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Valorization of residual yoghurt whey by lactic acid production: An optimized process

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Waste valorization practices have attracted a significant amount of attention in recent years with the aim of managing waste in the most sustainable way. Food waste constitutes a largely under-exploited residue from which a variety of valuable chemicals can be derived. The operation in global yoghurt market provokes frequently the reject and withdrawn of yoghurt derivates out of shelf life. This work comprises a contribution on the valorisation of this high polluting waste of the dairy industry, based on the production of lactic acid using residual yoghurt whey as raw material. Response surface methodology (RSM) based on central composite design (CCD) was used to evaluate the effects of fermentation parameters for lactic acid production by Lactococcus lactis subsp. in batch experiments. The critical factors selected for the investigation were: sugars concentration (X_1), yeast extract concentration (X_2) and inoculum size (X_3). The experimental results were fitted with a second order polynomial equation by a regression analysis and 93% of the variation could be predicted by the model. A maximum lactic acid production (6.80 g/l) was obtained under following optimal conditions: sugars concentration 57 g/l, yeast extract concentration 10.8 g/l and inoculum size 10%. Moreover, the maximum of lactic acid production predicted by the model was 6.61 g/l. These results confirmed the validity of the model, and the experimental values were quite close to the predicted values.

***Keywords*:** Lactococcus lactis subsp. Lactis, whey, lactic acid, design of experiments, fermentation.

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Валоризация остаточной йогуртной сыворотки при производстве молочной кислоты: оптимизированный процесс

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Валоризация отходов при их обработке наиболее устойчивым методом стала более актуальной темой в последние годы. Пищевые отходы, из которых могут быть получены разнообразные ценные химические вещества,

значительно недоиспользуемы. На мировом рынке йогуртной промышленности, йогуртные производные часто неприемлемы и снижают срок годности. В этой работе изучена валоризация этих высоко загрязняющих отходов молочной промышленности на основе производства молочной кислоты с использованием остаточной йогуртной сыворотки в качестве сырья. Методология отклика поверхности (МПО) основанная на конструкции центральной составной (КЦС) были использованы для оценки последствий параметров ферментации для производства молочной кислоты Lactococcus Lactis в пакетных экспериментах. Критические факторы, выбранные для исследования были: концентрация сахара (X_1), концентрация дрожжевой экстракт (X_2) и размер посевной (X_3). Экспериментальные результаты были оснащены полиномиального уравнения второго порядка с помощью регрессионного анализа и 93% вариация могла быть предсказана с помощью модели. Максимальное производство молочной кислоты (6,80 г / л) было получено при следующих оптимальных условий: концентрации сахаров 57 г / л, дрожжевой экстракт концентрации 10,8 г / л и размер инокулята 10%. Кроме того, максимум производства молочной кислоты, предсказанной модели составляет 6,61 г / л. Эти результаты подтвердили применимость модели, и экспериментальные значения были весьма близки к предсказанным значениям.

Ключевые слова: Lactococcus лактис подвид Lactis, сыворотка, молочная кислота, планирование эксперимента, брожение.

Introduction

The out-of-date products are the pet peeve of the bosses of all the grocery stores. Besides reducing the margin of the company, these products arrived in their best-before date classify these business companies among the big producers of biowaste. Biowaste containing large amounts of organic matter like yoghurt is a valuable commodity. The future of yoghurt production, considering the environmental implications of increasing manufacture, involves the treatment of the dairy fermented products out of shelf life. The withdrawn and rejects from market of damaged yoghurts and drinking yoghurts or those over their sell-by date, create an important amount of human foodstuff waste which is unsuitable for sale. Yoghurt is a dairy product sweetened with high levels of conventional added sugars such as sucrose and glucose [1]. However, as far as we know, until now there are only a few systematic reports on residual yoghurt whey fermentation for production of lactic acid by bacteria [2].

Lactic acid is a most important product that has attracted a great deal of attention due to its widespread applications, mainly in food, pharmaceutical, textile, leather, chemical, cosmetic, and polymer industries. Presently, almost all lactic acid produced worldwide comes from the fermentative production [3–6]. Lactic acid production has been studied with various raw materials such as wheat straw [7], wheat bran [8], corn stover [9], starch [10], potato peal waste [11], cashew apple juice [12], and rice bran [13]. However, their potential use is limited by laborious steps such as simultaneous hydrolysis and fermentation with saccharifying enzymes [14].

Yoghurt whey is proposed here to be used as a source for lactic acid fermentation. The present work was, therefore, carried out to optimize the process conditions for efficient sugars conversion in yoghurt whey to lactic acid by *Lactococcus lactis* subsp. *lactis* by employing a central composite design (CCD) of response surface methodology (RSM).

Materials and methods

1. Microorganism

The *Lactococcus lactis* subsp. *lactis* strain, isolated from Algerian raw camel milk, was maintained frozen (in 20% v/v glycerol at — 20 °C). The strain was cultured in the Eliker broth.

2. Yoghurt whey preparation

Yoghurts expired date were previously mixed in order to obtain yoghurt whey by heat treatment at 94 °C for 20 mn. The mixture was centrifuged at 4,000 x g for 20 min at 4 °C and filtration of the supernatant through a 0,45 μm filter. Yoghurt whey was used essentially as the carbon source in the fermentation medium. Immediately prior to each experiment, an appropriate quantity yoghurt whey was diluted to desired concentration of sugars. Table 1 summarizes the characteristics of yoghurt whey used in fermentation experiments.

Table 1

Characteristics of yoghurt whey used in fermentation experiments

Characteristics	Protein concentration (gl^{-1})	Total sugars (gl^{-1})	pH (units)
Raw yoghurt whey	9.4	140	4.2
Yoghurt whey used as medium	1.3	33.18–66.82	6.0

3. Culture and fermentation conditions

The inoculum was prepared by transferring glycerol stock culture (1 ml) to an Erlenmeyer flask containing 100 ml of Elliker medium and incubated at 33.5 °C for 4 h (time to needed for microorganism to reach the exponential growth phase) on a rotary shaker (New Brunswick Scientific) at 200 rpm. Initial pH of medium was ajusted to 6 by adding NaOH (1N).

4. Analytical methods

The content of sugars was determined by using phenol sulphuric acid, as described by Dubois et al. (1956) [15]. The following experiemnts were carried out in triplicate.

Protein content was determined according to Bradford [16] using bovine serum albumin as standard.

The amount of lactic acid in fermentation broth was determined by AOAC methode [17]. One ml of phenolphthalein indicator (0.5% in 5% alcohol) was added to 25 ml of culture broth. This was titrated with 0.1 M NaOH for the appearance of pink colour. The titratable acidity was calculated as

percentage of lactic acid. Each millilitre of 1 N NaOH is equivalent to 90.08 mg of lactic acid.

5. Central Composite Design

The yoghurt whey was considered as the basal medium, and used for optimization by response surface methodology (RSM) using Central Composie Design (CDD). The level of three variables: sugars concentration, yeast extract concentration and inoculum size chosen for this study were optimized by the experimental plan. Design of the experiments was done with Minitab Statistical Software version 16 (Minitab Inc, State College, PA). As shown in Table 2 the three factors were designated as X_1, X_2, X_3 and prescribed into five differents levels (–α, –1, 0, +1, +α). A set of 20 experiments were carried out. All variables were taken at a central coded value considered as zero. The minimum and the maximum ranges of variables were investigated and the full experimentatl plan with respect of their values in actual and coded forme is listed in Table 3. For predicting the optimal point, a second-order polynomial model was fitted to correlate relationship between independent variables and response (lactic acid concentration). For the three factors, the equation is

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \\ + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2, \quad (1)$$

where Y is the predicted response; β_0 is model constant; β_1, β_2 and β_3 linear coefficients; β_{12}, β_{23}, β_{13} interaction coefficients; β_{11}, β_{22}, β_{33} squared coefficients.

The quality of fit of the polynomial model equation was expressed by the coefficient of determination R^2.

Results and discussion

1. Optimization of lactic acid production

The results of CDD experiments for studying the effects of three independent variables: sugars, yeast extract and inoculum size are presented in the Table 4 along with the mean predicted and observed response. A 2^3 factorial central composite experimental design with six axial points and six replications at the center point leading to a total number of 20 experiments was employed for the optimization of the parameters. The agreement between the lactic acid predicted by the model and the experimental data is very strong, with a difference less than 1.

By applying multiple regression analysis to the experimental data, the following second order polynomial equation was found to represent the lactic acid production adequately.

$$Y\,(\text{Lactate g/l}) = 6.54 + 0.30\,X_1 + 0.56\,X_2 + 0.16\,X_3 - \\ -0.25\,X_1^2 - 0.36\,X_2^2 - 0.21\,X_3^2 - 0.03\,X_1X_2 + \\ + 0.03\,X_1X_3 - 0.4\,X_2, \quad (2)$$

where Y is the predicted response and X_1, X_2, X_3 are coded levels of the factors for lactic acid production.

The regression equation (Eq. 2) showed that lactic acid production (Y) is a function of the sugars concentration (X_1); yeast extract concentration (X_2) and inoculum size (X_3).

The goodness of the fit of the model was checked by the determination coefficient (R^2). The coefficient of determination is an important tool in determining the degree of linear correlation of variables in regression analysis. It provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. The coefficient of determination (R^2) was calculated to be 0.93, indicating that 93 % of the variability in the response could be predicted by the model. The closer the R^2 is to 1, the stronger the model and the better it predicts the response [18]. Chauhan and Gupta [19] reported R^2 greater than 75 % as acceptable for fitting a model.

The significance of the fit of the lactic acid model was assessed by performing analysis of variance (ANOVA). A model F-value of 15.10 and a very low probability value (P-value = 0.000) imply significant model fit. The Lack of Fit F-value of 1.17 implies that there is insignificant lack of fit. The Lack of Fit P value of 0.434 implies that there is only 43.4 % chance that the Lack of Fit F-value could occur due to noise (Table 5). The significance of each coefficient was determined by student's t-test and p-values which are listed in Table 6. The larger the magnitude of the t-value and the smaller the p-value, the more significant is the corresponding coefficient [20, 21]. The P-values were used as a tool to check the significance of each coefficient, which also indicates the interaction effects between each independent variable [22]. The regression of all the linear term and quadratic coefficients were significant.

Table 2

Concentration ranges of the three components used in Central Composite Design

Variables	Codes	Coded levels				
		–α	–1	0	+1	+α
Sugars (g/l)	X_1	33.18	40	50	60	66.82
Yeast extract (g/l)	X_2	1.59	5	10	15	18.41
Inoculum size (%)	X_3	1.95	4	7	10	12.04

α = 1,682

Table 3

Experimental plan for optimization of lactic acid production using response surface methodology

Run	Sugars (X_1)		Yeast extract (X_2)		Inoculum size (X_3)	
	Actual	Coded	Actual	Coded	Actual	Coded
1	40	–1	5	–1	4	–1
2	60	+1	5	–1	4	–1
3	40	–1	15	+1	4	–1
4	60	+1	15	+1	4	–1
5	40	–1	5	–1	10	+1
6	60	+1	5	–1	10	+1
7	40	–1	15	+1	10	+1
8	60	+1	15	+1	10	+1
9	33.18	–α	10	0	7	0
10	66.82	+α	10	0	7	0
11	50	0	1.59	–α	7	0
12	50	0	18.41	+α	7	0
13	50	0	10	0	1.954	–α
14	50	0	10	0	12.046	+α
15	50	0	10	0	7	0
16	50	0	10	0	7	0
17	50	0	10	0	7	0
18	50	0	10	0	7	0
19	50	0	10	0	7	0
20	50	0	10	0	7	0

Table 4

Experimental and predicted values of lactic acid production recorded in the experimental set up of RSM

Run	Lactic acid (g/l)		
	Actual	Predicted	Difference
1	4.45	4.29	–0.16
2	4.95	4.88	0.07
3	6.43	6.26	0.17
4	6.93	6.74	0.19
5	5.44	5.35	0.09
6	6.18	6.07	0.11
7	5.94	5.73	0.21
8	6.45	6.33	0.12
9	5.10	5.33	–1.23
10	6.18	6.33	–0.15
11	4.45	4.56	–0.11
12	6.18	6.44	–0.26
13	5.44	5.65	–0.21
14	6.05	6.21	–0.16
15	6.93	6.54	0.39
16	6.68	6.54	0.14
17	6.68	6.54	0.14
18	6.43	6.54	–0.11
19	6.43	6.54	–0.11
20	6.18	6.54	–0.36

Table 5

ANOVA for response surface quadratic model

Source	DF	Sum of square	Mean sum of square	*F*-Value	*P* > *F*
Model	9	10.1342	1.12602	15.10	0.000
Residual (error)	10	0.7455	0.07455	—	—
Lack of Fit	5	0.4018	0.08035	1.17	0.434
Pure Error	5	0.3437	0.06875	—	—
Cor total	19	10.8797	—	—	—

Table 6

Results of regression analysis of the second-order polynomial model for optimization of lactic acid production

Factor	Coefficient estimate	Standard error	*t*-Ratio	*P*-Value
Intercept	6.54	0.111	58,76	0.000
X_1	0.30	0.073	4.030	0.002*
X_2	0.56	0.073	7.57	0.000**
X_3	0.16	0.073	2.25	0.048*
X_1X_2	–0.03	0.096	–0.29	0.77***
X_1X_3	0.03	0.096	0.32	0.75***
X_2X_3	–0.40	0.096	–4.13	0.002*
X_1^2	–0.25	0.071	–3.50	0.006*
X_2^2	–0.36	0.071	–5.09	0.000**
X_3^2	–0.21	0.071	–2,98	0.014*

* Significant at $P < 5\%$.

** Very significant.

*** Not significant.

Values of *P* less than 0.05 indicate the model terms are significant. From the regression model of lactic acid concentration, the model terms X_1, X_2, X_3, X_1^2, X_2^2, X_3^2 were significant with a probability of 95%. The term X_2X_3 was also significant indicating that there was interaction between yeast extract concentration and inoculum size. The results *show the importance* of the yeast extract on lactic acid production. Lactic acid bacteria are known for their fastidious nutritional requirements [23, 24]. Most Lactic acid bacteria species require complex nutrients, including amino acids, peptides, nucleotides, and vitamins, for their growth because they lack many biosynthetic capabilities [25, 26]. Nitrogen is very crucial for cell growth [27], and its optimization would be relevant for lactic acid production [28], which is a growth associated product [29]. The interaction between the terms X_1, X_2 and X_1, X_3, however, had no significant effect on the lactic acid produced during fermentation (Table 6).

2. Validation of the Model

The statistical model was validated by comparing model predicted results with those of repeated experiments carried out at the optimized conditions: sugars concentration 57 g/l (X_1); yeast extract concentration 10.8 g/l (X_2) and inoculum size 10% (X_3). The mean of the results obtained from three replications was close to that predicted by the model (the predict response for lactic acid production was 6.61 g/l and the actual response was 6.80 g/l) thus showing validity.

Conclusion

Yoghurt whey, a waste material, has been revealed as a suitable and direct substrate to lactic acid production by *Lactococcus lactis* subsp. The central composite design and response surface methodology enable the determination of optimal operating conditions for obtaining greater lactic acid production. The validity of the model was proved by fitting the values of the variables in the model equation and by actually carrying out the experiment at those values of the variables. The methodology as a whole proved to be quite adequate for the design and optimization of the process.

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