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New Approach for Lactose Transglycosylation into Galactooligosaccharides

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Abstract: The purpose of the research was to explore the feasibility of enzymatic catalysis with elements of microbial synthesis of the polymerization of lactose monomers into the galactooligosaccharides (GalOS) nanoclusters. The main objects of the study were the lactose solution (milk raw sugar) and the whey permeates subjected to electroactivation and fermentation by Lactokanestsin G20x and Ha-Lactase. To determine the carbohydrate composition of samples and finished products, the high-pressure liquid chromatography method was used. For the process monitoring, the freezing point depression method and the lactose transformation rate were applied. The directed and controlled types of synthesis of a universal agricultural bioecologic raw material of animal origin were examined in the context of solving a task of transglycosylation of the whey lactose into the GalOS. A biotechnology paradigm of the enzyme-catalyzed GalOS polymerization by [beta]-galactosidase in model solutions of milk sugar and industrial samples of the whey permeates was substantiated. The transglycosylation patterns with optimized parameters of the GalOS synthesis were established. The GalOS synthesis in the whey permeate had an optimum, which was observed at a temperature of (50±2)°C and a pH value of (6.4±0.2). On the basis of the research results, the Innovative Technology Platform (the priority was patented with know-how elements) of the GalOS-concentrates production was generated. The relevance of this work is due not only to the practical applicability of the GalOS-concentrates as prebiotic components and their biological value, but also to the need for the complex processing of lactose containing raw material.

Key words: Lactose-containing dairy raw material, ultrafiltration, whey permeate, transglycosylation, galacto-oligosaccharides, food wastes recycling

INTRODUCTION

In view of the well-known situation concerning the implementation of high technologies in the food industry, it seems appropriate to present the advances in the synthesis of galactooligosaccharides, which are poorly understood derivatives of lactose from milk, in a summary form, with the lighthouse principle and the logistics of dairy production to be applied (Diekmann and Lindhorst, 2002; Cho *et al.*, 2003; Tentony, 2003; Khramtsov *et al.*, 2014; Khramtsov, 2014; Gobinath and Prapulla, 2014; Vera *et al.*, 2014; Sangwan *et al.*, 2015). The applicability of the whey, a normal and continually renewable raw material resource, for the synthesis of the galactooligosaccharides is of particular interest (Mannucci, 2009; Khramtsov, 2011; Corzo-Martinez *et al.*, 2013; Gavrilov *et al.*, 2015). Moreover, this doctrine of the dairy industry modernization on the basis of the High Technology Platforms is implemented (Gorlov, 2015; Khramtsov, 2015).

Galactooligosaccharides (GalOS) are low-calorie non-cariogenic substances classified as indigestible oligosaccharides-prebiotics by the modern classification of biologically active dietary supplement (BADS). In addition, they contribute to improving the mineral absorption (especially of calcium) in the large intestine and play a role in moderating the intestinal carcinogenesis (Bruno-Barcena and Azcarate-Peril, 2015). The uniqueness of GalOS together with lactulose (Gorlov *et al.*, 2014) and lactosucrose as compared with other classes of indigestible oligosaccharides with prebiotic properties is the ability of their synthesis in lactose containing materials: lactose solutions of different quality categories, whey and its ultrafiltrates (Jovanovic-Malinovska *et al.*, 2012; Guerrero *et al.*, 2013; Dallas *et al.*, 2014; Rodriguez-Colinas *et al.*, 2014; Guerrero *et al.*, 2015; Padilla *et al.*, 2015).

The system analysis of data with respect to the methods of the GalOS synthesis helped to determine the trends

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of the GalOS technologies and the level of the patent activity in different countries. The most notable among them are the patents in Japan, the USA, Korea, Russia, France, Poland, China, Portugal, New Zealand, the United Kingdom, Norway, Canada and Germany (Lu *et al.*, 2008; Kovacs *et al.*, 2014). A remarkable patenting activity was observed in the 90-ies of XX century. As for the applicant companies, the large majority of them was Japanese, with about half of those patents to be held by the Yakult Honsha Company.
The purpose of the study was to develop innovative technologies for the GalOS concentrate production.

MATERIALS AND METHODS

The objects of the research were: lactose solution in electrically activated water with the predetermined pH value; whey permeate subjected to the electric activation; enzymatic preparations Lactokanestsin G20x and Ha-Lactase, which are transgalactosylation catalysts; lactose solutions and permeates in transgalactosylation; GalOS concentrates obtained from the lactose solution and the whey permeate; commercial GalOS preparation Vivinal® GOS (Netherlands); bacterial concentrates of lactobacilli.
Refined milk sugar and milk raw sugar premium were used for the experiments. Milk sugar was used for the model solutions in preliminary experiments and as one of the raw materials for the GalOS concentrate developed. Both refined sugar and milk raw sugar however have a high cost price. So, the whey ultrafiltrates (UF) were considered to be used in the GalOS-concentrates technology.
For the UF-permeates production, there was used cheese whey complying with the requirements of Government Standard GOST R 53438-2009 "Whey. Technical specifications". The ultrafiltration was carried out on the Test Unit M20 (GEA Group, USA), UF-membrane from Alfa Laval GR70PE-2517/48, at a temperature of (10±2)°C and a pressure of 4 bar.
Composition and properties of the cheese whey UF-permeate used in the experiments are shown in Table 1.
For the reagentless pH control of the lactose solutions and the whey permeate, the method of electrochemical activation was used.
The GalOS synthesis process was influenced by many factors. In order to determine the optimum process parameters of transgalactosylation in the lactose solutions and the whey permeate, the effect of temperature, active acidity, mass fraction of solids (input parameters) on the process efficiency and the GalOS output (output parameter) was studied.
Optimization of the results of the multifactorial experiment was conducted by the graphic response surfaces method for an output parameter and their cross sections using the program STATISTICA 6.0 (Stat Soft Inc.) and in accordance Johnson and Bhattacharyya (2010).

Table 1: Basic properties of the cheese whey UF permeate (mean±S.E.M)

Indicator	Value
Mass fraction of solids (%)	5.23±0.25
Mass fraction of lactose (%)	4.25±0.20
Mass fraction of ash (%)	0.401±0.012
Mass fraction of protein (%)	0.21±0.03
Density (kg/m^3)	1019±10
pH	5.95±0.15
Titratable acidity, °T	16±2
Merit factor (%)	81.7±5.1

RESULTS AND DISCUSSION

In the preliminary experiments (Rodnaya and Lodygin, 2011), the increasing in the lactose solubility was studied with respect to the optimal conditions for the action of the enzymes for the GalOS synthesis. The observations were conducted on the model lactose solutions (industrial milk sugar samples) using two [beta]-galactosidase samples: Lactokanestsin G20x and Ha-Lactase, with the electroactivation applied. Both preparations provided a directed and controlled synthesis of the GalOS nanoclusters from the lactose anomers of nanoscale (1 nm).
The analysis of the previous data showed that intensification of the GalOS synthesis can be potential due to: increase in the lactose solubility at the optimum temperature for the enzyme action taking into account the pH medium; purification of the reaction mixture from the lactose and sugar residue; use of new lactase producers, which have thermal stability and high transgalactosylation activity, by genetic engineering; application of immobilized enzymes and cells for the GalOS synthesis; increasing in the transgalactosylation selectivity by reducing the water activity (high lactose concentrations, the use of co-solvents); use of microwave radiation to increase the GalOS outcome; use of highly effective methods of GalOS separation from the reaction mixture and their purification; cost-cutting of production of the GalOS preparations and concentrates (Palai and Bhattacharya, 2013; Warmerdam *et al.*, 2014).
In order to develop a new technologies for the GalOS concentrate production from the whey permeate, the processes occurring in the interaction of lactose solutions in the acid fraction of the UF permeate subjected to the electrochemical activation and the yeast [beta]-galactosidase were studied. The GalOS synthesis was studied at temperatures of 40; 45; 50°C and pH values of 6.0; 6.5; 7.0. The temperature and pH values were chosen on the basis of the studies of the kinetics of the enzymatic GalOS synthesis in milk sugar solutions using Ha-Lactase. The research results are shown in Table 2. The enzyme dose (0.8%) was selected in accordance with the manufacturer's recommendations with respect to the whey.

Table 2: Changes in freezing temperatures for the whey permeate after the Ha-Lactase introduction

		Temperature °C					
pH	Time, Min.	40		45		50	
6.0	0	-0.425	-0.425	-0.541	-0.512	-0.465	-0.453
	30	-0.441	-0.441	-0.546	-0.506	-0.468	-0.463
	60	-0.425	-0.430	-0.540	-0.497	-0.443	-0.444
	90	-0.430	-0.435	-0.520	-0.498	-0.452	-0.446
	120	-0.440	-0.441	-0.448	-0.497	-0.464	-0.440
	150	-0.432	-0.435	-0.441	-0.505	-0.391	-0.414
	180	-0.439	-0.440	-0.442	-0.510	-0.448	-0.439
Polymerization degree (%)		100	98.8	122.7	103.0	118.9	109.4
		99.4	99.4	112.9	112.9	114.2	114.2
6.5	0	-0.417	-0.419	-0.448	-0.457	-0.515	-0.501
	30	-0.466	-0.489	-0.433	-0.457	-0.506	-0.495
	60	-0.470	-0.467	-0.455	-0.474	-0.495	-0.494
	90	-0.495	-0.498	-0.442	-0.461	-0.499	-0.438
	120	-0.499	-0.508	-0.467	-0.480	-0.469	-0.447
	150	-0.487	-0.503	-0.461	-0.474	-0.486	-0.485
	180	-0.494	-0.510	-0.459	-0.488	-0.507	-0.501
Polymerization degree (%)		89.5	89.7	103.5	100	109.81	114.38
		89.6	89.6	101.8	101.8	112.10	112.10
7.0	0	-0.511	-0.480	-0.514	-0.526	-0.564	-0.630
	30	-0.560	-0.535	-0.579	-0.633	-0.594	-0.659
	60	-0.604	-0.574	-0.615	-0.653	-0.595	-0.669
	90	-0.595	-0.573	-0.659	-0.642	-0.604	-0.679
	120	-0.615	-0.585	-0.646	-0.667	-0.584	-0.662
	150	-0.608	-0.584	-0.657	-0.662	-0.609	-0.669
	180	-0.598	-0.578	-0.661	-0.673	-0.603	-0.682
Polymerization degree (%)		91.3	89.7	88.8	83.1	96.6	95.6
		90.5	90.5	86.0	86.0	86.0	96.1

Table 3: Experimental results on optimizing the enzymatic GalOS synthesis in the whey permeate

Test [1]	Temperature °C	pH	Polymerization degree S_p (%)
1	45	6.0	100.00
2	45	7.0	95.11
3	55	6.0	100.32
4	55	7.0	101.06
5	43	6.5	97.54
6	57	6.5	100.19
7	50	5.8	110.02
8	50	7.2	94.45
9	50	6.5	109.81
10	50	6.5	114.38
11	50	6.5	95.69
12	50	6.5	123.32

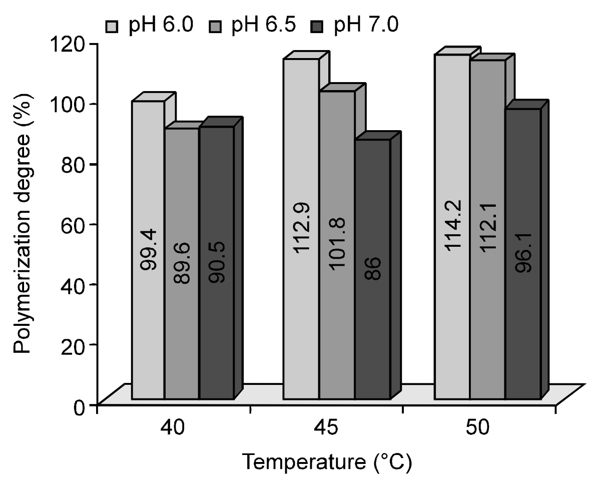

Fig. 1: Permeate pH and temperature effect on the GOS synthesis when using Ha-Lactase

A graph showing the visual patterns of joint influence of acidity (pH) and the fermentation temperature on the outcome of the hydrolysis and lactose transgalactosylation products is shown in Fig. 1.

Comparison of the study results of the lactose transgalactosylation in the milk sugar solutions and the whey permeate showed that in the second case, there was a steady increase in the polymerization degree with a fermentation temperature enhancement in the range studied. The highest yield of the lactose transgalactosylation resultants at a constant temperature was observed in the samples with a pH value of 6.0.

The results obtained served as a basis for a focused study of the joint effect of the technological factors on the yield of the lactose transgalactosylation resultants and for consistent patterns of a mathematical model of the enzymatic GalOS synthesis in the whey permeate.

On optimizing the parameters of the GalOS synthesis in the whey permeate, the temperature and pH values were considered as input factors. The mass fraction value of solids held constant at (20±2)% as well as the

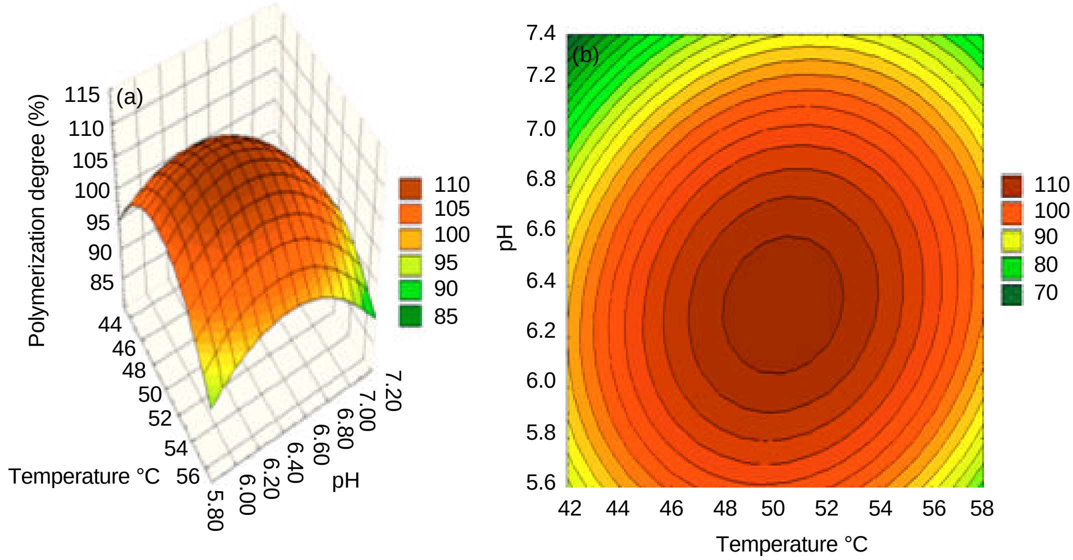

Fig. 2: Response surface of the output parameter (polymerization degree) (A) and its cross section (B)

Table 4: Operator model of the technology platform for the GalOS concentrate production

Subsystem	Operator	System elements
	Subsystem of the galactooligosaccharides concentrate production with the quality parameters complying with a standard	
A	10	Storage operator
	9	Prepackaging and packing operator
	Subsystem of the production of the thickened in-process product and ensuring a high quality product	
B	8	Cooling operator
	7	Thickening operator
	Subsystem of the fermentation	
C	6	Fermentation operator
	5	Operator of the electrochemical permeate activation
	Subsystem of the raw material preparation	
D	4	Whey ultrafiltration operator
	3	Operator of the fat and casein dust separation
	2	Dilution operator
	1	Operator of the electrochemical water activating

Table 5: Risks and control measures at each technological stage of the GalOS concentrate production

Technological stages of production	Stage modes	Effects on microflora and biological risks	Control measures and preventive actions
Separating	35-40°C	Down regulation of microorganisms, possible thermophilic and mesophilic bacteria growth	Compliance with the cleaning and disinfection procedures; use of good-quality raw materials
Ultrafiltration	50-55°C	Down regulation of microorganisms, reinoculation, thermophilic bacteria growth	Compliance with the cleaning and disinfection procedures
Electroactivation	55-60°C	Down regulation of microorganisms, reinoculation, thermophilic bacteria growth	Compliance with the cleaning and disinfection procedures
Fermentation	50-55°C	Seeding as a result of the enzyme introduction, thermophilic bacteria growth	Use of good-quality enzymes; compliance with the cleaning and disinfection procedures
Enzyme inactivating	70°C	Inhibition of vegetative cells of microorganisms	Compliance with the inactivating procedures
Packing in churns	6-8°C	Growth inhibition of microorganisms remaining after pasteurization and homogenization and caught with the equipment; reproduction of psychrophilic flora; seeding from equipment, air, workers' hands	Compliance with the rules of sanitation and personnel hygiene, the cleaning and disinfection procedures, the use of good-quality churns

application rates of the enzyme Ha-Lactase preparation -at 0.8%. The experimental results are shown in Table 3. Mathematical processing of the experimental data performed on the program Statistica 6.0 with real values helped to obtain the following process pattern regression equation:

$$z = -110.89 + 22.22x + 209.01y - 0.26x^2 + 0.56xy - 18.75y^2$$

where, z-polymerization degree, %; x-temperature, ^{0}C; y-pH.

Analysis of the equation showed that on the process optimization, both factors under the study should increase, with the pH to be more significant. The quadratic effects indicated the peak regions for the temperature and the pH. There were consistent synthesis patterns in the volumetric representation of the response surface and the peak regions were clearly expressed in its cross sections (Fig. 2).

The results of the experimental observations and their approximation with the mathematical pattern allowed for the conclusion that the GalOS synthesis in the whey permeate had an optimum, which was observed at a temperature of 50±2°C and a pH value of 6.4±0.2.

The research served as the basis for the Innovative Biotechnology Platform using the "input-output" principle as an operator model. Conversion of the raw material (whey) into an intermediate stock (permeate) and from the intermediate stock into the product (GalOS concentrate) was achieved by several operations, which together can be considered as a subsystem. The combined analysis of the information file, which is an element of high biotechnologies, subsystems of the Technology Platform for the GalOS concentrate production from the lactose containing raw material, is shown in Table 4.

The phased analysis of the operator process scheme of the GalOS concentrates production from the lactose containing raw material in accordance with the HACCP (Hazard Analysis and Critical Control Points) principles revealed the risks and control measures for their prevention that are presented in Table 5.

The experimental results confirmed the high innovation potential of the GalOS concentrates technology. The GalOS concentrate can be used as a functional supplement to various foods such as bakery, confectionery, as well as milk drinks for children, dietary and clinical nutrition (Foda *et al.*, 2009; Barile and Rastall, 2013). When used in a food, the final product contributes to better digestion of food, especially in case of the lactase deficiency.

Conclusion: The system analysis of the world patent situation with respect to one of the milk prebiotics-GalOS has shown its relevance in products for children, diet and nutritional therapy. The raw material for the GalOS synthesis is dairy lactose containing raw material (in practice, trivial whey) using ultrafiltration to obtain permeates with high lactose content. The procedure of lactose transglycosylation into the GalOS nanocluster is carried out by biotransformation by the [beta]-galactosidase (lactase) enzyme complex in specific conditions of molecular docking (high content of milk sugar), in contrast to the known process of hydrolysis to monosaccharides. The research allowed generating a highly innovative paradigm of high innovative Technological Platform of a directed synthesis of new generation products.

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