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Research Article

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Posted Date: March 25th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9066402/v1>

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Additional Declarations: No competing interests reported.

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Short running title: New functional fermented goji-based beverages

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Abstract

Goji berry fruits (*Lycium barbarum* L.) are recognized for their health benefits due to the presence of bioactive compounds such as organic acids, polyphenols, and carotenoids. The aim of this study was to develop and characterize new functional fermented goji berry juice beverages with lactic acid bacteria (*Lactiplantibacillus plantarum*), and yeast (*Saccharomyces cerevisiae*), applied in both monoculture and co-culture. All tested strains exhibited good growth properties in goji juice, maintaining high viability throughout the 15-day fermentation period. While *L. plantarum* drove a significant increase in acidity and phenolic content—enhancing antioxidant capacity of the fermented beverages—yeast fermentations were characterized by a sharp decrease in sugars. Carotenoid compounds increased mainly in yeast monocultures, remaining stable in co-cultures of yeast and lactic acid bacteria. Volatile profiling identified 24 compounds, dominated by acetic acid, ethyl octanoate and decanoate. The chemical composition of the beverages influenced the sensory profile, resulting in reduced sweetness and significant acidic notes, particularly in *L. plantarum* fermented juices. While this study successfully produced a novel functional fermented goji-based beverage, sensory analysis revealed a margin for improvement. This suggests that there is high potential for these innovative beverages to succeed through targeted product optimization, addressing the growing consumer interest in new functional fermented non-alcoholic drinks.

Keywords: fermentation, functional beverages, probiotics, bioactive compounds, health benefits.

1. Introduction

In recent years, food production and consumption trends have changed drastically, with consumer showing a greater demand for foods and beverages that enhance well-being and health¹. In this context, fermentation, one of the oldest food processing techniques, gains great importance because not only preserves food but also improves its nutritional, functional and sensory properties². Thus, fermentation offers a great potential for the development of new products, with improvements in the taste and quality of food ingredients being achieved through the coordinated use of multiple microorganisms³.

In this regard, fermented fruit juices are an attractive proposition due to the increased demand for beverages with high functional value and because they offer an alternative to traditional fruit juices^{4,5}. Furthermore, fruits and vegetables are a suitable fermentation matrix due to the high content of vitamins, minerals, dietary fibre, and antioxidant compounds^{6,7}. Probiotic lactic acid bacteria (LAB) have been used to obtain non-alcoholic beverages that can maintain and improve the nutritional and sensory quality, as well as prolong the shelf life of foods^{8,9}. *Lactobacillus plantarum*, recently renamed as *Lactiplantibacillus plantarum* (*L. plantarum*), is one of the most common LAB used in the fermentation due to the advantages for the bioprocessing of fruits and vegetables¹⁰. So, *L. plantarum* improved organoleptic perception¹¹, prolonged shelf life¹², and increased health benefits¹³ in fruit juices. On the other hand, fermentation with yeasts, usually *Saccharomyces cerevisiae* (*S. cerevisiae*), has traditionally been carried out to produce high quality wines, beers and ciders by transforming sugar into alcohol¹⁴. However, a large segment of consumers demands non-alcoholic and low-sugar fruit products derived from yeast-raised fruit. In addition, mixing the two microorganism types (lactic acid bacteria and yeasts) in fermentation can not only improve the taste of the beverage and synergistically enhance the health benefits of probiotics, but also increase the diversity of the beverages¹⁵.

77 Goji fruit (*Lycium barbarum* L.) contains many active compounds such as organic acids,
78 polyphenols and carotenoids, among others, responsible for its beneficial effects^{16,17}. However,
79 fresh goji berries have a high-water content, and their skin and cell walls are fragile, making
80 them susceptible to mechanical damage, which accelerates physiological processes leading to
81 a decrease in their nutritional and bioactive value¹⁸. Goji berry is mainly consumed in dried
82 forms, although its use is currently expanding to products such as tea infusions and juices.
83 Recently, Goji berry juice has attracted considerable attention due to its ease of preservation,
84 good taste, and health benefits; however, it has insufficient market competitiveness, so there is
85 a need to develop new processing techniques and strategies¹⁹.

86 In the literature, studies have been found on the application in goji berry juice of lactic acid
87 bacteria to produce probiotic drinks^{5,20,21}, and yeast to produce a beverage like wine²².
88 However, no literature has been found on the production of yeast-fermented drinks based on
89 goji juice with low ethanol production. Furthermore, no studies on the effects of the combined
90 application of lactic acid bacteria and yeast have been developed on goji juice. Composite strain
91 fermentation can improve the quality of fruit and vegetable juices and produce more active
92 ingredients through the synergistic action of multiple strains. In this sense, Liu et al.¹³ used
93 multiple bacterial strains to improve the production of bioactive compounds in fermented goji
94 juice; Chen et al.²³ fermented apple juice in stages, inoculating with *S. cerevisiae* and *L.*
95 *plantarum* to increase the content of bioactive compounds after fermentation.

96 The objective of this study was to develop and characterize novel fermented beverages based
97 on goji berry juice using *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae*, applied
98 both in monoculture and co-culture fermentations. To achieve this, the fermentation process
99 was monitored through the evaluation of pH, total acidity and total solid soluble). In addition,
100 the physicochemical and functional properties of the final products were assessed, along with
101 sensory acceptability, to determine their potential as functional fermented beverages.

2. Materials and methods

2.1. Fruits

Goji berry (*Lycium barbarum* L.) fruits were harvested at commercial maturity in May 2021 in the CICYTEX experimental field, located in Valle del Jerte (Cáceres, Extremadura, Spain) (39°54'31.7'' N, 5°55'18.4'' W). The collected fruits were immediately refrigerated (2 ± 1 °C) and transported to the CICYTEX-INTAEX facilities. Subsequently, the fruits were frozen at -80 °C for better preservation of the bioactive compounds and to avoid moisture loss, until further used.

2.2. Lactic acid bacterial and yeast strains and inoculum preparation

Two microorganisms were used; a lactic acid bacteria and a yeast (Table 1). The microorganisms were kept frozen at -80 °C until use. The frozen strains were seeded on plates of the appropriate medium: MRS agar for *L. plantarum* and YEPD agar for *S. cerevisiae*, and incubated for growth for 48 h at 25 °C. After this time, they were inoculated in liquid medium, using MRS medium broth for *L. plantarum* and YEPD medium broth for *S. cerevisiae*, and incubated for growth for 18 h at 25 °C under agitation; these cultures were used as inoculum in fermentations.

Table 1. Microorganisms used in the study.

Species	Strain	Origin
<i>Lactobacillus plantarum</i>	G2LB5	Cheese
<i>Saccharomyces cerevisiae</i>	881PA	Wine

2.3. Preparation of goji berry juice and fermentation

Frozen goji berries were crushed and vacuum-filtered at 2 °C to obtain the juice, which was then diluted with water to a total soluble solids (TSS) content of 2 %, 2,4 % of total sugars (1,2 % of glucose and 1,2% of fructose), to control alcohol formation during fermentation.

Subsequently, the juice was subjected to a high hydrostatic pressure (HPP) treatment at 600 MPa / 4 min to inactivate any microorganisms present. This non-thermal stabilisation treatment was chosen to preserve the phytochemical compounds. The resulting juice (GBJ) was stored at 4 °C until inoculation of microorganisms. All microorganisms were inoculated into goji juice at a concentration of 0.5 % of the final volume; samples were fermented at 20°C for 15 days in an incubator under static conditions. Three types of beverages were obtained: i) inoculated with *L. plantarum* (GBJL), ii) with *S. cerevisiae* (GBJS), and iii) a mixture (1:1) of both microorganisms (GBJLS). All fermentations were performed in triplicate. The physicochemical and microbiological analysis were carried out at 0, 2, 7, 12, and 15-day intervals. The phytochemical and volatile compounds were only analysed at 0 and 15-day intervals.

2.4. Microbiological analysis

For microbiological analysis, 10 mL of goji berry beverages were mixed with 90 mL of peptone water (OXOID, CM0009) and homogenized in a stomacher homogenizer. Serial 10-fold dilutions were prepared with the same diluent and duplicate 1 mL portions of the appropriate dilutions were spread on the following media: MRS agar (VWR Chemicals) for the enumeration of LAB, incubated at 30°C for 3 days and Potato Dextrose Agar (PDA) (OXOID,) for yeast, incubated at 25°C for 5 days.

2.5. Physicochemical analysis

The total soluble solids (TSS) content was measured at 20 °C with a portable digital refractometer (ATAGO Co., Ltd., Tokyo, Japan) and expressed as °Brix. For the pH and titratable acidity (TA) analysis, 3 mL of samples were mixed with 60 mL of deionised water and analyzed in a Mettler Toledo DL50 automatic titrator (Mettler Toledo, Barcelona, Spain) using 0.1 N NaOH for titration until pH 8.1. Results are expressed as percentage of malic acid.

2.6. Phytochemical content analysis

2.6.1. Sugars and organic acids

For sugar and organic acid profile analysis, 10 mL of goji berry beverages were centrifuged at 10800 rpm for 20 min at 4 °C²⁰, and then 2 ml of supernatant was filtered through a 0.22-mm nylon Filter (Agilent Technologies) for chromatographic analysis. An Agilent 1200 Liquid Chromatograph (Agilent Technologies, California, USA) coupled with a Refractive Index Detector (RID) (G-1362), and Diode Array Detection (DAD) (measuring at 210 nm) were used for sugar and organic acid analysis, respectively. Sugars separation was achieved in a RezexTM RPM-Monosaccharide PB+2 (300 x 7.8 mm, 8 mm) column with water as mobile phase and flow rate of 0.6 mL min⁻¹ according to the method described by Phenomenex® for saccharides determination

(<https://www.phenomenex.com/Application/Detail/5508?returnURL=/Application/Search&fsr=1>). The monosaccharide sugar (glucose, fructose, and sucrose) standards were obtained from Sigma Aldrich (Missouri, USA). The results were expressed as g sugar 100 mL⁻¹. For the organic acids analysis, a Rezex ROA-Organic Acid column (300 x 7.8 mm, 8 mm) with a mobile phase of 0.005 N sulfuric acid, and a flow rate of 0.5 mL min⁻¹ were employed according to Phenomenex® methods (<https://www.phenomenex.com/applications/single?appid=18812>). The organic acids (oxalic, citric, tartaric, malic, ascorbic, succinic, and fumaric acid) standards were obtained from Sigma Aldrich (Missouri, USA). Results were expressed as mg organic acid 100 mL⁻¹.

2.6.2. Determination of total phenolic content and antioxidant activity.

The total phenolic compounds were determined using the Folin-Ciocalteu reagent according to a protocol previously used by Wu et al. (2021)²⁴ slightly modified. For this, 10 mL of goji berry beverages were centrifuged at 10800 rpm for 20 min at 4 °C. Subsequently, 0.1 mL of the extract was mixed with 1 mL of Folin-Ciocalteu reagent (Panreac, Applichem), and 3 mL of sodium carbonate solution (200 g L⁻¹) until 10 ml with water volume flask. The absorbance

was read at 760 nm using a UV-Vis Shimadzu spectrophotometer (Kyoto, Japan) after allowing the solution to stand for 15 min. The total phenolic compounds were quantified from a calibration curve using gallic acid (GA) (Sigma Aldrich) and expressed as mg of gallic acid per L (mg GA L⁻¹). The antioxidant activity assay was also performed according to Wu et al. (2021) with minor modifications. 50 µl of the same extract, were pipetted into 2950 mL of DPPH solution. The absorbance was read at 517 nm after 1 h of incubation in darkness at room temperature. Trolox was used as a standard, and the antioxidant activity was expressed as mM of Trolox.

2.6.3. Individual phenolic compounds

Analysis of phenolic profile was performed according to Liu et al.¹³. For this purpose, 10 ml of each sample was extracted with 30 mL ethanol (80%, v/v) for 15 min at 4 °C, followed by centrifugation at 2500 g for 10 min. The residue was extracted again twice, and the supernatants were combined, evaporated to dryness at 45 °C, and reconstituted to 10 mL with methanol. For the chromatography analysis, 10 mL of the previous extract filtered through by 0.45 mm nylon Filter-Lab (Agilent Technologies), were injected into a 1100 Serie HPLC system (Agilent Technologies) equipped with a DAD and fluorescence detector to separate and identify the phenolic compounds. The chromatographic conditions for determination were as described Manzano Durán et al.²⁵. The identification of compounds was performed to compare the retention times and purity peak spectra with stock dissolution. Quantification was performed by chromatographic comparison with authentic markers. Regression analysis of the peak area was performed. The results are expressed as mg phenol L⁻¹.

2.6.4. Individual carotenoid compounds

The carotenoid compounds were extracted according to Zacarías-García et al.²⁶. For this purpose, 4 ml of fermented juice were extracted with 6 ml of extraction solution composed by methanol:acetone:dichloromethane (25:25:50 v/v/v) and 0.1% BHT (butylhydroxytoluene).

Subsequently, the sample was sonicated 5 min in an ultrasonic water bath (P-selecta Model 516) at room temperature, centrifuged at 4000 g for 5 min at 4 °C, and finally the organic phase was recovered. The aqueous phase was re-extracted with 2 ml of dichloromethane until the organic phase was colourless. The organic extracts obtained were combined and saponified with 10 ml of methanolic KOH (12%, w/v) for 90 min at room temperature and darkness. After this, 6 ml of 50 mM Tris-HCl pH 7.5 with 1 M NaCl buffer and 6 ml of dichloromethane were added. The mixture was stirred and centrifuged at 4000 rpm for 5 min at 4 °C. The aqueous phase was discarded, and the organic phase was filtered through Na₂SO₄ anhydrous. The extract was evaporated to dried and reconstituted in 2 mL of the extraction solution. This volume was filtered through a 0.22 mm nylon Filter-Lab (Agilent Technologies). For the individual carotenoid compounds analysis, 1.4 ml of the final filtered extract was injected in an Ultra-high liquid chromatography (UHPLC) (Agilent 1290, Agilent Technologies, CA, USA) with a Kinetex® XB-C18 (2.1 x 50 mm, 1.7 mm) (Phenomenex 100 Å) thermostatically controlled at 28 °C and coupled to a DAD detector (measuring at 460 nm) according to Bohoyo et al.²⁷. The mobile phase consisted of acetonitrile/water (85:15, v/v) (phase A), and acetonitrile/methanol/ethyl acetate (60:20:20, v/v/v) (phase B). The gradient elution conditions were 100 % phase A for 4 min, 100 % phase B at 4.17 min until 9 min, and 100 % phase A at 9.17 min until the end of the analysis (11 min). The results are expressed in mg L⁻¹.

2.7. Volatile organic compounds (VOCs) analysis

The volatile organic profile was analyzed in triplicate in the goji berry beverages unfermented and fermented at the end of fermentation (15 days) by gas chromatography-mass spectrometry (GC-MS). For this, 5 mL of beverage was placed in a 15 ml glass vial, and the volatile components were sampled from the headspace at 37 °C for 30 min. Each sample was equilibrated at 37 °C for 5 min using a CombiPAL autosample (CTC Analytics, Zwingen,

Switzerland). A solid phase microextraction (SPME) with a DVD/CAR/PDMS fibre (Supelco, Bellefonte, PA, USA) for volatile components extraction was used. After this, the volatile compounds were desorbed by inserting the SPME fibre into the injection port (set at 270 °C) of a Varian CP-3800 gas chromatograph coupled to a Varian Saturn 2200 MS mass spectrometer (Varian Inc., Palo Alto, CA, USA). A HP-5 capillary column (30 m x 0.32 mm x 0.25 mm, Agilent Technology, Santa Clara, CA, USA) and helium (1 mL min⁻¹) as the carrier gas were used. The oven temperature was set at 35 °C for 10 min, raised to 250 °C at a rate of 7 °C min⁻¹, and held for 5 min with a total running time of 45 min. The mass spectra were obtained by electronic impact at 70 eV, with one scan s⁻¹ over a range of 40-300 *m/z*. Compound identification was determined by comparison of their mass spectra and linear retention indexes (LRI) with those of the injected standards (Sigma Aldrich, St. Louis, MO, USA), or with mass spectra contained in the NIST standard reference database. For quantification, the analysis was performed by area comparison using an internal standard (1-pentanol, 4-methyl-) of known concentration. The results were expressed in mg mL⁻¹.

2.8. Descriptive sensory analysis

A sensory panel was set up in the tasting room at the CICYTEX research center (Extremadura, Spain). Evaluations were carried out by 10 trained tasters to analyze the characteristic (colour, aroma, taste, and texture) of the fermented beverages at the end of the fermentation (15 days). The unfermented goji juice was sensory evaluated at the beginning of the assay (0 day). A tasting sheet was used with an unstructured scale of 10 cm (0: non detected to 10: highest intensity) for each attribute evaluated²⁸. The aromatic attributes assessed were fruit and sour, and taste attributes were fruit, sweet, and sour. The data of the sensory assessment were expressed as median values. The results were considered valid when the coefficient of variation was less than 20 %. Each taster was informed through a structured document prior to the tasting evaluation (see Appendix 1) and were performed in accordance with the Ethics Committee of

the University of Extremadura for research involving human subjects, and with the 1964 Helsinki Declaration and its later amendments.

2.9. Statistical analysis

All analysis were performed in triplicate. Results are expressed as mean $\pm$ standard deviation. Normality was checked prior to ANOVA analysis by applying the Shapiro-Wilk test, and homoscedasticity was checked using the Levene test. For multiple comparisons, a Tukey's post hoc test was used when data showed normal distribution and homoscedasticity. When data did not show normal distribution a Kruskal-Wallis test was applied and a Welch ANOVA test followed by a Games-Howell post-hoc test was performed when variances were not equal. The degree of significance was set at $p < 0.05$. Also, dates were analyzed using an exploratory data model by making principal component analysis (PCA) to linearly transform to select a few significant variables. A heat map was also used for clustering volatile organic compounds of samples. All the analyses were accomplished using the XLSTAT-Pro 201,610 (Addinsoft 2009, Paris, France) statistical software package.

3. Results and discussion

3.1. Microbiological growth analysis

The Figure 1 shows microbial growth in goji berry juice during fermentation with *L. plantarum* lactic acid bacteria (GBJL), *S. cerevisiae* yeasts (GBJS), and a mixture of both microorganisms (GBJLS). Microbial growth in unfermented goji juice (GBJ) is also shown as control. The inoculation of each microorganism into the goji berry juice results in an initial lactic acid bacteria (LAB) count of approximately 7 log CFU/mL for GBJL and GBJLS, and an initial yeast count of approximately 6 log CFU/mL for GBJS and GBJLS. Regarding LAB counts, similar levels were found in both GBJL (inoculated with *L. plantarum*) and GBJLS (inoculated with a mixture of yeast and lactic acid bacteria) throughout fermentation. The lactic acid

bacteria populations were either maintained at the inoculated concentrations, or showed a slight increase, with minor differences between these two treatments. As expected, in GBJ the number of LAB remained very low (below 2 log CFU/mL) during fermentation, with a slight increase detected at the end of fermentation ($p < 0.05$). No lactic acid bacteria were found in GBJS on any day of fermentation. The survival of lactic acid bacteria is an important prerequisite for their probiotic effect²⁹. As shown in Figure 1, the number of viable bacteria after 15 days of fermentation reached 7.95 log CFU/g, suggesting that the nutrients in goji berry juice can satisfy the basic growth needs of *L. plantarum*⁵. Other studies involving goji juice fermented with selected lactic acid bacteria have reported similar results to those obtained in the current study²⁰.

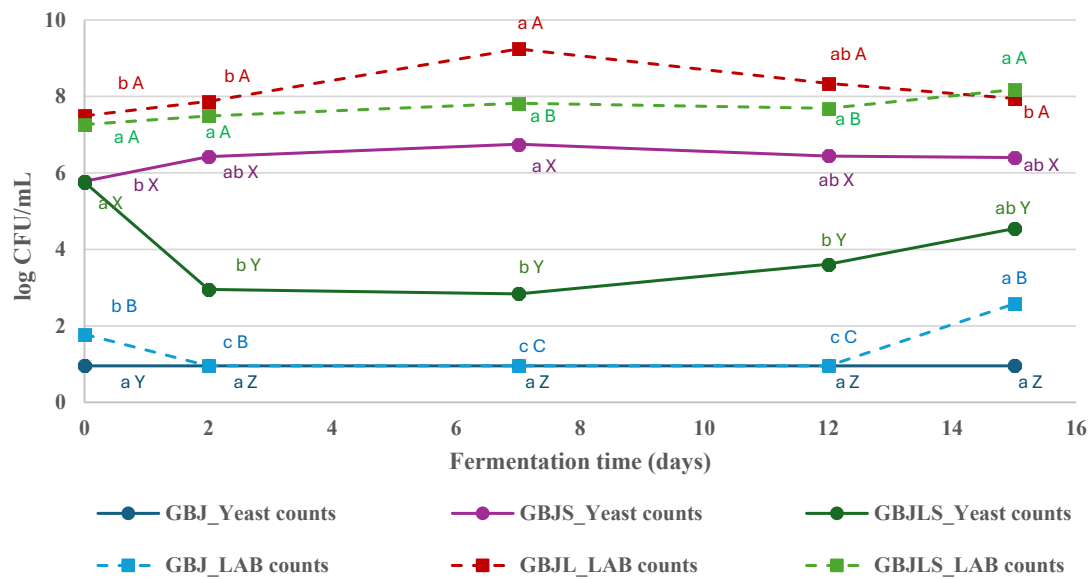


Figure 1. Analysis of lactic acid bacteria (LAB), and yeasts in goji juice fermented with *L. plantarum* (GBJL), *S. cerevisiae* (GBJS), *L. plantarum* + *S. cerevisiae* (GBJLS), and unfermented goji juice (GBJ). Different lower-case letters (a, b, c) indicate significant differences between the sampling date. Multiple comparisons were analysed following a Tukey Test for GBJL_LAB counts, GBJLS_LAB counts, GBJS_Yeast counts and GBJLS_Yeast counts, and a Kruskal-Wallis Test for GBJ_LAB counts and GBJ_Yeast counts at $p < 0.05$. Different capital letters (A, B, C) indicate significant differences between LAB counts of goji berry beverages at the same sampling date. Multiple comparisons were analysed following a Kruskal-Wallis Test for 0, 2, 7, 12, and 15 days at $p < 0.05$. Different capital letters (X, Y, Z) indicate significant differences between Yeast counts of goji berry beverages at the same sampling date. Multiple comparisons were analysed following a Kruskal-Wallis Test for 0, 2, 7, 12, and 15 days at $p < 0.05$.

Analysis of yeast concentration throughout the fermentation (Figure 1) revealed distinct behaviours among the samples. In GBJS, the yeast concentration increased significantly after 7 days of fermentation ($p < 0.05$) and remained stable at similar concentrations for the rest of the fermentation period. Conversely, GBJLS exhibited a significant decrease in yeast counts during the fermentation process ($p < 0.05$). Furthermore, the final yeast concentration was significantly higher in GBJS than in GBJLS ($p < 0.05$). In the control fermentation (GBJ), yeast counts remained very low and without significant differences throughout the fermentation. As expected, no yeasts were detected in the non-inoculated yeast fermentation (GBJL). The initial drop in yeast concentrations in GBJLS fermentation in the first 2 days is

likely attributable to the presence of LAB, which could inhibit the growth of *S. cerevisiae* yeasts³⁰. However, other studies have shown that the growth of yeast in fermented foods is favored by acidification of the environment created by LAB, and the yeast may provide growth factors such as vitamins and soluble nitrogen compounds to stimulate the growth of LAB³¹.

3.2. Changes of physicochemical properties during fermentation

As shown in Figure 2A, TA increased rapidly and significantly within the first 2 days in the fermentation processes involving *L. plantarum* lactic acid bacteria. The highest concentration was observed in GBJL, followed by GBJLS ($p < 0.05$). Consistent with these observations, a reduction in pH was observed across all types of fermentation processes (Figure 2B), but it was more significant and pronounced in the LAB-inoculated fermentations (GBJL and GBJLS). A decrease in pH was also noted in the fermentation inoculated with culture pure of *S. cerevisiae* (GBJS), and even in the unfermented control goji berry juice (GBJ). This behaviour, associated with the generation of organic acids during the fermentation process, mainly lactic acid in the case of fermentation inoculated with *L. plantarum*. Similarly, other studies have reported a decrease in pH in goji juices fermented with *Lacticaseibacillus rhamnosus* due to the generation of organic acids⁵.

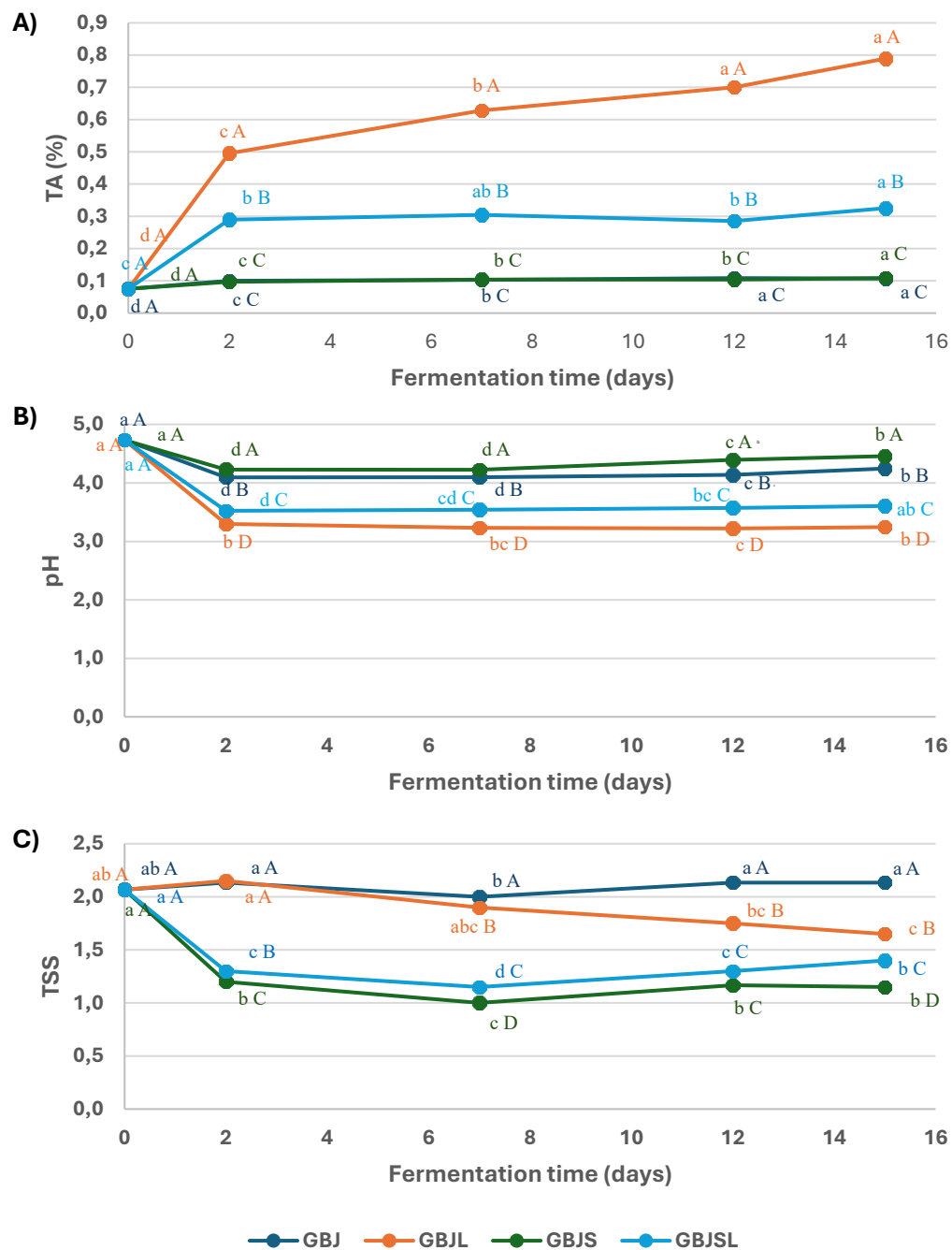


Figure 2. Changes in physicochemical properties, Total acidity (TA), pH, and Total solid soluble (TSS) content in goji juice during fermentation (at 0, 2, 7, 12, and 15 days). GBJ: goji berry juice, GBJL: goji berry juice inoculated with *L. plantarum*, GBJS: goji berry juice inoculated with *S. cerevisiae*, GBJSL: goji berry juice inoculated with *L. plantarum* and *S. cerevisiae*. Different lower-case letters indicate significant differences between different sampling dates for the same beverage. Multiple comparisons were analysed following a Tukey Test for TSS and Kruskal-Wallis Test for TA and pH at $p < 0.05$. Different capital letters indicate significant differences between goji berry beverages at the same sampling date. For TA and pH, multiple comparisons were analysed following a Kruskal-Wallis Test for 0, 2, 7, 12, and 15 days. For TSS, multiple comparisons were analysed following a Kruskal-Wallis Test for 0, 2 and 7 days, and a Tuckey Test for 12 and 15 days.

338

339 Significant differences were observed in the total soluble solids (TSS) content among the four
340 types of fermented goji juices (Figure 2C). Juices fermented with *S. cerevisiae* (GBJS and
341 GBJLS) showed a sharp decrease in TSS content the first 2 days of fermentation ($p < 0.05$).
342 Conversely, the TSS decreased slowly in GBJL, fermented only with *L. plantarum*, and
343 remained stable in the unfermented juice (GBJ). The observed decrease in TSS is attributed to
344 the consumption of sugars during the fermentation, which was more pronounced in
345 fermentations involving *S. cerevisiae*; this is consistent with reports by other authors regarding
346 yeast activity³². This rapid decrease produced by yeasts contrasts with the slower changes in
347 fermentation only inoculated with *L. plantarum*, where the production of organic acids could
348 have influenced TSS levels. These results align with those of other authors who also found that
349 the TSS of the juice fermented with lactic acid bacteria effectively remained stable or decreased
350 slowly^{5,33}.

351 **3.3. Reducing sugar and organic acid composition in fermented beverages**

352 Table 2 presents the sugar and organic acid composition of the beverages after 15 days of
353 incubation. The control juice GBJ exhibited the highest total content of reducing sugars (8.24
354 g L⁻¹), accounting for the highest levels of the three sugars analyzed, sucrose, glucose and
355 fructose. In comparison, fermentation of the goji juice with inoculated microorganisms resulted
356 in a significant decrease in total sugar content across all groups ($p < 0.05$). The juice fermented
357 with both microorganisms (GBJLS) showed the strongest decrease in total sugar content,
358 reaching only 1.43 g L⁻¹ ($p < 0.05$). This was followed by GBJS, inoculated only with *S.*
359 *cerevisiae*, which decreased to 2.19 g L⁻¹. Regarding individual sugar consumption, beverages
360 involving *S. cerevisiae* (GBJS and GBJLS) consumed the highest amount of glucose, which
361 was non-detectable at the end of fermentation. However, consumption of fructose was not as
362 pronounced in any of the beverages. Goji juice is considered a medium that contains enough

glucose and fructose²⁰, providing an adequate carbon source for the metabolic activity of microorganisms during fermentation of fruits and vegetables³⁴.

Table 2. Sugar and organic acid composition of goji berry beverages expressed as g L⁻¹ (mean $\pm$ SD) fermented to 15 days.

	GBJ	GBJL	GBJS	GBJLS
Sugars (g L⁻¹)				
Sucrose	0.10 $\pm$ 0.01 ^a	0.02 $\pm$ 0.01 ^c	0.03 $\pm$ 0.01 ^b	n.d.
Glucose	4.30 $\pm$ 0.08 ^a	2.49 $\pm$ 0.01 ^b	n.d.	n.d.
Fructose	3.84 $\pm$ 0.08 ^a	0.53 $\pm$ 0.15 ^d	2.16 $\pm$ 0.11 ^b	1.43 $\pm$ 0.07 ^c
Total	8.24 $\pm$ 0.16 ^a	3.04 $\pm$ 0.16 ^b	2.19 $\pm$ 0.10 ^c	1.43 $\pm$ 0.07 ^d
Organic Acids (mg 100 mL⁻¹)				
Oxalic	4.99 $\pm$ 0.01 ^c	5.23 $\pm$ 0.07 ^b	6.48 $\pm$ 0.13 ^a	5.32 $\pm$ 0.19 ^b
Citric	243.90 $\pm$ 0.06 ^b	248.47 $\pm$ 10.61 ^b	315.70 $\pm$ 0.27 ^a	187.33 $\pm$ 19.22 ^c
Tartaric	22.87 $\pm$ 1.87 ^{bc}	31.00 $\pm$ 1.36 ^{ab}	38.64 $\pm$ 3.85 ^a	18.84 $\pm$ 6.28 ^c
Malic	52.06 $\pm$ 8.31 ^b	55.83 $\pm$ 5.53 ^{ab}	106.56 $\pm$ 4.28 ^a	46.59 $\pm$ 11.67 ^b
Ascorbic	41.55 $\pm$ 1.77 ^a	25.71 $\pm$ 8.57 ^b	33.45 $\pm$ 0.37 ^b	n.d.
Lactic	70.36 $\pm$ 23.45 ^c	1446.05 $\pm$ 33.34 ^a	45.85 $\pm$ 13.27 ^c	524.28 $\pm$ 4.99 ^b
Total	435.74 $\pm$ 20.31 ^d	1812.28 $\pm$ 44.75 ^a	546.69 $\pm$ 12.95 ^c	782.37 $\pm$ 27.77 ^b

GBJ: unfermented goji berry juice; GBJL: goji berry juice fermented with *L. plantarum*; GBJS: goji berry juice fermented with *S. cerevisiae*; GBJLS: goji berry juice fermented with *L. plantarum* + *S. cerevisiae*.

n.d.: non detected.

Different superscript letters in the same row indicate significant differences among goji berry beverages.

Multiple comparisons were analyzed following a Tukey Test ($p < 0.05$) for fructose and citric and tartaric acids, a Kruskal-Wallis Test ($p < 0.05$) for sucrose, total sugars, oxalic, malic, ascorbic, lactic and total acids, and a t-Student Test ($p < 0.05$) for glucose.

Regarding the total organic acid content, the beverages involving lactic acid bacteria (GBJL and GBJLS) exhibited a significantly higher content (1812.3 and 782.4 mg 100 mL⁻¹) ($p < 0.05$). This substantial difference is primarily a consequence of lactic acid formation by *L. plantarum* during the fermentation process. Conversely, GBJ displayed the lowest total organic acid content (435.7 mg 100 mL⁻¹) ($p < 0.05$). The organic acids in GBJS (546.7 mg 100 mL⁻¹) which was inoculated with *S. cerevisiae*, were mainly citric and malic acids. These results are consistent with the previously reported data and show a relationship with TA, pH and TSS (Figure 2). The production of organic acids and other metabolites by microorganisms during

fermentation contributes to improved food taste and spoilage prevention^{35,36}. Moreover, the lactic acid produced by LAB may also be inhibited the growth of *S. cerevisiae* in GBJLS³⁰. Other common acids in fermented beverages, such as acetic acid, were not detected in any treatment using this specific method.

3.4. Total and individual phenolic content

Table 3 shows the total and individual content of the phenolic compounds identified in this study. The total phenol content (TPC) was significantly highest in the GBJL beverage compared to the other treatments ($p < 0.05$), while in the beverages GBJS and GBJLS, the TPC remained constant relative to GBJ. This is consistent with the results of antioxidant activity; the highest activity was found in GBJL, and the lowest in GBJ. Analyzing the individual phenolic compounds, the highest content corresponds to a significant increase in total hydroxycinnamic acid in fermentations with *L. plantarum*, GBJL and GBJLS, compared to the other treatments ($p < 0.05$). Similarly, the total of Flavonols showed the highest content in beverages fermented with *L. plantarum* (GBJL and GBJLS) ($p < 0.05$). Furthermore, and attributed to the influence of LAB, GBJLS showed the highest concentrations of Flavan-3-ols. When analyzing the individual phenolic compounds in fermentations inoculated with culture pure of *S. cerevisiae*, GBJS, only differences were observed in the total of Flavan-3-ols and p-Coumaric acid (hydroxycinnamic acids) compared to the other treatments; most of the hydroxycinnamic acids and total flavonols content in GBJS did not vary significantly with respect to GBJ ($p > 0.05$).

Table 3. Quantify of individual and total phenolic compounds, antioxidant activity, individual and total carotenoids compounds (mean $\pm$ SD) of goji juice fermented to 15 days.

	GBJ	GBJL	GBJS	GBJLS
Total phenolic compounds (TPC) (mg galic acid L ⁻¹)	4.52 $\pm$ 0.28 ^b	5.77 $\pm$ 0.03 ^a	4.65 $\pm$ 0.14 ^b	4.84 $\pm$ 0.42 ^{ab}
Antioxidant activity (mM Trolox)	0.78 $\pm$ 0.06 ^c	1.88 $\pm$ 0.03 ^a	1.21 $\pm$ 0.16 ^b	1.40 $\pm$ 0.03 ^b
Individual Phenolic compounds (mg phenol L ⁻¹)				
Hydroxycinnamic acids (total)	2.90 $\pm$ 0.18 ^b	7.59 $\pm$ 1.58 ^a	3.67 $\pm$ 0.19 ^b	6.00 $\pm$ 0.27 ^a

Chlorogenic acid (ChlA)	1.32 ± 0.15 ^c	7.26 ± 1.53 ^a	0.62 ± 0.01 ^c	4.46 ± 0.20 ^b
p-coumaroylquinic acid (pCouQ)	nd	nd	0.56 ± 0.10 ^b	0.81 ± 0.06 ^a
p-Coumaric acid (pCou)	1.35 ± 0.02 ^b	0.33 ± 0.05 ^c	2.50 ± 0.1 ^a	0.12 ± 0.01 ^d
Ferulic acid (t-Fer)	0.23 ± 0.01 ^b	nd	nd	0.61 ± 0.02 ^a
Flavonols (total)	1.12 ± 0.02 ^b	3.43 ± 0.46 ^a	2.90 ± 0.16 ^b	3.16 ± 0.06 ^a
Quercetin-3-rutinoside (Q3R)	1.12 ± 0.02 ^b	3.43 ± 0.46 ^a	2.90 ± 0.16 ^b	3.16 ± 0.06 ^a
Flavan-3-ols (total)	5.35 ± 0.34 ^b	nd	3.54 ± 0.65 ^c	18.64 ± 0.86 ^a
(+)-catechin (Cat)	1.89 ± 0.27 ^c	nd	3.54 ± 0.65 ^b	7.75 ± 0.18 ^a
(-)-epicatechin (Ecat)	nd	nd	nd	3.75 ± 0.21 ^a
Individual carotenoid compounds				
(mg L ⁻¹)				
Capsanthin (Cap)	0.41 ± 0.05 ^c	0.95 ± 0.42 ^b	5.25 ± 0.48 ^a	0.36 ± 0.12 ^c
Zeaxanthin (Zea)	10.70 ± 0.91 ^c	26.04 ± 2.35 ^b	138.12 ± 7.40 ^a	10.57 ± 3.84 ^c
Cryptoxanthin (Crp)	0.35 ± 0.04 ^b	1.09 ± 0.52 ^b	5.03 ± 0.30 ^a	0.38 ± 0.14 ^b
α-carotene (α-Car)	0.21 ± 0.01 ^c	1.30 ± 0.73 ^b	3.19 ± 0.06 ^a	0.44 ± 0.13 ^b

GBJ: unfermented goji berry juice; GBJL: goji berry juice fermented with *L. plantarum*; GBJS: goji berry juice fermented with *S. cerevisiae*; GBJLS: goji berry juice fermented with *L. plantarum* + *S. cerevisiae*.

n.d.: non detected.

Different superscript letters in the same row indicate significant differences among sprout quinoa varieties. No letters in the same row indicate that there are no significant differences between varieties. Multiple comparisons were analysed following a Tukey Test ($p < 0.05$) for Antioxidant activity, Hydroxycinnamic acids (total), TPC, ChlA, Cat and Zea, a Kruskal-Wallis Test ($p < 0.05$) for p-CouQ, p-Cou, t-Fer, Flavonols (total), Q3R, Flavan-3-ols (total), Ecat, PB1, Cap, Crp, α-Car and β-Car.

The observed increase in TPC in GBJL is consistent with other studies indicating that the reduction in pH during fermentation can stabilize phenolic acids, thereby explaining the higher content in beverages fermented with *L. plantarum*³⁷. The fermentation process significantly impacts the bioactivity of the juice by altering the structural composition of phenolic compounds. The metabolic activity of microorganisms leads to variations in final phenolic concentrations, which may either increase or decrease depending on the specific interactions involved^{38,39}. Some phenolic compounds could be transformed into others during fermentation, as was observed in the fermentation of mulberry juice with *L. plantarum*⁴⁰. Supporting these findings, other authors have also reported a significantly higher TPC, mainly phenolic acids and flavonoids, in goji juice fermented with *L. plantarum* compared to the unfermented juice^{13,23}. However, this impact is likely dependent on the specific microbial strain used^{5,41} found a decrease in TPC in goji juice fermented with a different strain of lactic acid bacteria (*Lactocaseibacillus rhamnosus*).

3.5. Antioxidant activity of beverages

The DPPH free radical scavenging capacity of fermented goji juices is significantly higher than non-fermented (Table 3). As noted earlier, these results align with the total phenolic content. GBJL showed the highest antioxidant activity (1.88 mM Trolox) followed by GBJLS and GBJS (1.40 and 1.21 mM Trolox, respectively) ($p < 0.05$) respect to GBJ (0.78 mM Trolox). This may be due to fermentation promoting the solubilization of active compounds, which in turn improves the juice's DPPH scavenging capacity⁴². While this effect was significant in all inoculated fermentations, it was most pronounced in the *L. plantarum* monocultures. Other authors associate fermentation with *L. plantarum* with an increased antioxidant capacity. Thus, Duan et al.²⁰ and Xie et al.²¹ reported that the antioxidant capacity of goji juice had significantly increased DPPH radical scavenging after fermentation with *L. plantarum*. This

increase in the antioxidant properties of goji juice after fermentation is associated with the accumulation of compounds with antioxidant activity after fermentation, which agrees with a higher content of TPC found in GBJL.

3.6. Individual carotenoid compounds

Table 3 summarizes the individual carotenoid concentrations found in the beverages; fermentations with inoculated microorganisms produced significant increase in all of carotenoid compounds analyzed (Capsanthin_Cap, Zeaxanthin_Zea, Cryptoxanthin_Crp, and α -caroteno_ α -Car) with respect to goji juice ($p < 0.05$). Fermentations inoculated only with *S. cerevisiae* (GBJS) showed the greatest differences. Zea, the major carotenoid compound in goji berries, was strongly increased in the GBJS beverage (138.1 mg L^{-1}) compared to the unfermented juice (10.7 mg L^{-1}). However, the increase in the carotenoid compounds content was not as pronounced in lactic bacteria fermentations, neither in mono-cultures nor in co-cultures with yeasts. Carotenoids are not only the main colorant in goji fruits but is also an important antioxidant component⁴³. The type of microorganisms, raw material composition and characteristics, fermentation conditions and so on may have a great impact on the stability of carotenoids in raw materials⁴⁴. Sovljanski et al.⁴⁵, have indicated that yeasts can produce significant amounts of carotenoids through fermentation.

3.7. Evaluation of volatile organic compounds

The volatile profile of the goji juice fermented by the different microorganisms is shown in Table 4. Esters represent the dominant family of volatile compounds ($0.25 - 34.0 \text{ mg/mL}$), followed by alcohols ($0.02 - 7.23 \text{ mg/mL}$) and aldehydic compounds ($0.07 - 2.24 \text{ mg/mL}$), although it depended on the type of fermentation. Esters, related to fruity and floral aromas, are normally produced by esterification reactions of microorganisms⁴⁶. They have a high volatility and low detection threshold, which allows their pleasant aromas to be perceived even at very low concentrations. The GBJS beverage showed the highest content of ester compounds

(34.0 mg/mL) followed by the GBJLS beverage (15.4 mg/mL). Ethyl octanoate was the major ester compound in the fermented juices involving yeast (GBJS and GBJLS) (20.6 and 8.17 mg/mL, respectively) and was not detected in the other juices. Similarly, ethyl decanoate, ethyl hexanoate, isoamyl acetate, and ethyl nonanoate, related to tropical flavors and sweetness, were found only in yeast-fermented juices. Another ester compound such as methyl salicylate was found in the unfermented GBJ (0.34 mg/mL), and in the GBJL (0.25 mg/mL).

Table 4. Quantification of volatile organic compounds (mg/mL) [mean (n = 3)] obtained by GC-MS in fermented goji juices at the end of fermentation by different microorganisms.

LRI	Volatile organic compounds	Id. Method	GBJ	GBJL	GBJS	GBJLS
Alcohols						
< 600	Ethanol	RF, RI, TI	nd	nd	3.15	3.00
622.2	1-Propanol, 2-methyl-(isobutanol)	RF, RI, TI	nd	nd	0.56	0.26
715.2	1-Butanol, 3-methyl- (isopentanol)	RI, TI	0.03	nd	3.25	2.95
873.5	1-Hexanol	RF, RI, TI	nd	nd	nd	0.14
981.9	1-Octene-3-ol	RI, TI	0.10	nd	nd	0.03
1113.6	Ethanol, 2-phenyl	RF, RI, TI	nd	nd	0.28	0.12
1274.8	1-Decanol	RI, TI	nd	0.02	nd	0.02
	TOTAL		0.13	0.02	7.23	6.53
Acids						
678.8	Acetic acid	RF, RI, TI	nd	0.40	nd	nd
1181.0	Octanoic acid	RF, RI, TI	nd	nd	0.14	0.23
1273.1	Nonanoic acid	RI, TI	nd	nd	nd	nd
1371.3	n-decanoic acid	RI, TI	nd	0.01	0.04	0.20
	TOTAL		nd	0.41	0.18	0.42
Esters						
617.0	Ethyl acetate	RF, RI, TI	nd	nd	nd	0.13
880.3	Isoamyl acetate	RF, RI, TI	nd	nd	1.26	0.75
999.8	Ethyl hexanoate	RF, RI, TI	nd	nd	2.98	1.13
1015.3	Hexyl acetate	RI, TI	nd	nd	nd	0.15
1196.3	Methyl salicylate	RI, TI	0.34	0.25	nd	nd
1198.2	Ethyl octanoate	RF, RI, TI	nd	nd	20.6	8.17
1298.6	Ethyl nonanoate	RF, RI, TI	nd	nd	0.16	0.13
1399.0	Ethyl decanoate	RI, TI	nd	nd	7.92	4.41
1599.5	Ethyl dodecanoate	RI, TI	0.01	nd	0.88	0.47
1391.2	4-decenoic acid, ethyl ester	RI, TI	nd	nd	0.23	0.07
	TOTAL		0.35	0.25	34.0	15.4
Aldehydes						
674.8	Pentanal	RF, RI, TI	0.06	nd	nd	nd
791.3	Hexanal	RF, RI, TI	1.61	nd	0.04	nd
1059.4	2-Octenal	RI, TI	0.20	nd	nd	nd
1104.1	Nonanal	RF, RI, TI	0.14	0.07	0.03	0.07
1296.8	2,4-Decadienal	RI, TI	0.22	nd	nd	nd
	TOTAL		2.24	0.07	0.08	0.07
Aromatic compounds						
1027.1	L-limonene	RF, RI, TI	0.11	nd	nd	0.54
1029.5	Eucalyptol	RI, TI	0.28	nd	nd	nd
1099.5	b-Linalool	RF, RI, TI	0.03	nd	nd	nd
1224.3	b-cyclocitral	RI, TI	0.06	nd	nd	nd
	TOTAL		0.48	nd	nd	0.54
Ketones						
988.5	6-methyl-5-Hepten-2-one	RI, TI	0.08	nd	nd	nd
	TOTAL		0.08	nd	nd	nd

GBJ: unfermented goji berry juice; GBJL: goji berry juice fermented with *L. plantarum*; GBJS: goji berry juice fermented with *S. cerevisiae*; GBJLS: goji berry juice fermented with *L. plantarum* + *S. cerevisiae*.
LRI: Lineal Retention Index; I.d. Method: method of identification (RF: mass spectrum and retention time identical with a reference compound; RI, mass spectrum and retention index from literature in agreement; TI, tentative identification by mass spectrum-NIST library). nd: non-detected.

Concerning aldehydic compounds, a low concentration can produce an acceptable flavor, while a high concentration produces an unpleasant odor⁴⁷. These compounds are easily reduced to alcohol or oxidised to fatty acids as fermentation proceeds⁴⁸. Kim et al.⁴⁷ found that the fermentation of bitter melon juice by *L. plantarum* can reduce the content of odors aldehyde, while increasing alcohol and acid, so that it has a more ideal flavor. Other authors also found a gradual decrease over time of aldehyde compounds such as furfural, phenylacetaldehyde and hexanal^{11,49}. This seems to have occurred in some fermentations where the concentration of aldehyde compounds was almost undetectable, increasing the content of alcoholic and acidic compounds. All this indicates that the aldehyde compounds were completely metabolized in the fermentation processes⁵⁰.

On the other hand, alcohol compounds, known for their powerful aromas, are also among the main volatile compounds in fermented beverages⁴⁶. These compounds can be derived from the fruit itself or produced by microbial metabolism, contributing to the aromatic profile of fermented juice. In our work, the major alcoholic compounds found were ethanol (3.15 and 3.00 mg/mL) and 1-butanol, 3-methyl- (isopentanol) (3.25 and 2.95 mg/mL), both in the juice fermented involving *S. cerevisiae* (GBJS and GBJLS, respectively). It is noteworthy that alcoholic compounds are practically absent in the unfermented GBJ and in fermented GBJL. During yeast fermentation, microorganisms break down glucose into pyruvate via the glycolytic pathway, and pyruvate is subsequently reduced to ethanol by the action of alcohol dehydrogenase⁴⁶. Hexanol, a characteristic alcohol in fruit juices that confers a floral and herbaceous aroma, appears in low concentration in GBJLS fermented juice. This is known to decrease levels during fermentation and is almost undetectable at the end of the process⁵¹ may

be due to its role as a precursor of other substances such as esters, contributing to the fresh fruit aromas characteristic of fermented beverages⁵².

Other components such as terpenoids play a crucial role in the aroma formation of fermented beverages. Eucalyptol and b-linalool are linear terpene alcohol commonly found in fruits with distinctive fruity and floral aroma and low detection threshold. In unfermented goji juice, L-limonene, eucalyptol, b-linalool and b-cyclocitral are present. However, only L-limonene was found in the GBJLS, and was not detected in the other fermented juices. Finally, ketones, due to their pungent odour characteristics, in low concentrations usually impart a pleasant aroma to the beverage⁵⁰. In our study, 6-methyl-5-hepten-2-one was only detected, although at very low concentrations (0.08 mg/mL) in the unfermented GBJ.

To further understand the differences in VOCs of goji juice fermented with different microorganisms, cluster analysis was performed using a heat map (Figure 3). According to the vertical direction of the heat map, all samples were classified into two main categories. Unfermented and *L. plantarum* fermented goji juice were clustered together to form a group (GBJ and GBJL in Figure 3). Cluster analysis shows that the microorganism used had a major influence on the volatile components of the juice; yeast-derived compounds dominated the final composition over those produced by lactic acid bacteria in the mixed fermentation. In general, the content of ester compounds such as ethyl nonanoate, ethyl decanoate, ethyl dodecanoate and isoamyl acetate, which are related to fruity aromas, was higher in goji juices fermented involving *S. cerevisiae* (GBJS and GBJLS). Only methyl salicylate was found in higher content in GBJ and GBJL. However, aldehydic compounds such as pentanal, hexanal, nonanal, 2-octenal and 2,4-decadienal were found in lower content in the juices fermented with the microorganism compared to the unfermented juice. This suggests an improvement in the aroma of fermented beverages according to Kim et al.⁴⁷ which indicated that higher concentrations of aldehyde compounds increase the unpleasant odor. Alcoholic compounds, primarily associated

with fruity, floral, and grassy aromas, were also predominantly found in fermented beverages. However, 1-octen-3-ol, associated with an unpleasant mushroom-like flavor⁵³, was found mainly in GBJ.

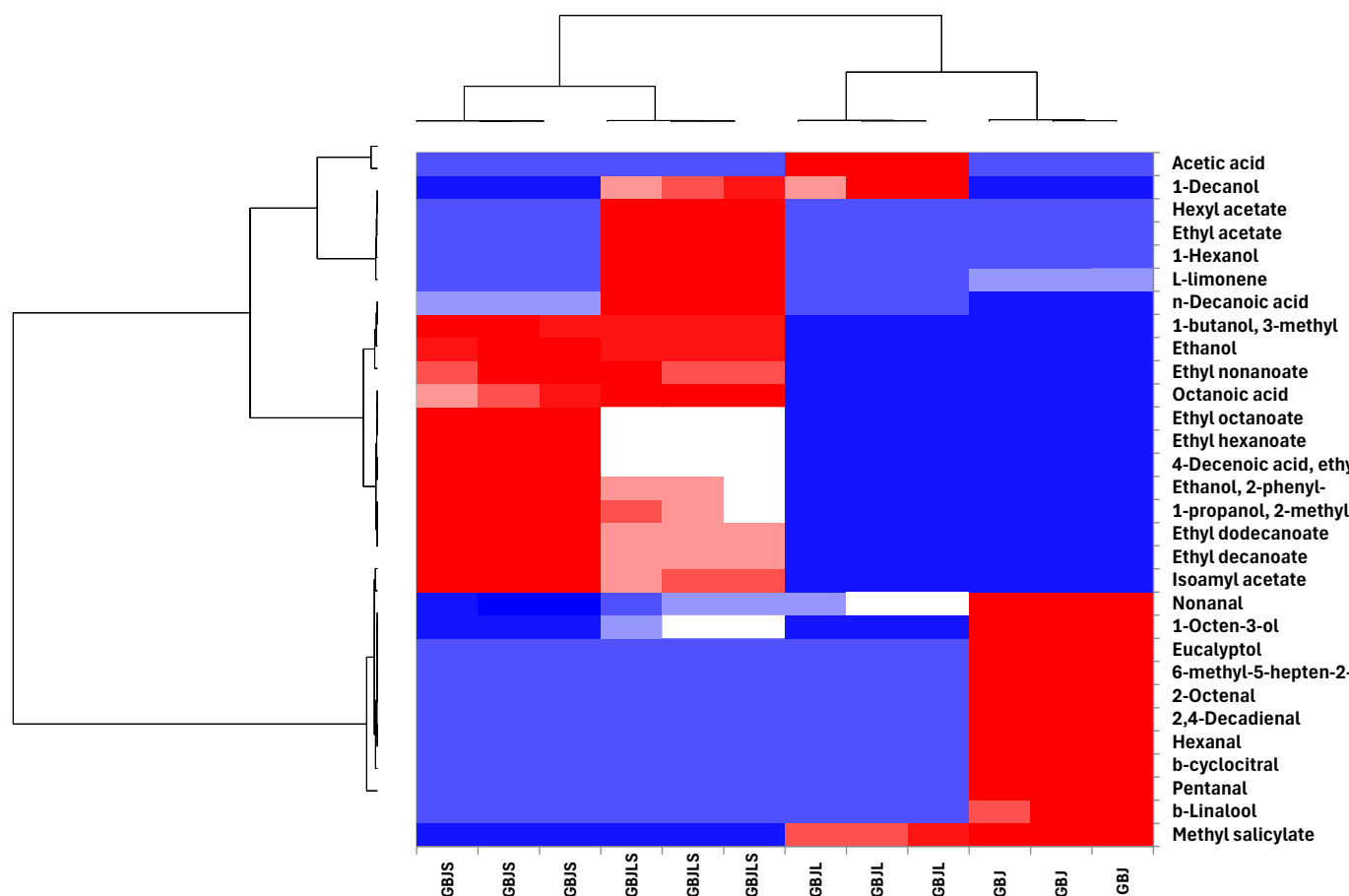
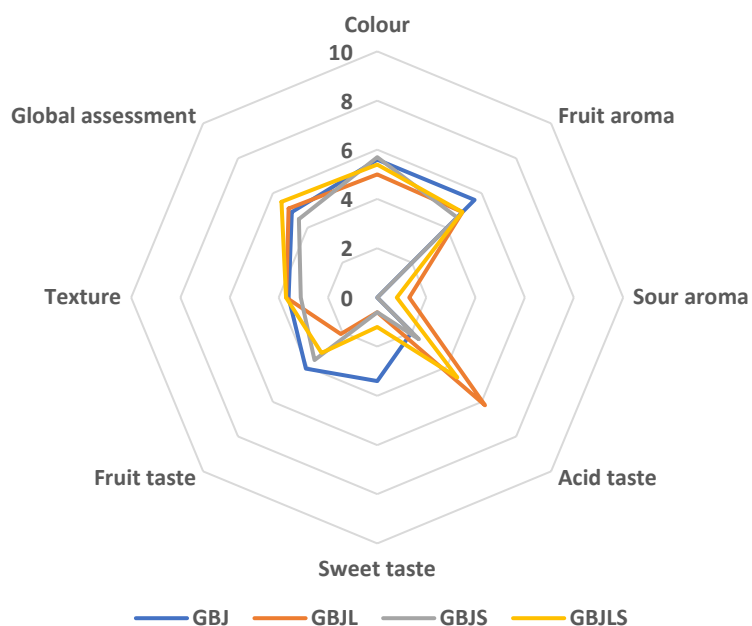


Figure 3. The hierarchical clustering heat map of the identified VOCs in all fermented beverages based on GC-MS analysis. GBJ: goji berry juice, GBJL: goji berry juice inoculated with *L. plantarum*, GBJS: goji berry juice inoculated with *S. cerevisiae*, GBJLS: goji berry juice inoculated with *L. plantarum* and *S. cerevisiae*.

3.8. Sensory Analysis of Fermented Beverages

Nutritional and functional characteristics influence the excellence of a food, however, the decision to accept or reject that food is closely related to the sensory appreciation of the consumer. Thus, a panel of tasters trained in the sensory analysis of food and beverages evaluated the fermented beverages with the different microorganisms.

540 A descriptive sensory analysis, which examines the colour, aroma (fruit and sour), taste (fruit,
541 sweet, and sour), and texture characteristics of the beverages was performed. A spider chart
542 was created for the evaluated attributes of the unfermented (GBJ) and fermented (GBJL, GBJS,
543 and GBJLS) juice (Figure 4). When the panelists were asked for the global assessment of the
544 unfermented and fermented beverages, no significant differences were found between the
545 beverages ($p > 0.05$). For all beverages, this parameter was approximately in the middle range
546 of the scale (4.9 ± 1.2 , 5.1 ± 0.4 , 4.2 ± 1.6 , 4.4 ± 1.8 for GBJ, GBJL, GBJS, and GBJLS,
547 respectively). This may be attributed to the fact that the consumption of low and non-alcoholic
548 fermented beverages is not yet widely consumed and is currently in a phase of growth and
549 acceptance⁵⁴. The attributes of colour and texture had similar acceptability between the samples
550 ($p > 0.05$), with an average value of 5.4 and 3.5, respectively. However, fermentation-related
551 sour aroma and acid taste were mainly detected in juices fermented with *L. plantarum* (BGJL
552 and BGJLS), with GBJL showing the highest intensity ($p < 0.05$). Thus, the lactic acid
553 production analytically observed for the GBJL and GBJLS fermented beverages (Table 2), the
554 main organic acid produced by the *L. plantarum* strains, was corroborated in the assessment of
555 acid taste by the tasting panel. A very significant difference observed was the perception of
556 sweet taste, which was significantly higher in the unfermented juice (GBJ) ($p < 0.05$). This
557 consists of significantly higher glucose and fructose content in GBJ ($p < 0.05$) (Table 2).



	Tukey Test				Kruskal Wallis Test			
	Colour	Acid	Fruit	Texture	Fruit	Sour	Sweet	Global assessment
GBJ	a	b	a	a	a	ab	a	a
GBJL	a	a	a	a	a	a	b	a
GBJS	a	b	a	a	a	b	b	a
GBJLS	a	ab	a	a	a	ab	b	a

Figure 4. Spider chart of the descriptors evaluated. Intensity scales: 0 (non detected) to 10 (highest intensity). Multiple comparisons were analysed following Tukey Test for Colour, acid and fruit taste, and texture. Kruskal Wallis Test for fruit and sour aroma, and sweet taste. All parameters at $p < 0.05$.

3.9. Multivariate Analyses: Principal Component Analysis (PCA)

A PCA analysis was performed to assess the variations among the goji berries beverages based on twenty-eight distinct parameters. The analysis successfully reduced the data dimensionality to four principal components, collectively accounting for 97.63% of the total variance.

Principal Component 1 (41.09 % of the variance) was positively associated with p-Cou, citric and malic acids, and Cap and Zea carotenoids compounds, and the t-Fer, ChlA and Ecat contents negatively influencing this component. Component 2 explained 28.63 % of the variance, showing positive correlations with Q3R and antioxidant activity, and a negative relationship with fructose and sucrose contents. Furthermore, Component 3 (25.41 % of the variance) was primarily defined by the positive contributions of p-CouQ and Cat, contrasted

by negative loading for glucose. Finally, Component 4 represented 2.41 % of the variance, driven positively by α -car, and negatively by ChlA (Supplementary Table 1).

As illustrated in Figure 5, the projection of the two primary components (PC1 vs. PC2) revealed the formation of three distinct clusters. The first group, comprising GBJ, was positioned in the positive quadrant of both components. This placement reflects significantly elevated levels of pCou, citric and malic acid, the carotenoids Cap and Zea, and Q3R, coupled with high antioxidant activity (Tables 2 and 3). The second cluster, represented by GBJL, showed positive PC1 but negative values for PC2; this profile is characterized by high concentrations of pCou, citric acid, malic acid, and the carotenoids Cap and Zea, as well as higher fructose and sucrose levels than the rest of the beverages. Finally, the third cluster grouped GBJL and GBJSL beverages, which were defined by negative PC1 and positive PC2. These beverages were distinguished by their higher ChlA and Q3R levels and increased antioxidant activity (Table 3).

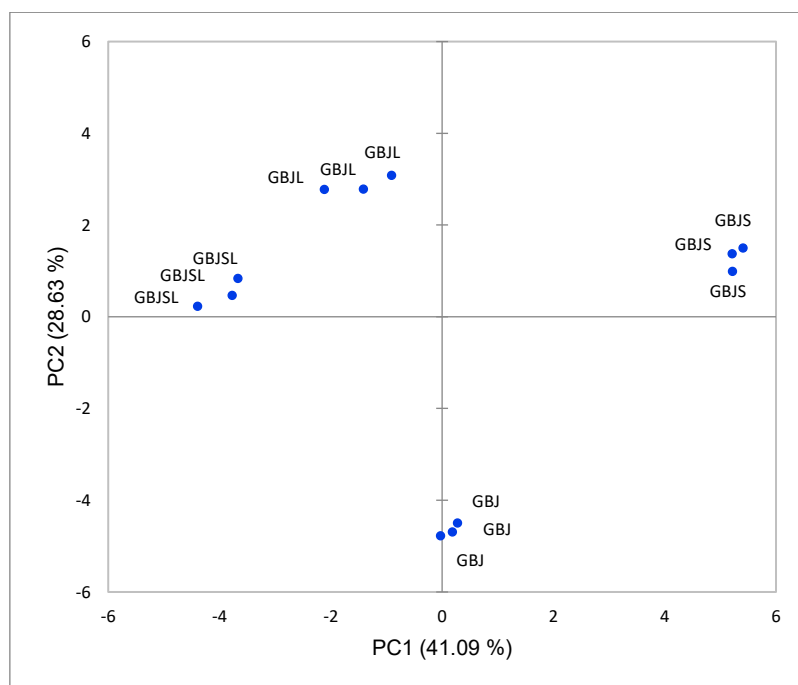


Figure 5. Principal component analysis (PCA) plotting components 1 and 2 for different goji beverages. GBJ: goji berry juice, GBJL: goji berry juice inoculated with *L. plantarum*, GBJS: goji berry juice inoculated with *S. cerevisiae*, GBJLS: goji berry juice inoculated with *L. plantarum* and *S. cerevisiae*.

4. Conclusions

The fermentation of goji juice using *L. plantarum* and *S. cerevisiae* represents a viable strategy for developing functional plant-based beverages with tailored nutritional and bioactive profiles. Goji juice provides an adequate medium for both microorganisms, which reach peak concentrations around the seventh day of fermentation and remain stable throughout the 15-day fermentation period. However, when used in co-inoculation, the lactic acid bacteria partially inhibit yeast growth during the first 48 hours. This initial two-day period is critical, as the most significant physicochemical changes occur across all fermentations conducted in this work, including a sharp decrease in pH and total soluble solids. Regarding bioactive properties, LAB is the primary driver of acidity and organic acid production, yielding the highest antioxidant activity and total phenol content. Conversely, the yeasts are the main drivers of sugar depletion—eliminating glucose—and the enhancement of the volatile profile through the synthesis of esters and alcohols. Furthermore, yeast-led fermentations significantly boost carotenoid levels, particularly zeaxanthin. Sensory results of the beverages show a significant margin for enhancing consumer preference regarding their acidity; this suggests that, alongside product optimization, consumer education is essential for the market success of these innovative beverages. Consequently, the selection of the fermentation starter depends strictly on the desired functional outcome. *L. plantarum* is the superior choice for a probiotic-rich beverage with maximum antioxidant capacity, whereas *S. cerevisiae* is ideal for a sugar-free product with high zeaxanthin content and a complex aromatic profile. Co-inoculation of both microorganisms offers a balanced compromise, maintaining high antioxidant levels while benefiting from the dominant aromatic influence of the yeast.

In conclusion, this research successfully yielded a functional beverage with favourable physicochemical properties and acceptable sensory results. Ultimately, this study demonstrates that by selecting specific microorganisms and controlling fermentation time—specifically

targeting the two-day mark for primary transformations—it is possible to design goji-based beverages with precise health-promoting and sensorial properties. While the current outcomes are promising, there are clear opportunities for optimization regarding the product’s organoleptic appeal. Therefore, further studies are necessary to optimize fermentation times, aiming to enhance the sensory profile and overall consumer acceptance of innovative functional goji-based beverages.

Funding

The authors would like to thank the Junta de Extremadura, the European FEDER Funds and the European Union Next GenerationEU (PRTR-C17.I1), and the Ministerio de Ciencia, Innovación y Universidades for the financial support (AGROALNEXT project). Open Access funding provided by Universidad de Extremadura (Transformative Agreement).

Clinical trial number

Not applicable

Conflict of interest

Authors declare no conflicts of interest.

Author contributions:

Francisco Pérez Nevado: Conceptualization, Supervision, Writing –original draft, Writing – review & editing. Rubén Magro Rubio: Methodology, Investigation. M^a Belén Ramos Alguijo: Methodology, Investigation. J. Carlos Solomando: Methodology, Investigation. M^a José Rodríguez Gómez: Conceptualization, Writing – review & editing, Writing – original draft, Validation, Methodology. Patricia Calvo Magro: Conceptualization, Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition.

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