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Short Communication

Multiple-objective optimization of lactic-fermentation parameters to obtain a functional-beverage candidate

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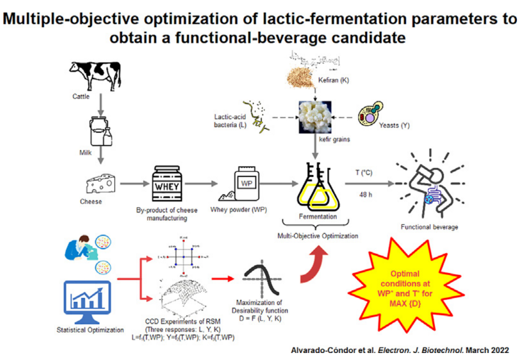
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Whey is the most abundant by-product of the cheese industry. It is estimated that it contains up to 55% of all nutrients of milk and therefore, it is considered a starting material for obtaining valuable products.

Results: The response surface methodology was used to find the combination of temperature (between 20 and 36°C), and the content of whey powder (37.5–77% (m/m)) to maximize the concentration of kefiran, the concentration of lactic acid bacteria (LAB) and yeast in the supernatant. After validating the quadratic models of each transformed response variable, it underwent a maximization procedure to find the optimal condition obtaining two maximum spaces at the temperature range of 28.5–29.7°C and 43.3% (m/m) of whey-powder content, or 28.0–28.3°C and 71.2% (m/m) of whey-powder content. The validation experiments were carried out for the first suggested optimal solution, through three repetitions under the same optimal conditions, and it was confirmed that there is no significant difference with the values provided by the model.

Conclusions: Physicochemical characteristics (protein, fat, acidity, lactose, viscosity, alcoholic content) under optimal conditions were evaluated and proved its compliance with the Ecuadorian and Andean community regulations. These results suggest that we are in the presence of a functional beverage candidate in which the contents of LAB and yeast (probiotics) and kefiran (prebiotic) were simultaneously maximized.

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1. Introduction

In Ecuador, around 31% of milk production is destined to the manufacture of cheeses, and whey is the main by-product obtained from the process. Whey constitutes between 80 and 90% of the total volume of milk used to obtain cheeses and takes with it between 50 and 55% of all nutrients present in milk [1,2].

To reduce the environmental impact that whey produces, numerous strategies have been suggested for its use [3]. Among others, those that allow its use as raw material to produce different products such as: functional foods [4], chemical precursors [5], biofuels [6], pharmaceuticals [7], cosmetics [8], etc. have prevailed.

On the other hand, kefir is a fermented milk drink similar to yoghurt, very popular in Eastern Europe and Asia [9], and that is produced by fermentation of milk with the kefir granule [10], the latter constituted by a symbiotic yeast-bacteria's consortium [11].

The main constituent of this granule is kefiran [12], a polysaccharide formed by bonds of glucose and galactose in approximately equal proportions and whose synthesis is attributed to *Lactobacillus kefiranofaciens* [13,14], one of the LAB probiotics present in the kefir granule [15,16]. Recent studies have shown several beneficial properties of this prebiotic [17,18].

Since it emerged in Japan in the mid-1980s, the concept of functional foods, today accepted, are those foods and beverages that provide consumers with direct health benefits, beyond their recognized nutritional properties [19]. In this sense, a beverage based on cheese whey fermented with kefir grains, a LAB, and yeast consortium, with a widely recognized probiotic effect [20,21,22], and the production by some of them, of certain exopolysaccharides (EPS), such as kefiran, with a recognized prebiotic effect [17,18,23,24], would be a good candidate to be a functional beverage.

Finally, the response surface methodology (RSM) is a popular statistical tool for the design of experiments widely used in industry [25,26]; and that allows optimizing a certain response variable and finding the combination of controllable factors or independent variables, which allows it to be achieved. The central composite design (CCD) is one of the most popular RSM arrangements and is based on distributing the experiments around a central point, distributing the rest of the experiments equidistant around it [27,28].

We aimed to create a functional beverage that is an optimal medium for proper growth of probiotics and prebiotics; such is the case, a culture medium made up of whey and nutrients that benefit their obtaining.

The objective of this research was to develop a fermentation bioprocess for obtaining a functional-beverage candidate from powdered whey and kefir grains, where the concentration of kefiran and the LAB and yeast contents are simultaneously maximized, and to find the values of temperature and content of powdered whey that allow the maximization of probiotics and prebiotics present in the fermentation supernatant.

2. Experimental

Fresh kefir granules from a local supplier (www.kefir.ec) were used. The granules were kept in fresh pasteurized milk at 4–8°C, changing it every two days. In each experiment, 100 g of culture medium was inoculated with 3.73% (m/m) of kefir granules as reported by others [29]. Before being inoculated, kefir granules were washed with abundant distilled water.

In all culture media, dissolved solids were maintained at 14% Brix, similarly as reported in other studies [30].

The amounts of whey powder (WP) used were from 37.5 to 77% (m/m) according to the conditions of the design of experiments [31]. To maintain 14% Brix in each variant, defined amounts of glucose at 77% (m/v) were added, as recommended elsewhere [30,32,33] for a duration of the fermentation process of about 48 h.

Additionally, the medium was supplemented with a 10X salt solution formed by 1% (m/v) of KH_2PO_4, 5% (m/v) $MgSO_4$ and 1% (m/v) $(NH_4)_2SO_4$.

All experiments were adjusted to pH 6.8 (near to the fresh milk pH), using 98% (v/v) H_2SO_4 or 0.1 M NaOH, as necessary; and lasted 48 h on an oscillating shaker at 100 rpm and controlling the temperature between 20 and 36°C, according to the values suggested by the CCD of experiments.

The response surface methodology was used to determine the effect of the independent variables of temperature and composition of whey powder in the medium, using the statistical package Design Expert 13 (Stat-Easy, Inc. Minneapolis, USA).

For the determination of kefiran in the samples, the phenol–sulfuric acid spectrophotometric method [34] was used (using a wavelength of 485 nm), employing glucose as a reference substance.

For the counting of lactic acid bacteria and yeasts, disposable plates were used with Man, Rugosa and Sharpe (MRS) media, and yeast extract-potato-dextrose agar (YPD), respectively. All plates were incubated at 30°C for 48 h and results expressed in (CFU/ml).

3. Results and discussion

The actual values of each transformed dependent response were fitted to quadratic models. Quadratic models were used for the concentration of kefiran, the concentration of LAB and yeasts, in which each response variable was transformed to a natural logarithm, in order to maintain the orthogonality of the models.

The second order statistical models used to experimentally estimate the transformed responses of concentration of kefiran, the concentration of LAB, and yeast during fermentation in terms of coded variables were:

$$(K + 10.00)^2 = 37763.49 - 5353.99 \cdot X_1 - 4148.32 \cdot X_2 - 6906.86 \cdot {X_1}^2 + 5282.45 \cdot {X_2}^2 \quad (1)$$

$$\ln(B + 10.00) = 19.58 + 1.01 \cdot X_1 + 0.3001 \cdot X_2 - 0.5773 \cdot X_1 X_2 - 0.7915 \cdot {X_1}^2 \quad (2)$$

$$\ln(Y + 10.00) = 19.23 + 0.3566 \cdot X_1 - 0.0039 \cdot X_2 - 0.8634 \cdot {X_1}^2 + 0.5207 \cdot {X_2}^2 \quad (3)$$

All terms of equations (1,2, and 3) are statistically significant (p value < 0.05), except those that were included to maintain the hierarchy of the chosen model.

The values of the response variables obtained, as well as their corresponding real value, show an adequate correspondence with the experimental values measured, the maximum relative error of these being 2, 7, and 1%, for the concentration of kefiran, LAB, and yeasts, respectively (Table 1).

All models were statistically significant (p value < 0.0001) and, therefore, are suitable to explore, within the experimental space, possible maximum values for the concentrations of kefiran, LAB and yeast.

Numerical optimization was carried out, looking for the conditions of temperature and content of powdered whey (those factors are labelled with a "*"), with which the simultaneously maximum values of the transformed responses of kefiran, LAB, and yeasts quadratic experimental models are reached with the maximum possible importance level (level 5), and eight possible solutions' findings were obtained, where two groups of optimal solutions are distinguished. The first four solutions are found between the temperatures of 28.5–29.7°C and a value of WP* = 43.3% (m/m) with a desirability around 0.82–0.83, and a second group whose optimal temperatures are between 28.0 and 28.3°C, for a WP* = 71.2% (m/m) with a desirability of 0.78.

In Fig. 1, two of the possible eight optimal solutions and their relationships to the responses are represented, including the values of its desirability function.

The relationship between the models obtained for kefiran concentration versus the yeast and LAB concentrations is interesting, under optimal conditions, observing an increase in kefiran concentration with increasing yeast concentration, and a decrease with increasing LAB concentration.

Other authors have observed this fact and the increase in the production of kefiran by *Lactobacillus kefiranofaciens* is associated with the presence and increase in the concentration of yeast in the medium [35,36], possibly associated with yeast metabolites that stimulate this bacterium to produce this polysaccharide, not able to be metabolized by yeasts. While the decrease in the concentration of kefiran, with the increase in the concentration of LAB, seems to be associated with the role of kefiran, as a possible reserve polysaccharide, in the event of adverse conditions that may exist in the culture

Table 1
Results of the CCD of experiments. The independent variables (X_1: Temperature and X_2: WP) and the real responses and the values obtained by the model of the dependent variables.

	Coded Variables		Actual Variables		Kefiran (*K*) mg/ml		LAB (*B*) $\times 10^8$ CFU/ml		Yeast (*Y*) $\times 10^8$ CFU/ml	
Run	X_1	X_2	Temp °C	WP, % (m/m)	Model	Actual	Model	Actual	Model	Actual
1	−1.414	0.000	20.0	57.3	167.55	167.54	0.16	0.16	0.24	0.24
2	−1.000	−1.000	22.3	43.3	203.64	203.64	0.22	0.21	1.12	1.12
3	−1.000	+1.000	22.3	71.2	183.25	183.25	1.27	1.27	1.11	1.11
4	0.000	0.000	28.0	57.3	184.33	185.13	3.19	3.09	2.25	2.25
5	0.000	+1.414	28.0	77.0	196.06	196.06	4.87	4.89	6.33	6.31
6	0.000	0.000	28.0	57.3	184.33	187.39	3.19	3.19	2.25	2.27
7	0.000	0.000	28.0	57.3	184.33	180.21	3.19	3.07	2.25	2.15
8	0.000	0.000	28.0	57.3	184.33	183.79	3.19	3.29	2.25	2.22
9	0.000	−1.414	28.0	37.5	222.79	222.80	2.09	2.23	6.40	6.37
10	0.000	0.000	28.0	57.3	184.33	185.05	3.19	3.03	2.25	2.31
11	+1.000	−1.000	33.7	43.3	176.90	176.90	5.23	5.21	2.29	2.28
12	+1.000	+1.000	33.7	71.2	153.21	153.21	3.01	3.13	2.27	2.26
13	+1.414	0.000	36.0	57.3	118.00	117.98	2.73	2.66	0.66	0.66

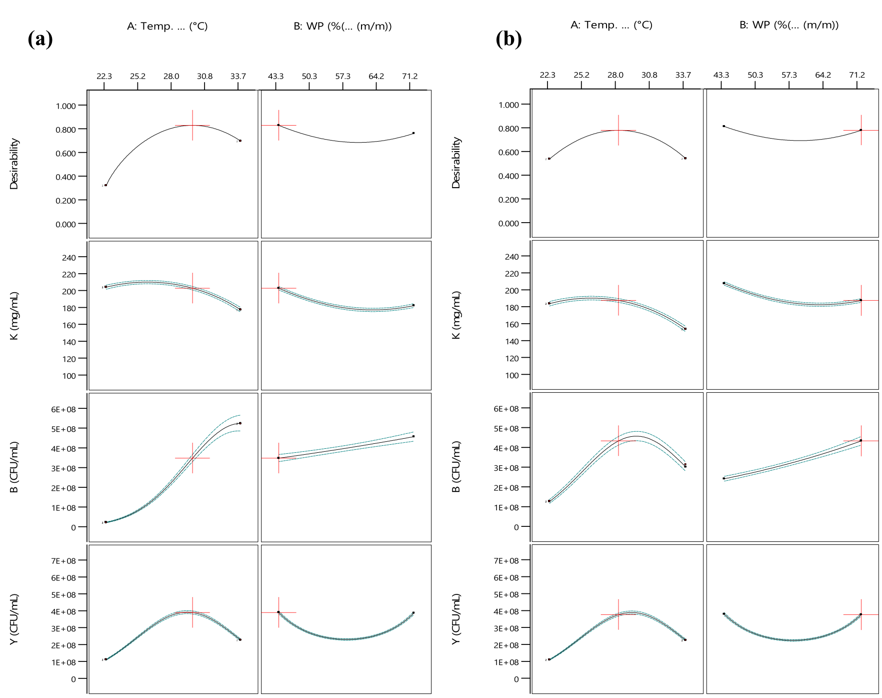

Fig. 1. Representation of two of the solutions found for numerical optimization and their relationship with the independent variables. (a) At 29.6°C and 43.3% (m/m); (b) At 28.1°C and 71.2% (m/m).

medium, these are reflected with a decrease in LAB concentration.

To produce kefiran alone, other authors report optimal temperatures at 24°C [25], at 25°C [37,38], at 30°C [32], and up to 33°C [31], although reports of optimal temperature values are scarce in the context of multi-objective optimization.

To validate the obtained models, three confirmation experiments were carried out under the conditions of the optimal solution (at 29.6°C and 43.3% (m/m)), showing values that are within the ranges predicted by the model (results not shown). Other determinations (fat, protein, ethyl alcohol, lactose, and viscosity content) required by Ecuadorian and Andean normative for fermented milk beverages were also carried out, finding that they were within the accepted values (result not shown).

RSM is a very useful tool to undertake multi-objective optimization studies, as has been corroborated in this work. Later studies could include sensorial analysis to find out other ingredients that should be added to this functional beverage to increase its acceptability by consumers without diminishing its nutraceutical properties.

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