Volatile profile of elderberry juice: Effect of lactic acid fermentation using L. plantarum, L. rhamnosus and L. casei strains

Volatile profile of elderberry juice: effect of lactic acid fermentation using *L. plantarum*, *L. rhamnosus* and *L. casei* strains

Annalisa Ricci, Martina Cirlini*, Alessia Levante, Chiara Dall'Asta, Gianni Galaverna and Camilla Lazzi*

Department of Food and Drug, University of Parma, 43124 Parma, Italy

*Corresponding authors

Camilla Lazzi; email: camilla.lazzi@unipr.it

Martina Cirlini; email: martina.cirlini@unipr.it

Abstract

In this study we explored, for the first time, the lactic acid fermentation of elderberry juice (EJ). A total of 15 strains isolated from dairy and plant matrices, belonging to *L. plantarum*, *L. rhamnosus* and *L. casei*, were used for fermentations. The volatile profile of started and unstarted EJ was characterized by HS-SPME/GC-MS technique after 48 hours of fermentation and 12 days of storage at 4°C. All *L. plantarum* and *L. rhamnosus* strains exhibited a good capacity of growth while not all *L. casei* strains showed the same ability. The aromatic profile of fermented juices were characterized by the presence of 82 volatile compounds pertaining to different classes: alcohols, terpenes and norisoprenoids, organic acids, ketones and esters. Elderberry juice fermented with *L. plantarum* strains showed an increase of total volatile compounds after 48 hours while the juices fermented with *L. rhamnosus* and *L. casei* exhibited a larger increase after the storage. The highest concentration of total volatile compounds were observed in EJ fermented with *L. plantarum* 285 isolated from dairy product. Ketones increased in all fermented juices both after fermentation and storage and the most concentrated were acetoin and diacetyl. The organic acids were also affected by lactic acid fermentation and the most abundant acids detected in fermented juices were acetic acid and isovaleric acid. Hexanol, 3-hexen-1-ol (Z) and 2-hexen-1-ol (E) were positively influenced during dairy lactic acid bacteria strains fermentation. The most represented esters were ethyl acetate, methyl isovalerate, isoamyl isovalerate and methyl salicylate, all correlated with fruit notes. Among terpenes and norisoprenoids, β-damascenone resulted among the main representatives with its typical note of elderberry. Furthermore, coupling obtained data with multivariate statistical analyses, as Principal Component Analysis (PCA) and Classification Trees (CT), it was possible to relate the characteristic volatile profile of samples with the different species and strains applied in this study.

Key words: elderberry juice, lactic acid fermentation, volatile compounds, HS-SPME/GC-MS, statistical analysis

1. Introduction

Elderberry (*Sambucus nigra L.)* is a native fruit-bearing shrub that grows wild on sunlight-exposed locations in several countries of Europe, Asia, North Africa and USA. In northern European countries elderberry is cultivated in small scale and its fruits are used for different industrial preparations: juices, wines, jams, jellies, and as colouring agent (Jensen et al., 2000). Elderberry juice can be used also in medicine preparations or in dietary supplements, because it is rich in sugar, organic acids and in secondary metabolites, like polyphenols, in particular anthocyanins (Veberic et al., 2009). The content of sugars and acids gives to elderberry the typical sweet and astringent taste, while its characteristic aroma is correlated to the presence of different volatile compounds (Jensen et al., 2000).

The aromatic profile of elderberry juice has been widely studied and it has been found associated to the presence of some particular volatiles such as 2-phenylethanol, phenylacetaldehyde, β-damascenone and ethyl-9-decenoate (Jensen et al., 2000; Kaack, 2008; Kaack at al., 2005; Salvador et al., 2016; Vítová et al., 2015). Other compounds such as hotrienol, linalool, and other terpenoids, can give to elderberry juice floral and floral-green notes, while the simultaneous presence of some alcohols and aldehydes, like hexanol, 1-octanol, (Z)-3-hexen-1-ol, (E)-2-hexenol, 1-hexanal and (E)-hexenal has been related to its fresh green note (Poll and Lewis, 1986). Moreover, alcohols together with some carboxylic acid esters resulted associated with its fruity odour, while the presence of carboxylic acids, ketones, alkanes and fatty acid esters is related to buttery, creamy and waxy notes (Kaack et al., 2005). However, the volatile profile is cultivar and genotype dependent and it is strongly modified by processing (Kaack, 2008; Kaack et al., 2005).

Fermentation is a well established processing technique aimed at obtaining several fruit derived products, such as alcoholic beverages, fruit vinegar, etc., but interest is growing about its possible use to modulate shelf-life extension of fruit products (Liang et al., 2017), to obtain added value

products from fruit wastes (Martinez-Avila et al., 2014), to produce natural flavour compounds (Cheetham, 2010) and to improve the nutritional quality (Marsha et al., 2014).

Among the fermentation processes, lactic acid fermentation of fruit is an innovative field whereby health, nutritional and organoleptic features of fruit can be enhanced (Di Cagno et al., 2013).

While the effect of lactic acid bacteria metabolism on the production of bioactive compounds during growth in fruit substrate is well studied (Di Cagno et al., 2011; Espirito-Santo et al., 2015; Filannino et al., 2013), the characterization of the volatile profile of the fermented juices is less investigated (Di Cagno et al., 2017, Filaninno et al., 2013). It is known that constituents of food (i.e. proteins, carbohydrates, and lipids) deliver precursors for the conversion to aroma compounds (Smid and Kleerebezem, 2014), but each microbial strain exerts different aroma-forming activities, being able to differently metabolize these substrates. Thus, LABs can be foreseen as microbial factories to produce flavour metabolites in fruit products characterized by new organoleptic properties and potential innovative nutritional value. It is therefore attractive to screen LAB isolates from different sources in order to explore the specific flavour formation capacities. Although autochthonous starters isolated from vegetal matrices are generally preferred as they are tailored for the specific plant matrix, (Di Cagno et al., 2013) the use of LABs from different origins could also widen the potential organoleptic enrichment and the possibility to produce innovative fruit products. In this study we described, for the first time, the lactic acid fermentation of elderberry juice and its effect on the volatile profile. To this aim 15 LAB strains belonging to *L. plantarum*, isolated both from plant and dairy environment, and *L. casei* and *L. rhamnosus* species, isolated from dairy environment, were used in fermentation process. To assess the aromatic potential of the strains in this matrix, the production of volatile compounds was monitored after fermentation and storage.

2. Materials and methods

2.1 Bacterial strains

Lactobacillus plantarum POM1 isolated from tomatoes, 1LE1 from pineapples, C1 from carrots, 1486 from Pecorino cheese, 285 from Brazilian cheese, *Lactobacillus rhamnosus* 1473 and 1019 from Parmigiano Reggiano cheese, 2360, 2140 and 2178 from Grana Padano cheese, *Lactobacillus casei* 2240, 2306 and 2246 from Parmigiano Reggiano cheese, 2057 and 2107 from Grana Padano cheese, were singly used as starters for fermentation. All *L. rhamnosus*, *L. casei* and two *L. plantarum* (1486 and 285) strains belong to the collection of Food and Drug Department, University of Parma, Italy; three *L. plantarum* strains (POM1, 1LE1, C1) were kindly given by the Department of Soil, Plant and Food Science, University of Bari, Italy. All bacterial strains were maintained as frozen stocks (-80 °C) in Man Rogosa Sharpe (MRS) medium (Oxoid, Milan, Italy) supplemented with 15% glycerol (w/v). The cultures were propagated three times with about 3% (v/v) inoculum in MRS and incubated in anaerobiosis (AnaeroGen, Oxoid) at 30 °C for 15 h for *L. plantarum* and at 37 °C for 15 h for *L. casei* and *L. rhamnosus*.

2.2 Fermentation and storage

A commercial pasteurized non filtered elderberry juice (EJ) was used as substrate for the fermentation process. The starter inoculum was prepared cultivating the strains until the late exponential phase (ca. 15 h), harvesting the cell by centrifugation (10,000 x g for 10 min at 4°C), washing twice with Ringer's solution (Oxoid, Mila, Italy), and finally re-suspending in sterile distilled water to a final concentration of 9.0 Log CFU/ml. Each culture were inoculated into EJ in order to reach 7 Log CFU/ml. The juice was fermented, at 30°C with *L. plantarum*, at 37°C with *L. rhamnosus* and *L. casei*, for 48 hours and after stored for 12 days at 4°C. Elderberry juice not added of starters (unstarted EJ) was incubated at 30°C and 37°C for 48 h and stored for 12 days at 4°C, and used as control.

2.3 Growth assay and pH evaluation

EJ samples were analysed at the beginning, at the end of fermentation process (48 h) and after the storage (14 days). Decimal dilutions of started EJ were made in quarter-strength Ringer solution (Oxoid, Milan, Italy) and plated in MRS agar incubating at 30 °C (when starter was *L. plantarum*) and at 37°C (when starters were *L. rhamnosus* and *L. casei*) for 48-72 h, under anaerobiosis. Microbial counts were carried out also in unstarted EJ as a control, using Plate Count Agar (PCA) medium (Oxoid, Milan, Italy) incubating at 30 °C for 48-72 h. The pH of the EJ samples was measured by pH meter (Mettler Toledo, Switzerland). Microbial counts and pH measurement were performed in triplicate.

2.4 Characterization of volatile profile of started and unstarted elderberry juices

The volatile profiles of the started and unstarted EJ were analysed at the beginning, after 48 hours (corresponding to the end of fermentation process) and after 12 days storage period. The volatile fraction of the samples was fully characterized by means of HS-SPME/GC-MS technique following the protocol reported by Dall'Asta et al. (2011) with slight modifications. Briefly, 2 ml of elderberry juice was placed in a 20 ml glass vial adding 5 µl of an aqueous toluene standard solution (100 µg/ml in 10 ml). Head space micro-extraction was performed for 30 min at 40°C after 15 min of equilibration time. For each analysis a SPME fiber coated with 50/30 µm of Divinylbenzene–Carboxen–Polydimethylsiloxane (DVB/Carboxen/PDMS) was used (Supelco, Bellefonte, PA, USA). Before the analyses, the fiber was conditioned by insertion into the GC–MS injector at 230°C for 2 min, analogously, the desorption of volatiles was accomplished exposing the fiber into the GC injector for 2 min at 230°C. GC–MS analyses were performed on a Thermo Scientific Trace 1300 gas chromatograph coupled to a Thermo Scientific ISQ single quadrupole mass spectrometer equipped with an electronic impact (EI) source. All samples were injected in splitless mode, maintaining the valve closed for 2 min. Helium was used as carrier gas with a total flow of 1 ml/min. The separation was performed on a SUPELCOWAX 10 capillary column

(Supelco, Bellefonte, PA, USA; 30 m × 0.25 mm x 0.25 µm) at programmed temperature starting from 50 °C for 3 min, increasing of 5 °C per minute to reach 200 °C and maintaining this final temperature for 12 min. The transfer line temperature was 250°C. The signal acquisition mode was full scan (from 41 m/z to 500 m/z). The main volatile compounds of elderberry juices were identified on the basis of their mass spectra compared with the library (NIST 14) mass spectra. Furthermore, in order to obtain a more confident identification, the linear retention indices (LRIs) were calculated on the basis of the retention times of a solution of C8–C20 alkanes analysed under the same conditions applied for sample analyses. The semi-quantification of all detected gas-chromatographic signals was performed on the basis of the use of an internal standard (toluene).

2.5 Chemometrics analysis

Volatile compounds concentrations were Z-transformed and used to build a heatplot in R environment (http://www.r-project.org), by using the cim function in mixOmics package. All data obtained as relative concentration of each detected volatile, calculated on the basis of reference standard (toluene), were statistically elaborated using SPSS Statistics 21.0 software (SPSS Inc., Chicago, IL). Principal component analysis (PCA) and Classification Trees (CT) were performed. Principal component analysis was performed using covariance matrix and the classification tree was grown with Gini criterion and miss-classification rates were obtained by a cross-validation leaving out 5 % of the samples.

In addition, one way ANOVA was used to compare the different results obtained for the samples fermented with *L. plantarum* species isolated from dairy and vegetable sources. In this case Tukey test was adopted and the results were considered different when $p < 0.05$.

3.Results and discussion

3.1 Fermentation and acidification of elderberry juice

A total of fifteen strains of three different species (*L. plantarum*, *L. rhamnosus* and *L. casei*) were used for single fermentation of elderberry juice. After 48 hours all the *L. plantarum* and *L. rhamnosus* strains, as well as *L. casei* 2246, 2107,2240 grew of about two log cycle (from ca. 7.20 ± 0.19 to 9.25 ± 0.22 Log CFU/ml) (Fig.1). On the other hand, cell densities of *L. casei* 2306 and 2057 remained unchanged from the original inocula. Considering the period of cold storage, cells viability remained stable in all fermented juices, with exception of *L. casei* 2306 which decreased of ca. 2 .0 Log CFU/ml after 12 days at 4°C (Fig. 1c). Lactic acid fermentation leads to only a moderate decrease of pH value, on account of the already low initial one (ca. 3.84). In particular, the pH value reached 3.6 ± 0.1 (mean value) at the end of fermentation (48h) and remained almost unchanged after 12 days of storage (3.58 ± 0.08). The different growth performance observed were ascribed to the different adaptability of the strains in stressful matrices and are in agreement with previous works. Despite this is the first study on fermented elderberry juice, other authors reported similar results, in terms of growth during fermentation and storage of fruit juices, with *L. plantarum* used as a starter (Filaninno et al., 2013; Mousavi et al., 2010). Differently from these studies, we tested both plant and dairy isolates and no difference in the adaptation to elderberry juice were observed among the strains. Compared to *L. plantarum*, few studies have explored the use of *L. casei* and *L. rhamnosus* to ferment a fruit substrate (Espirito-Santo et al., 2015; Pakbin et al., 2014). Indeed, these probiotic species were more frequently supplemented to fruit juices, which became vehicles of healthful microorganism, without a fermentation step, and generally only the viability during the storage was monitored. In this study we screened a wide set of strains, and we showed that not only *L. plantarum*, which is known to tolerate fruit environment, was able to ferment elderberry juice but also different strains of *L. rhamnosus* and *L. casei*. Interestingly, the *L. rhamnosus* and *L. casei* strains used in this work are all isolated from artisanal cheese, they overall showed a great ability to ferment the substrate and to maintain their viability during storage, differently from Espirito-Santo et al. (2015) which observed a better growth performance of commercial strains

3.2 Characterization of the volatile profile of fermented elderberry juice

The characterization of volatile composition of elderberry juice, both started and unstarted, was performed by HS-SPME/GC-MS technique. A total of 82 different compounds were detected in the headspace of all the considered samples. The fully identification of all the detected volatiles is reported in Table 1.

For the evaluation of the aromatic profile, not inoculated elderberry juice, maintained at 30°C and 37°C for 48 h and stored at 4°C for 12 days, was used as reference for all the fermented samples. The analyses were performed on both reference and inoculated samples after 48 h from the beginning of the fermentation process and after storage at 4°C.

The aromatic profile of fermented elderberry juice was mainly characterized by the following classes: alcohols (17.2%-52.3%), terpenes and norisoprenoids (17.2%-32.2%), organic acids (3.4%-41.1%), ketones (3.0%-28.5%), esters (0.95-5.6%) and, at a lower rate, by aldehydes (0.05% - 1.2%) (Fig.3, Table S1 and Table S2).

Figure 2, which reported the overall aromatic profile of each strain tested, highlights the different behaviour even among the strains belonging to the same species. The concentration of total volatile compounds ranged between 1.9 µg/ml (*L. rhamnosus* 2178) and 4.4 µg/ml (*L. plantarum* 285) after the fermentation and between 1.3 µg/ml (*L. rhamnosus* 1019) and 6.6 µg/ml (*L. plantarum* 285) after the storage.

Interestingly, the highest concentration of total volatiles was found in EJ fermented with *L. plantarum* 285, a dairy isolates. Moreover, at the end of the storage almost all dairy isolates had a higher concentration of volatile compounds compared to plant isolates. Another recent study proposed the use of strains isolated from different sources to improve flavor in fermented foods, specifically plant isolates for milk fermentation (Alemayehu et al., 2014). The use of strains not adapted to a specific niche could lead to induce different mechanism of stress response and it is

known that under stress condition food-related LAB enhance the production of aroma compounds (Papadimitriou et al., 2016).

Even if the microbial growth was similar in all strains during the fermentation and storage, a different trend in the production of volatile compounds were observed among the three species: elderberry juice fermented with *L. plantarum* strains showed an increase of total volatile compounds after 48 hours (compared to its control at 30°C) while for *L. casei* and *L. rhamnosus* a larger increase was evident upon storage (compared to its control at 37°C) (Fig.2).

Differently from all the other classes, ketones increased in all fermented juices both after fermentation and storage. In particular, total volatile ketones reached the value of 1.01 µg/ml in *L. rhamnosus* 2360 after 48 h and 1.96 µg/ml in EJ fermented with *L. rhamnosus* 2178 after storage (Fig. 2, Table S1 and Table S2). Diacetyl and acetoin were the ketones identified in greatest quantity. Also other authors (Di Cagno et al., 2017; Filannino et al., 2014), which analysed the volatile compounds in vegetable and fruit juices started with *L. plantarum* strains, found a high synthesis of diacetyl in several fermented juices. In this study the overall concentration of diacetyl was higher in EJ fermented with *L. casei* and *L. rhamnosus* compared to EJ fermented with *L. plantarum*, with exception of *L. plantarum* 1486, isolated from Pecorino cheese. The molecule of diacetyl can be bio-synthesized starting from citrate (Jyoti et al., 2003, 2004; Branen et al., 1971) present in many substrates such as fruits, even if it is usually associated with creamy and buttery note in dairy products (Curioni and Bosset, 2002). Acetoin could derive from the same pathway and small amounts of this compound have been also found in not fermented elderberry juice (Kaak et al., 2005).

Overall, an increase in organic acids concentration was observed after 48 hours using *L. plantarum* strains as starter (from 0.49 µg/ml to 1,32 µg/ml) and after storage period (from 0,11 µg/ml to 0,93 µg/ml) when *L. rhamnosus* and *L. casei* strains were used (Fig.2, Table S1 and Table S2). The most abundant acids detected in fermented juices were acetic acid and isovaleric acid. The first markedly increased in all fermented juices and it was produced mainly by *L. plantarum* strains especially by

L. plantarum 285 (1.18 µg/ml) after storage. Also Filannino et al. (2014) reported a markedly increased of acetic acid in fermented cherry, pineapple, carrot and tomato juices started with *L. plantarum* strains correlating its production with the acetate kinase route of phosphogluconate pathway. The production of acetic acid could be also related to the metabolism of citric acid, an organic acid present in good abundance in elderberry juice (Veberic et al., 2009). On the other hand, the increase of isovaleric acid, observed especially after the storage in juices fermented with dairy isolates, is probably due to the catabolism of leucine, that is present in elderberry fruit (Kunsch and Temperli, 1978).

After two days of fermentation, alcohols increased in all EJ fermented with *L. plantarum* strains (0.21 µg/ml - 1.49 µg/ml compared to the control) and in two *L. rhamnosus* (1.07 µg/ml in *L. rhamnosus* 1473 and of 0.44 µg/ml in *L. rhamnosus* 2360). After storage, an opposite trend was observed: only *L. plantarum* 4186 and 285, both dairy strains, showed higher amounts of alcohol compared to the control, whereas in all *L. casei* and *L. rhamnosus* a significant increase was shown (Fig. 2, Table S1 and Table S2). The most concentrated alcohols identified in fermented juices were: hexanol, 3-hexen-1-ol (Z), 2-hexen-1-ol (E), ethanol, 2-phenylmethanol, 2-phenylethanol and isoamyl alcohol. Hexanol, 3-hexen-1-ol (Z) and 2-hexen-1-ol (E), which are associated to green notes and represent the characteristic odour of elderberry juice; the highest amount occurred in EJ fermented with the strains 285 and 1473. A previous work reported an increase of these compounds in fermented table olives inoculated with *L. plantarum* strain, derived from linoleic and linolenic acids through lipoxygenase pathways (Sabatini et al., 2008) and due to the presence of fatty acids in the elderberry seeds (Dulf e al., 2013; Fazio et al., 2013), a similar metabolic conversion can be supposed.

Concerning the other alcohols produced, while the ethanol is produced during sugar degradation, 2-phenylmethanol, 2-phenylethanol and isoamyl alcohol, namely “fusel alcohol”, are originated by the catabolism of amino acids. In particular, isoamyl alcohol production is related with the catabolism of leucine by lactic acid bacteria (Smit et al., 2005). The increase of isoamyl alcohol and

the decrease of leucine was also reported during the fermentation with *L. plantarum* strains of carrot and tomato juices (Filaninno et al., 2014). On the other hand, 2-phenylmethanol and 2-phenylethanol, well-known constituent of elderberry juice with floral-like odour, were formed from the catabolism of phenylalanine.

Even though the concentration of esters did not show a wide increase in fermented juices after fermentation and storage (Fig.2, Table S1 and Table S2), the prevailing esters, as ethyl acetate, methyl isovalerate, isoamyl isovalerate, methyl salicylate, were characterized by positive fruity notes. In particular, isoamyl isovalerate (apple flavour), probably synthesized by the esterification of isoamyl alcohol and isovaleric acid, was high with *L. plantarum* 1LE1, after fermentation, and with *L. plantarum* 285, after storage.

Interestingly, among the class of terpenes and norisoprenoids, we observed an increase of limonene, β-linalool, β-damascenone and eugenol during fermentation and storage, especially when dairy isolates were used (Fig.2, Table S1 and Table S2). Indeed, β-linalool and β-damascenone reached the highest value in EJ fermented with strains from dairy origin, as *L. plantarum* 285 and *L. rhamnosus* 1473. The microbial activity determined a sharp increase of eugenol, in all fermented juices. The increase of these compound could be related with the ability of micro-organisms to produce glycosylases (Di Cagno et al., 2013, 2017; Sanni et al. 2002), able to cut the bond between terpenes and sugars, or to their possible ex novo production (Belviso et al., 2011; Yamada et al., 2015).

Finally, aldehyde class resulted fundamentally represented by isovaleraldehyde, with its characteristic ethereal and aldehydic notes, present in all fermented and stored samples but in lower quantities in respect to the unstarted juice (Table S1 and Table S2).

In order to highlight different behaviour between dairy and no dairy isolates, data collected from five *L. plantarum* strains were further analysed (Fig. 3). Significant differences in volatile compounds, especially after storage, were observed by ANOVA test as showed in Fig. 3. The variables with significant differences between the two groups of isolates were mainly ascribed to

compounds belonging to the class of terpenes and norisoprenoids, alcohols and ketones. To note, most of these compounds, (i.e. β-linalool, β-damascenone, 2-phenylethanol, hexanol, 3-hexen-1-ol) determined the characteristic aroma profile of elderberry (Kaack, 2008; Kaack at al., 2005; Poll and Lewis, 1986). Basing of these data, we can suggest that lactic fermentation of elderberry juice, employing dairy isolates, could be a valuable strategy to enrich or replace the original fruit aroma which often is compromised by processing.

3.3 Chemometrics analysis

Hierarchical cluster analysis was used as a preliminary method to assess if the volatile compounds profile associated to each sample could drive the clustering of the samples according to their Euclidean distance. As shown in the deriving heat-map (Fig. 4), the samples clustered according to the specific metabolic profile of the strains rather than to the species (leftmost bar) or the different stages of fermentation (48 h) or storage (12 days). The first cluster of the heat-map is characterised by strains that, upon a prolonged storage, are capable to develop a wide range of volatile compounds. The second cluster is represented by strains that are capable to synthetize a higher concentration of a certain compounds during fermentation, particularly alcohols terpenes and norisoprenoids, and in the case of strains 1473 and 285 the concentration remains high during storage. The third cluster groups together the strains that produces higher amounts of certain esters and ketons after 48 hours of fermentation. Finally, we have a cluster of samples with a lower concentration of volatile compounds, characterised by strains that show a high aromatic potential after 48 hours of fermentation (clusters II and III), but after a prolonged storage present a reduction of most of the volatile compounds.

In order to obtain more information on the parameters that could influence the differences and the similarities among the fermented EJ, the data collected from HS-SPME/GC-MS analysis were used as variable vectors for chemometric analysis. In particular, an unsupervised pattern recognition method (Principal Component Analysis, PCA) and a supervised technique (Classification tree, CT)

were used starting from the total volatile compounds dataset. Two separate analysis were performed to describe EJ after fermentation and storage. Starting from the total volatile compounds, two preliminary PCA were carried out to identify those variables that had higher influence on the characterization of fermented juices (Table 2 and Table 3) and consequently two final PCA were performed using only the previously selected variables.

In figure 5a and 5b the PCA score and loading plots related to samples after 48h of fermentation were reported. Applying PCA on the covariance matrix, two components were extracted representing together the 95% of total variance; component 1 explained the 56% of the variance while the last 39% was explained by component 2. The score plot showed that all the EJ started with *L. plantarum* strains isolated from fruit and vegetable (1LE1, POM1, C1), were clearly separated from the other samples and characterized by the presence of acetic acid. Also the EJ inoculated with *L. plantarum* 285 (dairy) was separated from the remaining samples, because characterized especially by the presence of alcohols, terpenes and norisoprenoids. The remaining EJs reported in the score plot (including controls, fermented juices with dairy isolates of *L. casei*, *L. rhamnosus* and *L. plantarum*) were characterized by the presence of ethyl acetate, ethyl isovalerate and isovaleric acid (Fig.5a, 5b and Table 2).

After storage an evolution in volatile profile was observed and the distribution of samples in the score plot was different from the 48h of fermentation (Fig. 5c). The first two PCA component accounted the 90% of the total variance, with component 1 and component 2 describing 54% and 36% of the variability, respectively. In particular, *L. rhamnosus* 1473 and *L. plantarum* 285 were characterized by compound with negative values on component 1, such as hexanol and (E)-2-hexen-1-ol (Fig. 5c, 5d and Table 3). On the other hands the variables with strongly positive values on the first component (3-carene, acetoin, isovaleric acid, ethyl acetate, diacetyl, methyl isovalerate, m-cymene, and phenol) were determinant to grouping the juices started with *L. casei* 2240, *L. plantarum* 4186, *L. rhamnosus* 2140 and 2178. The third group that we can observe in the score plot was composed by EJs fermented with *L. plantarum* strains (C1, POM1 and 1LE1), *L.*

rhamnosus (1019 and 2360) *L. casei* (2107 and 2246) and the controls, characterized by variables with values close to zero.

From the analysis of EJ after the fermentation period (48h) it can be observed that the increase of specific volatile compounds can be ascribed not only to the different species of LAB used for fermentation, but also to the specific strains and to their origin. Even if the disposition of samples on the score plot changed after the storage period, EJs started with *L. plantarum* 1LE1, POM1 and C1 (fruit and vegetable isolates) still remain separated from the EJ inoculated with *L. plantarum* 285 and 4186 (dairy isolates). In *L. plantarum* 285 fermented juice hexanol and (E)-2-hexen-1-ol remained determinant compounds for its discrimination also after the storage.

Subsequently, a classification model was applied in order to classify the different samples on the basis of the species used for the fermentation process. In particular, Classification Trees (CT) model was adopted using the concentration of the 82 volatile compounds detected as independent variables and considering as the dependent variables the 4 groups obtained dividing the samples according to the species used (1 = unstarted samples, 2 = samples started with *L. plantarum*, 3 = samples started with *L. casei*, 4 = samples started with *L. rhamnosus*). Two binary trees were built using Gini partitioning criterion: one considering samples analysed after the fermentation step (48 h) and the other one taking into account samples analysed after storage. The prediction ability of the model built to distinguish the samples submitted to fermentation process (48 h) was 87,4 % (Figure 6 A). The most significant variables in the classification were (in order of importance): acetic acid, methyl salicylate and isovaleraldehyde. In particular, acetic acid contributed to the separation between samples fermented using *L. plantarum* species from all the other samples (acetic acid > 16,9 %), while methyl salicilate distinguished the group formed by *L. rhamnosus* started juices from the other groups. Finally, the different amount of isovaleraldehyde contributed to the separation between control samples and *L. casei* started juices.

A separate CT was built considering the samples analysed after storage (Figure 6 B). In this case, a prediction ability value of 89.7 % was achieved. The most important variables in the classification

in order of importance were acetic acid, acetone, a terpenic compound and isovaleraldehyde. Also in this case acetic acid was effective in the separation between samples fermented with *L. plantarum* species from the other samples, but a better classification was achieved with the contribute of acetone, that concurred in the discrimination between group 2 and group 3. The terpenic compound was effective in the classification of the group formed by samples started with *L. rhamnosus* strains, while isovaleraldehyde contributed to the separation between control samples and *L. casei* started juices, as observed in the first case.

4. Conclusion

In this study we explored, for the first time, the lactic acid fermentation of elderberry juice (EJ).

We screened strains belonging to different species and, differently from other works focused on fruit juice fermentation, we focused the attention on the aromatic potential of strains isolated also from dairy environment. Despite bacterial growth represents one of the challenges for fruit juice fermentation, an optimal growth performance was observed after 48 hours, especially for *L. plantarum* and *L. rhamnosus*, which could be the best candidate for a potential starter. Furthermore, the EJ fermented with the first species reached an increase in total volatile compounds after 48 hours, while the latter species exhibited a larger production during storage.

The chemometric analysis permitted to better highlight the differences among the fermented EJs and the metabolic behaviour of the strains used. In particular, most of the volatiles associated with typical aroma of elderberry (terpenes and alcohols) were enriched after fermentation and storage with dairy isolates. Among these, one strain, *L. plantarum* 285, has shown interesting features, in terms of total aromatic potential as well as in the type of volatiles compounds produced. Finally, by CT analysis it was possible to relate the characteristic volatile profile of samples with the different species. In conclusion, beyond the knowledge acquired on a new fermentation substrate, elderberry juice, this study remarks the importance to explore the potential of strains isolated from non-plant niches in the field of lactic acid fermentation of fruit.

Acknowledgement

Authors acknowledge the Department of Soil, Plant and Food Science, University of Bari, Italy for providing *L. plantarum* strains (POM1, 1LE1, C1).

References

Alemayehu, D., Hannon, J.A., McAuliffe, O., Ross, R.P. (2014). Characterization of plant-derived lactococci on the basis of their volatile compounds profile when grown in milk. *International Journal of Food Microbiology*, 172, 57-61.

Belviso, S., Giordano, M., Dolci, P.,Zeppa, G. (2011). Degradation and biosynthesis of terpenoids by lactic acid bacteria isolated from cheese: first evidence. *Dairy Science & Technology*, 91, 227-236.

Bianchi, F., Careri, M., Mangia, A., Musci, M. (2007). Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: database creation and evaluation of precision and robustness. *Journal of Separation Science*, 30, 563-572.

Branen, A.L., Keenan, T.W. (1971). Diacetyl and Acetoin Production by *Lactobacillus casei*. *Applied Microbiology*, 22, 517-521.

Cavalli, J.F., Tomi, F., Bernardini, A.F., Casanova, J. (2003). Composition and chemical variability of the bark oil of *Cedrelopsis grevei H. Baillon* from Madagascar. *Flavour and Fragrance Journal*, 18, 532-538.

Cheetham, P. S. J., 2010. *Natural sources of flavours*. In Taylor, A. J.; Linforth, R. S. T. (Eds.), *Food Flavour Technology* (pp.127-177). Oxford: Wiley-Blackwell.

Chisholm, M.G., Wilson, M.A., and Gaskey, G.M. (2003). Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia Swingle*). *Flavour and Fragrance Journal*, 18, 106-115.

Choi, H.-S. (2003). Character impact odorants of citrus hallabong [(C. unshiu Marcov x C. sinensis Osbeck) x C. reticulata Blanco] cold-pressed peel oil. *Journal of Agricultural and Food Chemistry*, 51, 2687-2692.

Chung, T.Y., Eiserich, J.P., and Shibamoto, T. (1993). Volatile compounds isolated from edible Korean chamchwi (*Aster scaber Thunb*). *Journal of Agricultural and Food Chemistry*, 4, 1693-1697.

Cirlini, M., Dall'Asta, C., Silvanini, A., Beghè, D., Fabbri, A., Galaverna, G., Ganino, T. (2012). Volatile fingerprinting of chestnut flours from traditional Emilia Romagna (Italy) cultivars. *Food Chemistry*, 134, 662-668.

Cirlini, M., Mena, P., Tassotti, M., Herrlinger, K.A., Nieman, K.M., Dall'Asta, C., Del Rio, D. (2016). Phenolic and Volatile Composition of a Dry Spearmint (*Mentha spicata L.*) Extract. *Molecules*, 21 (8), 1007.

Cullere, L., Escudero, A., Cacho, J., Ferreira, V. (2004). Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry*, 52, 1653-1660.

Curiel, J.A., Pinto, D., Marzani, B., Filannino, P., Farris, G.A., Gobbetti, M., Rizzello, C.G. (2015). Lactic acid fermentation as a tool to enhance the antioxidant properties of *Myrtus communis* berries. *Microbial Cell Factories*, 14:67.

Curioni, P.M.G., Bosset, J.O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal*, 12, 959–984.

Dall'Asta, C., Cirlini, M., Morini, E., Galaverna, G. (2011). Brand-dependent volatile fingerprinting of Italian wines from Valpolicella. *Journal of Chromatography A*, 1218, 7557-7565.

Di Cagno, R., Coda, R., De angelis, M., Gobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology*, 33, 1-10.

Di Cagno, R., Filaninno, P., Gobbetti, M. (2017). Lactic acid fermentation drives the optimal volatile flavor-aroma profile of pomegranate juice. *International Journal of Food Microbiology*, 248, 56-62.

Di Cagno, R., Minervini, G., Rizzello, C.G., De Angelis, M., Gobbetti, M. (2011). Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. *Food Microbiology*, 28, 1062-1071.

Dulf, F.V., Oroian, I., Vodnar, D.C., Carmen Socaciu, C., Pintea, A. (2013). Lipid Classes and Fatty Acid Regiodistribution in Triacylglycerols of Seed Oils of Two *Sambucus* Species (*S. nigra L.* and *S. ebulus L.*). *Molecules*, 18, 11768-11782.

Espirito-Santo, A.P., Carlin, F., Renard, C.M.G.C. (2015). Apple, grape or orange juice: Which one offers the best substrate for lactobacilli growth?—A screening study on bacteria viability, superoxide dismutase activity, folates production and hedonic characteristics. *Food Research International*, 78, 352–360.

Fazio, A., Plastina, P., Meijerink, J., Witkamp, R.F., Gabriele, B. (2013). Comparative analyses of seeds of wild fruits of *Rubus* and *Sambucus* species from Southern Italy: Fatty acid composition of the oil, total phenolic content, antioxidant and anti-inflammatory properties of the methanolic extracts. *Food Chemistry*, 140, 817–824.

Ferreira, V., Aznar, M., Lopez, R., and Cacho, J. 2001. Quantitative gas chromatography-olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry*, 49, 4818-4824.

Filannino, P., Cardinali, G., Rizzello, C.G., Buchin, S., De Angelis, M., Gobbetti, M., Di Cagno, A. (2014). Metabolic response of *Lactobacillus plantarum* strains during fermentation and storage of vegetable and fruit juices. *Applied and Environmental Microbiology*, 80, 2206-2215.

Filannino, P., Azzi, L., Cavoski, I., Vincentini, O., Rizzello, C.G., Gobbetti, M., Di Cagno, R. (2013). Exploitation of the health-promoting and sensory properties of organic pomegranate (*Punica granatum L.*) juices through lactic acid fermentation. *International Journal of Food Microbiology*, 163, 184-192.

Goodner, K.L. (2008). Practical retention index models of OV-101, DB-1, DB-5, and DB-Wax for flavor and fragrance compounds. *LWT-Food Science and Technology*, 41, 951–958.

Jensen, K., Christensen, L.P., Hansen, M., Jørgensen U., Kaack K. (2000). Olfactory and quantitative analysis of volatiles in elderberry (*Sambucus nigra L.*) juice processed from seven cultivars. *Journal of the Science of Food and Agricolture*, 81, 237-244.

Jorgensen, U., Hansen, M., Christensen, L.P., Jensen K., Kaack, K., 2000. Olfactory and Quantitative Analysis of Aroma Compounds in Elder Flower (*Sambucus nigra L.*) Drink Processed from Five Cultivars. *Journal of Agricultural and Food Chemistry*, 48, 2376-2383.

Jyoti, B.D., Suresh, A.K., Venkatesh, K.V. (2003). Diacetyl production and growth of *Lactobacillus rhamnosus* on multiple substrates. *World Journal of Microbiology and Biotechnology*, 19, 509-514.

Jyoti, B.D., Suresha, A.K., Venkatesha, K.V., 2004. Effect of preculturing conditions on growth of *Lactobacillus rhamnosus* on medium containing glucose and citrate. *Microbiological Research*, 159, 35-42.

Kaack, K. (2008). Aroma composition and sensory quality of fruit juices processed from cultivars of elderberry (*Sambucus nigra L.*). *European Food Research and Technology*, 227, 45-56.

Kaack, K., Christensen, L.P., Hughes, M., Eder, R. (2005). The relationship between sensory quality and volatile compounds in raw juice processed from elderberries (*Sambucus nigra L.*). *European Food Research and Technology*, 221, 244-254.

Künsch, U., Temperli, A. (1978). Changes in free and protein-bound amino acids in elderberry fruit (*Sambucus nigra*) during maturation. *Journal of Agricultural and Food Chemistry*, 29, 1037-1040.

Liang, M., Min, Z., Bhesh, B., Zhongxue, G. (2017). Recent developments in novel shelf life extension technologies of fresh-cut fruits and vegetables. *Trends in Food Science and Technology*, 64, 23-38.

Marsha, A., J., Hill, C., Ross, R. P., Cottera, P. D. (2014). Fermented beverages with health-promoting potential: Past and future perspectives. *Trends in Food Science & Technology*, 38, 113-124.

Martinez-Avila, G. C. G., Aguilera, A. F., Saucedo, S., Rojas, R., Rodriguez, R., Aguilar, C. N. (2014). Fruit Wastes Fermentation for Phenolic Antioxidants Production and Their Application in Manufacture of Edible Coatings and Films. *Critical Reviews in Food Science and Nutrition*, 54, 303-311.

Mousavi, Z.E., Mousavi, S.M., Razavi, S.H., Emam-Djomeh, E., Kiani, H. (2010). Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World Journal of. Microbiology and Biotechnology*, 27, 123-128.

Nishimura, O. (1995). Identification of the characteristic odorants in fresh Rhizomes of ginger (*Zingiber officinale Roscoe*) using aroma extract dilution analysis and modified multidimensional gas chromatography-mass. *Journal of Agricultural and Food Chemistry*,43, 2941-2945.

Ong, P.K.C., Acree, T.E. (1999). Similarities in the aroma chemistry of Gewürztraminer variety wines and Lychee (*Litchi chinesis Sonn.*) fruit. *Journal of Agricultural and Food Chemistry*, 47, 665-670.

Pakbin, B., Razavi, S.H., Mahmoudi, R., Gajarbeygi, P. (2014). Producing Probiotic Peach Juice. *Biotechnology and Health Science*, 1(3).

Papadimitriou, K., Alegría, A., Bron, P.A., de Angelis, M., Gobbetti, M., Kleerebezem, M., Lemos, J.A., Linares, D.M., Ross, P., Stanton, C., Turroni, F., van Sinderen, D., Varmanen, P., Ventura, M., Zúñiga, M., Tsakalidou, E., Kok, J. (2016). Stress Physiology of Lactic Acid Bacteria. *Microbiology and Molecular Biology Reviews*, 80, 3837-890.

Pennarum, A.L., Prost, C., Demaimay, M. (2002). Identification and origin of the character- impact compounds of raw oyster *Crassostrea gigas*. *Journal of the Science of Food and Agricolture*, 82, 1652-1660.

Perricone, M., Bevilacqua, A., Altieri, C., Sinigaglia, M., Corbo, M.R. (2015). Challenges for the Production of Probiotic Fruit Juices. *Beverages* 1(2), 95-103.

Poll, L., Lewis, M.J. (1986). Volatile components of elderberry juice. *Lebensmittel-Wissenschaft & Technologie*, 19, 258-262.

Qian, M., Reineccius, G. (2003). Potent aroma compounds in Parmigiano Reggiano cheese studied using a dynamic headspace (purge-trap) method. *Flavour and Fragrance Journal*, 18, 252-259.

Rodriguez, H., Curiel, J.A., Landete, J.M., De Las Rivas, B., De Filipe, F.L., Gomez-Cordoveves, C., Mancheno, J.M., Munoz, R. (2009). Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology*, 132, 79-90.

Sabatini, N., Mucciarella, M.R., Marsilio, V. (2008). Volatile compounds in uninoculated and inoculated table olives with *Lactobacillus plantarum* (*Olea europea* L., cv. Moresca and Kalamata). *LWT-Food Science and Technology*, 41, 2017-2022.

Salvador, A.C., Rudnitskaya, A., Silvestre, A.J.D., Rocha, S.M. (2016). Metabolomic-Based Strategy for Fingerprinting of *Sambucus nigra L.* Berry Volatile Terpenoids and Norisoprenoids: Influence of Ripening and Cultivar. *Journal of Agricultural and Food Chemistry*, 64 5428-5438.

Sanni, A.I., Morlon-Guyot, J., Guyot, J.P. (2002). New efficient amylase-producing strains of *Lactobacillus plantarum* and *L. fermentum* isolated from different Nigerian traditional fermented foods. *International Journal of Food Microbiology*, 72, 53-62.

Sanz, C., Ansorena, D., Bello, J., and Cid, C.C. (2001). Optimizing headspace temperature and time sampling for identification of volatile compounds in ground roasted Arabica coffee. *Journal of Agricultural and Food Chemistry*, 49,1364-1369.

Smid, E.J., Kleerebezem, M. (2014). Production of Aroma Compounds in Lactic Fermentations. *Annual Review of Food Science and Technology*, 5, 313-26.

Smit, G., Bart A. Smit, B.A., Engels, W.J.M. (2005). Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiology Reviews*, 29, 591-610.

Umano, K., Nakahara, K., Shoji, A., and Shibamoto, T. (1999). Aroma chemicals isolated and identified from leaves of Aloe arborescens Mill. var. natalensis Berger. *Journal of Agricultural and Food Chemistry*, 47, 3702-3705.

Valim, M.F., Rouseff, R.L., Lin, J. (2003). Gas chromatographic-olfactometric aharacterization of aroma compounds in two types of cashew apple nectar. *Journal of Agricultural and Food Chemistry*, 51, 1010-1015.

Veberic, R., Jakopic, J., Stampar, F., Schmitzer V. (2009). European elderberry (*Sambucus nigra L.*) rich in sugars, organic acids, anthocyanins and selected polyphenols. *Food Chemistry*, 114, 511-515.

Vítová, E., Sůkalová, K., Mahdalová, M., Butorová, L., Babák, L., Matějíček, A. (2015). Comparison of flavour and volatile flavour compounds of mixed elderberry juices. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 63, 20.

Yamadaa, Y., Kuzuyama, T., Komatsua, M., Shin-ya, K., Satoshi Omura, S., David E. Cane D.S., Ikedaa, H. (2015). Terpene synthases are widely distributed in bacteria. *Proceedings of the National Academy of Sciences*, 112, 857-862.

www.flavornet.org

FIGURE CAPTIONS

Fig. 1. Viable cells concentration (Log CFU/ml) of strains, isolated from plant matrices (*L. plantarum* POM1, C1, 1LE1) and from dairy products (*L. plantarum* 4186, 285, *L. rhamnosus* 2178, 2140, 2360, 1473, 1019 and *L. casei* 2246, 2306, 2057, 2107, 2240), during fermentation of elderberry juice at 30°C and 37°C for 48 hours and further storage for 12 days. Data are the mean values of three different independent experiment ± standard deviation.

Fig. 2. Total volatile compounds and volatile profile, express in µg/ml, (alcohols, ketones, terpenes and norisoprenoids, acids and esters) of elderberry juices inoculated with *L. plantarum* (4186, POM1, C1, 1LE1, 285), *L. casei* (2107, 2240, 2246), *L. rhamnosus* (2360, 2178, 2140, 1473,1019) and unstarted juice (incubated at 30°C and 37°C) used as control. Different bars colour refer to 48 hours of fermentation (■) and further 12 days of refrigerate storage (■).

Fig. 3. Statistically significant different concentrations (express as µg/ml) of diacetyl (■), limonene (■), hexanol (■), (Z)-3-hexen-1-ol (■), β-linalool (□), α-terpineol (■), β-damascenone (■), 2-phenylmethanol (■), 2-phenylethanol (■), eugenol(■) between elderberry juices started with *L. plantarum* strains isolated from plant (no dairy) and from cheeses (dairy) after storage period. Statistical differences (p values < 0,05) between volatile compounds of elderberry juices started with dairy and no dairy strains were determined with ANOVA test.

Fig.4. Hierarchical clustering analysis and heat-map visualization of the volatile profiles of the elderberry juice fermented with the 15 *Lactobacillus* strains based on the Euclidean distance of the amount of each measured compound. The color scale represents the scaled abundance of each variable, denoted as d2 (squared Euclidean distance), with red indicating high abundance and blue indicating low abundance. The identification code of the strains is reported, together with a number

referring to the fermented samples (48h), or the samples measured after further storage (12d). The column bar is colored according to the species of the strains used in fermentation, the identified clusters are indicated with roman numbers. The compounds (C_) represented in the heat-map are numbered according to their peak numbers, as detailed in Table 1.

Fig.5. Principal component analysis (PCA), score plot and loading plot, based on volatile compounds with higher influence on elderberry juices characterization, identify after 48 hours (a and b) and after storage (c and d) in *L. plantarum* (POM1, C1, 1LE1, 4186 and 285), *L. casei* (2246, 2240, 2107), *L. rhamnosus* (1019, 1473, 2360, 2140 and 2178) fermented juices and in unstarted elderberry juice (elder 30°C, elder 37°C). Loading plots (b and d) show different variables: ea, ethyl acetate; di, diacetyl; mi, methyl isovalerate; ca, 3-carene; li, limonene; mc, m-cymene; ac, acetoin; oc, 2,3-octanedione; pe, 2-penten-1-ol; he, hexanol; ha, (E)-2-hexenyl-acetate; h3, (Z)-3-hexen-1-ol; ot, 3-octanol; h2, (E)-2-hexen-1-ol; lo, cis-linalol oxide; oe, 1-octen-3-ol; βl, β-linalool; ho, hotrienol; mh, 6-methyl-heptanol; dc, dihydro-citronellol; mo, 6-methyl-octanol; fu, 2-furanmethanol; ia, isovaleric acid; αt, α-terpineol; do, 3,4-dimetil octane; ms, methyl salicylate; pa, phenethyl acetate; cg, cis-geraniol; βd, β-damascenone; tg, trans-geraniol; tr, 1,2,4-Trimethyl-1H-indene; pm, 2- phenylmethanol; ph, 2-phenylethanol; cap, caproic acid like; dod, dodecanol; cl, cis-lanceol; phe, phenol; capr, caprylic acid; eu, eugenol; ei, ethyl isovalerate; hep, heptanol; ace, acetic acid; ep, 4-ethyl-phenol.

Fig. 6. Classification tree analysis (CT) performed on total volatile compounds, obtained after 48 hours (A) and further 12 days of refrigerate storage (B) of elderberry juice started with *L. plantarum* (POM1, C1, 1LE1, 4186 and 285), *L. casei* (2246, 2240,2107), *L. rhamnosus* (1019, 1473, 2360, 2140 and 2178) and unstarted juice. CT was carried out using CRT's method.

Figure 1

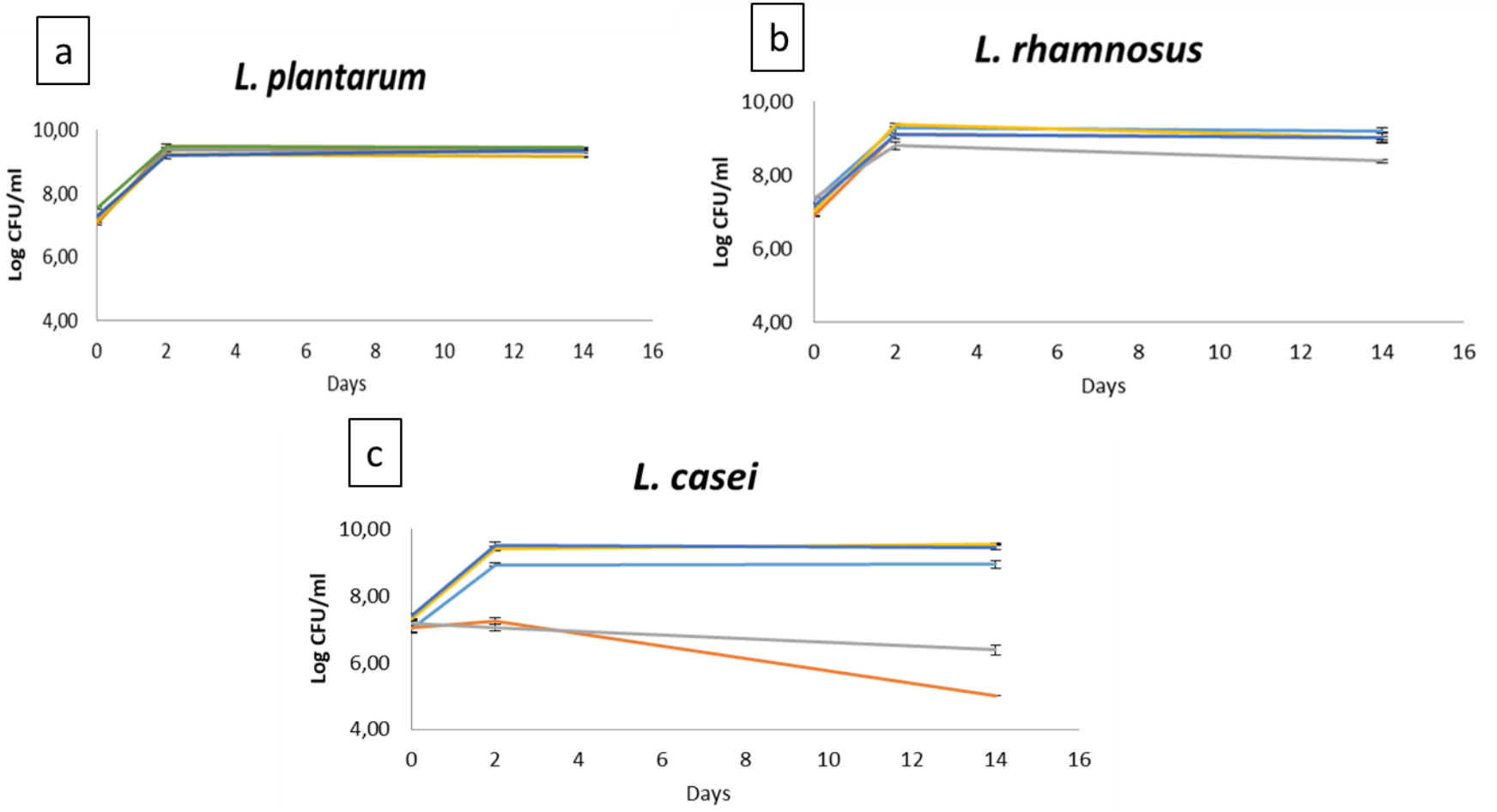

a
L. plantarum
Log CFU/ml
10,00
8,00
6,00
4,00
0
2
4
6
8
10
12
14
16
Days
b
L. rhamnosus
Log CFU/ml
10,00
8,00
6,00
4,00
0
2
4
6
8
10
12
14
16
Days
c
L. casei
Log CFU/ml
10,00
8,00
6,00
4,00
0
2
4
6
8
10
12
14
16
Days

Figure 2

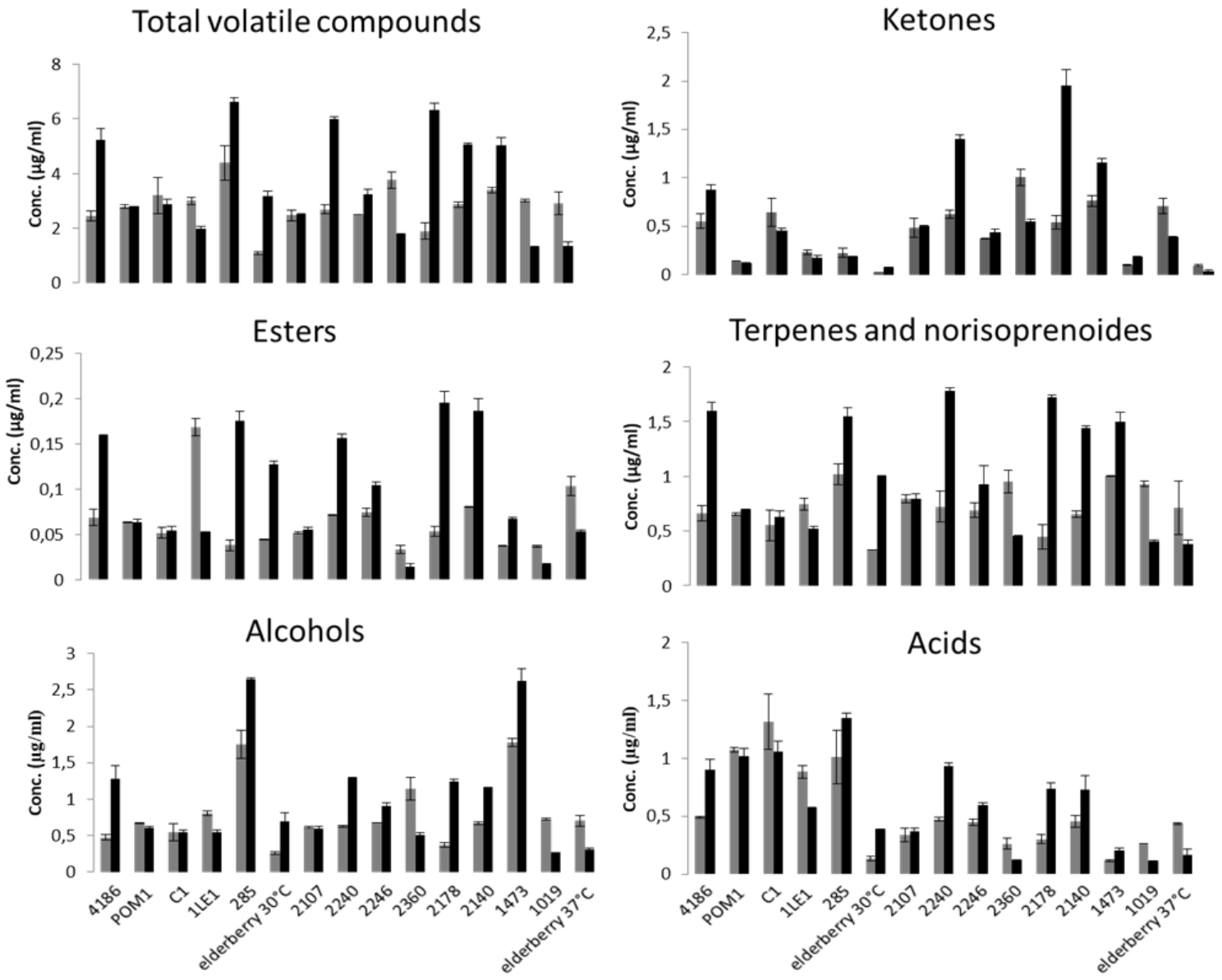

Total volatile compounds
Conc. (µg/ml)
0
2
4
6
8
Ketones
Conc. (µg/ml)
0
0,5
1
1,5
2
2,5
Esters
Conc. (µg/ml)
0
0,05
0,1
0,15
0,2
0,25
Terpenes and norisoprenoides
Conc. (µg/ml)
0
0,5
1
1,5
2
Alcohols
Conc. (µg/ml)
0
0,5
1
1,5
2
2,5
3
Acids
Conc. (µg/ml)
0
0,5
1
1,5
2
4186
POM1
C1
1LE1
285
elderberry 30°C
2107
2240
2246
2360
2178
2140
1473
1019
elderberry 37°C

Figure 3

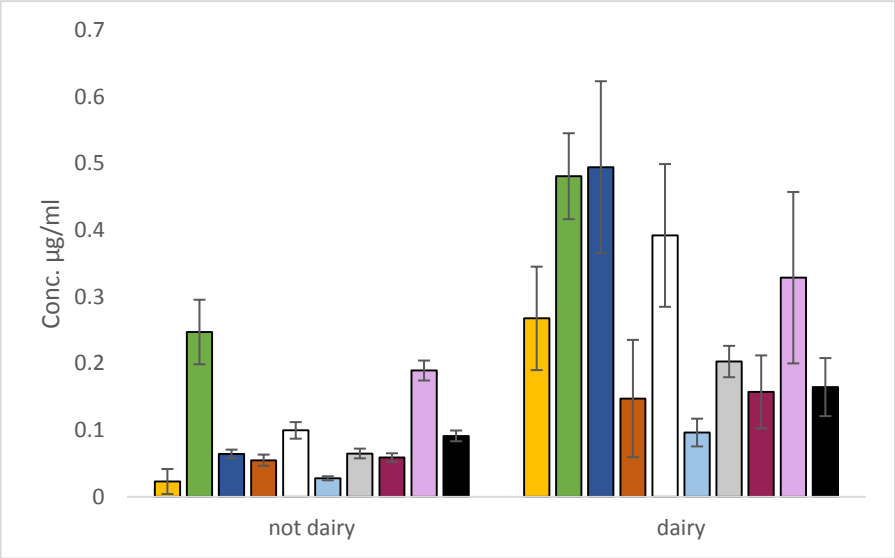


Figure 4

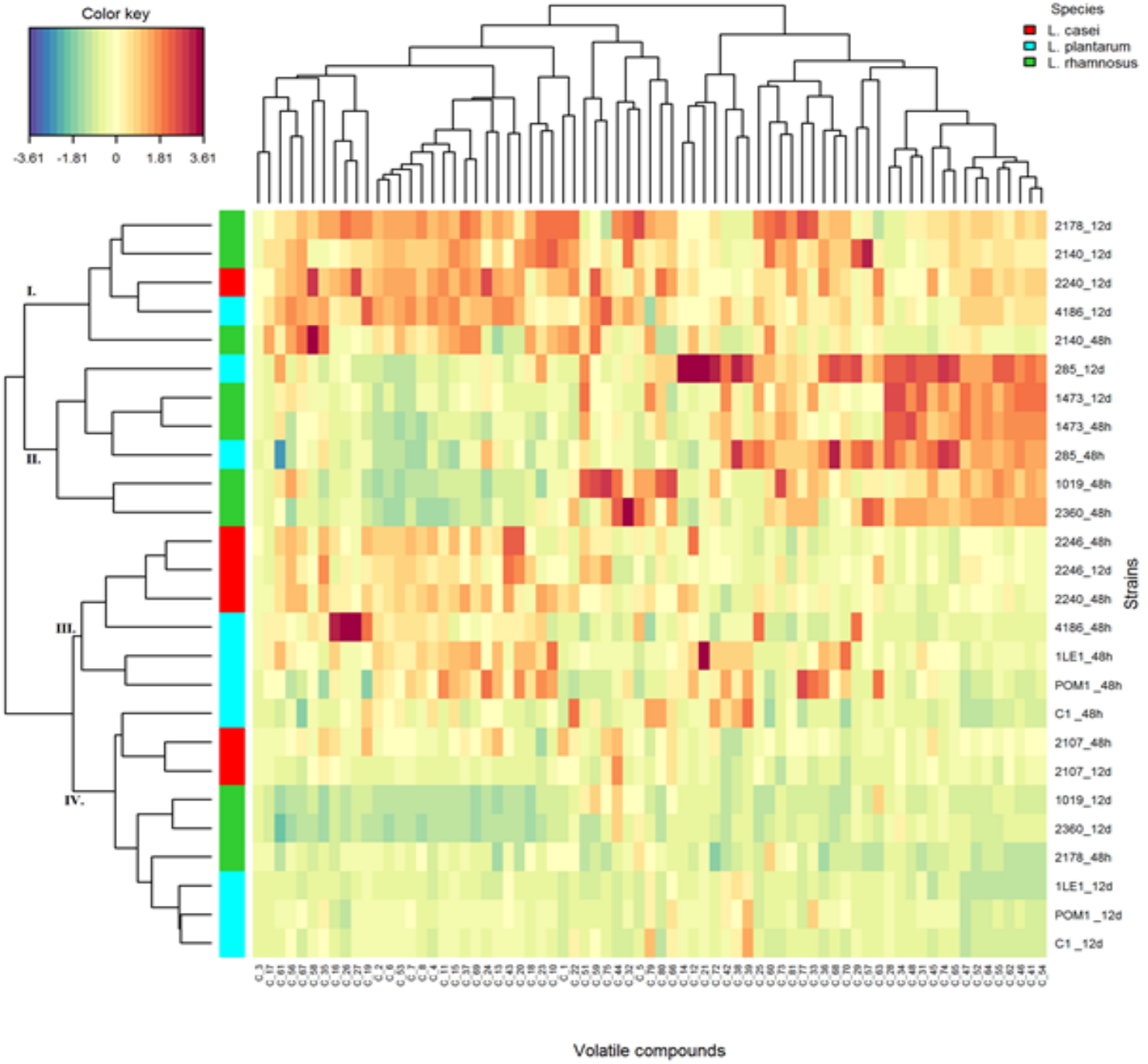

Color key
-3.61
-1.81
0
1.81
3.61
Species
L. casei
L. plantarum
L. rhamnosus
I.
II.
III.
IV.
2178_12d
2140_12d
2240_12d
4186_12d
2140_48h
285_12d
1473_12d
1473_48h
285_48h
1019_48h
2360_48h
2246_48h
2246_12d
2240_48h
4186_48h
1LE1_48h
POM1 _48h
C1 _48h
2107_48h
2107_12d
1019_12d
2360_12d
2178_48h
1LE1_12d
POM1 _12d
C1 _12d
Strains
Volatile compounds

Figure 5

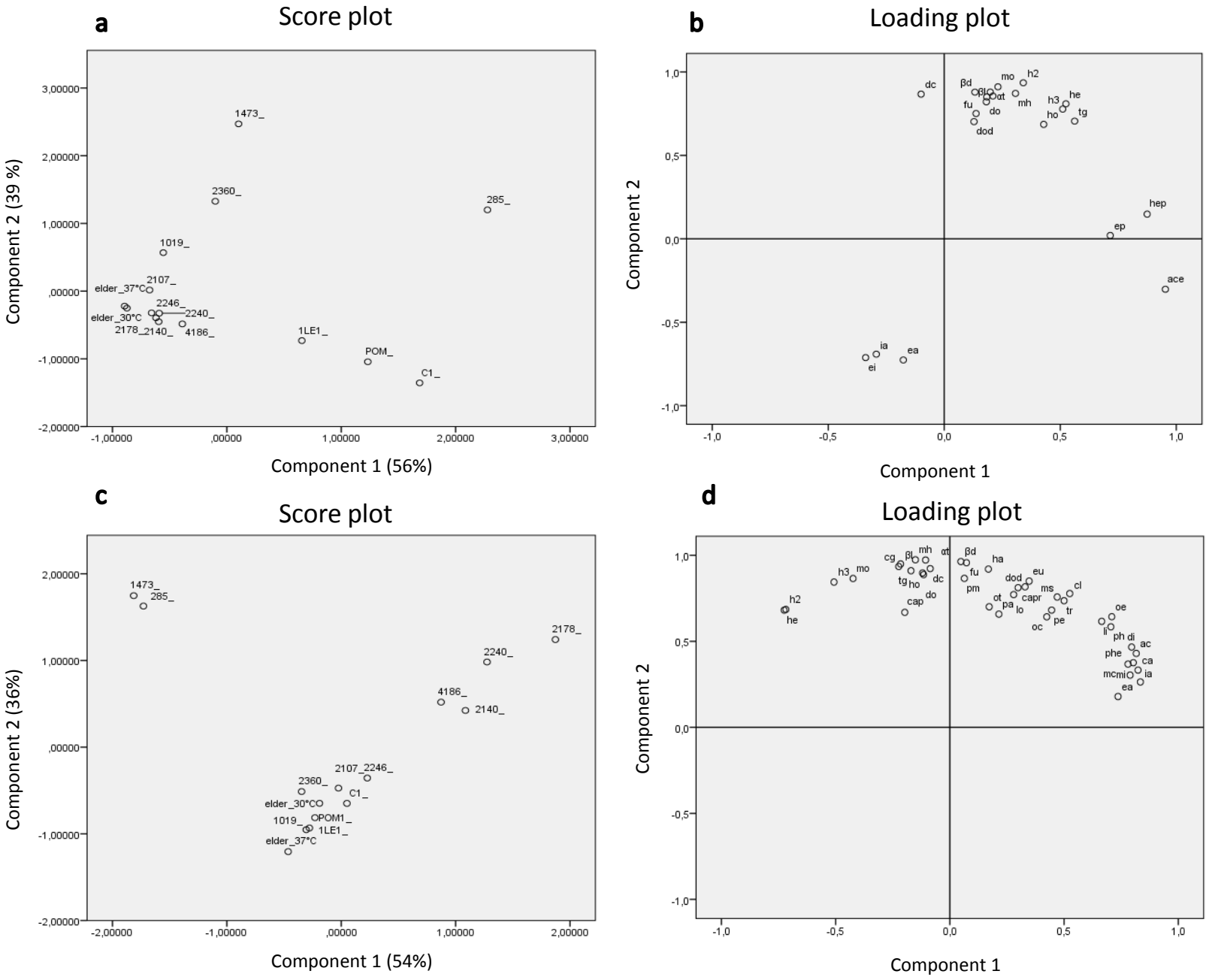

a
Score plot
Component 2 (39 %)
Component 1 (56%)
1473_
2360_
285_
1019_
2107_
elder_37°C
2246_
elder_30°C
2240_
2178_2140_
4186_
1LE1_
POM_
C1_
b
Loading plot
Component 2
Component 1
dc
βd
mo
h2
βl
αt
mh
h3
he
fu
do
ho
tg
dod
hep
ep
ace
ia
ea
ei
c
Score plot
Component 2 (36%)
Component 1 (54%)
1473_
285_
2178_
2240_
4186_
2140_
2107_2246_
2360_
C1_
elder_30°C
1019_
POM1_
1LE1_
elder_37°C
d
Loading plot
Component 2
Component 1
βd
ha
fu
h3
mo
cg
βl
mh
αt
tg
ho
dc
do
cap
h2
he
dod
eu
pm
ot
capr
ms
cl
pa
lo
tr
oc
pe
oe
li
ph
di
ac
phe
ca
mc
mi
ia
ea

Figure 6

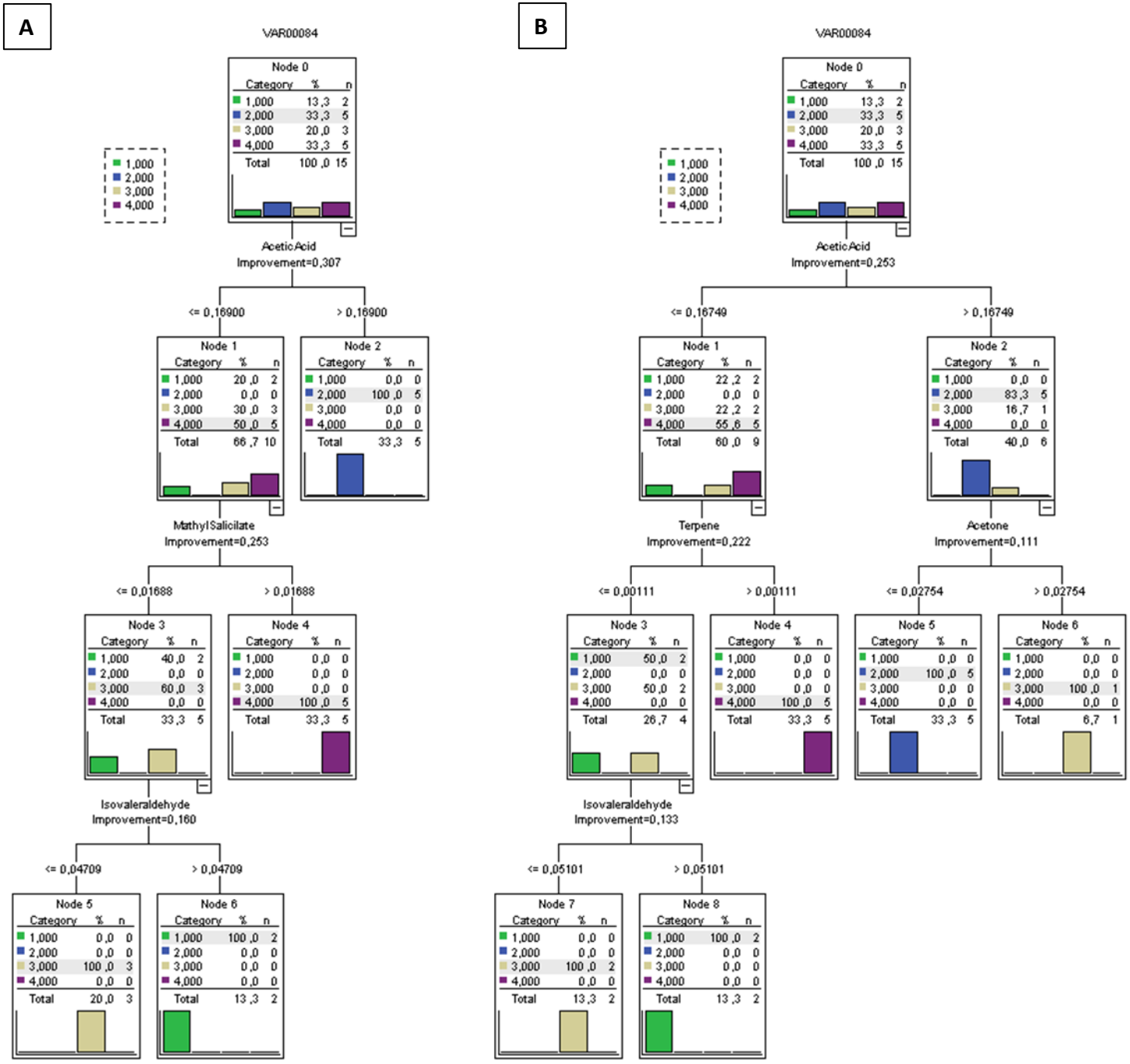

A
VAR00084
1,000
2,000
3,000
4,000
Node 0
Category % n
1,000 13,3 2
2,000 33,3 5
3,000 20,0 3
4,000 33,3 5
Total 100,0 15
AceticAcid
Improvement=0,307
<= 0,16900
> 0,16900
Node 1
Category % n
1,000 20,0 2
2,000 0,0 0
3,000 30,0 3
4,000 50,0 5
Total 66,7 10
Node 2
Category % n
1,000 0,0 0
2,000 100,0 5
3,000 0,0 0
4,000 0,0 0
Total 33,3 5
MethylSalicilate
Improvement=0,253
<= 0,01688
> 0,01688
Node 3
Category % n
1,000 40,0 2
2,000 0,0 0
3,000 60,0 3
4,000 0,0 0
Total 33,3 5
Node 4
Category % n
1,000 0,0 0
2,000 0,0 0
3,000 0,0 0
4,000 100,0 5
Total 33,3 5
Isovaleraldehyde
Improvement=0,160
<= 0,04709
> 0,04709
Node 5
Category % n
1,000 0,0 0
2,000 0,0 0
3,000 100,0 3
4,000 0,0 0
Total 20,0 3
Node 6
Category % n
1,000 100,0 2
2,000 0,0 0
3,000 0,0 0
4,000 0,0 0
Total 13,3 2
B
VAR00084
1,000
2,000
3,000
4,000
Node 0
Category % n
1,000 13,3 2
2,000 33,3 5
3,000 20,0 3
4,000 33,3 5
Total 100,0 15
AceticAcid
Improvement=0,253
<= 0,16749
> 0,16749
Node 1
Category % n
1,000 22,2 2
2,000 0,0 0
3,000 22,2 2
4,000 55,6 5
Total 60,0 9
Node 2
Category % n
1,000 0,0 0
2,000 83,3 5
3,000 16,7 1
4,000 0,0 0
Total 40,0 6
Terpene
Improvement=0,222
<= 0,00111
> 0,00111
Acetone
Improvement=0,111
<= 0,02754
> 0,02754
Node 3
Category % n
1,000 50,0 2
2,000 0,0 0
3,000 50,0 2
4,000 0,0 0
Total 26,7 4
Node 4
Category % n
1,000 0,0 0
2,000 0,0 0
3,000 0,0 0
4,000 100,0 5
Total 33,3 5
Node 5
Category % n
1,000 0,0 0
2,000 100,0 5
3,000 0,0 0
4,000 0,0 0
Total 33,3 5
Node 6
Category % n
1,000 0,0 0
2,000 0,0 0
3,000 100,0 1
4,000 0,0 0
Total 6,7 1
Isovaleraldehyde
Improvement=0,133
<= 0,05101
> 0,05101
Node 7
Category % n
1,000 0,0 0
2,000 0,0 0
3,000 100,0 2
4,000 0,0 0
Total 13,3 2
Node 8
Category % n
1,000 100,0 2
2,000 0,0 0
3,000 0,0 0
4,000 0,0 0
Total 13,3 2

Table 1.

Assignment of GC-MS signals and their relatives flavour notes.

Peak no.	Identification	Flavour note	LRI	Identification Method	Reference
1	Acetone	ethereal, apple, pear	776	MS	
2	Ethyl acetate	ethereal, fruity	855	MS + LRI	Dall'Asta et al. 2011
3	Isovaleraldehyde	ethereal, aldehydic	888	MS + LRI	Qian and Reineccius 2003
4	Ethanol	strong, alcoholic	903	MS + LRI	Goodner 2008
5	Diacetyl	strong, butter	973	MS + LRI	Ferreira et al. 2001
6	Methyl isovalerate	strong, apple, pineapple	1020	MS + LRI	Sanz et al. 2001
7	Terpene not specified		1059	MS	
8	Ethyl isovalerate	fruity, sweet, apple	1065	MS + LRI	Ferreira et al. 2001
9	Dimethyl disulfide	onion, cabbage, putrid	1073	MS + LRI	flavornet.org
10	Isopentyl acetate	sweet, fruity, banana	1117	MS + LRI	Ferreira et al. 2001
11	3-Carene	sweet, citrus, terpenic	1128	MS + LRI	Chung et al. 1993
12	Myrcene	peppery, spicy	1143	MS + LRI	Kaack 2005
13	Limonene	citrus	1175	MS + LRI	Cirlini et al. 2012
14	Eucalyptol	eucalyptus	1197	MS + LRI	Chisholm et al. 2003
15	Isoamyl alcohol	alcoholic, whiskey	1220	MS + LRI	Dall'Asta et al. 2011
16	γ-terpinene	oily, woody, terpenic, tropical	1227	MS + LRI	Cirlini et al. 2012
17	1-Pentanol	fermented	1251	MS + LRI	Cirlini et al. 2012
18	Styrene	swee,t balsam, floral	1252	MS + LRI	Bianchi et al. 2007
19	m-cymene		1258	MS + LRI	Cavalli et al. 2003
20	Terpinolene	fresh, woody, sweet, citrus	1264	MS + LRI	Choi 2003
21	Isoamyl isovalerate	sweet, fruity, green	1285	MS	
22	Acetoin	sweet, buttery, creamy, dairy	1300	MS + LRI	Bianchi et al. 2007
23	2,3-Octanedione	dill, cooked, broccoli	1322	MS + LRI	Bianchi et al. 2007
24	2-Penten-1-ol	green	1325	MS + LRI	Pennarum et al. 2002
25	(E)-2-Hexenyl-acetate	green, fruity	1329	MS + LRI	Jorgensen et al. 2002
26	Sulcatone	citrus	1336	MS + LRI	Chung et al. 1993
27	2,3,4,5-Tetramethyl-2-		1350	MS	

	cyclopenten-1-one				
28	Hexanol	herbal	1354	MS + LRI	Cirlini et al. 2012
29	(E)-3-Hexen-1-ol	green, leafy	1365	MS + LRI	Bianchi et al. 2007
30	Dimethyl trisulfide	sulfurous, cooked, onion	1375	MS	
31	(Z)-3-Hexen-1-ol	green, leafy	1386	MS + LRI	Bianchi et al. 2007
32	Herbal ketone	fresh, sweet, green, weedy	1388	MS	
33	3-Octanol	earthy, mushroom	1392	MS	
34	(E)-2-Hexen-1-ol	fruity, green, leafy	1407	MS + LRI	Bianchi et al. 2007
35	α-Ionene		1437	MS	
36	cis-Linalool oxide		1442	MS + LRI	Cirlini et al. 2012
37	1-Octen-3-ol	earthy	1449	MS + LRI	Cirlini et al. 2012
38	Heptanol	green	1455	MS + LRI	Cirlini et al. 2012
39	Acetic acid	sharp, pungent, vinegar	1470	MS + LRI	Bianchi et al. 2007
40	Ionone + Benzaldeide (co-elution)		1526	MS	
41	β-Linalool	floral	1547	MS + LRI	Cirlini et al. 2012
42	Octanol	waxy, green, orange	1555	MS + LRI	Dall'Asta et al. 2011
43	β-Caryophyllene	sweet, woody	1588	MS + LRI	Bianchi et al. 2007
44	2-Undecanone	waxy, fruity, creamy	1598	MS + LRI	Bianchi et al. 2007
45	Hotrienol	sweet, tropical	1609	MS + LRI	Kaack 2005
46	6-Methyl-heptanol		1612	MS	
47	Dihydro-citronellol	floral	1617	MS + LRI	Umano et al. 1999
48	6-Methyl-octanol		1626	MS	
49	Unknown (m/z 150, 107, 97, 69)		1644		
50	Unknown (m/z 111, 83, 69, 55)		1651		
51	Nonanol	fresh, fatty, floral	1657	MS + LRI	Dall'Asta et al. 2011
52	2-Furanmethanol	alcoholic, chemical, caramellic, bready	1665	MS + LRI	Dall'Asta et al. 2011
53	Isovaleric acid	stinky feet, cheese	1675	MS + LRI	Ferreira et al. 2001
54	α-Terpineol	pine, terpene, lilac	1695	MS + LRI	Cullere et al. 2004
55	3,4-Dimethyl octene		1700	MS	

56	Cadina-1(10)-4-diene		1751	MS	
57	β-damascenone "like"	woody, sweet, fruity, earthy	1758	MS	
58	β-citronellol (R)	floral	1763	MS +LRI	Ferreira et al. 2001
59	Terpene not specified		1773	MS	
60	Methyl salicylate	wintergreen mint	1778	MS + LRI	Kaack 2005
61	Phenethyl acetate	floral, rose, sweet, honey	1787	MS + LRI	Ong and Acree 1999
62	cis-Geraniol	sweet, floral, fruity, rose	1798	MS + LRI	Nishimura 1995
63	2-Tridecanone	fatty, waxy, dairy, milky	1806	MS	
64	β-damascenone	woody, sweet, fruity, earthy	1820	MS + LRI	Valim et al. 2003
65	trans-Geraniol	sweet, floral, fruity, rose	1845	MS + LRI	Goodner 2008
66	Caproic acid	sour, fatty, sweaty, cheesy	1855	MS + LRI	Ferreira et al. 2001
67	1,2,4-Trimethyl-1H-indene		1872	MS	
68	2- Phenylmethanol	floral	1879	MS + LRI	Chung et al. 1993
69	2-Phenylethanol	floral	1914	MS + LRI	Ferreira et al. 2001
70	3-(2,3,6,-trimethyl-1-cyclohexen-1-yl)-2-propenal		1926	MS	
71	3-(2,3,6,-trimethyl-1-cyclohexen-1-yl)-2-propenal "like"		1940	MS	
72	Caproic acid like		1953	MS	
73	Dodecanol	earthy, soapy, waxy, fatty	1963	MS + LRI	flavornet.org
74	Terpene not specified		1971	MS	
75	2-Hexenoic acid (E)	fruity, sweet, warm, herbal	1981	MS + LRI	flavornet.org
76	Unknown (m/z 159, 119, 105, 43)		1991		
77	cis-Lanceol		1995	MS	
78	Phenol	phenolic	2010	MS	
79	trans-Nerolidol	floral, green, citrus, woody	2035	MS	
80	Caprylic acid	fatty, waxy, rancid, oily	2062	MS + LRI	Ferreira et al. 2001
81	Eugenol	spicy	2157	MS + LRI	Cirlini et al. 2016
82	4-Ethyl-phenol	phenolic	2165	MS + LRI	Cirlini et al. 2016

Table 2.

Selected variables, obtained after 48 hours of fermentation, with higher loading on component 1 and component 2 either positive and negative.

Variables (48h)	**Component**	
	1	**2**
ethyl acetate	-0.089	-0.735
ethyl isovalerate	-0.250	-0.738
hexanol	0.453	0.854
(Z)-3-hexen-1-ol	0.244	0.827
(E)-2-hexen-1-ol	0.243	0.962
heptanol	0.853	0.244
acetic acid	0.974	-0.204
β-linalool	0.093	0.900
hotrienol	0.339	0.733
6-methyl-heptanol	0.204	0.902
dihydro-citronellol	-0.197	0.850
6-methyl-octanol	0.135	0.925
2-furanmethanol	0.047	0.755
isovaleric acid	-0.206	-0.725
α-Terpineol	0.109	0.872
3,4 dimetil octene	0.074	0.843
cis-geraniol	0.082	0.861
β-damascenone	0.026	0.887
trans-geraniol	0.484	0.749
dodecanol	0.042	0.714
4-ethyl-phenol	0.712	0.098

Table 3

Table 3.

Selected variables, obtained after 14 days of fermentation, with higher loading on component 1 and component 2 either positive and negative.

Variables (14d)	Component	
	1	**2**
ethyl acetate	0,703	0,305
diacetyl	0,748	0,539
methyl isovalerate	0,741	0,428
3-carene	0,762	0,469
limonene	0,558	0,727
m-cymene	0,709	0,500
acetoin	0,714	0,568
2,3-octanedione	0,269	0,759
2-penten-1-ol	0,334	0,750
hexanol	-0,792	0,536
(E)-2-hexenyl-acetate	0,047	0,916
(Z)-3-hexen-1-ol	-0,615	0,742
3-octanol	0,067	0,713
(E)-2-hexen-1-ol	-0,771	0,520
cis-linalol oxide	0,108	0,864
1-octen-3-ol	0,611	0,741
β-linalool	-0,307	0,857
hotrienol	-0,266	0,874
6-methyl-heptanol	-0,257	0,900
dihydro-citronellol	-0,157	0,836
6-methyl-octanol	-0,546	0,792
2-furanmethanol	-0,043	0,932
isovaleric acid	0,773	0,414
α-Terpineol	-0,204	0,899
3,4-dimetil octene	-0,250	0,874
methyl salicylate	0,340	0,836
phenethyl acetate	0,082	0,734
cis-geraniol	-0,340	0,866
β-damascenone	-0,052	0,917
trans-geraniol	-0,335	0,902
1,2,4-trimethyl-1H-indene	0,374	0,826
2- phenylmethanol	-0,123	0,929
2-phenylethanol	0,594	0,712
caproic acid like	-0,368	0,710
dodecanol	0,240	0,818
cis-lanceol	0,413	0,834
phenol	0,722	0,524
caprylic acid	0,210	0,813
eugenol	0,199	0,905

Supplementary material for online publication only

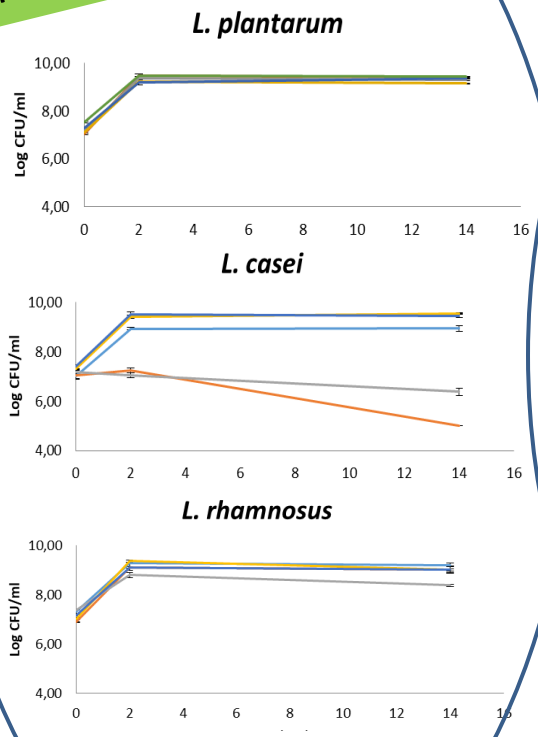
Click here to download Supplementary material for online publication only: supplementary tables.docx

*Graphical Abstract

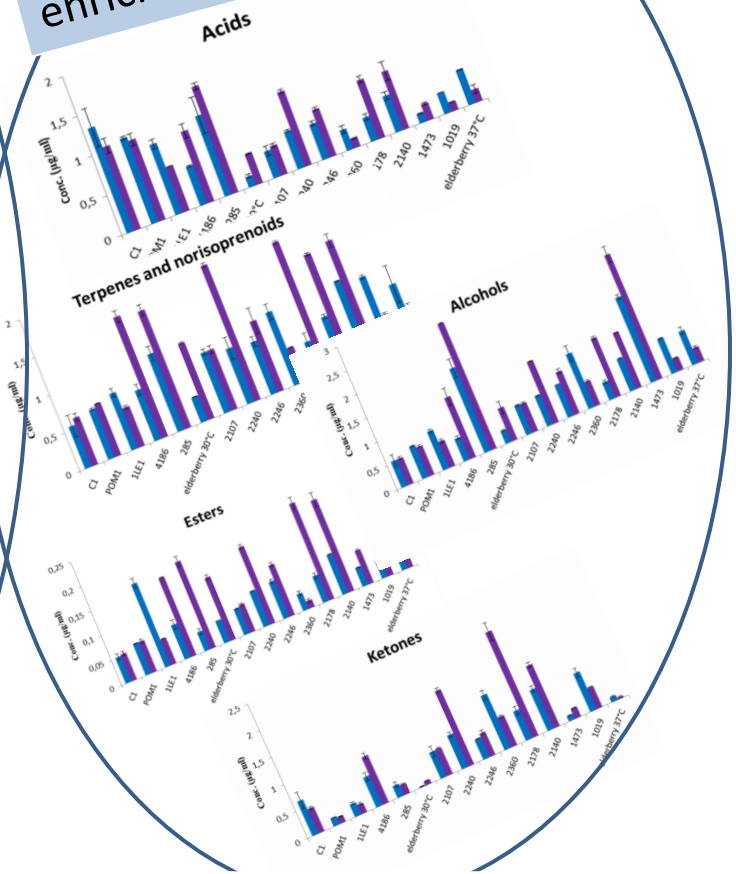
Elderberry juice

&

Elderberry allowed the growth of strains, also of dairy isolates



Typical aroma of elderberry enriched with dairy isolates



JUICE FERMENTATION

GROWTH ABILITY

VOLATILE PROFILES