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ASSESSMENT THE INFLUENCE OF THE MAIN TECHNOLOGICAL FACTORS ON YOGURT QUALITY

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Abstract: Yogurt is currently the most popular fermented milk product that is produced worldwide due to its multiple benefits for consumer health. Temperature on various technological phases is the determinant factor that influences the structure and texture of yogurt. Another important factor is the quality and dose of lactic bacteria used for starter cultures. By selecting lactic bacteria suitable for the substrate that ferments, at the optimal temperature for their metabolism, highly superior quality products can be obtained. The aim of this study is to highlight the combined effect of fermentation temperature and inoculum dose on finished yogurt under certain predetermined milk composition - raw material and starter culture quality. Yogurt samples were obtained at different fermentation temperatures (37 °C, 41 °C and 45 °C) using different amounts of inoculum (0.1 %, 0.2 % and 0.3 %). After 24 hours of storage at 4 °C the yogurt samples were analyzed for rheological and physico-chemical properties.

Keywords: *fermentation process, optimization, Response Surface Methodology, technological parameters*

INTRODUCTION

Yogurt is a popular fermented dairy product with a high biological and nutritional value obtained by coagulation of milk [1]. Acidification occurs by fermentation of lactose under the action of lactic bacteria that form acid lactic and are responsible for the process of coagulation and formation of milk gels [2, 3]. Microbial fermentation of dairy products is generally recognized for their beneficial effects on human health. Probiotics are live microorganisms that when administered in sufficient amounts confer a health benefit on the host [4]. The addition of probiotic cultures into diet improves digestion, nutrient absorption and promotes food safety and foods enriched with probiotics, prebiotics and synbiotics are recognized as functional foods [5].

In order to obtain a high quality yogurt, it is important to know the main factors of influence: milk - raw material, quality and dose of starter culture, technology and the technological equipment used as well as the technological parameters of the fermentation process. Industrially, the goal is to obtain a high viscosity yogurt, a stable product with a corresponding texture and flavour, under conditions of high production yields. One of the most important quality conditions for milk - raw material is the fat content, which is correlated in industrial practice with consumer needs. The manufacturers are interested in producing a wide range of dairy products of constant quality; hence, they need high quality cow's milk. Milk quality assessment is performed in terms of sensory, physicochemical and microbiological properties. In assessing milk quality specialists have sought to use the most appropriate and accurate analytical methods allowing the qualitative evidence of the finished product in time for disposition of raw material processing [6 – 8].

Another important factor in yogurt quality is the starter culture *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. The symbiosis phenomenon was studied by researchers, who observed a positive effect of these two cultures compared to each culture separately in terms of growth, acidification, production of flavours, and of proteolysis. Previous studies have shown that *Lactobacillus delbrueckii subsp. bulgaricus* is stimulated by CO_2 produced by the *Streptococcus thermophilus* and by formic acid, while *Streptococcus thermophilus* is stimulated by the small peptides and amino acids resulted from the metabolic activity of *Lactobacillus delbrueckii subsp. bulgaricus*. The amount of starter culture must be large enough to ensure correct acidification. Generally, manufacturers comply with the dosage indicated in the datasheet by lactic bacteria suppliers or make microprobes adapted to their own manufacturing conditions (raw material, technology and equipment) [9 – 12].

The transformation of milk into yogurt under the action of starter cultures is achieved by lactic fermentation at a certain optimal temperature determined by technological tests. The goal of this research is to optimize the yogurt manufacturing process.

The paper describes a study of the influence of the main technological factors (the fermentation temperature, the amount of starter culture, and the milk fat content) on the quality of yogurt and it aims to identify their optimum values, which can be later used in the industrial production of yogurt.

MATERIALS AND METHODS

Materials

The following raw materials were used for the preparation of yogurt under laboratory conditions: cow's milk with different fat content (2.5 %, 3.0 % and 3.5 %), from milk collection centre in Suceava county, Romania; lactic bacteria culture (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) was supplied by Enzymes & Derivates, Romania.

Yogurt preparation

In order to prepare the yogurt samples that were analyzed in this study, it was applied the method used by Dabija *et al.* (2018) [13]. The different types of cow's milk were first pasteurized at 90 °C for 15 minutes and cooled to 45 °C. After cooling, the milk was directly inoculated with the mentioned starter culture in different concentrations: 0.1 %, 0.2 % and 0.3 %. Then, the samples were agitated vigorously for a uniform distribution of the starter culture in the milk volume. The fermentation process was carried out in triplicate at 37 °C, 41 °C and 45 °C until a *p*H of 4.6 was reached in the finished product. After 24 hours of storage at 4 °C the yogurt samples were analysed in order to determine *p*H, syneresis, water holding capacity and rheological properties.

Methods

Syneresis (S) and water holding capacity (WHC) of yogurt samples were determined according to the method of Barkallah *et al.* [14]. Separation of the liquid phase from the gel is called syneresis. It may be spontaneous or may occur only when the gel is mechanically disrupted while cutting, agitating, or freezing. Thereby, syneresis is not desirable in yogurt and can negatively influence the consumer's acceptance of the product. For syneresis determination 100 mL of each sample was placed in a funnel lined with Whatman filter paper number 1. After 6 h of drainage, the volume of whey was measured and following formula was used to calculate susceptibility of syneresis:

$$S=(V_1/V_2)\times 100 \tag{1}$$

where: V_1 = volume of whey collected after drainage; V_2 = volume of yogurt sample.
Water holding capacity of yogurt samples was determined by the centrifugation of 5 g at 4500 × g (g - is the relative centrifugal force) for 15 min at 4 °C. The WHC was calculated as follows:

$$WHC\ (\%) = (1 - W_1/W_2)\times 100 \tag{2}$$

where: W_1 = weight of whey after centrifugation; W_2 = yogurt weight.
A *p*H portable F2 Standard METTLER TOLEDO device was used for the measurement of *p*H during fermentation, in finished yogurt and during storage.
All analyses were carried out in duplicate.
The rheological data for the yogurt samples were determined using the Thermo Haake Mars Rheometer, equipped with a 40 mm titanium geometry plate. The measurement temperature was 8 °C. To obtain the viscosity values, the variation of viscosity was analysed as a function of ascending and descending shear rate from 0.02 to 100 s^{-1}. For

the determination of elastic and viscous modules, there were conducted frequency dependency experiments from 0.05 to 10.0 Hz. Three determinations of each test were conducted for each sample replicate [13, 15].

Experimental design

Response surface methodology (RSM) is used for standardization of process. It is based on statistics and mathematics principles. It also generates regression equations. RSM is also utilized to study the influence of the variables on responses. Mathematical model describing the relation of variables and responses can also be generated using response surface methodology [16]. RSM with Box-Behnken design was used for the assessment of the relationships between experimental factors and obtained results. The low (-), middle (0), and high (+) levels of each variable used in design of experiments, as actual and coded values, were given in the Table 1.

Table 1. *Box Behnken design parameters (actual and coded values)*

Nr. crt.	Fermentation temperature [°C] - X_1 actual	Fermentation temperature [°C] - X_1 coded	Inoculation dose [%] - X_2 actual	Inoculation dose [%] - X_2 coded	Fat content of milk [%] - X_3 actual	Fat content of milk [%] - X_3 coded
1	37	-1	-1	0.1	0	3.0
2	37	-1	0	0.2	-1	2.5
3	37	-1	0	0.2	+1	3.5
4	37	-1	+1	0.3	0	3.0
5	41	0	-1	0.1	-1	2.5
6	41	0	-1	0.1	+1	3.5
7	41	0	0	0.2	0	3.0
8	41	0	+1	0.3	-1	2.5
9	41	0	+1	0.3	+1	3.5
10	45	+1	-1	0.1	0	3.0
11	45	+1	0	0.2	-1	2.5
12	45	+1	0	0.2	+1	3.5
13	45	+1	+1	0.3	0	3.0

Box-Behnken design with three variables like fermentation temperature, the amount of starter culture and the milk fat content at three different levels was conducted for predicting the *p*H, syneresis, WHC, dynamic viscosity (η at 100 s^{-1}), elastic modulus (G' at 1 Hz) and loss modulus (G'' at 1 Hz) in function of temperature (37 °C, 41 °C and 45 °C), inoculation dose (0.1 %, 0.2 % and 0.3 %) and fat content (2.5 %, 3.0 % and 3.5 %, respectively). The design was designed and made using Design Expert 10.0.7 (trial version). The model used to predict the evolution of extraction efficiency was a second-order (quadratic) polynomial response surface model which can be applied to fit the experimental results obtained by Box-Behnken design. The second-order (quadratic) polynomial response surface model which describes the relationship between the experimental results is:

$$y = b_0 + \sum_{i=1}^{n}(b_i x_i) + \sum_{i=1}^{n}(b_{ii} x_{ii}^2) + \sum_{ij=1}^{n}(b_{ij} x_i x_j) \quad (3)$$

where: y is the predicted response (pH, syneresis, WHC, dynamic viscosity (η at 100 s^{-1}), storage modulus (G' at 1 Hz) and loss modulus (G'' at 1 Hz)), x_i stands for the coded levels of the design variable (temperature, inoculation dose and fat content - Table 1), b_0 is a constant, b_i - linear effects, b_{ii} - quadratic effects and b_{ij}- interaction effects.

In order to predict the extraction of pH, syneresis, WHC, dynamic viscosity (η at 100 s^{-1}), storage modulus (G' at 1 Hz) and loss modulus (G'' at 1 Hz) it is very important that all parameters are simultaneously optimized. The desirability function approach is used to optimize the multiple characteristics concurrently [17]. In the desirability function approach, first each characteristic, y_i, is converted into an individual desirability function, d_i, which varies over the range:

$$0 \leq d_i \leq 1 \tag{4}$$

RESULTS AND DISCUSSION

The fermentation temperature exhibited an essential effect on yogurt quality and culture starter was selected as function of milk composition. A decrease in yogurt acidity was obtained at the temperature of 37 °C, compared to the temperature of 45 °C, according to Walstra *et al.* [18]. Also, the fermentation temperature increase led to lower water holding capacity, viscosity decreased, but higher syneresis, G' and G", being in accordance with the results obtained by Ozdemir & Kilic [19]. The amount of the starter cultures influenced the pH and viscoelastic properties of the finished product. This is in accordance with Abbasi *et al.* [20], where the rheological properties of acid gels were affected by different starter concentration and fermentation temperature which directly affected the rate of acidification [21, 22]. An increase in fat content determined the improvement of gel stability, WHC and viscosity, and decreased the yogurt syneresis [23].

Viscoelastic properties of yogurt

In our study the viscoelastic properties of yogurt samples in the linear viscoelastic region were evaluated. As it was expected, the yogurt behaved as a material with the elastically part much greater than the viscous one (G'>G'') [7]. Figure 1 (b) shows the influence of the inoculation doses on rheological properties and it can be observed that the optimum dose is 0.2 %, while at 0.3 % the rheological parameters decrease. The decrease of the rheological parameters at high inoculation doses is because of the powerful proteolysis activity of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* [24 – 26]. The fat content influenced positively the magnitude of all rheological parameters (Figure 1), this increase is due to the increase in the dry matter in the total product (with the decrease of the moisture content which increases the speed of the molecule and decreases the rheological parameters magnitude).

Application

Box-Behnken design for predicting the *p*H, syneresis, WHC, dynamic viscosity (η at 100 s^{-1}), storage modulus (G' at 1 Hz) and loss modulus (G" at 1 Hz).

Yogurt pH

The experimental values of the yogurt *p*H in function of temperature, inoculation dose and fat content of milk were fitted to quadratic equations using the Box-Behnken design; the statistical parameters (standard deviation, sum of squares, regression coefficients and F-value) of the model are presented in the Table 2. The quadratic equation of the *p*H modelling is presented in Equation 5:

$$pH = 4.50 - 0.06 \cdot X_1 - 0.01 \cdot X_2 + 0.02 \cdot X_3 + 0.04 \cdot X_1 \cdot X_2 + 0.02 \cdot X_1 \cdot X_3 -$$

$$-0.01 \cdot X_2 \cdot X_3 - 0.01 \cdot X_1^2 + 0.01 X_2^2 \quad (5)$$

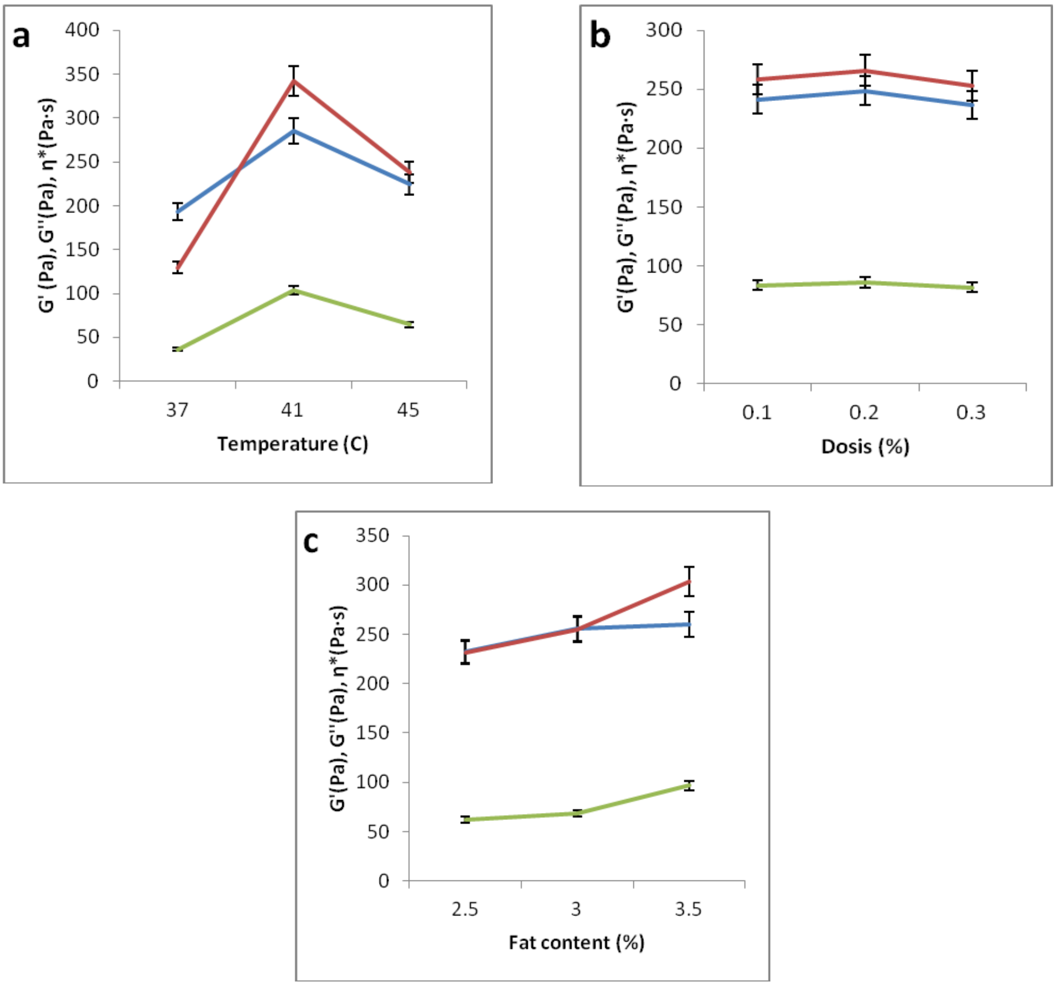

Figure 1. *Evolution of visco-elastical parameters (blue line - dynamic viscosity (Pa·s), red line - elastic modulus (Pa) and green line - loss modulus (Pa)) with temperature (a), inoculation doses (b) and fat content (c)*

Table 2. *Statistical parameters of physicochemical parameters prediction using Box Benken desigh based on temperature, innoculation dosis and fat content of milk*

Parameter	Standard deviation	Sum of squares	R^2	F-value (P)
*p*H	0.02	0.04	0.9235	9.38**
Syneresis [%]	0.84	132.7	0.9642	20.96***
WHC [%]	1.69	265.2	0.9300	10.33**
η [Pa·s]	0.01	0.02	0.9867	57.53***
G' [Pa]	24.9	12470	0.9670	21.81***
G" [Pa]	16.2	14430	0.8868	6.09*

* - P < 0.05, ** - P <0.01, ***-P<0.001

According to the data presented in the Table 2, the regression coefficients of the *p*H modelling is higher than 0.92 and the model is a significant one (P < 0.001). The ANOVA has been applied to check the influence of temperature, inoculation dose and fat content of milk, and their interactions on *p*H modelling; there can be observed that temperature, fat content and interaction between temperature and inoculation dose influence significantly the *p*H (P < 0.01), while the inoculation dose and all the other interactions do not influence significantly the *p*H modelling (P > 0.05). The evolution of the *p*H exp vs. *p*H pred. is presented in the Figure 2a.

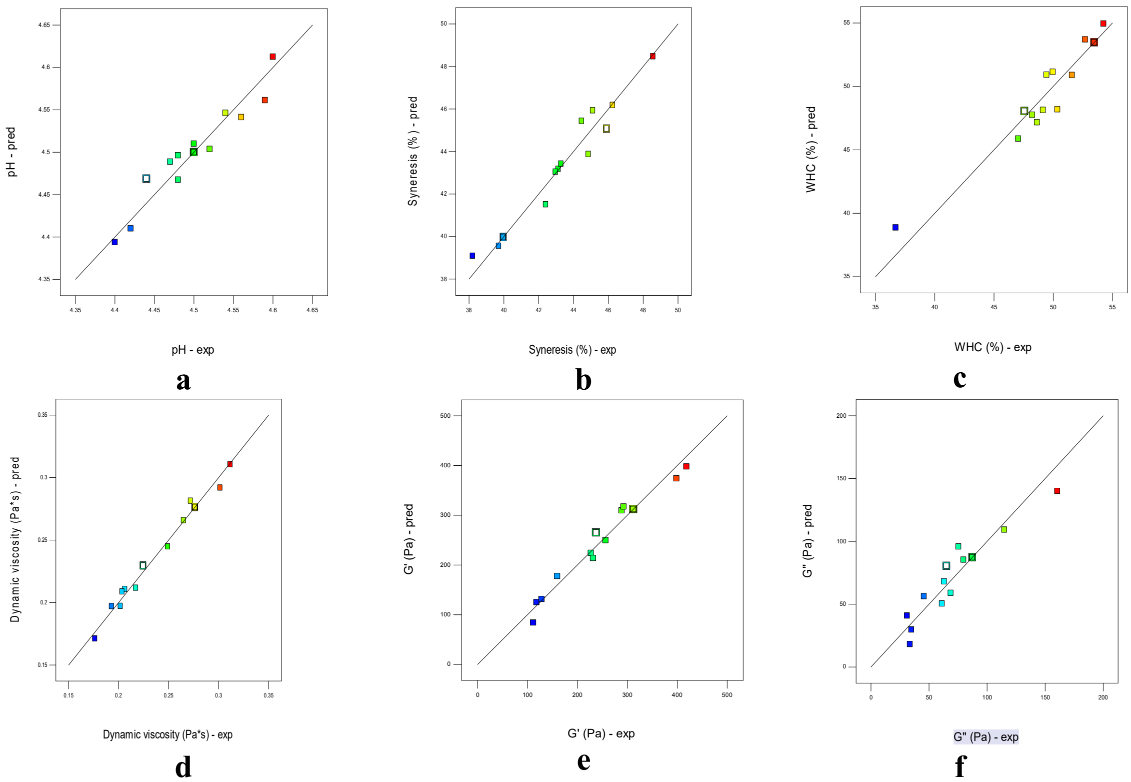

Figure 2. *Experimental vs. predicted values of: a) pH, b) syneresis, c) WHC, d) dynamic viscosity, e) storage modulus and f) loss modulus in function of fermentation temperature, inoculation dose and fat content using Box Behnken design*

Yogurt syneresis

The experimental values of the yogurt syneresis in function of temperature, inoculation dose and fat content of milk were fitted to quadratic equations using the Box-Behnken design; the statistical parameters (standard deviation, sum of squares, regression coefficients and F-value) of the model are presented in the Table 2. The quadratic equation of the syneresis modelling is presented in (Equation 6):

$$Syneresis = 39.97 + 1.14 \cdot X_1 - 0.36 \cdot X_2 - 1.57 \cdot X_3 - 0.01 \cdot X_1 \cdot X_2 - 0.13 \cdot X_1 \cdot X_2 +$$

$$+0.60 \cdot X_2 \cdot X_3 + 4.72 \cdot X_1^2 - 0.02 \cdot X_2^2 + 0.94 \cdot X_3^2 \tag{6}$$

According to the data presented in the Table 2, the regression coefficients of the syneresis modelling is higher than 0.96 and the model is a significant one ($P < 0.001$). The ANOVA has been applied to check the influence of the temperature, inoculation dose and fat content of milk, and their interactions on syneresis modelling; there can be observed that temperature, fat content of milk and interaction between temperature and temperature influence significantly the syneresis modelling ($P < 0.01$), while the inoculation dose and all the other interactions are not influence significantly the syneresis modelling ($P > 0.05$). The evolution of the syneresis exp vs. syneresis pred. is presented in Figure 2b.

Yogurt WHC water holding capacity

The experimental values of the yogurt WHC in function of temperature, inoculation dose and fat content of milk were fitted to quadratic equations using the Box-Behnken design; the statistical parameters (standard deviation, sum of squares, regression coefficients and F-value) of the model are presented in the Table 2. The quadratic equation of WHC modelling is presented in (Equation 7):

$$WHC = 53.47 - 2.25 \cdot X_1 + 0.38 \cdot X_2 + 2.40 \cdot X_3 + 0.26 \cdot X_1 \cdot X_2 + 2.19 \cdot X_1 \cdot X_3 -$$

$$-1.00 \cdot X_2 \cdot X_3 - 5.45 \cdot X_1^2 + 0.75 \cdot X_2^2 - 2.30 \cdot X_3^2 \tag{7}$$

According to the data presented in the Table 2, the regression coefficients of the WHC modelling is higher than 0.96 and the model is a significant one ($P < 0.001$). The ANOVA has been applied for checking the influence of the temperature, inoculation dose and fat content of milk, and their interactions on WHC modelling; there can be observed that temperature, fat content of milk and interaction between temperature and fat content of milk, interaction between temperature and temperature and interaction between fat and fat influence significantly WHC modelling ($P < 0.01$), while the inoculation dose and all the other interactions do not influence significantly the WHC modelling ($P > 0.05$). The evolution of the WHC exp vs WHC pred. is presented in the Figure 2c.

Yogurt dynamic viscosity

The experimental values of the yogurt dynamic viscosity in function of temperature, inoculation dose and fat content of milk were fitted to quadratic equations using the

Box-Behnken design; the statistical parameters (standard deviation, sum of squares, regression coefficients and F-value) of the model are presented in the Table 2. The quadratic equation of elastic modulus modelling is presented in (Equation 8):

$$\eta = 0.28 + 0.02 \cdot X_1 + 0.01 \cdot X_2 + 0.01 \cdot X_3 + 0.01 \cdot X_1 \cdot X_2 - 0.01 \cdot X_1 \cdot X_3 + \\ +0.01 \cdot X_2 \cdot X_3 - 0.01 \cdot X_1^2 + 0.01 \cdot X_2^2 + 0.01 \cdot X_3^2 \quad (8)$$

According to the data presented in the Table 2, the regression coefficients of the dynamic viscosity modelling is higher than 0.98 and the model is a significant one ($P < 0.001$). The ANOVA has been applied to check the influence of temperature, inoculation dose and fat content of milk, and their interactions on dynamic viscosity modelling; there can be observed that temperature, inoculation dose, fat content of milk and interaction between fermentation temperature and fermentation temperature influence significantly dynamic viscosity modelling ($P < 0.01$), while the rest of interactions do not influence significantly the dynamic viscosity modelling ($P > 0.05$). The evolution of the dynamic viscosity exp vs dynamic viscosity pred. is presented in Figure 2d.

Yogurt storage modulus G'

The experimental values of the yogurt loss modulus in function of temperature, inoculation dose and fat content were fitted to quadratic equations using the Box-Behnken design; the statistical parameters (standard deviation, sum of squares, regression coefficients and F-value) of the model are presented in the Table 2. The quadratic equation of loss modulus modelling is presented in (Equation 9):

$$G' = 87.3 + 14.1 \cdot X_1 - 5.1 \cdot X_2 + 17.0 \cdot X_3 + 0.4 \cdot X_1 \cdot X_2 - 2.0 \cdot X_1 \cdot X_3 - \\ -10.3 \cdot X_2 \cdot X_3 - 47.1 \cdot X_1^2 + 9.2 \cdot X_2^2 + 11.1 \cdot X_3^2 \quad (9)$$

According to the data presented in the Table 2, the regression coefficients of the storage modulus modelling is higher than 0.96 and the model is a significant one ($P < 0.001$). The ANOVA has been applied for checking the influence of the temperature, inoculation dose and fat content, and their interactions on storage modulus modelling; there can be observed that temperature, fat content and interaction between fermentation temperature and fermentation temperature influence significantly storage modulus modelling ($P < 0.01$), while the rest of interactions do not influence significantly the storage modulus modelling ($P > 0.05$). The evolution of the storage modulus exp vs storage modulus pred. is presented in the Figure 2e.

Yogurt loss modulus G"

The experimental values of the yogurt loss modulus in function of temperature, inoculation dose and fat content of milk were fitted to quadratic equations using the Box-Behnken design; the statistical parameters (standard deviation, sum of squares, regression coefficients and F-value) of the model are presented in the Table 2. The quadratic equation of loss modulus modelling is presented in (Equation 10):

$$G'' = 312.2 + 54.3 \cdot X_1 + 7.9 \cdot X_2 + 36.2 \cdot X_3 + 4.8 \cdot X_1 \cdot X_2 - 10.5 \cdot X_1 \cdot X_3 + \\ +4.0 \cdot X_2 \cdot X_3 - 147.1 \cdot X_1^2 + 17.5 \cdot X_2^2 + 20.0 \cdot X_3^2 \quad (10)$$

According to the data presented in the Table 2, the regression coefficients of the loss modulus modelling is higher than 0.96 and the model is a significant one ($P < 0.001$). The ANOVA has been applied for checking the influence of the temperature, inoculation dose and fat content of milk, and their interactions on loss modulus modelling; there can be observed that temperature, fat content of milk and interaction between fermentation temperature and fermentation temperature influence significantly storage modulus modelling ($P < 0.01$), while the rest of interactions do not influence significantly the loss modulus modelling ($P > 0.05$). The evolution of the loss modulus exp vs. loss modulus pred. is presented in Figure 2f.

Optimization

In order to achieve the proper characteristics of yogurt, we optimized the *p*H, syneresis and WHC as follows *p*H = 4.5, syneresis to be minimum and WHC, dynamic viscosity, storage modulus and loss modulus to be maximum, respectively. Accordingly, to the optimization process for achieving a *p*H of 4.5, syneresis of 39.63 %, WHC of 53.69 %, η = 0.311 Pa·s, G’ = 400.3 Pa and G” = 109.1 Pa the input parameters should be: fermentation temperature 41.24 °C, inoculation dose 0.3 % and fat content 3.50 % (D = 0.878).

CONCLUSIONS

Fermentation is the most important stage in the process of yogurt making. Identifying the factors that influence this technological operation is a challenge for manufacturers in the fermented milk products industry. The paper proposed an experimental model to optimize the main factors influencing the quality of yogurt - finished product. By applying this model in our research, we determined the optimal technological parameters to obtain a high-quality product and we could assess the quality of the milk used as raw material. The modelling of the pH, WHC, syneresis, dynamic viscosity, elastic modulus and loss modulus based on fermentation temperature, inoculation quantity and fat content reached higher regression coefficients ($R^2 > 0.88$). To achieve a *p*H of 4.5, syneresis of 39.63 %, WHC of 53.69 %, η = 0.311 Pa·s, G’ = 400.3 Pa and G’’ = 109.1 Pa the input parameters should be the following: fermentation temperature 41.24 °C, inoculation dose 0.3 % and fat content 3.50 % (D = 0.878).

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