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HPLC Determination of Inulin in Plant Materials

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Abstract

This study is oriented on the preparation of lactic acid fermented cabbage juices with addition of inulin preparation FRUTAFIT®. Vegetable juices were inoculated by lactic acid bacteria: Lactobacillus amylovorus, CCM 4380, Lactobacillus amylophilus CCM 7001, CCM 7039 Lactobacillus plantarum, Bifidobacterium longum CCM 4990, and a mixture of Lactobacillus plantarum CCM 7039 and Bifidobacterium longum CCM 4990 (ratio 1:1, v / v) and fermented 168 h at 21 °C. For determination of inulin preparation in the juices, the HPLC method with enzymatic pre-treatment was applied. At the first, inulin in the samples was decomposed to fructose by enzyme system FRUCTOZYME L. It was found that the enzyme in the fermented cabbage juices completely degraded inulin preparation at pH 4.5, room temperature 24 °C for 25 min. In the next step, the fructose released during inulin hydrolysis was determined using the HPLC method with RI detection.

Keywords: cabbage juice, FRUTAFIT®, inulinase, enzymatic hydrolysis

Introduction

Inulin is a naturally occurring storage polysaccharide present in numerous plants such as chicory root (Toneli et al. 2008). Naturally-occurring plant fructans are found as osmoregulators and storage carbohydrates in a variety of vegetables including onions, garlic, asparagus and artichokes, in fruits such as bananas, and in cereals (De Bruyn and Van Loo 1991; Van Loo et al. 1995). Chemically, inulin is a linear polydisperse fructan (degree of polymerization, DP, 2–60 or higher) consisting of fructose molecules linked by β(2-1) glycosidic bonds with, generally, a terminal glucose unit connected to the last fructose with a α(1-2) bond. Several inulin types occur in nature and they differ in the degree of

polymerization and molecular weight, depending on the source, the harvest time, and processing conditions (Chiavaro et al. 2007).

Inulin is frequently used as an additive in functional food articles, especially as substitute for lipid compounds and supplement for sugar. It can also be used in products with increased dietary fibre content, e.g. bread or food product, with a bifidogenic effect (Golob et al. 2004). The term "oligosaccharide" refers to a short chain of sugar molecules ("oligo" means "few" and "saccharide" means "sugar.") Fructo-oligosaccharides (FOS) and inulin, which are found in many vegetables, consist of short chains of fructose molecules (Yun and Song 1993)

The increasing use of inulin and FOS in the food industry was decisive for the development of several methods for the determination of inulin and FOS. Several direct determination methods for inulin and FOS using anionexchange chromatography equipped with pulsed amperometric detection (HPAEC-PAD) (Prosky and Hoebregs 1999; Van Waes et al. 1999) or refractive index detection have been reported (Vendrell-Pascuas et. al. 2000, Zuleta and Sambucetti 2001). According Prošek et al. (2003) HPTLC and HPLC-MS methods have been developed for quantitative determination of inulin in food products.

Inulinases usually applied for the hydrolysis of inulin are commercially available enzyme preparations (Novozyme SP 230 and fructanase mixture) containing exo- and endo-inulinase partially purified from Aspergillus niger. These enzyme preparations are usually contaminated with other enzymes that interfere with the measurement of inulin. Therefore, the inulin hydrolysis step in all reported enzymatic determination methods is carried out after treatment of the sample with a ß-amylase, pullulanase and/or amyloglucosidase. Since inulinase can also degrade sucrose, it is necessary in samples containing sucrose to determine/remove the sucrose (Chiavaro et al. 2007).

The work was focused on the preparation of lactic acid fermented vegetable juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by different strains of lactic acid bacteria. The goal of this study was also determination of optimal condition for hydrolysis of inulin preparation. Fructose released during enzymatic hydrolysis was subsequently determined by HPLC with RI detection.

Materials and Methods

Preparation of lactic acid fermented juices

The fresh cabbage was purchased in a local market in Slovakia. From the cabbage, the outer leaves were removed and the cabbage was chopped to small slices. The juices were obtained by pressing and filtration of crushed cabbage. The cabbage juices were prepared by addition of 2% prebiotic preparation FRUTAFIT® (Brenntag Slovakia s r.o., Bratislava, Slovak republic). After addition of prebiotic preparation, NaCl (0.5% addition) and D-glucose (2% addition) were added and the juices were inoculated by Lactobacillus plantarum CCM 7039, Lactobacillus amylophilus CCM 7001, Lactobacilus amylovorus CCM 4380, Bifidobacterium longum CCM 4990 and mixture of Lactobacillus plantarum CCM 7039 and Bifidobacterium longum CCM 4990 (ratio 1:1 v/v) (Faculty of Natural Sciences, Brno, Czech collection of microorganisms, Czech republic) at concentration 106 CFU cm-3. The adjusted juices were placed into 250 cm-3 sterile flasks (volume of juices in individual flask was 150 cm3).The flask were closed with sterile stoppers to ensure anaerobic condition. The juices were fermented in thermostat at 21 ºC ± 1 ºC for 168 h.

Enzymatic hydrolysis

For the determination of inulin, preceded by enzymatic hydrolysis of 2% of prebiotic preparation FRUTAFIT® contained in lactic acid fermented cabbage juices have been used commercially available enzyme FRUCTOZYME L (Novozymes, Slovak republic). Optimum hydrolysis conditions (time and pH) of the complete hydrolysis of inulin were detected at three pH values (4.0, 4.5 and 5.0) and at room temperature. For hydrolysis, McILvain buffer (composition) of a given pH was used. To determine the optimum conditions was used diluted enzyme (100 µl enzyme in 1.4 ml of McILvain buffer). Enzymatic hydrolysis prebiotic preparation was conducted for 40 min. In our designated intervals hydrolysis, the samples were taken and enzymatic reaction was stopped using a 0.5M solution Na2CO3.12H2O (ratio Na2CO3.12H2O to the reaction mixture was 1:1 v/v). Optimum pH and time of hydrolysis for enzyme was determined as the amount of released fructose by HPLC with RI detection

HPLC determination of lactic acid and fructose

Fructose and lactic acid were determined by HPLC methods according Marsilio et al. (2000). Analysis of samples was performed before and after enzymatic hydrolysis of inulin in samples of enzyme FRUCTOZYME L.

Results and Discussion

During the work, cabbage juices with 2% prebiotic preparation FRUTAFIT® were prepared. Conditions for the fermentation process and selection of suitable lactic acid bacteria were carried out according to the authors (Kohajdová and Karovičová 2004).
For the correct operation of lactic fermentation process, pH and lactic acid content were monitored. During fermentation the pH of fermented juices decreases from 6-6.5 to 3.8-4.5 (Holzapfel 2002). In course of fermentation process the pH declined in all samples of lactic acid fermented cabbage juices. The pH of the samples at the beginning of the fermentation process (0h) was 6.41 (sample B) during the fermentation and decreased to values in the range 3.8-3.88 (samples E respectively A). Graphical display of pH reduction during the fermentation process is described in Figure 1. Comparable results was achieved the authors Kohajdová a Karovičová (2004); Kohajdová et al. (2006) or Yoon et al. (2006) they fermented cabbage juices by microorganisms Lactobacilus plantarum CCM 7039 respectively Lactobacilus spp. juices (pH between 3.50 and 4.05).

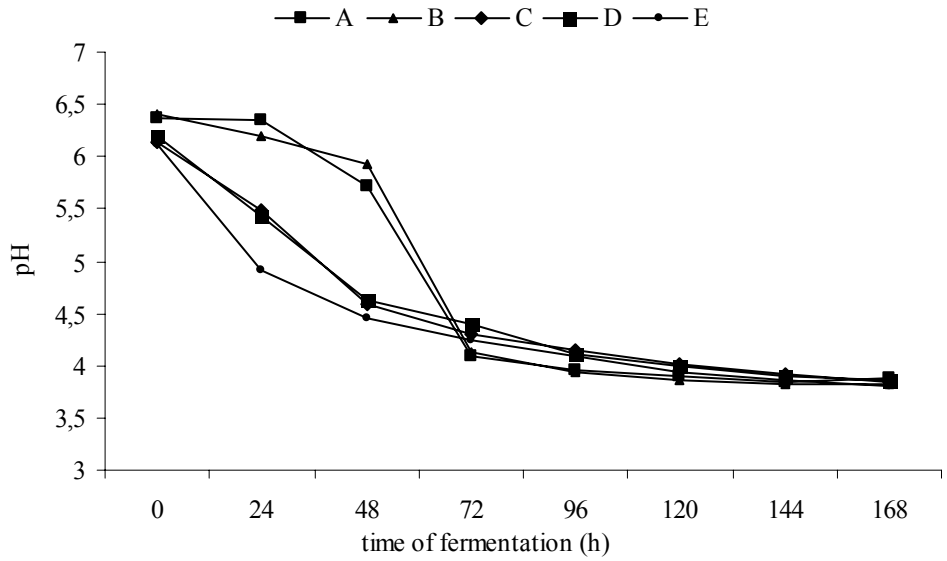

Figure 1. Relationship between the pH of lactic acid fermented cabbage juices and fermentation time

A – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus amylovorus CCM 4380
B – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus amylophilus CCM 7001
C – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus plantarum CCM 7039
D – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Bifidobacterium longum CCM 4990

E - lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by mixture of Lactobacillus plantarum CCM 7039 and Bifidobacterium longum CCM 4990 (ratio 1:1, v/v)

The decrease in pH during the fermentation process is the result of formation of organic acids, mainly lactic acid and acetic acid. The content of lactic acid during the fermentation process is illustrated in Figures 2. As we can see the highest lactic acid content was detected in a sample C (7.23 g.dm-3) fermented by mixture of Lactobacillus plantarum CCM 7039, while the lowest content of this acid was measured in the sample B fermented by Lactobacillus amylophilus CCM 7001 (2.96 g.dm-3).

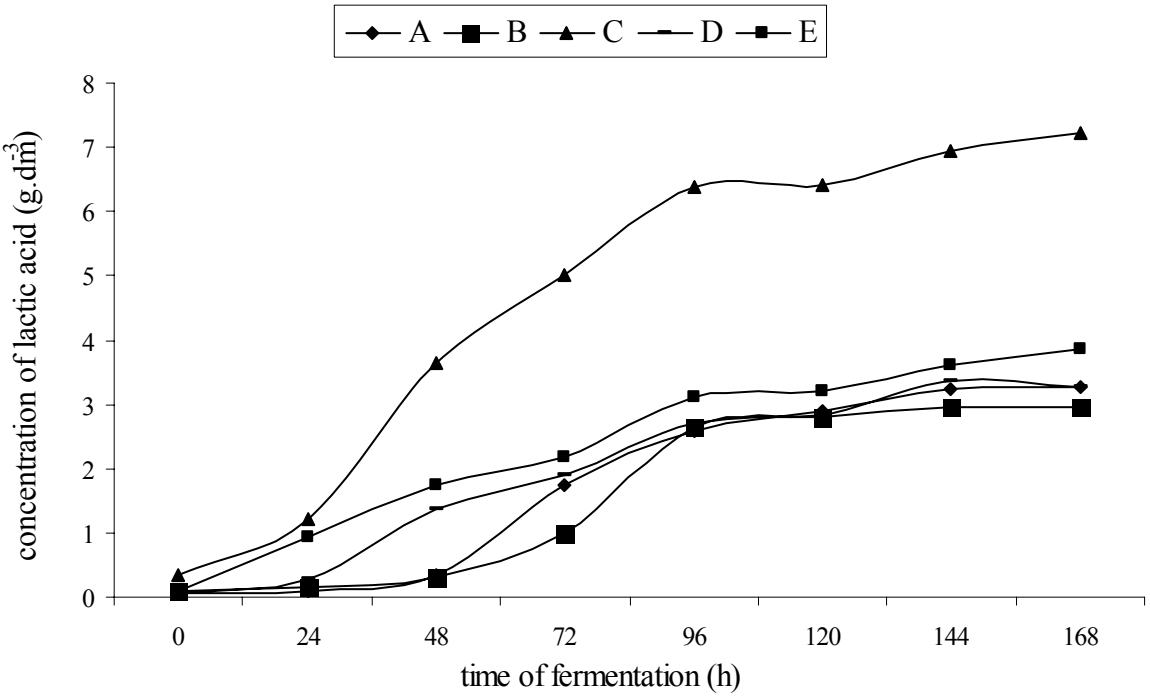

Figure 2. Relationship between the concentration of lactic acid fermented cabbage juices and fermentation time

A – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus amylovorus CCM 4380
B – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus amylophilus CCM 7001
C – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus plantarum CCM 7039
D – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Bifidobacterium longum CCM 4990

Part of this work was to determine the optimum conditions (pH and time) for the enzymatic hydrolysis of inulin preparation (FRUTAFIT®) with enzyme FRUCTOZYME L in samples of prepared fermented juices. Conditions for hydrolysis were detected in model solutions

containing McILvain buffer and 2% inulin. Hydrolysis was carried out in media with different pH values (4.0, 4.5, 5.0). Enzymatic hydrolysis was conducted at room temperature. The amount of released fructose was determined by HPLC with RI detection. The resulting fructose content released during the enzyme hydrolysis vs. time is shown in Figure 3.

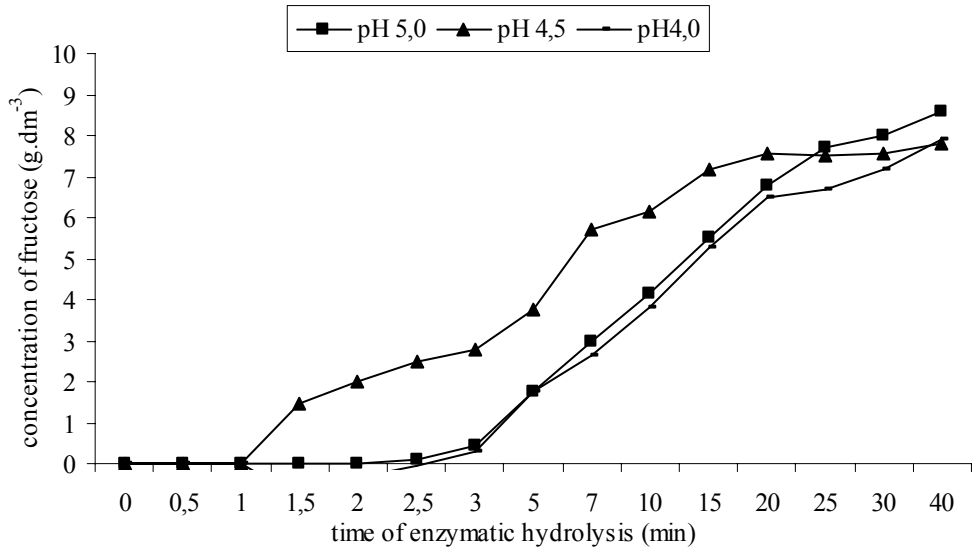

Figure 3. Relationship between the concentration of fructose and time of fermentation
A – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus amylovorus CCM 4380
B – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus amylophilus CCM 7001
C – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Lactobacillus plantarum CCM 7039
D – lactic acid fermented cabbage juice with 2% addition of prebiotic preparation FRUTAFIT® fermented by Bifidobacterium longum CCM 4990

The optimal conditions for hydrolysis of inulin preparation was achieved in the model McILvain buffer solution with pH 4.5 in the 25 minute hydrolysis. After this time and the pH of the solution did not alter the fructose content. Enzyme FRUCTOZYME L was also applied for the determination of inulin in meat products by Vendrell-Pascuas et al. (2000). These authors found that the most suitable conditions for enzymatic hydrolysis by the enzyme were pH 4.5 at 55 C for 30 minutes. After finding the most suitable conditions for enzymatic hydrolysis in model environments, enzyme was applied to the determination of inulin in lactic acid fermented cabbage juices. To determine whether during the fermentation process to maintain the added amount of inulin in the samples was determined amount of fructose at the start (0h) and at the end (168h) of the fermentation process in each set of samples fermented by lactic acid bacteria. Also, in those hours of the fermentation process, it was found the

amount of fructose released after enzymatic hydrolysis. Comparison of fructose before and after hydrolysis at the beginning and end of the fermentation process is shown in Table 1.

Table 1. Amount of fructose determined before and after enzymatic hydrolysis.

	The amount of fructose before the enzymatic hydrolysis	**The amount of fructose after the enzymatic hydrolysis**	**Concentration difference**
Time of fermentation	cF ($g.dm^{-3}$)	cHF ($mg.cm^{-3}$)	ci ($mg.cm^{-3}$)
Sample A			
0h	9.88	20.90	11.02
168h	7.90	19.14	11.24
Sample B			
0h	8.90	19.37	10.47
168h	7.45	17.70	10.25
Sample C			
0h	9.32	21.35	12.03
168h	7.89	20.21	12.32
Sample D			
0h	7.95	22.03	14.08
168h	5.96	19.90	13.94
Sample E			
0h	8.20	20.63	12.43
168h	5.68	18.21	12.53

RSD for all measurements was less than 5%

Conclusion

Lactic acid bacteria Lactobacillus amylovorus CCM 4380, Lactobacillus amylophilus CCM 7001, Lactobacillus plantarum CCM 7039, Bifidobacterium longum CCM 4990, and a mixture of Lactobacillus plantarum CCM 7039 and Bifidobacterium longum CCM 4990 (ratio 1:1, v / v) were used for lactic acid fermentation of cabbage juices with the addition of 2% inulin preparation FRUTAFIT®. It was concluded that the bacteria are unable to degradation of inulin. During the fermentation process did not alter the content of inulin. The content of inulin, was determined by HPLC with RI detection as the amount of released fructose. It was found that applied enzyme is capable of complete hydrolysis of inulin at room temperature 24°C, pH 4.5 over 25 min. Based on the results, we recommended Lactobacillus amylovorus CCM 4380, Lactobacillus amylophilus CCM 7001, Lactobacillus plantarum CCM 7039, Bifidobacterium longum CCM 4990, and a mixture of Lactobacillus plantarum CCM 7039 and Bifidobacterium longum CCM 4990 (ratio 1:1, v / v) for the preparation of

lactic acid fermented cabbage juices with the addition of 2% inulin. We also suggested the use of enzyme FRUCZOZYME L for degradation of preparation containing inulin.

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