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Functional prebiotic activity of inulin and fructooligosaccharides

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ABSTRACT

Fructooligosaccharides (FOS) consisted of 2-5 molecules of fructose and one molecule of glucose whereas inulin contained more than 5 molecules of fructose and one molecule of glucose at the reducing end. They are widely used in food industry as the prebiotic. The prebiotics stability is desirable to maintain their functional property. The stabilities of the synthesized prebiotic fructooligosaccharides (FOS) and extracted inulin from Jerusalem artichoke in the simulated processing conditions were determined using a prebiotic activity assay. The prebiotic activity scores were determined based on the change in cell biomass of *Lactobacillus acidophilus* TISTR1338 on the prebiotic relative to that of *Escherichia coli* TISTR 780 under the same processing conditions. The tested synthesized FOS were rather unstable. Also, it showed the reduction in prebiotic activity scores after exposure to high temperature (85°C) at all acidic pH levels. The extracted inulins were considered functionally stable in all tested processing conditions. This study presents the stability of the synthesized FOS and the extracted inulin under different simulated processing conditions to provide a better selection of prebiotics in different food applications.

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INTRODUCTION

Fructooligosaccharides (FOS) and inulin classified as prebiotics are of particular interest because they stimulate the growth of the probiotic bacteria in the colon (Gibson and Roberfroid, 1995). Inulin can be extracted from different plants such as chicory roots, Jerusalem artichoke and dahlia tuber; it contains a chain of fructose molecules connected together with one glucose molecule at the end. FOS can both extracted from plants such as garlic, banana, onion (Moshfegh *et al*., 1999) and produced from the fructosylation reaction of fructosyltransferase with sucrose (Sangeetha *et al*., 2005). To better apply these prebiotics in the foods, their stabilities against processing conditions such as pH and temperature are the primary concern. These severe processing conditions may degrade the FOS or inulin to the small components of mono- and disaccharides which do not have prebiotic properties. If these prebiotics can withstand the food processing conditions, they still remain their prebiotic activity by stimulating the growth of beneficial bacteria (Wang and Gibson, 1993). Huebner *et al.* (2008) evaluated the functional stability of different prebiotics before and after exposure to simulated food processing conditions. It has been presented that heating at low pH causes a reduction in prebiotic activity. Therefore, the aim of this work was to determine the prebiotic stability of the extracted inulin from Jerusalem artichoke and the synthesized FOS under different processing conditions.

MATERIALS AND METHODS

Lactobacillus acidophilus TISTR1338 and *E.coli* TISTR780 were used for this study. The *L. acidophilus* TISTR1338 and *E. coli* TISTR780 were grown in MRS medium agar which were sterilized by an autoclave at 121˚C for 15 min. The colony of *L. acidophilus* TISTR1338 and *E.coli* TISTR780 were streaked on plate and incubated at 37 ˚C for 18-24 h. The single colony of both cultures was kept at 4 ˚C. When used, the cultures were activated in 3 mL of MRS broth and incubated at 37°C overnight. All others chemicals were of analytical grade.

Prebiotics

The extracted inulin from Jerusalem artichoke and synthesized FOS used in this study are described below. Synthesized FOS product was prepared by the transfructosylation action of a β-fructosidase of fructotransferase enzyme on 200 g/L sucrose solution in 0.1 M acetate buffer pH 5.5 with 1000 U/mL of enzyme for a total reaction time of 20 h. The compositions of synthesized FOS were 15% of kestose, 35% of sucrose, 23% of fructose and 27% of glucose. The inulin was extracted from Jerusalem artichoke by hot water (water: Jerusalem artichoke at 2:1) at 80°C for 10 minutes.

Stability of prebiotics to simulated food processing conditions Effect of pH

The stability of prebiotics to different food conditions was performed according to the method of Huebner *et al.* (2008) with some modifications. The 19.3 mL of FOS (10% w/v FOS concentration) were added into 10.7 mL of 20 mM citrate–phosphate buffer. In the case of inulin, 0.6 g inulin (2% w/v inulin concentration) was added into 30 mL of 20 mM citrate–phosphate buffer. The pH was varied at pH 3.0, 4.0, 5.0 and 6.0 and 7.0 (control). Citrate–phosphate buffer was prepared using citric acid and disodium phosphate. Samples were filter-sterilized and held for 24 h at room temperature (25˚ C). After 24 h, the pH was adjusted to 7.0 with hydrochloric acid or sodium hydroxide. Samples were then filter-sterilized and stored frozen at -80˚C until used for the prebiotic activity assay. Each experiment was replicated three times. Statistical analyses were reported as mean value ± standard deviation.

Effect of heat at low pH

The pH was varied at pH 3.0, 4.0, 5.0, 6.0 and 7.0 (control). Samples were filter-sterilized and held at 85 ˚C for 30 min in an orbital water bath. Then the samples were removed, cooled, adjusted to pH 7.0 with hydrochloric acid or sodium hydroxide, filter-sterilized, and frozen at -80 ˚C until used for the prebiotic activity assay. Each experiment was replicated three times. Statistical analyses were reported as mean value ± standard deviation.

Prebiotic activity assay

The 0.3 mL of prebiotics was added to 2.7 mL of MRS Broth containing *L. acidophilus* or *E. coli* (without glucose as carbon source). The absorbance at 600 nm was measured at 0 h and 24 h. Then prebiotic activity assays were performed according to the procedure established by Huebner *et al.* (2008) with the following changes. The assay was performed with *L. acidophilus* TISTR1338 as a probiotic bacteria and *E.coli* TISTR 780 as an enteric bacteria. Cell densities were determined based on optical density (O.D.) at 600 nm. The prebiotic activity score was determined using the following equation:

$$\text{Prebiotic activity score} = \left[\frac{(\text{probiotic log O.D. on the prebiotic at 24 h} - \text{probiotic log O.D.on the prebiotic at 0 h})}{(\text{probiotic log O.D. on glucose at 24 h} - \text{probiotic log O.D.on glucose at 0 h})}\right] \left[-\frac{(\text{enteric log O.D.on the prebiotic at 24 h} - \text{enteric log O.D.on the prebiotic at 0 h})}{(\text{enteric log O.D.on glucose at 24 h} - \text{enteric log O.D.on glucose at 0 h})}\right]$$

RESULTS AND DISCUSSION

Effect of pH

Prebiotic activity scores after exposure to low pH are listed in Table 1. The prebiotic activity scores of the synthesized FOS were rather low compared to those of the extracted inulin in every pH studied. These may be due to the differences in composition of FOS and inulin. The synthesized FOS have low degree of polymerization (DP) (DP of 3: kestose) while the extracted inulin from Jerusalem artichoke have longer DP from 2-10. Both the synthesized FOS and extracted inulin had high prebiotic activity scores at lower pH indicating that these oligosaccharides may not easily be degraded at low pH conditions.

Table 1 Effect of pH on prebiotic activity scores of the extracted inulin from Jerusalem artichoke and the synthesized FOS

pH	Prebiotic activity score	
	Inulin	FOS
3	0.41±0.05	0.18±0.06
4	0.29±0.08	0.65±0.12
5	0.18±0.09	(-0.46) ± 0.02
6	0.32±0.08	(-0.52) ± 0.05
control	0.23±0.09	(-0.57) ± 0.07

Effect of heat at low pH

Prebiotic activity scores after heating at 85 ˚C for 30 min at different pH levels between 3 and 6 are shown in Table 2. The extracted inulin from Jerusalem artichoke had higher prebiotic activity scores than the synthesized FOS. As can be seen, all prebiotic activity scores of FOS at every pH were negative meaning that the *E. coli* grew better than the probiotic *L. acidophilus* which may imply that the synthesized FOS were easily hydrolyzed at these conditions (85°C). This also may be due to the fact that the chain of FOS is shorter than that of inulin, thus, it is easily hydrolyzed at low pH and high temperature. The synthesized FOS and the extracted inulin may be partially or fully degraded to glucose and fructose. Therefore, the applications of FOS and inulin in foods are rather different. FOS could be applied to dairy products since the pasteurization of milk and dairy products are performed at 7.1°C for 15s (Ryser, 2011). On the other hand, the extracted inulin is more stable to heat and low pH so it can be incorporated into different kinds of foods such as the salad dressings, crackers, bakery foods and other beverages (Huebner *et al*., 2008).

Table 2 Effect of heat at low pH on prebiotic activity scores of the extracted inulin from Jerusalem artichoke and the synthesized FOS

pH	Prebiotic activity scores	
	Inulin	FOS
3	1.55 ± 0.34	(-26.39)± 2.2
4	7.29 ± 4.04	(-37.37) ± 3.1
5	3.28 ± 2.21	(-31.91) ± 1.4
6	4.48 ± 3.05	(-17.72) ± 1.3
control	1.66 ± 0.15	(-8.22) ± 0.4

CONCLUSION

The prebiotic activity scores of the extracted inulin from Jerusalem artichoke and the synthesized FOS at different conditions were evaluated. The extracted inulin is more stable at the tested conditions (85°C, pH 3-6) than the synthesized FOS. Therefore, inulin can be applied to various types of foods while FOS can also be added into products with low pH and low heat treatment such as dairy products.

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