Effect of Spanish-style processing steps and inoculation with *Lactobacillus pentosus* starter culture on the volatile composition of cv. Manzanilla green olives

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Running title: Effect of Spanish-style olive processing on volatile compounds

Abstract

The effects of the main steps of Spanish-style processing (alkaline treatment and fermentation) on the volatile composition of cv. Manzanilla green olives were studied. Both spontaneous and controlled fermentations were considered. In the latter case, a *Lactobacillus pentosus* strain from green olive fermentation brine was used as starter culture. The volatile profile was determined by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS). Most of the volatile compounds detected in fresh olives decreased or were undetected after alkaline treatment, while several compounds (mostly acetic acid, 2-methylbutanoic acid, and ethanol) were formed as a result of this treatment. Over 50 new volatile components, mostly esters and phenols, appeared as a result of fermentation. The most outstanding finding was a considerable increase in 4-ethyl phenol (almost 100-fold increase) in inoculated olives compared to the uninoculated product. However, a sensory panel did not find significant differences in odor perception.

Keywords: table olives, processing, *Lactobacillus pentosus*, volatile composition, Manzanilla cultivar

36 **Highlights**

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- 38 ● The effects of processing steps on the volatiles of olives were revealed.
- 39 ● The alkaline treatment decreased considerably the amount and number of volatiles.
- 40 ● Fermentation enriched the volatile profile of olives, mainly with esters and phenols.
- 41 ● Fermentation with *Lactobacillus pentosus* led to a high level of 4-ethyl phenol.
- 42 ● The odors of inoculated and uninoculated samples were perceived as similar by a
- 43 panel.

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1. Introduction

Flavor is one of the key drivers in the consumer appreciation for the Spanish-style green table olive, which is considered the main fermented vegetable product in western countries (Breidt, McFeeters, Perez-Diaz, & Lee, 2013). Flavor formation in food fermentation processes is caused by the accumulation of volatile aroma compounds as well as the formation of compounds that are related to taste (bitterness, umami, sweetness, sourness, and saltiness) (Smid & Kleerebezem, 2014). Spanish-style green table olives contain a complex blend of volatile compounds, with phenols, alcohols, and acids being the predominant chemical classes (Cortés-Delgado et al., 2016; Sánchez et al., 2018). The volatile profile of this product can be influenced by a number of factors, including olive cultivar and production area (Cortés-Delgado et al., 2016; Garrido-Fernández, Montaña, Sánchez, Cortés-Delgado, & López-López, 2017), and post-fermentation and packing stages (Sánchez et al., 2018). The processing of Spanish-style green table olives consists of two main steps: (1) alkaline treatment including the operation of “cocido” (addition of 1.8-2.5%, w/v NaOH) and washing to remove the excess alkali, and (2) addition of brine (10-13%, w/v NaCl), where a fermentation process takes place (Rejano, Montaña, Casado, Sánchez, & de Castro, 2010). However, up to now, the effects of these steps separately on the volatile composition of olives have not been determined.

It has been generally established that lactic acid bacteria (LAB) are mainly responsible for the fermentation, with *Lactobacillus pentosus* y *Lactobacillus plantarum* being the predominant species (Hurtado, Reguant, Bordons, & Rozès, 2012). Many studies recommend the use of these species, in single or mixed combinations, as starter cultures in order to reduce the risk of unexpected growth of spoilage strains and to lead to a controlled and more predictable fermentation process (Ruiz-Barba, Cathcart,

Warner, & Jiménez-Díaz, 1994; Sánchez, Rejano, Montaña, & de Castro, 2001; De De Castro, Montaña, Casado, Sánchez, & Rejano, 2002; Leál-Sánchez et al., 2003; Hurtado et al., 2012; Ruiz-Barba & Jiménez-Díaz, 2012; Martorana et al., 2017). However, very few studies have been conducted to determine the effect of a starter culture inoculation on the volatile profile of the product. The selected strain in table olive processing, should have the ability to produce desirable aroma and taste through the production of volatile compounds, among other characteristics (Bonatsou, Tassou, Panagou, & Nychas, 2017). Panagou & Tassou (2006) studied the effect of inoculation with single strains of *L. pentosus* and *L. plantarum* on the volatile profile in Spanish-style green olives of the cv. Conservolea, but the number of compounds analyzed was quite limited and their identification was only based on retention times. More recently, a more complete analysis of the volatile compounds in green olives (cv. Nocellara del Belice) inoculated with a *L. pentosus* strain, based on headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS), was carried out by Martorana et al. (2017), showing significant differences both qualitative and quantitative in comparison with uninoculated olives.

The objectives of this work were (1) to determine the effects of alkaline treatment and fermentation steps on the volatile composition of olives, and (2) to determine the effect of inoculation with a strain of *L. pentosus* on the volatile profile of Spanish-style green olives in comparison with an uninoculated product. The study was carried out with olives of the Manzanilla cultivar, which is the most prominent cultivar dedicated to green table olives in Spain (ASEMESA, 2018).

2. Materials and Methods

2.1. Materials and chemicals

The olives were harvested in Arahal (Seville province, Spain) at their mature-green stage and transported to our laboratories to be processed. Processing was carried out in cylindrical fermenters made of polyethylene (5.2 kg fruits plus 3.4 L liquid capacity).

All volatile compounds used as reference standards were purchased from Sigma-Aldrich (St Louis, MO). The internal standard 2,3,5,6-d₄-4-ethylphenol was supplied by SPEX CertiPrep (Metuchen, New Jersey, USA). Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA). All other chemicals and solvents were of analytical or chromatographic grade from various suppliers (Panreac, Barcelona, Spain; VWR, Barcelona, Spain; Merck, Darmstadt, Germany).

2.2. Olive processing

The olives were divided into four fermenters (coded M1-M4) and alkali treated with 2.3 % (w/v) NaOH for 5.5 h. Then, the fruits were washed with tap water during 11 h and, finally, a brine with 12% NaCl (w/v) as initial concentration was used to sustain the fermentation. Two fermenters (M1 and M2) were left as spontaneous fermentation control and the other two (M3 and M4) were inoculated with the strain *Lactobacillus pentosus* LP99, originally isolated from green olive fermentation brines. Taking into account the initial high alkalinity of the brine, a massive inoculation was carried out in order to warrant the starter culture implantation from the beginning (Sánchez et al., 2001). Preparation of the inoculum was as follows. For propagation from stocks maintained at -80 °C, the strain was cultivated twice in MRS broth, the second time with 4.5% (w/v) NaCl to allow adaptation to the saline environment. After incubation at 32 °C for 30 h, the cultures were centrifuged, washed with saline (0.9 %

NaCl), and the washed pellets were re-suspended in the brine from the corresponding fermenters. A double inoculation was performed: ca. 2.1×10^8 cfu mL⁻¹ was the initial population first added at day 5, and then ca. 5.3×10^7 cfu mL⁻¹ seven days after, at day 12. No pH control by acid addition was carried out. However, the brines were supplemented with glucose at day 159 in order to attain the acidity and pH values to warrant a convenient final preservation.

2.3. Sampling

Brine samples from each fermenter were taken during fermentation to control the main physicochemical and microbiological characteristics. Analyses of volatile compounds in pulp were performed at three different times throughout the elaboration: before processing, just before brining (i.e. once olives were NaOH treated and washed), and after 7 months of brining.

2.4. Physico-chemical and microbiological analyses

The pH and titratable acidity of the brines were measured using a Metrohm 670 Titro processor (Herisau, Switzerland). Titratable acidity was determined by titrating to pH 8.3 with 0.2 mol L⁻¹ NaOH and expressed as lactic acid.

The viable and culturable populations of lactic acid bacteria (LAB), and yeast and moulds were determined by plating the brines and their decimal dilutions (in 0.9 % NaCl) with a Spiral Plater (Don Whitley Sci. Ltd., Shipley, England). The culture media used were de Man, Rogosa, Sharpe MRS agar (Biokar diagnostics, Beauvais, France) with and without 0.02 % sodium azide (Sigma-Aldrich), and oxytetracycline-glucose-yeast extract (Oxoid Ltd., Basingstoke, England) agar for the aforementioned groups,

respectively. MRS plates were incubated under anaerobic conditions (AnaeroGen, Oxoid). Plates were incubated at 32 °C for up to five days, and the numbers of colony forming units were counted with a Scan 500 (Interscience, St Nom la Bretèche, France) colony counter.

2.5. Analysis of volatile compounds

The olives (approximately 200 g) were pitted and homogenized, and 2.5 g of homogenized pulp were placed in a 15 mL glass vial. After the addition of a 7.5 mL NaCl solution (300 g L⁻¹), and 100 µL of 3-octanol (2 mg L⁻¹) as internal standard, the vial was closed and extraction by HS-SPME was performed with subsequent analysis by GC-MS, following the procedure reported by Sánchez et al. (2018). Concentrations were expressed as µg/kg of 3-octanol. Compound identification was based on mass spectra matching with the standard NIST 08 MS library and on the comparison of retention indices (RI) sourced from the NIST Standard Reference Database and from authentic reference standards when available. All analyses of the volatile compounds were made in triplicate.

In order to detect the volatile acids formed as a result of the alkaline treatment, and before extraction by HS-SPME, 150 µL of 2N HCl solution were added to the vial containing 2.5 g pulp and 7.5 mL of NaCl solution to adjust the pH to 4 units.

A quantitative analysis of 4-ethylphenol was carried out in the olive juice according to the method described by Pollnitz, Pardon, & Sefton (2000) for red wine. For olive juice preparation, the olives were pitted and homogenized using a mixer. Then the juice was obtained by filtration through cheesecloth and centrifugation of the filtrate at 20,000g for 15 min. A solution of 2,3,5,6-d₄-4-ethylphenol (100 µL, 10 µg/mL) in methanol was added to the olive juice (2.5 mL) in a screw cap vial. Diethyl ether-

pentane (1:2, 1 mL) was added and the mixture was shaken briefly. A portion of the organic layer was then transferred to a vial and an aliquot (1 μ L) was injected into the GC-MS system, using the splitless injection mode. The GC-MS system used was a 7890A GC coupled to mass detector model 5975C (Agilent Technologies, Santa Clara, CA). Separation was achieved on a VF-WAX MS capillary column (30 m x 0.25 mm x 0.25 μ m film thickness) from Agilent. The injector temperature was 200 $^{\circ}$ C, and the oven temperature program was as follows: isothermal for 1 min at 150 $^{\circ}$ C, then 150-210 $^{\circ}$ C at 10 $^{\circ}$ C min⁻¹, and then 210-240 $^{\circ}$ C at 40 $^{\circ}$ C min⁻¹ and held there for 15 min. The carrier gas was helium at a constant flow of 1 mL min⁻¹. For the mass selective detector conditions, the quadrupole, ion source and transfer line temperatures were maintained at 150, 230, and 250 $^{\circ}$ C, respectively. For quantification, mass spectra were recorded in the selective ion monitoring (SIM) mode. The ions monitored were: m/z 107 and 122 for 4-ethylphenol and m/z 111 and 126 for 2,3,5,6-d₄-4-ethylphenol. The ions used for quantitation were: m/z 122 for 4-ethylphenol and m/z 126 for the internal standard. The other ions were used as qualifiers. Replicate (n = 6) analysis of a sample (M1) gave a relative standard deviation (RSD) of 1.5%. To verify the accuracy, we carried out recovery tests by adding known amounts of 4-ethylphenol (0.2 and 1 μ g/mL) to a sample of olive juice that had been shown to contain 0.1 μ g/mL of 4-ethylphenol. The recovery rates were 100.7 and 97.7%, respectively.

2.6. Sensory evaluation of fermented olives

To assess whether the differences in concentration of 4-ethyl phenol among inoculated and uninoculated olives gave rise to perceivable differences in odor, the brines corresponding to samples M1 and M3 were evaluated using the triangle test with the protocol considerations described by ISO standard 4120:2004. The test was

performed by a panel of 18 judges (8 males and 10 females) with previous experience in the sensory analysis of table olives. The samples (5 mL) were presented in cups coded with three-digit random numbers and served to judges at 20 °C in individual booths under incandescent white lighting at the sensory laboratory of our department in the Instituto de la Grasa. Each comparison consisted of the judges smelling the three samples in the triangle test and choosing the sample that differed from the other two. The order of samples was randomly chosen.

2.7. Statistical analyses

The one-way analysis of variance (ANOVA) was applied to compare the means of individual concentrations of volatiles during processing. Prior to ANOVA, the homogeneity of variances was checked with the Levene test and the normality of the variables was checked by the Shapiro-Wilk test, both at a significance level of 5%. When these two conditions were fulfilled, the classic ANOVA (F-test) was applied and the Duncan test was used for mean comparisons. In case of heterogeneity of variances, assuming that normality was fulfilled, the Welch test was used to determine whether there was a significant difference among the means and the Games-Howell post hoc test was used for mean comparisons. Significant differences were determined at the $p < 0.05$ level. These statistical analyses were performed using SPSS software v. 23.0 (IBM Corp., Armonk, NY, USA).

Data from triangle test were analyzed using XLSTAT v. 2017 (Addinsoft, Paris, France). The probability of belonging to the statistical distribution of reference (binomial distribution) was calculated on the basis of the number of correct answers and the total number of responses using the Thurstonian model and the Clopper-Pearson method. The level required for significance was $\alpha = 0.05$.

3. Results and Discussion

3.1. Physico-chemical and microbiological changes during fermentation

Once the salt equilibrium between the brine and olive flesh was reached after brining, salt concentration was between 5.4-5.7 % in all fermenters at day 6, values which are suitable for an adequate process. The changes in pH and acidity, and LAB and yeast populations, as fermentation progressed are shown in Figures 1 and 2, respectively. Differences in pH and acidity between the uninoculated and inoculated fermenters were detected. The pH decrease was faster when inoculation was performed, and the acidity values corresponding to the inoculated brines were higher than those obtained when the starter was not applied. These results were not surprising since it is known that the application of a suitable lactobacillus culture improves the fermentation of table olives (Corsetti, Perpetuini, Schirone, Tofalo, & Suzzi, 2012; Martorana et al., 2017). Nevertheless, they confirm that the LAB populations shown in Figure 2 were effective in spite of the initial drop. It can also be seen in Figs 1 and 2 that the error bars (range of data) of points corresponding to the inoculated fermenters are, in general, shorter than those corresponding to the uninoculated fermenters, which supports the greater reproducibility that is achieved by using starter cultures.

3.2. Changes in volatile compounds during processing

The volatile compounds were identified and quantified before processing (fresh fruit), just before brining (i.e. once olives were NaOH treated + washed), and after 7 months of brining for spontaneous and controlled fermentations (Table 1).

3.2.1. Effect of alkaline treatment on volatile compounds

The volatile profile of the fresh fruit was composed of 51 compounds and was predominated by the chemical class of aldehydes/ketones, with 1-penten-3-one, (E)-2-hexenal, (E,E)-2,4-hexadienal, and phenylacetaldehyde being the most abundant compounds. Alcohols were found as the second main chemical class in fresh olives, and 1-penten-3-ol, (Z)-2-penten-1-ol and (Z)-3-hexen-1-ol were predominant. All the mentioned compounds, except phenylacetaldehyde, are known to be formed by endogenous olive enzymes through the lipoxygenase pathway from fatty acids (Reboredo-Rodríguez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2013). These enzymes would be activated during sample preparation for volatile analysis as a result of the operations of pitting and homogenization. Phenylacetaldehyde is known to be a degradation product of phenylalanine (Schwab, Davidovich-Rikanati, & Lewinsohn, 2008).

The volatile profile of the olives after alkaline treatment was composed of 41 compounds. Volatile acids were not detected due to the basic pH of the olives as a result of the NaOH treatment, but their presence was demonstrated by acidifying the sample prior to analysis by HS-SPME-GC-MS, as described in the Materials and Methods section. In this manner, in addition to acetic acid, 2-methylbutanoic acid and benzoic acid, which significantly increased their concentrations with respect to fresh fruit, the medium-chain fatty acids C6-C8 were detected (Table 1). Ethanol was also formed as a result of the alkaline treatment. It is known that acetic acid and ethanol are formed during this treatment in Spanish-style green olives, probably from fragmentation by the alkali of sugars (Sánchez, de Castro, Rejano, & Montaña, 2000). Other compounds found after alkaline treatment, although in smaller amounts, were isopentanol, 1-pentanol, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, octane, α -muurolene, and β -damascenone. Some of these compounds (1-pentanol, (E,E)-2,4-nonadienal, (E,E)-2,4-

decadienal, octane) are known secondary oxidation products from the autoxidation of fatty acids (Frankel, 1982). β -Damascenone can be formed by the degradation of the carotenoid neoxanthin (Winterhalter & Rouseff, 2002). On the other hand, the predominant alcohols and most aldehydes/ketones in fresh olives were decreased considerably or were undetected after the alkaline treatment, which can be attributed to the degradation or inactivation of endogenous enzymes caused by the treatment with NaOH. Salas, Williams, Harwood, & Sánchez (1999) found no activity of olive lipoxygenase above pH 7.5.

3.2.2. Effect of fermentation step on volatile compounds

Fermentation, both spontaneous and controlled with *L. pentosus* starter culture, resulted in more than a 2-fold increase in the number of volatiles compared to olives after the alkaline treatment (Table 1). In both cases, over 50 compounds from different chemical classes (around 60% of the total number of compounds identified in the fermented product) were formed *de novo* with fermentation, particularly esters and phenols. Among these compounds, p-cresol, 4-ethyl phenol, and ethyl acetate reached the highest concentrations. Propanoic acid was also formed in a relatively high amount, indicating probable growth of *Propionibacterium* species during fermentation (Montaño, Sánchez, Casado, de Castro, & Rejano, 2003). Several terpenes (linalool oxide, dihydroedulan, linalool, α -terpineol, α -farnesene, isogeraniol, geraniol) appeared during the fermentation step. These compounds can be considered as olive-derived compounds likely to have been released during fermentation from nonvolatile precursors. Thus, terpene glycosides could be hydrolyzed during fermentation by microbial glycosidase enzymes and by acidic conditions (pH $\approx$ 4) to form the free

volatile terpene compounds, as reported in the case of wine fermentation (Ebeler & Thorngate, 2009). In addition, several compounds present in olives after alkaline treatment increased their concentrations significantly during fermentation, mainly phenylethyl alcohol, benzyl alcohol, and (Z)-3-hexen-1-ol, which were three of the predominant compounds in the fermented product, which is in agreement with previous studies (Cortés-Delgado et al., 2016; Sánchez et al., 2018). On the other hand, most carbonyl compounds decreased significantly or disappeared with fermentation. The most noticeable exception was (E)-2-decenal, which increased with fermentation, and could be indicative of lipid oxidation (Jansen, 2015). The alcohols, phenols, esters, and acids formed during fermentation and which quantitatively constituted the majority of the volatile components in fermented olives (Cortés-Delgado et al., 2016; Sánchez et al., 2018), can be associated to the action of LAB and yeasts, the predominant microorganisms in table olive fermentations (Heperkan, 2013).

Four compounds (1-heptanol, 6-methyl-5-hepten-2-ol, isoamyl acetate, and methyl benzoate) were found in the inoculated olives, but were not detected in the uninoculated ones. On the contrary, hexanal and heptanal were only detected in the uninoculated olives. Thus, in total, the number of volatiles in inoculated and uninoculated olives was quite similar (85 and 84 compounds, respectively). The most outstanding difference between inoculated and uninoculated olives corresponded to the 4-ethyl phenol concentration (Table 1). When this compound was quantitatively analyzed, the values found in the olive juice were (mean $\pm$ SD, $\mu\text{g/L}$): 1791 ± 151 and 19 ± 2 for inoculated and uninoculated samples, respectively. The odor threshold of 4-ethyl phenol in water was found to be $42 \mu\text{g/L}$ (Pyysalo, Suihko, & Honkanen, 1977). This remarkable effect of *L. pentosus* LP99 on 4-ethyl phenol concentration was not found by Martorana et al. (2017), who used the strain *L. pentosus* OM13 in Spanish-

style olives of the Nocellara del Belice cultivar. This suggests that 4-ethyl phenol formation is strain-dependent, although olive cultivar could also have some contribution. It is well known that 4-ethyl phenol is generally formed by microorganisms via the decarboxylation of p-coumaric acid to give 4-vinyl phenol and further reduction of the latter compound (Dias et al., 2003; Rodríguez et al., 2009). 4-Ethyl phenol was previously detected in table olives (Cortés-Delgado et al., 2016; Sánchez et al., 2018; López-López, Sánchez, Cortés-Delgado, de Castro, & Montaña, 2018), although the quantitative determination of this compound was not carried out in any case. In olive oil, 4-ethyl phenol was found at low concentrations ($< 100 \mu\text{g/L}$) (Vichi et al., 2009). Off-odor in stored alperujo (i.e. the paste or semisolid waste generated from the two-phase extraction system of olive oil) was mainly attributed to the presence of 4-ethyl phenol, which was formed by lactic acid bacteria, particularly *L. pentosus* (de Castro, Asencio, Ruiz-Méndez, Romero, & Brenes, 2015). Yeasts classified as *Brettanomyces/Dekkera* spp. were associated with the formation of 4-ethyl phenol in wine (Lambrechts & Pretorius, 2000). If present at concentrations above its odor threshold ($440 \mu\text{g/L}$ in wine according to Campo, Ferreira, Escudero, Marqués, & Cacho, 2006), 4-ethyl phenol may impart “animal”, “horse sweat”, “leather”, “medicinal” aromas.

A sensory evaluation of the brines M1 and M3 from uninoculated and inoculated olives, respectively, using the triangle test was carried out. From 18 judgments, there were only 6 correct responses, which corresponded to a computed p-value of 0.588. As this p-value is greater than the significance level $\alpha = 0.05$, one cannot reject the null hypothesis, concluding that there were no significant differences between the brines M1 and M3 as assessed by smelling. This result appears to indicate that despite the relatively high concentration of 4-ethyl phenol in the inoculated sample, such

concentration would remain below (or would not be much higher than) the odor threshold of this compound in olive brine.

4. Conclusions

Our study revealed the effects of the two main steps of Spanish-style green olive processing (alkaline treatment and fermentation) on the volatile composition of olives. As a result of the alkaline treatment, several compounds (mostly acetic acid, 2-methylbutanoic acid, and ethanol) were produced, but the major volatiles in fresh olives were undetected or drastically decreased. Around 60% of the compounds identified in the fermented product were entirely produced during the fermentation step. Most of these compounds, which were dominated by esters and phenols, were presumably originated by microbial action. However, the knowledge of contribution of individual microbial groups and species (mainly lactic acid bacteria and yeasts) to the volatile profile of olives requires further studies.

Our study also confirmed the positive effect of using *L. pentosus* starter culture on the evolution of acidity and reproducibility in Spanish-style green olive processing. Regarding volatiles, from a qualitative point of view, it was evidenced that the volatile composition of inoculated olives was quite similar to that of uninoculated olives. The most outstanding effect that the strain *L. pentosus* LP99 had on the volatile composition of fermented olives was a considerable increase in 4-ethyl phenol (almost 100-fold increase) compared to uninoculated olives. However, this finding did not have a significant impact on the odor of fermented olives, as the samples were perceived as similar by a sensory panel.

Acknowledgements

This work was supported by the Ministry of Economy and Competitiveness from the Spanish government through Project AGL2014-54048-R, partially financed by the European Regional Development Fund (ERDF).

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Figure captions

Figure 1. Evolution of pH and titratable acidity throughout fermentation. Points are the means of duplicate fermenters. Where error bars (range of data) are not visible, determinations were within the symbols on the graph. ○ Uninoculated fermenters; □ Inoculated fermenters.

Figure 2. Microbial population of lactic acid bacteria and yeasts throughout fermentation. Points are the means of duplicate fermenters. Where error bars (range of data) are not visible, determinations were within the symbols on the graph. ○ Uninoculated fermenters; □ Inoculated fermenters.

Table 1. Changes in volatile composition during Spanish-style green table olive processing

Compound	IEC (m/z) ^a	RI (exp.) ^b	RI (lit.) ^c	ID ^d	Fresh fruit ^e	After alkaline treatment ^e	After spontaneous fermentation ^e	After <i>L. pentosus</i> fermentation ^e	<i>P</i> -value ^f
Acids									
Acetic acid	60	1460	1450	A	13 ± 2 a	ND (253 ± 50) ^g bc	184 ± 55 b	330 ± 103 c	0.002
Propanoic acid	74	1549	1536	A	ND	ND	38 ± 20 a	36 ± 10 a	0.890
2-Methylbutanoic acid	74	1680	1655	A	4.8 ± 0.6 a	ND (125 ± 25) ^g b	20 ± 10 a	25 ± 7 a	0.000
Hexanoic acid	73	1854	1850	A	ND	ND (5 ± 1) ^g a	4.3 ± 1.5 a	4.8 ± 1.5 a	0.849
Heptanoic acid	73	1959	1960	A	ND	ND (5.4 ± 0.7) ^g b	0.9 ± 0.4 a	1.7 ± 0.5 a	0.000
Octanoic acid	73	2067	2051	A	ND	ND (2.1 ± 0.1) ^g a	1.4 ± 0.6 a	2 ± 1 a	0.464
Decanoic acid	73	2277	2276	A	ND	ND	0.4 ± 0.1 a	0.7 ± 0.1 b	0.018
Benzoic acid	105	2436	2433	A	2.3 ± 0.5 a	ND (7 ± 1) ^g b	1.3 ± 0.9 a	1.7 ± 0.6 a	0.000
Alcohols									
Ethanol	45	935	937	A	ND	109 ± 18 a	155 ± 78 a	112 ± 46 a	0.588
1-Propanol	59	1039	1049	A	ND	ND	6 ± 1 a	9 ± 2 a	0.088
1-Penten-3-ol	57	1169	1158	A	137 ± 17	ND	ND	ND	
Isopentanol	55	1211	1217	A	ND	5 ± 0.4 a	21 ± 8 ab	13 ± 3 b	0.011
1-Pentanol	55	1255	1252	A	ND	3.5 ± 0.5	ND	ND	
(Z)-2-Penten-1-ol	45	1318	1318	A	201 ± 41	ND	ND	ND	
3-Methyl-2-buten-1-ol	71	1324	1322	A	ND	ND	1.8 ± 0.6 a	1.8 ± 0.8 a	0.988
1-Hexanol	56	1356	1360	A	13 ± 2 a	34 ± 2 b	15 ± 2 a	18 ± 4 a	0.001
(Z)-3-Hexen-1-ol	67	1385	1386	A	108 ± 17 c	18.2 ± 0.6 a	66 ± 13 b	87 ± 20 bc	0.002

1-Octen-3-ol	57	1454	1452	A	8 ± 1 a	4 ± 1 a	4.6 ± 0.8 a	5 ± 2 a	0.089
1-Heptanol	70	1458	1467	A	5.4 ± 0.5 a	4.9 ± 0.3 a	ND	7 ± 2 a	0.400
6-Methyl-5-hepten-2-ol	95	1466	1451	A	ND	ND	ND	4 ± 1	
2-Ethyl-1-hexanol	57	1491	1484	A	4.5 ± 0.2 a	3.6 ± 0.4 a	ND	ND	0.105
1-Octanol	84	1560	1553	A	7.0 ± 0.8 a	6 ± 1 a	9 ± 2 a	13 ± 4 a	0.051
(E)-2-Octen-1-ol	57	1613	1620	A	ND	ND	3.3 ± 0.8 a	5 ± 1 b	0.042
1-Nonanol	70	1661	1661	A	ND	ND	9 ± 2 a	13 ± 4 a	0.071
2-Decen-1-ol	57	1817	1812	B	ND	ND	20 ± 5 a	27 ± 8 a	0.177
Benzyl alcohol	108	1871	1865	A	11 ± 2 a	6 ± 1 a	84 ± 34 b	109 ± 30 b	0.002
Phenylethyl alcohol	91	1903	1912	A	37 ± 6 a	5 ± 1 a	311 ± 131 b	413 ± 126 b	0.002
Homovanillyl alcohol	137	2840	2830	B	ND	ND	1.3 ± 0.7 a	0.9 ± 0.4 a	0.390
Aldehydes/Ketones									
2-Methylbutanal	57	880	893	A	39 ± 7 a	43 ± 10 a	ND	ND	0.848
Pentanal	44	980	984	A	11 ± 1	ND	ND	ND	
1-Penten-3-one	55	1019	1019	A	492 ± 85	ND	ND	ND	
Hexanal	82	1071	1077	A	36 ± 4 c	3.5 ± 0.2 b	0.3 ± 0.1 a	ND	0.000
(E)-2-Pentenal	55	1126	1128	A	105 ± 21	ND	ND	ND	
(Z)-3-Hexenal	69	1152	1137	B	29 ± 3	ND	ND	ND	
Heptanal	70	1177	1182	A	5 ± 1 c	3.2 ± 0.5 b	0.6 ± 0.2 a	ND	0.000
(E)-2-Hexenal	83	1212	1200	A	530 ± 83	ND	ND	ND	
2-Vinyl-2-butenal	67	1283	1293	B	33 ± 5	ND	ND	ND	
Octanal	84	1284	1280	A	15 ± 3 b	3.9 ± 0.1 a	2.3 ± 0.4 a	3 ± 2 a	0.000
(E)-2-Heptenal	83	1313	1306	A	25 ± 5 b	ND	5 ± 2 a	9 ± 4 a	0.001
Nonanal	98	1388	1390	A	22 ± 8 c	9 ± 1 b	1.7 ± 0.5 a	2.0 ± 0.6 a	0.001
(E,E)-2,4-Hexadienal	81	1395	1395	A	372 ± 59	ND	ND	ND	
(E)-2-Octenal	55	1420	1434	A	4.8 ± 0.8 b	1.3 ± 0.3 a	1.3 ± 0.4 a	2 ± 1 a	0.018
(E,E)-2,4-Heptadienal	81	1458	1461	A	20 ± 3 b	7.1 ± 0.1 a	ND	ND	0.040
Decanal	57	1509	1510	B	ND	ND	0.5 ± 0.1 a	0.6 ± 0.2 a	0.890

Benzaldehyde	106	1511	1513	A	42 ± 1 b	23 ± 7 a	15 ± 7 a	14 ± 3 a	0.001
5-Methylfurfural	110	1567	1567	A	5.6 ± 0.1	ND	ND	ND	
Phenylacetaldehyde	91	1632	1638	A	255 ± 38 b	3.5 ± 1.5 a	ND	ND	0.003
(E)-2-Decenal	70	1633	1631	A	27 ± 4 b	9 ± 2 a	22 ± 3 b	28 ± 9 b	0.022
(E,E)-2,4-Nonadienal	81	1714	1701	B	ND	11 ± 2	ND	ND	
(E,E)-2,4-Decadienal	81	1799	1800	A	ND	2.8 ± 0.7 a	2.1 ± 0.6 a	2.1 ± 1 a	0.563
Esters									
Ethyl acetate	43	897	896	A	ND	ND	81 ± 37 a	60 ± 25 a	0.383
Propyl acetate	61	976	957	A	ND	ND	1.0 ± 0.2 a	1.4 ± 0.3 a	0.118
Methyl butanoate	87	989	989	A	1.6 ± 0.2 a	1.3 ± 0.2 a	ND	ND	0.342
Methyl 2-methylbutanoate	88	1010	1004	A	ND	ND	2.1 ± 0.9 a	2.8 ± 0.7 a	0.269
Methyl 3-methylbutanoate	74	1018	1011	A	ND	ND	0.6 ± 0.1 a	1.0 ± 0.2 b	0.026
Ethyl 2-methylbutanoate	102	1048	1052	A	ND	ND	13 ± 10 a	7 ± 4 a	0.340
Ethyl 3-methylbutanoate	88	1064	1064	A	ND	ND	3 ± 1 a	2.2 ± 0.9 a	0.340
Isoamyl acetate	43	1118	1121	A	ND	ND	ND	10 ± 11	
Methyl hexanoate	74	1185	1176	A	ND	ND	3 ± 2 a	4 ± 1 a	0.473
Ethyl hexanoate	88	1231	1232	A	ND	ND	6 ± 4 a	4 ± 2 a	0.442
Methyl lactate	45	1322	1314	A	ND	ND	3 ± 1 a	5 ± 1 b	0.012
Ethyl heptanoate	88	1326	1329	B	ND	ND	3 ± 2 a	3 ± 1 a	0.728
Methyl 6-heptenoate	74	1339	-	C	ND	ND	0.6 ± 0.3 a	0.8 ± 0.2 a	0.284
Ethyl lactate	75	1345	1358	A	ND	ND	3 ± 0.4 a	3 ± 1 a	0.795
Methyl octanoate	74	1387	1386	A	ND	ND	3 ± 2 a	5 ± 1 a	0.143
Ethyl octanoate	88	1432	1436	A	ND	ND	9 ± 7 a	6 ± 2 a	0.374
Methyl nonanoate	74	1488	1491	A	ND	ND	0.9 ± 0.2 a	1.5 ± 0.4 b	0.030
Methyl 2,5-dimethyl-3-furoate	139	1558	-	C	31 ± 5	ND	ND	ND	
Isobornyl acetate	95	1570	1568	A	16 ± 1	ND	ND	ND	
Methyl decanoate	87	1592	1590	A	ND	ND	0.6 ± 0.2 a	1.9 ± 0.6 b	0.008
Methyl benzoate	105	1611	1605	A	ND	ND	ND	1.7 ± 0.5	

Ethyl benzoate	105	1654	1651	A	ND	ND	4 ± 1 a	4 ± 2 a	0.840
Benzyl acetate	108	1721	1688	A	ND	ND	1.6 ± 0.5 a	5 ± 3 a	0.092
Methyl salicylate	120	1758	1755	A	28 ± 3 b	5 ± 1 a	3.0 ± 0.9 a	4 ± 1 a	0.000
Ethyl salicylate	120	1792	1788	A	ND	ND	1.4 ± 0.9 a	1.2 ± 0.9 a	0.791
Methyl hydrocinnamate	104	1833	1821	A	ND	ND	6 ± 1 a	10 ± 3 b	0.040
Ethyl hydrocinnamate	104	1873	1872	A	ND	ND	15 ± 6 a	12 ± 5 a	0.565
Triacetin	145	2074	2077	A	ND	ND	6 ± 2 a	6 ± 8 a	0.915
Hydrocarbons									
Octane	85	807	800	A	ND	4.4 ± 0.8 a	55 ± 15 b	51 ± 18 b	0.014
Decane	57	1001	1000	A	27 ± 4 b	5 ± 1 a	ND	ND	0.027
Toluene	91	1034	1036	A	26 ± 2 b	13 ± 1 a	ND	ND	0.004
Styrene	104	1249	1245	A	4.3 ± 0.6 a	ND	39 ± 12 a	37 ± 32 a	0.052
3-Ethylstyrene	117	1428	1424	B	19 ± 3	ND	ND	ND	
2-Bornene	121	1505	-	C	ND	ND	13 ± 3 a	25 ± 6 b	0.013
Phenols									
p-Creosol	138	1949	1960	A	ND	ND	207 ± 56 a	280 ± 69 a	0.157
Phenol	94	2006	1992	A	26 ± 3 b	4.3 ± 0.6 a	2.4 ± 0.7 a	4.1 ± 0.9 a	0.000
p-Ethyl guaiacol	137	2022	2033	A	ND	ND	5 ± 1 a	13 ± 6 a	0.075
p-Cresol	107	2083	2078	A	ND	ND	2.3 ± 0.7 a	4 ± 1 a	0.078
p-Propyl guaicol	137	2100	2115	A	ND	ND	1.7 ± 0.7 a	1.4 ± 0.6 a	0.455
Eugenol	164	2158	2167	A	ND	ND	0.9 ± 0.3 a	1.4 ± 0.4 a	0.100
4-Ethyl phenol	107	2175	2170	A	ND	ND	44 ± 13 a	5409 ± 1242 b	0.003
p-Vinyl guaiacol	150	2177	2181	B	ND	ND	1.1 ± 0.6 a	3 ± 2 a	0.093
Vanillin	151	2541	2551	A	ND	ND	3 ± 1 a	3 ± 1 a	0.583
Tyrosol	107	2805	2978	A	6 ± 2	3 ± 1	24 ± 15 a	18 ± 8 a	0.040
Terpenes									
Limonene	93	1182	1198	A	6.5 ± 0.3	ND	ND	ND	
(E)-β-Ocimene	93	1249	1233	A	7.1 ± 0.9 b	3.3 ± 0.9 a	2.0 ± 0.6 a	2.5 ± 0.7 a	0.000

[illegible]