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Production of prebiotics via reactions involving lactose as well as malic acid and citric acid

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Abstract. Prebiotics are such indigestible food ingredients that enter the colon and serve as nutrient for bifidobacteria and lactobacilli. Since fibres and oligosaccharides are the typical prebiotics, we produced prebiotics in our experiments with the reaction of lactose and malic acid as well as citric acid, where these reactions made use of an appropriate concentration of these substances, had an adequate duration, and were carried out under optimal temperature conditions. We determined the optimal parameters of the reaction, measured the loss of the starting materials as well as the increase in concentration of the end-product, and analysed the total sugar content of the hydrolysed prebiotics after hydrolysis by hydrochloric acid. In vitro experiments were performed to demonstrate our end-product's resistance to carbohydrate-degrading enzymes, which is a fundamental requirement for a prebiotic so that upon reaching the colon it can serve as nutrient for the probiotic bacteria found there.

Keywords and phrases: lactose, malic acid, citric acid, prebiotics, determination of sugar, enzymatic breakdown

1 Introduction

The nomenclature of probiotics (probiotics, prebiotics, and synbiotics) developed in the last two decades of the 20th century, and they became internationally standardized both in their designations and contents. We call probiotics all those human-friendly enteric bacteria that have multiple beneficial effects on the host organism's state of health. Prebiotics are all those natural nutrients that are typically the exclusive nutrients of probiotics, wherefore they facilitate the latter's multiplication and prevalence. Synbiotics mean the joint presence of pro- and prebiotics, that is, the effects of the two beneficial factors become cumulative, often synergistic. Subsequently, those dairy products have synbiotic qualities, for example, whose production process involves not only probiotics but one or more prebiotics as well. Prebiotics, previously termed bifidus or bifidogenic factors, are oligosaccharides built up from 2–9 simple sugars (monosaccharides). They are not metabolized in the organism, which is why they are intact (indigested) upon reaching the colon. These are dietary fibres – the finest of them due to their solubility in water. Besides functioning as dietary fibres, their true usefulness lies in serving as exclusive nutrients for probiotics. Since there is a small amount of digestible nutrients in the colon, a relative food shortage characterizes it, the consumed prebiotics thus setting the ground for the multiplication of human-friendly probiotics (*Csapó et al.*, 2016).

Prebiotics occur naturally in a number of foods. They are abundantly present in e.g. the roots of the Jerusalem artichoke and chicory, but they are also traceable in red onion, garlic, leek, artichoke, oatmeal, wheat, banana, milk, and matured cheese. Alimentary practices typically include industrially prepared pure products, which may be liquid concentrates and powders, the concentration of their active substance ranging between 40% and 95%. Natural industrial concentrates – depending on their constituent monosaccharides – may be e.g. galacto-, fructo-, malto-, or xylo-oligosaccharides. In the year 1995, they were already producing an annual amount of 80,000 tonnes of prebiotics, but production has increased to approx. 200,000 tonnes by today, marking the worldwide success of this special "elixir of life". About 40% of the amount produced is galacto-oligosaccharide (e.g. lactulose), whose basic ingredient is lactose (*Csapó et al.*, 2014a,b,c).

2 Literature review

Prebiotics are non-digestible polysaccharides and oligosaccharides, which, upon reaching the colon, inhibit Salmonella and Escherichia coli bacteria and promote the growth of bifidobacteria and lactic acid bacteria. The term prebiotic was originally coined by *Gibson* and *Roberfroid* in 1995. In 2004, *Gibson et al.* outlined the conditions required for a nutrient to have prebiotic qualities. These are as follows: they should resist gastric acid and the pepsin found therein, mammalian enzymes should not break them down in the gastrointestinal tract, and they should serve as nutrients for those bacteria in the gastrointestinal tract that contribute with their metabolites to humans' well-being and health. A great number of food ingredients meet these criteria – in 2007, *Stowel* grouped these ingredients as follows: inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), lactulose, and polydextrose, whereas isomalto-oligosaccharides, xylo-oligosaccharides (XOS), and lactitol were grouped in the category of emerging, potential prebiotics.

Prebiotics occur in plenty of foods. Chicory root, for example, contains fructo-oligosaccharides derived from inulin, while wheat bran contains arabinoxylo-oligosaccharides (AXOS) and xylo-oligosaccharides (XOS), widely adopted in nutrition (*Sabater-Molina et al.*, 2009; *Femia et al.*, 2010; *Xu et al.*, 2009). Mannitol, maltodextrin, raffinose, lactulose, and sorbitol are also prebiotics with health-protective effects (*Yeo* & *Liong*, 2010; *Vamanu* & *Vamanu*, 2010; *Mandal et al.*, 2009). Resistant, starchy seeds are also considered to be prebiotics, giving proof of several beneficial effects during their consumption. These are not digested and absorbed in the small intestine, but, upon reaching the colon, intestinal microflora can make use of them during fermentation, while short-chain fatty acids (SCHFA, propionic acid, butyric acid, valeric acid, and caproic acid) are formed, which, while reducing pH values, suppress the reproduction of putrefactive bacteria producing toxins (*Vaidya* & *Sheth*, 2010).

Such fermentable dietary fibres as the beta-glucan from rye, the rubbery polysaccharides of linseed and fenugreek may also be considered prebiotics, which are able to serve as basic ingredients for short-chain fatty acids, thus disposing of health-protective effects. Apart from the aforementioned, mannans can be found in large amounts in the wall of yeast cells, which are again prebiotics (*Lin et al.*, 2011).

Also, as a result of malnutrition, smoking, and alcoholism in modern age, there is a growing disease and mortality rate. Typical diseases of our age are chronic obesity, stomach and intestinal problems, diabetes, cardiovascular

diseases, cancer, and degenerative changes, whose numbers have been on a significant rise in the past few years. With a view to prevent or contain these diseases, a growing number of people have turned to foods containing health-protective prebiotics too, expecting substantial improvement from these with a view to their state of health.

Consumers are increasingly looking for low-carbohydrate, high-fibre and -protein foods, while there is a growing interest in prebiotic foods as well. A fine example for this are foods containing blackcurrant leaf extract in the form of powder, lactoferrin, and lutein, produced by several companies in large quantities worldwide. These products have significantly increased the number of bifidobacteria and lactobacilli in the colon, while substantially reducing the number of bacteroides and clostridia. Further, they have reduced beta-glucuronidase (GUS) and enhanced beta-galactosidase enzymatic activity in the small intestine, thus aiding the digestion of e.g. lactose in lactase-deficient humans. Therefore, these products can be truly considered prebiotics (*Molan et al.*, 2010).

As a result of wheat germ supplement applied for 20 days, the colon pH was significantly reduced, just as a strong decrease could be observed in the number of the clostridium population, whereas the amount of lactobacilli and bifidobacteria has significantly increased, indicating a considerable improvement in the quality of life of individuals consuming such products (*Matteuzzi et al.*, 2004). *Glover et al.* (2009) established that gum arabic has a beneficial effect on individuals' suffering from systolic blood pressure and diabetic renal failure. *Phillips* and *Phillips* (2011) found that administering 25 g of gum arabic preparations throughout a period of 8–12 weeks was highly beneficial to the condition of diabetic patients and significantly reduced systolic blood pressure.

Previously, we (*Csapó et al.*, 2014a,b,c) have performed the structural and quantitative analysis of exopolysaccharides and oligosaccharides produced by lactic acid bacteria, whereas our present work's ambition is to report on the results of our experiments where we produced prebiotics via the reaction of lactose as well as malic acid and citric acid. In our work, we have considered *Gaertner* and *Daytoni's* (1956) as well as *Antrim et al.'s* (2003) patent specification as reference material, who aimed at producing surfactants by creating ester linkages between carbohydrates and dicarboxylic acids and studied the mechanism of these reactions. Their methods were successfully tested with sugar alcohols, sugars, oligosaccharides, and polysaccharides as well.

3 Experimental objectives

Relying on literature results and our own previous research, we aimed at producing prebiotics, during which we established such linkages between lactose and malic acid as well as between lactose and citric acid that can resist acidic medium and the attack of carbohydrate-degrading enzymes in the human stomach and the forward section of the gastrointestinal tract and that get into the colon, where they function as nutrients for the probiotic microorganisms settled therein. Our goal was to determine the optimal reaction parameters, temperature, time, and reactant concentration, to measure the loss of the starting materials as well as the increase in concentration of the end-product, and to analyse the total sugar content of the hydrolysed prebiotics after hydrolysis by hydrochloric acid. We further aimed at performing in vitro experiments in order to demonstrate our end-product's resistance to carbohydrate-degrading enzymes, which is a fundamental requirement for a prebiotic.

4 Materials and methods

4.1 The materials used

Our experiments were performed with pharmaceutical-grade lactose, citric acid, and malic acid. The malic acid we used had a purity of 95%, containing less than 1% of fumaric acid and less than 0.05% of malonic acid. The quality certificate of the malic acid no E296 E can be downloaded from the following link: http://bbbb.hu/spec/almasav.jpg. The material that we used was in compliance with the pharmacopoeia of the USA, the EU, and Hungary as well as with the Hungarian Food Codex standards nos 1-2-89/107.

The citric acid used for our experiments was also food-grade, or, even better, pharmaceutical-grade citric acid monohydrate (E330), whose quality certificate can be downloaded from the following link: http://www.bbbb.hu/spec/Citrom.jpg, while its safety data sheet can be accessed from here: http://www.bbbb.hu/spec/citrombizt.jpg. Its CAS number is 5949-29-1, and its EU number is 201-069-1. As per the quality certificate, its citric acid monohydrate content is nearly 100%, it has a maximum water content of 8.8% and an oxalic acid content of less than 100 mg/kg. All of its parameters comply with the EU and the Hungarian Food Codex standards.

The lactose used in our experiments was a 95% pure, food-grade, finely pulverized D(+)-lactose 1-hydrate, isolated from bovine milk and spray-dried produced. Its quality was in compliance with the Ph.Eur 8.0 quality standards.

4.2 The applied analytical methods

As a method for monitoring the reaction of the lactose and malic acid as well as citric acid, we measured the lactose content as this seemed the easiest way to do it. The decrease in lactose content was indicative of the reaction since if the free glycosidic hydroxyl group of the lactose forms a bond, it will no longer manifest a reaction typical of reducing sugars. Lactose belongs to the group of reducing disaccharides, and it manifests Fehling's reaction. During the reaction, due to the aldehyde group of sugar, Cu^{+} ions are formed from Cu^{2+} ions – determining the amount of Cu^{+} ions lets us determine the exact sugar content. During the examination procedure, we measured out and introduced 2 g of sample material into a 100-cm^3 volumetric flask, added to it 50 cm3 of water, and subjected it to a one-hour-long shaking operation in a shaking apparatus. For the removal of substances interfering with the determination of sugar, we added 20–20 cm^3 of Carrez I and II solution. Afterwards, we filled it up to volume mark with 80% ethanol, shook it up, and filtered it. An amount of 20 cm^3 was separated from the filtrate, the bulk of the ethanol was evaporated, and the evaporation residue was flushed with distilled water into a 20-cm^3 volumetric flask, and, after cooling down, filled up to volume mark. Subsequently, this solution was used for the determination of the reducing sugar content. From the solution prepared this way, we removed an amount of 5 cm^3, put it into a 100-cm^3 Erlenmeyer flask, added to it 5 cm^3 of Luff-Schoorl reagent as well as a few pieces of pumice, brought it to boiling point within 2 minutes over open flames and by shaking it, boiled it for 10 minutes, and cooled it down immediately afterwards. The resulting copper(I)-oxide was iodometrically titrated using 0.1 mole of sodium thiosulphate solution, and lactose content was calculated from the amount decreased.

4.3 Engineering the reactions between malic acid and citric acid as well as lactose

In the first step, we added 20% of citric acid, while in the second step the same percentage of malic acid to the pharmacy-quality lactose. Following a thorough examination of the literature and the patents at our disposal, we ascertained that most reactions were performed at a temperature between 130 and 180 °C – therefore, we opted for 170 °C. The samples were blended in a mortar, ensuring their maximum homogeneity. Following this, the samples were distributed in quantities of approx. 10 g into glass vessels and heat treated for 5-10-20-30-40-50-60 minutes; after cooling down, the lactose content of the

samples was determined.

The following experiment investigated into the effect of temperature on the reaction between citric acid and malic acid as well as lactose. In the first stage, the samples containing 20% citric acid, 20% malic acid, and 80% lactose were treated at 130 °C for 30 minutes, in the second stage at 140 °C, in the third stage at 150 °C, in the fourth stage at 160 °C, while in the fifth stage at 170 °C. After cooling down, the lactose content of the samples was determined.

5 Results and conclusions

5.1 Effects of the duration of heat treatment at 170 °C on the reaction between lactose and carboxylic acids

Table 1 shows the effects of the duration of heat treatment at 170 °C on the reaction between lactose and citric acid.

Table 1: Effects of the duration of heat treatment at 170 °C on the reaction between lactose and citric acid

Sample designation	Sample	Heat treatment duration (min)	Lactose (%)
P1	80 g lactose + 20 g citric acid	5	73.6
P2	80 g lactose + 20 g citric acid	10	63.4
P3	80 g lactose + 20 g citric acid	20	48.4
P4	80 g lactose + 20 g citric acid	30	35.4
P5	80 g lactose + 20 g citric acid	40	21.9
P6	80 g lactose + 20 g citric acid	50	12.3
P7	80 g lactose + 20 g citric acid	60	7.1

Heat treatment temperature: 170 °C

By adding 20% of citric acid to the lactose and performing a heat treatment of 170 °C for various times, we established that upon heat treatment the initial white mixture changed to yellow in 5 minutes and turned to a brownish colour in 10 minutes, after which only its colour became increasingly darker, but its volume remained practically unaltered.

Table 2 shows the effects of the duration of heat treatment at 170 °C on the reaction between lactose and malic acid.

By adding 20% of malic acid to the lactose and performing heat treatment for various times, similarly to the case of citric acid, we found sample colour

almost unchanged in 5 minutes' time, within ten minutes, it took on a somewhat yellowish colour, in 20 minutes, it turned yellowish brown, and then it became increasingly swollen, while the last sample changed to dark brown.

Table 2: Effects of the duration of heat treatment at 170 °C on the reaction between lactose and malic acid

Sample designation	Sample	Heat treatment duration (min)	Lactose (%)
P8	80 g lactose + 20 g malic acid	5	70.6
P9	80 g lactose + 20 g malic acid	10	68.3
P10	80 g lactose + 20 g malic acid	20	52.1
P11	80 g lactose + 20 g malic acid	30	33.4
P12	80 g lactose + 20 g malic acid	40	25.3
P13	80 g lactose + 20 g malic acid	50	19.2
P14	80 g lactose + 20 g malic acid	60	16.4

Heat treatment temperature: 170 °C

5.2 Effects of heat treatment performed at various temperatures and for the same duration on the reaction between lactose and carboxylic acids

Table 3 shows the effects of heat treatment performed at various temperatures and for the same duration on the reaction between lactose and citric acid as well as between lactose and malic acid.

Upon a 30-minute heat treatment at 130 °C, the samples practically maintained their white colour; at 140 °C, both the samples containing citric acid and those containing malic acid changed to yellow; at 150 °C, this yellowing further intensified in both carboxyl acids; at 160 °C, the sample with citric acid as well as the one with malic acid took on a deeply brown colour; at 170 °C, the sample with citric acid formed a brown-coloured mass, just as the sample containing malic acid, which, however showed a less brownish discolouration.

Table 3: Effects of heat treatment performed at various temperatures and for the same duration on the reaction between lactose and citric acid as well as between lactose and malic acid

Sample designation	Sample	Heat treatment duration (min)	Lactose (%)
P15	80 g lactose + 20 g citric acid	130	78.2
P16	80 g lactose + 20 g malic acid	130	56.8
P17	80 g lactose + 20 g citric acid	140	48.1
P18	80 g lactose + 20 g malic acid	140	55.4
P19	80 g lactose + 20 g citric acid	150	33.4
P20	80 g lactose + 20 g malic acid	150	38.3
P21	80 g lactose + 20 g citric acid	160	25.6
P22	80 g lactose + 20 g malic acid	160	29.7
P23	80 g lactose + 20 g citric acid	170	16.4
P24	80 g lactose + 20 g malic acid	170	25.5

Treatment: 30 minutes

5.3 Discussion of results, conclusions

5.3.1 Time dependence, temperature dependence

The determination of lactose content allowed us to model what percentage of the originally 80% lactose turned into some kind of oligomer or polymer. If the lactose concentration significantly decreases during the reaction, it inevitably means that the applied hydroxycarboxylic acids reacted with the lactose in some way, creating molecules of various sizes during heat treatment.

The determination of lactose content performed with the use of 24 samples yielded the following results. In the first experiment, 20 g of citric acid was added to 80 g of lactose, while in the second experiment 20 g of malic acid was admixed with 80 g of lactose, whereafter heat treatment was carried out at 170 °C for a duration of 5, 10, 20, 30, 40, 50, and 60 minutes. A subsequent experiment investigated heat dependency, treating the above listed samples (citric acid, malic acid) at 130, 140, 150, 160, and 170 °C for 30 minutes. This time, we tried to identify the optimal temperature at which an adequate reaction can be produced between lactose and the various added carboxylic acids.

In the first case, we measured 79.1% from the control sample (a heat-treated mixture of erythrite and lactose), which in fact gives us the theoretical value.

Exposing the lactose with citric acid to heat treatment for 5 minutes, its quantity decreased to 73.6%, while upon a 60-minute heat treatment it decreased to 7.1%. So, it seems that with the application of citric acid 93% of the lactose transformed into some kind of oligomeric or polymeric compound.

In the second experiment, we measured a 70.6% lactose content for the sample containing malic acid and heat treated for 5 minutes, whereas the sample heat treated for 60 minutes had a lactose content of 16.4%, meaning that 83–84% of the lactose transformed into some sort of product during those 60 minutes of heat treatment. Therefore, we may conclude that both malic acid and citric acid are suitable for forming oligomers or polymers with lactose.

Regarding the temperature dependency of the reaction, we obtained the following results. In the sample with citric acid, only about 1.2% of the lactose was transformed upon a 30-minute heat treatment at 130 °C, whereas from the same sample we could only retrieve 16.4% of lactose upon a 170 °C heat treatment; so, more than 80% of the lactose transformed into oligomer or polymer. When repeating the experiment with malic acid, approximately 30% of the lactose was transformed at a temperature of 130 °C and in a period of 30 minutes, while at 170 °C this value increased to 70%.

The second experiment leads us to the conclusion that citric acid and malic acid alike proved to be 'eligible partners' in the formation of lactose oligomers or polymers at elevated temperature. 130 °C appears to be a temperature too low – a heat treatment performed at 160–170 °C and for 30 minutes (or perhaps at 150–160 °C for 1 hour) is what we consider optimal since it allows the bulk of the lactose to transform into a product. The experiment performed with erythrite clearly demonstrates that the applied thermal conditions and duration did not lead to lactose degradation as this experiment, where no reaction whatsoever was expected to take place between the erythrite and the lactose, resulted in almost the full retrieval of the lactose.

5.3.2 Determining the sugar content of the obtained prebiotic after hydrolysis by hydrochloric acid

Hydrolysis by hydrochloric acid was applied in an attempt to release lactose from its bonds in the obtained samples, which was followed by sugar content determination, allowing us to establish whether the lactose had integrated into some kind of non-reducing polymer, or perhaps something else occurred during the reaction. Following hydrolysis by hydrochloric acid, all sugar content determinations brought along positive results. The sample heat treated at 170 °C for 60 minutes in the presence of 20% citric acid had a lactose content of 7.1%,

which, subsequent to hydrolysis by hydrochloric acid, increased to 46.3% when measured for total sugar content. Following a heat treatment at 170 °C for 60 minutes in the presence of 20% malic acid, lactose content increased from 16.4% to 51.2% expressed in total sugar after hydrolysis by hydrochloric acid. Upon heat treatment at 170 °C for 30 minutes, lactose content increased from 16.4% to 54.9% in the presence of citric acid, whereas it increased from 25.5% to 53.8% in the presence of malic acid, expressed in total sugar content.

What conclusions can be drawn from these investigations? First of all, we can point out that most of the lactose did neither disappear nor degrade nor get damaged but got transformed into an oligomer or polymer that manifests Fehling's reaction to a minimum extent. However, when we used hydrochloric acid to transform the oligomers and polymers into mono- or disaccharides, the resulting sugar-like substances (most probably, glucose and galactose for the most part and lactose to a lesser extent) did manifest Fehling's reaction and could be determined as total sugar.

5.3.3 Enzymatic treatment of the 'obtained' prebiotic with *amylase*

The same samples were hydrolysed with *amylase* as well, thus modelling the reactions taking place in the forward section of the gastrointestinal tract. Following hydrolysis with *amylase*, total sugar content remained practically unchanged, meaning that *amylase* did not react naturally with disaccharide lactose and was not able to split the oligo- and polysaccharide derivatives. This way, the not yet identified, presumably oligomer or polymer product has all the conditions to become a prebiotic, that is, it is not degraded in the forward section of the gastrointestinal tract and most probably gets into the colon, where it can serve as a nutrient for the probiotics found there.

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References

[1] R. L. Antrim, F. W. Barresi, R. McPherson, J. I. Wang, *Dextrinized, saccharide-derivatized oligosaccharides*. United States Patent No 10/601, 912. 23 June 2003.

[2] J. Csapó, Cs. Albert, Zs. Csapóné Kiss, *Funkcionális élelmiszerek* [Functional foods]. Scientia Publishing House, Cluj-Napoca, (2016) 1–211.

[3] J. Csapó, É. Vargáné Visi, R. V. Salamon, Sz. Salamon, Zs. Csapóné Kiss, Tejsavbaktériumok által termelt exopoliszacharidok és oligoszacharidok szerkezeti és mennyiségi analízise [Structural and quantitative analysis of exopolysaccharides and oligosaccharides produced by lactic acid bacteria]. *Tejgazdaság*, 74. (2014c) 3–17.

[4] J. Csapó, R. V. Salamon, Sz. Salamon, Sz. Toró, Zs. Csapó-Kiss, Structural and quantitative analysis of exopolysaccharides produced by lactobacillus. I. Basic information, isolation, quantitative determination, molecular mass and monosaccharide composition. *Acta Universitatis Sapientiae*, *Alimentaria*, 7. (2014a) 5–20.

[5] J. Csapó, R. V. Salamon, Sz. Salamon, Sz. Toró, Zs. Csapó-Kiss, Structural and quantitative analysis of exopolysaccharides produced by lactobacillus. II. The connection status, configuration, phosphorous content, modification, structure of monosaccharides, exopolysaccharides in yogurt, galactooligosaccharides. *Acta Universitatis Sapientiae*, *Alimentaria*, 7. (2014b) 21–44.

[6] A. P. Femia, M. Salvadori, W. F. Broekaert, I. E. J. A. Francois, J. A. Delcour, Arabinoxylan-oligosaccharides (AXOS) reduce preneoplastic lesions in the colon of rats treated with 1,2-dimethylhydrazine (DMH). *European Journal of Nutrition*, 49. (2010) 127–132.

[7] V. R. Gaertner, E. L. Doerr, *Carbohydrate esters of carboxylic acids and methods of preparing same*. United States Patent No 579,719. 23 April 1956.

[8] G. R. Gibson, H. M. Probert, J. V. Loo, R. A. Rastall, M. B. Roberfroid, Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, 17. (2004) 259–275.

[9] G. R. Gibson, M. B. Roberfroid, Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*, 125. (1995) 1401–1412.

[10] D. A. Glover, K. Ushida, A. O. Phillips, S. G. Riley, Acacia (sen) SUPERGUM^{TM} (Gum arabic): An evaluation of potential health benefits in human subjects. *Food Hydrocolloids*, 23. (2009) 2410–2415.

[11] B. Lin, J. Gong, Q. Wang, S. Cui, H. Yu, B. Huang, In vitro assessment of the effects of dietary fibers on microbial fermentation and communities from large intestinal digesta of pigs. *Food Hydrocolloids*, 25. (2011) 180–188.

[12] V. Mandal, S. K. Sen, N. C. Mandal, Effect of prebiotics on bacteriocin production and cholesterol lowering activity of Pediococcus acidilactici LAB 5. *World Journal of Microbiology and Biotechnology*, 25. (2009) 1837–1841.

[13] D. Matteuzzi, E. Swennen, M. Rossi, T. Hartman, V. Lebet, Prebiotic effects of a wheat germ preparation in human healthy subjects. *Food Microbiology*, 21. (2004) 119–124.

[14] A-L. Molan, Z. Liu, M. Kruger, The ability of blackcurrant to positively modulate key markers of gastrointestinal function in rats. *World Journal of Microbiology and Biotechnology*, 26. (2010) 1735–1743.

[15] A. O. Phillips, G. O. Phillips, Biofunctional behaviour and health benefits of a specific gum Arabic. *Food Hydrocolloids*, 25. (2011) 165–169.

[16] M. Sabater-Molina, E. Larque, F. Torrella, S. Zamora, Dietary fructooligosaccharides and potential benefits on health. *Journal of Physiology and Biochemistry*, 65. (2009) 315–328.

[17] J. Stowell, Calorie control and weight management (Chapter 4). In: H. Mitchell (ed.), *Sweeteners and sugar alternatives in food technology.* Blackwell Publishing Ltd., (2007).

[18] R. H. Vaidya, M. K. Sheth, Processing and storage of Indian cereal and cereal products alters its resistant starch content. *Journal of Food Science and Technology*, 48. (2010) 622–627.

[19] E. Vamanu, A. Vamanu, The influence of prebiotics on bacteriocin synthesis using the strain *Lactobacillus paracasei* CMGB16. *African Journal of Microbiology Research*, 4. (2010) 534–537.

[20] B. Xu, Y. Wang, J. Li, Q. Lin, Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). *Fish Physiology and Biochemistry*, 35. (2009) 351–357.