4, the maximum rates of acidification in combinations 2B and 3B respectively were 0.043 1/h and 0.042 1/h, which were slightly lower than the one in combination 6 (0.052 1/h).

The coagulation time of the natural milk with these selected combinations varied in the range of 4h10min to 4h25min. The pH values of combinations 2B, 3B и 6 at the moment of coagulation were respectively 5.50 ± 0.03 , 5.53 ± 0.03 и 5.34 ± 0.04 . The titratable acidity of 3 combinations were in the range of 70 ± 0.4 to $80 \pm 0.4^\circ\text{T}$.

Another important factor for the production of Vietnamese products is the rate of post-acidification of the starter cultures. The mathematical model for kinetics of post-acidification was studied. The model's data were compared to the experimental data, which is shown in Figure 3.

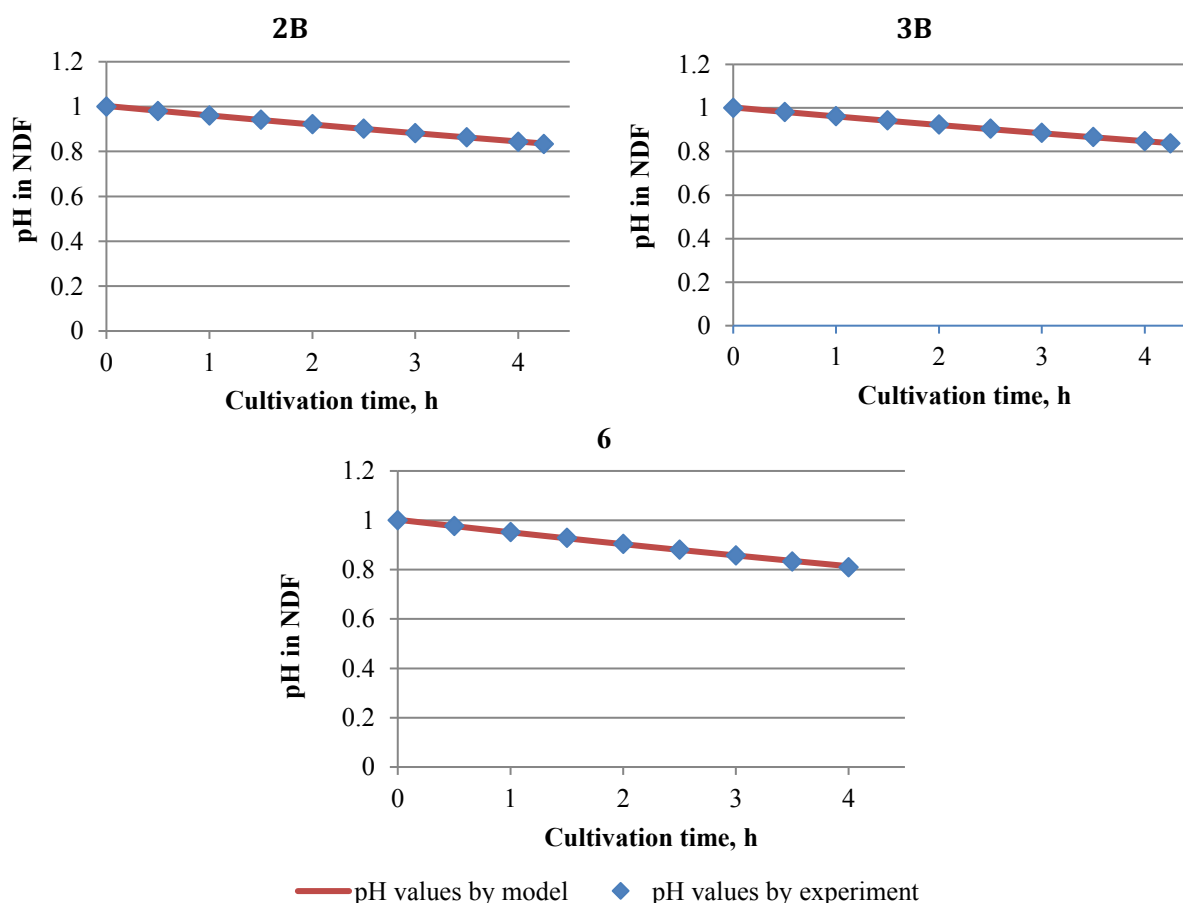


Figure 2. Comparison of dynamics of pH between experiment and model for starter cultures 2B, 3B and 6 used in yogurt from natural milk

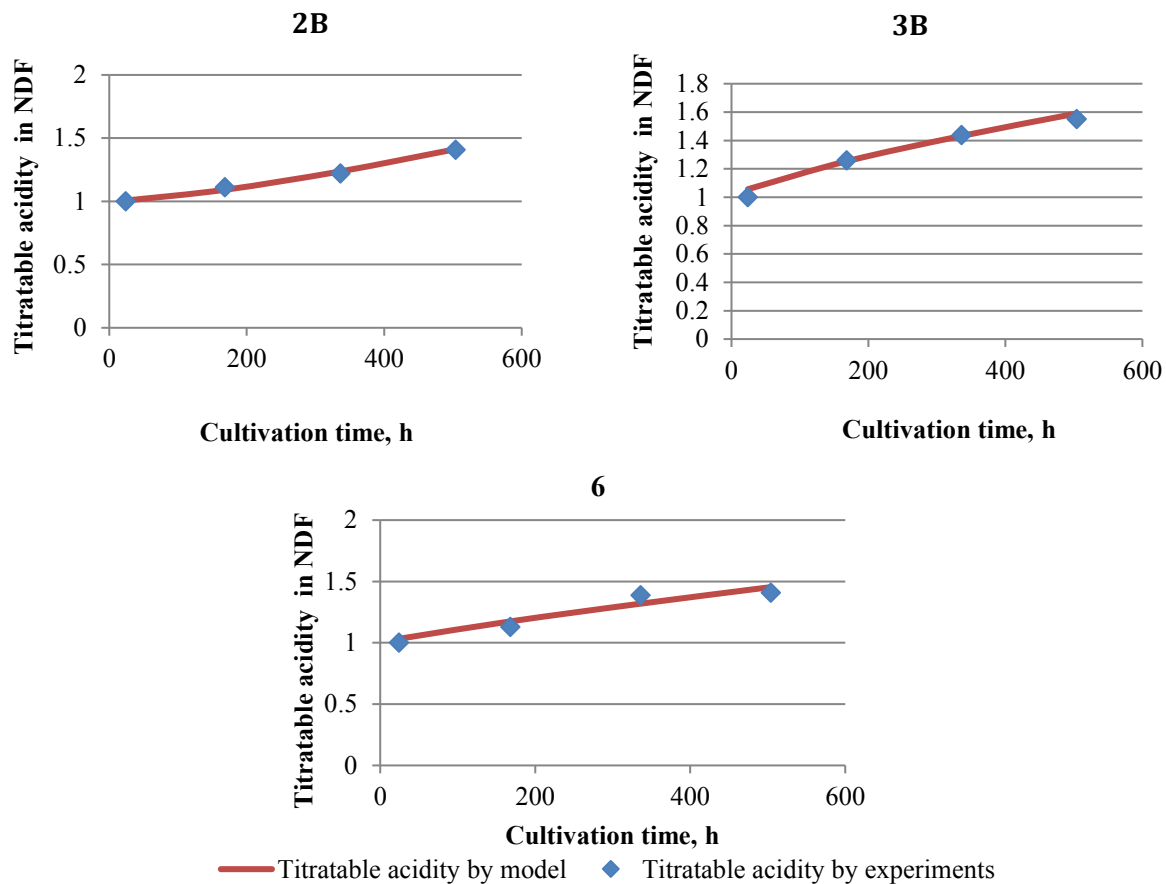


Figure 3. Comparison of dynamics of titratable acidity between experiment and model for starter culture 2B, 3B and 6 used in yogurt from natural milk

Table 4. The kinetic parameters for the models in formation of yogurt using raw cow's milk

Combination	V_m	$q_p, 1/h$	n	R^2	e
2B	0.043	1.04E-03	1.37	0.9926	0.018
3B	0.042	1.00E-03	0.77	0.9879	0.045
6	0.052	8.00E-04	0.87	0.8962	0.080

As shown in Figures 3 model's data were corresponded to experimental data. According to Table 3, combinations starter cultures 2B and 3B had similar rate of acidification ($1.04 \cdot 10^{-3}$ and $1.00 \cdot 10^{-3}$ 1/h). Combination 2B had highest exponent indicator (1.37), which exhibited the change of intensity of post-acidification process. The slightly higher rate of post-acidification in combination 2B and the high exponent indicator

showed the intensification of post-acidification in the early stage of storage of yogurt. For combinations 3B and 6, exponent indicators were similar at 0.87 and 0.77, showing that post-acidification process for those combinations was moderate during the storage period.

The rate of acidification for combination 6 was determined at $8 \cdot 10^{-4}$ 1/h.

From reviewed the cited literature, the main source for the production of Vietnamese yogurt is reconstituted whole milk (3% fat). In this case, yogurt was obtained from reconstituted whole milk using three combinations starter cultures 2B, 3B and 6. During the fermentation process, the rate of acidification, the dynamics of pH and titratable acidity at the moment of coagulation, the coagulation time and the rate of post-acidification were determined. The results are shown in Figure 4. The kinetics of model's parameters is shown in Table 5.

As shown in Figure 4, there's well fit between experimental data and model's data. The coagulation time of reconstituted whole milk with the selected starter cultures was shorter than the one of natural milk. According to Table 5, the higher rate of acidification was recorded – from 0.068 to 0.087 1/h. The shorter coagulation time and higher rate of acidification can be explained by the change

in structure and hydration characteristics of protein as a result of evaporating of milk to dryness.

The mathematical model of the rate of post-acidification and the comparison between the experimental data and model data were determined. The results are shown in Figure 5.

The presented results showed that the titratable acidity in non-dimensional type by the model was very well agreed with values by experiments. The combination 3B was characterized with highest rate of post-acidification ($1.40 \cdot 10^{-3}$ 1/h) and highest exponent indicator (1.25). It means for this combination starter culture there's more intensive process of post-acidification than the other two combinations.

For the starter cultures 2B and 6, lower rates of post-acidification were observed, respectively at $1.23 \cdot 10^{-3}$ 1/h and $9.3 \cdot 10^{-4}$ 1/h. The exponent indicators in both combinations are lower than 1 (0.77 and 0.97) which exhibit that the process of post-acidification proceeded with lower intensity.

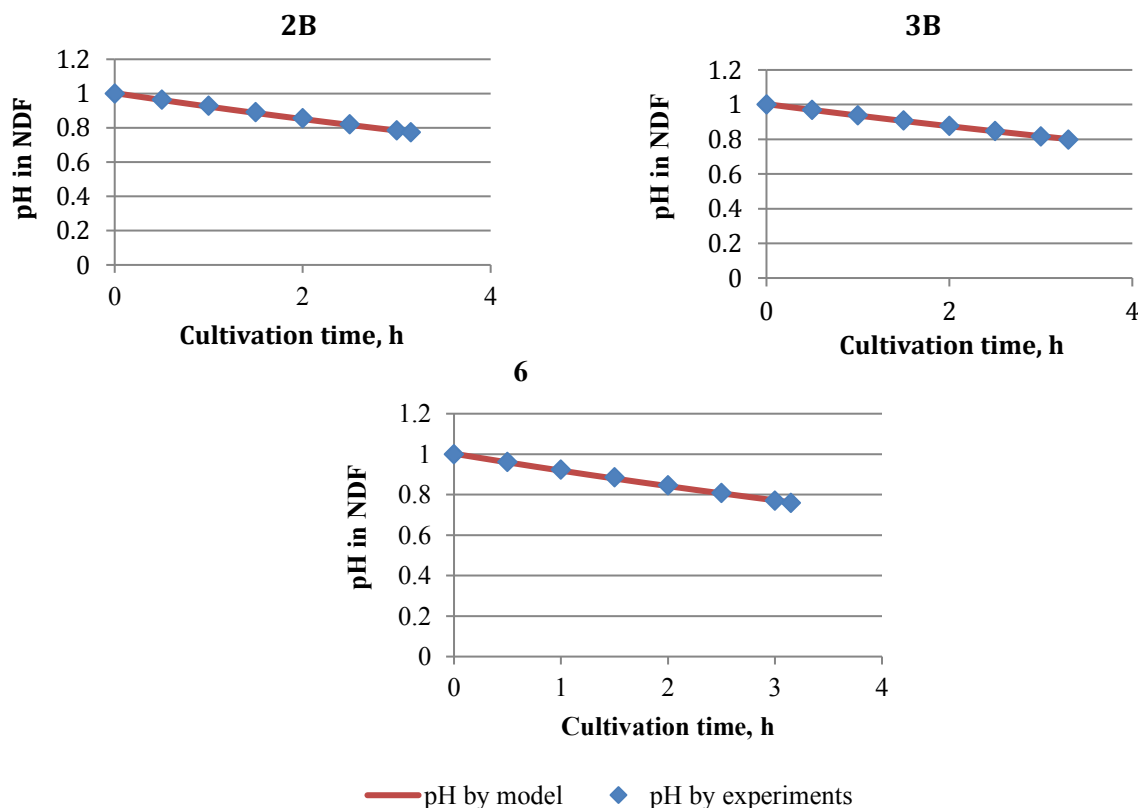


Figure 4. Comparison of dynamics of pH between experiment and model for starter cultures 2B, 3B and 6 used in yogurt from reconstituted whole milk

Table 5. The kinetic parameters for the models in formation of yogurt using reconstituted whole milk

Combination	V_m	$q_p, 1/h$	n	R^2	e
2B	0.082	1,23E-03	0.77	0.9359	0.090
3B	0.068	1.40E-03	1.25	0.9967	0.018
6	0.087	9.30E-04	0.97	0.9834	0.030

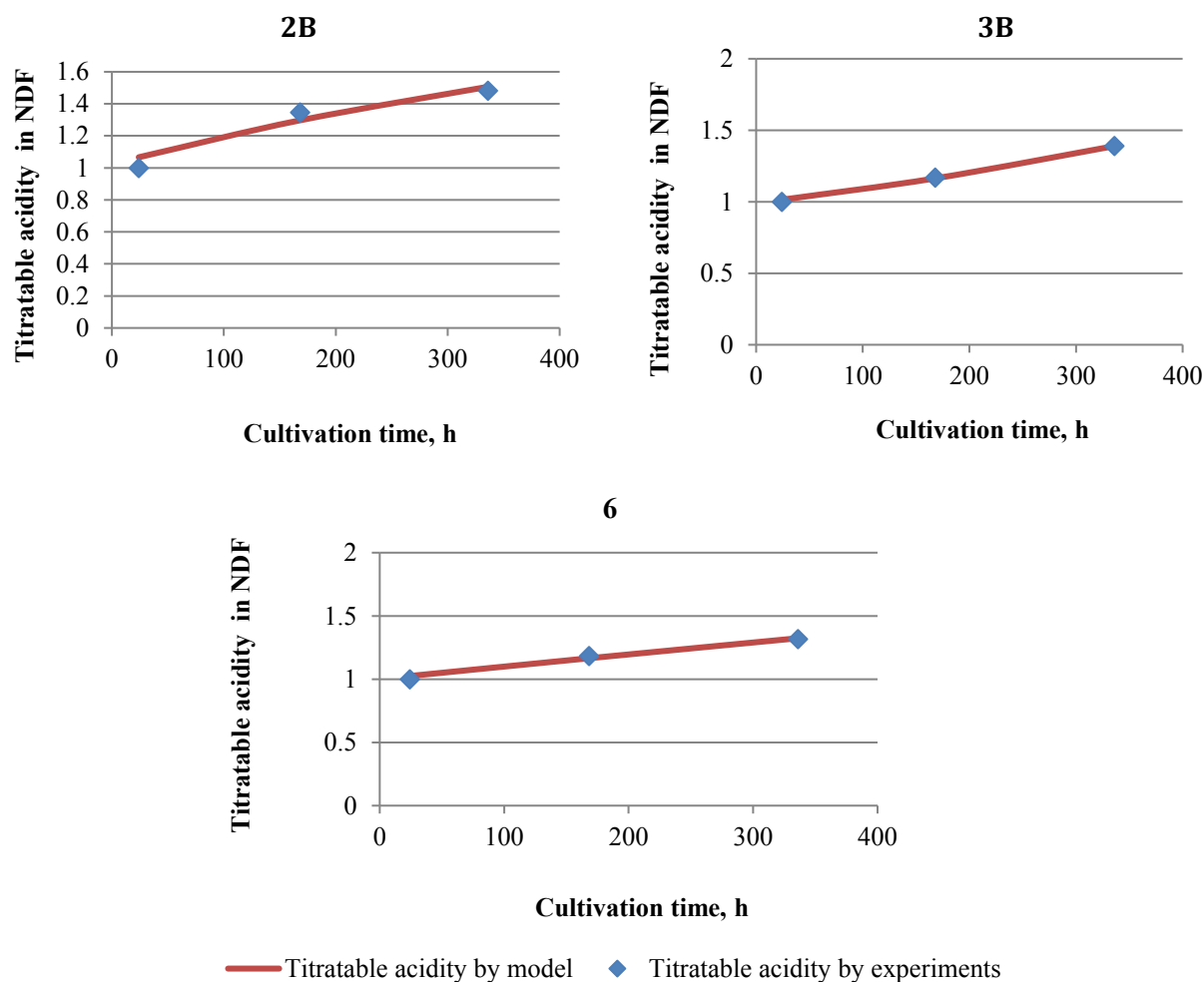


Figure 5. Comparison of dynamics of titratable acidity between experiment and model for starter culture 2B, 3B and 6 used in yogurt from reconstituted whole milk

Conclusions

Our examination of symbiotic starter cultures with selected strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* showed that the combinations starter cultures 2B, 3B and 6 are more suitable for production of Vietnamese yogurt.

As a result of mathematical modeling, we concluded that maximum rate of acidification at moment of coagulation and during storage was affected by the type of milk used in yogurt production. From that point of view, it allows us to select the appropriate starter cultures for various type of milk for production of Vietnamese yogurt.

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