



Physicochemical and microbiological characterisation of a novel functional beverage from water kefir grains and yacon roots

Lina E. Merino^{1,2}, Ana F. Moretti^{1,3}, Caterina Irazoqui^{1,2}, Florencia Franchi^{1,2}, Yanina Pavón^{4,5}, and Paula L. Bucci^{1,6,*}

¹National University of Hurlingham (UNAHUR), Laboratory for Research on New Foods and Nutrition (LINAN), Governor Vergara Avenue 2222, B1688 Villa Tesei, Province of Buenos Aires, Argentina

²Scientific Research Commission of the Province of Buenos Aires (CICPBA), 526 between 10 and 11, B1900 La Plata, Province of Buenos Aires, Argentina

³National Scientific and Technical Research Council (CONICET), Godoy Cruz 2290, C1425 Autonomous City of Buenos Aires

⁴National University of Rafaela, Santa Fe. Bv. President Julio A. Roca 989, S2300 Rafaela, Santa Fe

⁵National University of the Litoral, Santa Fe. Bv. Pellegrini 2750, S3000 Santa Fe de la Vera Cruz, Santa Fe

⁶Institute of Sustainable Processes, Department of Chemical Engineering and Environmental Technology, University of Valladolid, Plaza de Santa Cruz, 8, 47002, Valladolid, Spain

*Corresponding author: Institute of Sustainable Processes, Department of Chemical Engineering and Environmental Technology, University of Valladolid.
Email: paulalorena.bucci@uva.es

Abstract

The development of functional fermented beverages that combine complementary ingredients is a promising approach to enhance nutritional and health benefits. This study formulated and characterised a novel beverage from yacon (*Smallanthus sonchifolius*) using water kefir grains. Yacon was chosen for its high content of fructooligosaccharides (60% dry weight) and antioxidant capacity (58% 2,2-diphenyl-1-picrylhydrazyl, DPPH inhibition). Water kefir provided a probiotic culture rich in lactic acid bacteria and yeasts. Fermentation was conducted under controlled conditions, followed by evaluation of physicochemical, microbiological, and sensory properties. The final product exhibited moderate antioxidant activity (38% DPPH inhibition), phenolic content around 40 mg Gallic Acid Equivalents/L, GAE/L, and organic acids including lactic, acetic, and butyric acids, resulting in a pH of about 3.6. Its volatile profile indicated complex metabolic activity consistent with fermentation. Ethanol concentration remained low (0.3%). Microbial analysis revealed beneficial fermentative organisms, with counts of 7.44 log Colony Forming Units CFU/ml lactic acid bacteria, 8.00 log CFU/ml acetic acid bacteria, and 7.81 log CFU/ml yeasts. Sensory evaluation with 141 untrained panellists indicated that approximately half were willing to purchase the beverage. These results suggest that the combined use of water kefir and yacon produces a novel, health-promoting fermented beverage with potential benefits for consumers interested in digestive health.

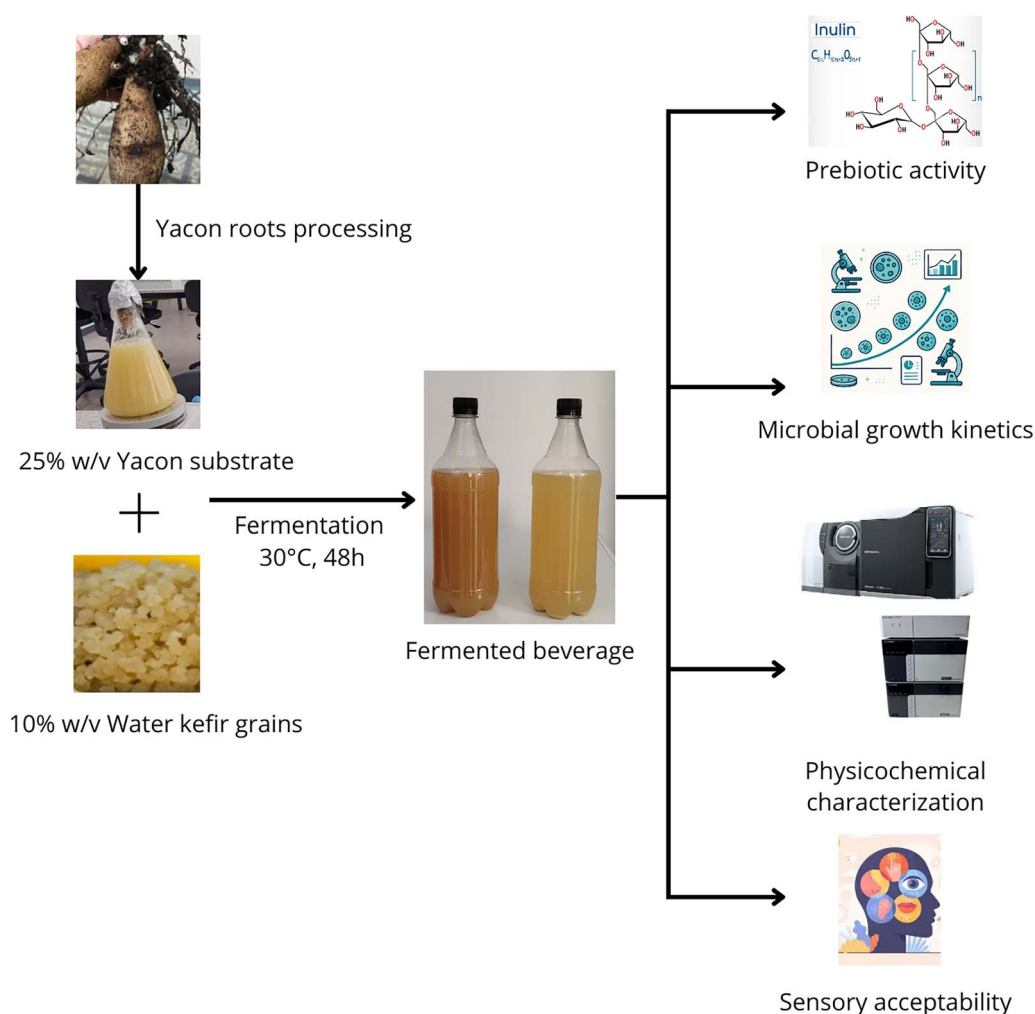
Keywords: antioxidant capacity, diabetes, functional foods, synbiotic, vegan, water kefir, yacon

Received: 20 March 2025. Accepted: 25 August 2025

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Graphical abstract



Introduction

Chronic diseases such as obesity are on the rise worldwide, driven by a complex interplay of genetic, physiological, environmental, and behavioural factors (WHO, 2023). This alarming trend is closely linked to the increased consumption of ultra-processed foods rich in calories, fats, sugars, and sodium, as well as increasingly sedentary lifestyles (Boushey et al., 2020). In this context, dietary interventions have become essential tools for preventing and managing these pathologies.

In recent years, global food trends have evolved towards improving the quality of life, with functional foods emerging as key contributors. These are foods that offer health benefits beyond basic nutrition by helping to prevent disease or promote well-being, often due to the presence of bioactive components with scientifically validated effects (Essa et al., 2023). Functional foods include probiotics (live microorganisms that confer health benefits when consumed in adequate amounts), prebiotics (nondigestible compounds that selectively stimulate beneficial gut bacteria), and synbiotics, which are combinations of both, harnessing the advantages of each (Yadav et al., 2022).

Among prebiotic-rich functional foods, yacon (*Smallanthus sonchifolius*) stands out as a promising Andean root crop. Native to regions from Colombia to northwestern Argentina and cultivated at altitudes ranging from 600 to 3,500 m, yacon is notable for

its high content of fructooligosaccharides (FOS) between 40.00% and 70.00% dry weight (Ojansivu et al., 2011), and inulin content was approximately 39.10% dry weight (Velásquez-Gamarra & Lozada-Urbano, 2022). Significant amounts of fructose, glucose, and sucrose have also been reported, with concentrations ranging from 4.13% to 26.00%, 1.96% to 10.01%, and 3.25% to 13.42% on a dry weight basis, respectively (Moscatto et al., 2006; Lobo et al., 2007; Habib et al., 2011; Choque Delgado et al., 2012). According to Palavecino Prpich et al. (2023), the composition of yacon juice showed values of moisture $72.70 \pm 0.17\%$ w/w, protein $1.09 \pm 0.09\%$ w/w, total lipids $0.84 \pm 0.08\%$ w/w, ash $1.02 \pm 0.03\%$ w/w, total dietary fibre $62.75 \pm 0.06\%$ w/w, FOS $58.90 \pm 0.07\%$ w/w, insoluble dietary fibre $3.85 \pm 0.05\%$ w/w, and carbohydrates $34.29 \pm 0.09\%$ w/w. In addition, yacon contains significant amounts of polyphenolic compounds including flavonoids, sesquiterpene lactones, and phenolic acids (Li et al., 2022; Plank et al., 2024; Silveira et al., 2024). These components have been associated with the stimulation of bifidobacteria in the colon, improved mineral absorption, enhanced gastrointestinal metabolism, and cholesterol regulation (Choque Delgado et al., 2012). Furthermore, studies have shown that yacon may reduce tumour proliferation (de Moura et al., 2012) and protect against DNA damage and cell proliferation (de Almeida et al., 2015) and support management of digestive and renal conditions

(Caballero Méndez & Colonia Pineda, 2018; Contreras-Puentes & Alviz-Amador, 2020).

Water kefir (WK) is a fermented, nondairy beverage produced by inoculating sugary solutions with kefir grains, which are composed of a symbiotic community of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts (LEV) embedded in a dextran matrix (Lynch et al., 2021). These microbial consortia drive acid-alcoholic fermentation, producing a variety of beneficial metabolites (Laureys & De Vuyst, 2014). Water kefir has been linked to numerous health benefits, including antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, gastroprotective, and cholesterol-lowering effects (Alsayadi et al., 2013; Aspiras et al., 2015; Rocha-Gomes et al., 2018; Brasil et al., 2019).

To date, various plant-based substrates have been evaluated for water kefir fermentation to produce beverages with unique functional and sensory properties (Corona et al., 2016; Gamba et al., 2019; Koh et al., 2018). The addition of fruits and vegetables such as carrot, fennel, melon, onion, tomato, and strawberry (Corona et al., 2016) and pumpkin (Koh et al., 2018) was also described. Recently, the addition of coconut extract (Alves et al., 2021), soy whey (Aziz et al., 2021), Russian olive fruit (Darvishzadeh et al., 2021), apple (Velázquez-Quirónes et al., 2021), red pitaya (Bueno et al., 2021), Cornelian cherry, hawthorn, red plum, rosehip and pomegranate (Ozcelik et al., 2021), and beet (Paredes et al., 2022) manifests the interest in the screening of new substrates for innovative fermented beverages. However, not all of these fermented beverages are suitable for individuals following specific dietary regimens, such as low-sugar diets.

Therefore, the primary objective of this research was to evaluate the feasibility of using yacon as a fermentable substrate for water kefir grain fermentation, with the goal of developing a novel synbiotic beverage that combines the benefits of both functional components. The study involved a comprehensive characterisation of the final beverage, including its physicochemical properties (such as pH, organic acids profile, and ethanol content) and microbiological dynamics (growth kinetics of lactic acid bacteria, acetic acid bacteria, and yeasts), as well as its functional properties (antioxidant capacity, prebiotic potential, and sensory acceptance). Ultimately, this work seeks to advance the development of an innovative synbiotic functional beverage with promising health-promoting effects, especially addressing the needs of the population affected by pathologies linked to high sugar intake.

Materials and methods

Materials

Water kefir grains (CMUNLP1) were obtained from the Microbiology Department at the National University of La Plata, Argentina. The grains were maintained in sugarcane solution at 4 °C and activated prior to use by incubation at 25 °C for 24 hr in fresh sugarcane solution. For fermentation, we used a methodology adapted from Gonda et al. (2019). Briefly, 30 ml of sugarcane solution was inoculated with 3 g of water kefir grains, maintaining a ratio that allows optimal microbial activity based on preliminary standardisation. The fermentation was conducted at 25 °C for 24 hr under static conditions. Yacon roots (*S. sonchifolius*) were sourced from a family farm in the community of Los Chorrillos, Jujuy, Argentina, harvested between July and August 2023. Only roots with normal appearance and no visible surface damage were selected. The following procedure was optimised in our laboratory. The tubers were washed and brushed in a 5% (v/v) sodium hypochlorite solution (commercial bleach, Clorox,

containing 5%–6% available chlorine) for disinfection, following standard food processing protocols. The roots were peeled and cut into approximately 1 cm³ cubes. These cubes were submerged in a 3% (w/v) citric acid solution (Sigma-Aldrich, analytical grade) for 2 min, drained, and centrifuged at 4,000 rpm for 5 min using a refrigerated centrifuge (Eppendorf 5810 R). Subsequently, the cubes were immersed in a 3% (w/v) ascorbic acid solution (Sigma-Aldrich, analytical grade) for an additional 2 min, drained, and centrifuged under the same conditions. Pretreated yacon cubes were blanched at 90 °C for 2 min in a temperature-controlled water bath (Julabo TW20), ensuring that the pH remained above 4.0 as monitored with a calibrated pH meter (Mettler Toledo SevenCompact). After blanching, samples were vacuum-packed using a FoodSaver V2860 vacuum sealer and stored at –20 °C until further processing.

Yacon-base substrate production

The pretreated yacon was crushed and dissolved in sterile potable water (25% w/v) by heating with stirring for 30 min at 78–83 °C (González-Gavilán et al., 2019; Arango Bedoya et al., 2008). It was subsequently filtered, and the yacon pieces were discarded. The yacon-based substrate was used immediately after production.

Kefir production

The fermented beverage (YK) was produced by inoculating drained water kefir grain at 10% w/v into the yacon-based substrate, without any other sugar source. In addition, a fermented beverage obtained from drained water kefir grain inoculated at 10% w/v into sugar water solution (4.5% w/v cane sugar) was performed as a control (water kefir, WK).

All fermentation processes were conducted at 30 °C for 48 hr (FAETA S.A.I.C.F.I. stove, model IS 23000C).

Chemical profile of water kefir and yacon roots

The chemical profile of water kefir and yacon was analysed using standard methods for total phenolic content (TPC) and total antioxidant capacity (TAC). For TPC, the Folin–Ciocalteu method, was employed by mixing 1 ml of sample with 5 ml of Folin–Ciocalteu reagent and 4 ml of sodium carbonate solution (7%), followed by absorbance measurement at 765 nm after 30 min using a UV-Vis spectrophotometer (Thermo Scientific Evolution 300), with a gallic acid standard curve (0–500 µg/ml gallic acid).

Results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g). TAC was assessed via the DPPH radical scavenging assay, with a Trolox standard curve (0–400 µM), where 0.2 ml of extract was mixed with 3.8 ml of DPPH solution (0.1 mM in methanol), and absorbance was measured at 517 nm after 30 min in the dark using the same spectrophotometer. Results were expressed as mg GAE/g. All measurements were conducted in triplicate ($n = 3$) to ensure reproducibility.

Volatile compounds were analysed using headspace gas chromatography–mass spectrometry (HS-GC-MS). Samples (5 ml for water kefir or 5 g for yacon) were placed in 20 ml headspace vials, sealed, and heated to 60 °C for 10 min in a headspace autosampler (Agilent Technologies 7697A). Volatile components were identified by injecting 1 ml of headspace gas into an HS-GC-MS system (Agilent Technologies 7890B GC coupled to a 5977A MSD) equipped with a DB-Wax column (Agilent, 30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed to start at 40 °C, increase by 5 °C/min to 220 °C, and compounds were identified by retention times and mass spectra compared to the NIST library, with quantification using an internal standard (2-hexanol).

Amino acid quantification was performed using high-performance liquid chromatography (HPLC) with precolumn derivatisation. Samples of water kefir and yacon roots were hydrolysed in 6 N hydrochloric acid (HCl) at 110 °C for 24 hr under an inert nitrogen atmosphere to prevent oxidation. After hydrolysis, the samples were neutralised with sodium hydroxide (NaOH), dried under vacuum, and reconstituted in ultrapure water. For derivatisation, o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) were used to label primary and secondary amino acids, respectively.

The HPLC analysis was conducted using an Agilent 1260 Infinity II system equipped with a quaternary pump, an autosampler, and a fluorescence detector (FLD). Chromatographic separation was achieved on a ZORBAX Eclipse Plus C18 column (4.6 × 250 mm, 5 µm particle size) maintained at 40 °C. The mobile phase consisted of Solvent A (0.1 M sodium acetate, pH 7.2) and Solvent B (acetonitrile:water:methanol, 45:45:10, v/v/v), following a linear gradient elution over 40 min. The flow rate was set at 1.0 ml/min, and the injection volume was 20 µl.

Fluorescence detection was performed with excitation and emission wavelengths of 340 and 450 nm, respectively. Amino acids were quantified by comparing the retention times and peak areas with authenticated standards (Sigma-Aldrich, USA) prepared under identical conditions. Results were expressed as mg of amino acid per g of dry sample, and all analyses were conducted in triplicate.

Fermentation and kefir grain biomass growth kinetics

Beverage's fermentation (yacon kefir, YK and WK) kinetics were evaluated by measuring pH (HACH HQxxD pHmeter) every hour for 25 hr at 30 °C.

To analyse kefir grain biomass growth, successive back sloppings were performed every 48 hr for 10 days. The increase in biomass was determined by weighing the wet grain using an analytical balance (Kretz, model EHB-3000).

Physicochemical characterisation of fermented beverages

Total soluble solid

A digital refractometer (Milwaukee, model MA871, Romania) was used to measure total soluble solids (TSSs). An aliquot (0.4 ml) of deionised water was placed on the prism well of the refractometer to obtain a standardised reading as per the manufacturer's recommendations. Following the removal of the deionised water, the refractometer was set to determine total solids in fermented beverages. An aliquot (0.4 ml) of each sample was placed in the prism well of the refractometer, and the result was displayed within a minute. The test was performed in duplicate.

Ash content

Ash content was determined by destroying the organic matter present in the sample through calcination and gravimetric analysis of the residue.

Empty capsules (iW) were weighed on an analytical balance (Radwag, model AS 220-R2, Poland). Approximately 30 ml of the sample (W) was added to each preweighed capsule and was dried in an oven at 150 °C for 90 min. The dried samples were then calcined for 2 hr at 550 °C in a muffle furnace (Tecnodalvo, model Dachel TD101). After calcination, the capsules were removed, cooled in a desiccator, and weighed again once they reached room temperature (fW). The ash content was calculated using the

following formula:

$$A\% = [(iW - fW) / W] * 100$$

where:

A%: Ash content expressed as a percentage.

iW: Weight of the empty capsule, in grams.

fW: Weight of the capsule with the sample after calcination, in grams.

W: Weight of the sample, in grams.

All samples were measured in duplicates. The results were expressed as % w/w.

Titrateable acidity

The samples were pretreated by heating for 5 min at 60–70 °C to eliminate carbon dioxide and were allowed to cool before analysis. For the titration, 25 ml of the sample was placed in a 100 ml Erlenmeyer flask and titrated with a 0.1 N sodium hydroxide solution (Merck, Darmstadt, Germany) using phenolphthalein as an indicator. All tests were performed in quadruplicate.

Titrateable acidity was calculated using the following formula:

$$A = [(Vol\ NaOH * N * eqW) / (W * 100)] * 100$$

where:

A: Titrateable acidity, expressed as grams of lactic acid per 100 ml of sample.

Vol NaOH: Volume of NaOH used in the titration, in millilitres.

N: Normality of the NaOH used in the titration.

eqW: Equivalent weight of lactic acid.

W: Weight of the sample, in grams.

Results were expressed as % w/v.

Ethanol content

The determinations of ethanol content were performed as described by the American Society of Brewing Chemists (ASBC (1979)). Samples were prepared by centrifuging 10 ml of each beverage at 5000 rpm for 10 min, followed by filtration through 0.45 µm sterile filters. The samples were stored refrigerated (4–6 °C) until analysis.

The ethanol content was analysed by gas chromatography using a liquid injection technique on a gas chromatograph (Agilent Technologies, Model 7890A, USA). A calibration curve was constructed using ethanol solutions of known concentrations ranging from 0% to 2.5% (v/v), with 1% (v/v) isopropanol as the internal standard. For measurement, 1 ml of the sample and 1 ml of the internal standard (1% n-propanol) were used. All tests were performed in quadruplicate.

Organic acid composition

Acetic, propionic, isovaleric, and butyric acid concentrations were analysed by gas chromatography at the Food Science and Technology Research and Development Center (CIDCA) using the liquid injection technique in a gas chromatograph (Agilent Technologies, Model 7890A, USA) and the DB FATWAX column. Standard solutions of acetic, propionic, and butyric acids were used for the calibration curve concentrations ranging from 0 to 100 mM (Sigma Aldrich, USA). The chromatograms were analysed using PeakFit v3.8 software (Systat Software Inc., San Jose, CA, USA).

Microbial growth kinetics

The counts of viable bacteria and yeasts in the fermentation products were determined by plating serial dilutions.

Lactic acid bacteria, acetic acid bacteria, and yeast concentration were determined by plating serial dilutions in tryptone (1 gl^{-1}) on DeMan, Rogosa, and Sharpe (MRS) agar, AAB medium agar (Carr & Passmore, 1979), and yeast glucose chloramphenicol (YGC) agar (Neogen, USA), respectively.

Microbial counts were determined at 10 min (zero time point), 4, 8, 24, and 48 hr of fermentation. Petri dishes were incubated aerobically at 30°C for 48 hr. Although some LAB species may prefer anaerobic conditions, most of the strains commonly associated with water kefir are aerotolerant and were adequately cultured under these conditions. The microbial concentration was expressed as CFU/ml. Two independent assays in duplicate per treatment were carried out.

Prebiotic activity determination

Lactobacilli growth

The growth of *Lactobacillus kefir* CIDCA 8321 and *Lactiplantibacillus plantarum* CIDCA 83114 was determined to evaluate the prebiotic activity of the yacon-based substrate.

Ten millilitres of MRS broth or yacon substrate was inoculated with $100 \mu\text{l}$ of lactobacilli strains and incubated for 48 hr at 30°C . Growth was evaluated using the plate count technique on MRS agar, incubating for 48 hr at 30°C .

B-Galactosidase activity

Enzymatic activity was determined according to Miller (1972). Briefly, in a sterile hemolysis tube containing 1 ml of saline solution, into which an ONPG (o-nitrophenyl- β -D-galactopyranoside) disc was placed and a single bacterial colony was inoculated. The tubes were incubated at 37°C for 4 hr, and enzymatic activity was quantified by measuring absorbance at 420 nm (Tille & Forbes, 2014). *Escherichia coli* and *Salmonella Typhimurium* were used as the negative and the positive controls, respectively. Results were calculated according to Blanco et al. (2001) expressed as EU.

Dietary fibre and inulin

For dietary fibre and inulin analysis, enzymatic-gravimetric methods were used. Total dietary fibre and inulin content were determined using the AOAC Official Method 2009.01. Although the method was not specifically validated for yacon-based matrices in this study, it has been widely used for high-FOS and inulin-rich foods. The analyses were carried out in triplicate with internal standards and recovery procedures to ensure reproducibility and accuracy in this matrix.

Total dietary fibre was measured by enzymatic digestion of samples (10 g) with α -amylase, protease, and amyloglucosidase (all from Sigma-Aldrich), followed by precipitation with ethanol, filtration, and weighing of the residue using an analytical balance (Mettler Toledo XS204). The inulin content was determined via high-performance liquid chromatography (HPLC) after hydrolysis of 1 g of sample with 0.2N hydrochloric acid at 80°C for 30 min. Chromatographic separation was achieved using an HPLC system (Shimadzu Prominence LC-20AT) with a C18 column (Agilent, $250 \text{ mm} \times 4.6 \text{ mm} \times 5 \mu\text{m}$) and acetonitrile:water (80:20) as the mobile phase, with detection at 210 nm using a UV detector (Shimadzu SPD-20A).

Sensory acceptability

The panel was formed by the community of the Universidad Nacional de Hurlingham, Argentina and was randomly selected. Each participant was asked to sign informed consent prior to taking part in the study, and the purpose of the activity was explained. It was clarified that participation was anonymous

and voluntary. Additionally, they declared themselves not to be allergic to any of the components of the drinks. Subsequently, they were invited to an evaluation room where individual spaces with controlled lighting and temperature conditions were provided. All samples were served randomly, coded, refrigerated, and in disposable white plastic cups with approximately 30 ml of the product. Potable water was used as a palate cleanser. The order of presentation of the samples was balanced among subjects.

For the attribute acceptability test, participants were instructed to observe, smell, and taste the samples and to indicate the degree of liking about appearance, odour, taste, and texture on a structured nine-point scale ranging from "dislike extremely" (1) and "like extremely" (9). Kefir samples (WK and YK) were considered acceptable if the mean scores were above 5 (neither like nor dislike). Purchase intention was also evaluated. In this case, participants answered a structured 5-point scale questionnaire ranging from "definitely would not buy" (1) to "definitely would buy" (5).

Statistical analysis

Statistical differences from the experimental determinations were evaluated using a two-factor analysis of variance (two-way ANOVA). Statistical analysis was performed using GraphPad Prism 8 for Windows version 8.01 (GraphPad Software, CA, USA).

Sensory acceptability results were statistically analysed using the t-Student test to evaluate significant differences ($p < .05$) using Statgraphics Centurion XV (Statpoint Inc., 2007).

Results

Chemical profile of water kefir and yacon roots

The chemical profile of WK and YK highlights their complementary bioactive properties, making them valuable ingredients for functional food development. Water kefir demonstrates moderate phenolic content (40 mg GAE/L) and antioxidant capacity (58% DPPH inhibition), reflecting its antioxidative potential influenced by fermentation conditions (Table 1). In contrast, YK presents a significantly higher phenolic content (40 mg GAE/100 g) and antioxidant capacity (38%) ($p < .01$), suggesting that the beverage has the potential to reduce oxidative damage at the cellular level due to its antioxidant compounds such as phenolic acids and organic acids, which are capable of scavenging free radicals. This inference is based on the antioxidant activity values obtained by the DPPH assay, as well as previous studies demonstrating the bioactivity of similar fermented matrices (Sabokbar et al., 2015). The volatile compound profile of WK, dominated by ethanol, acetic acid, and esters, adds complexity to its sensory attributes, while YK contributes sesquiterpene lactones and flavonoids that enhance its functional and aromatic properties. Both substrates are rich in essential amino acids, including glutamic and aspartic acid, which are crucial for cellular and neuronal functions, with YK exhibiting higher concentrations, making it a superior nutritional option. Organic acids, such as acetic and isovaleric acid, not only contribute to the flavour and preservation of these beverages but also play a key role in inhibiting pathogenic bacteria, thereby improving food safety. Additionally, YK is a rich source of dietary fibre and fructooligosaccharides (60% of its dry weight), which promote gut health and prebiotic activity, in contrast to WK's negligible fibre content. These attributes position WK and YK as promising candidates for functional beverages that support antioxidant intake, digestive health, and metabolic regulation, addressing the growing demand for health-promoting foods.

Table 1. Antioxidant activity of the fermented beverages (WK and YK) measured by DPPH assay. Values represent the percentage of free radical inhibition after 48 hr of fermentation. Bars represent mean $\pm$ SD ($n = 3$). Significant differences ($p < .01$) are indicated with “**.”

	Total phenolic content (TPC) (mg GAE/L)	Total antioxidant capacity (TAC) (%)
Water kefir	32 $\pm$ 0.7**	55 $\pm$ 4.1**
Yacon roots	48 $\pm$ 0.81**	69 $\pm$ 3.2**

Table 2. Amino acid profile (g/100 ml) of the fermented beverage produced with water kefir grains and yacon roots. Results are expressed as average values from three independent fermentations.

Amino Acid	Water kefir	Yacon roots
Alanine	1.5	2.2
Arginine	1.8	2.5
Aspartic acid	2.1	3.0
Cysteine	0.6	0.8
Glutamic acid	3.5	3.8
Glycine	1.2	1.9
Histidine	0.9	1.1
Isoleucine	1.3	2.0
Leucine	2.1	3.2
Lysine	1.7	2.8
Methionine	0.7	0.9
Phenylalanine	1.4	2.3
Proline	1.6	1.8
Serine	1.3	2.0
Threonine	1.5	2.4
Tryptophan	0.4	0.6
Tyrosine	1.0	1.4
Valine	1.6	2.9

The volatile profile of water kefir is dominated by compounds such as ethanol, acetic acid, lactic acid, and esters, which influence its sensory attributes and signify its metabolic complexity during fermentation. The volatile compounds in yacon, including sesquiterpene lactones and flavonoids, contribute to its characteristic aroma and bioactive profile.

The amino acid profile found in water kefir and yacon roots and the ANOVA results are shown in Table 2. In both water kefir and yacon, the highest concentrations of amino acids were those of glutamic acid, aspartic acid, and leucine, although, in yacon, these values were higher. High levels of some amino acids like glutamic acid are important because of their central role in neuronal function (Dalangin et al., 2020).

Fermentation and kefir grain biomass growth kinetics

The fermentation process in kefir is characterised by a decrease in pH values. The pH of both beverages decreased significantly during fermentation; however, the stabilisation pattern differed between them. Yacon roots exhibited a more rapid decrease and reached a stable pH by 24 hr, whereas WK continued to acidify until 48 hr. These differences were statistically significant ($p < .05$), confirming that the fermentation substrate influenced the acidification kinetics. At the beginning of fermentation at 30 °C, pH values were 5.70 ± 0.00 and 4.56 ± 0.00 for WK and YK, respectively. During the first 30 min postinoculation, these values

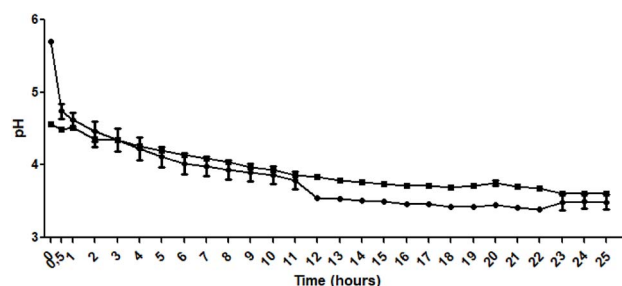


Figure 1. Changes in pH values during fermentation of yacon-based substrate (■) and cane sugar solution (●) inoculated with 10% w/v water kefir grains at 30 °C for 25 hr.

decreased to 4.74 ± 0.09 and 4.49 ± 0.01 , respectively. After 12 hr of fermentation, the pH stabilised at 3.45 for WK and 3.59 for YK, maintaining these levels until the end of the fermentation process (Figure 1). Moreover, the pH of WK and YK was below 4.5, a value that could be considered potentially safe from foodborne pathogens.

The substrate and its composition play an essential role in fermentation characteristics and, ultimately, the beverage properties. The most commonly used nutrient source for fermentation is sugar, mainly raw sugarcane (Gamba et al., 2021). However, the number of scientific reports in which water kefir grains are used for the fermentation of alternative substrates has increased in the last few years. The addition of fruits and vegetables such as black carrot, apple, grape, green cabbage (Agirman et al., 2024), yacon (Veeck et al., 2018), and pumpkin (Koh et al., 2018) has also been described. Agirman et al. (2024) reported that the pH values ranged from 3.71 ± 0.01 to 4.07 ± 0.00 after 24 hr of fermentation at 25 °C. A previous study demonstrated that water kefir prepared with yam extract, supplemented with brown sugar and sesame, achieved a pH of approximately 4.75 after 24 hr of fermentation at 22 °C (da Costa et al., 2018). Although fermentation parameters such as substrate, duration, and temperature impact the product's final pH (Caro Vélez & León Peláez, 2015; Cai et al., 2020), these results indicate that a yacon-based substrate may be suitable for supporting kefir grain fermentation.

Kefir grain mass production showed significant differences between the beverages (Figure 2). In the YK, the biomass of water kefir grains remained nearly constant across nine fermentation cycles, with values ranging from 10.0 ± 0.1 g to 11.85 ± 0.05 g, showing no statistically significant increase ($p > .05$). In contrast, grains fermented in a sucrose (sugar cane) solution (WK) increased significantly from 10.0 ± 2.6 g to 45.7 ± 0.3 g ($p < .01$). The lack of biomass growth in YK may be attributed to the lower content of simple fermentable sugars (e.g., glucose and sucrose) in yacon, which contains predominantly FOS. The sugar levels in the yacon-based substrate were adequate to support LAB growth, as indicated by stable CFU counts. However, the lower concentration of simple sugars compared to sucrose-based media may have limited exponential growth and matrix synthesis, affecting grain biomass expansion but not microbial viability. These complex carbohydrates may not support rapid microbial matrix synthesis to the same extent as sucrose. While the WK kefir grain mass quadrupled, the YK mass did not increase and remained at the initial values after nine successive backslippings. This phenomenon may be due to the fact that the use of yacon as a fermentation substrate results in either the lack of essential components for the production of the grain or conditions that do not favour it. Grain biomass increase is highly associated

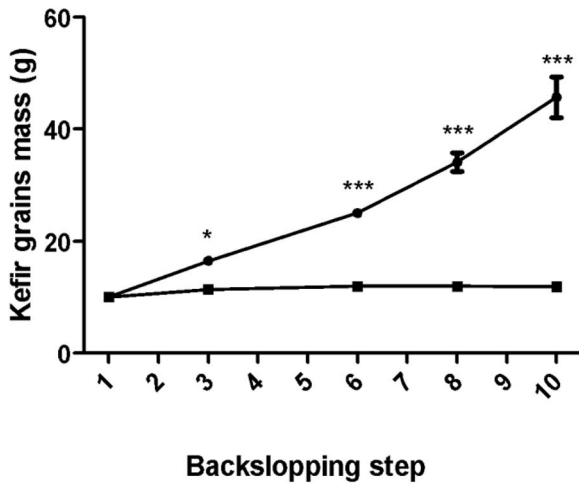


Figure 2. Biomass variation of water kefir grains during fermentation using two different substrates: sugar water (WK) (■) and yacon-based solution (YK) (●), across nine successive backsloppings over 10 days. Grain biomass increased significantly in WK, while remaining stable in YK, suggesting substrate-specific differences in microbial matrix synthesis. Significant differences ($p < .001$ and $p < .05$) are indicated with “***” and “*,” respectively.

with the synthesis of matrix components by microorganisms coexisting in the kefir grain. The WK grain consists of a diverse array of microbial species, each with distinct optimal pH values and growth temperatures. The main component of the grain matrix is a glucan named dextran (Fels et al., 2018). Although *L. hilgardii* is considered one of the key contributors to dextran production (Pidoux, 1989), its presence is not sufficient to promote grain growth (Gulitz et al., 2011). Low water kefir grain growth results in small water kefir grains with high viable counts of microorganisms, resulting in fast fermentation with low residual carbohydrate levels and high metabolite concentrations (Laureys & De Vuyst, 2017). Nevertheless, in relation to our research, it could be possible that the addition of an extra source of nutrients, such as dried figs, to the yacon substrate may be a viable alternative to enhance the potential for grain growth (Laureys et al., 2018). In this sense, these results could indicate the need to maintain a stock of granules that reproduce under optimal conditions when working with alternative substrates such as yacon, for the reuse of the grains over time in larger-scale or industrial production.

Physicochemical characterisation of fermented beverages

Regarding TSS content, WK initially exhibited a value of $8.2 \pm 0.5^\circ$ Bx, while YK displayed $2.5 \pm 0.1^\circ$ Bx. The difference in sugar content at the initial fermentation stage may influence the microbial count at the end of the process. After fermentation, the values decreased to $5.6 \pm 0.9^\circ$ Bx for WK and $1.2 \pm 0.1^\circ$ Bx for YK (Table 3). In both cases, a reduction in $^\circ$ Bx was observed, indicating sugar consumption due to microbial metabolism. According to these results, TSS determination indicates that YK contains less sugar available for fermentation, which correlates with reduced kefir grain biomass generation. To preserve kefir grain in optimal conditions, it is essential to maintain a constant supply of active kefir grain with sugar or an alternative substrate that supports kefir grain production.

Regarding the ash content (Table 3), values of 0.12 g/100 g were found for WK and 0.24 g/100 g for YK. Other authors have found similar ash contents in yacon, with values close to 0.3 g/100 g

Table 3. Physicochemical properties of the fermented beverages produced with water kefir grains using two substrates: traditional sugar-water (WK) and yacon-based solution (YK). Data are expressed as mean $\pm$ standard deviation. Asterisks (*) denote significant differences between WK and YK (Student's t-test, * $p < .05$; ** $p < .01$).

	Sugar consumption (%)	Ashes content (g/100 g)	pH	Lactic acid content (g/100 g)	Ethanol content (%)	Acetic acid content (mM)	Propionic acid content (mM)	Butyric acid content (mM)	Isovaleric acid content (mM)	Acetic acid/ethanol relation (mmol/mol)
Water kefir (WK)	31.71 ± 5.45	0.1198 ± 0.518	3.62 ± 0.25	0.666 ± 0.346	$0.290 \pm 0.009^{**}$	$27.30 \pm 0.49^{**}$	8.37 ± 0.30	$13.93 \pm 0.43^*$	n.d	$0.433 \pm 0.034^*$
Yacon kefir (YK)	$52.00 \pm 4.78^*$	0.239 ± 0.063	3.20 ± 0.23	0.649 ± 0.057	0.170 ± 0.00	40.20 ± 0.38	8.98 ± 0.29	7.64 ± 0.37	1.01 ± 0.26	2.610 ± 0.000

(Grau & Rea, 1997; Lachman et al., 2003; Vasconcelos et al., 2010). Among the minerals found, potassium, calcium, and magnesium were detected. Ash content in sugarcane ranges from 1% to 3% with mineral composition including elements such as calcium, potassium, iron, and magnesium (Sampaio et al., 2023). These minerals may influence the growth of the fermentative microbiota.

Acidity was similar for both WK and YK. The titratable acidity values were 0.6 g of lactic acid per 100 ml of sample, attributed to the growth of LAB, and no significant differences were observed. Regarding titratable acidity, lactic acid was identified as the predominant organic acid when yacon was used as a substrate (Rubio et al., 1993). The production of lactic acid can be attributed to the activity of lactic acid bacteria present in kefir grain, including *L. casei/paracasei*, *L. hilgardii*, *L. nageli*, *Lactococcus* sp., *Oenococcus* sp., *Streptococcus lactis* y *Leuconostoc mesenteroides* (Pidoux, 1989; Pidoux et al., 1990; Magalhães et al., 2010; Gultiz et al., 2013; Marsh et al., 2013; Laureys & De Vuyst, 2014, 2017; Laureys et al., 2016, 2018; Zanirati et al., 2015; Fiorda et al., 2017; Gamba et al., 2019; Talib et al., 2019; Romero-Luna et al., 2020).

Ethanol is produced by the metabolism of yeast. According to the results, ethanol content at the end of fermentation was affected by the fermentable substrate used. The ethanol content of the beverages was 0.29% in WK and 0.071% in YK. This lower ethanol content may be attributed to the fermentative activity of the AAB, potentially influenced by the chemical properties of yacon as a substrate. This aligns with previous studies suggesting that the availability of fermentable sugars and other substrate characteristics directly affect yeast and acid bacteria metabolic activity during fermentation (Fiorda et al., 2017; Laureys et al., 2018; Sy et al., 2023).

Additionally, both beverages comply with the requirements of the Argentine Food Code, which mandates that nonalcoholic beverages must not exceed an ethanol content of 0.5% v/v. Compliance with this regulation not only ensures the safety and acceptability of the product for a broader audience, including those who prefer nonalcoholic options, but also positions the beverage as a promising candidate in the functional foods market.

Organic acids are important in fermented foods not only for sensory characteristics and acceptability but also because they are natural biopreservatives. In this study, the identity and concentration of organic acids in WK and YK were determined. We found significant amounts of acids in the fermented beverages, which was expected since the TSS content decreased due to microbial fermentation and metabolic activity. The identified organic acids are listed in Table 3, where acetic acid was the predominant acid detected in both samples, with 27.3 mM for WK and 40.20 mM for YK. The amount of this metabolite is related to AAB found in these beverages. Also, propionic and butyric acids were found in WK while isovaleric acid was found in YK only. The presence of lactic, acetic, propionic, and butyric acids in the fermented beverage aligns with other studies on kefir-type fermentations (Fiorda et al., 2016; Gökirmaklı et al., 2023). Fermentation of different fruit-based media with WK culture showed that the substrate used influenced the type and proportion of organic acids produced (Zannini et al., 2023). Organic acid production by LAB is known to exert inhibitory effects on the growth of pathogenic microorganisms. Particularly, lactic acid and acetic acid have a strong inhibitory effect on Gram-negative bacteria (Moretti et al., 2019; Al-Mohammadi et al., 2021). These results highlight the safety of the beverage and its protective effect against pathogenic bacteria responsible for foodborne diseases (FBDs).

The concentration of microorganisms in kefir is determined by fermentation conditions such as substrate, temperature, and time. This microbial consortium in kefir will impact the metabolites profile in the beverages (Zannini et al., 2023). In this study, the effect of using sugarcane or yacon as a fermentation substrate on the concentration of viable microorganisms during the process was analysed. Growth curves of the three predominant microbial groups in kefir were generated (Figure 3A–C). Lactic acid bacteria populations grew and remained stable in both WK and YK beverages. After 48 hr, the LAB count in WK reached 7.83 ± 0.25 log CFU/ml while in YK, it was 7.44 ± 0.14 log CFU/ml, without a significant difference ($p < .05$).

In YK, the available sugar content, composed predominantly of FOS and minimal levels of glucose and sucrose, was adequate to sustain LAB viability, as CFU/ml counts remained above 7 log. However, the absence of high concentrations of simple sugars likely reduced the rate of microbial synthesis of extracellular polysaccharides, which may explain the lack of kefir grain biomass increase in this substrate.

Acid acetic bacteria growth was consistent between both samples during the fermentation, with similar counts observed in both beverages ($p < .05$), reaching values of 8 log CFU/ml, suggesting that they are equally resilient and capable of thriving under both conditions. This finding is also consistent with the amount of acetic acid found by HPLC in each beverage.

YK exhibited significantly higher yeast counts (7.81 ± 0.12 log CFU/ml) compared to WK (6.63 ± 0.20 log CFU/ml) after 48 hr of fermentation ($p < .001$), indicating that the yacon substrate provides favourable conditions for yeast proliferation. While the final pH of the beverage (~ 3.6) is low enough to inhibit many spoilage and pathogenic microorganisms, elevated yeast levels may compromise shelf life due to continued metabolic activity, CO₂ production, and potential flavour or texture alterations during storage. The shelf-life analysis of the fermented yacon-based beverage (KY) was conducted after 14 days of refrigerated storage. Microbiological counts revealed that LAB remained stable at 7.12 log CFU/ml, and yeasts were maintained at 7.25 log CFU/ml. These values indicate good microbiological stability and suggest that the probiotic viability is preserved under refrigerated conditions. However, given the high yeast population, continuous monitoring is advised to prevent secondary fermentation, which could affect sensory quality or safety during extended storage. Given their role in ethanol and aromatic compound production, the yeast population is crucial for preserving the sensory characteristics of kefir. Since the amount of ethanol found in YK was lower than in WK, it is possible that part of the ethanol produced by the yeasts was converted into acetic acid by the metabolism of AAB after 48 hr of fermentation. The literature reviewed indicated that AAB growth benefits acetic acid metabolism (Fiorda et al., 2017). These findings highlight the differential microbial responses to the substrates, with yacon favouring yeast, which may influence the overall sensory and functional properties of the respective beverages. The final counts of lactic acid bacteria and yeasts in WK and YK achieve the requirements for water kefir according to the recently updated Argentine Food Code (Chapter XIII, Resolution 7/2024), ensuring the quality and safety of the product. Furthermore, these results are consistent with those found by other authors. The amount and type of microorganisms in the fermented beverage depend on the substrate used (Gökirmaklı et al., 2023; Zannini et al., 2023), but counts of lactic acid and acetic acid bacteria and yeasts did not change significantly in different sugar solutions (Gamba et al., 2019).

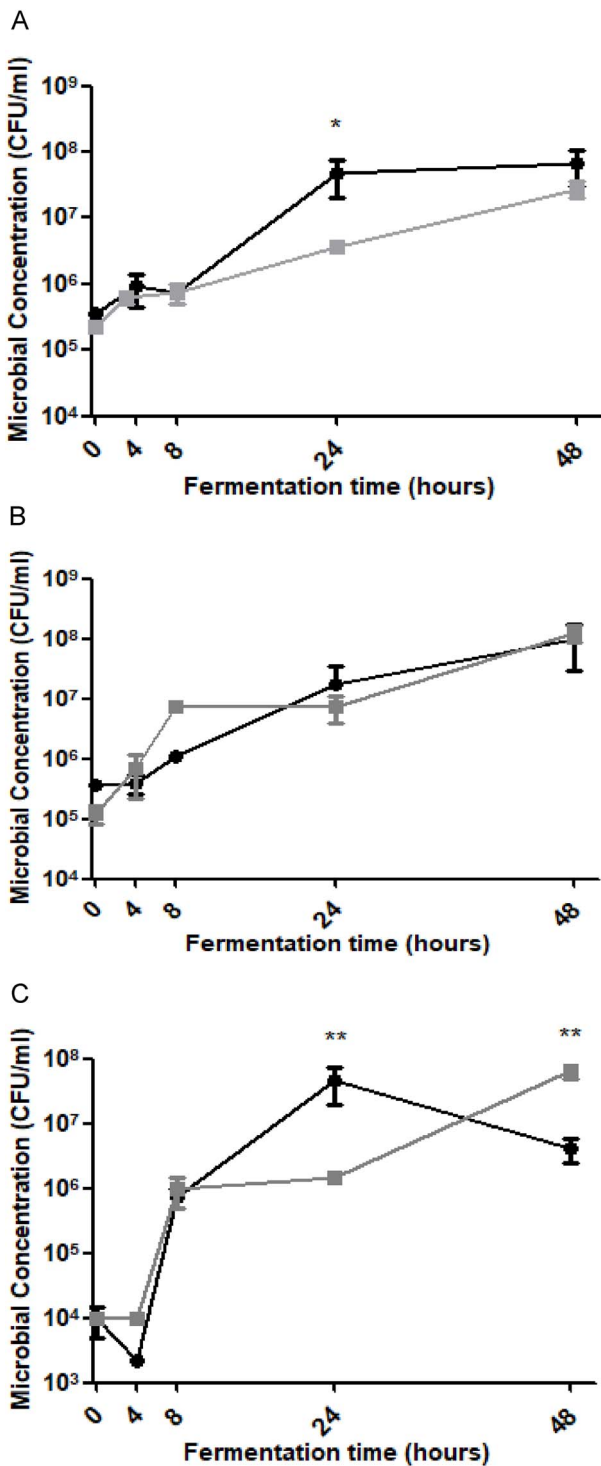


Figure 3. Microbial growth kinetics of (A) lactic acid bacteria, (B) acetic acid bacteria, and (C) yeasts in water kefir (WK, $\blacksquare$) and yacon kefir (YK, $\blacksquare$) beverages over 48 hr of fermentation at 30 °C. Results expressed as CFU/ml. Asterisks denote statistically significant differences between samples (* $p < .05$, ** $p < .001$). The curves highlight differential microbial dynamics based on the fermentation substrate.

Prebiotic activity determination

Prebiotic ingredients increase the growth of intestinal flora, improving its functionality. In this study, we analysed the ability of yacon to stimulate the growth of two strains of lactobacilli isolated from milk kefir grains, which have been previously

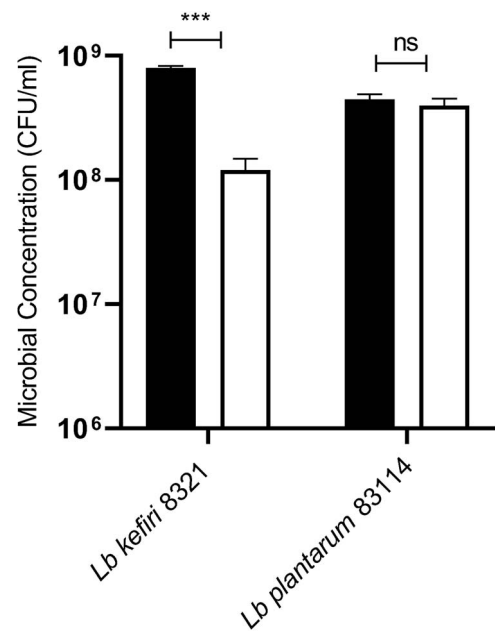


Figure 4. Growth of BAL strains *Lactobacillus kefir* CIDCA 8321 and *Lactiplantibacillus plantarum* CIDCA 83114 in MRS medium (black columns) and yacon-based substrate (white columns) after 48 hr of incubation at 30 °C. Data represent mean $\pm$ SD of CFU/ml from triplicate samples. Significant differences ($p < .001$) are indicated with “***”.

characterised for their probiotic potential (Kakis et al., 2013; Hugo et al., 2016; Merino et al., 2019, 2025; Cejas et al., 2017; Moretti et al., 2024). To evaluate yacon as a growth promoter for these strains of lactobacilli, it was compared with growth in MRS medium, which is generally used for the cultivation of these microorganisms, under the same culture conditions. Statistical analysis revealed no significant differences from *L. plantarum* CIDCA 83114 growth, while significant differences were observed from *L. kefir* CIDCA 8321 growth ($p < .001$) (Figure 4). These results suggest that a yacon-based substrate was an adequate medium for the growth of these microorganisms.

This study investigated the prebiotic activity of yacon roots, highlighting their exceptional potential as a dietary component for gut health. In addition to its high fibre content (10 g/100 g), yacon contains 40%–70% FOS of its dry matter. These prebiotic compounds enhance gut health by selectively promoting the growth of beneficial microbiota, such as lactobacilli and bifidobacteria. The fibre and FOS content also contribute to improved intestinal transit, enhanced mineral absorption, and reduced cholesterol levels.

Yacon's effectiveness as a substrate for specific probiotic strains is further demonstrated by its ability to stimulate β -galactosidase activity. For instance, *L. kefir* CIDCA 8321 exhibited significantly higher enzymatic activity (2.15×10^{-7} EU) compared to *L. plantarum* CIDCA 83114 (4.71×10^{-8} EU), underscoring yacon's role in supporting probiotic function and enhancing health-promoting properties.

The probiotic-rich matrix of water kefir, combined with its moderate antioxidant activity, complements the prebiotic properties of yacon. Together, yacon and water kefir present a synergistic approach to functional food development: Yacon supports gut microbiota balance and antioxidant defence, while WK enhances probiotic intake. This combination offers a comprehensive strategy to support digestive health and overall metabolic well-being when regularly incorporated into the diet.

Table 4. Sensory acceptability of the fermented beverage developed from water kefir grains and yacon roots. Results are presented as mean $\pm$ standard deviation for sensorial attributes including odour, taste, texture, and overall acceptance. The table also includes the percentage of respondents who expressed willingness to consume and/or purchase the beverage. Significant differences in column values ($p < .0001$) are indicated with “****.”

	Appearance	Odour	Taste	Texture	Purchase intention
Water kefir (WK)	6.4 $\pm$ 1.5	6.1 $\pm$ 1.3	6.5 $\pm$ 1.8	6.7 $\pm$ 1.6	3.2 $\pm$ 1.2
Yacon kefir (YK)	5.6 $\pm$ 1.7****	4.2 $\pm$ 1.6****	3.7 $\pm$ 1.3****	5.3 $\pm$ 1.6****	2.0 $\pm$ 0.9****

Sensory acceptability

A total of 141 consumers (104 female and 37 male) aged between 18 and 71 years were recruited from the community of the Universidad Nacional de Hurlingham (UNAHUR), Argentina. The gender imbalance observed among the sensory panel participants is likely influenced by broader demographic trends within the university. Specifically, the academic programs offered at UNAHUR have a predominantly female student population, representing approximately 65% of total enrollment. This demographic characteristic may have contributed to the higher proportion of female participants in the panel, rather than reflecting gender-based differences in sensory perception or interest in the study. The Check-All-That-Apply evaluation involved 21 sensory attributes covering appearance, aroma, taste, and texture, and YK was perceived as more intense in acidity (data not shown).

In Table 4, the average values and standard deviations for the acceptability of attributes and purchase intention are reported. Significant differences ($p < 0.05$) were observed among the samples regarding all attributes tested. In all attributes, the WK sample received the highest score (e.g., “like slightly/like moderately”), whereas in the case of YK, odour and taste were the least acceptable attributes (e.g., “dislike moderately/dislike slightly”).

Consumers are hesitant to purchase this type of beverage. About 48% of the participants would buy WK, while only 7% would buy YK. These results are related to the mean scores of purchase intention, with the WK sample showing higher averages.

WK is associated with an acidic, slightly sweet, fizzy, and slightly fermented aroma and taste (Gamba et al., 2019; Hsieh et al., 2012; Laureys et al., 2018, 2019). Studies have demonstrated that different grains, even when cultured under similar conditions, impart distinct flavour profiles to the final product, highlighting the significant influence of community structure on the organoleptic properties of the fermented beverage (Laureys & De Vuyst, 2017). Metabolite profiling of water kefir has previously attributed sweet notes to residual sugars, whereas acidic notes are derived from lactic and acetic acids (Laureys & De Vuyst, 2014; Magalhães et al., 2010). However, according to our results (Table 3), a higher concentration of acetic acid in YK could negatively affect the taste.

The production of volatile compounds associated with aroma and flavour (e.g., esters, higher alcohols, and higher aldehydes), as well as the microbial contributions to this process, remains less well understood. It is believed that these compounds are generated through multi-step conversion pathways originating from amino acid substrates present in the matrix (Hazelwood et al., 2008; Smid & Kleerebezem, 2014). Nevertheless, their identity and amounts vary depending on the microbiota of the grains and the composition of the medium (Laureys & De Vuyst, 2017). Based on the findings outlined in Table 2, both substrates exhibited a diverse amino acid profile, contributing to the enhanced sensory complexity of fermented beverages.

Conclusion

This study demonstrates the feasibility of producing a fermented functional beverage from yacon roots (*S. sonchifolius*) using WK grains as a starter culture, achieving a product with physico-chemical, microbiological, and functional characteristics suitable for consideration within the emerging market for plant-based fermented foods. The fermentation revealed differential microbial dynamics depending on the substrate used. Yacon is a substrate rich in dietary fibre and FOS, which significantly contribute to the generation of prebiotic compounds, highlighting its synbiotic potential when combined with the probiotic microbiota present in WK.

The final product effectively integrates prebiotics from yacon with probiotics present in the grains, forming a synbiotic matrix that promotes intestinal health and microbiota balance. It also complies with regulatory limits for alcohol content ($<0.5\%$ v/v), ensuring its suitability for consumption by diverse populations, including those seeking nonalcoholic products. Furthermore, its moderate antioxidant activity and complex volatile compound profile reinforce its functional and sensory potential.

The use of yacon as a fermentable substrate in WK grains offers significant advantages for industrial production, such as reduced costs due to the use of a low-cost plant resource and the elimination of the requirements linked to lactic fermentation, resulting in a light and naturally vegan beverage. The complementarity between the heterogeneous microbial composition of water kefir—composed primarily of LAB and yeast—and the nutritional profile of yacon, especially its high FOS content, contributes to an efficient fermentation process and the production of a product with integrated probiotic and prebiotic benefits.

However, the moderate sensory response observed in non-specialised consumers highlights the need to optimise organoleptic attributes to increase acceptability and facilitate market entry. Future strategies may include the incorporation of natural aromatic modulators (fruit extracts, citrus essential oils, and aromatic herbs), the optimisation of fermentation conditions (time, temperature, inoculation), and the addition of natural texturising agents to enhance sensory quality and the consumer experience. Addressing these factors could improve consumer appeal and facilitate the integration of such beverages into health-conscious diets.

In summary, this work provides relevant scientific evidence for the development of functional fermented beverages based on yacon and WK, which are low-sugar alternatives suitable for consumers with different dietary preferences. Future lines of research should prioritise extended sensory evaluation, microbiological and physicochemical stability during storage, as well as clinical studies to validate the product's physiological and functional effects, with a view to industrial scalability and potential public health impact.

Supplementary material

Supplementary material is available at *International Journal of Food Science and Technology* online.

Data availability

Data will be made available on request.

Author contributions

Lina Merino (Conceptualisation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualisation, Writing—original draft [equal]), Ana F. Moretti (Conceptualisation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing—original draft [equal]), Caterina Irazoqui (Conceptualisation, Formal analysis, Supervision, Validation, Writing—original draft [equal]), Florencia Franchi (Conceptualisation, Investigation, Methodology, Writing—original draft [equal]), Yanina Pavón (Formal analysis, Investigation, Methodology, Writing—review & editing [equal]), and Paula Bucci (Conceptualisation, Formal analysis, Investigation, Methodology, Supervision, Visualisation, Writing—review & editing [equal])

Lina Merino (Conceptualisation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualisation, Writing—original draft [equal]), Ana F. Moretti (Conceptualisation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing—original draft [equal]), Caterina Irazoqui (Conceptualisation, Formal analysis, Supervision, Validation, Writing—original draft [equal]), Florencia Franchi (Conceptualisation, Investigation, Methodology, Writing—original draft [equal]), Yanina Pavón (Formal analysis, Investigation, Methodology, Writing—review & editing [equal]), and Paula Bucci (Conceptualisation, Formal analysis [equal], Investigation [equal], Methodology [equal], Supervision [equal], Visualisation [equal], Writing—review & editing [equal])

Funding

The authors gratefully acknowledge the financial support for this work from the National University of Hurlingham (Argentina).

Conflicts of interest

None declared.

Acknowledgements

Water kefir grains (CMUNLP1) were provided by the Cátedra de Microbiología, Facultad de Ciencias Exactas, Universidad de La Plata (La Plata, Argentina).

We would like to thank Professor Graciela De Antoni of the National University of La Plata and CIDCA, who kindly provided *Lactobacillus kefir* CIDCA 8321 and *Lactiplantibacillus plantarum* CIDCA strains used in this study.

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