SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2024v45n1p131

Yacon (*Smallanthus sonchifolius*) beverage spontaneously fermented

Bebida de yacon (*Smallanthus sonchifolius*) fermentada espontaneamente

Carolina Paula Gouvêa de Souza[1]*; Ana Claudia Frasson Pretti[2]; Lílian Christiane da Silva Souza[2]; Luana dos Santos Nogueira[2]; Ana Carolina Bianco Gomes[1]; Rodrigo Scherer[3]; Mayara Fumiere Lemos[4]; Carmelita Zacchi Scolforo[5]; Patrícia Campos Bernardes[6]; Christiane Mileib Vasconcelos[3]

Highlights

Spontaneous fermentation enhanced the nutritional and functional value of beverages.
The beverages has had a considerable phenolic compounds amount as chlorogenic acid.
Beverage added citric acid exhibited a higher proportion of fructooligosaccharides.

Abstract

Yacon (*Smallanthus sonchifolius*) is a tuberous root with a high phenolic compounds and fibers content which has a prebiotic effect, both important for preventing and/or promoting the reduction of non-communicable chronic diseases risks. However, this root's shelf life is highly reduced and culminates the reduction of the beneficial to health compounds, which can be avoided by some strategies. In this sense, this work aimed to produce yacon beverages added with anti-browning agents (cysteine or citric acid) and evaluated the spontaneous fermentation during storage. Three yacon beverages have been produced with cysteine or citric acid (0.05% w/w) and a control beverage. Beverages were analyzed by microbiological counts, nutritional composition, physical-chemical characteristics, total phenolic compounds and phenolic acids, for 60 day storage. The data were evaluated by ANOVA and compared using the Duncan test ($p \leq 0.05$) or regression models were adjusted. Regarding microorganisms,

[1] Doctor's Students in Plant Biotechnology, Postgraduate Program in Plant Biotechnology, Food Biotechnology Laboratory, Universidade Vila Velha, UVV, Vila Velha, ES, Brazil. E-mail: carolina.gouvea2007@hotmail.com; anabiancogomes@gmail.com
[2] Nutrition Undergraduate Students, Universidade Vila Velha, UVV, Vila Velha, ES, Brazil. E-mail: claudia@pretti.com.br; lilianchriss@hotmail.com; luananogueira.nutri@gmail.com
[3] Profs. Drs., Postgraduate Program in Plant Biotechnology, Universidade Vila Velha, UVV, Vila Velha, ES, Brazil. E-mail: rodrigo.scherer@uvv.br; chrismileib@yahoo.com.br
[4] Drª in Pharmaceutical Sciences, Postgraduate Program in Pharmaceutical Sciences, Universidade Vila Velha, UVV, Vila Velha, ES, Brazil. E-mail: mayarafumierelemos@hotmail.com
[5] Profª. Drª, Nutrition Course, Universidade Vila Velha, UVV, Vila Velha, ES, Brazil. E-mail: carmelita.scolforo@uvv.br
[6] Prof. Dr., Postgraduate Program in Food Science and Technology, Food Engineering Department, Universidade Federal do Espírito Santo, UFES, Alegre, ES, Brazil. E-mail: paticbernardes@gmail.com
* Author for correspondence

Received: Aug. 01, 2023 Approved: Feb. 06, 2024

all beverages have reached 10^9 CFU.ml^{-1} after 30 day storage. To the anti-browning agents use, the differences were occasional; however, the citric acid beverage had a desirable pH (< 4.5) for a fermented beverage, which allows inhibition of undesirable bacteria and a higher proportional initial FOS amount and less variation with 30 and 60 day storage. However, the citric acid beverage have presented smaller amount of phenolic compounds like chlorogenic and gallic acids (230.37 ± 168.63 and 40.87 ± 1.32, respectively). So, we conclued that spontaneous fermentation was a preparation technique which has added nutritional and functionals values to the yacon beverage, in special to beverage added citric acid, with a higher proportion of FOS.
Key words: Fermentation. Anti-browning agents. Lactic acid bacteria. Fructooligosaccharides. Phenolic compounds.

Resumo

Yacon (*Smallanthus sonchifolius*), raiz tuberosa rica em compostos fenólicos e fibras, apresenta efeitos prebióticos benéficos na prevenção e/ou redução do risco de doenças crônicas não transmissíveis. No entanto, o seu curto prazo de validade leva a uma diminuição destes compostos promotores da saúde, o que pode ser evitado através de certas estratégias. Abordando isso, nosso estudo se concentrou na produção de bebidas de yacon com agentes antiescurecimento (cisteína ou ácido cítrico) e avaliação da fermentação espontânea durante o armazenamento. Foram elaboradas três bebidas de yacon: uma com cisteína outra com ácido cítrico (ambas com 0,05% p/p) e uma bebida controle. Durante um período de armazenamento de 60 dias, essas bebidas foram submetidas a análises de contagens microbiológicas, composição nutricional, características físico-químicas, compostos fenólicos totais e ácidos fenólicos. Os dados foram avaliados por meio da ANOVA e as comparações foram feitas pelo teste de Duncan ($p \leq 0,05$) ou por meio de modelos de regressão. Em relação aos microrganismos, todas as bebidas atingiram 10^9 UFC.mL^{-1} após 30 dias de armazenamento. O impacto dos agentes antiescurecimento variou, com a bebida de ácido cítrico mantendo um pH desejável (<4,5) propício para bebidas fermentadas, inibindo bactérias indesejáveis. Também apresentou maior quantidade proporcional inicial de frutooligossacarídeos e menor variação ao longo de 30 e 60 dias de armazenamento. No entanto, esta bebida continha teores mais baixos de compostos fenólicos, como ácidos clorogênico e gálico (230,37 ± 168,63 e 40,87 ± 1,32 µg.mL^{-1}, respectivamente). Concluindo, a fermentação espontânea provou ser uma técnica valiosa para aumentar o valor nutricional e funcional das bebidas de yacon, especialmente aquelas com adição de ácido cítrico, que apresentaram maior proporção de frutooligossacarídeos.
Palavras-chave: Fermentação. Agentes anti-escurecimento. Bactérias lácticas. Frutooligossacarídeos. Compostos fenólicos.

Introduction

Yacon (*Smallanthus sonchifolius*) belongs to the Asteraceae family originating in the Andes region and it has special interest properties in nutrition, medicine and health fields, as it provides nutrients and functionals compounds that promote health and well-being, and protective effect against diseases (Valentová & Ulrichová, 2003).

The main form of yacon consumption is related to the ingestion of tuberous roots *in natura,* which have a crunchy consistency and sweet taste (Simonovska et al., 2003; Reina et al., 2015). As for its composition, its fresh biomass consists of, in general, 83% to 90% of water, which reduces considerably its energy value (Santana & Cardoso, 2008).

Regarding dry matter, yacon roots have a large amount of fructooligosaccharides (FOS) and inulin, as well as phenolic compounds, such as chlorogenic acid, ferulic acid and caffeic acid (Pedreschi et al., 2003; Takenaka et al., 2003; Genta et al., 2005; Ojansivu & Lucia, 2011; M. D. F. Silva et al., 2017).

The phenolic compounds present in yacon may be at the origin of a few sensory inconveniences, due to their enzymatic or oxidative browning (J. A. R. Pereira et al., 2013; López-López et al., 2013). The enzymes can catalyze reactions with quinones, amino acids, peptides, and proteins, making the product visually less attractive because of the formation of dark pigments (Nicolas et al., 1993; Whitaker & Lee, 1995). This browning can be decelerated, or even inhibited, by adopting strategies which include the use of chemical agents, as citric acid or cysteine. These chemicals can act by mechanisms as lowering pH; complexing Cu^{2+} and Fe^{3+} cations existing in enzymas active site; stabilizing radicals and other chemical species formed during intermediates reaction steps, which prevents the formation of dark polymers (Ioannou & Ghoul, 2013).

FOS and inulin are non-digestible carbohydrates recognized as prebiotics, because they serve as fermentable substrates for the beneficial bacteria of intestinal colon (Macfarlane et al., 2006).

Given the yacon root composition, during its storage, occurs a fermentation that favors the microorganisms spontaneously growth, among them, lactic acid bacteria, with a prevalence of heterofermentation triggered by *Leuconostoc,* in a concentration of 2% NaCl, in addition to considerable amount of yeast (Reina et al., 2015).

Generally, lactic acid bacteria develop spontaneously in vegetables, with temperature, initial pH, nutrients and salt concentration (NaCl) being relevant factors for fermentation. Yeasts are more often used in alcoholic fermentation and, generally, they can ferment in both aerobic and anaerobic conditions (Chaud & Sgarbieri, 2006; A. M. Rodrigues & Sant'Anna, 2001).

The fermentation aims to improve food safety, nutritional value and sensory quality, and also increase storage time due to the metabolites production such as organic acids (lactic acid, acetic acid, formic acid and propionic acid), ethanol, bacteriocins, which can be associated with the addition of salt or drying to reduce water activity (Bourdichon et al., 2012; Reina et al., 2015).

So far, few or any studies have been identified on the physical-chemical, sensory

and microbiological characteristics triggered by the spontaneous fermentation of yacon root. In this context, the development of a beverage with functional claim properties, containing bioactive compounds, stimulating the microorganisms' spread with beneficial potential, can promote health benefits and become a product with innovative potential for consumers.

Therefore, the aim of the present study was to produce spontaneously fermented yacon beverages, using different anti-browning agents and to carry out the microbiological, nutritional, bioactive compounds and physical-chemical analysis during 60 days of storage.

Material and Methods

Experimental design

The experiment has been conducted following a completely randomized design (CRD) in a split-plot scheme, with anti-browning agents (cysteine, citric acid and control) located in the plot, and the storage time (0, 7, 15, 30, 45 and 60 days) in the subplots, with 3 repetitions, totaling 54 experimental units.

The citric acid and cysteine concentrations added in yacon beverages were 0.05% (w/v) defined according to preliminary tests and the study of Vasconcelos et al. (2015).

Preparation of yacon beverages

The roots were obtained from local market in the city of Vila Velha/ES. The preparation steps were based on the methodology described by Vasconcelos et al. (2015).

First of all, the roots have been selected to remove inappropriate parts for consumption, cleaned in running water and hand peeled. Then they have been cut into approximately 2 cm cylindrical slices and bleached at 100 ºC for 4 minutes with the amount anti-browning agent calculated for the yacon weight, considered 0.05% (w/w).

Afterwards, the slices were removed from the bleaching process and placed in a container containing water and ice in a ratio of 1:1 (w/v) yacon: water and ice. Then, the yacon was removed and ground with 2% NaCl, as used by Reina et al. (2015) in mixer (Walita Philips, Brazil). Then, the beverages were packed in impermeable plastic packaging, using vacuum sealer (Orved and Brock; Mod.-12), and kept refrigerated, approximately 10 °C during the storage time.

The control beverage has been prepared following all the steps, with exception of adding the anti-browning agent during the bleaching.

Microbiological count

In the microbiological analysis, were used selective culture media: *Plate Count Agar* (PCA) Biolog® for total bacteria, MRS *Lactobacillus* Kasvi® for lactic acid bacteria and Sabouraud Dextrose Kasvi® Agar for yeast and filamentous fungi. The media were prepared according to the manufacturer's guidance and the analyzes carried out according to the technique described by M. D. F. Silva et al. (2017), which consists of diluting the beverages in series, in saline solutions and plated. The counts were

expressed in Colony-Forming Units per gram of the sample (CFU/g).

Determination of nutritional composition

The moisture was determined by drying in an oven at 105 °C until constant weight; protein by the Kjeldahl method, using 5.75 as a nitrogen to protein conversion factor; ash by muffle incineration at 550 °C and total lipids by the Goldfish extraction method. The total carbohydrate content has been calculated by difference, subtracting from 100 the sum of the other nutritional composition constituents (moisture, protein, lipids and ashes) (Association of Official Analytical Chemists [AOAC], 2005).

The yacon beverage caloric value was calculated from the Atwater conversion factors: 4 $kcal.g^{-1}$ for proteins and carbohydrates and 9 $kcal.g^{-1}$ for lipids (Watt & Merrill, 1964).

Quantification of simple carbohydrates

Sucrose, fructose, glucose and mannitol from yacon fermented beverages have been determined using high performance liquid chromatography (HPLC) according to Evangelista et al. (2015) and C. F. Silva et al. (2013), with some adaptations.

Microtubes with 2 ml of each sample were placed in the centrifuge (Heraeus Megafuge 16R®) at 10,000 rpm for 10 minutes at 10 ºC. The supernatant was microfiltrated through a 0.2 µm cellulose acetate filter to remove the insoluble fraction. The sugar has been separated by a column (Aminez HPX-87C 250 cm x 4 mm) at 55 °C. A mobile phase consisting of 266 µl of sulfuric acid per liter of ultrapure water was used, at a 0.8 ml.minute-1 flow coefficient.

Twenty microliters of sample were injected and run for 35 min. Using the refractive index detector (RID), the compounds were identified by comparing the pattern retention times (mannitol, fructose, glucose and sucrose - Sigma Aldrich®) previously injected and quantified by the observed peak area in the chromatograms. The results have been expressed as the percentage of the peak area.

Physicochemical analysis

The pH was measured with the direct introduction of an electrode in the sample (AOAC, 2000). The content of total soluble solids (TSS) and density have been carried out according to the method AOAC (1997) using the RTP 20 ATC refractometer, with results being expressed in °Brix and $g.cm^{-3}$, respectively. The total titratable acidity was determined by titrating the beverage with 0.01 M sodium hydroxide solution until obtaining a pink color, following method AOAC (1997).

Determination of the total phenolic compounds content

The phenolic compounds content was determined by the method used by Bloor (2001), with some modifications. The extraction was performed with 1 g sample in falcon tube with light protection, adding 10 ml of methanol (MeOH):water (60:40 v/v) and then centrifuged at 10 min and 3500 rpm (Fanem® brand, model Excelsa i 2206). The supernatant was transferred to another tube and the volume was completed up to

15 ml. In a 96-well plate, an aliquot of 100 µl of the sample and 100 µl of Folin Ciocalteu (Sigma® - 20% v/v) reagent were added. Then, 100 µl of sodium carbonate at 7.5% (w/v) was added. After 30 min, the reading was performed at 765 nm in a SpectraMax® 190 spectrophotometer.

The calibration curve has been prepared using gallic acid as the standard, in concentrations from 0 to 1000 $mg.ml^{-1}$. The total phenolic compounds content was calculated from the standard curve obtained ($y = 0.2528 + 0.0698$, $R^2 = 0.9990$), and the results expressed in mg EAG per gram of beverage.

Determination of phenolic acids

For the identification of phenolic compounds (gallic acid, chlorogenic acid, caffeic acid, siringic acid, coumaric acid and ferulic acid), a high performance liquid chromatograph (Breeze, Waters) coupled to a UV detector (Waters 2489) and reverse phase column has been used C18 (150 x 4.6 mm; 5 µm; GL Sciences). The chromatographic conditions were based on the work described by Brunetto et al. (2007) modified. The beverages were filtered through a 0.45 µm membrane prior to HPLC injection. The compounds have been separated using 30% methanol with 0.1% (v/v) acetic acid as the mobile phase under isocratic elution. The runs had a 12-minute total time with a 1.4 $ml.minute^{-1}$ flow. Chromatograms have been monitored by UV at wavelengths of 274 nm for gallic acid and syringic acid, and 325 nm for chlorogenic acid, caffeic acid, p-coumaric acid and ferulic acid. The method used have had a linearity of $R^2 > 0.99$ for all compounds found, the Limit of Detection (LOD) and the Limit and Quantification (LOQ) have been in the range of 0.25 $\mu g.ml^{-1}$ to 0.52 $\mu g.\ ml^{-1}$ and 0.50 $\mu g.ml^{-1}$ to 1.03 $\mu g.ml^{-1}$, respectively.

Statistical analysis

The main effects (Anti-browning agents and Time) and interaction (Anti-browning agents*Time) on the variable responses have been analyzed using the Analysis of Variance (ANOVA), at 5% probability. For results with $p \leq 0.05$, referring to time, linear or quadratic regression equations have been used to analyze the effects of the independent variables on the responses (yi). For significant results ($p \leq 0.05$) regarding anti-browning agents, the averages have been compared by the Duncan test in the same probability. All statistical analyzes have been performed using the SAS software, online version.

Results and Discussion

Microbiological analysis

It hasn't been possible to observe a statistical difference ($p > 0.05$) between yacon beverages with and without anti-antibrowning agent, that is, regardless of cysteine or citric acid presence, all tended to have a similar behavior in relation to the amount of total microorganisms, lactic acid bacteria, yeasts and filamentous fungi, as seen in Table 1.

Table 1
Average values of microorganism growth (CFU.ml^{-1}) of fermented yacon beverages, in different culture media, during storage time

Analysis	Antibrowning agent	Day 7	Day 15	Day 30	Day 45	Day 60
Total bacteria	Control	5.2×10^6	2.8×10^7	1.1×10^9	1.3×10^{10}	6.4×10^9
	Cysteine	2.9×10^6	3.8×10^7	7.0×10^8	7.5×10^9	1.7×10^9
	Citric Acid	8.4×10^6	1.3×10^7	1.3×10^9	5.9×10^9	3.9×10^9
Lactic acid bacteria	Control	3.9×10^6	2.1×10^7	6.1×10^8	8.8×10^9	4.3×10^9
	Cysteine	4.3×10^6	4.2×10^7	6.1×10^8	1.3×10^{10}	1.4×10^{10}
	Citric Acid	4.9×10^6	1.6×10^7	6.0×10^8	7.8×10^9	3.7×10^9
Yeasts and filamentous fungi	Control	7.0×10^5	3.9×10^7	3.6×10^8	1.1×10^{10}	2.3×10^9
	Cysteine	1.1×10^6	1.8×10^7	2.1×10^8	7.5×10^9	3.2×10^9
	Citric Acid	8.5×10^6	1.9×10^7	8.4×10^8	4.9×10^9	3.8×10^9

Despite the similar growth, regardless of the beverage type, in the three culture media, there was a difference ($p \leq 0.05$) in the amount of microorganisms, during the storage time. However, it wasn't possible to adjust an equation model to explain growth as a storage time function.

According to the fermentation kinetics, there is a time for the microorganisms to adapt to the medium, a phase in which they are in a latent state. After this period, the microorganisms' multiplication occurs at a higher speed, given their higher metabolic activity (Kim et al., 2016). The beverages reached their peak of multiplication between 30 and 45 day storage, with 10^9 CFU.ml^{-1} for the 3 culture media and in the 3 yacon beverages analyzed, that is, the citric acid or cysteine addition wasn't a relevant quantitative factor to favoring or inhibiting the microorganisms' growth. However, the NaCl addition may have influenced this multiplication.

Normally, in vegetable fermentations, spontaneous growth of native lactic acid bacteria (LAB) occurs with variations in the amount depending on temperature, initial pH, nutrients and salt (NaCl) concentration (Fleming et al., 1995). NaCl favors the LABs' growth that produce lactic acid and, consequently, reduce the pH of the medium, inhibiting the proliferation of competing undesirable bacteria (Rodríguez et al., 2009a).

The study of fermented yacon conducted by Reina et al. (2015) has found similar results with a LAB predominance (10^9 CFU.g^{-1}) for 30 day fermentation. Three types of *Leuconostoc* spp. have been identified, namely *mesenteroids, pseudomesenteroides* and *citreum.* As deteriorating bacterias were found, in the same study, *Staphylococcus warningeri* and *Enterobacteriaceae.* Staphylococcus were detected in the fresh yacon only, and *Enterobacteriaceae* decreased after 48 h of fermentation reaching numbers below the limits of detection as the pH decreased.

Krüger et al. (2008) reported that for microorganisms to guarantee functional effects, it is necessary to produce viable cultures in effective concentrations, estimating it to be between 10^8 to 10^{11} CFU.g^{-1} of product. Other authors claim that the amount of surviving probiotic bacteria in the final product must be above 10^6 CFU.g^{-1} of product (Kalchayanand et al., 2002; Mattila-Sandholm et al., 2002; Maruyama et al., 2006).

Determination of nutritional composition

The yacon beverage nutritional composition was determined only at time 0, that is, right after processing. Therefore, they weren't undergoing the fermentation process yet.

The proximate composition averages and the yacon beverages total caloric value treated with anti-browning agents can be seen in Table 2.

Table 2
Average and standard deviation of the proximate composition results (g.100 g^{-1}) and total caloric value (TCV - kcal.100 g^{-1}) of yacon beverages with and without anti-browning agents, right after being processed

Constituintes	Control	Citric Acid	Cysteine
Moisture	83.64 ± 1.79^b	91.11 ± 2.38^a	91.42 ± 0.94^a
Carbohydrates	8.85 ± 1.22^a	6.54 ± 0.454^b	7.45 ± 0.60^b
Protein	0.15 ± 0.05^a	0.15 ± 0.03^a	0.19 ± 0.04^a
Lipids	1.34 ± 0.69^a	1.47 ± 0.64^a	1.53 ± 0.50^a
Ash	2.02 ± 0.45^a	1.73 ± 0.46^a	1.79 ± 0.45^a
TCV	35.57 ± 4.87^a	26.44 ± 1.93^b	30.19 ± 2.42^b

Different letters on the same line differ, at 5% probability, by the Duncan Test.

Moisture and carbohydrates varied significantly ($p \leq 0.05$), possibly due to the intrinsic variation in the roots humidity content. However, the values found are in line with the literature, where studies show a high moisture content of the root, which may exceed 70% of its fresh weight, giving a low energy value (Borges et al., 2012; Gusso et al., 2015; Marcon et al., 2000).

As expected, yacon beverages are predominantly constituted by carbohydrates (Kotovicz, 2011; Santana & Cardoso, 2008), including glucose, fructose and sucrose, which can be used as carbon sources for microorganisms to produce other compounds such as lactic and acetic acids (Reina et al., 2015), as a result of fermentation.

Yacon beverages had low protein amounts, lipids and ash, with no significant differences ($p > 0.05$) between them. Protein levels can also be reduced due to possible hydrolysis during the fermentation process (Asquieri et al., 2008).

The beverages nutritional composition is similar to the studies by Lachman et al. (2003) and Asquieri et al. (2020) with fresh yacon, which found a variation of 3.33 to 3.50% for ash, 0.15 to 4.29 for protein, 0.80 to 1.50 for lipids, however, it is important to note that the yacon composition can vary depending on the cultivation time, environmental conditions in the place where it was cultivated, harvest period, time, temperature and post-harvest storage conditions; factors which cause variations, especially in the amount of digestible carbohydrates and fibers, in addition to other compounds when compared to the literature (Vilhena et al., 2000; Graefe et al., 2004).

The yacon root has an energy value considered low due to the high water concentration and lower when compared to other roots and tubers, which have starch as an energy source, such as cassava and potatoes that have around 92 kcal (Lewu et al., 2010). Among the sugar found in yacon, there are the monosaccharides fructose and glucose; sucrose disaccharide; in addition to the FOS and inulin fibers (Ricarte et al., 2020; Vanini et al., 2009), and these fibers don't directly provide calories to the body.

FOS and inulin present in yacon are characterized as soluble dietary fibers with beneficial intestinal functions for the individual, in addition to the selective growth stimulation and activity of health-promoting intestinal bacteria, especially bifidobacteria. Given these characteristics, yacon intake by people with diabetes includes increased glucose absorption in peripheral tissues, decreased gluconeogenesis, better tolerance to insulin in the liver and increased insulin secretion in the pancreas (Russo et al., 2015; Caetano et al., 2016).

Regarding these simple carbohydrates and fibers, their amounts were analyzed in yacon beverages with 0, 30 and 60 days of storage. During the chromatographic analysis in beverages, the sucrose and mannitol presence weren't identified by the methodology used.

The chromatographic areas results obtained have been compared with the total area of each beverage, in the respective time, and presented as percentages. Likewise, the total curve areas at times 30 and 60 were compared to the area at time 0 to assess how much the total carbohydrate content increased or reduced (Table 3).

Table 3
Percentage values related to the chromatographic curves area generated in determination of yacon beverages carbohydrates with and without anti-browning agents, during storage, and proportional values (Pv) of the beverages total area in the 30 and 60 day storage, regarding the time 0

Beverage	Carbohydrates	Percentage values for each Storage Time				
		0	30	60	Pv_{0-30}	Pv_{0-60}
Control	Glucose	4.70	0.95	ND	+14.4%	- 2.8%
	Fructose	10.72	ND	ND		
	FOS	84.59	99.05	100.00		
Cysteine	Glucose	35.83	ND	ND	+17.7%	- 4.8%
	Fructose	15.11	ND	ND		
	FOS	49.06	100.00	100.00		
Citric Acid	Glucose	3.38	ND	ND	- 5.0%	- 2.5%
	Fructose	6.53	ND	ND		
	FOS	90.09	100.00	100.00		

ND: not determined

In all the beverages there is a predominance FOS, as expected, as this is the main constituent in the yacon roots, after water (Ojansivu & Lucia, 2011; Vasconcelos et al., 2015; M. D. F. Silva et al., 2017). There is also a reduction, especially, of fructose and glucose by the storage time, and in the evaluation carried out after 30 days, the amount of these carbohydrates wasn't detected, except for the control beverage at low levels, suggesting consumption by the microorganisms present.

Reina et al. (2015) evaluated spontaneously fermented yacon and also observed a reduction in the glucose and fructose concentrations in 2 day fermentation, of 4.3 and 2.7 times, respectively. However, after a 7 day fermentation they haven't detected fructose and, after 30 days, they also did not detect glucose, corroborating our findings. Most lactic acid bacteria obtain energy from the metabolism of sugar molecules alone (Madigan et al., 2010).

FOS chains are unstable under certain process conditions, and their final composition in the product depends on the steps used in processing, such as temperature, pH and storage time, in addition to the food matrix and degree polymerization (DP) of FOS (Vega & Zuniga-Hansen, 2015; D. Campos et al., 2016; Topolska et al., 2017).

Bleaching impairs the stability of FOS due to the hydrolysis mainly of low DP molecules, constituted of GF3, GF4 and GF5 (GF - glucose-fructose) (D. Campos et al., 2016). Thus, the higher amount of fructose in relation to glucose at time 0, may be the result of hydrolysis present in processing steps. In addition, during the beverages fermentation process occurs the pH reduction, which is consistent with the involvement of protons in the degradation process and, therefore,

with an acid catalysis mechanism of the FOS. These chains are unstable under acidic conditions (Matusek et al., 2009), which also contributes to their reduction during storage.

However, there is an increase in the carbohydrates amount (higher total area) in time 30 (Pv0-30) for control and added cysteine beverages. Theses carbohydrate can be considered FOS, because the glucose and fructose amounts haven't been detected.

The LABs belonging to the Lactobacillus, Streptococcus, Leuconostoc and Weissella genera are reported to produce fructosyltransferases (FTFs) (Monsan et al., 2001; Tieking et al., 2003; Malik et al., 2009; Malang et al., 2015). These enzymes can catalyze the transfer fructose from both sucrose and raffinose to variable acceptors. The presence of different monosaccharides, disaccharides and oligosaccharides as acceptors of transfer activity, it can lead to the FOS synthesis (S. A. F. T. Van Hijum et al., 2001). The enzyme nature, the acceptors type and concentration, the temperature and pH of the reaction influence the amount and molecular masses of the synthesized oligosaccharides (S. Van Hijum et al., 2002; Korakli et al., 2003; Korakli & Vogel, 2006). As FOS are unstable at pH, this may have been the limiting factor for the production and hydrolysis of chains that showed low reduction at 60 day storage. This result agrees with microbiological growth, which has shown a tendency to stabilize between 30 and 45 day storage.

Some fungi and yeasts species are also capable of FOS synthesizing, besides bacteria (Antosova & Polakovic, 2001). Elevated amounts of yeasts have been found in the beverages, however, it still hasn't been identified which ones are present in yacon fermentation.

The citric acid beverage initially had a higher FOS proportion and less oscillation in the carbohydrate content during 60 day fermentation. Several LABs can decompose citric acid to produce lactic acid, but also acetic acid and other products (García-Martínez et al., 2012; Moreno & Peinado, 2012), suggesting the use of the carbohydrates in this beverage, and also of citric acid as fermentation substrate.

The results obtained here indicate that it is possible to have a beverage with until 60 day storage with FOS presence, that has a prebiotic claim, and LAB. However, further studies are needed to identify the LAB present and, maybe confirm the possibility of a symbiotic product.

Physicochemical analysis

The averages and standard deviation of pH, total soluble solids (TSS), acidity and density of fermented yacon beverages treated with anti-browning agents can be seen in Table 4.

Table 4
Average and standard deviation of the physical-chemical analysis of yacon beverages with and without anti-browning agents

Analysis	Control	Citric Acid	Cysteine
pH	4.38 ± 0.82^{ab}	4.13 ± 0.44^{b}	4.52 ± 0.72^{a}
Acidity (ml.g^{-1})	3.12 ± 1.33^{a}	2.54 ± 1.05^{a}	2.94 ± 1.79^{a}
TSS (ºBrix)	6.18 ± 0.77^{a}	5.43 ± 0.88^{a}	5.67 ± 0.72^{a}
Density (g.cm^{-3})	1.04 ± 0.01^{a}	1.04 ± 0.01^{a}	1.04 ± 0.01^{a}

Different letters on the same line differ, at 5% probability, by the Duncan Test.

Only pH has shown a significant difference (p≤0.05) between beverages with and without anti-browning agents. The fermented beverage with the citric acid addition obtained a lower mean pH than the beverage with cysteine (p≤0.05), probably due to the strong acidification capacity with its low pKa values (between 3.12 and 3.13), contrasting with cysteine which has a pka of 8.3, so its pH was close to the control beverage (Rosa et al., 2007; O. R. L. Rodrigues et al., 2014).

The pH variation observed in yacon beverages showed an optimal pH range, from 4 to 4.5, to conduct a good fermentation and required by safety guidelines, inhibiting the growth of pathogenic microorganisms (Lopes et al., 2005; Reina et al., 2015). The pH still varied in function the time and in the interaction Anti-browning agents*Time, however, for none of these variation sources it was possible to adjust linear or quadratic equations, so, have been plotted a graph with the average results obtained. To evaluate the influence of time in relation to each beverage pH, the graph in Figure 1 was plotted.

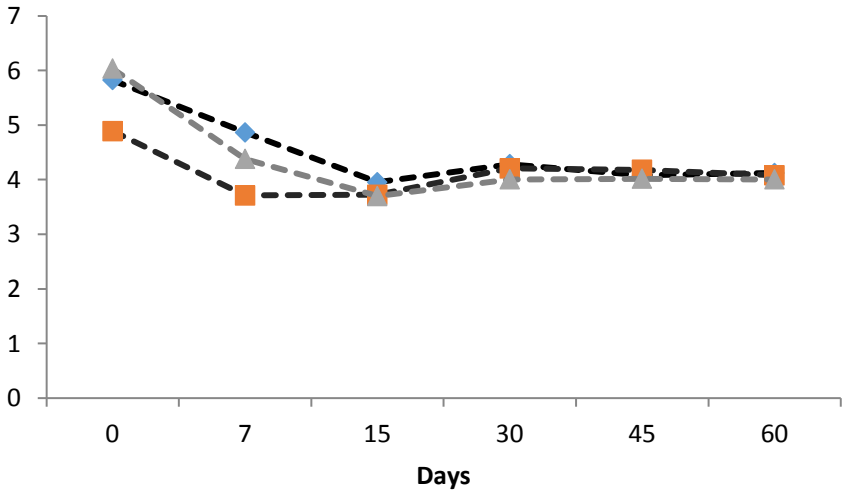

Figure 1. pH variation, during storage, for each yacon beverage.
-- ■ -- Beverage with cysteine; ... ♦ ... Beverage with citric acid; -- ► -- Control beverage.

It is possible to observe a gradual reduction in the fermented beverage pH during the storage period, especially for control and cysteine beverages, which had a higher initial pH. This decrease coincided with the microorganisms' growth, reaching a balance after 15 day fermentation, regardless of the anti-browning agent used. Similar results have been found in the fermented yacon study that started with a pH of 5.77 and after 7 day fermentation, the pH reached 3.71, a decrease directly related to the lactic acid bacteria growth (Reina et al., 2015). In other food this pH reduction behavior has been also observed as in milk fermented by L. casei, from 5.59 to 4.60 (Hu et al., 2018) and in yogurts from 4.51 to 4.40, both after 28 day storage at 4 °C (Akalın et al., 2004) confirming the bacteria active metabolism even at refrigerated temperatures.

Figure 2 shows the yacon beverages behavior, independent of anti-browning agent used, as a time function, for the physical-chemical analysis performed.

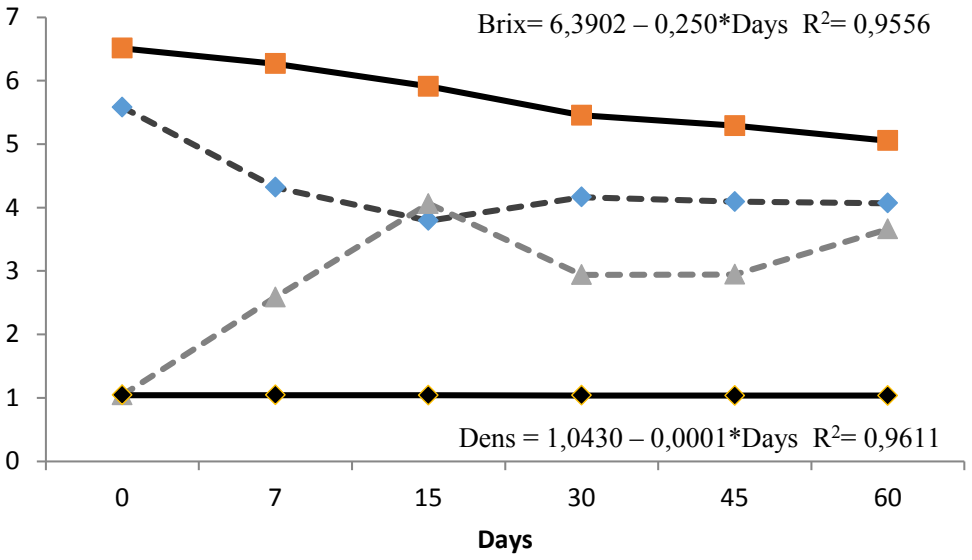

Figure 2. Behavior of the yacon beverages physical-chemical analysis, during the storage time. __ ♦ __ TSS (ºBrix); --■-- pH; ..►.. Acidity (ml. 100 g^{-1} of beverage); __ ♦ __ Density (g.cm-³).
º Brix = 6.3902 – 0.250*Days; R^2 = 0.9556
Density = 1.0430 – 0.0001*Days; R^2 = 0.9611

Along the pH decrease, there was a rapid acidity increase at the beginning of the yacon beverage fermentation, which may favor the deteriorating bacteria reduction, such as *Clostridium.* Slow acidification, oppositely, limits the fermentation process due to the butyric bacterias growth (Kohajdová et al., 2006) which are important, but in cheese making.

During the sugar fermentation used as a carbon source, the acetic and lactic acid metabolites are originated, characteristically produced by heterofermentative *L. mesenteroides* bacteria in natural fermentation (Saha & Racine, 2011).

Yacon fermented beverages hasn't significantly differed to TSS content ($p>0.05$). Similar values have been found in study by Muniz et al. (2002) who showed a gradual reduction in Brix during ciriguela and mangaba wort fermentation period, which stabilized between 5.4 and 6.5 ºBrix. The TSS reduction during storage time occurs due to the use of sugar as a substrate for microbiological fermentation by lactic acid bacteria and yeasts present in beverages (Yoon et al., 2006).

The storage temperature, approximately 10 °C, may have made slower and more gradual substrate consumption and metabolism. Similar results have been found in studies from 7 to 18 days with the yeasts addition in umbu fermented by Paula et al. (2012), in the vegetable juice fermented by Kohajdová et al. (2006) and in the orange fermented by Corazza et al. (2001).

The density has shown a slight reduction during the storage period. It is due to the fact that the density is directly related to the TSS content (Rizzon et al., 2005), which in the yacon fermented beverage is predominantly constituted by carbohydrates.

Determination of total phenolic compounds and phenolic acids

In addition to the total phenolic compounds content, 6 compounds have been individually evaluated - gallic acid, syringic acid, chlorogenic acid, caffeic acid, p-coumaric acid and ferulic acid - however, it has been possible to identify and quantify only 2 in yacon beverages, being gallic and chlorogenic acids. The total phenolic compounds content, as well as gallic and chlorogenic acid, statistically differed between yacon beverages ($p\leq0.05$) (Table 5).

Table 5
Average and standard content deviation of the total phenolic compounds (TPC - mg EAG.g^{-1}), gallic acid (µg.ml^{-1}) and chlorogenic acid (µg.ml^{-1}) in yacon beverages with and without anti-browning agents

Analysis	Control	Citric Acid	Cysteine
TPC	0.52 ± 0.20^{a}	0.38 ± 0.16^{b}	0.48 ± 0.20^{ab}
Gallic Acid	285.44 ± 3.54^{a}	40.87 ± 1.32^{c}	198.30 ± 2.01^{b}
Chlorogenic Acid	434.20 ± 52.58^{a}	230.37 ± 168.63^{c}	251.16 ± 138.13^{b}

Different letters on the same line differ, at 5% probability, by the Duncan Test.

The average phenolic compound content of yacon fermented beverages has shown lower values compared to the amount found in fresh matter (about 2 $mg.g^{-1}$), reported by other authors (Gusso et al., 2015; Simonovska et al., 2003).

Some microorganisms' species, such as *Lactobacillus fermentum,* are able to metabolize phenolic compounds in fermented food by decarboxylation and/or reduction activity (F. M. Campos et al., 2009; Svensson et al., 2010). Despite this lower value, the levels are close to some fruits such as passion fruit (0.21 mg $EAG.g^{-1}$), guava (0.83 mg $EAG.g^{-1}$), papaya (0.53 mg $EAG.g^{-1}$) and pineapple (0.38 mg $EAG.g^{-1}$) (Rocha et al., 2011; Alves et al., 2017).

The control beverage had the highest levels ($p \leq 0.05$) of phenolic compounds, gallic acid and chlorogen. Only similarly to the beverage with cysteine in the total phenolic compounds content ($p > 0.05$).

During processing and storage, the cell structures which contain the enzymes and phenolic substrates in separate compartments are disrupted. These oxidative enzymes then begin to catalyze the oxidation of phenolic compounds to quinones, which in turn polymerize to form dark colored compounds, called melanoidins, thus leading to phenolic compounds degradation (D. I. A. Pereira & Gibson, 2002; Mizobutsi et al., 2010). This browning can be seen in Figure 3.

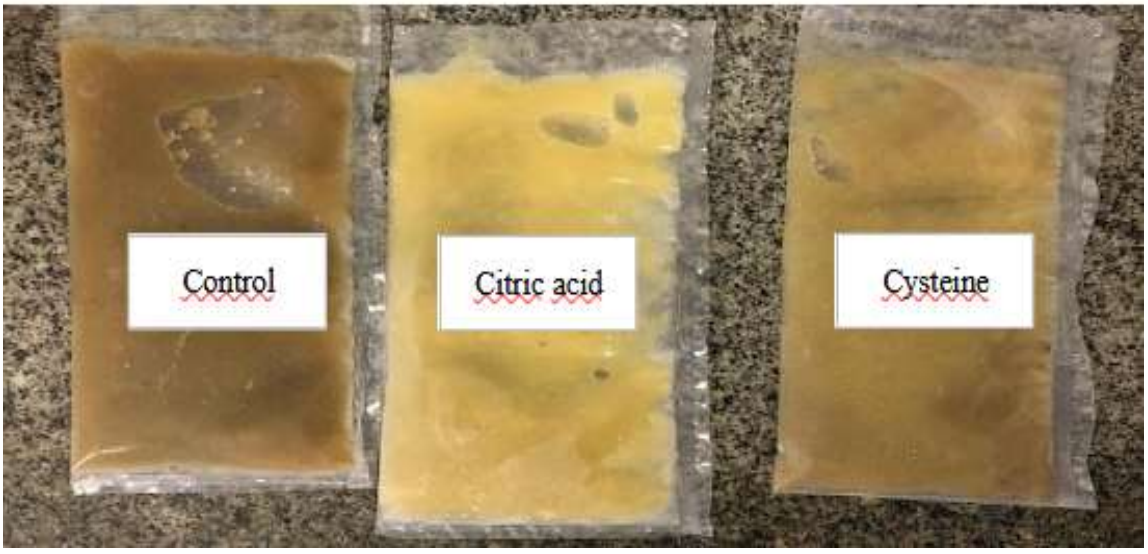

Figure 3. Beverages prepared without (control) or with a browning agent (citric acid or cysteine), after 60 days of storage.

It is possible that oxidative enzymes inactivation in the citric acid or cysteine presence has occurred, avoiding the beverages browning and, consequently, the phenolic compounds preservation (F. M. Campos et al., 2009; Sekwati-Monang & Gänzle, 2011). However, lactic acid bacteria, an important part of the microbiota of fermented food and vegetable beverages (Di Cagno et al., 2013; Reina et al., 2015), as found in yacon beverages, have the ability to metabolize antioxidants, affecting the phenolic compounds profile, gallic acid and chlorogenic acid (Rodríguez et al., 2009b; Cueva et al., 2010; Curiel et al., 2010; Filannino et al., 2015; J. A. R. Pereira et al., 2016; Lago & Noreña, 2017).

The storage time also affected ($p \leq 0.05$) the beverages phenolic compounds content, in general, regardless of the beverage, but it wasn't possible to adjust a linear or quadratic equation model to explain this behavior, being its tendency plotted on a graph (Figure 4).

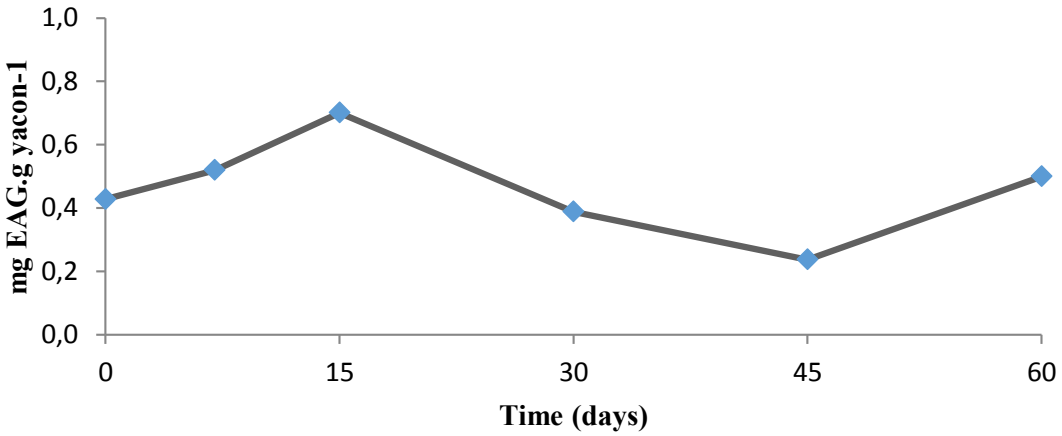

Figure 4. Average phenolic content variation (mg EAG.g yacon^{-1}) of the yacon fermented beverage as a storage time function.

There was an increase in the amount of phenolics up to the 15 th storage day and later that period, a tendency of reducing these levels, culminating in a gentle oscillation. The oxidative enzymes, besides the active form present in the cytosol, are also found latently, both in the cytosol and in plastids, isolated from the rest of the cell (Sellés-Marchart et al., 2006; Queiroz et al., 2011; Carvalho & Orlanda, 2017). These enzymes in latent form and plastids which are still intact, when stored under refrigeration, can undergo changes over time, leading to the plastids rupture and phenolic compounds oxidation. Associated with this, there is also the secondary cells metabolism that leads to cycles which form phenolic compounds, and probably these cycles may have been activated during cold storage and by the stress caused during the beverage preparation (Ding et al., 2001; Ferreres et al., 2009).

Chlorogenic acid also decreased by storage time ($p \leq 0.05$), in the interaction (Anti-browning agents*Time) and in relation to the

general behavior (Time), being possible to observe through the adjusted regression models (Table 6). It is important to note that gallic acid has been found only in Time 0, not being detected in other analysis days in any beverage.

Table 6
Regression model for chlorogenic acid (μg.ml^{-1}) of yacon fermented beverages as a time function (T) and their respective determination coefficients (R^2) and probability levels (p)

Beverage	Regression Model	R^2	Prob > F
Control	493.3827- 1.9728*T	0.9999	<0.0001
Cysteine	378.9044 - 4.2582*T	0.6459	<0.0001
Citric Acid	424.6194 - 6.4748*T	0.9999	<0.0001
General behavior	432.3022 - 4.2353*T	0.9439	0.0003

The food intake containing chlorogenic acid doesn't confer many benefits without its metabolism. The human gatrointestinal mucosa doesn't have esterases capable of hydrolyzing the esterified acids, significantly reducing the efficiency of absorption of this acid in the gastric lumen and small intestine (Manach et al., 2004; Farah et al., 2008). However, in the yacon beverage this acid is metabolized to form hydroxycinnamic acid by microorganisms during the fermentation time (Olthof et al., 2001; Adam et al., 2002; Rechner et al., 2002). Thus, the chlorogenic acid hydrolysis makes it more bioavailable to the body, which in turn, will be better absorbed by the gastrointestinal tract promoting health benefits.

Despite these results, yacon fermented beverages has shown a considerable amount of phenolic compounds, including chlorogenic and gallic acids, which have important antioxidant activity (Simonovska et al., 2003; Valentová & Ulrichová, 2003). The food intake containing phenolic compounds brings benefits by helping in anti-inflammatory, antiplatelet activity, in addition to preventing the action of free radicals (M. L. C. Silva et al., 2010; Caleja et al., 2017; Taamalli et al., 2019; Yan et al., 2019) and, possibly, these benefits can be observed with this fermented beverage consumption spontaneously.

Conclusion

Spontaneous fermentation was a preparation technique which has added nutritional and functionals values to the yacon beverage, especially for the citric acid beverage that showed greater preservation of FOS, a prebiotic fibers. Regarding microorganisms, all beverages have reached 10^9 CFU.ml^{-1} after 30 day storage. However, it is necessary the identify these microorganisms to infer a probiotic effect.

Regarding the use of anti-browning agents, the differences have been occasional, however, the citric acid beverage has shown a desirable pH (< 4.5) for a fermented beverage, which allows undesirable bacteria inhibition

and a higher proportional initial FOS amount and less variation with 30 and 60 day storage.

The beverages has had a considerable phenolic compounds amount (0.38 to 0.52 mg EAG.g^{-1}) such as chlorogenic acid (230.37 to 434.20 µg.ml^{-1}), which confer important antioxidant activity that bring several health benefits. The citric acid beverage presented a more interesting visual color than the control and cysteine beverages, reinforcing its better performance.

References

Adam, A., Crespy, V., Levrat-Verny, M. A., Leenhardt, F., Leuillet, M., Demigné, C., & Rémésy, C. (2002). The bioavailability of ferulic acid is governed primarily by the food matrix rather than its metabolism in intestine and liver in rats. *Journal of Nutrition, 132*(7), 1962-1968. doi: 10.1093/jn/132.7.1962

Akalın, A. S., Fenderya, S., & Akbulut, N. (2004). Viability and activity of bifidobacteria in yoghurt containing fructooligosaccharide during refrigerated storage. *International Journal of Food Science & Technology, 39*(6), 613-621. doi:10.1111/j.1365-2621.2004.00829.x

Alves, A. M., Dias, T., Hassimotto, N. M. A., & Naves, M. M. V. (2017). Ascorbic acid and phenolic contents, antioxidant capacity and flavonoids composition of Brazilian savannah native fruits. *Food Science and Technology, 37*(4), 564-569. doi: 10.1590/1678-457x.26716

Antosova, M., & Polakovic, M. (2001). Fructosyltransferases: the enzymes catalyzing production of fructooligosaccharides. *Chemical Papers, 55*(6), 350-358.

Asquieri, E. R., Nishi, A. C. F., Batista, R. D., & Asquieri, E. M. A. R. (2020). Yacon extract drying (Smallanthus sonchifolius) by Spray Dryer: effect of the different carrier agents and evaluation of the levels of fructooligosaccharides and phenolic compounds. *Research, Society and Development, 9*(7), e591974521. doi: 10.33448/rsd-v9i7.4521

Asquieri, E. R., Rabelo, A. M. S., & Silva, A. G. M. (2008). Fermented jackfruit: study on its physicochemical and sensorial characteristics. *Ciência e Tecnologia de Alimentos, 28*(4), 881-887. doi: 10.1590/S0101-20 612008000400018

Association of Official Analytical Chemists (1997). *Association of official analytical chemists international official methods of analysis* (16nd ed.). AOAC.

Association of Official Analytical Chemists (2000). *Official methods of analysis.* Univ. Michigan, Assoc. Off. Analytical Chem.

Association of Official Analytical Chemists (2005). *Official methods of analysis.* Int. (18nd ed.). AOAC.

Bloor, S. J. (2001). Overview of methods for analysis and identification of flavonoids, *Methods in Enzymology, 335,* 3-14. doi: 10.1016/S0076-6879(01)35227-8

Borges, J. T. S., Pirozi, M. R., Paula, C. D., Vidigal, J. G., Silva, N. A. S., & Caliman, F. R. B. (2012). Yacon na alimentação humana: aspectos nutricionais, funcionais, utilização e toxicidade. *Scientia Amazonia, 1*(3), 3-16.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P.,

Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I. B., Prajapati, J. B., Seto, Y., Ter Schure, E., Van Boven, A., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S., & Hansen, E. B. (2012). Food fermentations: microorganisms with technological beneficial use. *International Journal of Food Microbiology, 154*(3), 87-97. doi: 10.1016/j.ijfoodmicro.2011.12.030

Brunetto, M. del R., Gutiérrez, L., Delgado, Y., Gallignani, M., Zambrano, A., Gómez, Á., Ramos, G., & Romero, C. (2007). Determination of theobromine, theophylline and caffeine in cocoa samples by a high-performance liquid chromatographic method with on-line sample cleanup in a switching-column system. *Food Chemistry, 100*(2), 459-467. doi: 10.1016/j.foodchem.2005.10.007

Caetano, B. F. R., Moura, N. A., Almeida, A. P. S., Dias, M. C., Sivieri, K., & Barbisan, L. F. (2016). Yacon (Smallanthus sonchifolius) as a food supplement: health-promoting benefits of fructooligosaccharides. *Nutrients, 8*(7), 436-449. doi: 10.3390/nu8070436

Caleja, C., Ribeiro, A., Barreiro, M. F., & Ferreira, I. C. F. R. (2017). Phenolic compounds as nutraceuticals or functional food ingredients. *Current Pharmaceutical Desing, 23*(19), 2787-2806. doi: 10.2174/1381612822666161227153906

Campos, D., Aguilar-Galvez, A., & Pedreschi, R. (2016). Stability of fructooligosaccharides, sugars and colour of yacon (*Smallanthus sonchifolius*) roots during blanching and drying. *International Journal of Food Science and Technology, 51*(5), 1177-1185. doi: 10.1111/ijfs.13074

Campos, F. M., Couto, J. A., Figueiredo, A. R., Tóth, I. V., Rangel, A. O. S. S., & Hogg, T. A. (2009). Cell membrane damage induced by phenolic acids on wine lactic acid bacteria. *International Journal of Food Microbiology, 135*(2), 144-151. doi:10.1016/j.ijfoodmicro.2009.07.031

Carvalho, J. O., & Orlanda, J. F. F. (2017). Heat stability and effect of pH on enzyme activity of polyphenol oxidase in buriti (*Mauritia flexuosa Linnaeus* f.) fruit extract. *Food Chemistry, 233*(15), 159-163. doi: 10.1016/j.foodchem.2017.04.101

Chaud, S. G., & Sgarbieri, V. C. (2006). Propriedades funcionais (tecnológicas) da parede celular de leveduras da fermentação alcoólica e das frações glicana, manana e glicoproteína. *Ciência e Tecnologia de Alimentos, 26*(2), 369-379. doi: 10.1590/S0101-20612006000200020

Corazza, M. L., Rodrigues, D. G., & Nozaki, J. (2001). Preparação e caracterização do vinho de laranja. *Química Nova, 24*(4), 449-452. doi: 10.1590/S0100-40422001000400004

Cueva, C., Moreno-Arribas, M. V., Martín-Álvarez, P. J., Bills, G., Vicente, M. F., Basilio, A., Rivas, C. L., Requena, T., Rodríguez, J. M., & Bartolomé, B. (2010). Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. *Research in. Microbiology, 161*(5), 372-382. doi: 10.1016/j.resmic.2010.04.006

Curiel, J. A., Rodríguez, H., Landete, J. M., Rivas, B., & Muñoz, R. (2010). Ability of *Lactobacillus brevis* strains to degrade food phenolic acids. *Food Chemistry, 120*(1), 225-229. doi: 10.1016/j.foodchem.2009. 10.012

Di Cagno, R., Coda, R., De Angelis, M., & Gobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology, 33*(1), 1-10. doi: 10.1016/j.fm.2012.09.003

Ding, C. K., Chachin, K., Ueda, Y., Imahori, Y., & Wang, C. Y. (2001). Metabolism of phenolic compounds during loquat fruit development. *Journal of Agricultural and Food Chemistry, 49*(6), 2883-2888. doi: 10. 1021/jf0101253

Evangelista, S. R., Miguel, M. G. C. P., Silva, C. F., Pinheiro, A. C. M., & Schwan, R. F. (2015). Microbiological diversity associated with the spontaneous wet method of coffee fermentation. *International Journal of Food Microbiology, 210,* 102-112. doi: 10.1016/j.ijfoodmicro.2015.06.008

Farah, A., Monteiro, M., Donangelo, C. M., & Lafay, S. (2008). Chlorogenic acids from green coffee extract are highly bioavailable in humans. *The Journal of Nutrition, 138*(12), 2309-2315. doi: 10.3945/jn.108.09 5554

Ferreres, F., Gomes, D., Valentão, P., Gonçalves, R., Pio, R., Chagas, E. A., Seabra, R. M., & Andrade, P. B. (2009). Improved loquat (*Eriobotrya japonica* Lindl.) cultivars: variation of phenolics and antioxidative potential. *Food Chemistry, 114*(3), 1019-1027. doi: 10.1016/j. foodchem.2008.10.065

Filannino, P., Bai, Y., Di Cagno, R., Gobbetti, M., & Gänzle, M. G. (2015). Metabolism of phenolic compounds by *Lactobacillus* spp. during fermentation of cherry juice and broccoli puree. *Food Microbiology, 46*, 272-279. doi: 10.1016/j.fm.2014.08.018

Fleming, H. P., McDonald, L. C., McFeeters, R. F., Thompson, R. L., & Humphries, E. G. (1995). Fermentation of cucumbers without sodium chloride. *Journal of Food Science, 60*(2), 312-315. doi: 10. 1111/ j.1365-2621.1995.tb05662.x

García-Martínez, T., Puig-Pujol, A., Peinado, R. A., Moreno, J., & Mauricio, J. C. (2012). Potential use of wine yeasts immobilized on *Penicillium chrysogenum* for ethanol production. *Journal of Chemical Technology & Biotechnology, 87*(3), 351-359. doi: 10.1002/jctb.2725

Genta, S. B., Cabrera, W. M., Grau, A., & Sánchez, S. S. (2005). Subchronic 4-month oral toxicity study of dried *Smallanthus sonchifolius* (yacon) roots as a diet supplement in rats. *Food and Chemical Toxicology, 43*(11), 1657-1665. doi: 10.1016/j.fct.2005.05.007

Graefe, S., Hermann, M., Manrique, I., Golombek, S., & Bürkert, A. (2004). Effects of post-harvest treatments on the carbohydrate composition of yacon roots in the Peruvian Andes. *Fields Crops Research, 86*(10), 157-165. doi: 10.1016/j.fcr.2003.08.003

Gusso, A. P., Mattanna, P., & Richards, N. (2015). Yacon: benefícios à saúde e aplicações tecnológicas. *Ciência Rural, 4545*, 912-919. doi: 10.1590/0103-8478 cr20140963

Hu, K., Jin, G. J., Mei, W. C., Li, T., & Tao, Y. S. (2018). Increase of medium-chain fatty acid ethyl ester content in mixed H. uvarum/S. cerevisiae fermentation leads to wine fruity aroma enhancement. *Food Chemistry, 239*, 495-501. doi: 10.1016/j. foodchem.2017.06.151

Ioannou, I., & Ghoul, M. (2013). Prevention of enzymatic browning in fruit and vegetables. *European Scientific Journal, 9*(30), 310-341. doi: 10.19044/ESJ.2013.V9N30P%P

Kalchayanand, N., Frethem, C., Dunne, P., Sikes, A., & Ray, B. (2002). Hydrostatic pressure and bacteriocin-triggered cell wall lysis of *Leuconostoc mesenteroides. Innovative Food Science & Emerging Technologies, 3*(1), 33-40. doi: 10.1016/S1466-8564(02)00004-8

Kim, B., Hong, V. M., Yang, J., Hyun, H., Im, J. J., Hwang, J., Yoon, S., & Kim, J. E. (2016). A review of fermented foods with beneficial effects on brain and cognitive function. *Preventive Nutrition and Food Science, 21*(4), 297-309. doi: 10.3746/pnf.2016.21.4.297

Kohajdová, Z., Karovičová, J., & Greifová, M. (2006). Lactic acid fermentation of some vegetable juices. *Journal of Food and Nutrition Research, 45*(3), 115-119. doi: 10.17221/3878-HORTSCI

Korakli, M., & Vogel, R. F. (2006). Structure/function relationship of homopolysaccharide producing glycansucrases and therapeutic potential of their synthesised glycans. *Applied Microbiology and Biotechnology, 71*(6), 790-803. doi: 10.1007/s00253-006-0469-4.

Korakli, M., Pavlovic, M., Gänzle, M. G., & Vogel, R. F. (2003). Exopolysaccharide and kestose production by *Lactobacillus sanfranciscensis* LTH2590. *Applied and Environmental Microbiology, 69*(4), 2073-2079. doi: 10.1128/AEM.69.4.2073-2079.2003

Kotovicz, V. (2011). *Otimização da desidratação osmótica e secagem do yacon (Polymnia sonchifolia).* Dissertação de mestrado, Tecnologia de Alimentos, Universidade Federal do Paraná, Curitiba, PR, Brasil.

Krüger, R., Kempka, A. P., Oliveira, D., Valduga, E., Cansian, R. L., Treichel, H., & Di Luccio, M. (2008). Desenvolvimento de uma bebida láctea probiótica utilizando como substratos soro de leite e extrato hidrossolúvel de soja. *Alimentos e Nutrição, 19*(1), 43-53.

Lachman, J., Fernández, E. C., & Orsák, M. (2003) Yacon [*Smallanthus sonchifolia* (Poepp. et Endl.) H. Robinson] chemical composition and use-a review. *Plant Soil and Environment, 49*(6),283-290. doi: 10.17221/4126-PSE

Lago, C. C., & Noreña, C. P. Z. (2017). Thermodynamic and kinetics study of phenolics degradation and color of yacon (*Smallanthus sonchifolius*) microparticles under accelerated storage conditions. *Journal of Food Science and Technology, 54*(13), 4197-4204. doi: 10.1007/s13197-017-2887-y

Lewu, M. N., Adebola, P. O., & Afolayan, A. J. (2010). Comparative assessment of the nutritional value of commercially available cocoyam and potato tubers in South Africa. *Journal of Food Quality, 33*(4), 461-476. doi: 10.1111/j.1745-4557.2010.00325.x

Lopes, R. V. V., Rocha, A. S., Silva, F. L. H., & Gouveia, J. P. G. (2005). Aplicação do planejamento fatorial para otimização do estudo da produção de fermentado do fruto da palma forrageira. *Revista Brasileira de Produtos Agroindustriais,*

7(5), 25-32. doi: 10.1590/S0100-4042 2008000500024

López-López, M., Veja-Espinoza, A., Ayón-Reyna, L., LópezValenzuela, J., & Veja-Garcia, M. (2013). Combined effect of hot water dipping treatment, N-acetylcysteine and calcium on quality retention and enzymatic activity of fresh-cut apple. *Journal of Food, Agriculture & Environment, 11*(2), 243-248. doi: 10.1234/4.2013.4257

Macfarlane, S., Macfarlane, G. T., & Cummings, J. H. (2006). Prebiotics in the gastrointestinal tract. *Alimentary Pharmacology & Therapeutics, 24*(5), 701-714. doi: 10.1111/j.1365-2036.20 06.03042.x

Madigan, M. T., Martinko, J. M., Dunlap, P. V., & Clark, D. P. (2010). *Microbiologia de brock* (12nd. ed.). Ed. Artmed.

Malang, S.K., Maina, N.H., Schwab, C., Tenkanen, M., & Lacroix, C. (2015). Characterization of exopolysaccharide and ropy capsular polysaccharide formation by Weissella. *Food Microbiology, 46,* 418-427. doi: 10.1016/j.fm.2014.08.022

Malik, A., Radji, M., Kralj, S., & Dijkhuizen, L. (2009). Screening of lactic acid bacteria from Indonesia reveals glucansucrase and fructansucrase genes in two different Weissella confusa strains from soya. *FEMS Microbiology Letters, 300*(1), 131-138. doi: doi.org/10.1111/j.1574-6968.2009.01772.x

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinicanl Nutrition, 79,* (5) 727-747. doi: 10.1093/ajcn/79.5.727

Marcon, L. D. N., Moraes, L. F. S., Cruz, B. C. S., Teixeira, M. D. O., Bruno, T. C. V., Ribeiro, I. E., Marshall, M. R., Kim, J., & Wei, C. I. (2000). *Enzymatic browning in fruits, vegetables and seafoods.* FAO: Food Science and Human Nutrition.

Maruyama, L. Y., Cardarelli, H. R., Buriti, F. C. A., & Saad, S. M. I. (2006). Textura instrumental de queijo petit-suisse potencialmente probiótico: Influência de diferentes combinações de gomas. *Ciência e Tecnologia de Alimentos, 26*(2), 386-393. doi: 10.1590/S0101-20612006000200022

Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., & Saarela, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal, 12*(2), 173-182. doi: 10. 1016/ S0958-6946(01)00099-1

Matusek, A., Merész, P., Le, T. K. D., & Örsi, F. (2009). Effect of temperature and pH on the degradation of fructo-oligosaccharides. *European Food Research and Technology, 228*(3), 355-365. doi: 10.1007/s00217-008-0941-8

Mizobutsi, G. P., Finger, F. L., Ribeiro, R. A., Puschmann, R., Neves, L. L. M., & Mota, W. F. (2010). Effect of pH and temperature on peroxidase and polyphenoloxidase activities of litchi pericarp. *Scientia Agricola, 67*(2), 213-217. doi: 10.1590/ s0103-90162010000200013

Monsan, P., Bozonnet, S., Albenne, C., Joucla, G., Willemot, R. M., & Remaud-Siméon, M. (2001). Homopolysaccharides from lactic acid bacteria. *International Dairy Journal, 11*(9), 675-685. doi: 10. 1016/S0958-6946(01)00113-3

Moreno, J., & Peinado, R. (2012). *Enological chemistry.* Academic Press.

Muniz, C. R., Borges, M. F., Abreu, F. A. P., Nassu, R. T., & Freitas, C. A. S. (2002). Bebidas fermentadas a partir de frutos tropicais. *Boletim do Centro de Pesquisa e Processamento de Alimentos, 20*(2), 309-322. doi: 10.5380/cep.v20i2.1256

Nicolas, J., Cheynier, V., Fleuriet, A., & Rouet-Mayer, M.-A. (1993). Polyphenols and enzymatic browning. *Polyphenolic phenomena* (pp. 165-175). Paris.

Ojansivu, I., & Lucia, C. (2011). Yacon, a new source of prebiotic oligosaccharides with a history of safe use. *Trends in Food Science & Technology, 22*(1), 40-46. doi: 10.1016/j.tifs.2010.11.005

Olthof, M. R., Hollman, P. C. H., & Katan, M. B. (2001). Chlorogenic acid and caffeic acid are absorbed in humans. *The Journal of Nutrition, 131*(1), 66-71. doi: 10.1093/jn/131.1.66

Paula, B. D., Carvalho, C. D ., Matta, V. M. D., Menezes, J. D. S., Lima, P. D. C., Pinto, C. O., & Conceição, L. E. M. G. (2012). Produção e caracterização físico-química de fermentado de umbu. *Ciência Rural, 42*(9), 1688-1693. doi: 10.1590/S0103-84782012000900027

Pedreschi, R., Campos, D., Naratto, G., Chirinos, R., & Cisneros-Zevallos, L. (2003). Andean Yacon root (*Smallanthus sonchifolius* Poepp. Endl) fructooligosaccharides as a potential novel source of prebiotics. *Journal of Agricultural and Food Chemistry, 51*(18), 5278-84. doi: 10.1021/jf0344744

Pereira, D. I. A., & Gibson, G. R. (2002). Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Critical Reviews in Biochemistry and Molecular Biology, 37*(4), 259-281. doi: 10.1080/10409230290771519

Pereira, J. A. R., Barcelos, M. F. P., Pereira, M. C. A., & Ferreira, E. B. (2013). Studies of chemical and enzymatic characteristics of yacon (*Smallanthus sonchifolius*) and its flours. *Food Science and Technology, 33*(1), 75-83. doi: 10.1590/S0101-20612013005000020

Pereira, J. A. R., Teixeira, M. C., Saczk, A. A., Barcelos, M. F. P., Oliveira, M. F., & Abreu, W. C. (2016). Total antioxidant activity of yacon tubers cultivated in Brazil. *Ciência e Agrotecnologia, 40*(5), 596-605. doi: 10.1590/1413-70542016405009416

Queiroz, C., Silva, A. J. R. da, Lopes, M. L. M., Fialho, E., & Valente-Mesquita, V. L. (2011). Polyphenol oxidase activity, phenolic acid composition and browning in cashew apple (*Anacardium occidentale*, L.) after processing. *Food Chemistry, 125*(1), 128-132. doi: 10.1016/J.FOODCHEM.2010.08.048

Rechner, A. R., Kuhnle, G., Bremner, P., Hubbard, G. P., Moore, K. P., & Rice-Evans, C. A. (2002). The metabolic fate of dietary polyphenols in humans. *Free Radical Biology and Medicine, 33*(2), 220-235. doi: 10.1016/s0891-5849(02)00877-8

Reina, L. D., Pérez-Díaz, I. M., Breidt, F., Azcarate-Peril, M. A., Medina, E., & Butz, N. (2015). Characterization of the microbial diversity in yacon spontaneous fermentation. *International Journal of Food Microbiology, 203,* 35-40. doi: 10.1016/j.ijfoodmicro.2015.03.007

Ricarte, D., Júlio, B. L. A., Zocateli, G. A. F., Barreto, R. L. F., Guimarães, M., Ferreira, R. S., & Guimarães, N. S. (2020). Análise sensorial de preparações com batata yacon: revisão sistemática. *HU Revista, 45*(4), 431-440. doi: 10.34019/1982-8047.2019.v45.28419

Rizzon, L. A., Bernardi, J., & Miele, A. (2005). Características analíticas dos sucos de maçã Gala, Golden Delicious e Fuji. *Food Science and Technology, 25*(4), 750-756. doi: 10.1590/S0101-20612005000400020

Rocha, W. S., Lopes, R. M., Silva, D. B., Vieira, R. F., Silva, J. P., & Agostini-Costa, T. S. (2011). Total phenolics and condensed tannins in native fruits from brazilian savanna. *Revista Brasileira de Fruticultura, 33*(4), 1215-1221. doi: 10.1590/S0100-29452011000400021

Rodrigues, A. M., & Sant'Anna, E. S. (2001). Efeito do cloreto de sódio na produção de proteínas (*Saccharomyces cerevisiae*) em fermentação semi-sólida. *Food Science and Technology, 121*(1), 57-62. doi: 10.1590/S0101-20612001000100013

Rodrigues, O. R. L., Asquieri, E. R., & Orsi, D. C. (2014). Prevention of enzymatic browning of yacon flour by the combined use of anti-browning agents and the study of its chemical composition. *Food Science and Technology, 34*(2), 275-280. doi: 10.1590/fst.2014.0045

Rodríguez, H., Curiel, J.A., Landete, J.M., Rivas, B., Felipe., F.L., Gómez-Cordovés, C., & Muñoz, R. (2009a). International journal of food microbiology food phenolics and lactic acid bacteria. *International Journal of Food Microbiology, 132*(2-3), 79-90. doi: 10.1016/j.ijfoodmicro.2009.03.025

Rodríguez, H., Curiel, J. A., Landete, J. M., Rivas, B., Felipe, F. L., Gómez-Cordovés, C., Mancheño, J. M., & Muñoz, R. (2009b). Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology, 132*(2-3), 79-90. doi: 10.1016/j.ijfoodmicro.2009.03.025

Rosa, J. S., Godoy, R. L. O., Oiano, J., Neto, Campos, R. S., Matta, V. M., Freire, C. A., Silva, A. S., & Souza, R. S. (2007). Desenvolvimento de um método de análise de vitamina C em alimentos por cromatografa líquida de alta eficiência e exclusão iônica. *Food Science and Technology, 27*(4), 837-846. doi: 10.1590/S0101-20612007000400025

Russo, D., Valentão, P., Andrade, P. B., Fernandez, E. C., & Milella, L. (2015). Evaluation of antioxidant, antidiabetic and anticholinesterase activities of *Smallanthus sonchifolius* landraces and correlation with their phytochemical profiles. *International Journal of Molecular Sciences, 16*(8), 17696-17718. doi: 10.3390/ijms160817696

Saha, B. C., & Racine, F. M. (2011). Biotechnological production of mannitol and its applications. *Applied Microbiology and Biotechnology, 89*(4), 879-891. doi: 10.1007/s00253-010-2979-3

Santana, I., & Cardoso, M. H. (2008). Raiz tuberosa de yacon (*Smallanthus sonchifolius*): potencialidade de cultivo, aspectos tecnológicos e nutricionais. *Ciência Rural, 38*(3), 898-905. doi: 10.1590/S0103-84782008000300050

Sekwati-Monang, B., & Gänzle, M. G. (2011). Microbiological and chemical characterisation of ting, a sorghum-based sourdough product from

Botswana. *International Journal of Food Microbiology, 150*(2-3), 115-121. doi:10.1016/j.ijfoodmicro.2011.07.021

Sellés-Marchart, S., Casado-Vela, J., & Bru-Martínez, R. (2006). Isolation of a latent polyphenol oxidase from loquat fruit (*Eriobotrya japonica* Lindl.): Kinetic characterization and comparison with the active form. *Archives of Biochemistry and Biophysics, 446*(2), 175-185. doi: 10.1016/j.abb.2005.12.004

Silva, C. F., Vilela, D. M., Cordeiro, C. S., Duarte, W. F., Dias, D. R., & Schwan, R. F. (2013). Evaluation of a potential starter culture for enhance quality of coffee fermentation. *World Journal of Microbiology and Biotechnology, 29*(2013), 235-247. doi: 10.1007/s11274-012-1175-2

Silva, M. D. F., Dionísio, A. P., Carioca, A. A. F., Adriano, L. S., Pinto, C. O., Abreu, F. A. P., Wurlitzer, N. J., Araújo, I. M., Garruti, D. S., & Pontes, D. F. (2017). Yacon syrup: food applications and impact on satiety in healthy volunteers. *Food Research International, 100*(1), 460-467. doi: 10.1016/j.foodres.2017. 07.035

Silva,M.L.C.,Costa,R.S.,Santana,A.S.,&Koblitz, M. G. B. (2010). Compostos fenólicos, carotenóides e atividade antioxidante em produtos vegetais. *Semina: Ciências Agrárias, 31*(3), 669-681. doi: 10.5433 /1679-0359.2010v31n3p669

Simonovska, B., Vovk, I., Andrenšek, S., Valentová, K., & Ulrichová, J. (2003). Investigation of phenolic acids in yacon (*Smallanthus sonchifolius*) leaves and tubers. *Journal of Chromatography A, 1016*(1), 89-98. doi: 10.1016/S0021-9673(03)01183-X

Svensson, L., Sekwati-Monang, B., Lutz, D. L., Schieber, A., & Ganzle, M. G. (2010). Phenolic acids and flavonoids in nonfermented and fermented red sorghum (*Sorghum bicolor* (L.) Moench). *Journal of Agricultural and Food Chemistry, 58*(16), 9214-9220. doi: 10. 1021/jf101504v

Taamalli, A., Contreras, M. D. M., Abu-Reidah, I. M., Trabelsi, N., & Ben Youssef, N. (2019). Quality of phenolic compounds: occurrence, health benefits, and applications in food industry. *Journal of Food Quality, 2019,* 1-2. doi: 10.1155/2019/9594646

Takenaka, M., Yan, X., Ono, H., Yoshida, M., Nagata, T., & Nakanishi, T. (2003). Caffeic acid derivatives in the roots of yacon (*Smallanthus sonchifolius*). *Journal of Agricultural and Food Chemistry, 51*(3), 793-796. doi: 10.1021/jf020735i

Tieking, M., Korakli, M., Ehrmann, M. A., Gänzle, M. G., & Vogel, R. F. (2003). In situ production of EPS by intestinal and cereal isolates of lactic acid bacteria during sourdough fermentation. *Applied Environmental Microbiology, 69*(2), 945-952. doi: 10.1128/aem.69.2.945-952.20 03

Topolska, K., Filipiak-Florkiewicz, A., Florkiewicz, A., & Cieslik, E. (2017). Fructan stability in strawberry sorbets in dependence on their source and the period of storage. *European Food Research and Technology, 243,* 701-709. doi: 10.1007/s00217-016-2783-0

Valentová, K., & Ulrichová, J. (2003). *Smallanthus sonchifolius* and *Lepidium meyenii* - prospective Andean crops

for the prevention of chronic diseases. *Biomedical Papers, 147*(2), 119-130. PMID: 15037892

Van Hijum, S. A. F. T., Bonting, K., van der Maarel, M. J. E. C., & Dijkhuizen, L. (2001). Purification of a novel fructosyltransferase from *Lactobacillus reuteri* strain 121 and characterization of the levan produced. *FEMS Microbiology Letters, 205*(2), 323-328. doi: 10.1111/j.1574-6968.2001.tb10967.x

Van Hijum, S., Van Geel-Schutten, G. H., Rahaoui, H., Van Der Maarel, M., & Dijkhuizen, L. (2002). Characterization of a novel fructosyltransferase from *Lactobacillus reuteri* that synthesizes high-molecular-weight inulin and inulin oligosaccharides. *Applied and Environmental Microbiology, 68*(9), 4390-4398. doi:10.1128/AEM.68.9.4390-4398.2002

Vanini, M., Barbieri, R. L., Ceolin, T., Heck, R. M., & Mesquita, M. K. (2009). A relação do tubérculo andino yacon com a saúde humana. *Ciência, Cuidado e Saúde, 8,* 92-96. doi: 10.4025/cienccuidsaude.v8i0.9723

Vasconcelos, C. M., Oliveira, E. B., Rossi, S. N., Arantes, L. F., Puschmann, R., & Chaves, J. B. P. (2015). Evaluating strategies to control enzymatic browning of minimally processed yacon (*Smallanthus sonchifolius*). *Food Bioprocess Technology, 8*(9), 1982-1994. doi: 10.1007/s11947-015-1558-0

Vega, R., & Zuniga-Hansen, M. E. (2015). The effect of processing conditions on the stability of fructooligosaccharides in acidic food products. *Food Chemistry, 173*(15), 784-789. doi: 10.1016/j.foodchem.2014.10.119

Vilhena, S. M. C., Câmara, F. L. A., & Kakihara, S. T. (2000). O cultivo de yacon no Brasil. *Horticultura Brasileira, 18*(1), 5-8. doi: 10.1590/S0102-05362000000100002

Watt, B. K., & Merrill, A. L. (1964). *Composition of foods: raw, processed, prepared.* (Agricultural Research Service, 198, Agriculture Handbook, 8). Consumer and Food Economics Research Division.

Whitaker, J. R., & Lee, C. Y. (1995). Recent advances in chemistry of enzymatic browning: an overview. In C. Y. Lee, & J. R. Whitaker (Eds.), *Enzymatic browning and its prevention* (pp. 2-7). Washington, ACS: Symp.Ser.600.

Yan, M. R., Welch, R., Rush, E. C., Xiang, X., & Wang, X. (2019). A sustainable wholesome foodstuff; health effects and potential dietotherapy applications of yacon. *Nutrients, 11*(11), 1-16. doi: 10.3390/nu11112 632

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2006). Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology, 97*(12), 1427-1430. doi: 10.1016/j.biortech.2005.06.018