MODELING AND OPTIMIZATION OF LACTIC FERMENTATION PROCESS IN THE PRESENCE OF ANIONIC CLAYS USING EXPERIMENTS DESIGN

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Lucrarea prezintă un studiu privind modelarea şi optimizarea procesului de fermentare lactică în prezenţa argilelor anionice utilizând programarea factorială a experimentelor. S-a utilizat un program factorial tip 33 (trei parametri şi trei nivele de variaţie). Parametrii variabili urmăriti au fost: temperatura de pasteurizare a laptelui, raportul solid:lichid, durata fermentării, iar funcţia de răspuns este reprezentată de aciditatea mediului de fermentaţie. Rezultatele experimentale obţinute au permis evidenţierea efectelor parametrilor variabili asupra cineticii procesului. Aciditatea optimă a mediului de fermentaţie s-a obţinut la temperatura de pasteurizare de 80,5 0C, pentru un raport solid-lichid de 0,15 g argilă anionică/ mL lapte şi la o durată de fermentaţie de aproximativ 5 ore.

This paper presents a study on modeling and optimization of lactic fermentation process in the presence of anionic clays using experiments design. It is used a 3^3 type experiment design (three parameters and three levels of variation). Variable parameters of the process were: pasteurization temperature of milk, solid-liquid ratio, fermentation duration; the response function was the acidity of the fermentation media. The experimental results evidencing the effects of the operating parameters on the lactic fermentation process in the presence of anionic clays. Experimental set-up shown an optimum acidity at a pasteurization temperature of 80,5 ^{0}C, a solid-liquid ratio of 0.15 g mL anionic clay/ milk and a fermentation duration around of 5 hours.

Keywords: lactic fermentation process, anionic clays, acidity, experiment design, optimization

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1. Introduction

Lactic fermentation is an anaerobic process in which carbohydrates with fermentation capacity are metabolized by the enzyme produced by microorganisms, in lactic acid, the main fermentation product and, in secondary products such as: diactyl, acetoyne, acetic acid, ethyl alcohol and CO_2 [1].

Fermented dairy products result from fresh or skimmed milk fermentation and curdling, under the enzymatic action of lactic acid bacteria cultures.

The milk lactic fermentation stages are:

- hydrolysis of lactose into glucose and galactose [2] according to the reaction:

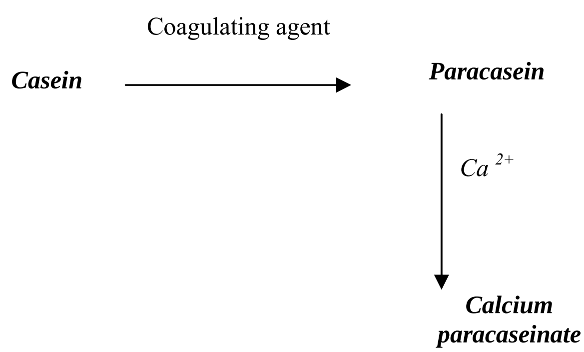
$$\underset{Lactose}{C_{12}H_{22}O_{11}} + \underset{Water}{H_2O} \rightarrow \underset{Glucose}{C_6H_{12}O_6} + \underset{Galactose}{C_6H_{12}O_5}$$

- decomposition of glucose, using different paths, to lactic acid, respectively:

$$\underset{Glucose}{2C_6H_{12}O_6} + \underset{Water}{H_2O} \rightarrow \underset{Lactic\ acid}{2\,CH_3{-}CHOH-COOH} + \underset{Ethyl\ alcohol}{CH_3-CH_2OH} + \underset{Aceticacid}{CH_3-COOH} + CO_2 + H_2$$

Lactic acid resulted from lactic fermentation has an important role in the fermented milk products technology, influencing the degree of casein coagulation by increasing the milk acidity.

Coagulation process consists in a pronounced reticulation (formation of stable chemical bonds) of casein protein globules in a three-dimensional structure, semi-solid and gelatinous (paracasein), which include fat particles and whey [3], according to the following reaction scheme:



Coagulation is a biochemical process and its mechanism depends on the nature of coagulation agent, type of the enzymes used and runs under certain conditions of temperature and medium acidity.

In the classical technology of obtaining of the dairy products, cell growth and metabolism of lactic bacteria are inhibited by progressive decrease of pH in fermentation medium, followed by the conversion of lactose into lactic acid and lactic acid radical accumulation.

These two combined effects determine the decreasing efficiency of cell growth and a reduced metabolism, causing the reduction of the yield production of fermented dairy products.

In classical fermentation technologies, the fermentation product (lactate anion) is not removed, being the main inhibitor of cell development in the fermentation stage.

So, the main disadvantage of the classical method and for all known methods used for acid dairy products preparation, is the large microbial mass losses during fermentation process, causing low productivity in lactic acid and reducing growth rate of cells population. This aspect also influences the amount and cost of selected cultures.

In the literature are presented various techniques to improve the classical technology of lactic acid obtaining and its salts.

To reduce the fermentation inhibition by product, Friedman and Saden (1980) used dialysis systems immersed in fermentation medium, which remove lactic acid from it [4]; San Martin and colab., (1992) have removed the lactic acid from fermentation medium by a liquid-liquid extraction system using amines [5]; Yabannavar and Wang (1991) have studied the production of lactic acid with Lactobacillus delbrueckii by fermentative-extraction method using tertiary amines (336 Alanin and oleic alcohol) [6]; Heriban V. (1993) has used for the extraction of lactic acid from fermentation medium a combined electro-dialysis method using ion exchange technology; this technique allows to obtain a 85% yield of lactic acid, comparatively with 50% yield obtained by the classical technology, which use as a method of disposal of acid lactic acid the precipitation of calcium lactate [7].

In this context, the research presented in this paper is focused on studying the removal of lactic acid from fermentation medium by its retention on porous anionic clay matrix-type during the fermentation process.

It is important to note that in literature there are not studies related to the use of porous matrices, such as anionic clays, in the biotechnologies used to obtain fermented dairy products.

Anionic clays (AA) are mixed structured hydroxides, double layered, synthetic or natural, having between layers spaces which contain exchangeable anions and water molecules [8]. Depending on the composition and mineralogical structure are used different names for these compounds, most frequently terms are *hydrotalcite (HT) and layer double hydroxides (LDH).*

Anionic clays have a structure consisting on similar layers type with minerals like *brucite* (flat or layered), positively charged, in which the bivalent cations are substituted by trivalent cations in an octahedral coordination.

Anionic clays can be represented by the following general formula:

$$\mathbf{M(II)M(III)A\text{-}HT= [M(II)_{1-x}M(III)_x(OH)_2]^{x+}(A^{n-}x/n).mH_2O},$$

where: **M (II)** is the bivalent cations (ex. Mg^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} or Ca^{2+});

M(III) is the trivalent cations (ex. Al^{3+}, Cr^{3+}, Mn^{3+}, Fe^{3+}, Co^{3+} or La^{3+});

$\mathbf{A^{n-}}$ the interlayered anion (ex. inorganic anions: F^-, Cl^-, Br^-, I^-, $(ClO_4)^-$, $(ClO_3)^-$, $(NO_3)^-$, $(CO_3)^{2-}$, $(SO_4)^{2-}$, $(S_2O_3)^{2-}$, $(CrO_4)^{2-}$, $[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$;

x = M(III)/(M(II)+M(III));

m is the number of water molecules.

Due to their specific properties, anionic clays are used as catalysts [9], adsorbents [10, 11], anion exchangers [12], and in medicine [13], as bio-organic and bio-inorganic composite materials [14] etc..

2. Experimental design set-up

The experimental design have as purpose to obtain: quantitative relations such as $y = f(x_1, x_2, x_3, \ldots)$; the mathematical models associated to the processes; systematically, efficiently and economically investigation of significant factors that influence a process respectively [15,16]. These methods allow studying the influence of many variables and the interaction between them.

Experimental design involves the assignation of a procedure for selecting the number of experiments, the conditions for their achievement in order to answer to the asked problem with sufficient precision. Factorial design experiments involve simultaneous variation, after a certain level, of all parameters which are considered to influence the process [17, 11].

The most frequently used experimental design programs are those of k^n type, where k is the number of levels variation of parameters and n is the number of parameters.

The objectives of this study were selected on the basis of the lactic fermentation process technological aspects, in the presence of anionic clays. Those are:

- modeling and optimization of lactic fermentation in the presence of anionic clays using a design of experimental data (DOE), evidencing the effects of operating parameters values (x_1, x_2, x_3) on the lactic fermentation process and interaction between them;

- determining the equation, that describes the studied process, in a polynomial type, with the coefficients attached to independent variables (factors) which are being determined by regression analysis.

3. Results and discussions

Modeling of lactic fermentation process in the presence of anionic clays was realized by using a 3^3 type experimental design (three parameters and three levels of variation) which require the performing of 27 experiments.

By establishing procedures for the selection of the number and the conditions of experiences achieving, it can be obtain a sufficient accuracy of the model build on the experimental data.

The constant parameters of the study are: fermentation temperature, amount of starter culture used for milk inoculation and raw materials. Experimental study variables are: pasteurization temperature of milk, X_1 (T), the ratio solid : liquid (S: L) between the quantity of anionic clay and volume of milk, X_2 (R) and fermentation duration (h), X_3 (τ).

The real variables ranges and their correlation with dimensionless values are presented in table 1.

Table 1

Variation level of parameters for the response function

Response function	Parameter	Code	Minimum value (-1)	Average value X_i^{med} (0)	Maximum value (+1)	Unit variation ΔX_i
Acidity (**Y**)	Temperature, **T** (^{0}C)	X_1	65	80	95	15
	Anionic clay /milk ratio, **R** (g/mL)	X_2	0,05	0,1	0,15	0,05
	Duration, τ (h)	X_3	2	4	6	2

Experiments were performed on cow milk, whose physical-chemical characteristics are presented in table 2.

Table 2

Physical-chemical values of milk - raw material

S ample	cidity [^{0}T]	at content %]	ensity [kg/m^3]	P rotein [%]	D ry substance [%]	L actose [%]	F reezing point
ilk	8	.45	029	3 .25	1 2.50	5 .15	5 10

Preparation of anionic clay with ratio $Mg / Al = 2 : 1$ was performed by co-precipitation method using the corresponding nitrates, as raw materials [9].

Experimental steps taken to achieve experimental lactic fermentation were:

- pasteurization of milk at different temperatures: 65, 80, 95 ^{0}C, for 30 minutes;
- inoculation cooling temperature (45 ^{0}C);
- inoculation with starter culture (3%), Yo-Flex YC-X11 produced by CHR HANSEN company, which contains *Streptococcus Thermophilus* and *Lactobacillus delbruekii subsp. bulgaricus*.
- thermo - stating at 42 ^{0}C, for 2, 4, 6 hours.

For each set of experiments were used 100 ml inoculated fresh milk mixed with different amounts of anionic clay: 0.05 g (-1), 0.1 g (0), 0.5 g (1). At the end of lactic fermentation was determined the acidity according to STAS 6353-85, for the considered samples [18].

The factorial program takes into account the experiments carried out in the centre areas of variation of different process parameters. Table 3 shows the correspondence between the selected process parameters and the mathematical variables studied by DOE, considering the response function, Y, obtained values.

Table 3

Acidity values according to the adopted criteria

o. crt.	**Variation levels of parameters, x**			**Response function**
	Temperature, T (^{0}C)	**Anionic clay/milk ratio, R (g/mL)**	**Duration, τ (h)**	**Acidity (^{o}T)**
	x_1 (X_1)	**x_2 (X_2)**	**x_3 (X_3)**	**Y**
	-1 (65)	-1 (0.05)	-1 (4)	94,5
			0 (6)	102,1
			+1 (8)	107,3

			-1 (4)	95,3
		0 (0.1)	0 (6)	98,6
			+1 (8)	104,3
			-1 (4)	94,1
		+1 (0.15)	0 (6)	100,5
			+1 (8)	99,16
0			-1 (4)	95,6
1		-1 (0.05)	0 (6)	103,8
2			+1 (8)	105,3
3			-1 (4)	94
4	0 (80)	0 (0.1)	0 (6)	95
5			+1 (8)	98,6
6			-1 (4)	90,5
7		+1 (0.15)	0 (6)	93,5
8			+1 (8)	98
9			-1 (4)	101,3
0		-1 (0.05)	0 (6)	135
1			+1 (8)	111
2			-1 (4)	94,3
3	+1 (95)	0 (0.1)	0 (6)	105
4			+1 (8)	107,5
5			-1 (4)	95,16
6		+1 (0.15)	0 (6)	96,6
7			+1 (8)	103,6

3.1. Mathematical model

The mathematical model associated to lactic fermentation process and experimentally conducted by factorial design consists in determining the coefficients of a polynomial function, as:

$$Y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + a_3 \cdot x_3 + a_{12} \cdot x_1 \cdot x_2 + a_{13} \cdot x_1 \cdot x_3 + a_{23} \cdot x_2 \cdot x_3 + \\ + a_{11} \cdot x_1^2 + a_{22} \cdot x_2^2 + a_{33} \cdot x_3^2 \tag{1}$$

where: Y is the response function, x_1, x_2, x_3 are the process variables, a_0 ... a_{123} are the coefficients of regression function.

The polynomial model accuracy depends on the accuracy of experimental data obtained. Based on polynomial regression were determined the coefficients for the response function, ***"acidity"***. The values of the regression function coefficients are presented in table 4.

Table 4

Values of the regression function of coefficients

o. crt	**Co efficients**	**Value s**
	a_0	98.21 3
	a_1	2.978
	a_2	-4.71
	a_3	4.444
	a_{11}	5.373
	a_{22}	2.323
	a_{33}	- 3.927
	a_{12}	- 3.483
	a_{13}	0.373
0	a_{23}	- 0.933
1	a_{123}	0.81

Substituting the coefficients of the equation (1) with values presented in table 4 is obtained the following mathematical model for response function "***acidity***":

$$Y = 98{,}213 + 2{,}978 \cdot x_1 - 4{,}71 \cdot x_2 + 4{,}44 \cdot x_3 + 5{,}37 \cdot x_1^2 + 2{,}32 \cdot x_2^2 - 3{,}29 \cdot x_3^2 - \\ - 3{,}48 \cdot x_1 x_2 + 0{,}37 x_1 x_3 - 0{,}93 x_2 x_3 + 0{,}81\ x_1 x_2 x_3 \tag{2}$$

To validate this mathematical model it must analyze the effects of the parameters on the regression function and the accordance with the experimental data.

3.2. Simulation of the effects

To simulate the effects induced by the variable parameters on the response function, we consider the absolute values and signs of each coefficient, obtained by experimental design (table 5).

Table 5

The simulation of effects of the coefficients on the regression function

r. crt	**Variables**	**Effect on the regression function**
	x_1	++
	x_2	---
	x_3	+++
	x_1x_2	--
	x_1x_3	+
	x_2x_3	-
	$x_1x_2x_3$	++

Absolute value of each coefficient indicates the intensity of variable effect or those interactions. Signs of coefficients give meaning to each effect, as shown below:

High favorable:+ + + High unfavorable: - - -

Favorable: + + Without effect: 0 Unfavorable: – –

Low favorable:+ Low unfavorable: –

3.3 Analysis of the effects of regression functions coefficients

To visualize the type of interactions between variables of the regression function (2) was represented in 3-D coordinates the response function, ”acidity” , Y for different combinations of process variables (Figures 1-3).

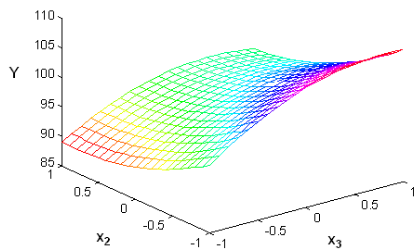


Fig. 1. Influence of ratio S : L and the fermentation time on acidity of yogurt fermentation temperature is zero

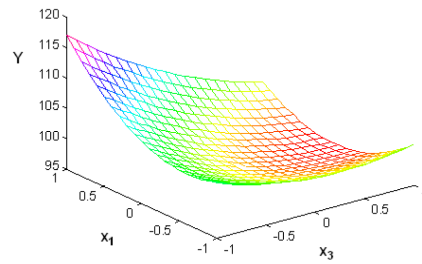


Fig. 2. Influence of fermentation time and fermentation temperature on acidity of when yoghurt when ratio S : L is zero

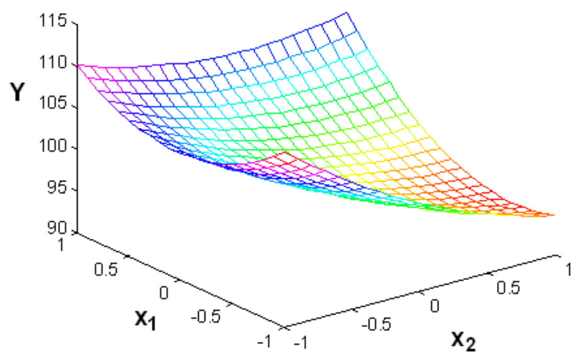


Fig. 3. Influence of ratio S:L and the fermentation temperature on acidity of yogurt when fermentation time is zero

Analyzing the shape of the regression function (relation 2) and the graphs presented in Figures 1-3 it can be seen that conjugated variables x_1 and x_3 have a favorable effect on the fermentation process, while individual variable x_3 bring a disadvantage on the process; the variable x_2 mostly affects the response function. Interaction between variables x_1 and x_2 affects negatively the process.

Quadratic terms coefficients, x_1^2 and x_2^2 are positive, determine a minimum for regression function and the response function surface corresponding to the mathematical model is convex.

3.4. Simplification of the mathematical model

To simplify the mathematical model was applied the *t-student* test. This involves removing the coefficients with the lowest share, considering that they can not have a significant role in the developed model. For this purpose, two measurements are repeated in the centre area (0, 0, 0) - table 6.

Table 6

Values measured in the centre field (0,0,0)

o. crt	**Measu rements**	**1**
	I	5,5
	II	6
	III	6,5

There will be calculate the average value of the acidity of three standard samples in the central point of the field (0,0,0):

$$y^0_{med} = \frac{\sum_{i=1}^{3} y_i^0}{3} = \frac{95.5 + 96 + 96.5}{3} = 96 \quad (3)$$

Knowing that the number of standard samples, n is 3, was calculated the mathematical model error:

$$\varepsilon^2 = \sum_{i=1}^{n} \frac{\left(y_i^0 - y_{med}^0\right)^2}{n-1} = 0.25 \quad (4)$$

$$\varepsilon = \sqrt{\varepsilon^2} = \sqrt{0.25} = 0.5 \quad (5)$$

Knowing that the total number of experiments, N, is 27, can be identified the significance of the model coefficients on the response function, with the following equation:

$$S = \frac{\varepsilon}{\sqrt{N}} = \frac{0.5}{5{,}196} = 0.096 \quad (6)$$

The significance of the coefficients is assessed with t-student test using the relation: $t_j = \left|a_j\right| / S$. The values of the t-student test for each coefficient are presented in table 7.

Table 7

Values of t-student test

t_j	**t** **0**	**1**	**2**	**3**	**12**	**23**	**13**	**11**	**22**	**33**	**123**
C alculated value	1 ,021x10^3	0,95	8,95	6,18	6,2	,7	,88	5,84	4,14	0,8	,41

After eliminating insignificant terms (a_{23}, a_{13}, a_{123}), identified by t-student test, the final mathematical model, characteristic to lactic fermentation process in the presence of anionic clays, is:

$$Y = 98{,}213 + 2{,}978 \cdot x_1 - 4{,}71 \cdot x_2 + 4{,}44 \cdot x_3 + 5{,}37 \cdot x_1^2 + 2{,}32 \cdot x_2^2 - 3{,}29 \cdot x_3^2 - 3{,}48 \cdot x_1 x_2 \qquad (7)$$

or, explained with corresponding physical variables:

$$A = 98{,}213 + 2{,}978 \cdot T - 4{,}71 \cdot R + 4{,}44 \cdot \tau + 5{,}37 \cdot T^2 + 2{,}32 \cdot R^2 - 3{,}29 \cdot \tau^2 - 3{,}48 \cdot T \cdot R \qquad (8)$$

3.5. Optimization of the mathematical model

The Lagrange method is used to obtain the optimal points for the developed mathematical model. Optimum points (minimum) obtained for the response function „acidity” are: $x_1 = 0.035$, $x_2 = 1.125$, $x_3 = 0.438$.

Optimal points in real coordinates are obtained using the following relation:

$$X_i^{optim} = \Delta X \cdot x_i + X_{med} \qquad (9)$$

The results obtained for the optimal points, using the simplified model, correspond to values: X_1= 80,525; X_2= 0,156; X_3= 4,876.

The final results concluded that the optimum acidity is obtained at a pasteurization temperature of 80.5 ^{0}C, a ratio S: L = 0.15 and a fermentation duration of approximately 5 hours.

4. Conclusions

Using the experimental design for modeling and optimization of lactic fermentation process in the presence of anionic clays has demonstrated the viability of this technique for analyzing experimental data.

Factorial experimental design permits to estimate the effects of the determinant parameters of lactic fermentation process and of the interactions between them. Based on experimental data and using DOE technique was developed an equation that shows response function (the acidity of fermentation medium) as a polynomial, where the coefficients attached, independent variables, can be determined by regression analysis.

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