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Original Research Article

Investigating the Combined Effects of Physicochemical Conditions on Functional Properties of Two strains of Lactic Acid Bacteria

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ABSTRACT

Lactic acid bacteria (LAB) are widely used in the food industry due to their interesting technological activities and beneficial effects on human health. In technological processes, physicochemical conditions often appear in combination. To ensure the viability of LAB and improve their metabolic and functional properties, adjustment of optimal physicochemical conditions should be considered. In this study, the combined effects of temperature, hydrogen potential (pH), and sodium chloride concentration, on two functional properties, proteolysis and acidification, of two strains of LAB, *Lactococcus lactis* (LCL) and *Enterococcus faecium* (CHT4), were investigated. Both activities were assayed at the end of growth of the two strains at different values of the three factors using a central composite design (CCD). Graphical analysis of results transcribed as isoresponse contour plots showed that temperature positively affected the LCL strain's proteolysis at 40°C. In contrast, the CHT4 strain's proteolysis was affected by pH of about 7 and temperature of around 40°C in decreasing order. The LCL strain's acidification was positively affected by reducing the salt concentration in the medium to about 2 to 3% combined with a temperature above 37°C; in contrast, the CHT4 strain's acidification was affected by temperature (37 to 40°C), and pH (6.2 to 7). Statistical analysis of the results was used to generate mathematical models describing both activities according to the most significant factors. Applying these models when using the strains on an industrial scale will optimize food production conditions and improve their organoleptic quality, with health benefits that meet consumer expectations.

***Keywords*:** *Lactococcus lactis*, *Enterococcus aecium*, Physicochemical factors, Functional properties, Central composite design.

Introduction

Lactic Acid Bacteria (LAB) have been used in food manufacturing for centuries, due to their safety, potential technological properties, and positive effect on shelf life.[1,2] Before microorganisms were known and isolated, spontaneous lactic fermentation was applied to preserve food and obtain products with desired sensory properties.[3] Proteolysis is one of the physiological properties of LAB that has attained important knowledge. The proteolytic system of lactic acid bacteria is related to the casein use and supplies the cells with essential amino acids during their growth in milk. Lactic acid bacteria and their proteolytic system contribute to the pleasant organoleptic properties of fermented milk products such as yogurt, cheese, and butter.[4,5] This system comprises proteinases, peptidases, and specific transport proteins. Proteinases cleave casein into peptides, then peptidases (intracellular) degrade peptides into amino acids and smaller peptides. Transport systems transfer amino acids and peptides across the cytoplasmic membrane.[6] Lactic acid bacteria can also produce lactic acid, an organic compound produced via fermentation of different carbohydrate sources. It provides leading roles in the food industry. It is used as a food preservative, fermentation agent, acidulant, flavor enhancer and decontaminant, antioxidant, probiotic activity, and cryoprotectant.[7,8,9]

Both proteolysis and lactic acid production vary according to bacterial species and environmental conditions. Therefore, to ensure the survival of lactic acid bacteria and achieve their full potential, the adjustment of optimal physico-chemical conditions must be considered. Consequently, there has been a growing interest in using mathematical models to describe the growth and metabolism of LAB in response to environmental conditions. Predictive microbiology makes use of both primary and secondary models. These models can be used to optimize industrial processes that anticipate the use of technological strains by utilizing validated mathematical models that have received preliminary consensus from the International Organization for Standardization.[10] Primary models describe how the microbial population changes over time. Secondary models describe how the primary model parameters respond to changes in environmental conditions.[11] Square root models are the most commonly used secondary models for describing the effects of physicochemical conditions on microorganism growth and metabolism. Many studies have looked at the individual effects of these physicochemical factors on LAB's metabolic pathways, but little is known about their combined effects. In this study the combined effects of three physicochemical factors (temperature, pH, and NaCl concentration) on two technological and functional properties (proteolysis and acidification) of two strains of lactic acid bacteria:

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Lactococcus lactis (LCL) and *Enterococcus faecium* (CHT4) have been investigated. A multifactorial approach was used to assess the individual effects of the three factors, as well as their interactions on the two activities to optimize them simultaneously. Isoresponse contour plots, based on statistical principles, have been generated; they provide a two-dimensional view that shows the areas of optimal variation of each activity as a function of the factor combinations (T-pH), (T, NaCl), and (pH-NaCl). These areas of interest represent the maxima of these responses and define the experimental domain in which the relevant conditions will result in maximum proteolysis and lactic acid production by both stains. Second-order mathematical models describing responses as a function of the most influential factors have been developed. Considering the importance of LAB in food production and their potential health-promoting impact, knowledge of the effects of environmental factors can lead to optimizing food production and obtaining LAB strains with the greatest potential as starter cultures because they have optimized fermentation characteristics. Hence the study aimed to propose a mathematical approach for modeling and optimizing the two responses which will provide large-scale phenomics data for both strains, as requested by the dairy industry to expand their product portfolio.

Materials and Methods

Bacterial strains, media, and growth conditions

Two strains of lactic acid bacteria from the culture collection of the Laboratory of Biology of Microorganisms and Biotechnology (LBMB), University of Oran 1, Ahmed Ben-Bella, Oran, Algeria, were used: *Lactococcus lactis* (LCL) strain (INRA collection, Jouy en Josas, France) and *Enterococcus faecium* (CHT4) strain isolated from camel milk (Timimoun, Southern Algeria). Reactivation of both strains was carried out in M17 broth[12] for the LCL strain and in MRS broth[13] for the CHT4 strain from stored cultures, which were kept at -20°C with 80% (v/v) glycerol(MP Biomedicals™ Glycerol, 80% sterile solution). After 12 h of growth at 30°C, the strains were used for further work. Sterilized skimmed milk inoculated with 1% of each precultured strain was used to culture the strains and assess their proteolytic and acidifying activities.

Experimental design

The activities of the two strains were monitored with three different variables: incubation temperature ranging from 16 to 44 °C, environmental pH ranging from 5.4 to 7, and sodium chloride concentration ranging from 2 to 4% and 2 to 6% for the LCL and CHT4 strains, respectively. Both strains were grown at different values of the three variables using a Central Composite Design (CCD) of 17 runs with three factors varying to five levels.[14]

Table 1 presents temperature, pH, and NaCl concentration values, both coded and real, from the 17 runs of the CCD. The latter was divided into three blocks. The first block is a 2^k factorial design that combines two-level factors, denoted as +1 and -1. The second block consists of the 2k axial points noted as -α and +α. The final block includes points at the center of the experimental domain noted as 0 and repeated n_0 times to estimate the repeatability variance. There are a total of ($2^k + 2k + n_0$) combinations, where k is the number of factors (k = 3), and n_0 is the number of repetitions in the experimental field ($n_0 = 3$). Various factors were assigned at each level to facilitate the statistical analysis: $-\alpha, -1, 0, +1, +\alpha$, where ($\alpha = 2$) (Table 2).

Table 1: Central Composite presenting the real and coded values of the factors

Blocks	Runs	Coded values			Real values			
		x_1	x_2	x_3	Temperature (°C)	pH	NaCl (%)	
							LCL	CHT4
1	1	-1	-1	-1	23	5.8	2.5	3
	2	+1	-1	-1	37	5.8	2.5	3
	3	-1	+1	-1	23	6.6	2.5	3
	4	+1	+1	-1	37	6.6	2.5	3
	5	-1	-1	+1	23	5.8	3.5	5
	6	+1	-1	+1	37	5.8	3.5	5
	7	-1	+1	+1	23	6.6	3.5	5
	8	+1	+1	+1	37	6.6	3.5	5
2	9	-2	0	0	16	6.2	3	4
	10	+2	0	0	44	6.2	3	4
	11	0	-2	0	30	5.4	3	4
	12	0	+2	0	30	7	3	4
	13	0	0	-2	30	6.2	2	2
	14	0	0	+2	30	6.2	4	6
3	15	0	0	0	30	6.2	3	4
	16	0	0	0	30	6.2	3	4
	17	0	0	0	30	6.2	3	4

x_1: temperature (°C), x_2: Hydrogen potential , x_3: sodium chloride concentration

Table 2: Correlation between coded and real factor levels

Factors	Levels (coded and real)				
	-2	-1	0	+1	+2
Temperature (°C)	16	23	30	37	44
pH	5.4	5.8	6.2	6.6	7
NaCl (%)	2	2.5	3	3.5	4

Proteolytic activity
The proteolytic activity of both strains (expressed as mg tyrosine eq/ l) was measured in skimmed milk after 24 h of incubation for the 17 runs of the experimental design. A spectrophotometer (UV/Vis SP-200, COLE-PARMER®) at 750 nm was used to measure absorbance at regular intervals of 1 h using the Folin reagent (1.9-2.1 N, density:1,24 g/cm^3 at 20 °C), according to the method described by Lowry *et al*.[15]

Acidifying activity
The Dornic acidity (1°D = 0.1 g lactic acid/liter) of both strains was determined in skimmed milk after 24 h of incubation for the 17 runs of the CCD using the method described by Accolas *et al*.[16] which involved titrating 10 ml of culture samples with NaOH (1/9N) in the presence of phenolphthalein indicator (1% in alcohol) at regular 1h intervals.

Statistical analysis
The responses generated in each CCD run for the dependent variables (proteolysis and acidification) were fitted by a second-order model to the independent variables (temperature, pH, and NaCl concentration) using the following quadratic polynomial equation (1):

$$y = \beta 0 + \beta 1x1 + \beta 2x2 + \beta 3x3 + \beta 11x12 + \beta 22x22 + \beta 33x32 + \beta 12x1x2 + \beta 13x1x3 + \beta 23x2x3 \quad (1)$$

Where y is the dependent variable to be modeled, β_0 is the constant, β_1, β_2, and β_3 are the linear coefficients; β_{11}, β_{22}, and β_{33} are quadratic coefficients; β_{12}, β_{13}, and β_{23} are the interaction coefficients; and x_1, x_2, x_3 are the independent variables.
In this study, all experimental data were analyzed using STATISTICA ® version 10 statistical software (StatSoft, Inc. (2011) USA). This software uses equation1 to perform all the calculations and plot the following two types of graphs. Pareto diagram, which is a bar diagram showing graphically the absolute values of linear, quadratic, and interaction factors' effects, ranked from the highest to the lowest. It also includes a reference line to indicate statistically significant effects, the bars of effects that cross the reference line are statistically significant at the 0.05 level. Isoresponse contour plots, which are a projection of the three-dimensional response surface in the horizontal plane; were used to graphically relate the response y to two factors simultaneously, the third factor was fixed at a constant level. These representations show the optima of the experimental design according to the factor combinations (T-pH), (T, NaCl), and (pH-NaCl) and define the experimental domain in which the relevant conditions will result in maximum proteolysis and lactic acid production by both stains.
As well as the graphical analysis, a statistical analysis was carried out to assess the effects of the three physicochemical factors on the studied responses, the P-value was calculated and compared to a previously defined "α" significance level (typically 5%). Factors with a significance level of less than 95% ($P>0.05$) must be excluded from the final models. These were generated through regression analysis. The established models' validity and robustness were assessed using the following indices: coefficient of determination (R^2) and mean squared error (MSE). The lower the MSE, the more accurately the model describes the data.[17] The two indices were obtained through an analysis of variance (ANOVA).

Results and discussion

Reactivation of bacterial strains
The two strains of lactic acid bacteria, namely *Lactococcus lactis* (LCL) and *Enterococcus faecium* (CHT4), were first reactivated from stored cultures in M17 and MRS broth medium, respectively. The strain's growth was evidenced by the development of turbidity. Precultured strains were then inoculated into sterile skimmed milk to evaluate their proteolytic and acidifying activity. Growth in this medium was indicated by coagulation. Results are shown in Figure 1.
Table 3 summarizes the results of the two strains' proteolysis and acidification after 24 h of incubation for the 17 CCD runs.

Proteolytic activity
The results indicate that both strains could release tyrosine during the incubation period. Tyrosine results from the protease enzyme's activity during the degradation of protein substrates found in the medium. Various environmental factors, for each strain, appear to influence this protease activity. The Pareto diagram (Figure 2) depicts the effects of the three factors on the proteolytic activity of the lactococcal strain LCL. At the chosen confidence level ($P<0.05$), temperature had a positive linear effect on proteolysis. Furthermore, as shown by the isoresponse contour plot (Figure 3) relating to (T-pH) interaction, the increase in proteolytic activity is temperature dependent, with the highest activity (16 mg tyrosine eq/l) obtained at an incubation temperature of about 40°C to 45°C. The effects of pH and NaCl concentration appear to be minimal (at least within the range of values considered in this study).

Table 3: Results of proteolysis and acidification of both strains, for the 17 runs of the CCD.

Runs	LCL		CHT4	
	Proteolysis (mg tyrosine eq/ l)	Acidification (°D)	Proteolysis (mg tyrosine eq/ l)	Acidification (°D)
1	8.32	71.5	9.98	35.64
2	11.64	72.25	10.6	47.5
3	9.64	71.98	12.64	36.6
4	12.64	72.2	12.9	52.47
5	9.32	57.42	9.98	35.66
6	11.32	70.25	10.64	47.52
7	8.98	57.42	12.6	36.63
8	13.98	70.35	12.98	52.4
9	7.98	63.36	10.98	27.74
10	13.98	70.5	11.64	59.4
11	11.64	66.33	8.64	37.62
12	11.2	70.29	13.8	46.68
13	10.31	72.3	11.3	45.98
14	10.64	54.45	11.4	45.9
15	11.32	69.3	11.32	46.1
16	11.3	68.9	11.4	45.85
17	11.4	69.4	11.36	45.9

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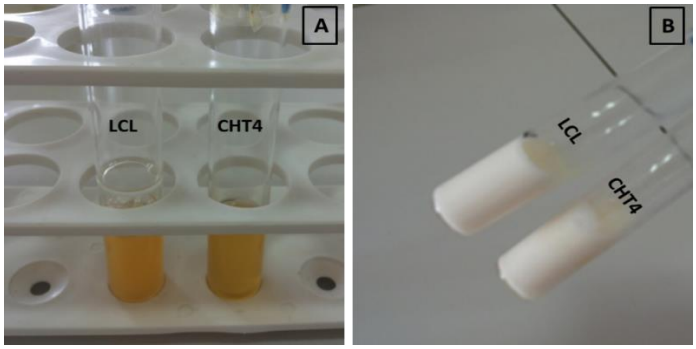

Figure 1: Precultures of strains in M17 and MRS broths (A); in skimmed milk medium (B)

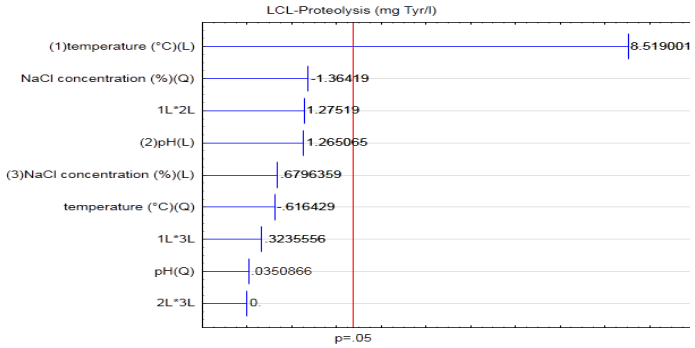

Figure 2: Pareto diagram showing the effects of variables on LCL strain proteolysis

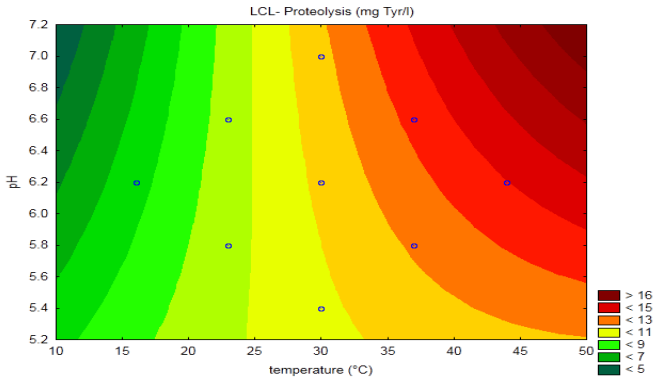

Figure 3: Isoresponse contour plot showing the effect of the interaction [T-pH] on LCL strain proteolysis

F□□□□□ □: Response surfaces for the growth rate (□, h
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andtemperature and Na-lactate (c

The regression and variance analysis results in Table 4 indicate that temperature had the most significant effect on the evaluated response (3.16500 (x_2)) with a (P-value=0.000061) less than 0.05. The other variables had no significant effects on strain proteolysis and were excluded from the final model. The findings also show that the coefficient of determination was greater than 0.90 (R^2 = 0.91), indicating that the established model is robust; additionally, the lower MSE (0.55212) indicates that the model has fewer errors and makes more precise predictions. Eq. (2) represents the mathematical model resulting from the experimental design that predicts the variation of proteolysis as a function of temperature (x_1), pH (x_2) and NaCl concentration (x_3):

The simplified Eq. (3) represents the final model, which only considers the significant effect of temperature:

$$LCL - Proteolysis = 11.22739 + 3.16500x1 + 0.47000\,x2 + 0.25250x3 - 0.20815x12 + 0.01185x22 - 0.46065x32 + 0.67000x1x2 + 0.17000x1x3 + 0.00000x2x3 \quad (2)$$

In this model, temperature appears to have the greatest positive impact on LCL proteolysis; the highest activity in milk was observed at 40°C. This result is consistent with that of de Giori *et al.*[18] who demonstrated that maximum proteolysis of *Lactococcus lactis* strains was achieved at 45°C. This finding indicates that, under certain conditions, the temperature required for growth is not always the same as that required for the development of a specific enzymatic activity. According to Hugenholtz *et al.*[19] and Monnet *et al.*[20] *Lactococcus lactis* proteolysis is primarily dependent on the presence of extracellular proteases. Its production appears to be subject to a regulatory system that depends not only on the composition of the medium but also on surrounding physicochemical factors such as temperature. The latter, according to Smid *et al.* [21] can activate energy-dependent peptide transport systems. The LCL strain can serve as an industrial starter culture. To achieve maximum proteolysis, this study proposes increasing the incubation temperature to around 40°C. Indeed, the proteolytic activity of *Lactococcus lactis* starter cultures is required for cell growth in milk to produce lactic acid at the required rate for fermented product production. It also influences the rheological and organoleptic properties of cheese during the ripening process. According to Thomas and Pritchard,[22] some industrial starter cultures made up of *L. lactis* strains have good fermentation capability and flavor, implying that they have high economic value.

In contrast to the LCL strain, proteolytic activity of enterococcal strain CHT4 was found to be influenced by a linear term of pH and a linear term of temperature in decreasing order, as shown by the Pareto diagram (Figure 4). The isoresponse contour plot (Figure 5A), which describes the effects of the interaction (T-pH) on this physiological activity, shows that increasing the pH of the medium to about 7 increases the strain's proteolysis and can reach a maximum of about 14 mg tyrosine eq/l. Temperature had a less pronounced effect, though there is a linear increase in strain proteolysis with increasing incubation temperature at around 40°C. These findings support the findings of previous studies,[23,24,25] as pH and temperature can influence enterococci's proteolytic activity. As demonstrated by Van der Zant and Nelson,[26] the proteolytic behavior of *Enterococcus faecium* strain in milk medium could be explained by the presence of an enzymatic system active at pH 6.60 and 45°C.

According to Cowman *et al.* [27] pH variations have a significant impact on enterococci proteolysis because pH changes the hydrogen-ion equilibrium at the active center or alters the active structure of the protease enzyme. As a result, the significant decrease in enzymatic activity observed at low pH may indicate that this factor primarily determines the development of this activity. *Enterococcus faecium* is one of the most common enterococci found in cheese,[28] including raw and pasteurized milk-based cheeses from goats, sheep, and cows.[29] Thus, determining the optimal conditions for improving the strain's proteolysis will make it promising for the ripening of many cheese varieties, milk protein hydrolysis, and the release of amino acids and small peptides, which are precursors to numerous aromatic compounds. Moreover, in this study, proteolysis of the CHT4 strain was independent of NaCl concentration, as shown in the isoresponse contour plot (Figure 5B) describing the effect of the interaction (T-NaCl), where the strain showed a greater capacity to grow at a high concentration of NaCl than the LCL strain. Indeed, this strain has been characterized by a high osmotolerance potential, as demonstrated by several studies, since it was isolated from salt-rich camel milk from southern Algeria.[30] This

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osmotolerance could be a significant advantage for potential CHT4 strain applications in the production of certain cheeses, such as cheddar, which is salted during the ripening process.

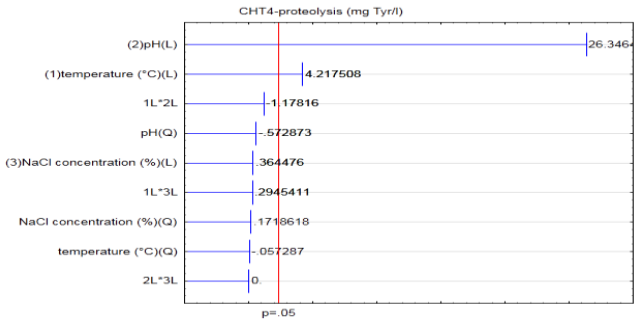

Figure 4: Pareto diagram showing the effects of variables on CHT4 strain proteolysis

The regression analysis presented in Table 5 statistically confirms that only two factors, pH and temperature, had significant effects on strain proteolysis and were taken into account for the establishment of the final model. The latter is given by Eq. (4):

$$CHT4 - proteolysis = 11.44000 + 0.40500[T] + 2.53000[pH] \quad (4)$$

The lower MSE (0.3689) indicates that the model has fewer errors. Furthermore, the coefficient of determination (R^2 = 0.99) indicates that the established model is well-adjusted and robust, explaining a greater proportion (99%) of the total variation.

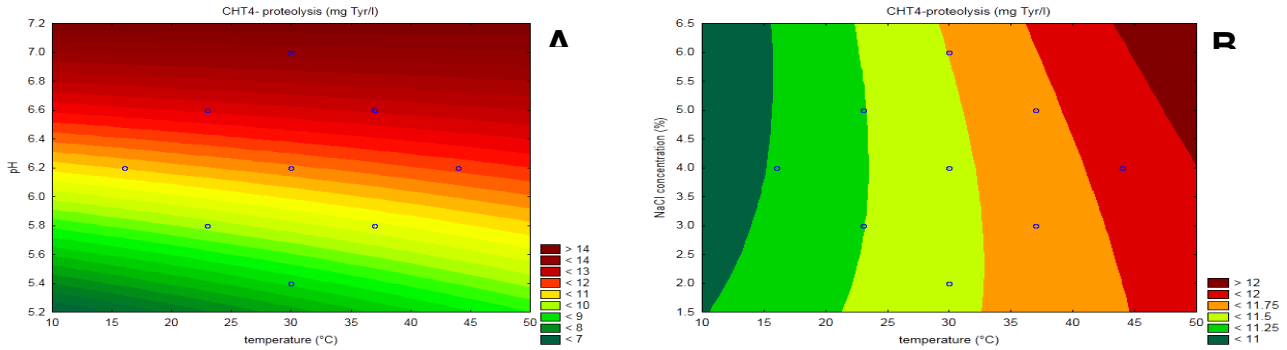

Figure 5: Isoresponse contour plots showing the effect of the interaction [T-pH] (A) and [T-NaCl] (B) on CHT4 strain proteolysis

Table 4: Regression and variance analysis, for LCL strain proteolysis

Factors	Coefficients	*P-value*	MSE [a]	R^2 [b]
Constant	11.22739*	0.000000*	0.55212	0.91
x_1Temperature (°C) (L)	3.16500*	0.000061*		
Temperature (°C) (Q)	-0.20815	0.557118		
x_2 pH (L)	0.47000	0.246332		
pH (Q)	0.01185	0.972990		
x_3 NaCl (%) (L)	0.25250	0.518570		
NaCl (%) (Q)	-0.46065	0.214745		
x_1 x_2	0.67000	0.242931		
x_1 x_3	0.17000	0.755726		
x_2 x_3	0.00000	1.000000		

*: statistically significant (P<0.05)
[a] MSE: mean squared error
[b] R^2: coefficient of determination

Table 5: Regression and variance analysis, for CHT4 strain proteolysis

Factors	Coefficients	*P-value*	MSE [a]	R^2 [b]
Constant	11.44000*	0.000000*	0.3689	0.99
x_1Temperature (°C) (L)	0.40500*	0.003949*		
Temperature (°C) (Q)	-0.00500	0.955917		
x_2 pH (L)	2.53000*	0.000000*		
pH (Q)	-0.05000	0.584655		
x_3 NaCl (%) (L)	0.03500	0.726264		
NaCl (%) (Q)	0.01500	0.868409		
x_1 x_2	-0.16000	0.277227		
x_1 x_3	0.04000	0.776888		
x_2 x_3	0.00000	1.000000		

*: statistically significant (P<0.05)
[a] MSE: mean squared error
[b] R^2: coefficient of determination

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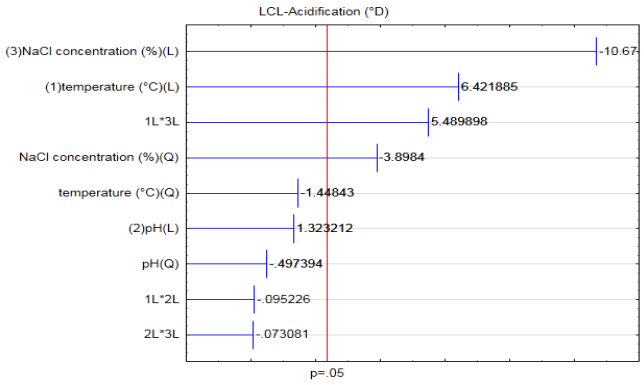

Figure 6: Pareto diagram showing the effects of variables on LCL strain acidifying activity

Acidifying activity

The acidifying activity of the LCL strain appears to be influenced according to the Pareto diagram (Figure 6), specifically by a linear term of NaCl concentration (with a negative sign), a linear term of temperature, the interaction (temperature-NaCl), and the quadratic term of NaCl concentration (NaClxNaCl). These variables had the greatest impact on this response. Lactic acid production increases linearly with increasing temperature and decreasing NaCl concentration, as shown in Figure 7(A) and Figure 7 (B), respectively. The maximum production of this acid (70°D) occurs at an incubation temperature greater than 37°C, combined with a reduced NaCl concentration in the medium of about 2–3%. The effect of pH on this activity was relatively weak although increasing pH causes an increase in acidification.

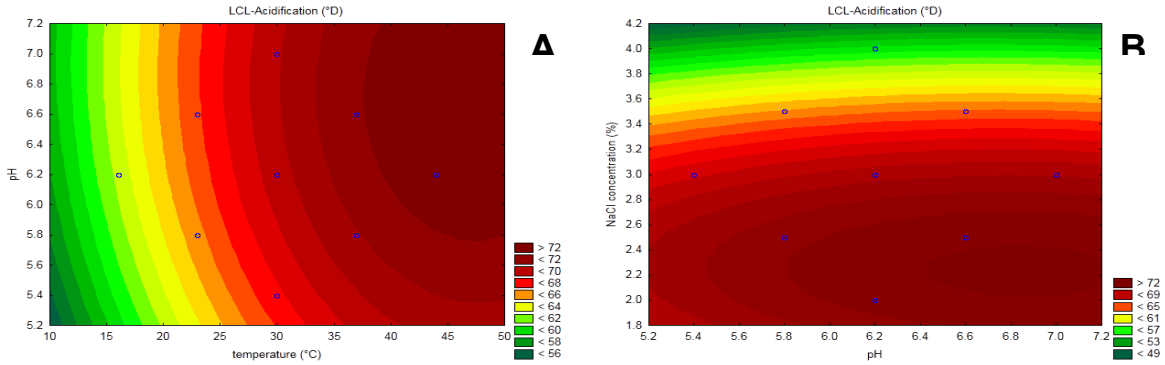

Figure 7: Isoresponse contour plots showing the effect of the interaction [T-pH] (A) and [pH-NaCl] (B) on LCL strain acidifying activity

In general, lactic acid production was very low at high NaCl concentrations, which was exacerbated by decreasing temperature. According to Thomas,[31] a phenomenon of decoupling between energy production and cellular metabolism can occur when the medium contains a high salt concentration. Turner and Thomas,[32] cited temperature and high salt concentration as factors that can inhibit the *Lactococcus lactis* strain's growth and lactic acid production. In fact, in biological systems, temperature and NaCl concentration can have a significant impact on lactic acid production by influencing the rate of biochemical reactions and enzyme activity. These findings are confirmed by the regression analysis shown in Table 6. This analysis identified the three factors with the most significant effects on the response (P<0.05): positive linear and quadratic terms of decreasing salt concentration, increasing temperature, and their interaction. The final model with four significant terms is given by Eq. (5):

$$LCL - acidification = 69.53652 + 5.12625[T] - 8.52375[NaCl] - 2.82837[NaCl]2 + 6.19750\ [T][NaCl] \quad (5)$$

The two indices generated by the analysis of variance, MSE=2.5488, and R^2 = 0.96, show a strong correlation between reality and experimentation. The model is thus well-adjusted and robust. Thus, this study proposes adjusting both temperatures above 37°C and NaCl concentrations between 2 and 3% to maximize lactic acid production by the LCL strain. This will allow the strain to be used as a starter culture, as *Lactococcus lactis* strains are frequently used in the production of cheese, butter, and other fermented kinds of milk, depending on their fermentation performance and the desired properties of the finished product.

In contrast to the LCL strain, the CHT4 strain's lactic acid production appears to be unaffected by salt concentration. Indeed, as illustrated in the Pareto diagram (Figure 8), this response is influenced by the linear terms of temperature and pH. This is illustrated in the isoresponse (Figure 9A), which shows the interaction of temperature and pH, implying that maximum acidification (60°D) was achieved at incubation temperatures above 37°C. Figure 9 (B), which depicts the (pH-NaCl) interaction, demonstrates that adjusting the pH of the medium between 6.2 and 7 can also maximize this response.

The regression analysis is presented in Table 7. It can be seen that temperature has the greatest influence on the production of lactic acid by the CHT4 strain. Linear and quadratic pH effects were also observed. This table also shows that the variance analysis yielded R^2 and MSE values of 0.98 and 2.0167, respectively. The R^2 value indicates that the independent variables accounted for 98% of the results, with only 2% of the variation not explained by the model, confirming its robustness. The final model is given by Eq. (6):

$$CHT4 - acidification = 45.48043 + 14.83500[T] + 3.73750[pH] - 2.01739[pH]2 \quad (6)$$

These findings are in good agreement with those obtained by Morandi *et al.*[33] who found that the metabolic activity of enterococci is influenced by temperature and pH, with the amount of lactic acid produced being higher at 37°C than at 25°C and decreasing similarly with increasing acidity. The positive effect of increasing the incubation temperature on the acidifying activity of the CHT4 strain can be attributed to the activation of transport systems and sugar metabolism,

as well as the stimulation of enzyme activity involved in fermentation pathways and sugar bioconversion to lactic acid. Similarly, adjusting the medium's pH to around 7 is critical to improving this response. These findings will make it possible to suggest adjustments to both factors to increase lactic acid production. The latter is well-known for its use as a preservative, acidifier, or food additive in a variety of products, thanks to its light acidic taste that does not mask natural aromatic flavors. Furthermore, the strain's osmotolerance appears to be advantageous for industrial applications. According to Jensen *et al.*[34] enterococci's salt tolerance and resistance to other environmental factors contribute to the faster development of lactic acid in the early curing stages of cheeses. Cogan *et al.*[35] reported that the rate of acid production is a crucial factor when selecting a starter culture.

Table 6: Regression and variance analysis, for LCL strain acidifying activity

Factors	Coefficients	*P-value*	MSE [a]	R^{2} [b]
Constant	69.53652*	0.000000*	2.5488	0.96
x_1Temperature (°C)(L)	5.12625*	0.000360*		
Temperature (°C) (Q)	-1.05087	0.190763		
x_2 pH (L)	1.05625	0.227347		
pH (Q)	-0.36087	0.634153		
x_3 NaCl (%) (L)	-8.52375*	0.000014*		
NaCl (%) (Q)	-2.82837*	0.005911*		
x_1 x_2	-0.10750	0.926804		
x_1 x_3	6.19750*	0.000916*		
x_2 x_3	-0.08250	0.943786		

*: statistically significant (P<0.05)
[a] MSE: mean squared error
[b] R^2: coefficient of determination

Table 7: Regression and variance analysis, for CHT4 strain acidifying activity

Factors	Coefficients	*P-value*	MSE [a]	R^{2} [b]
Constant	45.48043*	0.000000*	2.0167	0.98
x_1Temperature (°C) (L)	14.83500*	0.000000*		
Temperature (°C) (Q)	-1.30739	0.082421		
x_2 pH (L)	3.73750*	0.001169*		
pH (Q)	-2.01739*	0.016704*		
x_3 NaCl (%) (L)	-0.020000	0.978315		
NaCl (%) (Q)	-0.12239	0.854969		
x_1 x_2	-1.98000	0.089258		
x_1 x_3	-0.02500	0.980833		
x_2 x_3	-0.02000	0.984666		

*: statistically significant (P<0.05)
[a] MSE: mean squared error
[b] R^2: coefficient of determination

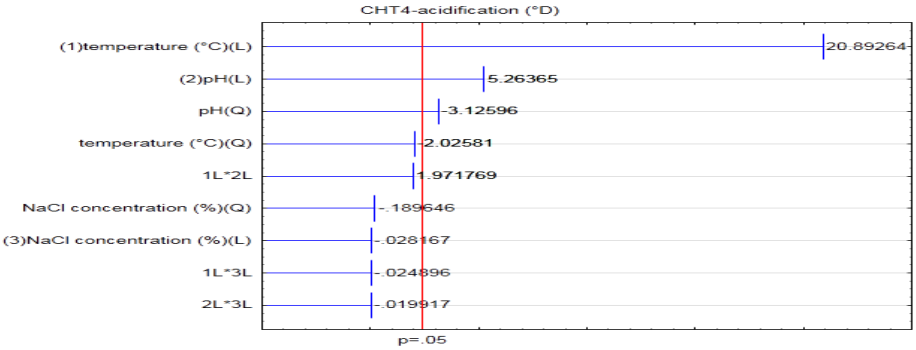

Figure 8: Pareto diagram showing the effects of variables on CHT4 strain acidifying activity

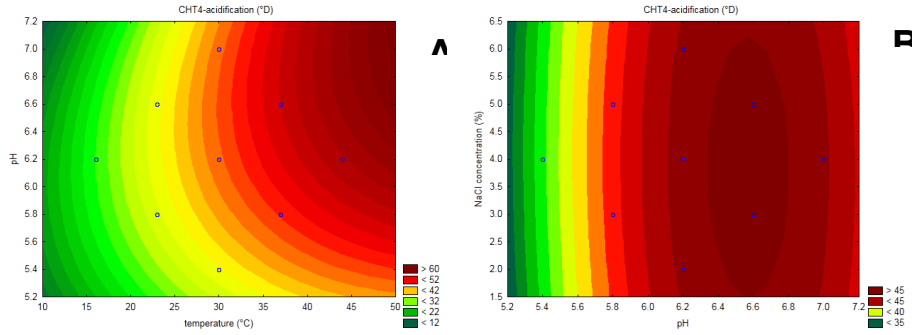

Figure 9: Isoresponse contour plots showing the effect of the interaction [T-pH](A) and [pH-NaCl] (B) on CHT4 strain acidifying activity

Conclusion

Dracaena spicata Roxb. is a potential source of polyphenol-like The present study proposes a mathematical modeling approach to enhance proteolysis and lactic acid production of two strains of lactic acid bacteria. The isoresponse contour plots method showed the ability to adequately represent the responses as a function of the physicochemical factors. The established polynomial models provided high-quality predictions and described the best conditions for achieving higher levels of both activities. This will enable the optimization of fermentation and production processes when both strains are used on an industrial scale. However, these results must be validated on a pre-industrial scale before considering the large-scale use of the two strains.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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