

Original article

Development of synbiotic vegan beverages: probiotic viability, sensory profile, consumers' acceptance and functional stabilityEstefanía Valero-Cases,^{1*} María José Frutos¹ & Francisca Pérez-Llamas²

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Summary The development of synbiotic vegan drinks expands the probiotic food options on the market as an alternative to dairy products and with no added sugars. This study aimed to investigate the impact of fermentation and inulin addition in mixed carrot-orange juice with *Lactiplantibacillus plantarum* (LP) on the sensory profile, consumer acceptance and functional stability. The results demonstrated that all fermented juices had high antioxidant capacity (AOC) and were a rich source of bioactive compounds such as β -carotene and phenolics. However, only the β -carotene showed a high correlation with the AOC. Inulin could improve LP survival, remaining at high concentration during the 21 days of storage. Only fermented juices with 2% inulin showed a positive correlation with sweet taste and had the best impact on sensorial properties. Fermentation with LP and inulin addition could contribute to the improvement of orange flavour and taste. Penalty analysis showed that the control-unfermented juices were the samples with a clear need for improvement with respect to the fermented ones. Therefore, carrot-orange juices represent a promising vegan food matrix for probiotic delivery.

Keywords Antioxidant capacity, functional compounds, inulin, lactic acid bacteria, prebiotics, probiotic drinks.

Introduction

Nowadays, consumers demand healthier food options, such as the fermented foods containing probiotics that are live microorganisms that inhabit the gut and when administered in adequate amounts, confer a health benefit to the host (Hill *et al.*, 2014). However, most fermented foods are dairy products and cannot be consumed by certain sectors of the population, such as people with allergies to milk proteins, lactose intolerance, vegetarians and vegans or due to sustainability, lifestyle and dietary reasons (Valero-Cases *et al.*, 2020). As a result, research has been carried out using cereals, seeds, pseudocereals, fruits and vegetables (García *et al.*, 2022; Marnpae *et al.*, 2022; Ramos *et al.*, 2022; Uruc *et al.*, 2022). However, there is always a need for further research because probiotic survival and functional properties can be affected by the food matrix, the addition of prebiotics which are 'a substrate that is selectively utilised by host microorganisms conferring a health benefit' (Gibson *et al.*, 2017), processing and storage conditions. At the same

time, probiotics can also have an impact on the quality characteristics of the processed food (sensory, functional and physicochemical parameters) (Pimentel *et al.*, 2021). Therefore, for the development of probiotic vegan foods, it is essential to choose a suitable matrix as a good vehicle to deliver probiotics that are also rich in functional compounds and with good sensory quality. At the same time, to ensure that the food is vegan, the probiotic strain must be isolated from a plant matrix. Carrots are well-known vegetables consumed on a daily basis and with many functional compounds such as vitamins, minerals, carotenoids, phenolic compounds, flavonoids and anthocyanins (in purple carrots) (Anjani *et al.*, 2022). β -carotene has an important antioxidant activity and prevents the development of cardiovascular disease, cancer and oxidative stress (Celitan *et al.*, 2022). Moreover, it represents the main source of vitamin A in human diet. However, these compounds cannot be biosynthesised by the human body and can be consumed as a part of the diet or through dietary supplements (Kot *et al.*, 2018; Gies *et al.*, 2019). At the same time, orange juice has high contents of polyphenols, carotenoids and ascorbic acid and is consumed worldwide (Castello *et al.*, 2020). Therefore, mixtures of

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carrot and orange juice could have sensory advantages and a mutual complementary effect between probiotic survival and functional and sensory properties. At the same time, inulin is used in the food industry with many possibilities as dietary fibre (prebiotic), which can improve probiotic survival, texture (thickener and gelling) and taste in food while being low in calories and suitable for diabetics (Aidoo *et al.*, 2015). With this, the use of inulin could help to improve the sensory parameters of synbiotic vegan juices and the viability of probiotics. Therefore, the appropriate choice of a vegan probiotic strain, the adequate viability of the probiotic during the shelf life of the vegan beverage, the stabilisation of product quality and the understanding of consumer preferences are essential for the development of a functional vegan fermented beverage. Therefore, taking into account all the above aspects, the aim of the present study was to develop synbiotic vegan drinks with carrot-orange juice and inulin (1 and 2%) as substrate, without sugar supplementation, to evaluate the sensory profile (descriptive and effective analysis) and functional properties (antioxidant capacity, total phenols and β -carotene content, probiotic viability) of the different vegan drink formulations.

Materials and methods

Probiotic culture preparation

In the study, *Lactiplantibacillus plantarum* CECT 220 (LP) isolated from corn silage, was used for its good tolerability to gastrointestinal conditions, adhesion properties and improvement of intestinal epithelial cells (Valero-Cases *et al.*, 2017b; Valero-Cases & Frutos, 2017b), which was obtained in lyophilised form from the Spanish Type Culture Collection (CECT, Valencia, Spain). The bacterial strain was grown from frozen glycerol stocks on Man Rogosa and Sharpe (MRS) agar for 48 h at 37 °C according to Valero-Cases *et al.* (2017a). Single colonies were inoculated in MRS broth and incubated at 37 °C for 48 h. Then, to obtain a biomass of approximately 8 Log Colony Forming Units per millilitre (CFU/mL), the preinoculum (1% v/v) was inoculated into MRS broth for 24 h at 37 °C. The LP was harvested by centrifugation at 4 °C during 10 min at 2000 $\times g$ and washed with sterile phosphate saline (PBS) (Oxoid, Madrid, Spain) and stored in glycerol 10% (v/v) at -80 °C until use.

Production of the mixed probiotic carrot and orange beverages

The mixed vegetable juice was produced according to Valero-Cases & Frutos (2017b) with some modifications. The carrot juice (*Daucus carota* L.) cv. Nantesa

and orange juice (*Citrus sinensis* L.) cv. Valencia-Late was mixed at 60:40 ratio. Xanthan gum (0.25% w/v) (Guinama; Valencia, Spain) was added as a stabiliser. The juices were supplemented with different concentrations of artichoke inulin with a degree of polymerisation (DP) 10 (Farma-química; Málaga, Spain) to obtain the following formulations: carrot-orange juice without inulin (COJ0%), carrot-orange juice with 1% inulin (COJ1%) and carrot-orange juice with 2% inulin (COJ2%). Nonfermented and inulin-free juices were kept as control samples (CJ). The juices were pasteurised at 90 °C for 5 min in a water bath and cooled to 37 °C in an ice bath. Then, the different juices (COJ0%, COJ1% and COJ2%) were inoculated with *Lactiplantibacillus plantarum* (10⁶ CFU/mL) and fermented at 37 °C for 24 h. After incubation time, all samples of vegan juices (CJ, COJ0%, COJ1% and COJ2%) were stored during 21 days at 4 °C. The samples were analysed at 0, 1, 7, 14 and 21 days. Different independent replicates of each formulation were carried out for the storage testing and for the descriptive and hedonic analyses at the end of storage.

Lactiplantibacillus plantarum viability and the study of moulds and yeast contamination in the beverages

Lactiplantibacillus plantarum viability was determined during the storage period. Aliquots of each fermented beverage were taken and serial dilutions were spread in triplicate on MRS agar plates that were incubated at 37 °C for 48 h. The results were expressed as Log CFU/mL of each fermented beverage. Moulds and yeasts were determined by taking 1 mL of the aliquot from appropriate dilutions, put on Petrifilm™ (3M; Madrid, Spain) and count plates and incubated aerobically at 25 °C for 2–5 days. The results were expressed as Log CFU/mL.

Sensory analysis

Descriptive analysis

The descriptive sensory tests of the fermented juices were performed by a trained panel that consisted of twelve panellists (five men and seven women) from the Agro-Food Technology Department of the Miguel Hernández University (Orihuela, Alicante, Spain), with long experience in sensory analysis. The panellists participated in seven training sessions of 1 h carried out in a standardised test room (ISO 8589:2010). During the two first sessions, the samples were served and each panellist described the differences and similarities in the appearance, aroma, flavour and texture among the samples. Next, the panel leader initiated a discussion among the panellists, to select, describe and define the sensory attributes for the fermented and unfermented mixed carrot and orange juices (Table 1). During

Table 1 Definitions, references and intensity of the descriptor used for the sensory profiling of the mixed carrot and orange juices supplemented with different concentration of inulin

Attributes	Descriptor	Definition	Reference and intensities
Appearance	Colour	Intensity of colour inside scale Pantone®	Pantone® colour 137C = 1 Pantone® colour 1495C = 5 Pantone® colour 152C = 9
	Turbidity	Lack of clarity of juices	Aquarius® = 1 Commercial peach juices (Don Simon®) = 10
Aroma	Orange	Aroma of orange peel	Orange peel = 10
	Carrot	Aroma of fresh carrot	Fresh carrot (unpeeled) = 10
	Fermented	Aroma of products has been submitted a fermentation process	Sauerkraut Vegetalia = 9.5
Flavour	Sweet taste	The taste stimulated by sugar presence	Sucrose solution 40 g/L = 9.5
	Lactic taste	The taste stimulated by lactic acid presence	Lactic acid solution 1.5 g/L = 9.5
	Citric taste	The taste stimulated by citric acid presence	Citric acid solution 1 g/L = 9.5
	Orange	Taste associated with orange juice	Orange juice freshly squeezed = 10
	Carrot	Taste associated with orange carrot	Carrot juice freshly liquefied = 10
Texture	Body	Sensation of covering the mouth	Commercial peach juices (Don Simon®) = 10

the following four sessions, the panellists individually were trained to identify and quantify the sensory attributes using the reference samples (Table 1). In the last training session, the four different juices (COJ, COJ0%, COJ1% and COJ2%) were coded with 3-digit randomised numbers and served in clear plastic cups at 4 °C in a randomised order. Each juice was evaluated using the reference samples to quantify the intensity of each attribute using a 10-point numerical scale (1 = weak and 10 = strong) with 0.5 increments.

Acceptance test and purchase intention

The acceptance (colour, aroma, sweet taste, acid taste, overall flavour, body texture, general acceptance) and purchase intention of the different fermented and non-fermented juices (COJ, COJ0%, COJ1%, COJ2%) were evaluated by 104 untrained panellists (65 women and 39 men aged between 18- and 55-year-olds). The consumers were recruited among graduates and employees of the High Polytechnic School of the Miguel Hernández University (Orihuela, Alicante, Spain). The main requirement was being regular consumers of juices. The tests were performed in a standardised test room. To clean the mouth before and between tests, water at room temperature was served in each individual cabin. During the session, 20 mL of each sample was served at 4 °C in clear plastic cups, coded with 3-digit random numbers and in a random order. The consumers evaluated the samples using a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely) to evaluate the global acceptance together with questions on the intensity of the attributes (acceptance of colour, aroma, sweet taste, acid taste, overall flavour, body and general acceptance) using a Just About Right (JAR) scale. The purchase intent was evaluated using a 5-point scale (5 = surely would buy, 1 = surely would not buy).

Biochemical analysis

Determination of β -carotene content

For the analysis, 2.5 mL of juice was mixed with the extraction medium (2.5 mL of hexane, 1.25 mL acetone and 1.25 mL ethanol) and homogenised in an Ultra-Turrax® (T25 digital) at 10 000 rpm for 1 min at 4 °C. Then, it was centrifuged at 15 000 $\times g$ for 20 min at 4 °C. The change in absorbance was measured using an UV-vis spectrophotometer (Hewlett Packard mod. 8453), at 450 nm. The carotene content was determined using the Beer-Lambert Law. The carotene concentration was calculated using the extinction coefficient 2505 of β -carotene in hexane (De Ritter & Purcell, 1981). The blank was hexane. The results were expressed as mg/100 mL.

Antioxidant activity

The scavenging capacity rate of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical was determined based on the method of Cano *et al.* (1998). The change in absorbance was measured using an UV-vis spectrophotometer (Hewlett Packard mod. 8453), at 414 nm. The calibration curve was prepared with known concentrations of ascorbic acid and results were expressed as mg of Vitamin C equivalents/L of juice.

Determination of total polyphenolic compounds

Total polyphenolic compounds (TPC) were determined using the Folin-Ciocalteu assay (Wood *et al.*, 2002).

The change in absorbance was measured using an UV-vis spectrophotometer at 756 nm. The standard curve was made with pyrogallol. The results were expressed as mg of pyrogallol equivalents/L of juice.

Statistical analysis

All experiments and analyses were conducted in triplicate. The results were expressed as mean \pm standard deviation. The mean comparison was performed using analysis of variance (ANOVA) followed by a Duncan test ($P < 0.05$), using SPSS v 21.0 software package (SPSS Inc., Chicago, IL, USA). To determine the correlation between antioxidant capacity and antioxidant compounds (total polyphenolic compounds and β -carotene), Pearson's correlation coefficients were calculated with $P < 0.05$. Principal component analysis (PCA) was performed in the QDA attributes for fermented beverages with different concentrations of inulin using XLSTAT Software (version 2016.02; Addinsoft, New York, NY, USA).

Results and discussion

Viability of *L. plantarum* after fermentation and during the storage in carrot-orange drinks

Lactiplantibacillus plantarum concentrations increased by more than two Log units after 24 h of fermentation compared with the initial inoculate concentration (6 Log CFU/mL) during beverage preparation (Fig. 1). All fermented samples showed around 8.65 Log CFU/mL of viable cells indicating that the inulin supplementation did not have an effect on the metabolism of the probiotic during the fermentation step. At the same time, as expected, the physicochemical parameters showed a pH reduction while the acidity values increased. The acidity increment was related to the lactic acid production during fermentation (data not shown), according to a previous study with *L. plantarum* in similar matrices (Valero-Cases & Frutos, 2017b). Inulin addition did not affect the fermentation process as it was shown in previous studies with similar matrices and goat-fermented milk (Valero-Cases & Frutos, 2017b; Galdino *et al.*, 2021). However, the inulin addition in nectar-type beverages showed an improvement in the *L. acidophilus* viability during 36 h of fermentation (Valero-Cases & Frutos, 2017a). Therefore, the fermentation of prebiotics as a source of energy during fermentation could depend on the matrices and on the type of probiotic used.

At 7 days of the storage period (4 °C), the viability of *L. plantarum* showed an increase in all the fermented vegan beverages compared with the concentration at the end of fermentation time reaching values around 8.74 Log CFU/mL. This cell concentration remained without significant differences after the 14 days of the storage period. During these weeks, the inulin

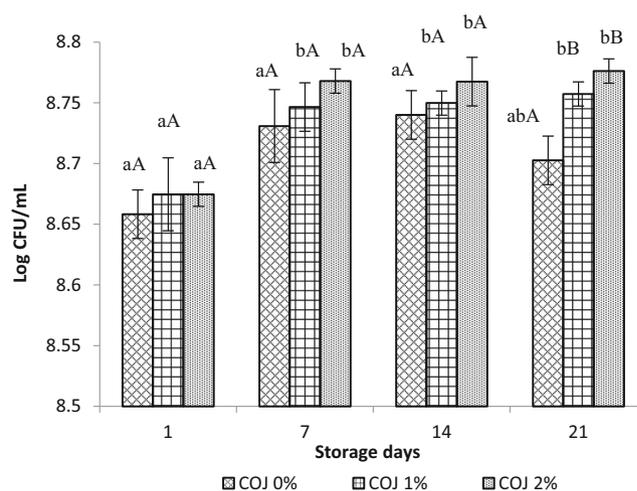


Figure 1 Viability of *L. plantarum* during storage period in different fermented mixed carrot and orange juices. COJ0%—carrot-orange juice without inulin; COJ1%—carrot-orange juice with 1% inulin; COJ2%—carrot-orange juice with 2% inulin. ^{a,b,c}Different lowercase letters for each beverage denote significant differences during storage period ($P < 0.05$). ^{A,B,C}Different capital letters for each beverage denote significant differences among the different fermented beverages ($P < 0.05$).

concentration did not improve probiotic viability, because all the fermented samples remained without significant differences between them. However, at the end of the storage period (21 days), the fermented samples without inulin showed a significant decrease in the probiotic viability to the same concentration that at the beginning of storage (8.65 Log CFU/mL). However, the fermented samples supplemented with inulin showed the higher viability of *L. plantarum* without significant differences between COJ1% and COJ2%. This can be related to the rapid metabolism of simple sugars (monosaccharides) during fermentation and during the first days of storage, exhausting their availability at the end of the refrigerated storage period, thus promoting the metabolism of inulin (Valero-Cases & Frutos, 2017b; Alves *et al.*, 2021). Therefore, the addition of inulin to these fermented samples can help the survival of *L. plantarum* to remain at higher concentrations during cold storage. At the same time, the presence of moulds and yeasts was not detected (data not shown) in any of the juice samples elaborated during the entire storage period. This indicates that the beverages have been produced in accordance with good hygienic practices.

Descriptive analysis

The sensory attributes and the mean intensity values for the formulations of orange and carrot juices are

shown in Table 2. The panellists by comparison with a colour reference scale (Pantone®) (Table 1) evaluated the colour of the samples. The results showed that there was no significant difference ($P > 0.05$) among the juices for the colour, with values *ca.* 5, corresponding to the colour reference 1495C on the Pantone® scale. However, a significant increase ($P < 0.05$) in the turbidity of the juices was observed as the inulin concentration increased. With respect to fermented aroma, the panellists did not detect high fermented aroma differences between the fermented samples, with scores (*ca.* 4.5) that were significantly higher ($P < 0.05$) than the values of control samples as expected. At the same time, a weak lactic flavour (*ca.* 2.40) was detected in all the fermented samples, without significant differences due to the production of lactic acid during fermentation, being the main organic acid produced. However, citric flavour was the most significant flavour detected in all samples, presenting higher values (approx. 5) than lactic flavour. It may be related to terpenoids as the predominant chemical group found in fresh and processed orange juice where limonene represents 6.3% of the total volatile profile of the processed juice (Perestrelo *et al.*, 2019).

The aroma and flavour perceptions were higher for orange than for carrot attribute in all the fermented samples. The COJ2% presented the highest ($P < 0.05$) orange aroma and flavour intensities. In previous studies, it has been reported that the addition of prebiotics

to foods could increase the citric aroma retention and the citrus taste intensity in dairy desserts (Arcia *et al.*, 2011). In other studies, on the sensory assays of fermented malt beverages, the ones with higher acetaldehyde concentration were the best accepted, this compound being related to fruit, citric and green tastes and aromas (Gómez-Míguez *et al.*, 2007; Koutinas *et al.*, 2007). Therefore, the fermentation with LP together with inulin addition could contribute to the improvement of orange flavour and taste respect to control samples.

In relation to the sweet taste attribute, the COJ2% presented the highest value while the addition of 1% of inulin did not increase significantly the sweetness perception by the panellists with respect to the control and to the fermented samples without inulin. Inulin is a slightly sweet carbohydrate with a sweetness intensity of 30% of the sweetness power of sucrose (Franck, 2002). In our study, the concentrations of 2% of inulin promoted an increase in the sweet taste in these juices while the addition of 1% did not trigger any change with respect to the sweetness of the control.

The scores for the body texture were high in all juices (values higher than 7) when compared with the peach juice as attribute reference (Table 1). This could be mainly due to the use of xanthan gum as a stabiliser, which could improve the viscosity of the beverages (Zhu *et al.*, 2020). At the same time, panellists perceived a higher increase ($P < 0.05$) of body texture in samples with inulin (COJ1% and COJ2%) with respect to COJ and COJ0% and they qualified this perception as good when they were asked for further comments.

The PCA confirmed QDA results due to the first and second principal components and described 95.85% of the variance (Fig. 2). The PC1 explained 75.18% of the variance between the samples and attributes. The following interactions between the different attributes were shown: fermented aroma and lactic taste; orange aroma with orange taste and body with turbidity. At the same time, these attributes were inversely related to colour, carrot aroma, citric taste and carrot taste. PC2 showed 20.67% of the variance and explained the sweet taste and carrot aroma attributes showing an inverse relationship between them. These attributes can be explained by the difference between the fermented samples with or without inulin (COJ2% and COJ0%, respectively). Only the fermented sample with 2% of inulin addition showed a positive correlation with sweet taste. This correlation indicates that the addition of 2% inulin to fermented mixed carrot and orange juices leads to an increase in the sweet taste perception in fermented samples and potentially increases consumer acceptance. However, the fermented sample without inulin supplementation (COJ0%) was correlated with carrot aroma. On the

Table 2 Attribute means for each fermented mixed beverage with different concentration of inulin in QDA (scale used ranged from 0 = no intensity to 10 = extremely strong intensity)

Attributes	Descriptor	CJ	COJ0%	COJ1%	COJ2%
Appearance	Colour	5.21 ^a	5.21 ^a	5.14 ^a	5.07 ^a
	Turbidity	7.43 ^a	7.93 ^{ab}	8.43 ^b	9.12 ^c
Aroma	Orange	4.92 ^a	5.86 ^b	5.71 ^b	6.29 ^c
	Carrot	2.50 ^a	2.79 ^a	2.50 ^a	2.43 ^a
	Fermented	0.14 ^a	4.57 ^b	4.64 ^b	4.07 ^b
Flavour	Sweet taste	5.07 ^a	4.93 ^a	4.93 ^a	6.86 ^b
	Lactic taste	0.21 ^a	2.43 ^b	2.36 ^b	2.28 ^b
	Citric taste	5.57 ^b	5.21 ^{ab}	5.14 ^a	5.14 ^a
	Orange	2.43 ^a	4.14 ^b	4.43 ^b	5.29 ^b
	Carrot	4.07 ^b	3.36 ^b	3.36 ^b	2.71 ^a
Texture	Body	7.50 ^a	8.21 ^b	8.86 ^c	9.36 ^c

Mean \pm standard deviation. Values with the same lowercase letters, within the same column, were not statistically different at the $P < 0.05$ level among of mixed carrot and orange juices.

CJ, control juice; COJ0%, carrot-orange juice without inulin; COJ1%, carrot-orange juice with 1% inulin; COJ2%, carrot-orange juice with 2% inulin.

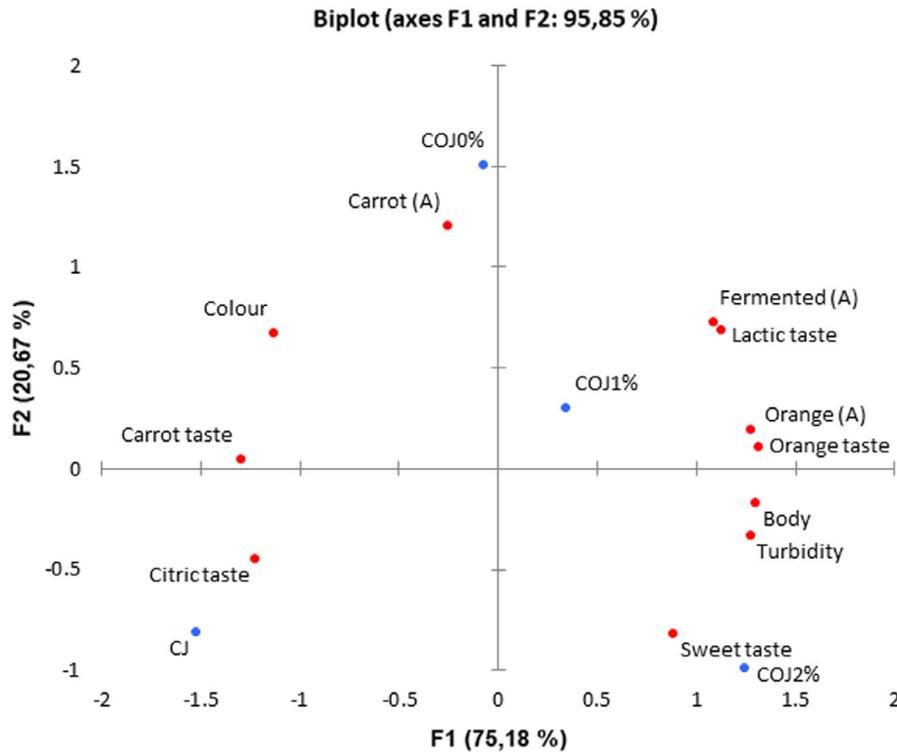


Figure 2 Principal component analysis (PCA) of mean sensory ratings of QDA attributes for fermented beverages with different concentration of inulin. CJ—control juice; COJ0%—carrot-orange juice without inulin; COJ1%—carrot-orange juice with 1% inulin; COJ2%—carrot-orange juice with 2% inulin.

other hand, the unfermented control samples were highly correlated with the citric taste and inversely related with fermented aroma and lactic taste attributes more characteristic of the fermented samples. PCA did not show a similarity between any of the samples. Placing each of them at different ends except for COJ1% placed by PCA close to the origin.

Sensory acceptability and purchase intention test of carrot-orange synbiotic drinks

For the commercialisation of novel foods, it is necessary to know the acceptance and preference of consumers. For this reason, this type of study is applied in recent investigations where new foods are developed (García-Gómez *et al.*, 2021; Alinovi *et al.*, 2023; Samakradhamrongthai *et al.*, 2023). The acceptability and purchase intent of CJ, COJ0%, COJ1% and COJ2% are shown in Table 3. Among the untrained panellists the frequency of juice consumption was 19.2% for daily consumption, 29.8% several times per week, 16.3% for moderate consumption (once a week), 6.7% for occasional consumption (1–3 times a month) and 5.8% reported no consumption. Consumers demand healthy foods with pleasant sensory attributes,

but the flavour and acid taste attributes can be adversely changed by fermentation (Valero-Cases & Frutos, 2017a). Nevertheless, it is important to point out that there was not any adverse change in those parameters in the fermented juices studied with respect to the control ones. Regarding the consumer acceptance of fermented juices, no negative scoring was reported for these attributes (flavour and acid taste). The results showed that the fermentation of mixed carrot and orange juice with LP and the addition of inulin did not modify ($P > 0.05$) the acceptance with respect to colour, flavour and acid taste in fermented samples compared with the control juices. Therefore, the lactic acid attribute detected and quantified by the trained panel did not have any influence on the acceptance of fermented juices. The general acceptance, purchase intention and acceptance of the other sensory attributes (sweetness, flavour, body texture), was higher in COJ2% than in the other formulations. Inulin is a low-calorie sweetener and its addition to food can balance the lack of sweet taste improving its acceptability (Villegas *et al.*, 2010; Arcia *et al.*, 2011). In the present study, the addition of 2% of inulin led to an increased acceptance in sweet taste with respect to the control juices. It was also observed that the

Table 3 Influence of fermentation and inulin addition on the acceptability and purchase intent of mixed carrot-orange juices

Juices	Parameters							
	Colour	Aroma	Sweet taste	Acid taste	Overall flavour	Body texture	General acceptance	Purchase intent
CJ	6.88 ± 1.42 ^a	7.41 ± 1.45 ^a	5.90 ± 1.72 ^a	5.95 ± 1.61 ^a	6.54 ± 1.83 ^a	5.73 ± 1.52 ^a	6.39 ± 1.63 ^a	3.02 ± 1.11 ^a
COJ0%	7.10 ± 1.43 ^a	7.26 ± 1.55 ^a	6.10 ± 1.53 ^{ab}	6.45 ± 1.47 ^a	6.69 ± 1.68 ^a	5.52 ± 1.42 ^a	6.43 ± 1.59 ^a	3.24 ± 1.10 ^a
COJ1%	7.07 ± 1.10 ^a	7.22 ± 1.42 ^a	6.12 ± 1.68 ^{ab}	6.00 ± 1.45 ^a	6.73 ± 1.75 ^a	5.95 ± 1.43 ^{ab}	6.80 ± 1.79 ^a	3.27 ± 1.05 ^a
COJ2%	7.27 ± 1.37 ^a	7.37 ± 1.18 ^a	6.71 ± 1.29 ^b	6.51 ± 1.32 ^a	7.63 ± 1.37 ^b	6.54 ± 1.29 ^b	7.54 ± 1.31 ^b	4.12 ± 0.78 ^b

Mean ± standard deviation. Values with the same lowercase letters, within the same column, were not statistically different at the $P < 0.05$ level among of mixed carrot and orange juices.

CJ, control juice; COJ0%, carrot-orange juice without inulin; COJ1%, carrot-orange juice with 1% inulin; COJ2%, carrot-orange juice with 2% inulin.

consumers reported a better acceptance for the body texture in fermented juices with 2% of inulin than in the COJ and COJ0%. These results are in accordance with the descriptive sensory profile, where COJ2% showed the highest sweet taste and the juices with inulin presented a higher body texture than the juices without inulin (Table 1). Furthermore, the addition of 2% of inulin contributed to an increased general acceptance and consumers indicated that they 'liked very much' (values near 8 on a 9-point scale) the juices with the highest inulin concentration (COJ2%) while for the other juices, they liked moderately (values near 7 in a 9-point scale). At the same time, the purchase intention for juices containing more inulin (COJ2%) was the highest (value of 4.12 on a 5-point scale). In previous studies, the acceptance of clarified apple juice with activated *L. paracasei* spp. *paracasei* and oligo-fructose was positively influenced by sweet aroma and taste and negatively by darker colour and apple flavour and aroma after 2 days of storage at 4 °C (Pimentel *et al.*, 2015). On the other hand, malt beverages fermented with LP were evaluated after 10 h of fermentation at 37 °C, reporting greater acceptance than nonfermented ones (Salmerón *et al.*, 2015). However, other studies reported that the fermented bergamot juice, and carrot and orange nectars exhibited good sensory features (Valero-Cases & Frutos, 2017a; Hashemi & Jafarpour, 2020). Therefore, the sensory acceptance of fermented beverages would depend on different factors such as the vegetable matrix, the type of LAB used, the fermentation time and the type and concentration of the prebiotic compound.

Penalty analysis of carrot-orange synbiotic drinks

Penalty analysis is performed to understand the relationship between general consumer acceptances and attribute intensity scores of the JAR questions. It is a very popular method in the food industry that helps to choose those attributes that are susceptible to the improvement of processed products (Pagès *et al.*, 2014). Attributes that have a negative impact on the

acceptance of the sample for at least 20% of consumers and that have a drop of at least 1 point in flavour are considered susceptible to improvement (Lipan *et al.*, 2019; Clemente-Villalba *et al.*, 2021). The results of penalty analysis showed that the control-unfermented juices were the samples with a clear need for improvement with respect to the fermented juices (Fig. 3). The results demonstrated that the CJ needed an improvement in the increase of aroma, sweet taste, body texture, acid taste and overall flavour. At the same time, the results in COJ0% showed fewer attributes susceptible to improvement with respect to the CJ such as the increase in aroma, overall flavour and body texture. However, the fermented juices with the inulin addition showed an improvement in the results of penalty analysis. COJ1% and COJ2% showed only the need for an increase in overall flavour and acid taste, respectively. Therefore, knowing the preferences of consumers, the development of new formulations of vegetable drinks with inulin, can expand the existing market offering probiotic products that could satisfy the demands of consumers for vegetable probiotic products.

β-Carotene content of carrot-orange synbiotic drinks

The β-carotene content was measured for fermented and unfermented carrot-orange beverages (Table 4). The β-carotene content was around 7.5 mg/100 mL in all samples before fermentation. However, after incubation period (24 h at 37 °C), there was a significant decrease in the β-carotene concentration in all juices compared with the amounts prior to fermentation. The β-carotene content varied significantly between fermented and unfermented juices. The higher β-carotene content was found in control samples (7.06 mg β-carotene/100 mL of juice) while in fermented juices there was no significant difference between the β-carotene content (6.25–5.97 mg β-carotene/100 mL of juice). The decrease in β-carotene content after the incubation period could be due to the lowest pH in the fermented samples due to fermentation. This is in line with previous studies that reported that β-carotene was less stable

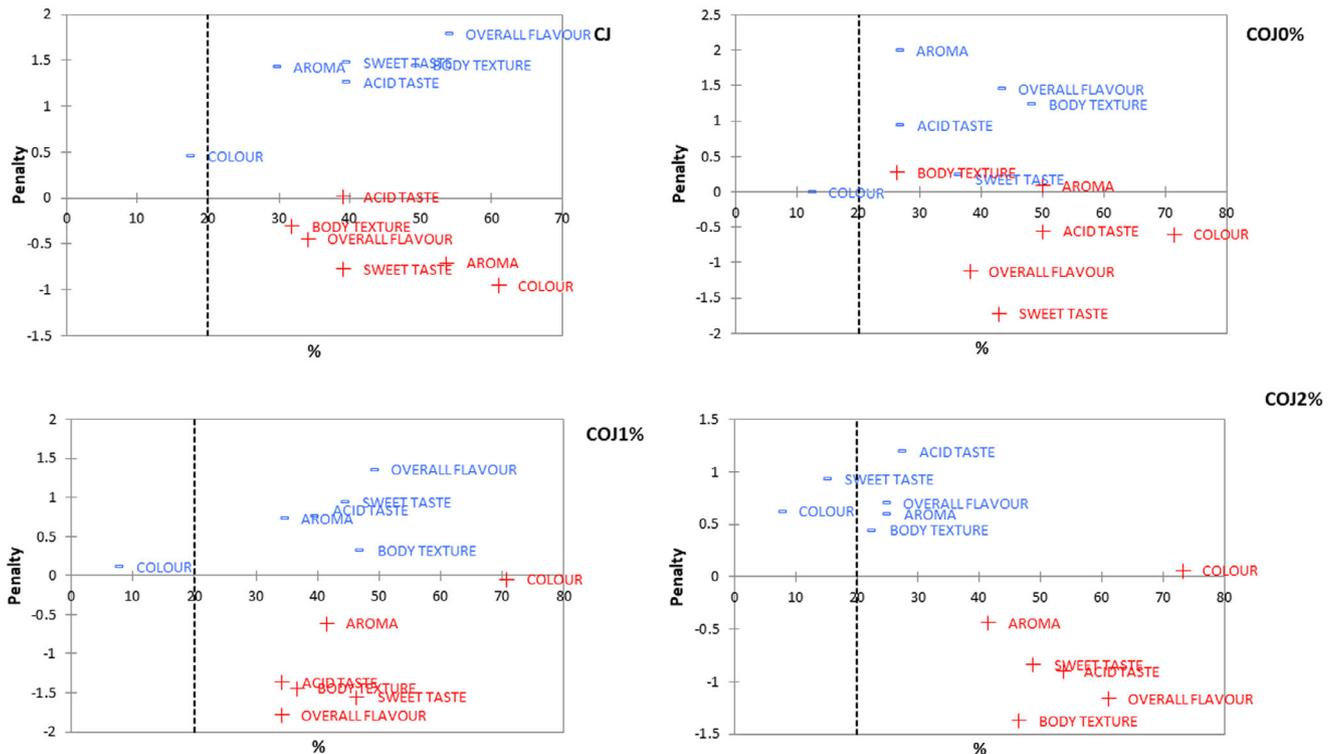


Figure 3 Penalty analysis of intensities of attributes evaluated by consumers. Sample code indicated at the top right of each figure: CJ—control juice; COJ0%—carrot-orange juice without inulin; COJ1%—carrot-orange juice with 1% inulin; COJ2%—carrot-orange juice with 2% inulin. Too low intensity is indicated by the symbol ‘-’, and too high intensity is indicated by the symbol ‘+’.

at lower pHs (3.0 and 4.0) than at higher pHs (6.0 and 7.0) (Kot *et al.*, 2018; Ding *et al.*, 2020). However, the presence of different concentrations of inulin did not cause any negative influence on the β -carotene content during the incubation period. Therefore, the pH values play an important role in carotenoid content during fermentation time. Consequently, the fermentation process could be standardised by monitoring pH to help maintain the β -carotene content. However, during storage period, the comparison between the different fermented juices with control juices showed that all fermented samples presented higher β -carotene stability than the control juices. However, after 14 days of the storage period, the values in all fermented juices did not show any differences with respect to the control juices. The values remained at high and stable concentrations until the end of storage for all fermented samples. Therefore, using the retinol activity equivalent (RAE) as an estimate of vitamin A in the fermented drinks, 100 mL of fermented beverages provides about 55.5% of vitamin A over the Recommended Daily Allowance (RDA) for an adult. At the same time, the β -carotene is necessary for visual adaptation to darkness, maintenance of mucous membranes and skin (Chung *et al.*, 2020).

Total polyphenolic content of carrot-orange synbiotic drinks

Table 4 shows the total polyphenolic content (TPC) for the samples COJ; COJ0%; COJ1% and COJ2% during 30 days of refrigerated storage at 4 °C. After incubation period and during the first 7 days of storage, TPC did not show significant differences between all samples. On the contrary, at 14 and 21 days of storage, the highest values were found for the fermented juices with 2% of inulin. However, TPC was stable in all samples during the last week of storage. Hence, the fermentation and the addition of inulin did not affect negatively to TPC. These results are in agreement with the values shown in fermented sweet potato, with *L. plantarum*, where phenolic concentration did not change during fermentation (Panda & Ray, 2007). Di Cagno *et al.* (2011), did not show any differences in the total polyphenolic concentrations in smoothies after fermentation and remained constant during the first 15 days of storage. However, a higher concentration was observed in apple juices and, oppositely, in fermented cherimoya juices was reported a lower TPC after fermentation (Li *et al.*, 2018; Isas *et al.*, 2020). Therefore, the metabolism of lactic acid bacteria can

Table 4 β -carotene content (mg β -carotene/100 mL of juice), TPC (mg pyrogallol equivalent/L of juice) and AOC (mg of vitamin C equivalent/L of juice)

	Days	CJ	COJ0%	COJ1%	COJ2%
β -carotene	0	7.67 \pm 0.38 ^{aD}	7.55 \pm 0.78 ^{aB}	7.63 \pm 0.38 ^{aB}	7.68 \pm 0.38 ^{aB}
	1	7.06 \pm 0.35 ^{bC}	6.25 \pm 0.31 ^{aA}	5.97 \pm 0.30 ^{aA}	6.22 \pm 0.15 ^{aA}
	7	6.70 \pm 0.33 ^{bBC}	6.00 \pm 0.30 ^{aA}	5.90 \pm 0.30 ^{aA}	6.07 \pm 0.14 ^{aA}
	14	6.00 \pm 0.30 ^{aA}	6.04 \pm 0.30 ^{aA}	5.68 \pm 0.28 ^{aA}	5.90 \pm 0.29 ^{aA}
	21	5.82 \pm 0.29 ^{aA}	6.02 \pm 0.28 ^{aA}	5.70 \pm 0.28 ^{aA}	5.90 \pm 0.30 ^{aA}
TPC	0	42.20 \pm 2.11 ^{aA}	42.12 \pm 2.10 ^{aBC}	44.72 \pm 2.23 ^{aC}	42.90 \pm 2.14 ^{aBC}
	1	41.70 \pm 2.08 ^{aA}	45.95 \pm 2.29 ^{aC}	43.39 \pm 2.16 ^{aC}	43.39 \pm 2.16 ^{aBC}
	7	41.60 \pm 2.08 ^{aA}	45.05 \pm 2.25 ^{aC}	45.35 \pm 2.27 ^{aC}	43.99 \pm 2.20 ^{aBC}
	14	39.25 \pm 1.00 ^{aA}	41.79 \pm 1.68 ^{bBC}	42.87 \pm 1.75 ^{bBC}	44.98 \pm 0.53 ^{cBC}
	21	39.40 \pm 1.41 ^{aA}	39.38 \pm 1.58 ^{aB}	40.78 \pm 0.79 ^{abAB}	42.42 \pm 0.63 ^{bB}
AOC	0	300.3 \pm 15.01 ^{aD}	300.4 \pm 15.02 ^{aC}	300.7 \pm 15.03 ^{aC}	289.5 \pm 14.50 ^{aB}
	1	281.2 \pm 14.06 ^{aCD}	279.1 \pm 13.95 ^{aBC}	262.3 \pm 13.11 ^{aB}	267.2 \pm 13.36 ^{aAB}
	7	271.3 \pm 13.57 ^{bBC}	260.4 \pm 13.02 ^{bAB}	233.7 \pm 11.69 ^{aA}	271.3 \pm 13.57 ^{bAB}
	14	250.1 \pm 12.50 ^{aBC}	246.2 \pm 12.31 ^{aA}	243.5 \pm 12.17 ^{aAB}	260.5 \pm 13.02 ^{aA}
	21	262.7 \pm 13.13 ^{aA}	264.6 \pm 13.23 ^{aAB}	257.5 \pm 12.88 ^{aAB}	259.5 \pm 13.01 ^{aA}

Values are expressed as mean \pm SD ($n = 3$). ^{a,b,c}Different lowercase superscript letters in the same row denote significant differences between samples ($P < 0.05$). ^{A,B,C}Different superscript capital letters in the same column denote significant differences during storage period ($P < 0.05$).

AOC, antioxidant capacity; CJ, control juice; COJ0%, carrot-orange juice without inulin; COJ1%, carrot-orange juice with 1% inulin; COJ2%, carrot-orange juice with 2% inulin; TPC, total polyphenolic compounds.

increase TPC by metabolising phenolic compounds into simpler structures. On the contrary, the pH of the plant matrix and its decrease during fermentation can affect the stability of TPCs. Therefore, it is important to carry out the study for each matrix and probiotic involved in the development of the vegetable drink.

Antioxidant activity of carrot-orange synbiotic drinks

Table 4 shows the antioxidant capacity for the samples COJ; COJ0%; COJ1% and COJ2% during 30 days of refrigerated storage at 4 °C. The ABTS method is usually applied for the determination of the lipophilic and hydrophilic antioxidant activities in vegetable materials. This method is highly sensible and stable and is based on the capacity of antioxidants for quenching the cation radical ABTS^{•+} (Arnao *et al.*, 2001; Contreras-Calderón *et al.*, 2011). The antioxidant activity values after 24 h of incubation at 37 °C did not show any significant differences from the initial values (*ca.* 300 mg vitamin C equivalent/L) and to the control juices, with a similar trend during the 21 days of storage. However, a decrease in antioxidant capacity was observed throughout storage. Thus, fermentation with LP did not significantly improve the ABTS scavenging activity of fermented juices compared with that of nonfermented ones.

A Pearson correlation between the antioxidant capacity measured by ABTS and the polyphenolic compound content was very low ($r < 0.3$) for all samples. This is in agreement with other studies with

smoothies, fruits and buckwheat extracts, where a positive correlation between the total polyphenolic compounds and the antioxidant capacity was not found (Sun, 2005; Ikram *et al.*, 2009; Di Cagno *et al.*, 2011). However, the antioxidant capacity and β -carotene showed a higher positive correlation in all samples during storage: COJ; COJ0%; COJ1% and COJ2% ($r = 0.89$; $r = 0.85$; $r = 0.84$ and $r = 0.92$, respectively). Previous studies also showed the AOC positive correlation with β -carotene and with other antioxidant compounds such as ascorbic acid (Ikram *et al.*, 2009).

Conclusions

The results indicated that the probiotic carrot-orange vegan beverages were good consumer choices due to good acceptance and high stability of the functional compounds together with high probiotic viability compared to the control beverages. At the same time, the inulin in the synbiotic juices improved the survival of *L. plantarum* during the storage period. The antioxidant capacity and β -carotene contents showed a high correlation in all samples. According to all acceptability scores and purchase intention, synbiotic vegan juices with 2% inulin were the most preferred by consumers. The results of penalty analysis showed the undesirable sensory properties in the different samples, with the control-unfermented juices being the samples with a clear need for sensory improvement with respect to the fermented juices. Therefore, the pleasant or unpleasant attribute perception of this type of

beverages is an important issue for the development and/or reformulation of fermented vegetable drinks. Therefore, the results are useful for the improvement of vegetable-based fermented drinks according to consumer demands.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical approval

Ethics approval was not required for this research.

Author contributions

Estefanía Valero-Cases: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **María José Frutos:** Conceptualization (equal); supervision (equal); writing – review and editing (equal). **Francisca Pérez-Llamas:** Supervision (equal); writing – review and editing (equal).

Data availability statement

The data that support the findings of this study are available upon request from the corresponding author.

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