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Response surface methodology as a tool for modelling galacto-oligosaccharide production

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The experiments reported in this research paper describe the effects of β-galactosidase enzyme dose and cheese whey amount, on the maximum concentration and yield of galacto-oligosaccahride (GOS) and reaction time. The experimental plan was based on central composite rotational design (CCRD) and modelled by response surface methodology (RSM). The results indicate that the proposed mathematical models could adequately describe the concentration and yield of GOS and the reaction time within the limits of the factors that are being investigated. The variance analysis shows high values of coefficients of determination (>0·97) while no significant lack of fit was evident. Hence, the models could be employed to select reaction conditions applied in the manufacture of products enriched in bioactive compounds with high value-added.

Keywords: Galacto-oligosaccharides, β-galactosidase enzyme, milk-whey mixtures, central composite design, response surface methodology.

Galacto-oligosaccharides (GOS) are non-digestible oligosaccharides which are now recognised as prebiotic food ingredients of considerable interest (Lamsal, 2012). They are produced as a result of a transgalactosylation reaction catalysed by β-galactosidase enzymes (EC 3.2.1.23) from lactose, in which two or more galactose units and just one unit of terminal glucose are condensed by different glycosidic linkages. Therefore, the resulting product is a complex mixture of oligosaccharides with different degrees of polymerisation (Gänzle, 2012; Rodriguez-Colinas et al. 2012).

Great efforts have been made to analyse the variables that affect the production of GOS (concentrations of enzyme and substrate, origin of enzyme, reaction time, pH, and temperature, among others) which are discussed in several reviews (Lamsal, 2012; Intanon et al. 2014). In particular, in a previous work, we studied the individual effects of lactose concentration and dose of enzyme using lactose solution as medium of reaction (Vénica et al. 2015); we found that the GOS values increased with the augment of starting lactose concentration, and the increases of the dose of enzyme led to maximum amounts of GOS in a shorter reaction time, for each level of initial lactose tested. However, the interactive effect of both factors was not evaluated. In this sense, the RSM that consists of a group of mathematical and statistical techniques based on the fit of polynomial models to the experimental data, is widely used to describe its behaviour and to make statistical previsions (Bezerra et al. 2008). In particular, González-Delgado et al. (2016), Martins et al. (2014), Fai et al. (2015) and Lisboa et al. (2012) modelled by RSM the concentration and yield of GOS using commercial and microbial β-galactosidase enzymes and different substrates as reaction media, among other factors (pH, lactose concentration, enzyme dose, temperature, inoculum quantity and reaction time, etc). Nevertheless, according to our knowledge, the time in which the maximum GOS is obtained has not yet been modelled. The process time is an important variable for the industry, and improved knowledge of this parameter is a topic of great interest in the development of new and innovative dairy products enriched in GOS by the incorporation of exogenous β-galactosidase enzyme into the traditional manufacture (in situ approach). Cheese whey powder is widely employed as an ingredient in the preparation of these dairy foods.

The aim of this work was to model the effects of the β-galactosidase enzyme dose and cheese whey amount, which were varied according to a central composite design, on the maximum concentration and yield of GOS and reaction time, by response surface methodology.

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Materials and methods

Materials

Partially demineralised whey powder at 40% (DWP) was generously supplied by García Hnos. Agroindustrial S.R.L. (Santa Fe, Argentina); the composition (w/w) declared was carbohydrates (80·0%), protein (11·0%), fat (2·0%), and minerals (1·3%). UHT milk (Milkaut S.A., Santa Fe, Argentina) which composition (w/v) was carbohydrates (4·8%), protein (3·0%) and fat (3·0%), was also employed. Commercial food grade β-galactosidase enzyme (E) derived from *K. lactis*, YNL-2 GODO (50 000 U ONPG/g) was granted from Shusei Company Limited (Tokyo, Japan).

Enzymatic reactions

The experiments were carried out in screw-cup flasks (500 ml). Mixtures for each run were prepared by manually dissolving the DWP in the amounts defined by the experimental design and diluting with UHT milk to 400 ml. Flasks were kept at room temperature for approximately 30 min and heated at 60 ± 2 °C in order to ensure adequate dissolution. Then, they were cooled to 40 ± 1 °C and the enzyme was added at the setted dose according to the experimental design. Reaction systems were incubated in a water bath at 40 ± 1 °C for a period of 3 h. Assuming that the enzyme was added at zero time, aliquots (10 ml) were withdrawn at 10 min intervals and kept in a boiling water bath for 8 min to inactivate the enzyme and to stop the reaction. Samples were stored at −18 °C until the moment of the carbohydrate analysis.

The physicochemical composition of mixtures ranged from 6·66 to 6·43, from 14·4 to 25·2 g/100 g, from 3·7 to 5·0 g/100 g and from 6·5 to 15·4 g/100 g for pH, total solids, protein and lactose, respectively.

Chromatographic estimation of carbohydrates

The identification and quantification of sugars: lactose, glucose, galactose and GOS, was performed by HPLC with refractive index detection (HPLC-IR). A Perkin Elmer (Norwalk, USA) chromatograph, equipped with a Aminex HPX-87H column (300 × 7·8 mm) and a cation H^+ micro-guard cartridge (Bio-Rad Laboratories, Hercules, USA), a quaternary pump, an on-line degasser, a refractive index detector and a column oven (Series Flexar), and Chromera® software (Perkin Elmer, Norwalk, USA) were used for the analyses. Sugars were eluted with 10 mM H_2SO_4, at a flow rate of 0·6 ml/min, at 65 °C. Before injection, the samples 2·5 g were diluted with 10 mM H_2SO_4 to 25 ml, homogenised and centrifuged at 15 000 ***g***/20 min/4 °C. The supernatant was filtered through 0·45 µm membrane (Millex, Millipore, Brazil) and injected into the equipment, using a loop of 60 µl (Vénica et al. 2015). Quantification was performed by external calibration using suitable standards (Sigma-Aldrich, Saint Louise, USA).

Experimental design

In order to study the conditions for GOS synthesis a CCRD for two independent variables was performed. The independent variables (factors) were enzyme dose and amount of DWP, and the dependent variables (responses) taken into account were maximum concentration and yield of GOS produced and the reaction time in which the maximum GOS is reached. Each factor in the design was studied at five different levels which comprise two fractional points (−1 and +1), one central point (0) and two axial points encoded as $-\alpha$ and $+\alpha$, as shown in Table 1. Four replicates at the central point were performed to evaluate the pure error. The ranges of the variables were determined on the basis of our previous experiments, in order to obtain a matrix with high content of lactose to promote the synthesis of GOS. The full experimental plan resulted in a total of 12 runs, whose levels are listed in Table 2.

GOS yield was defined as weight per cent of the starting lactose content in the reaction system calculated according to Fai et al. (2015), as follows:

$$\text{GOS yield (\%)} = \text{GOS(g/100 g)/initial lactose(g/100 g)} \times 100 \quad (1)$$

For modelling the responses, a second order polynomial function was fitted to the experimental results. For two factors, the equation is given as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{12}X_1X_2 \quad (2)$$

where Y is the predicted response output of maximum GOS concentration, maximum GOS yield or reaction time for obtaining maximum GOS; b_0 is the intercept; b_1 and b_2 are the linear coefficients; b_{11} and b_{22} are the quadratic coefficients; b_{12} is the cross-product coefficients; and X_1 and X_2 are the enzyme dose and amount of DWP, respectively. The three-dimensional (3D) surface graphs were built to visualise the main and interactive effects of the independent variables on the dependent ones.

Data analysis

A software Design Expert version 7.0.0 (Stat-Ease Inc., Minneapolis, MN, USA) was used to design the experiment and process the results by analysis of variance (ANOVA). The quality of fit of the models was expressed by the coefficients of determination (R^2 and adjusted R^2) and the statistical significance was tested by the F-test.

Results and discussion

Evolution of GOS production

The carbohydrate profiles (GOS, lactose, glucose and galactose) during the time course of reaction of the 12 runs of the CCRD were followed by HPLC-IR. At initial reaction

Table 1. Experimental range and levels of the independent variables used for response surface central composite rotable design

Variable		Range	Levels of variable studied				
			−α (−1·41)	−1	0	+1	+α (+1·41)
X_1	Enzyme (g/l)	0·50–1·50	0·29	0·50	1·00	1·50	1·71
X_2	DWP (g/100 ml)	5·00–15·00	2·93	5·00	10·00	15·00	17·07

DWP, demineralised whey powder

Table 2. Full experimental central composite rotable design with level of variables and observed and predicted responses

Run	Variables		Responses					
	E (g/l)	DWP (g/100 ml)	Maximum GOS (g/100 g)		Maximum GOS yield (%)		Reaction time (min) to obtain maximum GOS (Ln Time)	
			Observed	Predicted	Observed	Predicted	Observed	Predicted
1	0·50	5·00	0·89	0·88	11·27	11·27	60 (4·09)	66·0 (4·19)
2	0·50	15·00	2·42	2·44	17·71	17·65	150 (5·01)	153·9 (5·04)
3	1·50	5·00	0·90	0·92	11·3	11·64	30 (3·40)	30·8 (3·43)
4	1·50	15·00	2·30	2·32	16·81	16·74	50 (3·91)	47·8 (3·87)
5	1·00	2·93	0·65	0·66	9·98	9·97	40 (3·69)	39·5 (3·67)
6	1·00	17·07	2·78	2·75	18·02	18·09	90 (4·50)	98·0 (4·58)
7	0·29	10·00	1·61	1·60	14·79	14·81	130 (4·87)	123·2 (4·81)
8	1·71	10·00	1·57	1·55	14·40	14·43	30 (3·40)	31·4 (3·45)
9	1·00	10·00	1·59	1·58	14·56	14·43	60 (4·09)	62·2 (4·13)
10	1·00	10·00	1·58	1·58	14·45	14·43	60 (4·09)	62·2 (4·13)
11	1·00	10·00	1·58	1·58	14·45	14·43	70 (4·25)	62·2 (4·13)
12	1·00	10·00	1·55	1·58	14·24	14·43	70 (4·25)	62·2 (4·13)

DWP, demineralised whey poder.
GOS yield (%), GOS (g/100 g)/initial lactose (g/100 g) × 100.

time, for all mixtures, GOS was not detected, the glucose and galactose concentrations were low (<0·26 g/100 g) and lactose was the main carbohydrate present at the levels mentioned below in the description of enzymatic reactions. As the reactions took place, lactose was consumed and glucose and galactose were released by hydrolytic activity of the enzyme and the GOS was synthesised by transgalactosylation. The galactose contents were always lower than glucose, because the galactose molecules are condensed for the GOS formation. Its presence was already evident at 10 min of incubation in all runs. As example, Fig. 1 is shows the carbohydrate evolution from 0 to 100 min for the central point of design (mean values of the four replicates: runs 9–12). In particular, at 10 min of reaction, the mean values of GOS, galactose, glucose and lactose were 0·85, 1·27, 1·66 and 7·89 g/100 g, respectively. GOS increased through time until the maximum was reached at 60–70 min (mean value 1·57 g/100 g), after which a gentle decline occurred due to hydrolytic processes. A similar behaviour in relation to GOS evolution after the maximum level reached was observed in the runs 1, 4 and 6. In these cases the ratio of DWP/E ranged from 10 to 17. In the runs 3, 5 and 8 (DWP/E ratio: 3–6) a more pronounced decrease was found. Nevertheless, in the runs 2 and 7 (DWP/E ratio: 30–35) the maximum GOS concentration remained unchanged until the end of incubation. Table 2 shows the observed responses for maximum concentration and yield of GOS, and their corresponding reaction time. It can be seen that the values depend on the two factors studied. Time, concentration and yield of GOS ranged from 30 to 150 min, from 0·65 to 2·78 g/100 g and from 9·98 to 18·02%, respectively. In particular, the maximum level and yield of GOS: 2·78 g/100 g and 18·02%, respectively, were achieved at 90 min of incubation using 1·00 g/l of enzyme and with the upper amount of DWP employed (run 6).

Building of the response surface models

The influence of the combinations between the variables enzyme dose and whey content on the GOS production were investigated in detail by CCRD and responses were modelled by RSM.

Based on ANOVA (Table 3), polynomial regression models describing the maximum concentration and yield of GOS and the corresponding time as functions of independent variables (E and DWP), were established. The best fitting models were determined with backward elimination whereby insignificant factors ($P > 0·10$) were removed. The natural logarithmic (Ln) transformation for the reaction time response was necessary to improve the fit of the

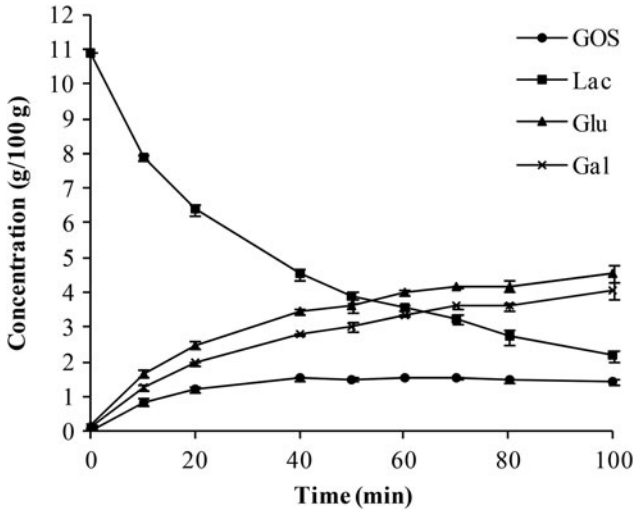

Fig. 1. Evolution of carbohydrates composition as time elapsed under conditions of central point of experimental design (enzyme: 1 g/l, DWP: 10 g/100 ml). Values were mean ± standard deviation of 4 runs of central point.

model to the data. The equations generated are given bellow:

$$\text{GOS(g/100 g)} = 0 \cdot 238 + 0 \cdot 112X_1 + 0 \cdot 112X_2 - 0 \cdot 015X_1X_2 + 0 \cdot 002{X_2}^2 \quad (3)$$

$$\text{GOS yield(\%)} = 7 \cdot 280 - 0 \cdot 223X_1 + 0 \cdot 860X_2 - 0 \cdot 127X_1X_2 + 0 \cdot 389{X_1}^2 - 0 \cdot 008{X_2}^2 \quad (4)$$

$$\text{Ln Time(min)} = 4 \cdot 047 - 0 \cdot 561X_1 + 0 \cdot 105X_2 - 0 \cdot 040X_1X_2 \quad (5)$$

where X_1, enzyme dose (g/l); X_2, demineralised whey powder (g/100 ml); Ln, natural logarithmic.

As can be seen, the variables enzyme dose and whey content at the levels studied affected the responses evaluated. The positive coefficients indicated a constructive effect on the concentration and yield of GOS and reaction time, while the negative coefficients affected the responses in a reverse manner. Taking into account the *P*-values, for GOS concentration (Eq. (3)), the amount of DWP had the greatest effect, followed by the DWP quadratic term and to a lesser extent the interaction between E and DWP and the enzyme. For GOS yield (Eq. (4)), the significance of the factors in decreasing order was DWP, ExDWP, DWP^2, E and E^2. Meanwhile, for reaction time (Eq. (5)), both factors studied had a similar and very significant effect followed by the interaction term (ExDWP).

The ANOVA results show that the three models obtained were significant ($P < 0{\cdot}0001$) while no significant lack of fit was evident ($P > 0{\cdot}1$) within the experimental ranges studied. The high values (close to 1) for R^2 (between 0·9992 and 0·9801) and adjusted R^2 (between 0·9986 and 0·9726) denote a satisfactory correlation between observed

Table 3. Analysis of variance (ANOVA) for the fitted polynomial model for the concentration and yield of GOS and for natural logarithmic transformed reaction time, in the peak of maximum GOS synthesis

	Maximum GOS concentration					Maximum GOS yield					Natural logarithmic (reaction time)				
Source	Sq	Df	Mq	*F*	*P*-v	Sq	Df	Mq	*F*	*P*-v	Sq	Df	Mq	*F*	*P*-v
Model	3·8954	4	0·9738	1822·44	< 0·0001	58·8215	5	11·7643	876·59	< 0·0001	2·7369	3	0·9123	131·38	< 0·0001
X_1 = E	0·0023	1	0·0023	4·33	0·0826	0·1104	1	0·1104	8·23	0·0350	1·8677	1	1·8677	268·96	< 0·0001
X_2 = DWP	3·3495	1	3·3495	6268·11	< 0·0001	49·4867	1	49·4867	3687·41	< 0·0001	0·8281	1	0·8281	119·26	< 0·0001
E × DWP	0·0035	1	0·0035	6·56	0·0428	0·2430	1	0·2430	18·10	0·0081	0·0411	1	0·0411	5·92	0·0410
E^2	—	—	—	—	—	0·0568	1	0·0568	4·23	0·0947	—	—	—	—	—
DWP^2	0·0264	1	0·0264	49·44	0·0004	0·2343	1	0·2343	17·46	0·0087	—	—	—	—	—
Residual	0·0032	6	0·0005			0·0671	5	0·0134			0·0555	8	0·0069		
Lack of fit	0·0026	3	0·0009	4·22	0·1338	0·0150	2	0·0075	0·43	0·6836	0·0318	5	0·0064	0·80	0·6139
Pure error	0·0006	3	0·0002			0·0521	3	0·0173			0·0238	3	0·0079		
Cor total	3·8986	10	0·9738			58·8886	10	11·7643			2·7925	11	0·9123		
R^2			0·9992					0·9989					0·9801		
R^2_{aj}			0·9986					0·9977					0·9726		
Adeq prec			134·53					94·94					33·46		

Sq: sum of squares; Df: degrees of freedom; Mq: mean square; *F*: *F* value; *P*-v: *P*-value > 0·1 no significant; E: enzyme; DWP: demineralised whey powder.

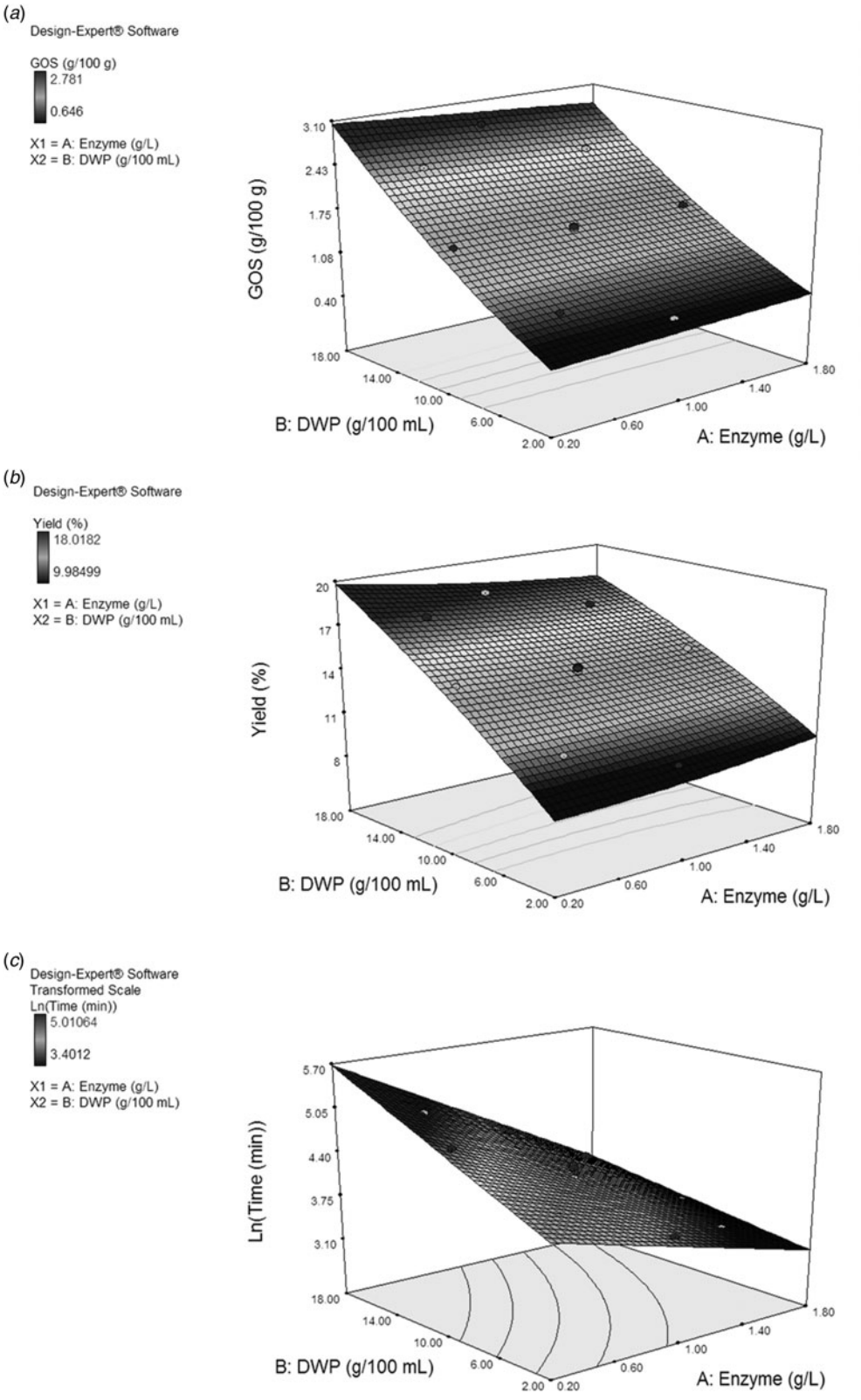

Fig. 2. Response surface graph showing interaction effect of enzyme dose and DWP amount on (a) GOS concentration, (b) GOS yield (%) (GOS (g/100 g)/initial lactose (g/100 g) × 100) and (c) reaction time on the peak of maximum GOS synthesised.

and predicted values. The adequate precision ratios of the models were 134·53, 94·94 and 33·46 for maximum concentration and yield of GOS and reaction time, respectively, indicating an adequate signal for the models (ratios greater than 4 are desirable). Therefore, the predictive polynomial models can satisfactorily explain the maximum concentration and yield of GOS and the time of reaction as functions of the two factors studied under the given experimental domain. The predicted responses obtained for 12 runs are summarised in Table 2.

The mutual interaction of enzyme dose and DWP amount on the concentration and yield of GOS and reaction time can be better understood by the 3D response surface graphs (Fig. 2). Slightly deformed planes are observed for all responses. In the case of concentration and yield of GOS (Fig. 2a and b, respectively), the effect of variables was quite similar in the experimental domain. The values of both responses increased with the increase of DWP amount and, due to the role of interaction term, the maxima were obtained at the lowest enzyme dose for the highest DWP amount and in the opposite vertex (at the highest enzyme dose for the lowest amount of DWP). For the case of Ln Time (Fig. 2c), a strong decrease was evident with the augmentation of the enzyme dose and with the decrease of DWP amount. Likewise, it is observed that the time decreases to a greater extent in the upper limit of DWP compared to the lower limit, and in the lower limit of enzyme in comparison with the upper limit studied, due to the interaction term.

Discussion

The results obtaining in this work concerning to the increment in GOS production with the increase of DWP amount (from 2·9 to 17·1 g/100 g corresponding to 6·5 and 15·4 g/100 g of lactose, respectively) and the diminution of the reaction time in which the maximum GOS was reached as the dose of GODO YNL-2 enzyme was increased (0·29–1·71 g/l), were similar to those found previously by Vénica et al. (2015). However, on that occasion we evaluated the evolution of GOS during 3 h of incubation from lactose solution (5–20 g/100 ml) in buffer phosphate using lower enzyme levels (0·16–0·40 g/l) than used in the present study. It is important to note that in some reaction conditions (low levels of enzyme and high lactose concentration), the maximum GOS content was not reached. In relation to the enzyme effect on GOS synthesis, slightly divergent results were found by some authors. Chockchaisawasdee et al. (2004) observed the same amount of GOS regardless of the dose of enzyme used, while Lisboa et al. (2012) noted an increase in GOS content as the amount of enzyme increased. On the other hand, some authors obtained models to predict GOS production studying different process variables by the application of RSM. González-Delgado et al. (2016) obtained 12·18% of GOS yield with Lactozym Pure 6500 l from *K. lactis* (5 U/ml) and lactose solution (250 g/l) at 3 h of reaction, 40 °C and pH 7·0. Martins et al. (2014) evaluated the GOS formation from reconstituted whole milk powder containing 90 g/l of lactose and using a mix of β-galactosidases Lactomax Flex (*K. lactis* and *Aspergillus niger*); they found the maximum GOS of 0·49 g/100 ml with 0·44 g/l of enzyme at 43 °C. Lisboa et al. (2012) reported GOS values of 11·98 g/100 ml and yield of 29·9% at 4 h and 40 °C working with cheese whey (400 g/l of lactose) and Lactozym 3000 l from *K. lactis* (10 U/ml). Otherwise, Fai et al. (2015) optimised the conditions of transgalactosylase activity by *Pseudozyma tsukubaensis* in a medium containing lactose among other components; 7·37 g/100 ml and 28·3% of concentration and yield of GOS, respectively, were obtained from 260 g/l of lactose at 24 h of fermentation.

In our work, the maximum content and yield of GOS and their corresponding time of incubation found experimentally were 2·78 g/100 g, 18·02% and 90 min, respectively, which were achieved with 1·00 g/l of enzyme and 17·07 g/100 ml of DWP. The corresponding values predicted by the models were 2·75 g/100 g, 18·09% and 98 min; the relative deviations between observed and predicted values (standard deviation/mean × 100) were lower than 0·8, 0·3 and 6·1%, respectively. The predicted residual sum of squares (PRESS) is a measure of how well the model fits each point in the design (Myers et al. 2016); the low PRESS values obtained (0·02, 0·28 and 0·14 for the maximum content and yield of GOS and reaction time, respectively) indicated an adequate fit of the models to the experimental data.

Furthermore, it is important to highlight that in this work we obtained a model to predict the time at which the maximum GOS is achieved, allowing one to know when the reaction should be stopped to prevent the GOS hydrolysis. Based on these results, experimental design and RSM have proved to be effective tools in establishing the reaction conditions to obtain high amount of GOS in a dairy matrix, which could be applied in the manufacture of products with high value-added for potential prebiotic capacity.

Conclusion

In this work the GOS production was modelled with the use of central composite rotational design by response surface methodology, studying the combined effects of enzyme dose and amount of cheese whey. The good results obtained demonstrated that this statistical tool is suitable to predict the maximum concentration and yield of GOS and reaction time. For each combination of enzyme dose and amount of cheese whey, it is possible to know the time in which GOS production is maximum. This information is of great industrial interest for obtaining dairy products enriched in GOS by in situ synthesis, especially since the dairy matrix used in this work is widely employed in the preparation of different dairy foods. In future studies we will focus on the application of these models to obtain high-GOS yogurt, cheese and dessert, thus contributing to the development

of food with high value-added for the potential prebiotic capacity of GOS.

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References

Bezerra MA, Santelli RE, Oliveira EP, Villar LS & Escaleira LA 2008 Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* **76** 965–977

Chockchaisawasdee S, Athanasopoulos VI, Niranjan K & Rastall RA 2004 Synthesis of galacto-oligosaccharide from lactose using beta-galactosidase from *Kluyveromyces lactis*: studies on batch and continuous UF membrane-fitted bioreactors. *Biotechnology and Bioengineering* **89** 434–443

Fai AEC, Simiqueli APR, Ghiselli G & Pastore GM 2015 Sequential optimization approach for prebiotic galacto-oligosaccharides synthesis by *Pseudozyma tsukubaensis* and *Pichia kluyveri*. *LWT – Food Science and Technology* **63** 1214–1219

Gänzle MG 2012 Enzymatic synthesis of galacto-oligosaccharides and other lactose derivatives (hetero-oligosaccharides) from lactose. *International Dairy Journal* **22** 116–122

González-Delgado I, López-Muñoz M-J, Morales G & Segura Y 2016 Optimisation of the synthesis of high galacto-oligosaccharides (GOS) from lactose with β-galactosidase from *Kluyveromyces lactis*. *International Dairy Journal* **61** 211–219

Intanon M, Arreola SL, Pham NH, Kneifel W, Haltrich D & Nguyen TH 2014 Nature and biosynthesis of galacto-oligosaccharides related to oligosaccharides in human breast milk. *FEMS Microbiology Letters* **353** 89–97

Lamsal BP 2012 Production, health aspects and potential food uses of dairy prebiotic galacto-oligosaccharides. *Journal of the Science of Food and Agriculture* **92** 2020–2028

Lisboa CR, de Simoni Martinez L, Trindade RA, de Almeida Costa FA, de Medeiros Burkert JF & Burkert CAV 2012 Response surface methodology applied to the enzymatic synthesis of galacto-oligosaccharides from cheese whey. *Food Science and Biotechnology* **21** 1519–1524

Martins AR, Manera AP, de Medeiros Burkert JF & Veiga Burkert CA 2014 Improving galacto-oligosaccharide content in the production of lactose-reduced yogurt. *International Journal of Engineering Research and Applications* **4** 84–89

Myers RH, Montgomery DC & Anderson-Cook CM 2016 *Response Surface Methodology: Process and Product Optimization Using Designed Experiments, 4th edition*. United States of America: John Wiley & Sons

Rodriguez-Colinas B, Poveda A, Jimenez-Barbero J, Ballesteros AO & Plou FJ 2012 Galacto-oligosaccharide synthesis from lactose solution or skim milk using the β-galactosidase from *Bacillus circulans*. *Journal of Agricultural and Food Chemistry* **60** 6391–6398

Vénica CI, Bergamini CV, Rebechi SR & Perotti MC 2015 Galacto-oligosaccharides formation during manufacture of different varieties of yogurt. Stability through storage. *LWT – Food Science and Technology* **63** 198–205