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Effect of processing conditions on the prebiotic activity of commercial prebiotics

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Abstract

The functional prebiotic stability of fructooligosaccharides (FOS) and inulin was determined using a prebiotic activity assay. Prebiotic activity scores were determined based on the change in cell biomass of *Lactobacillus paracasei* 1195 on the prebiotic relative to that of *Escherichia coli* under equivalent conditions. Prebiotics were dissolved in citrate–phosphate buffer solutions (10% FOS or 2% inulin), and then exposed to each of three treatments simulating food processing conditions: low pH (pH 3–6), heat at low pH (30 min at 85 °C, pH 4–7), and Maillard reaction conditions (up to 6 h at 85 °C with 1% glycine, pH 7). Prebiotics were considered functionally stable if their score was unchanged after treatment. In general, only heating at low pH caused a significant reduction in prebiotic activity, with one of the FOS products being the least stable. The other conditions caused little change in activity. These results provide a basis for selecting prebiotics for use as functional food ingredients and for predicting the extent to which processing affects prebiotic activity.

Keywords: Fructooligosaccharides; Inulin; Prebiotics; Probiotics

1. Introduction

Prebiotics, such as fructooligosaccharides (FOS) and inulin, are defined as "nondigestible food ingredient(s) that beneficially affects host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson & Roberfroid, 1995). Lactobacilli and bifidobacteria are among the intestinal bacteria stimulated by prebiotics and some strains of these bacteria have been added to foods as probiotic cultures. In addition, prebiotics are increasingly added to yogurt and other fermented dairy products, as well as a wide array of other foods. Ultimately, it is the ability of prebiotics to withstand food processing conditions that allows for these carbohydrates to reach the colon intact, resulting in selective enrichment of colonic bacteria by providing these bacteria with a competitive advantage (Wang & Gibson, 1993). Therefore, prebiotics alone or combined with probiotic bacteria in the form of synbiotics are acknowledged as having the capability to influence and improve the gastrointestinal health of humans (Tuohy, Probert, Smejkal, & Gibson, 2003).

For prebiotics to serve as functional food ingredients, they must be chemically stable to food processing treatments, such as heat, low pH, and Maillard reaction conditions. Several studies have evaluated the chemical stability of prebiotics to heat and/or acidic conditions. Dry heating of inulin from chicory or Jerusalem artichoke for up to 60 min at temperatures between 135 and 195 °C, resulted in significant degradation ranging between 20% and 100% and led to the formation of new products, likely to be di-D-fructose dianhydrides (Bohm, Kaiser, Trebstein, & Henle, 2005; Bohm, Kleessen, & Henle, 2006). Furthermore, in buffered solutions, FOS have been found to be degraded at increased temperature and decreased pH (L'Homme, Arbelot, Puigserver, & Biagini, 2003).

Even though a prebiotic may be chemically changed, its functional activity may remain the same or be enhanced after the processing treatment. As reported by Bohm et al. (2006), inulin heated at 195 °C for 30 min resulted in complete degradation of the fructan chains and the

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formation of new low-molecular weight products (possibly di-D-fructose dianhydrides). Although the heated inulin had been chemically altered, when added to a mixed fecal culture it showed significantly greater stimulation of the growth of bifidobacteria and Enterobacteriaceae, and a significant decrease in the growth of potential pathogens (Bohm et al., 2006). Therefore, an effective evaluation of the stability of a prebiotic to processing conditions should account for both the chemical and functional stability.

Functional activity of prebiotics has been described previously by several groups (Huebner, Wehling, & Hutkins, 2007; Olano-Martin, Gibson, & Rastall, 2002; Palframan, Gibson, & Rastall, 2003; Sanz, Gibson, & Rastall, 2005; Sanz et al., 2005; Vulevic, Rastall, & Gibson, 2004). As defined by Huebner et al. (2007), the prebiotic activity reflects the ability of a given substrate to support the growth of an organism relative to other organisms and relative to growth on a non-prebiotic substrate, such as glucose. Therefore, carbohydrates have a positive prebiotic activity score if they are (i) metabolized as well as glucose by probiotic strains; and (ii) are selectively metabolized by probiotics but not by other intestinal bacteria. Prebiotics will be functionally stable if their prebiotic activity before and after food processing conditions remains the same or has increased. Thus, the aim of this work was to evaluate the functional stability of commercial prebiotics, using the prebiotic activity assay developed by Huebner et al. (2007), following exposure to three types of simulated food processing conditions. Ultimately, these results may be useful in identifying prebiotics that could be added to dairy and other foods.

2. Materials and methods

2.1. Bacterial strains

Lactobacillus paracasei 1195 (University of Nebraska, Lincoln, NE, USA); *Escherichia coli* ECOR 1, *E. coli* ECOR 2, and *E. coli* ECOR 22 (*E. coli* Reference Collection, University of Rochester, Rochester, NY, USA) were used for this study. The specific test strain, *L. paracasei* 1195, was selected because it had high prebiotic activity scores for the commercial prebiotics in the previous study by Huebner et al. (2007) and because it is a well-characterized strain. The *E. coli* strains were chosen to represent the enteric portion of the commensal biota and were also used in the Huebner et al. (2007) study. The *Lactobacillus* culture was maintained at −80 °C in MRS Broth (Difco Laboratories, Sparks, MD, USA) containing 15% (w/v) glycerol and *E. coli* cultures were maintained at −80 °C in Tryptic Soy Broth (TSB; Difco Laboratories) containing 15% (w/v) glycerol. For the prebiotic activity assays, frozen cultures were streaked onto MRS agar, for the *Lactobacillus* culture, or Tryptic Soy Agar (TSA), for *E. coli*, followed by incubation at 37 °C for 24–48 h at ambient atmosphere. Then, one colony from each plate was transferred into 10 mL of MRS broth or TSB and incubated overnight. For the *E. coli* strains, an additional transfer of 1% (v/v) was made from a TSB overnight culture into 10 mL of M9 Minimal Medium broth (Atlas, 1993) and incubated overnight.

2.2. Commercial prebiotics

The commercial prebiotics used in this study are described in Table 1. The two FOS products, NutraFlora P-95 and Raftilose P95, were obtained from GTC Nutrition (Golden, CO, USA) and Orafti Group (Tienen, Belgium), respectively. These products are distinguished based on their method of manufacture and their final composition. The former are enzymatically synthesized from sucrose and consist of glucose (G) linked by α-1,2 bonds to two or more β-2,1-linked fructose units (F), forming a mixture of GF_2, GF_3, and GF_4. The Raftilose P95 FOS is produced by partial enzymatic hydrolysis of inulin, yielding mostly linear β-2,1-linked fructose oligosaccharides as well as some GF_n oligosaccharides. The two inulin products, both derived from chicory included Inulin-S and Raftiline HP, were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Orafti Group, respectively.

2.3. Stability of oligosaccharides to simulated food processing conditions

If prebiotics are to serve as functional food ingredients, they must be able to withstand thermal and pH conditions likely to occur during food processing. To assess functional activity of FOS and inulin, experiments were performed using buffered prebiotic solutions exposed to reaction conditions described below. The prebiotic activity score was determined for each prebiotic subjected to no

Table 1
Commercial prebiotic carbohydrates used in this study

Commercial prebiotics	Chemical structure[a]	Degree polymerization (DP)	Purity (%)
NutraFlora P-95	Gluα1-2-[βFru-2-1]*n*	2–4	97% FOS[b]
Raftilose P95	Gluα1-2-[βFru-2-1]*n* and [βFru2-1]*n*	2–7	⩾95% FOS[b]
Inulin-S	Gluα1-2-[βFru-2-1]*n*	2–60	>99% Inulin[b]
Raftiline HP	Gluα1-2-[βFru-2-1]*n*	>23 (average)	>99% Inulin[b]

[a]Glu, glucose; Fru, fructose; FOS, fructooligosaccharides.
[b]Based on manufacturer's analysis.