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In vitro evaluation of yacon (*Smallanthus sonchifolius*) tuber flour prebiotic potential

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ABSTRACT

Yacon [*Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson; *Asteraceae*] roots have been shown to be a source of prebiotic compounds. However, there are no known studies concerning processed yacon roots. The objective of this study was to investigate the potential prebiotic activity of yacon tuber flour. For this purpose, an aqueous extract was tested for selection of yacon incorporation and sterilization method and selection of the most favourable concentration to be tested for prebiotic activity. Once these conditions were identified, the potential prebiotic activity of the yacon extract was evaluated by determination of viable cell numbers and metabolic activity against four probiotic strains, namely, *Enterococcus faecium* 32, *Bifidobacterium animalis* Bo, *Lactobacillus acidophilus* Ki and *Lactobacillus casei* L26). Results showed that the best incorporation and sterilization method was to autoclave the supernatant, resultant from the yacon tuber flour suspension, at 121 °C for 20 min and add it to sterilized basal medium. For the confirmation of potential prebiotic activity, de Man–Rogosa–Sharpe (MRS) medium without a conventional carbon source (negative control), with 2% (w/v) glucose *per se* (positive control) and associated with 1% (w/v) yacon tuber flour were chosen. Yacon tuber flour revealed a potential prebiotic activity upon the growth of the probiotic strains tested, probably due to its fructooligosaccharides (FOS) content.

1. Introduction

A dietary prebiotic is a selectively fermented ingredient that results in “selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host” (Roberfroid et al., 2010). Their beneficial effects for the consumers are not only limited to the growth stimulation of beneficial intestinal microbiota, but they have also been associated with several other health benefits, including the modulation of the immune system (Wang et al., 2012), regulation of metabolic disorders related to obesity and increase in the bioavailability of minerals, among others (Charalampopoulos and Rastall, 2012). From a technological standpoint, prebiotics also play several roles, particularly in the formulation of functional foods, which are associated with demonstrated physiological benefits (Al-Sheraji et al., 2013). For example, the sweetening power of some of these ingredients (e.g. inulin) may be used in the development of low calorie or low sugar products, since the amount of sugar added in their formulation can be lower (Cruz et al., 2013), and they have also been used as fat replacers (Pimentel et al., 2013).

Yacon [*Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson; *Asteraceae*], which is classified as a fruit in local Andean

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markets, has tubers with delicious sweet flavour that may be consumed peeled in fruit salads, steamed or fried.

Yacon is a productive crop with root dry matter (DM) yields in soils of moderate fertility exceeding 10 t/ha in 6–8 mo (Hermann et al., 1999), it is effortless to grow under hot or cold conditions and has no problems with pests or diseases due to protective effects of its di- and sesquiterpenes (Lachman et al., 2003). For these reasons yacon can be considered a promising and interesting crop with high economic and environmental value.

The major storage compounds in yacon tubers are fructans with low glucose content (Valentová and Ulrichová, 2003); it also lacks starch, which makes it potentially beneficial in the diet of diabetics (Yan et al., 1999). The fructans' structure is rich in inulin-type compounds, i.e. β (2 → 1) fructofuranosylsaccharose, similar to other *Asteraceae* species, e.g. Jerusalem artichoke (Valentová and Ulrichová, 2003).

Despite its high fructan productivity, yacon is unlikely to become a source of purified dietetic sweeteners or fructose products due to the lack of industrial scale production and protectionism of sugar markets (Hermann et al., 1999). So, it is important to explore more relevant biological properties of yacon in order to stimulate the industry to valorize its fructan richness.

Having tissues rich in FOS with low degree of polymerization, yacon may be a potential prebiotic source. Yacon roots FOS have indeed been shown to be metabolized by bifidobacteria (Pedreschi et al., 2003) or to promote the growth of bifidobacteria and lactobacilli, and the consequent production of short-chain fatty acids (SCFAs) (Campos et al., 2012). However, these studies have been conducted with yacon roots and, to the best of our knowledge, there are no known studies concerning processed yacon roots.

So, the main objective of this research work was to study the potential prebiotic properties of yacon tuber flour. For this purpose, the selection of the most favourable yacon incorporation and sterilization method was initially performed, followed by the selection of the yacon tuber flour concentration to be used in further studies, where the evaluation of prebiotic potential was performed by determination of viable cell numbers and associated metabolic activity.

2. Materials and methods

2.1. *Yacon tuber flour*

Yacon tuberous roots were obtained directly from the local producers at Campinas (SP) city, Brazil. Afterwards, they were carefully washed under running water, packed in cotton fiber bags and autoclaved at 121 °C for 20 min. Subsequently, sterilized samples were cooled down at room temperature, peeled, cross-cut and homogenized. Next, they were packed in inox trays, frozen and lyophilized. The lyophilized material was then mashed in a mortar, thus obtaining yacon tuber flour. Its composition expressed on a dry matter basis (g/100 g), included approximately, 2.56 protein, 0.59 lipids, 93.12 carbohydrates and 3.73 ash content.

2.2. *Microorganisms*

The strains used to evaluate potential prebiotic activity of yacon tuber flour included two *Enterococcus* strains isolated from traditional "Terrincho" cheese and identified as *Enterococcus faecium* 32 and *E. durans* 37, with validated safety and probiotic potential (Pimentel et al., 2012, 2003), and several commercial probiotic strains—*Lactobacillus acidophilus* Ki and LAFTI® L10, *Lactobacillus casei* ssp. *paracasei* LAFTI® L26, *Bifidobacterium animalis* Bb12 and Bo, and *B. animalis* ssp. *lactis* LAFTI® B94. *L. acidophilus* Ki and *B. animalis* Bo, previously isolated from fermented milk, were obtained from CSK (The Netherlands), as ultrafrozen concentrates; *B. animalis* Bb12 was obtained from Christian Hansen (Denmark) as lyophilized cultures, and *L. acidophilus* L10, *L. casei* L26 and *B. animalis* B94 were obtained from DSM Food Specialties (Australia) as freeze-dried concentrated starter cultures.

The aforementioned microorganisms were reactivated, and pre-cultures were prepared in de Man–Rogosa–Sharpe (MRS; Biokar Diagnostics, France) broth and incubated overnight at 37 °C. Except for *E. faecium* 32, *E. durans* 37 and *L. casei* L26, MRS was supplemented with filter-sterilized 0.5 g/L of L-cysteine·HCl (Fluka, Switzerland) to lower the redox potential, and incubated in a plastic anaerobic jar with an AnaeroGen sachet (an atmosphere generation system from Oxoid, England), to achieve anaerobic conditions.

2.3. *Media*

The basal medium used for the evaluation of prebiotic properties of the yacon tuber flour was MRS broth prepared by mixture of the different compounds, in order to enable carbon source substitution, i.e. 10 g/L of tryptone (Sigma-Aldrich, USA), 8 g/L of meat extract (Merck, Germany), 4 g/L of yeast extract (Biokar Diagnostics), 2 g/L of di-potassium hydrogen phosphate (Merck), 1 g/L of tween 80 (Merck), 5 g/L of sodium acetate (Merck), 2 g/L of ammonium citrate tribasic (Sigma-Aldrich), 0.2 g/L of magnesium sulfate (Merck), 0.04 g/L of manganese sulfate (Sigma-Aldrich) and 20 g/L of respective carbon source. Based on MRS composition three different basal media were prepared, i.e. MRS without conventional carbon source, MRS with 2% (w/v) glucose (Fluka) and MRS with 2% (w/v) lactose (Merck, Germany). These were combined with either 2% (w/v) FOS (Orafti® P95; Orafti, Belgium) or [0.5, 1 and 2% (w/v)] of yacon tuber flour. Glucose at 2% (w/v) was selected since it is the carbon source (and concentration) present in the commercial growth medium (MRS) for the growth of probiotic microorganisms. Lactose and FOS were also employed, since lactose is also a sugar that these bacteria preferentially metabolize, and FOS is a traditional commercial prebiotic, known to have a growth enhancement effect upon these probiotics. Both glucose and lactose were used, in order to assess the role of yacon tuber flour as a substitute (when present as the single carbon source) or as an additive carbon source (in addition to the ones already present in the growth media). FOS was used as a reference prebiotic to compare with the prebiotic potential of the yacon tuber flour.

Except for *E. faecium* 32, *E. durans* 37 and *L. casei* L26, all media were supplemented with filter-sterilized 0.5 g/L of L-cysteine·HCl and incubated at 37 °C under anaerobic conditions as previously described.

2.4. *Evaluation of prebiotic activity*

The evaluation of the potential prebiotic activity of yacon tuber flour included three subsequent steps, namely: (i) a preliminary screening of the yacon addition and sterilization method in order to select the protocol, which would have no effect on the structure and activity of yacon tuber flour for further experiments; (ii) three different concentrations of yacon