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Lactase production by *Saccharomyces fragilis* IZ 275 using different carbon sources

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ABSTRACT

This study sought to create a better fermentation medium to maximize lactase production by *Saccharomyces fragilis* IZ 275 using different carbon sources, including reconstituted powdered cheese whey. A factorial design 2^4 was applied to evaluate the significant effects of variables which compose the fermentation medium. Then, a steepest descent-ascent design was applied to obtain the maximum activity. A Rotational Central Composite Design (RCCD) 2^4 was made to optimize the fermentation medium. We verified that the cheese whey, a by-product of the dairy industry, can be employed as an excellent fermentation medium by yeast, within the bioeconomy concept and used by the dairy industry as product with additional value. The employed methodology is an efficient tool in the optimization process for β-galactosidase production. In the optimized fermentation medium, the maximum production of β-galactosidase (54.68 U/mL) by *S. fragilis* IZ 275 is obtained with 14 g/L sucrose, 17.7 g/L reconstituted powdered cheese whey, 5.14 g/L yeast extract and 8.85 g/L peptone.

Keywords: β-galactosidase, Cheese whey, environmental damage.

Produção de lactase por *Saccharomyces fragilis* IZ 275 usando diferentes fontes de carbono

RESUMO

O presente trabalho teve como objetivo estudar a melhor composição do meio de fermentação para máxima produção de lactase por *Saccharomyces fragilis* IZ 275 usando diferentes fontes de carbono incluindo o soro de queijo em pó reconstituído. Primeiramente, um delineamento fatorial 2^4 foi aplicado para verificar dentre as variáveis estudadas as que apresentavam efeito significativo. Logo após, foi aplicado um delineamento de máxima inclinação ascendente e máxima inclinação descendente para obter a máxima atividade. Para

otimizar o meio de fermentação, foi então, realizado um delineamento Composto Central Rotacional (DCCR) $2^{4.}$ Foi observado que o soro de queijo, um subproduto da indústria de lacticínios, e altamente poluidor para o meio ambiente, pode ser empregado como excelente meio de fermentação por leveduras, dentro do conceito de bioeconomia com valor agregado. O emprego da metodologia é uma ferramenta eficiente para otimizar o processo de produção da β-galactosidase. No meio de fermentação otimizado, a máxima produção de β-galactosidase (54,68 U/mL) por *S. fragilis* IZ 275 é obtida com 14 g/L sacarose, 17,7 g/L soro de queijo em pó reconstituído, 5,14 g/L extrato de levedura e 8,85 g/L peptona.

Palavras-chave: β-galactosidase, impacto ambiental, soro de queijo.

1. INTRODUCTION

β-D-galactosidase (EC 3.2.1.23; β-D-galactoside galactohydrolase) or lacatase has several applications in the food industry (Jones *et al.*, 2017). It is an intracellular enzyme that hydrolyzes lactose, a disaccharide present in milk and dairy products, into its two monosaccharides units, galactose and glucose, which are easily absorbed in most organisms, including humans (Anisha, 2017; Panesar *et al.*, 2006). Some people are lactose intolerant and cannot digest lactose properly due to an inactive intestinal lactase enzyme. Common symptoms of lactose intolerance are intestinal-dysfunction gas, abdominal pain, and diarrhea. The sugar is found in mammalian milk at a concentration of 3-8% (w/v), and has low solubility and sweetness. (Perini *et al.*, 2013; Cardoso *et al.*, 2017).

One of the main sources for the production of lactase are yeasts (Mlichová and Rosenberg, 2006) by the submerged fermentation process (Anisha, 2017). Several factors influence the production of lactase, such as temperature, pH, incubation time and fermentation medium. The optimization of the fermentation medium is critical for β-galactosidase production (Jones *et al.*, 2017). and some works have described these factors that influence the β-galactosidase production (Karlapudi *et al.*, 2018; Venkateswarulu *et al.*, 2017). However, the best composition of fermentation medium for maximizing the lactase production by yeast has not been yet developed.

Cheese whey produced during cheese-making or during the coagulation of milk casein process presents as principal components, lactose (70–72% of the total solids), whey proteins (8%–10%) and minerals (12–15%) (Yadav *et al.*, 2015). Over the last few decades, the dairy industry has explored different alternatives to exploit the valuable components of cheese whey. The world production of whey (El-Tanboly *et al.*, 2017) is estimated to be around 180 to 190 x 10^6 ton / year, and causes serious socio-economic and environmental problems, since much of this amount is discarded in the environment. Furthermore, Lopes *et al.* (2018) found that, in comparison to domestic sewage, cheese whey can be 100 times more polluting. One alternative to help remediate these problems would be the application of cheese whey as the fermentation medium for lactase production by microorganisms within the concept of circular economy or bioeconomy (Ranta *et al.*, 2018; López-Gómez *et al.*, 2019). This will promote the integration of economic activities and environmental wellbeing in a sustainable way.

Considering these aspects, we studied the creation of a better composition of fermentation medium to maximize lactase production by *Saccharomyces fragilis* IZ 275 using different carbon sources, including reconstituted powdered cheese whey.

2. MATERIAL AND METHODS

2.1. Microorganism and inoculum

Saccharomyces fragilis IZ 275 yeast was used for the study and collected in the Collection of Tropical Cultures (WDCM 69 885 number). The yeast was maintained in tubes containing

PDA (Potato Dextrose Agar, Acumedia) and stored at 4°C, and was reactivated in a medium containing (w/v) malt extract (2%) yeast extract (0.5%) autoclaved at 121°C for 15 min and then incubated at 30°C for 48 h on an orbital shaker (Tecnal®, TE-420). The inoculum was performed by using a Neubauer chamber at a count of 1 x 10^7 cells/mL. An inoculum concentration of 10% v/v was used in relation to the culture medium.

2.2. Extraction and analysis of β-galactosidase

Samples of 80 mL fermentation medium were centrifuged (Eppendorf centrifuge 5804R, Germany) under conditions of 27,200 x *g*, 4°C for 5 min. The supernatant was resuspended in 0.1 M phosphate buffer, pH 6.6, and centrifuged again under the above conditions. The new precipitate was solubilized in 10 mL of the same buffer, to which 0.2 mL of chloroform was added. This mixture was incubated at 37°C under agitation of 150 rpm for 17 h. The suspension was then centrifuged and the supernatant used to determine the enzymatic activity. The enzymatic activity was determined using the o-nitrophenyl-β-D-galactopyranoside substrate (ONPG) following the methodology described in the Food Chemical Codex (National Academy of Sciences, 1996) with some modifications. The ONPG (1.25 mM) substrate dissolved in 0.05 M sodium phosphate buffer (pH 7.0) was used. The amount of substrate and enzyme used were 2 mL and 0.5 mL, respectively. At the time zero, 0.5 mL of enzyme solution was added to the ONPG solution and incubated for 5 min. The assay was stopped by the addition of 0.5 mL sodium carbonate 1 M, and the absorbance was determined in spectrophotometer (Biochrom libra S22 Cambridge England) at 420 nm. One enzymatic unit was defined as the quantity of enzyme that would liberate 1 mM of o-nitrophenol from ONPG per minute under the assay conditions. Enzymatic unit was calculated using the following Equation 1:

$$\text{Unit/mL} = \text{A x dilution factor}/\varepsilon \text{ x time x enzyme solution} \quad (1)$$

Where A was the absorbance at 420 nm, dilution factor was the fold dilution of the enzyme solution, enzyme solution was the amount of enzyme (mL) undergoing the reaction, ε was the extinction coefficient (determined from the o-nitrophenol standard curve) and time was the incubation time (15 min).

2.3. Experiment 1: screening experiments to investigate the composition of medium fermentation

First, initial experiments were performed to evaluate the significant effects of variables which compose the fermentation medium used for β-galactosidase production by *Saccharomyces fragilis* IZ 275 yeast. A factorial design 2^4 was applied with eight variables and three replicates at the central point totaling 19 assays. The coded independent variables (x_1, x_2, x_3, x_4, x_5, x_6, x_7 and x_8) and uncoded variables (X_1 = g/L lactose, X_2 = g/L sucrose, X_3 = g/L glucose, X_4 = g/L cheese whey, X_5 = g/L yeast extract, X_6 = g/L peptone, X_7 = g/L $MgSO_4$ and X_8 = g/L K_2HPO_4) are shown in Table 1 with their variation levels.

The fermentation media were run in 250 mL Erlenmeyer flasks containing 100 mL of each, incubated on the orbital shaker (Tecnal, TE-420) at 180 rpm and with pH initial 6.8, at 30°C temperature for 72 h according to the literature (Kumar *et al.*, 2012). The β-galactosidase production was evaluated in terms by β-galactosidase activity and its response function as follows: Y_1 (β-galactosidase activity, U/mL). The model equation was as follows: $Y_1 = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \beta_5x_5 + \beta_6x_6 + \beta_7x_7 + \beta_8x_8 + e$

Where Y_1 (response function), x_1, x_2, x_3, x_4, x_5, x_6, x_7 and x_8 (coded variables), β (estimated coefficients for each term of the response surface model). The response functions (Y_1) were used to perform regression analyses and analysis of variance (ANOVA) for the regression and were performed using STATISTICA 7.0 software (StatSoft Inc., 2007).

Table 1. Independent variables and levels of variation in factorial design 2^4.

Independent Variables	Levels		
	-1	**0**	**1**
Lactose (g/L) X_1	1	5.5	10
Sucrose (g/L) X_2	1	5.5	10
Glucose (g/L) X_3	1	5.5	10
Cheese whey (g/L) X_4	1	5.5	10
Yeast extract (g/L) X_5	0.5	2.75	5
Peptone (g/L) X_6	0.5	2.75	5
$MgSO_4$ (g/L) X_7	0.5	2.75	5
K_2HPO_4 (g/L) X_8	0.5	2.75	5

2.4. Experiment 2: Method of steepest ascent-descent design to investigate the maximum increase of β-galactosidase activity

From the results of factorial design and to obtain the maximum increase of β-galactosidase activity, a steepest descent-ascent design was applied (Montgomery, 2011). The independent variables were stabilized as follows: sucrose and cheese whey ranged from 6 to 16 g/L; yeast extract, peptone and $MgSO_4$ ranged from 2 to 8 g/L; the variables lactose and glucose ranged from null to 10 g/L and K_2PHO_4 ranged from null to 5 g/L (Table 2).

2.5. Experiment 3: Rotational Central Composite Design (RCCD) 2^4 to optimize the medium fermentation to β-galactosidase production and model validation

From the results of steepest ascent-descent design and to optimize the fermentation medium for β-galactosidase production, a third experiment was performed. In this third step, a Rotational Central Composite Design (RCCD) 2^4 was applied with two central points and eight axial points, for orthogonal, totaling 26 assays. The coded independent variables (x_1, x_2, x_3, x_4) and uncoded variables (X_1 = sucrose g/L, X_2 = cheese whey g/L, X_3 = yeast extract g/L and X_4 = peptone g/L) are shown in Table 3 with their variation levels. The coded independent variables (x_5, x_6, x_7, x_8) and uncoded variables (X_5 = lactose g/L, X_6 = glucose g/L, X_7 = $MgSO_4$ g/L and X_8 = K_2HPO_4 = g/L) that did not show significance in the factorial design were stabilized according to the steepest ascent-descent design (Table 2).

For each assay, the fermentation media were run in 250 mL Erlenmeyer flasks containing 100 mL of each, incubated on the orbital shaker (Tecnal, TE-420) at 180 rpm and with pH initial 6.8 at 30°C temperature for 72 h. β-galactosidase production was evaluated in terms of β-galactosidase activity by its response function as follows: Y_2 (β-galactosidase activity, U/mL). The model equation was as follows: $Y_2 = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 + e$.

Where Y_2 (response function), x_1, x_2, x_3 and x_4 (coded variables), β (estimated coefficients for each term of the response surface model) and e = pure error. The response functions (Y_2) were used to perform regression analyses and analysis of variance (ANOVA) for the regression. The equation model was fitted to experimental data to yield the proposed model. Response surface graphs were generated. All executed analysis and response surfaces were performed with STATISTICA 7.0 software (StatSoft Inc., 2007). After response surface analysis for maximum β-galactosidase activity, the proposed model was validated by performing new assays in triplicate. The results ($Y_{exp.}$) were compared with the estimated response ($y\hat{}_1$) by Student's t-test ($p < 0.05$).

Table 2. Steepest ascent-descent design to the maximum increase in terms of β-galactosidase production.

Assays	Sucrose g/L	Whey Cheese g/L	Yeast extract g/L	Peptone g/L	$MgSO_4$ g/L	Lactose g/L	Glucose g/L	K_2PO_4 g/L	β-galactosidase activity U/mL
1	6	6	2	2	2	10	10	5	17.21
2	8	8	4	4	4	10	10	5	34.84
3	12	12	6	6	6	10	10	5	26.98
4	14	14	7	7	7	10	10	5	39.81
5	16	16	8	8	8	10	10	5	27.03
6	6	6	2	2	2	1	1	0.5	1.57
7	8	8	4	4	4	1	1	0.5	13.51
8	12	12	6	6	6	1	1	0.5	19.61
9	14	14	7	7	7	1	1	0.5	23.36
10	16	16	8	8	8	1	1	0.5	20.84
11	6	6	2	2	2	5.5	5.5	2.75	10.67
12	8	8	4	4	4	5.5	5.5	2.75	18.61
13	12	12	6	6	6	5.5	5.5	2.75	16.25
14	14	14	7	7	7	5.5	5.5	2.75	18.61
15	16	16	8	8	8	5.5	5.5	2.75	13.46
16	6	6	2	2	2	null	null	null	2.46
17	8	8	4	4	4	null	null	null	7.09
18	12	12	6	6	6	null	null	null	13.29
19	14	14	7	7	7	null	null	null	14.12
20	16	16	8	8	8	null	null	null	17.39

Table 3. Independent variables and levels of variation in RCCD 2^4.

Independent Variables	Levels				
	-1.48	-1	0	1	1.48
Sucrose (g/L) X_1	10.29	11.5	14	16.5	17.70
Cheese Whey (g/L) X_2	10.29	11.5	14	16.5	17.70
Yeast Extract (g/L) X_3	5.14	5.75	7	8.25	8.85
Peptone (g/L) X_4	5.14	5.75	7	8.25	8.85

3. RESULTS AND DISCUSSION

3.1. Factorial design 2^4 to investigate the composition of fermentation medium

The first experiment was performed using a factorial design of 2^4 for evaluating the effects of significant variables which compose the fermentation medium of *Saccharomyces fragillis* IZ 275 during β-galactosidase production. According to ANOVA and regression analysis, only the independent variables X_4 (cheese whey) and X_5 (yeast extract) were shown to have a significant effect on the response function Y_1 (β-galactosidase activity, U/mL). None of the other independent variables had a significant effect and the coefficient of determination (R^2) was 0.75. The proposed model could be described as follows:

$Y_1 = 6.23 + 5.58\ x_4 + 8.72x_5$. Thus, the β-galactosidase activity was 20.53 U/mL. The highest β-galactosidase activity (Y_1) was obtained in the assay 16 (Y_1 = 28.42 U/mL) (Table 4) when the independent variables were shown in the maximum levels. This correspond to using X_1 (lactose); X_2 (sucrose); X_3 (glucose) and X_4 (cheese whey) at a concentration of 10 g/L and while using X_5 (yeast extract), X_6 (peptone), X_7 ($MgSO_4$) and X_8 (K_2HPO_4) at 5 g/L concentration. This observation suggested that, although not significant to the model, the investigated variables influenced the β-galactosidase activity. It was decided to make a new regression analysis and ANOVA including only the significant independent variables. Our results confirmed that the response function Y_1 decreased (data not shown), indicating that the other independent variables (x_1, x_2, x_3, x_6, x_7 and x_8) were not in their optimal regions earlier and they were important to explain the model. The complete model can now be described as follows: $Y_1 = 6.23 + 0.21x_1 + 3.23x_2 + 0.94x_3 + 5.58x_4 + 8.72x_5 + 3.81x_6 + 4.70x_7 - 0.55x_8$.

3.2. Method of steepest ascent-descent design to investigate the maximum increase in the β-galactosidase production

According to the results of the steepest ascent-descent design (Table 2), it was observed that assay 4 yielded maximum value for β-galactosidase activity (39.81 U/mL). This corresponds when, the concentration of independent variables sucrose (X_2) and cheese whey (X_4) were 14 g/L, yeast extract (X_5), peptone (X_6) and $MgSO_4$ (X_7) were 7 g/L and lactose (X_1), glucose (X_3) and K_2HPO_4 (X_8) were at a concentration of 10 g/L, 10 g/L and 5 g/L, respectively i.e. when present at their maximum levels. Also, the values of β-galactosidase activity were lower (1.57 U/mL and 2.46 U/mL, respectively) when the levels of independent variables decreased, as shown in assays 6 and 16. The results from the steepest ascent-descent design and from factorial design 2^4 suggest that the non-significant independent variables (lactose, sucrose, glucose, peptone, $MgSO_4$ and K_2HPO_4) contribute to the increase in β-galactosidase production. Therefore, to optimize the composition of fermentation medium for the β-galactosidase production and the model validation, all variables were considered.

3.3. Rotational Central Composite Design (RCCD) 2^4 to optimize the fermentation medium to the β-galactosidase production by Saccharomyces fragilis IZ 275 and model validation

ANOVA and regression analysis were used to evaluate the effects of variables X_1 (sucrose, g/L), X_2 (whey cheese, g/L), X_3 (yeast extract, g/L) and X_4 (peptone, g/L). The linear effects of variables X_1, X_2, X_3 and X_4 were found to be significant. The quadratic effects of variables X_2 and X_3 and the interactions among the variables X_1X_2, X_1X_4 and X_2X_3 were also significant. According to Table 5 (ANOVA), the model showed a lack of fit, which was not significant ($p > 0.05$) and an R^2 of 0.77, indicating that 77% of the experimental results adequately matched the proposed model. Thus, the model can be used for predictive purposes and can be described as follows: $Y_2 = 43.54 + 4.97x_1 + 4.69x_2 - 5.61x_2^2 - 4.96x_3 + 4.55x_3^2 + 2.51x_4 - 2.84x_1x_2 - 5.24x_1x_4 - 2.66x_2x_3$.

Table 4. Factorial Design 2^4 to evaluate the fermentation medium and response function Y_1 (β-galactosidase activity, U/mL).

Assays	$x_1(X_1)$	$x_2(X_2)$	$x_3(X_3)$	$x_4(X_4)$	$x_5(X_5)$	$x_6(X_6)$	$x_7(X_7)$	x_8 (X_8)	Y_1
1	-1(1)	-1(1)	-1(1)	-1(1)	-1(0.5)	-1(0.5)	-1(0.5)	-1(0.5)	1.80
2	1(10)	-1(1)	-1(1)	-1(1)	-1(0.5)	1(5)	1(5)	1(5)	1.01
3	-1(1)	1(10)	-1(1)	-1(1)	1(5)	-1(0.5)	1(5)	1(5)	6.22
4	1(10)	1(10)	-1(1)	-1(1)	1(5)	1(5)	-1(0.5)	-1(0.5)	6.49
5	-1(1)	-1(1)	-1(1)	-1(1)	1(5)	1(5)	1(5)	-1(0.5)	10.63
6	1(10)	-1(1)	1(10)	-1(1)	1(5)	-1(0.5)	-1(0.5)	1(5)	0.20
7	-1(1)	1(10)	1(10)	-1(1)	-1(0.5)	1(5)	-1(0.5)	1(5)	0.71
8	1(10)	1(10)	1(10)	-1(1)	-1(0.5)	-1(0.5)	1(5)	-1(0.5)	2.25
9	-1(1)	-1(1)	-1(1)	1(10)	1(5)	1(5)	-1(0.5)	1(5)	10.74
10	1(10)	-1(1)	-1(1)	1(10)	1(5)	-1(0.5)	1(5)	-1(0.5)	12.51
11	-1(1)	1(10)	-1(1)	1(10)	-1(0.5)	1(5)	1(5)	-1(0.5)	8.21
12	1(10)	1(10)	-1(1)	1(10)	-1(0.5)	-1(0.5)	-1(0.5)	1(5)	0.92
13	-1(1.0)	-1(1)	1(10)	1(10)	-1(0.5)	-1(0.5)	1(5)	1(5)	1.19
14	1(10)	-1(1)	1(10)	1(10)	-1(0.5)	1(5)	-1(0.5)	-1(0.5)	0.66
15	-1(1)	1(10)	1(10)	1(10)	1(5)	-1(0.5)	-1(0.5)	-1(0.5)	11.31
16	1(10)	1(10)	1(10)	1(10)	1(5)	1(5)	1(5)	1(5)	28.42
17	0(5.5)	0(5.5)	0(5.5)	0(5.5)	0(2.75)	0(2.75)	0(2.75)	0(2.75)	7.05
18	0(5.5)	0(5.5)	0(5.5)	0(5.5)	0(2.75)	0(2.75)	0(2.75)	0(2.75)	6.89
19	0(5.5)	0(5.5)	0(5.5)	0(5.5)	0(2.75)	0(2.75)	0(2.75)	0(2.75)	6.93

Considering the complete model, the value of β-galactosidase activity was found to be 32.87 U/mL, and therefore an increase of 62% in the response was obtained.

Table 5. ANOVA for β-galactosidase activity produced by *Saccharomyces fragilis* IZ 275.

Source of variation	SS	DF	MS	F	*p* value*
X_1	126.06	1	126.06	919.43	0.021*
X_1^2	20.46	1	20.46	149.21	0.052
X_2	112.21	1	112.21	818.41	0.222*
X_2^2	76.03	1	76.03	554.53	0.027*
X_3	125.64	1	125.64	916.34	0.021*
X_3^2	50.11	1	50.11	365.49	0.033*
X_4	32.11	1	32.11	234.17	0.042*
X_4^2	0.40	1	0.40	2.94	0.336
X_1X_2	32.18	1	32.18	234.72	0.041*
X_1X_3	7.09	1	7.09	51.68	0.088
X_1X_4	109.68	1	109.68	799.98	0.022*
X_2X_3	28.34	1	28.34	206.72	0.044*
X_2X_4	0.43	1	0.43	3.12	0.328
X_3X_4	0.19	1	0.19	1.39	0.448
Lack of Fit	215.14	10	21.51	156.92	0.062
Pure Error	0.14	1	0.14		
Total SS	936.20	25			

In Table 6, the β-galactosidase activity in assay 21 was found to be greater (52.84 U/mL) during which the conditions were as follows: 14 g/L sucrose, 14 g/L cheese whey, 5.14 g/L yeast extract and 7 g/L peptone. Other variables (lactose, glucose, $MgSO_4$ and K_2HPO_4) were stabilized at maximum levels. Assay 1 showed a lower value for β-galactosidase activity (29.62 U/mL), for which all variables analysed were at a minimum level, except for lactose, glucose, $MgSO_4$ and K_2HPO_4.

After analyzing the mathematical model, response function Y_2 and response surface (Figure 1a), it was observed that there was a region with maximum β-galactosidase activity when x_1(X_1) was -1.48 (10.29 g/L) and x_2 is between -1 and +1 or X_2 was between 11.5 and 16.5 g/L, suggesting that the cheese whey, rich in lactose, promotes the production of enzyme by *Saccharomyces fragilis* IZ 275. In Figure 1b, there is a region in which the β-galactosidase activity is greater when x_1 and x_3 is -1.48 or X_1 is 10.29 g/L and x_3 is 5.14 g/L. Figure 1c indicates two regions with maximum β-galactosidase activity when x_1 is +1.48 or 17.70 g/L and x_4 is -1.48 or 5.14 g/L and the other regions in which x_1 is -1.48 (10.29 g/L) and x_4 +1.48 (8.85 g/L). In Figure 1d, there is a region which the maximum β-galactosidase activity is observed when cheese whey, x_2, ranged from 0 to 1.48, regardless of yeast extract concentration; In Figure 1e, the maximum β-galactosidase activity is observed when the x_2 ranged from 0 (center point) to + 1.48 and in the Figure 1f when peptone, x_4, is 1.48 or X_4 is 8.85 g/L.

An assay in triplicate, which coincided with the points (X_1 = 13.99 g/L; X_2 = 17.7 g/L; X_3 = 5.14 g/L and X_4 = 8.85 g/L), was performed experimentally, and the result was $YM_{exp.}$ = 54.68 U/mL. The results, which did not differ by the t-test ($p > 0.05$), confirm the validity of the proposed model. The results showed that for maximum β-galactosidase production by *Saccharomyces fragilis* IZ 275, the following conditions should be used: X_1 = 14 g/L, X_2 = 17.7 g/L, X_3 = 5.14 g/L, X_4 = 7 g/L, X_5 and X_6 = 10 g/L, X_7 = 7 g/L and X_8 = 5 g/L obtaining the value of 52.84 U/mL β-galactosidase activity.

The data obtained from the present study draws attention towards two points: 1) the variables studied are critical for lactase production by *Saccharomyces fragilis* IZ 275; and, 2) cheese whey, a by-product of the milk and dairy industry, is an important medium for the

growth of yeast. Thus, cheese whey could be re-used in the fermentation industry instead of being discarded as a pollutant as per the concept of the circular economy or bioeconomy (Ranta *et al.*, 2018; López-Gómez *et al.*, 2019). The maximum β-galactosidase activity (28.42 U/mL) obtained in factorial design was observed with 10 g/L of lactose, sucrose, glucose and cheese whey and of variables yeast extract, peptone, $MgSO_4$ and K_2HPO_4 5 g/L. In the steepest ascent-descent design, the maximum β-galactosidase activity obtained (39.81 U/mL) for sucrose and cheese whey were 14 g/L, yeast extract, peptone and $MgSO_4$ were 7 g/L, lactose and glucose were 10 g/L, and for K_2HPO_4 was 5 g/L . In the optimized conditions, the maximum β-galactosidase activity was 52.84 U/mL and obtained with 17.7 cheese whey, 14 g/L of sucrose, 5.14 g/L of yeast extract, 7 g/L peptone and $MgSO_4$, 10 g/L lactose and glucose and 5 g/L K_2HPO_4. The study clearly showed that the Central Rotational Composite Design (CRCC) and Response Surface (RSM) are efficient tools to optimize the composition of fermentation medium and lactase production increased 53.78% after optimization.

Table 6. RDCC to optimize the medium fermentation to the β-galactosidase production by *Saccharomyces fragilis IZ 275* and response function Y_2 (β-galactosidase activity, U/mL).

Assays	(x_1) X_1	(x_2) X_2	(x_3) X_3	(x_4) X_4	Lactose	Glucose	$MgSO_4$	K_2HPO_4	Y_2
1	(-1) 11.5	(-1) 11.5	(-1) 5.75	(-1) 5.75	10	10	7	5	29.62
2	(-1) 11.5	(-1) 11.5	(-1) 5.75	(1) 8.25	10	10	7	5	41.06
3	(-1) 11.5	(-1) 11.5	(1) 8.25	(-1) 5.75	10	10	7	5	33.29
4	(-1) 11.5	(-1) 11.5	(1) 8.25	(1) 8.25	10	10	7	5	35.73
5	(-1) 11.5	(1) 16.5	(-1) 5.75	(-1) 5.75	10	10	7	5	42.89
6	(-1) 11.5	(1) 16.5	(-1) 5.75	(1) 8.25	10	10	7	5	51.97
7	(-1) 11.5	(1) 16.5	(1) 8.25	(-1) 5.75	10	10	7	5	35.47
8	(-1) 11.5	(1) 16.5	(1) 8.25	(1) 8.25	10	10	7	5	35.91
9	(1) 16.5	(-1) 11.5	(-1) 5.75	(-1) 5.75	10	10	7	5	51.09
10	(1) 16.5	(-1) 11.5	(-1) 5.75	(1) 8.25	10	10	7	5	39.92
11	(1) 16.5	(-1) 11.5	(1) 8.25	(-1) 5.75	10	10	7	5	41.23
12	(1) 16.5	(-1) 11.5	(1) 8.25	(1) 8.25	10	10	7	5	42.28
13	(1) 16.5	(1) 16.5	(-1) 5.75	(-1) 5.75	10	10	7	5	49.35
14	(1) 16.5	(1) 16.5	(-1) 5.75	(1) 8.25	10	10	7	5	43.33
15	(1) 16.5	(1) 16.5	(1) 8.25	(-1) 5.75	10	10	7	5	44.02
16	(1) 16.5	(1) 16.5	(1) 8.25	(1) 8.25	10	10	7	5	41.67
17	(-1.48) 10.29	(0)14	(0) 7	(0) 7	10	10	7	5	39.66
18	(1.48) 17.70	(0)14	(0) 7	(0) 7	10	10	7	5	42.19
19	(0) 14	(-1.48) 10.29	(0) 7	(0) 7	10	10	7	5	32.07
20	(0) 14	(1.48) 17.70	(0) 7	(0) 7	10	10	7	5	43.85
21	(0) 14	(0) 14	(-1.48) 5.14	(0) 7	10	10	7	5	52.84
22	(0) 14	(0) 14	(1.48) 8.85	(0) 7	10	10	7	5	45.42
23	(0) 14	(0) 14	0	(-1.48) 5.14	10	10	7	5	36.69
24	(0)14	(0) 14	(0)7	(1.48) 8.85	10	10	7	5	50.66
25	(0) 14	(0) 14	(0)7	(0) 7	10	10	7	5	42.19
26	(0) 14	(0) 14	(0)7	(0) 7	10	10	7	5	42.72

X_1 (sucrose, g/L); X_2 (cheese whey, g/L), X_3 (yeast extract, g/L), X_4 (peptone, g/L).

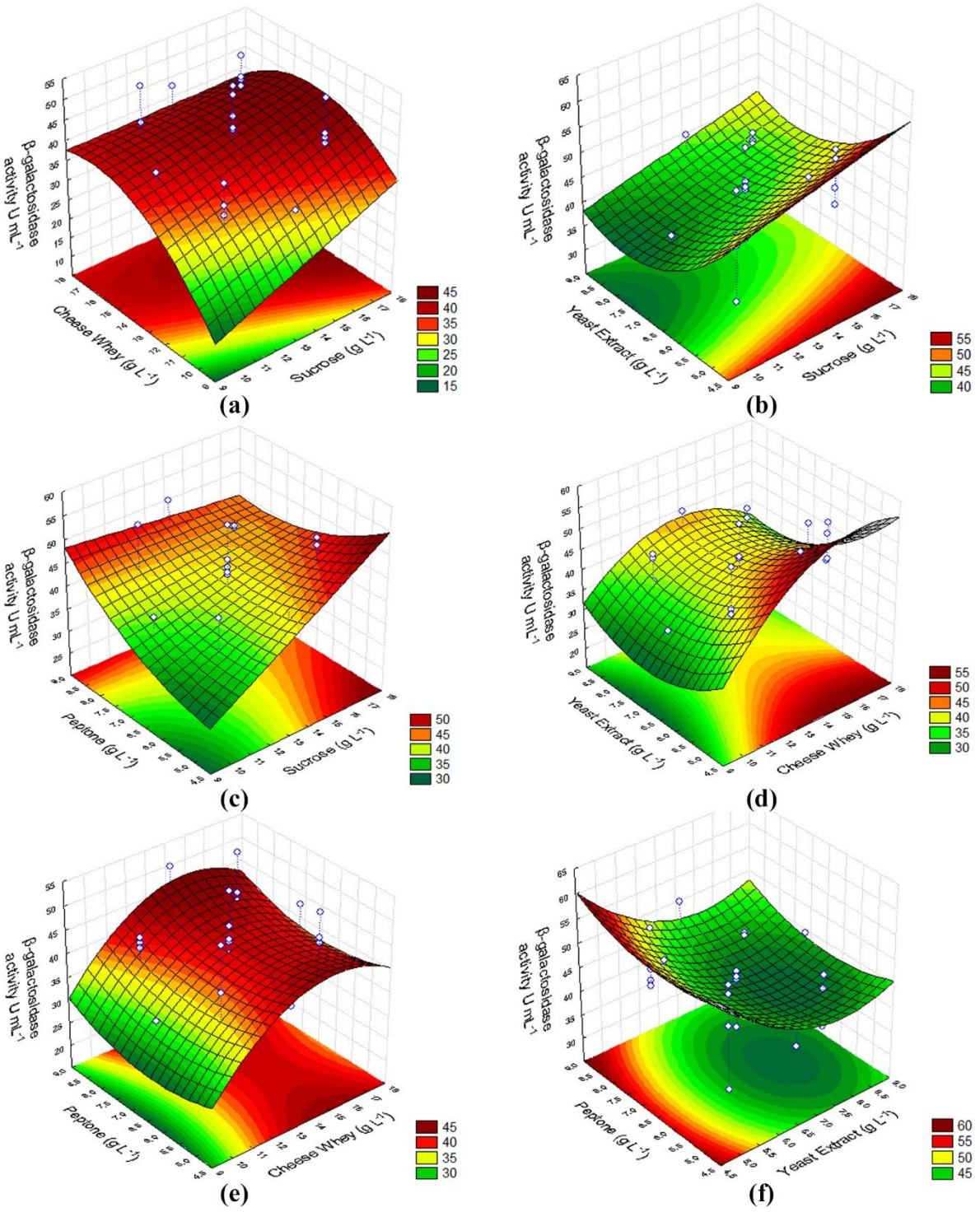

Figure 1. Response surface to β-galactosidase activity as function cheese whey and sucrose (a), sucrose and yeast extract (b); peptone and sucrose (c); cheese whey and yeast extract (d) and peptone and yeast extract (e).

It is very important to determine the composition of fermentation medium for maximum lactase production. There are few studies described in the literature that show the β-galactosidase production using different fermentations. Bosso *et al.* (2019) worked with microfiltrated cheese whey permeate as substrate for *Saccharomyces fragilis* IZ 275 yeast for the production of beta-galactosidase. Kumari *et al.* (2019) concluded that the use of cheese whey for β-galactosidase production improves the economics of the process, and the problems associated with its disposal. Manera *et al.* (2008) obtained by CRCC the maximum β-

galactosidase (10.7 U/mL) activity by *K. marxianus* CCT 7082 and used as fermentation medium lactose, yeast extract and $(NH_4)_2SO_4$. Venkateswarulu *et al.* (2017) concluded that the submerged fermentation with *Bacillus subtilis* strain VUVD001 produced lactase activity of 63.54 U/mL in optimized medium. The authors observed that the activity was threefold higher in comparison to an unoptimized medium and confirmed that the designed medium was useful for producing higher yields of lactase. Ahmed *et al.* (2016) revealed that the strain *Lactobacillus sp*. KLSA 22 isolated from cheese whey was the best potential source of lactase. Cheese whey, a dairy waste, is utilized as a carbon substrate for the enhanced enzyme production at low-cost. Obviously, the value of β-galactosidase activity can range according to microorganisms, culture conditions and fermentation medium; however, it was clear from this work that cheese whey is an excellent culture medium for *Saccharomyces fragilis* IZ 275 and could be employed by the food industry for β-galactosidase production. In a recent study, Murari *et al.* (2019) optimized bioethanol production from cheese whey using *Kluyveromyces marxianus* URM 7404. The authors described that cheese whey can be employed for ethanol production, reducing the environmental damages caused by this by-product.

4. CONCLUSION

The experimental design methodology applied in this study and the response surface are efficient tools in the optimization of fermentation medium for β-galactosidase production by *Saccharomyces fragilis* IZ 275. In the optimized fermentation medium, the maximum production of β-galactosidase is obtained. Cheese whey, a by-product of the dairy industry, can be employed as an excellent fermentation medium by yeast, within the bioeconomy concept and can be used by the dairy industry as a by-product with additional value.

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