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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) fructofuranosyl-saccharose, 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

tuber flour were then tested upon 8 probiotic strains in order to select the most favourable concentration to be confirmed later by measurement of viable cell numbers and metabolic activity of selected bacterial strains; and (iii) potential prebiotic activity was finally assessed upon four probiotic strains, i.e. *E. faecium* 32, *B. animalis* Bo, *L. acidophilus* Ki and *L. casei* L26 in MRS without conventional carbon source, with 2% (w/v) glucose and with 2% (w/v) glucose associated with 1% (w/v) yacon tuber flour. Each of these three steps is detailed in the following sections.

2.4.1. *Selection of yacon incorporation and sterilization method*

In order to assess the best yacon incorporation and sterilization method the growth of *E. faecium* 32 and *E. durans* 37 in basal MRS—without conventional carbon source, with 2% (w/v) glucose and with 2% (w/v) lactose, was compared with yacon solution (1% (w/v)) added according to three different sterilization methods and with 1% (w/v) FOS. The FOS had a purity of 93.2% (with glucose, fructose and sucrose as impurities) and a DP between 2 and 8. At this stage only *Enterococcus* strains were utilized, as they are less fastidious microorganisms.

In what concerns the sterilization strategy, a 5% (w/v) yacon solution was centrifuged at 4.000 rpm for 25 min. The resulting supernatant was added to the basal medium at 1% (w/v) according to three different treatments: (i) filter-sterilized and added to sterilized basal media, (ii) autoclaved at 121 °C for 20 min and added to sterilized basal media, and (iii) addition to basal media and autoclaved at 121 °C for 20 min.

Media were then inoculated with 2% (v/v) of *E. faecium* 32 and *E. durans* 37 (grown overnight at 37 °C in MRS with 2% (w/v) glucose) and transferred (in triplicate) to a 96-well microplate. The microplate was then incubated during 24 h at 37 °C and monitored by measuring absorbance (Abs) at 655 nm (680 Microplate Reader; Bio-Rad, USA), using Microplate Manager 5.2.1 software (Bio-Rad). Specific growth rates were determined (by determination of the slope of the trend line, of the viable cell numbers in the log phase of the growth curves) and maximum growth (maximum absorbance) was assessed, to compare the results of the different growth conditions.

All subsequent tests were carried out with the simplest sterilization method that least affected yacon structure and functionality, i.e. autoclaving of yacon supernatant at 121 °C for 20 min followed by addition to sterilized basal media.

2.4.2. *Selection of yacon tuber flour concentration*

In order to select the yacon tuber flour concentration that exhibited the best growth-promoting activity a screening in microplate was performed according to a factorial plan encompassing three different media—MRS without conventional carbon source, with 2% (w/v) glucose and with 2% (w/v) lactose, with three different concentrations of yacon tuber flour (0.5%, 1% and 2% (w/v)), and compared with 1% (w/v) FOS. Each medium was inoculated with the aforementioned eight probiotic strains.

A 10% (w/v) yacon tuber flour solution was centrifuged at 4.000 rpm for 25 min. The resulting supernatant was autoclaved at 121 °C for 20 min and added to sterilized basal media (MRS—without conventional carbon source, with 2% (w/v) glucose and with 2% (w/v) lactose) at 0.5, 1 and 2% (w/v). Each experimental medium was then inoculated at 2% (v/v) with each probiotic bacterium and transferred (in triplicate) to a 96-well microplate. 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 the wells were covered with autoclave-sterilized liquid paraffin (Merck, Germany), to avoid the presence of oxygen. The microplate was then incubated during 24 h at 37 °C and monitored by measuring Abs at 655 nm. Specific growth rates were calculated (by determination of the slope of the trend line, of the viable cell numbers in the log phase of the growth curves) and maximum growth (maximum absorbance) was assessed, in order to compare the obtained results.

2.4.3. *Evaluation of prebiotic activity by determination of viable cell numbers and metabolic activity*

For the confirmation of potential prebiotic activity by growth and metabolic activity achievements, four probiotic strains were chosen, namely *E. faecium* 32, *B. animalis* Bo, *L. acidophilus* Ki and *L. casei* L26. Different probiotic bacteria were tested, since the carbon source utilization can differ, according to the microorganism. Growth curves were monitored by enumeration of viable cell numbers (and growth rates were determined as described above) as well as by optical density assessment, and bacterial metabolism was assessed by pH measurement and determination of organic acids production and sugars consumption by High Performance Liquid Chromatography (HPLC) analysis. Optical density was measured at 650 nm, using an Ultraviolet–visible (UV–vis) spectrophotometer (UV mini 1240; Shimadzu, Japan) in the range of 0–0.8. For this assay, two replicas were analysed.

In what concerns the growth curves of *E. faecium* 32 and *L. casei* L26, MRS without conventional carbon source and with 2% (w/v) glucose—per se or associated with 1% (w/v) yacon flour, were inoculated at 2% (v/v), in duplicate. Inoculated media were then transferred to 10 mL sterile tubes, incubated in a water bath at 37 °C under agitation, and sampled at 0, 4, 6, 8, 10, 24 and 48 h. For *B. animalis* Bo and *L. acidophilus* Ki some modifications were included: in order to assure anaerobic conditions some complementary procedures were performed, namely, media were supplemented with filter-sterilized 0.5 g/L of L-cysteine·HCl, the sterile tubes were filled to the top (to avoid the presence of oxygen), and sampling at 6 h was replaced by 12 h of incubation. At each sampling time, decimal dilutions were prepared byusing aqueous 1 g/L peptone (Sigma-Aldrich) added with 8.5 g/L sodium chloride (Panreac, Spain). Bacterial populations were enumerated according to Miles and Misra (1938) method on MRS agar plates (Biokar Diagnostics) (supplemented with filter-sterilized 0.5 g/L of L-cysteine·HCl, in the case of *B. animalis* Bo and *L. acidophilus* Ki), in duplicate, following appropriate incubation.

HPLC analysis was performed according to Zeppa et al. (2001), with slight changes—*viz.* 2 g of each sample were diluted in 10 mL of sulphuric acid 13 mM (95–97% (p.a.), from Merck), homogenized with an Ultra-Turrax (T18 Basic; IKA Works, Inc, USA) at 18.000 rpm for 3 min and centrifuged at 4.000 rpm for 10 min at 4 °C (Universal 32R; Hettich, Germany). The resulting supernatant was then filtered with filter paper no. 1 (V. Reis, Portugal), and immediately prior to injection, with a 0.22 μm filter (Orange Scientific, Belgium).

The HPLC system consisted of a LaChrom L-7100 pump (Merck-Hitachi, Germany); an ion exchange Aminex HPX-87H Column (300 × 7.8 mm) (Bio-Rad), which was maintained at 65 °C (L-7350 Column Oven; LaChrom, Merck-Hitachi); and two detectors in series, refractive index (L-7490 RI Detector; LaChrom, Merck-Hitachi) to determine sugars and spectrophotometry to analyse organic acids (220 nm) (L-7400 UV

Detector; LaChrom, Merck-Hitachi). The mobile phase used was 13 mM sulphuric acid at a flow rate of 0.8 mL/min. The running time was 30 min, and the injection volume was 50 μL. Data were collected and analysed by a D-7000 Interface (LaChrom, Merck-Hitachi) and HPLC System Manager 3.1.1 software (Merck-Hitachi). For each of the different conditions tested two samples were analyzed, and each sample was injected in duplicate.

2.5. Statistical analyses

Statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL, USA). The homoschedascity assumption was met, hence analysis of variance (ANOVA), with a 95% confidence interval, was applied to every dependent parameter, in order to assess differences between the different media tested. The Tukey test, with a 95% confidence interval, was used for the pair-wise comparisons. Correlation between the parameters was determined by Spearman's rho, with a 99% confidence interval.

3. Results

3.1. Evaluation of prebiotic activity

3.1.1. Selection of yacon incorporation and sterilization method

The results (Table 1) pertaining to the selection of the yacon incorporation and sterilization method revealed that there were no significant differences between the maximum growth and specific growth rates of the different incorporation and sterilization methods. Based on this observation, and also because of its practicality, the method where the supernatant, obtained from the yacon solution centrifuged at 4.000 rpm for 25 min, was autoclaved at 121 °C for 20 min and added to sterilized basal media was selected for further studies.

3.1.2. Selection of yacon tuber flour concentration

The results concerning the selection of yacon tuber flour concentration (Table 2), for both *Enterococcus* strains, revealed that yacon tuber flour extract had a more effective growth-promoting effect than FOS, when added as the sole carbon source to MRS culture medium. When used as an additional carbon source, besides glucose or lactose, no significant improvement ($p > 0.05$) was observed in comparison with a conventional carbon source. Regarding *Lactobacillus* strains, yacon tuber flour extract also showed a more effective growth-promoting effect than FOS upon *L. acidophilus* Ki, and a similar effect on *L. casei* L26, when added as the sole carbon source to MRS. Unlike the *Enterococcus* strains, *Lactobacillus* strains showed some differences ($p < 0.05$) between the three yacon tuber flour concentrations tested as far as growth was concerned, and differences were most relevant for *L. acidophilus* L10 with yacon tuber flour at 2% (w/v) showing the best growth-promoting effect. Similarly to *Enterococcus* strains, when yacon tuber flour was used as an additional carbon source, besides glucose or lactose, there was no significant ($p > 0.05$) improvement in growth behavior when compared with a conventional carbon source. The microplate system used in this screening step probably did not allow attaining the complete absence of oxygen (even though paraffin was used for that purpose) and therefore no growth of *Bifidobacterium* was observed. Taking into account the abovementioned results, MRS without conventional carbon source and MRS with—*per se* or with 2% (w/v) glucose associated with 1% (w/v) yacon tuber flour extract were chosen for the confirmation of prebiotic activity in the next experiment.

3.1.3. Evaluation of prebiotic activity by determination of viable cell numbers and metabolic activity

The prebiotic activity of yacon tuber flour extract (YF1%) was studied on 4 strains in either basal MRS medium without glucose (MRS-WG) or MRS containing 2% (w/v) glucose (MRS-G); specific growth rates are listed in Table 3, whereas viable cell numbers and metabolic activity are represented in Table 4 and Fig. 1. In Table 4 only the 0, 10 and 24 h results are shown (48 h is not included in the table, since no relevant changes were observed between 24 and 48 h).

E. faecium 32 specific growth rates (Table 3) and viable cell numbers throughout 48 h incubation (Fig. 1a) revealed that MRS-WG medium alone or containing yacon tuber flour at 1% (w/v) as the sole carbon source (MRS-WG + YF1%), had a similar behavior ($p > 0.05$, no significant differences were observed except for CFU/mL at 10 and 48 h), and were the best growth media among the four tested. Absorbance values (Fig. 1b) presented a different scenario: MRS-WG + YF1% was the best growth medium whereas MRS-WG showed the worst behavior. Although absorbance values were similar for the first 4 h of incubation, after 4 h the absence of an available carbon source led to a decrease in the growth curve. Variation in sugars' concentrations (Table 4) showed that in MRS-WG + YF1%, fructose was consumed within the first 6 h, which is in agreement with the results obtained for viable cell numbers, absorbance and pH variation (Fig. 1 a and b, and Table 4, respectively). In MRS-G alone or containing yacon flour as an additional carbon source (MRS-G + YF1%) a similar behavior in the sugar consumption profile (Table 4) was observed. Most of the glucose and fructose present in the media were consumed within the first 12 h. The variation in pH values showed a similar trend to the one obtained for absorbance values but in an opposite direction, i.e. MRS-WG + YF1% revealed the sharpest decrease in pH during the logarithmic growth phase, whereas MRS-WG reported less than 0.5 units variation in pH. There was, as expected, a high negative correlation ($r = -0.926$) between pH variation and the variation in lactic acid concentration (Table 4). Citric acid (Table 4) was almost totally consumed within the first 4 h of incubation, corresponding to the period when growth was observed. Furthermore, in MRS-WG + YF1%, citric acid consumption was also registered, but to a less extent. Strangely, acetic acid concentration (Table 4) remained virtually stable, except for a slight decrease in MRS-WG + YF1%, a very slight increase in MRS-WG, and an even slighter one in MRS-G + YF1%.

Specific growth rates (Table 3) and viable cell numbers (Fig. 1c) of *L. casei* L26 showed that MRS-WG + YF1% reported a similar behavior ($p > 0.05$) to that of MRS-G. Furthermore, in MRS-G + YF1%, there was no significant improvement ($p > 0.05$) in growth enhancing capacity (specific growth rates and viable cell numbers) when compared with either MRS-G or MRS-WG + YF1%. These results are in agreement with the pH results (Table 4), but only partially with the ones obtained for absorbance measurements (Fig. 1d). MRS-WG + YF1%, originated lower absorbance values (at maximum growth and stationary phases) and higher pH values than MRS-G, whereas MRS-G + YF1% generated similar values to the ones obtained in MRS-G. As expected, the variation in absorbance values was positively correlated ($r = 0.951$) with the variation in lactic acid concentration (Table 4) and negatively correlated with

Table 1 – Specific growth rates and maximum growth for the two *Enterococcus* strains tested in the different media, with different yacon sterilization and incorporation methods: (i) filter-sterilized and added to sterilized basal media, (ii) autoclaved at 121 °C for 20 min and added to sterilized basal media, and (iii) addition to basal media and autoclaved at 121 °C for 20 min.

Strain	Yacon sterilization and incorporation method	Specific growth rate (h^{-1})			Maximum growth (absorbance)		
		Medium carbon source %(w/v)			Medium carbon source %(w/v)		
		Without	Glucose 2%	Lactose 2%	Without	Glucose 2%	Lactose 2%
E. faecium 32	No yacon	0.083 ± 0.009^{ab}	0.036 ± 0.003^{a}	0.054 ± 0.002^{a}	1.12 ± 0.01^{a}	1.26 ± 0.08^{a}	1.07 ± 0.04^{a}
	i	0.094 ± 0.019^{a}	0.035 ± 0.000^{a}	0.052 ± 0.006^{a}	1.07 ± 0.05^{a}	1.27 ± 0.03^{a}	1.03 ± 0.06^{a}
	ii	0.080 ± 0.006^{ab}	0.031 ± 0.003^{a}	0.046 ± 0.007^{a}	1.10 ± 0.03^{a}	1.21 ± 0.04^{a}	1.09 ± 0.03^{a}
	iii	0.084 ± 0.001^{ab}	0.020 ± 0.006^{b}	0.052 ± 0.006^{a}	1.13 ± 0.00^{a}	1.12 ± 0.07^{a}	1.08 ± 0.00^{a}
	FOS 2%	0.068 ± 0.003^{b}	0.032 ± 0.002^{a}	0.040 ± 0.005^{a}	1.00 ± 0.06^{a}	1.20 ± 0.03^{a}	1.01 ± 0.00^{a}
E. durans 37	No yacon	0.105 ± 0.010^{a}	0.080 ± 0.015^{a}	0.097 ± 0.008^{ab}	0.95 ± 0.02^{a}	1.16 ± 0.02^{a}	1.08 ± 0.05^{a}
	i	0.145 ± 0.010^{bc}	0.081 ± 0.002^{a}	0.106 ± 0.001^{a}	1.14 ± 0.09^{b}	1.13 ± 0.03^{a}	1.02 ± 0.03^{ab}
	ii	0.150 ± 0.002^{c}	0.068 ± 0.008^{ab}	0.078 ± 0.008^{bc}	1.18 ± 0.03^{b}	1.11 ± 0.04^{a}	0.94 ± 0.01^{c}
	iii	0.137 ± 0.026^{abc}	0.054 ± 0.007^{b}	0.077 ± 0.007^{c}	1.13 ± 0.04^{bc}	1.10 ± 0.09^{a}	0.94 ± 0.02^{bc}
	FOS 2%	0.111 ± 0.011^{ab}	0.070 ± 0.006^{ab}	0.077 ± 0.010^{c}	1.01 ± 0.03^{ac}	1.03 ± 0.09^{a}	1.00 ± 0.01^{bc}

Note: For each strain and each medium carbon source, values with the same superscript letters show no significant difference ($p < 0.05$).

pH evolution ($r = -0.932$) (Table 4). The variation in lactic acid concentration was negatively correlated with pH evolution ($r = -0.948$). In addition, in MRS-WG + YF1% maximum growth was reached after 6 h whereas, for both MRS-G and MRS-G + YF1% it only occurred after 10 h (Fig. 1c and d). These results are in accordance with the ones obtained for sugars' concentrations variation (Table 4) where yacons' fructose is consumed within 6 h in MRS-WG + YF1%, while for MRS-G, glucose is mostly consumed within the first 10 h. In MRS-G + YF1% the highest rate of consumption of both sugars occurred within the first 10 h. Regarding acetic and citric acids (Table 4), a slight increase in acetic acid was observed in both MRS-WG

Table 2 – Specific growth rates and maximum growth for the *Enterococcus* and *Lactobacillus* strains tested in the different media, with different yacon concentrations.

Strain	Added carbon source %(w/v)	Specific growth rate (h^{-1})			Maximum growth (absorbance)		
		Medium carbon source %(w/v)			Medium carbon source %(w/v)		
		Without	Glucose 2%	Lactose 2%	Without	Glucose 2%	Lactose 2%
E. faecium 32	None	0.134 ± 0.004^{a}	0.068 ± 0.006^{a}	0.116 ± 0.012^{a}	0.93 ± 0.03^{a}	1.14 ± 0.02^{a}	1.13 ± 0.07^{a}
	Yacon 0.5%	0.199 ± 0.003^{b}	0.063 ± 0.004^{ab}	0.111 ± 0.005^{ab}	1.18 ± 0.04^{b}	1.08 ± 0.03^{a}	1.10 ± 0.06^{ab}
	Yacon 1%	0.182 ± 0.004^{bc}	0.053 ± 0.006^{bc}	0.105 ± 0.004^{ab}	1.14 ± 0.01^{b}	1.05 ± 0.06^{a}	1.14 ± 0.03^{a}
	Yacon 2%	0.170 ± 0.011^{c}	0.041 ± 0.001^{c}	0.091 ± 0.002^{bc}	1.16 ± 0.04^{b}	1.03 ± 0.02^{a}	1.14 ± 0.03^{a}
	FOS 2%	0.124 ± 0.011^{a}	0.054 ± 0.005^{b}	0.079 ± 0.012^{c}	0.85 ± 0.05^{a}	1.01 ± 0.09^{a}	0.98 ± 0.08^{b}
E. durans 37	None	0.086 ± 0.003^{a}	0.066 ± 0.002^{a}	0.082 ± 0.005^{ab}	0.87 ± 0.06^{a}	1.13 ± 0.02^{a}	1.02 ± 0.01^{a}
	Yacon 0.5%	0.136 ± 0.002^{b}	0.065 ± 0.001^{a}	0.087 ± 0.002^{bc}	1.16 ± 0.03^{b}	1.17 ± 0.04^{a}	1.08 ± 0.05^{ab}
	Yacon 1%	0.143 ± 0.005^{b}	0.058 ± 0.003^{ab}	0.097 ± 0.004^{c}	1.19 ± 0.03^{b}	1.14 ± 0.03^{a}	1.18 ± 0.02^{c}
	Yacon 2%	0.130 ± 0.015^{b}	0.050 ± 0.005^{b}	0.082 ± 0.007^{ab}	1.25 ± 0.06^{b}	1.14 ± 0.04^{a}	1.15 ± 0.05^{bc}
	FOS 2%	0.095 ± 0.03^{a}	0.057 ± 0.005^{ab}	0.071 ± 0.004^{a}	0.92 ± 0.03^{a}	1.08 ± 0.08^{a}	1.03 ± 0.03^{a}
L. casei L26	None	0.032 ± 0.007^{a}	0.103 ± 0.008^{a}	0.082 ± 0.002^{ab}	0.99 ± 0.07^{a}	1.77 ± 0.08^{a}	1.82 ± 0.04^{a}
	Yacon 0.5%	0.061 ± 0.009^{b}	0.099 ± 0.004^{ab}	0.070 ± 0.004^{a}	1.26 ± 0.04^{b}	1.82 ± 0.07^{a}	1.52 ± 0.08^{b}
	Yacon 1%	0.103 ± 0.009^{c}	0.103 ± 0.004^{a}	0.074 ± 0.002^{a}	1.52 ± 0.08^{c}	1.73 ± 0.05^{a}	1.63 ± 0.02^{bc}
	Yacon 2%	0.122 ± 0.002^{c}	0.089 ± 0.005^{b}	0.091 ± 0.004^{b}	1.70 ± 0.00^{d}	1.87 ± 0.04^{a}	1.77 ± 0.02^{ac}
	FOS 2%	0.132 ± 0.019^{c}	0.104 ± 0.000^{a}	0.076 ± 0.010^{a}	1.70 ± 0.05^{d}	1.82 ± 0.03^{a}	1.62 ± 0.12^{bc}
L. acidophilus Ki	None	0.025 ± 0.005^{a}	0.046 ± 0.005^{a}	0.039 ± 0.006^{a}	0.96 ± 0.03^{a}	1.46 ± 0.09^{a}	1.10 ± 0.05^{a}
	Yacon 0.5%	0.044 ± 0.003^{b}	0.047 ± 0.003^{a}	0.043 ± 0.003^{ab}	1.23 ± 0.07^{b}	1.44 ± 0.07^{a}	1.22 ± 0.10^{ab}
	Yacon 1%	0.060 ± 0.004^{c}	0.051 ± 0.007^{a}	0.054 ± 0.007^{b}	1.35 ± 0.00^{c}	1.50 ± 0.02^{a}	1.32 ± 0.05^{b}
	Yacon 2%	0.059 ± 0.008^{c}	0.042 ± 0.006^{a}	0.039 ± 0.006^{a}	1.42 ± 0.00^{c}	1.44 ± 0.07^{a}	1.23 ± 0.09^{ab}
	FOS 2%	0.041 ± 0.006^{b}	0.041 ± 0.004^{a}	0.036 ± 0.002^{a}	1.13 ± 0.02^{d}	1.43 ± 0.06^{a}	1.11 ± 0.04^{a}
L. acidophilus L10	None	0.036 ± 0.006^{a}	0.138 ± 0.003^{a}	0.114 ± 0.001^{a}	0.88 ± 0.07^{a}	1.94 ± 0.02^{a}	1.73 ± 0.08^{a}
	Yacon 0.5%	0.079 ± 0.003^{b}	0.133 ± 0.008^{ab}	0.128 ± 0.013^{a}	1.22 ± 0.11^{b}	1.90 ± 0.06^{a}	1.81 ± 0.11^{a}
	Yacon 1%	0.095 ± 0.001^{c}	0.122 ± 0.006^{bc}	0.125 ± 0.002^{a}	1.27 ± 0.02^{b}	1.86 ± 0.05^{a}	1.87 ± 0.04^{a}
	Yacon 2%	0.129 ± 0.003^{d}	0.116 ± 0.007^{c}	0.120 ± 0.002^{a}	1.55 ± 0.04^{c}	1.84 ± 0.03^{a}	1.81 ± 0.04^{a}
	FOS 2%	0.053 ± 0.003^{e}	0.129 ± 0.004^{abc}	0.122 ± 0.004^{a}	1.02 ± 0.07^{a}	1.87 ± 0.02^{a}	1.82 ± 0.02^{a}

Note: For each strain and each medium carbon source, values with the same superscript letters show no significant difference ($p < 0.05$).

Table 3 – Specific growth rates for the four probiotic strains tested in the different carbon sources – glucose and yacon flour, simple or in combination, added to basal MRS culture medium.

Medium carbon source %(w/v)	Specific growth rate (h^{-1})			
	E. faecium 32	*L. casei* L26	*L. acidophilus* Ki	*B. animalis* Bo
Without glucose	0.487 ± 0.018^{a}	0.192 ± 0.000^{a}	0.110 ± 0.037^{a}	0.107 ± 0.030^{a}
Yacon 1%	0.470 ± 0.065^{a}	0.348 ± 0.019^{b}	0.164 ± 0.010^{ab}	0.242 ± 0.003^{b}
Glucose 2%	0.284 ± 0.026^{b}	0.355 ± 0.008^{b}	0.181 ± 0.010^{ab}	0.178 ± 0.003^{c}
Glucose 2% + Yacon 1%	0.275 ± 0.018^{b}	0.313 ± 0.020^{b}	0.212 ± 0.012^{b}	0.194 ± 0.005^{bc}

Note: For each strain, values with the same superscript letters show no significant difference ($p < 0.05$).

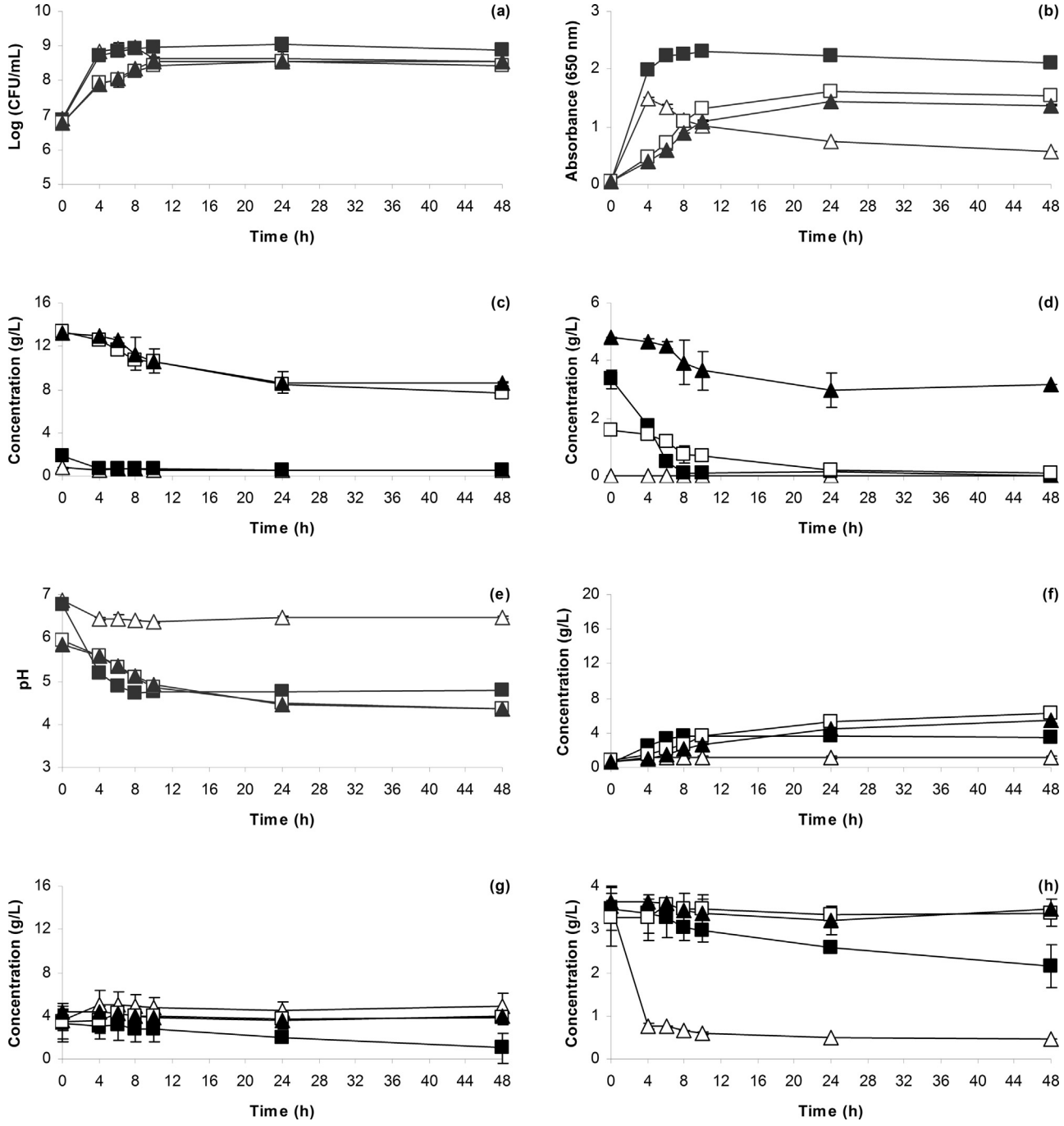

Fig. 1 – Growth curves in MRS-WG (△), MRS-WG + YF1% (■), MRS-G (□) and MRS-G + YF1% (▲), of *Enterococcus faecium* 32 (a and b), *Lactobacillus casei* L26 (c and d), *Lactobacillus acidophilus* Ki (e and f) and *Bifidobacterium animalis* Bo (g and h).

Table 4 – Variation of the pH, glucose, fructose, lactic, acetic and citric acids concentrations, for the four probiotic strains tested in the different MRS culture media.

Strain	Parameter	Incubation time (h)	MRS-WG	MRS-WG + YF1%	MRS-G	MRS-G + YF1%
E. faecium 32	pH	0	6.88 ± 0.04	6.78 ± 0.01	5.93 ± 0.03	5.84 ± 0.01
		10	6.36 ± 0.02	4.74 ± 0.01	4.86 ± 0.02	4.92 ± 0.02
		24	6.46 ± 0.05	4.76 ± 0.01	4.49 ± 0.01	4.47 ± 0.01
	Glucose	0	0.73 ± 0.04	1.82 ± 0.25	13.34 ± 0.01	13.28 ± 0.09
		10	0.58 ± 0.01	0.67 ± 0.01	10.62 ± 0.01	10.63 ± 1.08
		24	0.58 ± 0.02	0.59 ± 0.11	8.48 ± 0.27	8.65 ± 1.02
	Fructose	0	0.00 ± 0.01	3.37 ± 0.32	1.59 ± 0.01	4.81 ± 0.03
		10	0.00 ± 0.01	0.12 ± 0.01	0.71 ± 0.01	3.65 ± 0.66
		24	0.00 ± 0.01	0.17 ± 0.01	0.20 ± 0.01	2.98 ± 0.61
	Lactic acid	0	0.65 ± 0.06	0.42 ± 0.02	0.81 ± 0.02	0.64 ± 0.01
		10	1.19 ± 0.11	3.56 ± 0.56	3.60 ± 0.04	2.70 ± 0.06
		24	1.19 ± 0.13	3.58 ± 0.57	5.32 ± 0.15	4.45 ± 0.15
	Acetic acid	0	3.61 ± 1.00	3.37 ± 1.54	3.40 ± 1.78	4.39 ± 0.06
		10	4.74 ± 0.91	2.75 ± 1.12	3.97 ± 0.62	3.88 ± 0.68
		24	4.56 ± 0.72	2.03 ± 0.01	3.76 ± 0.34	3.51 ± 0.77
	Citric acid	0	3.55 ± 0.30	3.48 ± 0.51	3.28 ± 0.68	3.64 ± 0.03
		10	0.59 ± 0.02	2.98 ± 0.27	3.46 ± 0.33	3.38 ± 0.33
		24	0.49 ± 0.01	2.57 ± 0.01	3.34 ± 0.21	3.21 ± 0.35
L. casei L26	pH	0	6.67 ± 0.01	6.60 ± 0.03	5.90 ± 0.04	5.83 ± 0.01
		10	6.13 ± 0.01	4.47 ± 0.01	3.99 ± 0.01	3.98 ± 0.01
		24	6.20 ± 0.03	4.54 ± 0.01	3.81 ± 0.01	3.66 ± 0.01
	Glucose	0	0.77 ± 0.01	1.12 ± 0.27	13.18 ± 0.01	12.62 ± 0.39
		10	0.60 ± 0.09	0.58 ± 0.13	5.50 ± 0.64	7.82 ± 0.47
		24	0.66 ± 0.02	0.67 ± 0.01	3.30 ± 0.61	4.60 ± 0.47
	Fructose	0	0.00 ± 0.01	2.90 ± 0.01	1.28 ± 0.01	4.14 ± 0.17
		10	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	1.97 ± 0.37
		24	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	0.19 ± 0.04
	Lactic acid	0	1.15 ± 0.01	0.61 ± 0.01	1.49 ± 0.01	1.12 ± 0.05
		10	1.12 ± 0.12	5.28 ± 0.47	9.28 ± 0.01	8.68 ± 0.56
		24	1.19 ± 0.03	6.08 ± 0.34	11.42 ± 0.97	13.73 ± 0.36
	Acetic acid	0	5.51 ± 0.01	1.87 ± 0.59	3.93 ± 0.01	3.13 ± 0.20
		10	4.17 ± 0.67	3.50 ± 0.64	3.31 ± 0.01	2.99 ± 0.14
		24	5.10 ± 0.96	5.22 ± 0.34	3.27 ± 0.15	2.97 ± 0.03
	Citric acid	0	3.27 ± 0.01	2.53 ± 0.29	3.43 ± 0.01	3.16 ± 0.10
		10	1.06 ± 0.05	2.13 ± 0.58	3.05 ± 0.01	3.12 ± 0.10
		24	1.23 ± 0.33	1.07 ± 0.16	2.90 ± 0.08	3.00 ± 0.14
L. acidophilus Ki	pH	0	6.63 ± 0.02	6.53 ± 0.01	6.26 ± 0.02	6.20 ± 0.01
		10	6.04 ± 0.01	4.52 ± 0.01	4.27 ± 0.01	4.26 ± 0.01
		24	6.04 ± 0.02	4.57 ± 0.01	4.01 ± 0.04	4.00 ± 0.01
	Glucose	0	0.72 ± 0.01	1.64 ± 0.03	13.97 ± 0.95	14.59 ± 1.40
		10	0.61 ± 0.01	0.53 ± 0.03	7.08 ± 0.04	8.03 ± 0.97
		24	0.53 ± 0.01	0.00 ± 0.01	4.61 ± 0.42	5.24 ± 1.46
	Fructose	0	0.00 ± 0.01	3.15 ± 0.05	0.23 ± 0.06	2.99 ± 0.41
		10	0.00 ± 0.01	0.04 ± 0.02	0.10 ± 0.03	2.71 ± 0.39
		24	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	1.90 ± 0.23
	Lactic acid	0	0.58 ± 0.01	0.55 ± 0.01	0.67 ± 0.01	0.68 ± 0.01
		10	1.18 ± 0.01	5.37 ± 0.01	7.27 ± 0.01	7.49 ± 0.01
		24	1.24 ± 0.05	5.23 ± 0.11	9.72 ± 0.19	9.50 ± 0.17
	Acetic acid	0	5.09 ± 0.01	4.17 ± 0.01	4.40 ± 0.01	4.56 ± 0.01
		10	4.92 ± 0.01	4.79 ± 0.01	4.49 ± 0.01	4.84 ± 0.01
		24	3.91 ± 0.22	4.89 ± 0.03	4.08 ± 0.03	3.54 ± 0.09
	Citric acid	0	3.54 ± 0.01	3.30 ± 0.01	2.86 ± 0.01	3.58 ± 0.01
		10	3.36 ± 0.01	2.70 ± 0.01	2.63 ± 0.01	3.19 ± 0.01
		24	3.16 ± 0.11	0.77 ± 0.07	2.06 ± 0.07	2.39 ± 0.04
B. animalis Bo	pH	0	6.44 ± 0.03	6.35 ± 0.01	5.63 ± 0.01	5.53 ± 0.02
		10	6.30 ± 0.02	5.30 ± 0.03	4.99 ± 0.01	4.77 ± 0.01
		24	6.43 ± 0.02	5.24 ± 0.01	4.54 ± 0.03	4.45 ± 0.01
	Glucose	0	0.78 ± 0.03	1.70 ± 0.01	12.81 ± 0.01	13.20 ± 0.01
		10	0.80 ± 0.12	0.99 ± 0.13	10.92 ± 0.78	10.30 ± 0.48
		24	0.76 ± 0.14	0.71 ± 0.01	6.93 ± 0.78	6.83 ± 0.50
	Fructose	0	0.00 ± 0.01	3.13 ± 0.06	0.84 ± 0.16	3.20 ± 0.04
		10	0.00 ± 0.01	3.16 ± 0.22	1.39 ± 0.17	4.14 ± 0.30
		24	0.00 ± 0.01	3.25 ± 0.06	1.41 ± 0.14	4.16 ± 0.33
	Lactic acid	0	0.46 ± 0.03	0.39 ± 0.01	0.58 ± 0.01	0.52 ± 0.01
		10	0.50 ± 0.01	0.57 ± 0.01	0.94 ± 0.01	1.11 ± 0.05
		24	0.50 ± 0.01	0.59 ± 0.01	2.27 ± 0.12	2.51 ± 0.04

Table 4 – (*Continued*)

Strain	Parameter	Incubation time (h)	MRS-WG	MRS-WG + YF1%	MRS-G	MRS-G + YF1%
	Acetic acid	0	4.56 ± 0.19	3.93 ± 0.01	4.51 ± 0.06	3.91 ± 0.05
		10	5.06 ± 0.58	5.62 ± 0.44	6.66 ± 0.31	6.58 ± 0.02
		24	4.93 ± 0.82	7.12 ± 0.43	10.89 ± 0.25	10.86 ± 0.17
	Citric acid	0	3.32 ± 0.06	3.23 ± 0.01	2.93 ± 0.04	2.78 ± 0.03
		10	3.38 ± 0.35	3.17 ± 0.23	3.51 ± 0.19	3.36 ± 0.08
		24	3.18 ± 0.35	3.18 ± 0.04	3.62 ± 0.08	3.47 ± 0.18

and MRS-WG + YF1%, concomitant with a decrease in citric acid. The other two media showed no variation in both lactic and acetic acids' concentrations.

MRS-WG revealed, as expected, to be the less efficient growth medium independently of the parameter (specific growth rate or viable cell numbers) considered (Table 3 and Fig. 1c, respectively).

In what concerns *L. acidophilus* Ki, specific growth rates (Table 3) and viable cell numbers (Fig. 1e) showed a slightly better performance in MRS-G + YF1%, although it was only significantly different ($p < 0.05$) on MRS-WG (0.212 against 0.110, respectively). Upon 24 h, viable cell numbers decreased abruptly in all media except in MRS-WG + YF1%. Absorbance values (Fig. 1f), pH evolution (Table 4) and variation in lactic acid concentrations (Table 4) followed similar trends to those observed for viable cell numbers—all three culture media containing a carbon source revealed similar values, except for a slightly lower growth in MRS-WG + YF1%. Absorbance and pH values reported also showed that, in MRS-WG + YF1%, maximum growth was reached after 8 h, while for the two other mentioned media this only occurred after 12 h. In MRS-WG + YF1% fructose was totally consumed within the first 8 h and glucose was used within 12 h for the other two mentioned media (Table 4).

As previously observed, MRS-WG revealed to be the least efficient medium. It presented the lowest specific growth rate (Table 3), viable cell numbers (Fig. 1e) and there was practically no variation in both absorbance (Fig. 1f) and pH values (Table 4).

Results pertaining to *B. animalis* Bo (Fig. 1g and h, and Table 3) are distinguishable from those obtained for the other three strains under assessment; in fact, the largest impact of yacon tuber flour on bacterial growth was observed for *B. animalis* Bo. Its specific growth rate (Table 3) more than duplicated in MRS-WG + YF1% as compared to the control (MRS-WG) where no carbon source was readily available. In MRS-G + YF1%, the results were less favourable, but slightly better than in MRS-G (specific growth rate is almost 1.5 fold lower than in MRS-WG + YF1%, Table 3); nevertheless, it is important to note that results were not significantly ($p > 0.05$) different. MRS-WG revealed the worst results. These results uphold those obtained for viable cell numbers (Fig. 1g), where MRS-WG + YF1% and MRS-G + YF1%, were the first to reach maximum growth (10 h), followed by MRS-G (12 h). After 24 h a decrease in viable cell numbers was observed in all media. This decrease was more prominent in MRS-WG + YF1% and MRS-WG. Absorbance values (Fig. 1h) presented a different trend; the best results were obtained in MRS-G + YF1% (absorbance values increased up to 24 h) followed closely by MRS-G (absorbance values increased up to 48 h, time at which the same value was reached in both cases). In MRS-WG + YF1% *B. animalis* growth was not very high and it only grew until 12 h and no variation in fructose was detected (Table 4) throughout the incubation time. In MRS-G *B. animalis* Bo consumed the majority of glucose within the first 8 h; a similar trend was observed in MRS-G + YF1%. The acidification capacity reflected in the variation in pH values presents a similar behavior to that obtained by viable cell numbers. A higher increase was observed in acetate than in lactate concentrations (proportion of 3:1), and this occurred mainly in the MRS media where glucose was present. In what concerns citric acid (Table 4) no significant variation ($p > 0.05$) was observed in its concentrations, as expected.

4. Discussion

In this research work, the prebiotic potential of yacon tuber flour has been shown using an *in vitro* model evaluation. Differences in fermentation capacity of different carbon sources (both conventional and yacon tuber flour derived) were however denoted among species and reflect the specific metabolic behavior. It should be taken into account that the final products of fermentation depend on the substrate, in addition to the tested bacterial strain.

Fermentation of YF1%, reflected in the lactate/acetate production was highest for *L. casei* L26, followed closely by *L. acidophilus* Ki and *B. animalis* Bo; *E. faecium* 32 presented the lowest values. The production of these organic acids resulted from the specific and different fermentation metabolisms of the probiotic strains. *Lactobacillus* (Holzapfel and Schillinger, 2002; Østlie et al., 2003) and *Enteroccocus* (Domig et al., 2003; Vasiljevic and Shah, 2008) strains are homofermentative organisms and their main organic acid produced is lactic acid (Ray and Bhunia, 2008; Vasiljevic and Shah, 2008). Besides lactic acid, acetic acid can also be produced from degradation of citrate by citrate metabolism (Johnson and Steele, 1997; Freitas et al., 1999; Sarantinopoulos et al., 2001; Østlie et al., 2003), which was the case of MRS-WG + YF1% for *L. casei* L26 and *L. acidophilus* Ki, and MRS-WG for *E. faecium* 32. As a heterofermentative organism, *B. animalis* Bo produces hexose fermentation metabolism, and in addition to lactic acid also produces acetic acid (Vasiljevic and Shah, 2008). These results are in agreement with the sugars' consumption profiles, except for *B. animalis* Bo, which presented, apparently, somewhat unexpected results; Shah (2000) reported the utilization of both fructose and glucose by *Bifidobacterium* ssp, yet, this was not the case in our study. However, the results obtained can be explained by the ability of *B. animalis* Bo to ferment FOS from yacon tuber flour, similarly to what Pedreschi et al. (2003) found for *B. bifidum* ATCC 15696, utilizing Reinforced Clostridial Medium (RCM) with 1% glucose. The fermentation results are also in agreement with the pH values, as

the increase in organic acids' concentrations correlated with a decrease in the pH values of the different media.

In the present study it was shown that YF1% exerts a very significant effect on *B. animalis* Bo growth. This effect can be observed in the viable cells numbers and absorbance values, and it can be explained, as previously mentioned, by the capacity of *B. animalis* Bo to ferment FOS from yacon tuber flour. For both *L. casei* L26 and *L. acidophilus* Ki, YF1% enabled a growth similar to that of MRS-G, which can be a consequence of the presence of glucose and fructose in the YF1%, both of which can be metabolized by these probiotics, as already reported by Shah (2000), for *L. acidophilus*. For *E. faecium* 32, YF1% promoted a growth similar to that obtained for MRS-WG, which was the best growth medium for this strain, by providing available carbon sources (glucose and fructose).

As such, yacon tuber flour revealed a potential prebiotic effect upon growth of all the four probiotic strains tested, when used as a single carbon source. When used as an additional carbon source no growth enhancement was observed. In the presence of glucose, a simple carbon source, probiotics are able to metabolize it faster and reach maximum growth more quickly, not requiring the more complex polysaccharides from yacon tuber flour compounds to obtain energy. However, viable cell numbers when yacon tuber flour was used as a single carbon source were similar to those obtained when only the traditional carbon source was available. Moreover, yacon flour at 1% (w/v) was able to generate similar growth as that promoted by the traditional carbon source (glucose) at 2% (w/v).

5. Conclusion

Based on the above considerations it is possible to conclude that yacon tuber flour possesses interesting compounds that are easily metabolized and may stimulate the growth of beneficial bacteria, proving its potential as a prebiotic. Based on the results reported herein, i.e. enhancement of probiotic bacteria growth and metabolism by the increase of organic acid production, including acetate (SCFA), and combined with scientific evidences of yacon tuber safety and health effects, it can be stated that yacon tuber flour has the potential for being used by the food industry as an ingredient for the production of functional food products. This will stimulate the industry toward the valorization of yacon tubers as an added-value ingredient. Further studies are however required to prove yacon tuber flour's prebiotic potential with more diverse probiotic strains and in human volunteers and to establish the sensory profile and consumer acceptance of this product.

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