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ANALYSIS OF FERMENTED LACTIC ACID DAIRY PRODUCTS ENRICHED WITH INULIN-TYPE FRUCTANS

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Abstract

The production of functional lactic acid dairy products constantly increases nowadays. They are usually used to enrich with soluble dietary fibers that enhance their functional characteristics. The aim of the current research was to investigate the lactic acid dairy products enriched with inulin-type fructans with different degree of polymerization and to analyse the fructooligosacharides and inulin content in them after lactic acid fermentation. For the yoghurt preparation inulin and fructooligosacharides extracted from tubers of Helianthus tuberosus L. and commercially available inulin from chicory were used. The amount of fructans from inulin-type was determined by spectrophotometric, TLC and HPLC-RID methods. The results from the analysis showed that after lactic acid fermentation the content of inulin changed in a very small amount and the obtained product possessed improved healthy and potential prebiotic effect.

Keywords: *fructoligosaccharides, inulin, Helianthus tuberosus L., yoghurt, HPLC-RID analysis*

INTRODUCTION

Inulin is a fructan, that consist mainly of β-(2→1) fructofuranosyl units (Fm) and usually, but not always ended with a terminal α-glycopyranose unit (1→2) (GFn) (Van Laere and Van Den Ende, 2002) (Figure 1). Its degree of polymerization (DP) varies from 2 to 70 (De Leenheer and Hoebregs, 1994). Fructooligosaccharides (FOSs) are a subgroup of inulin, with DP 2-10 (Niness, 1999).

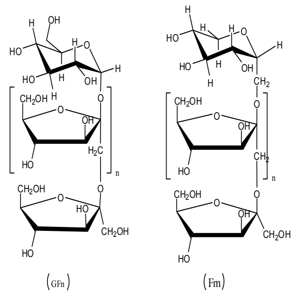

Figure1. Chemical structure of inulin

The primary plant sources of inulin are Jerusalem artichoke (*Helianthus tuberosus* L.) and chicory. Its content in Jerusalem artichoke varies in the range of 7 and 20 % on a fresh weight basis and >75% on a dry weight basis (De Leenheer, 1996).

During the last decade, the application of inulin and fructooligosacharides in food constantly increases due to their diverse range of potential health benefits (Niness, 1999; Roberfroid, 2005). Because of the β-configuration of the anomeric C_2 in the fructose monomers inulin-type fructans are resistant to hydrolysis by human small intestinal digestive enzymes, being therefore classified as dietary fibers (Roberfroid, 2005). They are selectively fermented by β-fructofuranosidase-producing bacteria. FOS and inulin were considered as potential prebiotic ingredients in foods (Gibson and Roberfroid, 1995). They offer a unique combination of nutritional properties and important technological benefits, which depends on the degree of polymerization DP. Therefore, fractions with variable DP can be used to formulate special food products (Yi et al., 2010).

Inulin is legally classified as a food ingredient and it is used in foods to improve organoleptic characteristics, to replace fats and carbohy-drates and to enrich products with dietary fibers. Inulin and FOS are often used to improve the functional properties of dairy products as replace fat and provide creamy mouthfeel in low-fat dairy products such as drinks, yogurts, dips, cream cheese, and process cheese (Franck, 2002). This makes inulin favorable as an ingredient for dietetic food (Roberfroid, 1999; Roberfroid, 2002; Roberfroid, 2005).

Many reports are dedicated to enrichment of yoghurt with inulin or/and fructooligosacharides extracted from chicory or Jerusalem artichoke as prebiotic food ingredients (Hempel et al, 2007, Paseephol T., Sherkat F., 2009). Yogurt is one of the most popular dairy products, obtained as the result of lactic acid fermentation of the milk. It is a traditional product in the everyday meal of Bulgarian people. The addition of inulin-type fructan influences the texture of fermented lactic acid product (Yi et al., 2010; Khalifa et al, 2011). They not only improve the textural properties but also enrich the product with soluble dietary fibers. The incorporation of inulin in dairy products is connected with the health benefits of this fructans, together with their immunomodulating and functional properties (Barclay et al., 2010).

The increasing application of inulin and FOS in the food industry needs the methods for their quantitative determination for food labeling and control of production process. Several methods are available in the literature for quantification of inulin-type fructans in milk or yogurt. Due to the high protein content, analysis of inulin in dairy products remains a challenge. Most of the spectrophotometric methods are based on enzyme hydrolysis with endo- and exo-inulinase (Borromei et al. 2010). Most of them are based on the quantification of fructose and glucose released after the enzymatic hydrolysis of inulin. Analysis with HPTLC was recommended by AOAC (Simonovska, 2000). The other analytical methods are based on HPLC separation with ELSD detection (Kristo, 2011) or using anion-exchange chromategraphy equipped with pulsed amperometric detection (HPAEC-PAD) (Yi et al., 2010; Borromei et al. 2010). Another developed method for FOS analysis in yoghurt is MALDI-TOF MS (Borromei et al. 2009).

The production of yoghurt prepared with traditional Bulgarian starter culture and dietary fibers extracted from Jerusalem artichoke tubers will be considered as a new functional dairy product with improved health benefits. Until now no report were available about enrichment of traditional Bulgarian yoghurt with FOS and inulin fractionated from *Helianthus tuberosus* L.

Therefore, the aim of the current study was to analyse inulin-type fructans with different degree of polymerization in yoghurt after lactic acid fermentation.

MATERIALS AND METHODS

Fructooligosacharides (DP 4-8) and inulin were extracted from tubers of previously investigated varieties *Helianthus tuberosus* L. (Petkova et al. 2013). The sequential extractions by ultrasonic irradiation, previously described by Peshlova et al. (2013) were applied. The obtained fractions were cleaned by ion-exchange resin and evaporated under reduce pressure. The resulting inulin and FOS from *Helianthus tuberosus L.* were dissolved in milk in final concentration 1 % and were used to prepare yoghurt enriched with inulin-type fructan. Inulin and fructooligosacharides from Jerusalem artichoke tubers were compared with commercial chicory root inulin Raftiline HP (DP 25) for preparation of yoghurts. Comparative study of yoghurt prepared with 1 % chicory inulin were carried out. Fructans were added to milk in the form of a solution prior to pasteurization. Standardized milk with 2.5 % fat was inoculated with 2 % *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus* as previously described by the authors (Vlaseva et al., 2013).

The moisture content in yoghurt was determinated by drying the samples at 102 °C for 4 hours (AOAC, 2007).

The changes of inulin content were followed on the first and twentieth after coagulation. Prior to analysis the samples were well homogenized.

The extraction of inulin and sugars from yoghurt was done as follows: 10 g of yoghurt were extracted with 30 ml boiling deionized water for 10 min. The proteins were precipitated by addition of 5 ml Carrez I reagent ($K_4Fe(CN)_6 \times 3H_2O$, 15 g/100 ml) and

5 ml Carrez II reagent ($Zn(CH_3COO)_2 \times 2H_2O$, 30 g/100 ml). The sample was filtered through 0.45 µm paper filter, transferred in 50 ml volumetric flask and made up to the mark with deionized water.

The carbohydrate content in yoghurt were analyzed by TLC analysis. For determination of inulin-type fructan in the obtained water extracts spectrophotometric and HPLC-RID method were performed (Petkova and Denev, 2013).

Chromatographic separation was performed on HPLC Shimadzu, coupled with LC-20AD pump, refractive index detector Shimadzu RID-10A. The control of the system, data acquisition, and data analysis were under the control of the software program LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan). The HPLC separation was carried out on a Shodex® Sugar SP0810 with Pb^{2+} a guard column (50 × 9.2 mm i.d.) and an analytical column (300 mm ×8.0 mm i.d.) at 85 °C. The column was placed in thermostat LCO 102 (ECOM, Czech Republic). The mobile phase was deionized water, vacuum-filtered through 0.2 µm membranes (Germany) before use. All samples were passed through the cellulose acetate filter with pore size 0.2 µm (Sartorius AG, Goettingen, Germany) before injection into the HPLC column.

Statistical analysis was performed by Excel 2010. Treatment means were considered significantly different ($P<0.05$).

RESULTS AND DISCUSSIONS

The changes in carbohydrate composition of fermented lactic acid dairy products enriched with inulin-type fructans were shown (Figure 3). In water extracts of yoghurts enriched with inulin the presence of fructose, galactose, and lactose, FOS and inulin similar to the used standards was observed. It was found that for the storage period of twenty days only the levels of lactose was significantly changed.

The HPLC-RID chromatograms of analyzed inulin in yogurts were presented (Figure 4 and Figure 5). The total fructan content of analyzed samples lactic acid fermented dairy products were expressed as fructose equivalent. Their content in FOS is reduced twice due to the lactic acid fermentation process, while the quantities of inulin isolated from Jerusalem artichoke and commercial one remained significantly constant (Table 1).

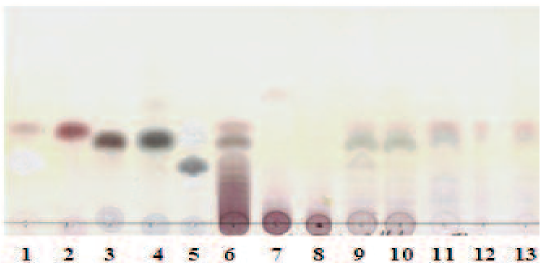

Figure 3. TLC chromatogram of water extracts from yogurt enriched with inulin, where 1. glucose, 2. fructose, 3. sucrose, 4. galactose, 5. lact 1 2 3 4 5 6 7 8 9 10 11 12 13 artichoke, 8. inulin, 9. 10. water extract from yoghu m chicory, respectively

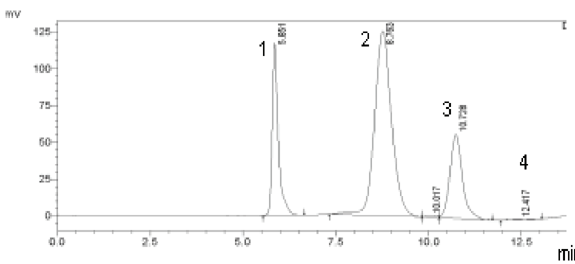

Figure 4. HPLC chromatogram of yogurt enriched with inulin extracted from tubers of Jerusalem artichoke (*Helinathus tubersus* L.), where 1. inulin, 2. lactose, 3. galactose, 4. fructose

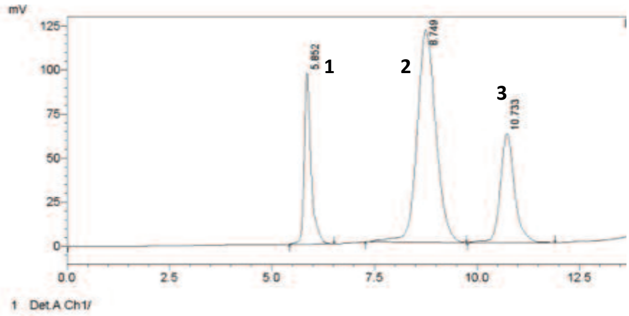

Figure 5. HPLC chromatogram of yogurt enriched with chicory inulin Raftiline HP (DP = 25), where 1. inulin, 2. lactose, 3. galactose

Table 1. Changes in inulin-type fructan content in yoghurt during storage at 4°C, g/100g dw[1] (Mean±SD[2]; n=3).

Sample	Inulin	Fructose	Total fructans	Inulin	Fructose	Total fructans[3]
	1 day after coagulation			20 day after coagulation		
Yoghurt wih 1 % FOS from *Helianthus tuberosus* L.	0.43	0.23	0.82	0.37	0.40	0.80
Yoghurt with 1% inulin from *Helianthus tuberosus* L.	0.90	-	0.92	0.83	0.09	0.90
Yoghurt with 1% chicory inulin Raftiline HP	1.00	-	1.00	0.86	0.08	0.98

[1]dry weight, [2]standard deviation, [3]express as fructose equivalent

On 20 days small amount of fructose 0.09g/100 g appeared in yogurt enriched with inulin with higher DP. FOS due to their shorther fructan chains was more preferable to be fermeted by the strains used for lactic acid fermentation. The small changes in FOS and inulin level could be explained with patrician hydrolysis due to the pH of the dairy products or to the ability of used microorganism to metabolized them. According to Kaplan and Hutkins *Lactobacillus bulgaricus* fermented inulin, whereas *Streptococcus thermophilus* is FOS non-fermentable (Borromei et al. 2010).

According to Kaplan and Hutkins (2000), Ivgantova et al., (2012) all studied *Lactobacillus* strains fermented FOS in different manner. These results show the differences in the ability to metabolize FOS by the isolated strains, which could be owed to different production levels of β-fructofuranosidase to break up the *β* (2→1) bonds presented in FOS. Makras et al. reported that lactobacilli have different capability to ferment fructooligosacharides and inulin and they conclude that not all probiotics degrade inulin in higher degree of polymerization (Borromei et al. 2009).

CONCLUSIONS

From the results obtained during the analysis of lactic-acid fermented dairy products enriched with fibers, we can conclude that the levels of inulin and fructooligosacharides in them remained constant. Therefore, the bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* used for lactic acid fermentation process were not capable to ferment inulin and FOS. Analysis of yoghurt enriched with inulin-type fructans extracted from tubers of Jerusalem artichoke (*Helianthus tuberosus* L.*)* presented a new functional food with improved nutritional values due to the presence of soluble fibers in it. On the basis of our investigation and considerably constant level of fructans added in lactic-acid fermented dairy products, we recommend application of inulin and FOS in preparation of functional dairy products. The final product will characterized with improved healthy effect because of fibers, presence of lactic acids and typical Bulgarian yoghurt starter culture.

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