



# Impact of *Lactobacillus rhamnosus* fermentation and blending strategy on the biochemical and aroma profile of pomegranate juice beverages

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## ABSTRACT

This study investigated the effect of incorporating different percentages of fermented pomegranate juice on physicochemical, sensory and volatile compound properties of resulting blends, as well as their impact on consumer acceptance. *L. rhamnosus* demonstrated excellent viability, reaching up to 8.59 Log CFU/mL in 100 % fermented pomegranate juice, supporting its potential as a base for probiotic beverages. Fermentation significantly altered physicochemical properties (pH and titratable acidity) and the organic acid profile, increasing lactic acid concentration and decreasing malic acid. Volatile compound analysis revealed distinct aroma profile in fermented versus unfermented juices, with fermentation generating new volatile compounds that increased aroma complexity. Blending fermented and unfermented juice modified the volatile profile, offering a strategy to optimize aroma characteristics. Sensory analysis demonstrated that fermentation and blending influence the sensory attributes of pomegranate juice. While 100 % fermented juice had less acceptable sensory characteristics, blending allowed for sensory profile adjustments and improved overall acceptance. A blending containing of 60 % fermented juice emerged as the most promising ratio, balancing favorable sensory characteristics and probiotic viability. This research highlights fermentation and blending as effective strategies to modulate both the sensory and functional properties of pomegranate juice.

## 1. Introduction

Pomegranate juice has gained significant attention in recent years due to its important nutritional values and health promoting properties, largely attributed to its rich content of polyphenols and other bioactive compounds (Akyidiz et al., 2020). The *Mollar de Elche* pomegranate cultivar is the most important Spanish pomegranate cultivar and its juices are consumed both fresh and processed. Beyond its inherent nutritional value, consumer demand is shifting towards foods with additional health benefits, such as those associated to probiotics. This trend is exacerbated by the growing consumption of plant-based foods, driven by health concerns (lactose intolerance, cholesterol, milk protein allergies), ideological beliefs, and the rise of vegetarianism and veganism (Isas et al., 2025). In response to these changing consumer needs, the development of plant-based fermented probiotic beverages has emerged as a promising alternative to fulfill current market demands.

A crucial aspect of creating probiotic plant-based drinks is the careful selection of the food matrix to ensure probiotic viability. The chosen

matrix should favor probiotic survival, maintaining a colony-forming unit (CFU) count of 6–7 Log per gram or milliliter of food at the time of consumption (Valero-Cases et al., 2020). This often involves the use of lactic acid bacteria, known for their probiotic potential and ability to produce antimicrobial compounds relevant to food safety (Tariq et al., 2024). Pomegranate juice has shown great potential in this regard, as its fermentation has been found to enhance the bioavailability of the phenolic compounds, suggesting their potential prebiotic effect that benefits lactic acid bacteria (Valero-Cases et al., 2017a). This indicates that pomegranate juice can serve as an ideal matrix for developing probiotic plant-based beverages. However, the success of functional foods in the market is dependent upon not only on their functional properties but also on their physicochemical and sensory characteristics, which significantly influence consumer appeal (Salmeron et al., 2009). Flavour is a key factor in food quality assessment due to its direct influence on consumer choice and approval (Salman et al., 2025). An et al. (2022), highlighted that the aroma quality of fermented beverages can be altered by the volatile compounds produced during fermentation, reinforcing the need to know these compounds for flavor improvement.

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In this regard, the strategy of blending pomegranate juice with fermented juice presents a novel approach to modulate the sensory profile of the final product, allowing precise control of the balance between sweetness, acidity, and other key sensory attributes.

The central hypothesis of this study is that the strategic implementation of pomegranate juice blending with different concentrations of fermented juice enables the targeted modulation of the final product's sensory and physicochemical profile, thereby improving consumer acceptability and enhancing its probiotic activity. This research stands out for its novelty in investigating the combined effects of *Lactobacillus rhamnosus* fermentation and blending on the sensory properties, probiotic viability, and volatile compound profile of pomegranate juice. Specifically, this study aims to: Evaluate the impact of *L. rhamnosus* fermentation and blending on the physicochemical and sensory properties of pomegranate juice, analyze the volatile compound profile of fermented pomegranate juice and establish correlations with its sensory attributes and determine the viability of *L. rhamnosus* in fermented pomegranate juice. The findings from this study are anticipated to provide valuable insights for the food and beverage industry, contributing to the development of innovative fermented pomegranate juice products with optimized sensory and functional properties.

## 2. Material and methods

### 2.1. Bacterial strain and culture preparation

*L. rhamnosus* HN001 obtained from Larbus S.A. (Madrid, Spain). The lyophilized probiotic strain was resuspended in 10 mL of the Man Rogosa Sharpe (MRS) broth (Oxoid; Madrid, Spain) at 37 °C for 24 h to prepare the pre-inoculum. Subsequently, 1 mL of the pre-inoculum was transferred to 100 mL of MRS broth and incubated at 37 °C for 24 h to obtain an initial biomass about 8 Log Colony Forming Units per mL (CFU/mL). Following incubation, the bacterial cells were harvested by centrifugation at 2000×g for 10 min at 4 °C. The resulting cell pellet was washed with sterile phosphate-buffered saline (PBS) and then resuspended in PBS to achieve a concentrated cell suspension of approximately 9 Log CFU/mL. This concentrated cell suspension was stored at -80 °C with glycerol until further use in the fermentation experiments.

### 2.2. Fermentation of probiotic pomegranate juice

Pomegranate juice cv. *Mollar de Elche* was provided by Probeltebio (Murcia, Spain) in 4.5 Kg aseptic bags. The juice was accompanied by an analytical certificate confirming its compliance with microbiological standards for *Salmonella* sp., *Escherichia coli*, total plate count, yeast, and mold.

For fermentation, pomegranate juice was transferred to sterile borosilicate glass bottles with polypropylene screw caps (1000 mL) and inoculated with LR at an initial concentration of 6 Log CFU/mL. The inoculum was prepared as previously described. The inoculated juice was then incubated at 37 °C for 24 h (Valero-Cases et al., 2023). After 24-h of fermentation period, four different pomegranate juice (PJ) formulations were prepared. These were obtained by blending unfermented pomegranate juices (CPJ) and fermented pomegranate juices (FPJ) in the following volumetric ratios: 100:0 (control, unfermented PJ "CPJ") served as the unfermented control; 80:20 (fermented PJ 20 % "FPJ20"); 40:60 (fermented PJ 60 % "FPJ60") and 0:100 (fermented PJ 100 % "FPJ100"). All juice formulations were subsequently stored at 4 °C until further use.

### 2.3. Viability of *L. rhamnosus* after fermentation time

The *L. rhamnosus* viability after 24 of fermentation time in pomegranate juice was measured by plate count on MRS agar. Serial dilutions were prepared in 0.1 % peptone water using 10 mL of each juice sample. These dilutions were then spread plate onto MRS agar and incubated at

37 °C for 48 h. After the incubation period, the viability of *L. rhamnosus* was calculated and the results were expressed as Log CFU/mL.

### 2.4. Chromatographic identification and quantification of sugars and organic acids

The identification and quantification of sugars, and organic acids in probiotic pomegranate juices were determined simultaneously by High Performance Liquid Chromatography (HPLC), as describe by Valero-Cases and Frutos (2017b). The samples were diluted 1:10 (v/v) with ultrapure water and stirred for 30 min. Then, the samples were centrifuged (10000×g for 10 min at 4 °C) and the supernatants were filtered (0.45 µm, Millipore, Spain). The determination was made using a Hewlett Packard HP 1100 liquid chromatograph equipped with a column Supelcogel C-610H (30 cm × 7.8 mm) (Supelco) (Sigma; Madrid, Spain) and a guard column Supelco guard C-610H (5 cm × 4.6 mm) (Supelco) (Sigma; Madrid, Spain). A refractive index detector (RID G1362A) was used for the analysis of sugars and inulin. Acids were monitored at 210 nm with a visible-ultraviolet (vis-UV) diode array detector (DAD G1315A). The mobile phase was orthophosphoric acid at 0.1 g 100 mL<sup>-1</sup> and the injection volume was 20 µL with a flow rate of 0.5 mL min<sup>-1</sup> under isocratic conditions. Quantification was performed using calibration curves prepared with standards for sugars (glucose, fructose, and sucrose), inulin and organic acids (malic, lactic and citric and acids) (Sigma; Madrid, Spain). Results were expressed as g/L.

### 2.5. Physicochemical analysis

Total acidity (TA) and pH were measured simultaneously using an automatic titrator (TritoMatic®, Crison) calibrated with pH 4.01 and 7.00 buffer solutions at 25 °C. TA was determined by titration with a 0.1 N NaOH solution using a mixture of 1 g of juice and 9 mL ultrapure water. The results were expressed as a percent lactic acid. Soluble solids content was determined using a digital refractometer (Hanna®, model HI96812) on scale of 0–75 % and expressed as °Brix.

The CIEL\*a\*b\* color parameters were determined using a colorimeter (Minolta®, model CR300). The equipment was calibrated with the white plate provided by Minolta. The L\* parameter represent clarity, ranging from 0 (black) to 100 (white). The a\* values represent greenness (-) to redness (+) tones, and the b\* values represent blueness (-) to yellowness (+) of each of the samples.

### 2.6. Chromatographic analysis of volatile compounds by GC-MS

Volatile compounds were extracted using the protocol by (Clemente-Villalba et al., 2020), with slight modifications. Volatile compounds of pomegranate juices were extracted using the headspace solid-phase micro-extraction method (HS-SPME). Briefly, 15 ml of each sample were mixed with 1.5 g of NaCl and 2 µL of internal standard (benzyl acetate), placed in 50 mL vials with polypropylene caps and PTFE/silicone septa, and constantly stirred. Then, a 50/30 p.m. DVB/CAR/PDMS fiber (length of 2 cm) was exposed to the sample headspace for 50 min at 40 °C. Volatile extractions were run in triplicate.

The separation and identification of the volatile compounds were performed using the column and GC-MS conditions previously described (Lipan et al., 2020). The equipment used consisted of a gas chromatogram Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) coupled with a Shimadzu mass spectrometer detector (MS) QP-5050A and with a DBWax column (30 m length × 0.25 mm internal diameter × 0.5 µm thickness, J&W Scientific, Folsom, California, USA). Finally, a gas chromatograph, Shimadzu GC-2010, with a flame ionization detector (FID) was used for the quantification of the volatile composition of samples, based on the use of 2 µL of internal standard, benzyl acetate. The extraction experiments and volatile studies were run in triplicate. The identification of the volatile compounds was performed using three methods: (a) retention indices, (b) retention times of standards, and (c)

mass spectra (authentic chemicals and NIST69 spectral library collection) (NIST, 2020).

## 2.7. Sensory analysis

Sensory analysis of different probiotic pomegranate juices was performed as described previously Valero-Cases and Frutos (2023) with 118 untrained panelists (67 women and 51 men age range 20–67 years-old). The consumers were recruited among employees and graduates of the High Polytechnic School of the Miguel Hernández University (Orihuela, Alicante, Spain). The tests were performed in a standardized test room. During the session, 30 mL of each sample were served at 4 °C in a random order in transparent plastic glasses (with tree digit coded). To clean the mouth before and between tests, water at room temperature was served in each individual cabin. The panelist used a 9-point hedonic scale (1 = dislike very much to 9 = like very much) to answer about acceptance of color, flavor, aroma, acidity, sweetness and total acceptance. The consumer study was performed according to the principles established by the Declaration of Helsinki, and the survey was approved by the UMH research ethics committee.

## 2.8. Statistical analysis

All experiments and analysis were repeated for three times. The results were expressed as mean  $\pm$  standard deviation. The mean comparison was performed using analysis of variance (ANOVA) followed by a Tukey test to evaluate the significant differences ( $p < 0.05$ ), using SPSS v 22.0 software package (SPSS Inc., Chicago-Illinois-USA).

## 3. Results and discussions

### 3.1. Viability of *Lactobacillus rhamnosus*

The viability of *L. rhamnosus* (LR) in different blends of fermented and unfermented pomegranate juice was investigated (Table 1). As expected, LR counts correlated positively with the amount of fermented juice in the blends, demonstrating the direct relationship between fermented juice concentration and probiotic load. The concentration of probiotics in 100 % fermented juice (FPJ100), exhibited the highest probiotic viability at 8.59 Log CFU/mL. This result exceeds the growth kinetics observed in previous studies with other lactic acid bacteria. For example, strains of *Lactiplantibacillus paraplantarum* CRL2051 and *Lactiplantibacillus plantarum* CRL2030 required 48 h to reach similar counts (Isas et al., 2023). In addition, strains of *Bifidobacterium bifidum*, *L. plantarum*, *B. longum* subsp. *infantis* and *L. acidophilus* showed a growth of 7.26–7.78 Log CFU/mL in pomegranate juice in 24 h

(Valero-Cases et al., 2017b), being this growth lower than that observed with *L. rhamnosus* in the present study. These findings suggest that *L. rhamnosus* could be a more efficient option for pomegranate juice fermentation compared to other strains.

Blending fermented and unfermented juices significantly impacted LR concentrations. The blending with 60 % of fermented pomegranate juice (FPJ60) maintained a high viability of 8.07 Log CFU/mL and FPJ20 showed the lowest concentration at 6.96 Log CFU/mL. However, all blends exceeded the recommended probiotic level for consumption ( $10^6$ – $10^7$  CFU/mL), highlighting the potential of blended pomegranate juices as effective probiotic carriers (Valero-Cases et al., 2020).

Brix, pH, and total titratable acidity (TTA) are determinants of probiotic viability and sensory acceptability (Table 1). The implemented blending strategy proved effective in modulating both pH and TTA. Significant differences ( $p < 0.05$ ) in both parameters were observed across the different juices' blends. The addition of 20 % fermented juice did not significantly alter the pH compared to the unfermented control (pH 3.8), likely due to buffering capacity of the unfermented pomegranate juice. However, with increasing the proportion of fermented juice (60 % and 100 %), the pH decreased markedly (3.78 and 3.62, respectively) and TTA increased. While a pH of 3.6 is generally acceptable for many fermented beverages, its potential impact on sensory attributes, particularly acidity, should be carefully considered. The ability to adjust pH levels by combining fermented and unfermented juices allows for a balance between acidity and palatability, ensuring probiotic viability and creating a product that meets functional and sensory requirements. The direct correlation between the proportion of fermented juice and the modulation of these physicochemical parameters is essential to optimize the properties of the final product.

Fermentation-derived organic acids also contribute to increase TTA and decrease pH. Lactic, malic, and citric acid concentrations varied across different formulations (Table 1). Malic acid was identified as the predominant organic acid in all samples. Lactic acid concentrations, however, showed a significant increase ( $p < 0.05$ ) with increasing proportions of fermented juice, reaching the highest levels in FPJ100 and FPJ60 (1.73 and 1.99 g/L, respectively). The amount of lactic acid in fermented juices, is as a result of the activity of LR, and it is consistent with the malolactic fermentation, converting malic acid to lactic acid. Thus, malic acid concentrations decreased in FPJ60 and FPJ20, further supporting the occurrence of malolactic fermentation. These findings highlight the critical role of LR in lactic acid production during pomegranate juice fermentation and its subsequent impact on the final product's acidity and overall sensory profile. The interaction between these organic acids and other juice components would contribute to the complex sensory characteristics of the blended beverages. At the same time, fermentation resulted in a progressive decrease in sucrose, with

**Table 1**

Bacterial account, pH, Total acidity, TSS, colour, sugar and organic content of developed pomegranate juice with different concentration of fermented juice.

Properties	Units	Pomegranate juices samples			
		CPJ <sup>a</sup>	FPJ20	FPJ 60	FPJ100
Bacterial count	Log CFU/mL	Nd	7.01 $\pm$ 0.15c	8.07 $\pm$ 0.04b	8.59 $\pm$ 0.02a
pH		3.87 $\pm$ 0.01a	3.85 $\pm$ 0.03 a	3.78 $\pm$ 0.04b	3.62 $\pm$ 0.05c
Total acidity (TA)	% citric acid (CPJ)	0.62 $\pm$ 0.02a	0.68 $\pm$ 0.03a	0.75 $\pm$ 0.03b	0.81 $\pm$ 0.01c
	% lactic acid (FPJ)				
TSS	°Brix	15.10 $\pm$ 0.05a	15.24 $\pm$ 0.01a	15.64 $\pm$ 0.02b	15.97 $\pm$ 0.01c
L <sup>a</sup>		37.57 $\pm$ 0.08d	35.82 $\pm$ 0.13c	34.34 $\pm$ 0.28b	33.18 $\pm$ 0.13a
a <sup>a</sup>		4.20 $\pm$ 0.04d	3.79 $\pm$ 0.01 c	3.23 $\pm$ 0.01b	2.97 $\pm$ 0.09a
b <sup>a</sup>		2.53 $\pm$ 0.02d	1.85 $\pm$ 0.02c	1.15 $\pm$ 0.01b	0.83 $\pm$ 0.08a
Sucrose	(g/L)	4.63 $\pm$ 0.07d	2.39 $\pm$ 0.01c	1.33 $\pm$ 0.01b	1.11 $\pm$ 0.02a
Glucose		34.07 $\pm$ 0.14d	33.68 $\pm$ 0.01c	33.16 $\pm$ 0.05b	32.84 $\pm$ 0.01a
Fructose		41.16 $\pm$ 0.16b	40.93 $\pm$ 0.03b	39.09 $\pm$ 0.04a	39.79 $\pm$ 0.12a
Citric acid		6.03 $\pm$ 0.04a	5.94 $\pm$ 0.01a	5.92 $\pm$ 0.02a	6.02 $\pm$ 0.04a
Malic acid		10.72 $\pm$ 0.04b	10.68 $\pm$ 0.30b	10.15 $\pm$ 0.20 ab	9.81 $\pm$ 0.07a
Lactic acid		nd	1.53 $\pm$ 0.01a	1.73 $\pm$ 0.01b	1.99 $\pm$ 0.08c

<sup>a</sup> CPJ: control pomegranate juice; FPJ20: Pomegranate juice with 20 % of fermented juices; FPJ60: Pomegranate juice with 60 % of fermented juices; FPJ100: Fermented pomegranate juice. Different letters in same row indicate significant differences between the means ( $p \leq 0.05$ ).

the lowest levels in FPJ100 (1.11 g/L), confirming that *L. rhamnosus* uses this disaccharide as its main energy source. Reintroduction of unfermented juice into the blends (FPJ60 and FPJ20) increased the sucrose concentration (1.33 and 2.39 g/L, respectively) and the control juice (4.63 g/L), which is predictable given that this juice has not been fermented. Interestingly, monosaccharides showed lower fermentation compared to sucrose, suggesting a metabolic preference of *L. rhamnosus* for this disaccharide. According to the data, the levels of both carbohydrates studied decreased significantly ( $p < 0.05$ ) compared to the unfermented control sample. In previous studies, in bergamot, pomegranate, carrot and orange juice, the *Lactobacillus* strains used for fermentation used simple carbohydrates (glucose and sucrose) as carbon and energy sources for growth (Hashemi & Jafarpour, 2020; Musavi et al., 2013; Valero-Cases et al., 2023). Therefore, the metabolism of sugars by *Lactobacillus* varies significantly between strains and is influenced by factors such as fermentation time and substrate type.

### 3.2. Colour coordinates

Analysis of the CIE Lab\* colour coordinates revealed significant differences between the pomegranate juice samples, indicating that both fermentation and blending with unfermented juice significant impact the visual properties of the final product (Table 1). The CPJ juice exhibited the highest brightness ( $L = 37.57$ ), suggesting that fermentation tends to decrease the clarity or transparency of the juice. The reduction in  $L^*$  can be attributed to increased turbidity, a phenomenon commonly associated with the growth of lactic acid bacteria (Meng et al., 2022). As the percentage of fermented juice in the blends increases, lightness progressively decreases, reaching its lowest value in the FPJ100 with  $L^* = 33.18$ . The values of  $a^*$  (indicating the intensity from red to green) and  $b^*$  (representing the intensity from yellow to blue) followed a similar trend to the lightness. CPJ juice had the highest values for both parameters ( $a^* = 4.20$ ,  $b^* = 2.53$ ), resulting in greater intensity of red and yellow tones. As the fermentation decreases percentage in the blends, the values of  $a^*$  and  $b^*$  increased. This suggests that blends approach the color characteristics of the fermented control

**Table 2**

Identification and descriptors of volatile compound found in the developed pomegranate juice with different concentration of fermented juice.

Code	Volatile Compound	CF	RT	KI (EXP)	KI (LIT)	p-value	ANOVA <sup>1</sup>	Concentration ( $\mu\text{g/L juice}$ )				Descriptors
								CPJ	FPJ20	FPJ60	FPJ100	
V1	Ethanol	Alcohol	3.10	926	932	0.9878	NS	4,58	4,29	4,39	4,57	Alcoholic
V2	Isoamyl acetate	Ester	7.24	1117	1126	0.6312	NS	0,81	1,10	1,06	0,92	Sweet, fruity, banana
V3	2-Heptanone	Ketone	9.06	1176	1185	0.0181	*	0,13 b	0,54 b	0,84	2,57 a	Cheese, fruity, coconut, waxy
V4	Limonene	Terpene	9.19	1180	1189	0.2911	NS	10,99	8,32	13,60	6,13	Orange, citrus, sweet
V5	t-2-Hexenal	Aldehyde	10.10	1211	1216	0.0089	**	0,57	0,75 a	0,42	0,27 c	Fresh green leafy, fruity, vegetable
V6	g-Terpinene	Terpene	10.72	1234	1240	0.2027	NS	1,65	1,32	2,08	0,84	Citrus, lime, oily, green
V7	p-Cymene	Terpene	11.54	1263	1268	0.2157	NS	1,42	1,13	1,66	0,74	Rancid, woody, citrus, spicy
V8	2-Octanone	Ketone	12.10	1284	1284	0.0208	*	1,01 b	1,57	1,43 b	3,22 a	Dairy, waxy, cheese, woody, mushroom, yeast
V9	Methyl heptanoate	Ester	12.21	1287	1288	0.2866	NS	3,31	3,04	4,22	2,06	Sweet, fruity, green
V10	2-Heptanol	Alcohol	13.18	1323	1321	0.0147	*	0,05 b	1,00 b	1,34 b	4,14 a	Fruity, green, earthy, bitter
V11	Ethyl heptanoate	Ester	13.56	1337	1331	0.1369	NS	4,58	3,65	5,76	2,05	Fruity, pineapple, banana, spicy, oily
V12	1-Hexanol	Alcohol	14.12	1357	1357	0.0313	*	4,39	7,79 a	3,61 b	3,87 b	Green, fruity, apple-skin, oily
V13	Heptyl acetate	Ester	14.73	1379	1370	0.1420	NS	1,53	1,26	1,93	0,77	Green, fatty, spicy, citrus, soapy
V14	cis-3-Hexenol	Alcohol	15.00	1389	1390	0.0925	NS	2,76	5,16	3,18	5,29	Fresh, green, raw fruity, pungent
V15	2-Nonanone	Ketone	15.13	1394	1393	0.0013	**	0,69 b	3,38 b	8,42 a	11,15 a	Cheesy, fruity, dairy, dirty, buttery
V16	Methyl octanoate	Ester	15.18	1396	1387	0.4547	NS	2,90	2,34	4,26	3,70	Green, fruity, waxy, aldehydic, fatty
V17	Nonanal	Aldehyde	15.27	1399	1397	0.7898	NS	4,02	5,01	4,78	6,67	Waxy, fatty, citrus, floral
V18	Hexyl butyrate	Ester	15.93	1421	1414	0.1828	NS	1,40	1,11	1,65	0,67	Green, waxy, soapy, fruity
V19	1-Octen-3-ol	Alcohol	16.93	1454	1447	0.6343	NS	1,33	1,76	1,85	1,39	Mushroom, earthy, fungal, green, oily
V20	2-Ethyl-1-hexanol	Alcohol	18,04	1491	1480	0.0641	*	0,40 b	1,75	1,77	2,92 a	Fatty, sweet, fruity
V21	Decanal	Aldehyde	18,31	1500	1503	0.1165	NS	0,78	2,50	1,37	1,10	Waxy, fatty, citrus, orange
V22	Benzaldehyde	Aldehyde	18,93	1521	1520	0.0002	***	0,79 c	1,33 c	2,19 b	3,68 a	Sweet, oily, almond, cherry, nutty
V23	Linalool	Terpene	19,76	1549	1549	0.6241	NS	0,72	0,90	1,06	0,92	Citrus, orange, lemon, floral, waxy
V24	Methyl decanoate	Ester	21,13	1596	1590	0.4108	NS	1,33	0,93	1,53	0,72	Fatty, oily, fruity
V25	Terpinen-4-ol	Terpene	21,23	1599	1599	0.0064	**	2,16 c	4,94	8,05	9,87 a	Woody, earthy, clove, spicy
V26	Furfuryl alcohol	Alcohol	23,00	1661	1665	0.9752	NS	0,93	1,01	1,03	1,11	Burnt, sweet, caramellic
V27	$\alpha$ -Terpineol	Terpene	23,97	1695	1692	0.4398	NS	3,87	4,57	4,60	5,00	Citrus, woody, lemon, lime
V28	2-Tridecanone	Ketone	27,22	1832	1836	0.0138	*	0,27 b	1,09	1,73 a	2,23 a	Fatty, earthy, dairy, cheesy
V29	Ethyl dodecanoate	Ester	28,21	1857	1850	0.6583	NS	2,35	1,57	1,99	2,12	Waxy, soapy, floral, dairy, fruity
V30	Hexanoic acid	Acid	29,59	1893	1885	0.0069	**	1,33 b	1,83 b	2,51 b	5,40 a	Cheesy, fruity, fatty, goaty
V31	Phenylethyl alcohol	Alcohol	29,87	1900	1904	0.3857	NS	4,05	2,20	3,15	5,85	Floral, sweet, rose
V32	1-Dodecanol	Alcohol	31,47	1972	1975	0.3222	NS	0,64	0,93	1,37	1,33	Soapy, waxy, earthy, fatty
V33	Eugenol	Acid	36,48	2121	2158	0.0046	**	0,05 b	0,47 b	1,16 b	2,38 a	Sweet, spicy, clove, woody
V34	Nonanoic acid	Acid	37,86	2174	2174	0.5002	NS	1,45	1,72	1,59	2,50	Fatty, cheese, dairy products, waxy

CF, Chemical Family; RT, Retention Time; KI, Kovats Index; <sup>1</sup>NS: not significant at  $p > 0.05$ ; \*, \*\*, and \*\*\*, significant at  $p < 0.05$ , 0.01 and 0.001, respectively; <sup>1</sup>Values (mean of 3 replications) followed by the same letter, within the same row and factor, were not significantly different ( $p > 0.05$ ). Tukey's least significant difference test.

juice, leading to an increase in the intensity of red and yellow tones. These findings indicate that fermentation-induced modifications, potentially including pH changes, contribute to the observed alterations in juice color and brightness (Akyildiz et al., 2020). The observed correlation between color changes and the proportion of fermented juice underlines the importance of this process in the visual properties of the final product. The ability to modulate these visual characteristics by blending fermented and unfermented juices offers a valuable tool for optimizing the appearance of the final product and, consequently, its consumer acceptance.

### 3.3. Volatile profile of fermented pomegranate juice: impact of fermentation on aroma compounds

A comprehensive analysis of volatile compounds was performed in unfermented pomegranate juices, fermented, and different blends of both pomegranate juices to elucidate the impact of fermentation on the aroma profile. Thirty-four volatile compounds were identified and quantified, representing five chemical families: alcohols ( $n = 9$ ), esters ( $n = 8$ ), terpenes ( $n = 6$ ), ketones ( $n = 4$ ), aldehydes ( $n = 4$ ), and acids ( $n = 3$ ) (Table 2). Significant quantitative differences in volatile composition were observed across samples, revealing the influence of fermentation on the development of aroma-active compounds (Table 2).

Limonene, terpinen-4-ol, 2-nonanone, and nonanal were identified as the main volatile components in all samples (Table 2), consistent with previous reports on pomegranate juice volatiles (Di Cagno et al., 2017; Mantzourani et al., 2020). Limonene, a dominant terpene often associated with citrus notes, did not show significant variation among samples. This aligns with previous findings where limonene was also found as a major and stable component (Andreu-Sevilla et al., 2013).

Terpinen-4-ol, the second most abundant compound, exhibited a significant increase in concentration with increasing fermentation percentage (from  $2.16 \mu\text{g L}^{-1}$  in the control to  $9.87 \mu\text{g L}^{-1}$  in the 100 % fermented juice). This observation suggests a direct correlation between fermentation and terpinen-4-ol production. While previous research has outlined various formation pathways for the formation of terpinen-4-ol and  $\alpha$ -terpineol from limonene and linalool, involving heat treatment, acidic conditions, and biological processes (Pérez-López et al., 2006), our results indicate a possible biotransformation of limonene by *L. rhamnosus* or a shift in equilibrium caused by the acidic environment produced during fermentation. Further investigation is required to determine the specific mechanism responsible for the increased terpinen-4-ol concentration.

Ketones, exemplified by 2-nonanone, also demonstrated a significant increase with higher fermentation percentages (ranging from  $0.69 \mu\text{g L}^{-1}$  in the control to  $11.15 \mu\text{g L}^{-1}$  in the 100 % fermented sample). This trend was also presented by other ketones, including 2-heptanone, 2-octanone, and 2-tridecanone. Ketones are often associated with fruity, dairy and buttery flavors, which are frequently observed in dairy or dairy fermented products. However, consumer acceptance of these flavour notes can be variable and product-specific (Di Cagno et al., 2017). The observed increase in ketones during fermentation may be attributed to microbial activity, potentially through the degradation of fatty acids.

Beyond these major compounds, other volatiles showed significant differences across samples. Benzaldehyde, 2-heptanol, 2-ethyl-1-hexanol, hexanoic acid and eugenol increased in fermented samples. Notably, 2-ethyl-1-hexanol, associated with floral attributes, was directly proportional to the fermented juice concentration, reaching  $2.92 \mu\text{g L}^{-1}$  in the 100 % fermented juice compared to  $0.40 \mu\text{g L}^{-1}$  in the control. Benzaldehyde, known for its bitter almond, nutty, and cherry-like notes, also increased with fermentation (from 0.79 to  $3.68 \mu\text{g L}^{-1}$ ). While benzaldehyde can contribute desirable flavor complexity, it is important to acknowledge that high concentrations of aldehydes, particularly in conjunction with high acidity, have been negatively correlated with sensory acceptance in pomegranate juice (Mantzourani

et al., 2020). Therefore, careful monitoring and control of benzaldehyde levels during fermentation are crucial for optimizing product quality.

Eugenol and hexanoic acid also showed positive and significant correlations with the increase in the percentage of fermented juice. Eugenol (associated with spicy, clove-like aromas) increased from  $0.05 \mu\text{g L}^{-1}$  in the control to  $2.38 \mu\text{g L}^{-1}$  in FPJ100, and hexanoic acid (with acidic and fatty aromas) from  $1.33 \mu\text{g L}^{-1}$  in the control to  $5.40 \mu\text{g L}^{-1}$  in FPJ100.

The sensory impact of hexanoic acid is complex. At low concentrations, it can contribute to desirable flavour nuances, adding complexity and a subtle cheesy or fatty note. However, at higher concentrations, as observed in the 100 % fermented juice, it can contribute to off-flavours described as sour, sweaty, or goaty, having a potential negative impact on the overall sensory acceptance. The sensory descriptors listed (sour, fatty, sweaty, cheesy) highlight the importance of controlling hexanoic acid levels, as these are not typically desirable attributes in a fruit juice beverage. It is in this context that the present study becomes particularly relevant. By exploring the fermentation of pomegranate juice using *L. rhamnosus* and, crucially, by combining different proportions of fermented and unfermented juice in blends, this research offers the possibility of modulating the concentration of both desirable volatile compounds (such as eugenol) and undesirable ones (such as hexanoic acid). This modulation could be of utmost importance for optimizing the sensory profile of the final product, without compromising beneficial probiotic activity.

### 3.4. Impact of fermentation and blending on sensory perception

Consumer study results are presented in Fig. 1, showing statistically significant differences ( $p < 0.05$ ) across eight sensory attributes of the different pomegranate juice blends.

The consumer study revealed that FPJ100, the fully fermented juice, received the lowest colour score, with a significant difference compared to all other samples. This suggests that the fermentation process significantly alters the colour of the juice, making it less attractive to consumers. This finding, related to the colour measurement data, results in a lighter and more intensely coloured juice, which may appear less fresh compared to unfermented or partially fermented juices. The decrease in consumer preference for the colour of FPJ100 could be attributed to various fermentation-induced changes, such as pigment oxidation, browning reactions, or the formation of new chromophore compounds. These changes could result in a less desirable appearance, affecting consumer perception. Fortunately, blending strategy mitigated this negative

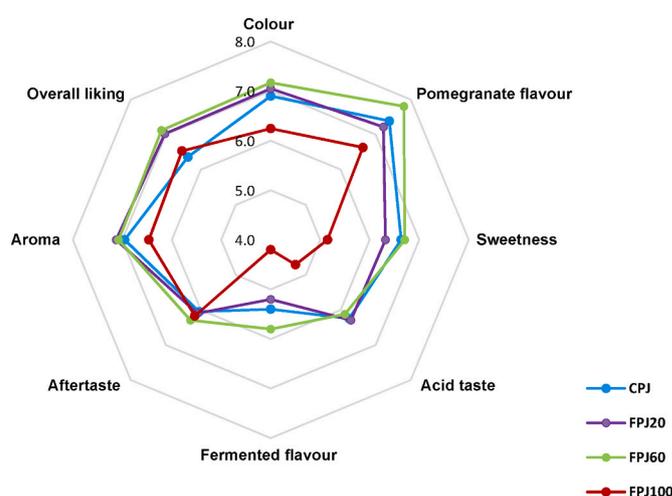


Fig. 1. Results of sensory analysis of pomegranate juices with different concentration of fermented juice. CPJ: control pomegranate juice; FPJ20: Pomegranate juice with 20 % of fermented juices; FPJ60: Pomegranate juice with 60 % of fermented juices; FPJ100: Fermented pomegranate juice.

impact. The blended juices (FPJ60 and FPJ20) received higher colour scores in sensory analysis, indicating that the addition of unfermented juice helped maintain a more visually appealing colour. This direct assessment of the proportion of unfermented juice and the improvement in colour perception was corroborated by instrumental measurements, where the blended juices showed higher  $L^*$ ,  $a^*$  and  $b^*$  values than to FPJ100, approaching the preferred colour of the unfermented control juice. Therefore, blending fermented and unfermented juice offers a promising strategy to modulate colour and optimize consumer visual acceptance.

Flavour scores also varied significantly among samples. FPJ60 received the highest score, significantly different from all other samples, while FPJ100 received the lowest. These results indicate that blend with 60 % fermented juice (FPJ60) can improve overall flavour compared to both fully fermented juice (FPJ100) and unfermented juice (CPJ). This direct trade-off between blend ratio and flavor preference is a key finding.

Regarding sweetness, all samples, except FPJ100, were well-perceived, with ratings above 7. However, a statistically significant difference ( $p < 0.05$ ) was observed in sweetness perception. FPJ60 score significantly higher on sweetness than FPJ100, suggesting that the amount of unfermented juice in the blend significantly influences perceived sweetness, being the 60 % fermented juice blend being perceived as the preferred regarding sweetness. The inverse trade-off between fermentation percentage and perceived sweetness is evident, as lactic acid bacteria (LAB) metabolize sugars (Table 1), which reduces sweetness. The preference for sweetness in the 60 % fermented juice blend highlights the importance of balancing sugar consumption by LAB with the residual sweetness of the unfermented juice to maximize acceptability.

Concerning the acidity attribute, no significant differences were observed between the control and samples with 20 % (FPJ20) and 60 % (FPJ60) fermented juices. This indicates that the blending strategy was successful in preventing a perceptible increase in acidity compared to the unfermented juice, which is a positive aspect in the development of probiotic beverages. However, the 100 % fermented juice (FPJ100) showed the lowest acceptance for this attribute. This correlates with the higher sugar consumption and organic acid production by the *L. rhamnosus* in FPJ100, as reflected in the physicochemical results (Table 1). Lactic acid bacteria (LAB) metabolize mainly glucose and fructose, generating organic acids and aromatic compounds that modify the flavour profile of foods (Hashemi & Jafarpour, 2020). Interestingly, FPJ60 was not perceived as excessively acidic, suggesting that other compounds (such as sweetness or the balance of other volatiles) could be masking or attenuating the acidity perception contributing to its high flavor score.

Aftertaste ratings did not differ significantly ( $p > 0.05$ ) between samples. This suggests that neither the fermentation process nor the addition of unfermented juice had a significant impact on the perceived aftertaste of pomegranate juice. This lack of significant evaluation could be indicated that aftertaste is a sensory attribute that is less sensitive to changes induced by fermentation and blending in this specific matrix.

The results indicate that blending the juices does not significantly alter the acidity perception when compared to the unfermented juice, which can be considered a positive aspect in the development of probiotic beverages. In contrast, the PJF100 % juice showed a negative impact on aroma compared to the rest of the samples. This difference suggests that 100 % fermented juices with *L. rhamnosus* may not generate an optimal aroma profile for pomegranate juice. These sensory results are highly consistent with the previously discussed volatile compound analysis. For example, the lower flavour, aroma, and overall acceptance scores for FPJ100 can be attributed to higher concentrations of volatile compounds such as hexanoic acid, which, at elevated concentrations (as observed in FPJ100), can contribute to off-flavours described as sour, sweaty, or goatish. This direct correlation between hexanoic acid concentration and low sensory acceptability in FPJ100 is

crucial for understanding consumer preferences. In contrast, modulating these compounds through blending offers an advantage.

In terms of overall acceptability, the blended juices, FPJ20 (7.122) and FPJ60 (7.024), obtained the highest scores with no significant differences between them. This suggests that blending pomegranate juice with different concentrations of fermented juice improves overall acceptability compared to conventional pomegranate juice. Therefore, partial blending of pomegranate juice with fermented juice offers an optimal balance between potential probiotic benefits and desirable sensory characteristics. This strategy is a promising approach to modulate sensory profiles, especially acidity, sweetness and overall flavor, thus improving overall consumer acceptability and commercial viability of the product.

### 3.5. Penalty analysis and its correlation with sensory and volatile compound data in fermented pomegranate juice

The penalty analysis revealed distinct areas for improvement across the different pomegranate juice samples, highlighting the potential of blending fermented and unfermented juices to modulate the sensory profile and enhance overall acceptability. FPJ20 and FPJ60 achieved higher overall acceptability scores than FPJ100, suggesting that blending can effectively balance sensory attributes (Fig. 2). Sweet taste emerged as a key attribute requiring careful control during both fermentation and blending. Both CPJ and FPJ20 were flagged for insufficient sweet taste intensity, indicating that fermentation can influence sweetness perception. Similarly, the fermented flavour and post-flavour were perceived as too low in FPJ100, correlating with lower ratings for these attributes in the overall sensory profile. This suggests that complete fermentation, as implemented in FPJ100, may not be sufficiently developing these characteristic flavours of fermentation to a desirable level, or that conversely, other negative attributes mask their perception. The acid taste intensity and the overall pomegranate flavor were also crucial to the acceptability of the fermented pomegranate juice. FPJ60 was identified as needing improvement in both attributes, suggesting that the fermentation and blending process requires optimization to achieve a balanced flavour profile. Specifically, while consumers indicated a need for increased "acid taste" in FPJ60, volatile compound analysis revealed high concentrations of citrus-associated volatiles, which typically contribute to perceived acidity. This apparent discrepancy is likely due to the complex interaction and synergy of volatile compounds and their sensory impact. FPJ60 also contained high levels of compounds like 2-nonanone, nonanal, and terpinen-4-ol, associated with fermented flavours. These dominant flavor notes, and potentially less desirable in a fruit drink, could be masked or overwhelmed the perception of the underlying acidity. Essentially, while the chemical components contributing to acidity were present, they did not have a positive or prominent impact on the overall flavour profile, leading consumers to perceive a lack of "acid taste." This observation underscores the importance of considering sensory perception as a whole, rather than focusing solely on individual chemical components. The interaction between different volatile compounds can produce complex masking or synergistic effects, which ultimately influencing the overall sensory perception. In the case of FPJ60, high levels of rancid and fermented flavour compounds likely altered the perceived balance, resulting in the perception of insufficient acidity despite the presence of acid-related volatiles. These penalty analysis results correlate reasonably well with the previously presented overall sensory analysis. They demonstrate that blending represents a promising approach to refining the sensory profile of fermented pomegranate juice, but careful attention must be paid to the complex interactions of volatile compounds to achieve optimal balance and consumer acceptance. The positive correlation between the blends (FPJ20 and FPJ60) and the higher overall acceptability (with higher scores) compared to FPJ100 demonstrate the effectiveness of this strategy. Ultimately, the partial blending of pomegranate juice with fermented juice offers a desirable

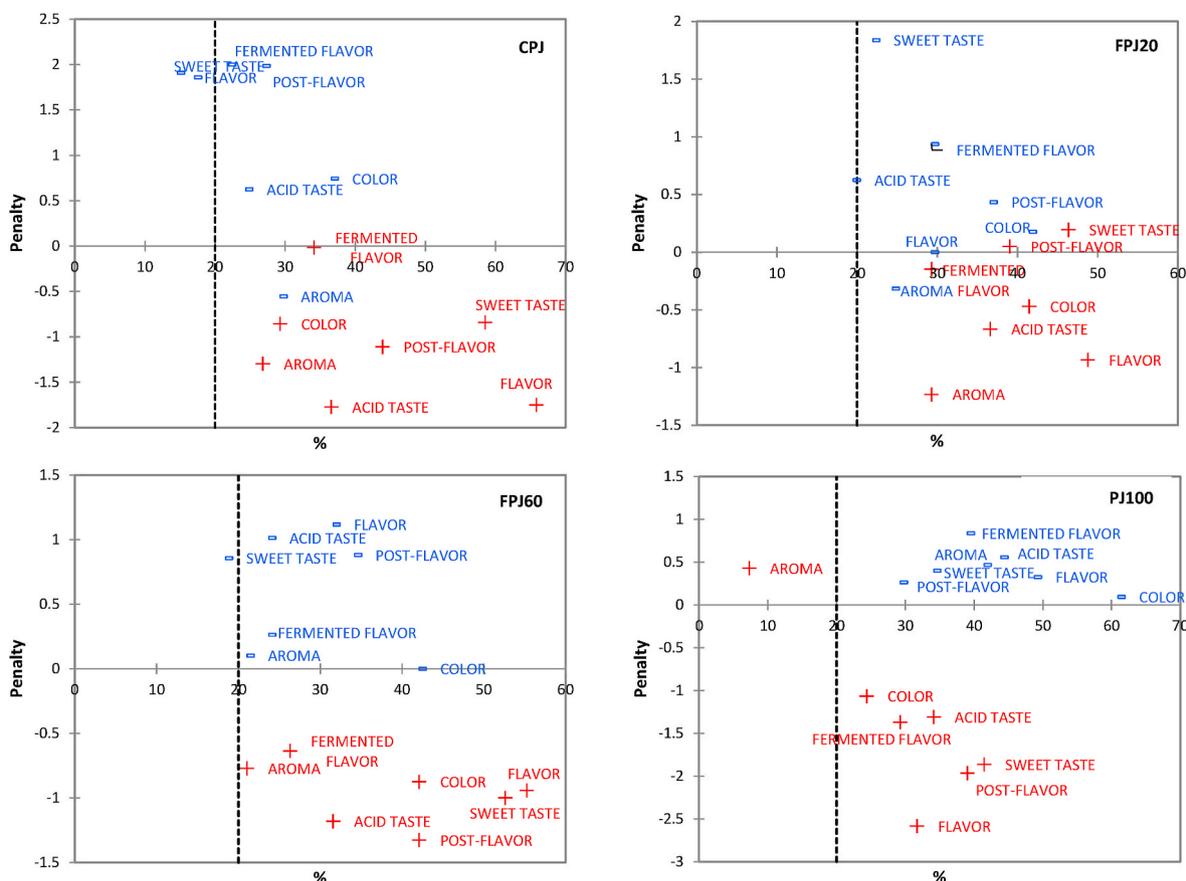


Fig. 2. Penalty analysis of intensities of attributes evaluated by consumers. Sample code indicated at the top right of each figure: CPJ: control pomegranate juice; FPJ20: Pomegranate juice with 20 % of fermented juices; FPJ60: Pomegranate juice with 60 % of fermented juices; FPJ100: Fermented pomegranate juice. Too low intensity is indicated by the symbol “-”, and too high intensity is indicated by the symbol “+”.

balance between probiotic benefits and sensory characteristics.

#### 4. Conclusions

This study successfully demonstrates the great potential of *L. rhamnosus* (LR) as a viable probiotic culture for the fermentation of pomegranate juice. The strategy of blending fermented pomegranate juice with unfermented juice proved to be a powerful tool for modulating the physicochemical and sensory characteristics of the final product without compromising the desired probiotic load. Furthermore, fermentation and blending significantly influenced the volatile compound profile, altering the beverages' aromatic composition. Compounds such as terpinen-4-ol and various ketones showed a direct correlation with the degree of fermentation, contributing to aroma complexity. Sensory and penalty analysis revealed that blends with 20 % and particularly 60 % fermented juice (FPJ20 and FPJ60) achieved an optimal balance between probiotic functionality and sensory acceptability, obtaining the highest scores in the consumer study. This underscores the importance of modulating sensory attributes, such as sweetness, acidity, and aroma, through strategic blending of juices. Ultimately, this work not only validates pomegranate juice as an excellent matrix for the development of probiotic beverages with *L. rhamnosus*, but also establishes an innovative approach to optimize its sensory characteristics. Blending fermented and unfermented juices represents a promising strategy for the food industry, enabling the development of functional products with health benefits and high consumer acceptance.

#### CRedit authorship contribution statement

Estefanía Valero-Cases: Writing – review & editing, Writing –

original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Jesús Clemente-Villalba:** Writing – original draft, Formal analysis. **María José Frutos:** Writing – review & editing, Validation.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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