ORANGE PEEL FERMENTATION USING *LACTOBACILLUS PLANTARUM*: MICROBIOLOGICAL ANALYSIS AND PHYSICO-CHEMICAL CHARACTERIZATION

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KEYWORDS

By-products, lactic-acid fermentation, blanching, texture, volatile compounds, *msayer*

ABSTRACT

To find an innovative use for orange peels discarded in the orange juice making process, a fermentative process was assessed using a *Lactobacillus plantarum* strain. Blanched or rinsed peels were submerged in a 5% NaCl-3% inoculated sucrose brine for 10 days. Total soluble solids, pH, sugars, and total aerobic and anaerobic counts were determined in the brines to characterize the process. The final products were characterized by instrumental texture, color, and volatile composition. The blanching pretreatment had a significative effect on the whole process and the final product characteristics. Anaerobic bacteria total counts were significantly higher in the blanched samples during the whole fermentation, and pH decreased significantly slower in these samples than in the rinsed ones. Rinsed samples were characterized by higher aerobic total counts, higher sucrose consumption, and higher glucose, fructose and polyalcohols production. Texture was softer in the pretreated samples, probably due to the blanching process rather than the fermentation. The volatile composition was quite similar between samples, although it was different from the one of the raw orange peels due to a significant decrease on various volatile compounds.

1. INTRODUCTION

Global orange production (*Citrus sinensis*) was over 98 million t in the 2020 / 2021 season, and orange juice production was estimated over 1.6 million t (USDA, 2021) meaning the generation of an enormous amount of by-products (Jiménez-Castro *et al.*, 2020). Different studies have focused on the conversion of citrus waste into value-added products, but most of the proposed solutions continue producing new by-products, making necessary to improve citrus waste management (Bustamente *et al.*, 2019; Gavahian *et al.*, 2018; Razola-Diaz *et al.*, 2021; Ricci *et al.*, 2019; Sharma *et al.*, 2017). Fermentation has been considered an interesting technique for by-product transformation, a simple and valuable biotechnological method to maintain and/or increase the shelf life and the nutritional and sensory properties of different vegetables and fruits. Additionally, spontaneous fermentation has been shown to be useful in transforming lemons, and also by-products of orange juice making, into a new product called *msayer* (Aayah *et al.*, 2010; Bousmaha *et al.*, 2006; Deba-Rementeria *et al.*, 2021). Although research is scarce in this matter, the product obtained using orange peels from orange juice making and fermented during 40 to 90 days has been characterized using physico-chemical, microbiological, and sensory analyses. After 40 days of fermentation in a 5% NaCl and 3% sucrose (w/v) brine, the transformed orange peels were considered a new ingredient appropriate from a sensory standpoint (Deba-Rementeria *et al.,* 2021). Although the valorization of the peels was clear in this study, the fermentation process was long, and orange *msayer* made in a 5% NaCl brine was perceived as “too bitter”. To shorten the process, some authors suggested using a driven fermentation by a lactic bacterium and a yeast purified from a previous *msayer* making process, but the product was not characterized this study and only microbiological analyses were conducted (Bousmaha *et al.,* 2006).

Under favorable conditions of anaerobiosis, water activity, salt concentration, and temperature, a spontaneous lactic-acid fermentation can be adequately developed in raw plant matrices such as cucumbers, cabbage or radish (Di Cagno *et al.,* 2013). Because of the presence of a natural lactic bacterial microflora, lactic-acid fermentation occurs “spontaneously” in these products when immersed in a brine (Swain *et al*., 2014). In addition, the presence of organic acids and low pH values of lactic fermented products (pH below 4.5) make these fermentations very interesting in terms of food safety (Stoll *et al.,* 2020). However, depending on the predominant microbiota of

68 the raw fruit or vegetable, and the presence of bacteria growth inhibitors (e.g.: limonene of the
69 citrus fruit peels; Ruiz & Flotats, 2014) different microorganisms could drive the fermentation
70 process (e.g.: yeast species), and the characteristics of the final products will be different from
71 the ones coming from acid lactic fermentations. Lemon *msayer* and orange peel *msayer*
72 fermentations have been reported to be mainly driven by yeast species such as *Candida* and
73 *Saccharomycetales* (Aayah *et al.,* 2010; Deba-Rementeria *et al.,* 2021).
74 Using starters for conducting controlled fermentation processes is a common practice to increase
75 homogeneity of the process and the final products. Ricci *et al.* (2019) and Bustamante *et al.*
76 (2019) have recently reported that orange peel was an appropriate matrix for growing lactic acid
77 bacteria, but orange peels had to be pretreated before the fermentation process to reduce the
78 citrus essential oils antimicrobial properties. In general, essential oil extraction methods are
79 laborious, expensive and/or energy consuming (Razola-Díaz *et al.,* 2021), but blanching could be
80 used to decrease the presence of some potential inhibitors (e.g.: limonene). Blanching is generally
81 used before freezing and other unit operations in fruits and vegetables to diminish microbiota,
82 preserve color of green vegetables, and denaturalize some enzymes (Brown, 2010). In the
83 present study, blanching was chosen as a pretreatment to remove competitive microbiota and
84 decrease the presence of other potential inhibitors (Fernández-López *et al.,* 2004; Abadi *et al.,*
85 2018).
86 The aim of the present study was to characterize the orange peels' fermentation process when
87 driven by a commercial *Lactobacillus plantarum* strain. During the study, different physico-
88 chemical analyses were conducted to characterize the process and the final product, and lactic
89 acid bacteria growth was determined by microbiological traditional methods (plate count agar).

90 **2. MATERIALS AND METHODS**

91 **2.1. Reagents and culture media**

92 Ultrapure water (Type I, 18.2 mΩ-cm) was from an Elga Purelab Flex 3 (ELGA LabWater, UK).
93 Analytical standards of inositol, erythritol, lactitol, glucose (1000 mg L^{-1}), fructose, sucrose, all
94 analytical quality grade, were supplied by Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).
95 Sodium hydroxide solution (1 mol L^{-1}) and sodium acetate anhydrous (>99%) (HPLC mobile
96 phase) were supplied by Panreac AppliChem (Panreac AppliChem, Barcelona, Spain) and
97 Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), respectively. Peptone saline solution, Man,

Rogosa and Sharpe (MRS) agar and Nutrient Agar were from VWR, (VWR Inc, Germany). The alkane standard mixture for Gas Chromatography-Mass Spectrometry compounds identification was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

2.2. Samples

Orange peel discarded from making orange juice in Basque Culinary Center (Basque Country, Spain) restaurant were used for the present study (*Citrus sinensis,* Navel Lane late cultivar). The juice was made in a Zumex V machine (Zumex®, Valencia, Spain), which cut the oranges in halves for making juice; the obtained de-juiced halve peels were cut in 2 x 2 cm pieces and orange pulp was removed. Peels which were significantly damaged by the machine, or had irregular shapes, were discarded.

To study the effect of a blanching pretreatment on the orange *msayer* making, half of the peel samples were immersed (ratio of approximately 175 g in 1 L of water) for 10 minutes in boiling water. Although diverse time/temperature combinations have been studied in green vegetables blanching (Mnkeni *et al*., 1999), not much literature can be found about *Citrus sinensis* water blanching. Some authors have reported used a blanching process of 95 ºC during 5 min to treat lime by-products (Kuljarachanan *et al*., 2009). A 10-min process was chosen for the present process to favor a great diminish of the microbiota present in the peel and to improve fermentability by the later inoculated lactic acid bacteria (Abadi *et al*., 2018; Breidt and Fleming, 2000; Fernández-López *et al*., 2004). The other half of the orange peels were rinsed in a strainer with tap water at room temperature (~21 ºC).

The bacterial strain chosen in the preset study for inoculation was *Lactobacillus plantarum* (Harvest LB-1 culture *L. plantarum* pure culture lyophilized; CHR Hansen, Denmark), a facultative heterofermentative microorganism which has been isolated in different vegetable and fruit fermentations (Swain *et al.,* 2014; Razola-Díaz *et al.,* 2021). The commercial strain was directly inoculated as suggested by the distributor: 0.08 g L^{-1} directly into the sterilized brine (5% NaCl and 3% sucrose), without prior reactivation.

After the orange peels pretreatment (blanching or rinsing) and the brines inoculation, the fermentation process was similar for all samples. Approximately 175 g (mean = 175.3 g; SD = 0.4) of pretreated (PT) or not-pretreated peels (NPT) were weighed and placed in 500 mL glass jars (Bormioli Rocco, Italy) and full covered with the inoculated 300 mL brine (Bousmaha *et al.,*

2006). Peels were kept flooded in the brine using a glass weight. Filled jars were closed with plastic lids with an airlock (Sterikap®, UK) and placed in a dark room with controlled temperature (21 ± 2 °C) throughout experiment (10 days). Samples preparation was done in triplicate and their analyses conducted as indicated below.

2.3. Brine sampling

Brine samples were taken using sterile 10 mL disposable syringes at 0, 12, 24, 36, 48, 60, 84, 168 and 240 hours to characterize, using microbiological and physico-chemical analyses, the driven fermentation process.

2.3.1. Microbiological analyses

Brine samples were diluted in tenfold series in saline peptone solution (0.85% NaCl and 1% peptone) and plated onto different culture media to quantify the presence of different microorganisms using a traditional Plate Count Agar (PCA) method (ISO 4833-1:2013) (AENOR, 2014): Nutrient Agar for total viable colonies in aerobic conditions (37 °C for 48 h) and Man Rogosa Sharpe (MRS) agar for lactic acid bacteria (37 °C for 72 h). The colony counts were then calculated, and results were presented as log10-transformed data of the average of 3 replications.

2.3.2. Determination of pH and Total Soluble Solids (TSS)

The pH of the original brine and the brine samples was measured with an electronic pH meter Crison Basic 20 (Crison Instruments SA, Spain). The total soluble solids of the brines were measured at 20 ± 0.5 °C using a refractometer Digital Handheld Refractometer VWR® (VWR Inc, Germany), results were obtained in °Brix. Samples were analyzed in triplicate.

2.3.3. Sugars and polyalcohols determination using High Performance Liquid Chromatography-Pulsed Amperometric Detector (HPLC-PAD)

Sugars and polyalcohols determinations were conducted as previously reported (Deba-Rementeria *et al.,* 2021). Briefly, brine samples were diluted 1:50 (v/v) with ultrapure water at 60 °C, then shaken for 30 min and centrifugated at 2367 g for 10 min. The supernatant was filtered using a 25 mm, 0.45 µm nylon VWR® Syrenge Filter (VWR Inc, Germany). Twenty-five µL of each sample were injected in a Knauer HPLC system (Knauer Scientific Devices GmbH, Germany). The equipment was coupled to an amperometric detector 945 Professional Detector Vario (Metrohm AG, Herisau, Suiza) in pulsed amperometric mode. Standards for inositol, erythritol, lactitol, glucose, fructose, and sucrose, were used to identify by retention time and

quantify the presence of the different compounds using calibration curves for each of the aforementioned pure standards. Samples were analyzed in triplicate.

2.4. Orange *msayer* characterization

The original orange peels (control) and the 10-days fermented peels (orange *msayer*) were characterized using the instrumental texture, color and volatile composition analyses described below.

2.4.1. Instrumental texture: cutting force

Msayer and the original orange-peel hardness determinations were done using a Texture Analyzer Aname (Microstable system, UK). The pieces, already with a 2 × 2 cm shape, were cut with an HDP/BSK blade set probe (Warner-Bratzler with rectangular slot blade). The load cell was calibrated to 295 N and the cut was made at a speed of 1 mm s^{-1} (Deba-Rementeria *et al.,* 2021). Peak cutting force was considered as the first peak force in g. A total of 15 replications were carried out for each sample.

2.4.2. Instrumental color

The color of orange peels and *msayer* was measured with a Chroma Meter CR 400 (Konica Minolta, Inc, Japan) using the CIE *L*a*b** color space and an illuminant D65 and a 10° observer as references. Data were expressed with the *L*, a*, b** values and then, chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$], Hue angle [$H = \tan^{-1}(b^*/a^*)$], and the total color change (ΔE) of orange peels after fermentation [$\Delta E^* = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2}$], using crude orange peel values as reference (L_0^*, a_0^*, b_0^*), were calculated (Cserhalmi *et al*., 2006). Twenty different measurements were made for each sample.

2.4.3. Volatile composition (solid phase microextraction/gas chromatography-mass spectrometry)

The volatile composition of the samples was determined using headspace solid phase micro-extraction (HS-SPME) using the same conditions as previously reported for citrus fruits and citrus beer by Cano-Lamadrid *et al.* (2018) and Zapata *et al.* (2019) respectively. A total of 0.3 g of milled sample was weighted, and ultrapure water (10 mL), NaCl (1.0 g) and benzyl acetate (5 µL of 1000 mg L^{-1}, internal standard for semi-quantification of compounds) were added into a 40 mL vial with polypropylene caps and PTFE/silicone septa. The vial was placed in an AOC-6000 Plus autosampler (Shimadzu Corporation, Kyoto, Japan) and, after 5 min of equilibration time, a 50/30

μm DVB/CAR/PDMS fiber was exposed to the sample headspace for 50 min at 40 °C and stirred at 250 rpm. The DVB/CAR/PDMS fiber was chosen because previous studies had reported its suitability for orange juice analysis (Rega *et al.,* 2003). The separation and identification of compounds was conducted by GC2030 (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA), with a Sapiens X5MS (Teknokroma, Barcelona, Spain) column of 30 m × 0.25 mm i.d., 0.25 μm film thickness, and coupled with a Mass Spectrometer detector (TQ8040 NX triple quadrupole mass spectrometer; Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). GC temperature and pressure program and MS conditions were the ones reported by Deba-Rementería *et al.* (2021). Retention indexes of a commercial alkane standard mixture (Sigma-Aldrich, Steinheim, Germany) were used to identify the compounds, as well as the NIST 17 Mass Spectral and Retention Index Libraries. The identification was considered tentative when it was based only on mass spectral data, and only compounds with spectra similarity >90% were considered as correct hits. Linear retention similarity filter was set at ± 10 units. Samples were analyzed in triplicate.

2.5. Data analyses

A two-way ANOVA test was conducted with the brines data using 'process' (blanching pretreatment, PT, *vs* rinsing or non-pretreatment, NPT) and 'time' as factors or independent variables, followed by a post-hoc test (Tukey HSD). A one-way ANOVA followed by a post-hoc test (Tukey HSD) was conducted with the data from the original orange peels as well as the fermented samples, using 'product' as independent variable. All data analyses were conducted using the statistical package XLSTAT Version 2009.6.03 (Addinsoft, USA) (Addinsoft, 2019). Differences among samples were considered significant when $p < 0.05$ unless otherwise stated.

3. RESULTS AND DISCUSSIÓN

pH results showed significant differences ($p < 0.05$) among samples for both 'process' and 'time' factors, and for the interaction 'process*factor'. The pH values sharply decreased from over 5.5 during the first 12 h of fermentation, being below 4.0 thereafter in all samples (Figure 1). pH in the rinsed samples (NPT) was significantly lower than in the blanched samples (PT) after 12 h. This may have affected the viability of *L. plantarum*, because pH values below 4 have been considered non-optimal for lactic acid bacteria growth (Ricci *et al*., 2019). However, final values were similar (≈ 3.3) in both PT and NPT samples at the end of the process. pH is a critical indicator

of fermentation progress and was probably related to the presence and metabolic activity of the inoculated *Lactobacillus plantarum* (Xiong *et al*., 2016). pH dynamics of the present fermentation process were similar to those reported by Yang *et al.* (2018) for fruit beverages made using two *L. plantarum* strains. *L. plantarum* has been reported as having higher acid tolerance, and higher survival rate at late stages on fermentation than other lactic bacteria (Siezen & van Hylckama, 2011), but some pH maintenance has been recommended at the initial stages of vegetable fermentation process to provide the optimum conditions for these bacteria development (Ricci *et al*., 2019).

Total Soluble Solids (TSS) were also significantly different among samples for both 'process' and 'time' factors, and for the interaction 'process*factor'. Initial TSS were similar in PT and NPT samples (8.3 ± 0.4) and significantly decreased ($p < 0.05$) during the first 12 h of fermentation, reaching minimums of 7.3 ± 0.1 (PT) and 7.6 ± 0.4 (NPT) ºBrix. The reduction of TSS at the beginning of the fermentation (first 12h) was significantly higher and faster in the PT samples than in the NPT samples; then, TSS increased in both samples, highlighting a higher increment in the NPT samples, but with a similar TSS content at the end of the process, and which was also close to the initial TSS content (mean = 8.1 ± 0.4 º Brix; $p \geq 0.05$).

Results of both total aerobic and total anaerobic counts (TAeC; TAnC respectively) showed significant differences among samples for 'process' and 'time' factors, and for the interaction 'process*time'. Figure 1 shows the evolution of the total counts in the pretreated (PT) and not-pretreated (NPT) samples. TAnC were similar ($p \geq 0.05$) in both samples at time 0 (after inoculation), but significantly higher in the blanched samples from 12 h until the end of the whole process. TAnC significantly increased in the PT samples during the first 36 hours of fermentation, while decreased in the NPT samples from the beginning of the process. Nevertheless, TAeC were significantly different at the beginning of fermentation, being TAeC almost inexistant in PT samples while significantly present in the NPT samples.

Results suggested that the microbiota present in the orange peels acted as competitor in the NPT fermentation process, obstructing the development of the inoculated strain. Other authors have reported that yeast dominated lemons and orange peels fermentation processes when added in a brine (Aayah *et al*., 2010; Deba-Rementeria *et al*., 2021). During the present study, blanching and inoculation were used to favor a process driven by acid lactic bacteria. Results showed that

using blanching was useful to significantly reduce the total aerobic counts at the beginning of the fermentation (Figure 1), with a clear predominance of anaerobic bacteria during the first week of the process. On the contrary, the NPT fermentation process was driven by a mixture of anaerobic and aerobic microorganisms, probably with a significant presence of the previously reported yeast species (Aayah *et al*., 2010).

Results of sugars content in the brines showed significant differences among samples for 'process' and 'time' factors, as well as for the interaction 'process*time'. Sucrose, which had been supplemented into the brines as a nutrient, significantly decreased from the beginning of the process, although from 60 h the detriment was significantly higher in the NPT samples (Figure 2). Both glucose and fructose concentration significantly increased in the NPT samples from the beginning of the fermentation process, but only glucose concentration significantly increased in the PT samples, probably contributing to the TSS increment. Glucose concentration was significantly lower in the PT samples than the NPT samples from 36 h until 168 h, when it became similar until the end of the process. Fructose concentration was significantly lower in the PT samples from 24 h until the end of fermentation. Sucrose has been reported as the preferred carbohydrate source of lactic acid bacteria, prior to glucose or fructose (Cho *et al*., 2006; Xiong *et al*., 2014), but the main difference among the PT and NPT samples was found in the fructose utilization, highlighting by a greater utilization of fructose as carbon source by the anaerobic bacteria present in the PT samples. Previous studies in which *L. plantarum* was used together with other lactic acid bacteria to ferment pomegranate juice showed that *L. plantarum* and *L. delbruekii* were more capable to consume higher amount of substrate than other bacteria and that glucose was preferred over fructose as energy source (Mousavi *et al*., 2010).

A significant increment of some polyalcohols such as inositol, erythritol and sorbitol was detected during the fermentation process (Table 1). Significant differences were found among samples for 'process' and 'time' factors, and for the interaction 'time*process'. In general, a higher concentration of the 3 polyalcohols was detected in NPT samples, although inositol concentration was similar at the end of the fermentation process in both PT and NPT samples. Sorbitol was only detected in the NPT samples. Polyalcohols are noncyclic hydrogenates carbohydrates coming from the reduction of carbohydrates (Wittmann & Liao, 2017). Sorbitol, derived from D-glucose, is produced by several yeasts and bacteria, except from lactic acid bacteria (Ortiz *et al*., 2013).

278 Therefore, the presence of sorbitol in NPT samples and absence of this polyalcohol in the PT
279 samples confirmed the differences among the microbial populations present in these samples.
280 Erythritol, also found in the study conducted by Deba-Rementeria *et al*. (2021) could be produced
281 by osmophilic yeast as osmoprotectant in the NPT samples, but was also present in the PT
282 samples, suggesting the capability of *L. plantarum* to produce this polyalcohol (Vrancken *et al*.,
283 2010).
284 The main impact that the whole process had on the original orange peels was found in the texture
285 of PT samples and was probably due to the blanching process. The one-way ANOVA instrumental
286 texture analysis showed significant differences among the original orange peel and the 10-days
287 fermented samples (orange *msayer*), as well as significant differences between PT and NPT
288 samples (Table 2). The PT samples were the softest ones, followed by the original orange peel
289 samples; the NPT samples were the hardest ones. Deba-Rementeria *et al*. (2021) reported that
290 hardness of orange peels fermented in a brine with a 5% NaCl were higher than the one of the
291 unfermented peels; similar results have been found in the present research in NPT samples
292 (Table 2). Different salts have been used to maintain the textural properties of pickled and stored
293 vegetables (McFeeters & Pérez-Díaz, 2010). However, hardness significantly decreased in the
294 PT samples, as thermal processes such as blanching of fruits and vegetables are known to result
295 in softening (Palermo *et al*., 2013; Van Buggenhout *et al*., 2009). It has been reported that pectin
296 chains can be split into shorter fragments by hydrolysis of their glycosidic bonds during heating
297 (Gierschner, 1981), and pectin means a 3.5-5.5 % of wet weight of orange peels (De Cindio *et*
298 *al*., 2016). The softening of the peels could have led to a greater access of the lactic acid bacteria
299 to the different peel components and, therefore, a greater flavor transformation.
300 Instrumental color was significantly different ($p < 0.05$) among products. In general, as shown in
301 Table 3, although the 3 samples were significantly different ($p < 0.05$) in some parameters (*L**, *a**
302 and Hue), the PT samples were not different from the original orange peels in the *b** coordinate
303 and Chroma. The ΔE values, which expressed the total color change of the orange peel after the
304 whole process fermentation, showed that the sample's color was significantly different from the
305 original one, being $\Delta E^* = 10.4$ and 14.7 for PT and NPT samples respectively. ΔE^* has been
306 reported to shown differences between treated and untreated samples when being over 0.5 in
307 citrus juices (slightly noticeable from 0.5 - 1.5, noticeable from 1.5 - 3.0, well visible from 3.0 - 6.0,

and great from 6.0) (Cserhalmi *et al*., 2006). Deba-Rementeria *et al*. (2021) reported that fermentation had a significative effect on orange peel color parameters; NPT samples showed similar results as the ones reported by these authors: higher *L**, *b**, Hue and Chroma values. Although differences were found among all samples, PT samples color was closer than NPT samples in all color coordinates (Table 3) and ΔE was lower in PT orange peels; these results suggested that blanching could be an interesting approach to preserve the original orange color as seen in other blanched vegetables (Penfield & Campbell, 1990). Also, it is possible that the fermentative process driven by lactic-acid bacteria was less aggressive than yeast's one on the color transformation of the raw material.

Table 4 shows the volatile composition of the orange *msayer* samples and the original orange peels. Over 40 compounds were identified in all samples, but results of the one-way ANOVA showed significant differences among samples only for some of them; the main differences were found between the original orange peel and the fermented samples, independently on the fermentation process (PT or NPT). Original orange peel was characterized by a higher concentration of aldehydes and terpenes, which seemed to decrease during the fermentation process. The main difference between the PT and NPT samples was a higher concentration of 1-decanol in the NPT samples, probably due to the differences in the microbiological activity during the fermentation process. Volatile composition of the orange peel and the fermented products was characterized by a high terpenoid content, as previously reported for citrus fruits (González-Mas *et al*., 2019). Limonene was the main component in all samples, meaning a 93% of the volatile composition and being in accordance with the 85-99% previously reported for orange essential oils (Ruiz & Flotats, 2014). Although the proportion of limonene was the same in all samples, the amount of some volatile compounds, mainly aldehydes and terpenes was significantly higher in the orange peels before fermenting. Bevilacqua *et al*. (2010) showed that limonene and citrus extract had an antimicrobial effect; for that reason, Mantzouridou *et al*. (2015) suggested to decrease its content by autoclaving to develop a proper fermentation of orange peels by yeast species. Although some samples were blanched for 10 min during the present study (PT samples), limonene content in PT samples was like the one of NPT samples, suggesting that the fermentation was the reason of the reduction of this, and all other compounds.

Finally, alcohols significantly increased in NPT samples, probably due to the presence of yeast as reported by Deba-Rementería *et al*. (2021).

3. CONCLUSIONS

Results of the present study suggested that using blanching as a pretreatment was useful to allow *Lactobacillus plantarum* to drive the fermentation process of orange peels, mainly due to a decrease on the original peels microbiota. Total anaerobic counts were significantly different at the end of the process for pretreated and non-pretreated samples, with a significantly higher anaerobic counts in the PT samples. The 10-min blanching followed by a driven fermentation of orange peels led to a softer product with a color closer to the one of original orange peels. Volatile composition of both PT and NPT samples was similar, but a general decrease was detected if compared with the composition of the orange peels. Sensory analyses of the final products should be conducted to determine the main differences in flavor perception between PT and NPT samples, because the detriment of the volatile composition could be considered interesting in a product generally not consumed by its very intense flavor and bitterness (orange peel). Also, further studies must be conducted on the inclusion of these food products adapted from specific culinary cultures in other ones, promoting the use of by-products as food ingredients wherever they are produced.

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504 **Table 1.** Results of two-way ANOVA and post-hoc analysis (Tukey HSD) for polyalcohols content
505 grouped by 'time' and 'treatment'. Values followed by different letters within the same column
506 were significantly different.

Time (h)	Treatment	POLYALCOHOL (mg / mL brine)		
		INOSITOL	ERYTHRITOL	SORBITOL
0	Pretreated	38.2 ± 3.9 g	n.d.	n.d.
	Not pretreated	98.7 ± 4.0 g	31.9 ± 0.9 ef	n.d.
12	Pretreated	345 ± 77 ef	42.8 ± 2.7 ef	n.d.
	Not pretreated	332 ± 29 f	53.4 ± 2.8 cde	n.d.
24	Pretreated	453 ± 17 cd	48.3 ± 1.0 ef	n.d.
	Not pretreated	461 ± 15 cd	72.4 ± 2.5 de	35.4 ± 1.0 bc
36	Pretreated	455 ± 7 cd	48.3 ± 0.9 ef	n.d.
	Not pretreated	553 ± 43 abc	75.3 ± 15.8 cde	37.8 ± 1.1 bc
48	Pretreated	486 ± 57 cd	52.1 ± 4.0 ef	n.d.
	Not pretreated	590 ± 32 ab	73.4 ± 11.1 de	38.6 ± 1.4 bc
60	Pretreated	463 ± 13 cd	52.1 ± 0.9 ef	n.d.
	Not pretreated	613 ± 17 a	85.7 ± 15.1 cde	38.4 ± 0.7 bc
84	Pretreated	456 ± 24 cd	58.9 ± 11.8 ef	n.d.
	Not pretreated	604 ± 3 a	133 ± 51 c	45.4 ± 15.2 bc
168	Pretreated	445 ± 31 de	73.9 ± 7.4 de	n.d.
	Not pretreated	627 ± 3 a	232 ± 7 b	97.4 ± 53.6 ab
240	Pretreated	503 ± 49 bcd	118 ± 36 cd	n.d.
	Not pretreated	600 ± 15 ab	297 ± 18 a	112 ± 67 a
p-value		<0.0001	<0.0001	0.004

507

508 **Table 2.** Results of ANOVA and post-hoc analysis (Tukey HSD) of the fermented orange
509 (pretreated and not treated samples) and original orange peel (control) texture. Legend: samples
510 within the same column followed by different letters were significantly different.

Sample	Texture: Cutting force (g)
Control (orange peels)	3827 ± 1304 b
Pretreated	990 ± 370 c
Not pretreated	10515 ± 3555 a
p-value	<0.001

511

512 **Table 3.** Results of ANOVA and post-hoc analysis (Tukey HSD) of the fermented orange
513 (pretreated and not treated samples) and original orange peel (control) color parameters. Legend:
514 *L**, lightness; *a**, red/green coordinate, *b**, yellow/blue coordinate; Chroma, color intensity; Hue°,
515 Color tonality. Samples within the same column followed by different letters were significantly
516 different.

Sample	**L* (D65)**	***a****	***b****	**Chroma**	**Hue°**
Orange peel	65.7 ± 2.1 b	28.5 ± 3.9 a	66.6 ± 2.7 b	72.5 ± 1.7 b	66.8 ± 3.5 c
Pretreated (PT)	63.1 ± 2.4 c	18.7 ± 4.8 b	68.4 ± 3.7 b	71.1 ± 3.8 b	74.7 ± 4.0 b
Not pretreated (NPT)	67.5 ± 2.3 a	16.2 ± 5.8 c	74.5 ± 3.4 a	76.4 ± 3.5 a	77.8 ± 4.0 a
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

517

518 **Table 4.** Volatile composition of samples pretreated and not treated. Legend: values followed by
519 different letters were significantly different (Tukey HSD) and indicated in bold. Retention index of
520 literature from NIST (2021) References of the odor thresholds indicated with [numbers].

Compound	RT	KI (EXP)	KI (Lit)	Semi-quantification (mg mL^{-1})			p-value	OT (µg L^{-1})
				Control	PT	NPT		[Ref]¤
1-Octanol	19.62	1069	1069	**10.8 a**	**2.95 b**	**8.69 ab**	0.041	110-130 [1]
1-Nonanol	25.71	1159	1160	1.24	1.64	1.80	0.425	50 [1]
1-Decanol	32.35	1266	1268	**6.14 b**	**10.7 b**	**22.0 a**	0.000	210 [2]
		Total alcohols		**18.2**	**15.3**	**32.7**		
Hexanal	5.82	800	800	**2.89 a**	**0.03 b**	**0.09 b**	0.002	4.5-5 [1]
2-Hexenal	7.63	848	848	**0.57 a**	**0.05 b**	**0.03 b**	0.004	17 [1]
Octanal	15.23	1002	1002	**27.81 a**	**0.31 b**	**0.47 b**	0.002	0.7 [1]
Nonanal	21.88	1103	1103	**10.01 a**	**1.96 b**	**2.33 b**	0.006	1 [1]
Decanal	28.73	1204	1204	**97.0 a**	**30.8 b**	**46.8 ab**	0.025	0.1 - 2 [1]
Undecanal	34.58	1305	1305	0.98	1.19	1.19	0.796	5 [1]
		Total aldehydes		**139**	**34.4**	**50.9**		
α-Pinene	11.31	931	931	12.5	11.7	10.2	0.794	6 [1]
Sabinene	13.45	970	970	23.1	9.35	7.78	0.106	
β-Pinene	13.73	975	975	1.25	0.86	0.67	0.312	140 [1]
β-Myrcene	14.43	988	987	89.9	67.7	67.5	0.546	13-15 [1]
α-Phellandrene	15.41	1005	1005	0.86	1.20	0.79	0.316	
δ-3-Carene	15.59	1007	1007	**4.2 ab**	**7.7 a**	**2.89 b**	0.047	
α-Terpinene	16.11	1015	1015	3.33	1.70	2.48	0.145	
Limonene	16.99	1029	1029	5042	3026	3147	0.201	10 [1]
E-β-Ocymene	18.02	1044	1044	2.42	2.42	2.05	0.830	
γ-Terpinene	18.76	1056	1056	10.8	4.93	7.73	0.106	1000 [3]
Terpinolene	20.56	1083	1083	3.39	2.99	2.20	0.408	200 [1]
Linalool	21.55	1098	1098	**29.9 a**	**7.11 b**	**7.84 b**	0.020	6 [1]
Citronellal	26.52	1171	1172	0.40	0.06	0.061	0.123	300 [4]
4-Terpineol	27.86	1191	1191	**2.93 b**	**2.68 b**	**4.90 a**	0.049	330-350 [1]
α-Terpineol	27.94	1192	1192	**1.83 a**	**0.23 b**	**0.67 b**	0.008	330-350 [1]
Citronellol	30.00	1226	1226	5.04	1.65	2.36	0.051	40 [1]
Neral	30.62	1236	1235	**1.61 a**	**0.07 b**	**0.04 b**	0.005	
Carvone	30.90	1241	1241	**11.0 a**	**0.26 b**	**0.53 b**	0.003	50 [1]
Geranial	32.67	1271	1270	**3.82 a**	**0.23 b**	**0.06 b**	0.005	32 [1]
Citronellyl acetate	36.75	1348	1348	0.38	0.22	0.74	0.390	
Neryl acetate	37.16	1356	1357	1.39	1.22	2.42	0.322	1.6 [3]
		Total terpenes		**5253**	**3151**	**3270**		

521 **Table 4.** Continuation.

Compound	RT	KI (EXP)	KI (Lit)	Semi-quantification (mg mL^{-1})			p-value	OT* (µg L^{-1})
				Control	PT	NPT		[Ref]*
Butyl butyrate	14.79	994	994	**0.78 a**	**0.06 b**	**0.04 b**	0.002	100 [1]
Hexyl butanoate	26.99	1178	1178	**0.50 a**	**0.05 b**	**0.20 ab**	0.040	250 [1]
Octyl acetate	29.04	1209	1210	3.50	1.70	4.53	0.092	12 [1]
Total esters	**4.78**	**1.80**	**4.76**					
p-Cymene	16.57	1022	1022	0.83	0.21	0.40	0.067	
Total benzene derivatives				**0.83**	**0.21**	**0.41**		
Z-Caryophyllene	38.74	1387	1383	0.61	1.39	2.70	0.113	
Tetradecane	39.66	1406	1400	7.23	6.89	12.3	0.061	64 [1]
E-Caryophyllene	40.15	1417	1417	0.30	2.22	0.85	0.085	
E-β-Farnesene	41.68	1451	1450	0.43	1.97	1.73	0.367	
Germacrene D	42.97	1479	1479	0.07	0.75	0.38	0.192	
Valencene	43.46	1490	1490	4.61	19.60	8.95	0.080	
y-Gurjunene	43.59	1493	1491	0.04	1.01	0.40	0.133	
Farnesene	43.96	1501	1500	0.08	1.75	0.63	0.138	
δ-Cadinene	44.57	1516	1516	0.61	2.98	1.47	0.153	
Total sesquiterpenes				**14.0**	**38.6**	**20.4**		
TOTAL VOLATILE COMPODUNS				**5430**	**3241**	**3379**		

522 ¤ [1] Leffingwell & Cantrell (2004); [2] National Library of Medicine (2021); [3] Niu *et al*. (2020) [4]

523 Miller (2019).

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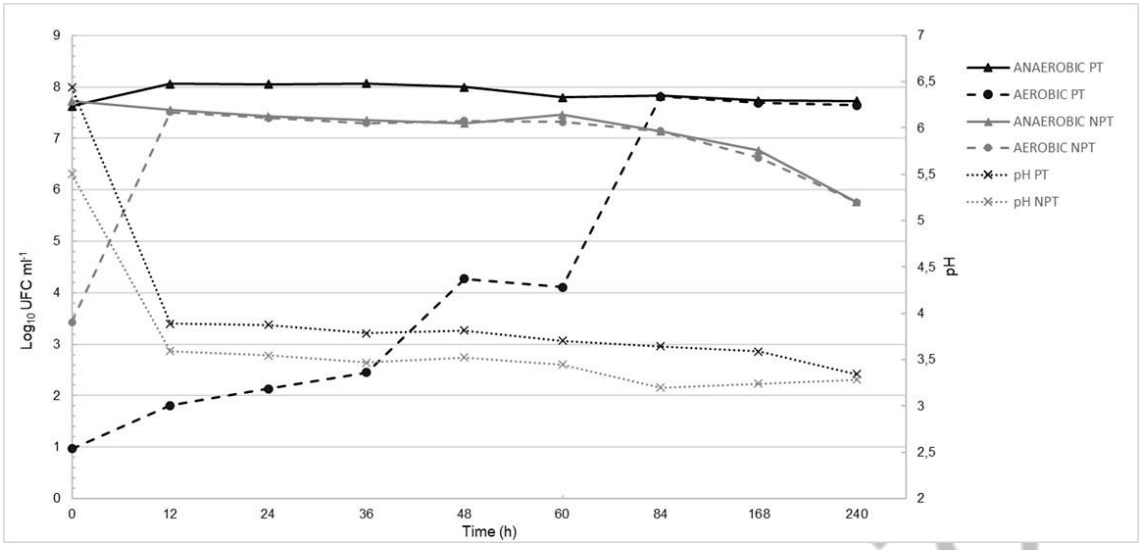
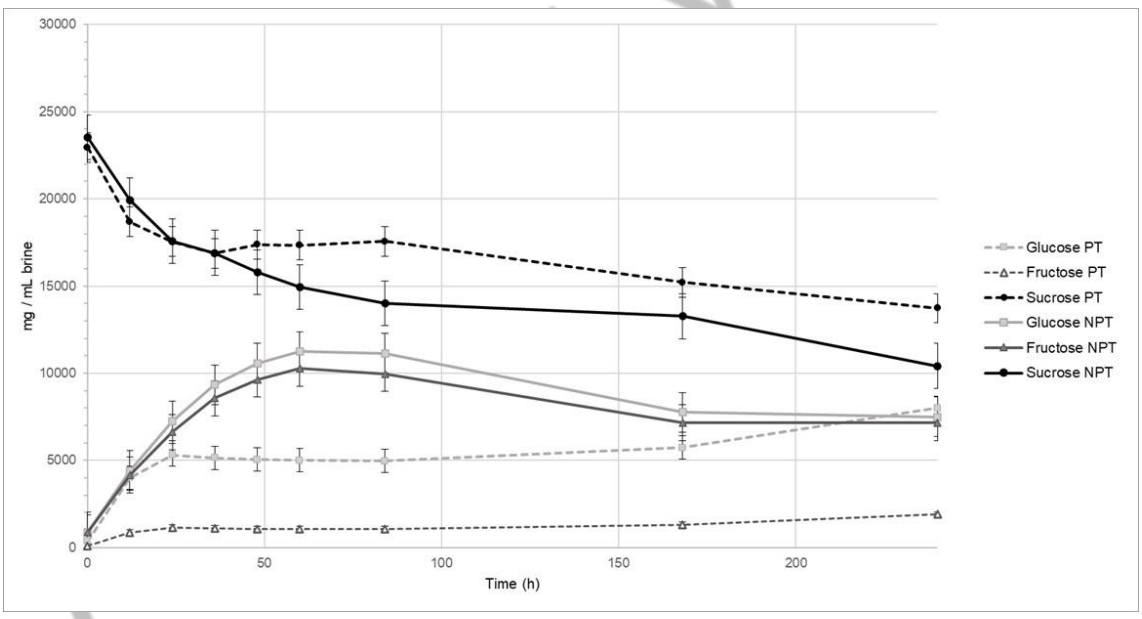


526

527 **Figure 1.** Total anaerobic and aerobic count (expressed as Log10 of TC), and pH values in

528 pretreated samples (PT) and not pretreated samples (NPT).

529



530

531 **Figure 2.** HPLC results showing sugars concertation (glucose, fructose, and sucrose) in PT and

532 PNT samples along time.

533

534

535