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FULL PAPER

Kombucha based synbiotic beverage using Yacon (*Smallanthus sonchifolius*) as a fermentation substrate: development and sensorial analysis

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Abstract

Probiotic and prebiotic beverages are promoters of the growth and maintenance of healthy intestinal microbiota composition. However, a drink that gathers the therapeutic attributes of Yacon with symbiotic multiple yeast-bacteria colonies, like Kombucha, has not yet been explored and appears to be potentially beneficial for human health. The objectives of this work were to develop a low-sugar Kombucha beverage using Yacon as fermentation substrate, labelled as Yacon-Kombucha (YK), with physical-chemical properties and sensorial acceptability compatible with the traditional Sugar-Kombucha (SK). Two beverages were prepared following standard procedures and fermented for 14 days. The pH, acidity, Brix and glucose content were evaluated at days 0, 4, 7, 9 and 14. The sensorial panel used a hedonic scale of 7 points considering colour, aroma, taste and global acceptability attributes for SK and YK final product. At the end of fermentation, obtained were beverages in which YK was less acidic and had the lowest glucose content when compared to SK. The sensorial analysis revealed no significant differences between the two samples for the tested attributes, also no rejection was observed. However, the scores received for both drinks were moderate, with the Index of acceptability for SK as 75% and YK 72%. Improvements in the aroma and taste are necessary for an accessible yacon-Kombucha beverage. The development of a low-sugar synbiotic beverage was possible and it may present benefits to gut microbiota and other health properties and can be consumed by individuals with diabetes.

1. Introduction

Functional beverages added with live microorganisms (probiotics) and non-digestible food or ingredients that modulate the intestinal microbiome (prebiotics) have been considered as an increasingly important topic on human nutrition, exerting together a synergetic effect (synbiotics) on health promotion. Foods with these properties are part of the history of food and human technology development. Products like yogurt and wine are typically cited as the oldest in the Western world, while soy and tea are part of the Eastern traditional fermented foods (Gasbarrini *et al.*, 2016).

Kombucha is an ancestral beverage of Asian origin that has a slightly fizzy carbonated, sweet, and acidic taste, resembling cider, and is resulting in the fermentation of sugars with green or black tea (*Camelia sinensis*) by a synbiotic culture containing yeasts and bacteria. Known for its healthy properties and becoming a popular drink recently, it is rich in assorted types of metabolites and nutrients that benefit human health and gut microbiota (Jayabalan *et al.*, 2014; Leal *et al.*, 2018; Villarreal-Soto *et al.*, 2018).

A Synbiotic Culture of Bacteria and Yeasts (also known as SCOBY) has a microbiological composition of several fermentation yeasts and acetic acid-producing bacteria and a floating cellulose network on the surface of tea broth. The biochemical properties of this fermentation are complex. The anaerobic ethanol fermentation, the anaerobic organic acid fermentation and the aerobic oxidation of ethanol occur simultaneously along an oxygen gradient to produce acetic, lactic and gluconic acids as main chemical compounds (Jayabalan *et al.*, 2014; Villarreal-Soto *et al.*, 2018). The organoleptic properties and nutritional quality of the final product are dependent on the origin of the SCOBY associated with other variables related to production, for example, time and temperature of fermentation, as well as types and concentrations of the tea leaves and sugars used in the starter (Villarreal-Soto

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et al., 2018).

In order to occur, the fermentation process must contain sources of carbon (from sugars) and nitrogen (from tea), so that SCOBY can metabolize and produce the characteristic chemical compounds. This usually occurs by using brown or white refined sugar as substrates, but other sweet ingredients rich in carbohydrates are also used, such as honey, maple syrup, date syrup, coconut sugar, beet or sugar cane molasses (Malbaša *et al.*, 2008; Jayabalan *et al.*, 2014; Watawana *et al.*, 2017; Villarreal-Soto *et al.*, 2018; Khosravi *et al.*, 2019; Muhialdin *et al.*, 2019; Perry *et al.*, 2020).

All of those aforementioned types of substrates are rich in sucrose, glucose and fructose, and for that reason, even if residual on final fermentation, may restrict the Kombucha consumption of those who have limitations on and in which would benefit most from its health effects, as people with diabetes for instance (Jayabalan *et al.*, 2014; Leal *et al.*, 2018). In order to make this product accessible to the public, alternative carbon sources must be used to promote fermentation without leaving high amounts of glucose-like residues.

Yacon (*Smallanthus sonchifolius*) is a root native to the Andean regions of South America, considered a functional prebiotic food with positive technological and nutritional characteristics. Water content reaches up to 90% of its total fresh weight and dry matter content has up to 70% of fructooligosaccharides (FOS), which is its storage carbohydrate type. Generally, FOS can be naturally hydrolysed to simple sugars by the action of the enzyme fructan hydrolase, which converts it into fructose and glucose depending on the time and temperature of post-harvest and storage conditions (Santana and Cardoso, 2008).

FOS has long been characterized as a prebiotic molecule of a linear chain of fructose that varies the number between 2 and 10. It is classified as a probiotic molecule as it reaches the intestinal colon intact before going through microbial fermentation. There, it stands out for promoting a bifidogenic activity, which is a selective stimulation for the bifidobacteria proliferation in the human colon. The end products of FOS fermentation are generally short-chain acids, such as acetate, butyrate, propionate and lactate, mostly considered for their health-promoting benefits in cases of chronic diseases (Caetano *et al.*, 2016).

Developing a drink using fructooligosaccharides as a fermentation substrate has been the subject of recent researches using a single probiotic medium, such as *Bifidobacterium* (Manzoni *et al.*, 2012) or *Lactobacillus* (Dahal *et al.*, 2020). However, a drink that gathers the therapeutic attributes of Yacon with synbiotic multiple yeast-bacteria colonies, like Kombucha, has not been yet explored and appears to be potentially beneficial for human health. The evaluation of acceptability is important to place it before the consumer market. Hence, the objectives of this work were to develop a low-sugar Kombucha beverage using Yacon extract as fermentation substrate and compare the physical-chemical properties and sensorial acceptability with the traditional Sugar-Kombucha drink.

2. Materials and methods

2.1 Development of beverages

The production of the beverages was performed at the Pharmacognosy and Bromatology Laboratory. The drink was handled in an environment maintaining the hygienic standard of preparation and procedures were modified and adapted from Jayabalan *et al.* (2014). A SCOBY consortium of microorganisms was provided by a homemade cultivator in the city of Brasília, Brazil. Different Tea bases were used to receive the colonies labelled as Sugar-Kombucha (SK) and Yacon-Kombucha (YK). In the first (SK), it was used 1000 mL of before boiling mineral water (92°C), 10 g of green tea leaves and 120 g of common white table sugar. The second (YK) infusion was prepared with 600 mL of before boiling mineral water (92°C) and 10 g of green tea. Leaves were set in infusion for 5 mins, filtered, transferred to two previously sterilized 3-liter glass jars and were let chill to room temperature. Meanwhile, approximately 1000 g of Yacon was processed in a centrifugal juicer to obtain 400 mL of its extract that was added to the YK Tea base at room temperature. Afterwards, the SCOBY culture and remaining original broth (about 300 mL) were divided into two samples for respective cultivation. Jars were covered with multipurpose kitchen cloths wrapped with rubber bands for fixation and to ensure the oxygenation of the colonies. The Kombucha was then stored in a cupboard sheltered from light at uncontrolled room temperature (ranging from 25 to 30°C) and left in the fermentation process for 14 days.

2.2 Physicochemical analysis

Samples containing 20 to 50 mL aliquots were reserved on days 0, 4, 7, 9 and 14, for the SK and YK preparations, and stored in sterile flasks at constant refrigeration (6 to 10°C). Subsequently were assessed for the physicochemical tests in triplicates.

Potential of hydrogen (pH) values were determined by using a calibrated pH meter, read after stabilized. For titratable acidity (TA) the samples were prepared with 5 mL of liquid sample in a 250 mL Erlenmeyer flask, with 100 mL of distilled water and a drop of 1% phenolphthalein indicator added. Then, samples were titrated with standardized 0.1 N NaOH until permanent pink colour holds for 30 s. Acidity was calculated as acid content % mass/volume of the sample as calculated by Jayasena and Cameron (2008).

Degrees Brix (°Bx) was used to measure the amount of total soluble solids (TSS) in a sucrose solution and was measured in a refractometer with a temperature correction scale. Glucose content was determined by enzymatic spectrophotometry according to the manufacturer's instructions (Labtest®, Brazil). Before the procedure, samples were diluted at 1:10 in distilled water. After the read, converted results from mg/dL to g/100 mL.

2.3 Sensorial analysis

The sensory panel was carried out with 20 non-trained volunteers, individually tested in an isolated room with appropriate illumination. Two samples were presented to each individual: 30 mL of the Sugar-Kombucha (SK) drink and 30 mL of the Yacon-Kombucha (YK) drink, both at room temperature. Samples were codified and presented in a randomized way for each participant. A hedonic scale expressed as like or dislike in a 7-point scale (1 for "Dislike very much" and 7 for "Like very much"), for the following evaluated attributes: colour, aroma, taste and global acceptance. The index of acceptability (IA%) for each attribute was calculated according to Teixeira *et al.* (1987) as: IA% = [(mean attribute score × 100)/ maximum scale score]. Rejection was assumed if IA% < 70% (Teixeira *et al.*, 1987). All participants voluntarily signed the consent form and the study was approved by the Research Ethics Committee of the University.

2.4 Data analysis

The data were arranged in a spreadsheet, treated and analysed using Microsoft Excel®. The analyses were performed by descriptive statistics and t-test for sensorial analysis, considering results with statistical difference with a significance level of 5%.

3. Results and discussion

3.1 Physicochemical properties: less acidity and low-glucose beverage

Here the development of a low-sugar Kombucha with the healthy and technological properties of Yacon FOS was studied. It is known that the fermentation of traditional Kombucha depends on many factors, mostly as temperature, pH, time, quality and concentration of tea and sugar substrates, as reviewed by Villarreal-Soto *et al*. (2018). The physicochemical characteristics of a product are essential for its development and acceptance.

The results indicated that the longer the time left in fermentation, the greater the final acidity of Sugar-Kombucha (SK) and Yacon-Kombucha (YK) products, both for pH (Figure 1), and titratable acidity (Figure 2). It was observed that SK and YK started at day zero with nearly the same pH and acidity. As the fermentation process increased, the drinks became more acidic, as expected (Watawana *et al.* 2017; Villarreal-Soto *et al.*, 2018). However, at the end of the 14 days, YK was less acidic than SK.

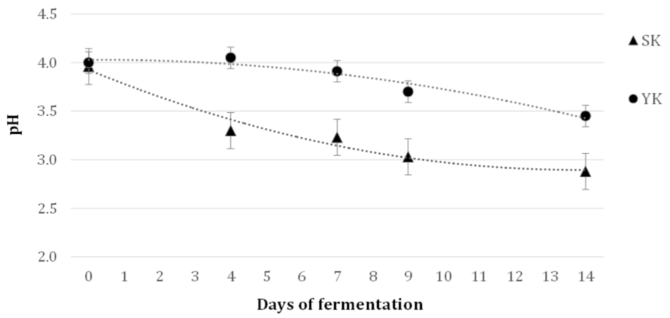

Figure 1. pH measurements of Sugar-Kombucha (SK) and Yacon-Kombucha (YK) during 14 days of fermentation. Error bars stands for standard deviations of replicates

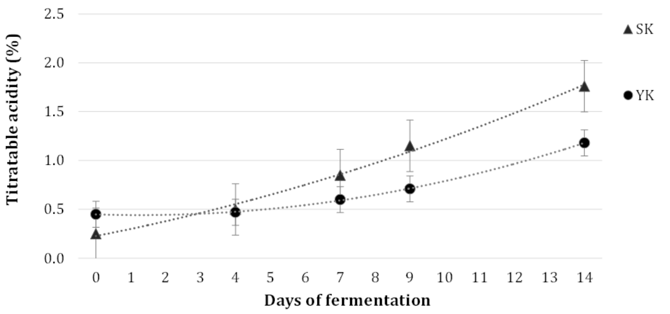

Figure 2. Titratable acidity (%) of Sugar-Kombucha (SK) and Yacon-Kombucha (YK) during 14 days of fermentation. Error bars stands for standard deviations of replicates.

The phenomenon that occurred for soluble solids (Figure 3) and glucose content (Figure 4), were rather different from that in acidity. Despite the increase in acidity for SK and YK, the °Bx remained stable with during the total fermentation period, as observed by others (Quiao-Won and Teves, 2018; Khosravi *et al.*, 2019; Santos *et al.* 2019). However, from the beginning, the °Bx for SK was about 4 times greater than YK, and they remained without much change throughout the 14 days.

In regular sugar-Kombucha fermentation, the initial hydrolysis of sucrose is attributed to the action of yeasts breaking it down into glucose and fructose. As fermentation progresses, fructose is used anaerobically to

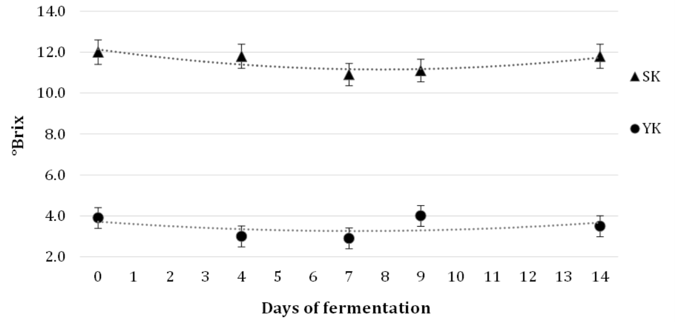

Figure 3. Total soluble solids (°Bx %) in Sugar-Kombucha (SK) and Yacon-Kombucha (YK) during 14 days of fermentation. Error bars stands for standard deviations of replicates.

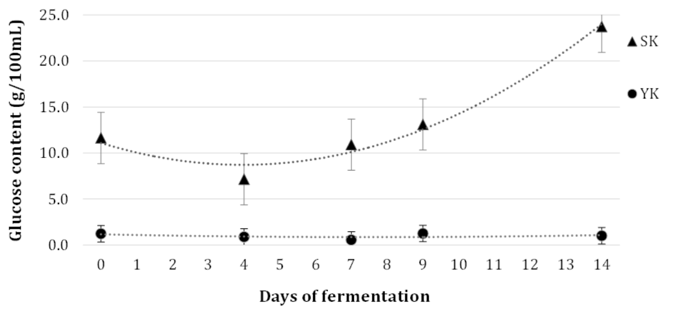

Figure 4. Glucose content (g/100 mL) of Sugar-Kombucha (SK) and Yacon-Kombucha (YK) during 14 days of fermentation. Error bars stands for standard deviations of replicates.

produce ethanol and, later, acetic acid, while acetic bacteria use glucose and ethanol to produce gluconic acid and acetic acid, respectively (Chen and Liu, 2000; Villarreal-Soto *et al.*, 2018). This allows acid concentrations to increase and consequently lower the pH, as observed here for both SK and YK. However, for the Yacon, the hydrolysis of FOS in acid medium mainly generated fructose residues (Blecker *et al.*, 2002), which then required additional steps in metabolic pathways to generate glucose (Villarreal-Soto *et al.*, 2018). Hence, the present data points to this argument, since the YK drink was less acidic than SK. In addition, the fact of less total soluble solid present in the YK may also be a factor in which contributed to less acidity.

Analysing the by-products of sucrose hydrolysis in SK, it can be noted that the glucose content is higher on the 14th day of fermentation, which suggests that fructose is preferred as a carbon source by yeasts. This corroborates other studies, which analysed the kinetics of sucrose fermentation and obtained under all conditions a higher glucose value than fructose in Kombucha fermentation (Chen and Liu, 2000; Khosravi *et al.*, 2019). In the present study, Yacon main carbohydrate FOS is a linear molecule of fructose, and hence would be expected a very low concentration of glucose, as in fact was observed. Muhialdin *et al.* (2019) showed that along 14 days of fermentation, using different sugar sources, the total soluble solids (TSS) had a high decrease in the sample prepared with white sugar (from 10 to 7) but moderate in samples with Coconut Palm (7.8 to 6.87) and Molasses Sugar (8.73 to 7.33). These differences can be explained by the different cultivation conditions and initial concentrations of the starter culture (Chen and Liu, 2000; Khosravi *et al.*, 2019; Muhialdin *et al.*, 2019). In any case, the different conditions required for the fermentation may reflect the acceptability of the product by consumers.

3.2 Sensorial analysis: acceptance without differences

The Sensory panel consisted of 20 non-trained volunteers (55% male) that 50% reported not knowing the drink, and 50% had never consumed it before. As for Yacon, 55% reported not knowing what Yacon was, 35% never had consumed Yacon before and 10% have consumed it before. Table 1 presents the average scores attributed by the volunteers. It was observed that there was no significant difference regarding the acceptance of colour, aroma, taste and global acceptance. Despite this, it was found that the YK drink was less ranked in all attributes, however was well accepted within the limit of the IA% index. Likewise, the SK drink performed better, but also close to the minimum acceptance limits.

This result may be explained due to the fact that volunteers were not familiar with Kombucha or Yacon consumption. However, the acceptability of Yacon or Kombucha products has been moderate in other studies. Gramza-Michałowska *et al.* (2016) observed moderate acceptability in different Kombucha brews and Salvador *et al.* (2012) also observed moderate acceptability (73.5% to 79.0% IA%) in pure and spiced Yacon jam products. Similarly to the present study, Dahal *et al.* (2020) prepared a fermented juice of Yacon with

Table 1. Scores in a 7-point scale (mean ± standard deviation) and index of acceptability (IA%) assigned by panellist for Sugar-Kombucha and Yacon-Kombucha

Sample	Sugar-Kombucha		Yacon-Kombucha		p-value
Attribute	Score	IA %	Score	IA %	
Color	5.90±0.79	84.29	5.75±0.97	82.14	0.330
Aroma	5.10±1.07	72.86	4.95±0.89	70.71	0.614
Taste	5.10±1.17	72.86	4.90±1.07	70.00	0.428
Global Acceptance	5.25±1.02	75.00	5.05±1.05	72.14	0.408

P-value stands for t-test comparison of means between groups. Differences were assumed as statistically significant at $p<0.05$.

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Lactobacillus acidophilus that received good acceptance, even with flavour and mouthfeel attributes receiving the lowest scores.

Since the taste of YK was the attribute with the lowest score, some suggestions for improving the acceptance index in future research shall be adopted. This includes a higher concentration of Yacon extract to be corresponding to TTS of sugar-based Kombucha, addition of non-caloric sweeteners and testing the acceptance by different times of fermentation.

The ratio of °Bx/Acid was used by Jayasena and Cameron (2008) to evaluate the quality of table grapefruits and found a positive correlation with consumer acceptability. Even though they are non-related products with differentiated sensory characteristics and consuming public, it is suggested that this ratio, or an equivalent, should be used in future research on acceptance of fermented drinks, in order to establish a better relationship between general acceptance, fermentation time and physicochemical characteristics of this type of beverage.

4. Conclusion

The development of an acceptable Kombucha drink prepared with Yacon and without sugar was possible due to fructose-rich FOS content, capable of providing conditions for fermentation. It is important that more projects seek to investigate the total chemical composition, analysing the beverages by accurate analytical techniques such as mass spectrometry and NMR spectroscopy. This will allow for the improvement of sensorial characteristics of this drink, which combines the therapeutic attributes of Kombucha and Yacon in a single synbiotic product, with the potential to benefit human health.

Conflict of interest

The authors declare no conflict of interest.

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