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Lactic Acid Fermentation:
a traditional process for new applications

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Abstract

Fruit and vegetable harbour different microbial species and among them lactic acid bacteria were ranged from 2 and 4 Log CFU/g. *Leuconostoc, Lactobacillus, Weissella, Enterococcus* and *Pediococcus* genera can be found, however *Lactobacillus plantarum* as versatile species, is often encountered and employed for fruit and vegetable fermentations. Lactic acid fermentation can be applied using microorganisms as cell factories, for the production of interesting compounds as enzymes, flavouring agents, metabolites, etc. Moreover, can leads to the transformation of raw materials into final products (fermented food) contributing to shelf life extension, microbial safety and to the implementation of organoleptic, technological and nutritional properties. Some chemicals and physical parameters, such as pH, the concentration of sugars and organic acids, make, especially fruits, an hostile environment for microorganisms. Generally lactic acid bacteria has great biodiversity at species and strain level, derived from their ability to adapt in different environment. In adverse conditions, microorganisms shift towards specific metabolic pathways, using non-conventional carbon source, exploiting alternative substrates to improve stress tolerance. This attempt to adapt can resulted in flavours formation, phenolic compounds metabolism and molecules production.

Based on these assumptions the aim of the present thesis was to exploit lactic acid bacteria in order to valorise different plant matrices, from fruit juices to food by-products. An overview on the metabolism of LAB strains, of plant but especially dairy origin, during fermentation, was reported. Two types of fruit juices, elderberry and cherry, and one by-product, orange peels, were used for fermentations in order to study the metabolism of the different strains applied, to modulate/improve the organoleptic and nutritional properties of juices and to produce lactic acid, a valuable chemical compound, from orange by-products.

Chapter 1

Introduction and aim of the thesis

Fermentation

The word fermentation originated from the Latin verb "*fervere*" that means to boil and describe the action of yeast in a fruit matrix. The boiling appearance was due to the liberation of carbon dioxide from microorganism, through the metabolism of sugars contained in the substrate.

Fermentation is a catabolic process, which results in the production of reduced pyridine nucleotides (NADH and NADPH) which must be reoxidized (Stanbury et al., 1995). The reduced pyridine nucleotide oxidation is coupled with a reduction of an organic compound, which is often a product of a catabolic pathway. Through this process prokaryotic cells are able to reduce pyruvate into a wide range of final products such as lactate, acetate, ethanol, butanol, etc. Therefore the term fermentation is used to mean a process for the generation of energy in which organic compounds play the role of electron donors and electron acceptors. Fermentation can be employed using microorganisms as cell factories, for the production of interesting compounds as enzymes, flavouring agents, metabolites, etc. (Bosma et al., 2017). Moreover, fermentation can leads to the transformation of raw materials into final products (fermented food) contributing to shelf life extension, microbial safety and to the implementation of organoleptic, technological and nutritional properties (Di Cagno et al., 2013). For these reasons the products obtained can be of industrial interest since they can be used in food, pharmaceutical, nutraceutical, chemical areas, etc.

Mainly, two type of fermentations are currently used: submerged and solid state fermentation. Submerged fermentation, also called liquid fermentation, employ free liquid substrates, in which the compounds produced by microorganisms were released. This method is suited especially for bacteria which require high moisture content to survive (Subramaniyam & Vimala, 2012). On the other hand, solid state fermentation is a process in which microorganisms grow in a solid substrate with a low presence of water, in an adsorbed state or complexed with the solid matrix. However, inside the substrate, enough moisture to support growth and metabolism of microorganism must be

present (Bhargav et al., 2008; Panday et al., 2003). Considering the limited quantity of water and the theoretical classification of water activity, only fungi and yeast should be suitable for this process. However the experience have taught that also bacterial cultures can be employed in this process showing good performances (Wee et al., 2006; Panday et al., 2003).

Lactic acid Bacteria

Lactic acid bacteria (LAB) gather an heterogeneous group of microorganisms characterized by the production of lactic acid as the main end-product of carbohydrates fermentation (Carr et al., 2002). They are gram-positive, acid-tolerant, both bacilli or cocci bacteria that have been used for a long time in fermented food leading them to the status of GRAS (generally recognized as safe) by the US Food and Drug Authority (FDA) and earning a place on the QPS (qualified presumption of safety) list of European Food Safety Authority (EFSA, 2016) (Hill et al., 2018).

Variations in LAB metabolic products have yielded three categories of fermentation: homofermentation, mixed-acid metabolism and heterofermentation. Homofermentative LAB ferment sugars by the Embden-Meyerhof-Parnas (EMP) pathway to pyruvate then converted into lactic acid by lactate dehydrogenase. In particular conditions, such as variations in carbon concentration, the homolactic pathway can shift to a mixed-acid metabolism, leading to the production of formate, acetate, ethanol and/or CO_2 in addition to lactate. Heterofermentative LAB ferment sugars generally by the phosphoketolase pathway (PKP). Fermentation of pentoses leads to the formation of pyruvate and acetyl-phosphate converted into lactate and acetate, respectively, while from hexoses lactate, CO_2 , and ethanol are produced (Mozzi et al., 2010).

These microorganisms are widespread in different environment, rich in carbohydrates and/or protein, as milk and dairy products, vegetables, meat products, and/or can be added as a starter.

Thanks to the huge enzymatic portfolio they can contribute to the organoleptic, nutritional and technological properties of fermented food. In addition, as a consequence of acidification, LAB can exert a role in foods and feeds preservation. Usually LAB can also be found as ubiquitous microflora in human and animals mucous where they are considered health-promoters. Indeed is suggested the use of LAB to supply a flora able to compete with potential pathogenic organisms to prevent disease. For this reason some LAB strains belong to different species, such as *Lactobacillus plantarum, Lactobacillus casei group, Lactobacillus acidophilus* etc. are often used as probiotics (Fijan, 2014; Mozzi et al., 2010). Sugars are the primary carbon and energy source used by LAB lead to the formation of lactate alone or in combination of other organic acid and ethanol (Mozzi et al., 2010). Moreover, LAB can also obtain energy from the amino acids catabolism, especially in nutrient-limited conditions, helping the microorganism to maintain pH-homeostasis. Through different pathways starting from amino acids, aromatic and flavour enhancing compounds can be released highlighting the important role of LAB in fermented food and food industry (Fijan, 2014). Among amino acids those containing branched chain, aromatic rings and sulfur are the most important source for the production of flavour compounds (Mozzi et al., 2010). Furthermore, LAB were able to metabolize citrate, exploited specific permeases, and convert it into pyruvate and carbon dioxide. Subsequently pyruvate can be converted in different end-products such as lactate, acetate, ethanol and into aromatic compounds like acetoin, diacetyl and butanediol (Ganzle et al., 2015; Neves et al., 2005).

Lactobacillus casei group

The *Lactobacillus* genus, which currently contains 204 species (http://www.bacterio.net/lactobacillus.html), is the largest group among LAB. Within this genus, the facultative heterofermentative species *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus*

rhamnosus, which are closely phylogenetically and phenotypically related, are regarded together as the *Lactobacillus casei* group (Savo Sardaro et al., 2016; Felis & Dellaglio, 2007). The strains belonging to this group have a great potential related to their applicability in industry and health. Indeed they are widely used in the fermentation of food products and they contribute to the implementation of flavour and texture. In addition they are able to produce bioactive compounds which can give health benefits after their consumption (Dietrich et al., 2014). Further, demonstrating the beneficial effect by in vivo studies, various strains belonging to this group are recognized as probiotics (Hill et al., 2018). It is known that *L. casei* group can supply benefits for a lot of health conditions, even if the mechanisms are not completely clarified. Possible mechanisms can be related to the modulation of immune system, to the competition with pathogenic strains for binding site or to the production of antimicrobial compounds (Bermudez-Brito et al., 2012). Probiotic strains have exhibited the capacity to reduce the risk of developing allergies and they seem to have a potential beneficial effect on brain functions, exploiting the brain-gut axis. In addition they are recognized to have a role in the management of body weight and different strains of *L. casei* have revealed a good ability to prevent the antibiotic associated diarrhoea (Hill et al., 2018). Moreover, the assumption of *L. rhamnosus* was correlated with beneficial effect during the therapy for the eradication of *Helicobacter pylori* and during the treatment of irritable bowel syndrome (Guarino et al., 2009).

Lactobacillus plantarum

L. plantarum is a widespread LAB isolated from different environmental niches such as dairy, meat and a lot of fermented vegetables such as olives, cocoa beans, sauerkraut, wine, etc. It is also often found in gastrointestinal-tract of human species. *L. plantarum* possess many genes involved in sugar transport (Mozzi et al., 2010) and is able to ferment a lot of them (De Vries et al., 2006), in

addition can synthetize all amino acids excepted leucine, isoleucine and valine (Mozzi et al., 2010). A great ability in the production of enzymes involved in the metabolism of phenolic compounds have been shown. Indeed phenolic acid decarboxylase can transform phenolic acids into their vinyl derivates and phenylpropionic acids (Rodriguez et al., 2009) and tannase is involved in the degradation of hydrolyzable tannins and tannic acid (Vaquero et al., 2004). These activities allow the adaptation in plant matrices rich in polyphenols (Filannino et al., 2018; Rodríguez et al., 2009). Despite most vegetable fermentations are spontaneous, *L. plantarum* is the commercial starter frequently used in the fermentation of vegetable food products. For example *L. plantarum* with *Lactobacillus pentosus* are regarded as the main species used as starter in guided olive fermentations and, historically, it is also involved in sauerkraut and cucumber fermentations (Rodríguez et el., 2009).

Moreover, the ability to survive thought the gastrointestinal tract, to colonize the human and other mammals gut and its positive physiological effect was reported in literature. A large number of surface-anchored protein were found in this species suggesting its ability in the association with different substrates, potentials for growth (Kleerebezem et al., 2003). Different strains were able to adhere to human colonic cells, this ability create a competition between *L. plantarum* and pathogenic bacteria, so, in this way, the host can be protected from infections. This species also have a positive effect on subjects affected by irritable bowel syndrome. Moreover the assumption of *L. plantarum* lead to the decrease of Gram negative anaerobic bacteria, of enteropathogenic bacteria and to the reduction of LDL-cholesterol and fibrinogen levels in blood, risk factors for coronary artery disease (De Vries et al., 2006).

Fruit and vegetables lactic acid fermentation

The consumption of fruit and vegetables is encouraged for the prevention of chronical diseases. Following this direction the consumers were increasingly directed to the use of fresh-like fruit and vegetables and to the consumption of ready-to-eat and ready-to-drink products. In order to offer safety products, with a long shelf-life, and with high nutritional and organoleptic features, lactic acid fermentation could be a valid strategy which can be applied to reach these purposes (Marsh et al., 2014). So, in the last years, the interest on fruits and vegetables lactic acid fermentation is increasing (Espirito-Santo et al., 2015; Di Cagno et al., 2013). Moreover the growing quantity of waste and/or by-products produced in all food life cycle, from agriculture to households, lead to the loss of valuable materials resulting in environmental and economic management problems. In Europe the total food loss is about 89 million tonnes, 179 kg per capita, 42% of them is produced by households, 39% losses occur in the food manufacturing industry, 14% pertains to food sector while 5% is lost along distribution chain (Mirabella et al., 2014). A great part of these residues (about the 40% of the total quantity) derived from fruits and vegetables, among them bananas, apples, oranges and melon represent more than 73% of the generated wastes (WRAP, 2008). However a lot of them could be reused or recovery (Mirabella et al., 2014), applying fermentation as suitable method, also employing lactic acid bacteria (Wang et al., 2015).

Fruits and vegetables matrices can host a microbial population ranged between 5 and 7 Log CFU/g, among them, 2-4 Log CFU/g are represented by lactic acid bacteria (Di Cagno et al., 2013). Different species belong to *Leuconostoc, Lactobacillus, Weissella, Enterococcus*, and *Pediococcus* genera can be found, but *L. plantarum* is the species most frequently present. Therefore, when favourable conditions (water activity, salt concentrations and temperature) occur in the raw materials, spontaneous lactic acid fermentation can take place, reflecting the composition of autochthonous microflora (Di Cagno et al., 2013). Nevertheless, the inadequate inhibition of

spoilage and pathogenic microorganisms, the not predictable sensory and nutritional variations and the risk to failure fermentation are factors that can lead to favour selected starters instead spontaneous fermentation. The starter cultures chosen should possess strain-specific metabolic traits conducing to technological, probiotic, sensory, and antimicrobial positive attribute (Di Cagno et al., 2013).

Some chemicals and physical parameters, such as pH, the concentration of sugars and organic acids, make, especially fruit, an hostile environment for microorganisms (Papadimitriou et al., 2016; Nualkaekul & Charalampopoulos, 2011; Filannino et al., 2014). To adapt, in these adverse conditions, bacteria should adopt specific metabolic pathways, involved in the use of non-conventional carbon source, in the exploitation of alternative substrates or in a global stress response (Filannino et al., 2014). Since *L. plantarum* is considered as the *Lactobacillus* most adapted in fruit and vegetable is also the most studied. To adapt in fruits, alternative energy pathway can be employ resulting in amino acids catabolism, malolactic fermentation, and synthesis of volatile compounds. Branched chain amino acids can be used to obtain ATP energy, the amino acid is converted into the corresponding α-ketoacids, while α-ketoglutarate was converted in glutamic acid, and then the ketoacids in aldehydes and alcohols (Smit et al., 2005). In addition the decarboxylation of malic acid, observed in high content in fruits, lead to an intracellular pH increase and to the reducing power synthesis (Papadimitriou et al., 2016; Filannino et al., 2014).

Other plant components, which can exert an antimicrobial activity, also against LAB, are phenolic compounds. They are plant secondary metabolites, exerting health benefits and also having a sensory impact on food. To avoid their adverse activity, in particular observed on cytoplasmic membrane and cell wall (Filannino et al., 2018; Rodriguez et al., 2009), LAB metabolize them in a species strain-dependent manner. Therefore, the metabolism of phenolics is a mechanism to detoxify them and to decrease their antimicrobial activity. Recently the used of hydroxicinnamic

acids as external acceptor of electrons, allowing the cofactor recycling, and giving metabolic energy to the cells in stressful conditions, was proposed (Filannino et al., 2018; Filannino et al., 2014). So, phenolics metabolism can give an advantage for the cell but also confer health effects for humans (Kwaw et al., 2018). Indeed it is recognized that the beneficial effects of these compounds depends, in part, on their microbial conversion that can occur in plant fermentation but also during human digestion (Filannino et al., 2018; Filannino et al., 2015; Zhao et al., 2016).

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http://www.bacterio.net/ lactobacillus.html (25th October 2018)

Aim of the thesis

This Ph.D. project was aimed to exploit lactic acid bacteria (LAB) in order to valorise different plant matrices, from fruit juices to food by-products. In this context, elderberry and cherry juices have been employed as substrates for submerged fermentation in order to modulate/improve their organoleptic and nutritional properties. Elderberry, grows spontaneously in the North of Italy and Europe, is a matrix rich in bioactive compounds such as polyphenols and it was never exploited for lactic acid fermentation. Instead, cherry was already used as substrate for lactic acid fermentation but a large set of LAB, deriving from dairy products, was never investigated.

On the other hand, orange by-products have been utilised in solid state fermentation for the production of lactic acid, a valuable chemical compound for different applications. The choise of sweet orange for lactic acid fermentation was based on different reasons: i) it is the citrus fruit most produced and consumed worldwide, ii) Spain and Italy are the main producer countries in Europe with a great quantity of residues discarded after processing. iii) For the high cost of non-renewable raw material, frequently used in industry, new low cost processes are necessary for lactic acid production and solid state fermentation can be a valid stategy to avoid this problem.

Since a lack of information, on the potentiality of strains not isolated from plant matrices, was observed in literature, the present work was focused to study the performances of plant and non-plant isolates in fruit fermentation.

Chapter 2

The effect of lactic acid bacteria on elderberry juice

Chapter 2.1

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

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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 h 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 was 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 h 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 the main representative 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.

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 et 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 & 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; Filannino 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 & 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.

Materials and methods

Bacterial strains

Lactobacillus plantarum POM1 isolated from tomatoes, 1LE1 from pineapples, C1 from carrots, 4186 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. case*i 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*.

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 Log CFU/ml. Each culture was 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 h 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.

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.

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 h (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× 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).

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 $p < 0.05$.

Results and discussion

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 h 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) (Table 1).

Table 1. Viable cells concentration (Log CFU/mL) of strains during fermentation of elderberry juiceat 30 °C and 37 °C for 48 h and further storage for 12 days. Data are the mean values of three different independent experiment ± standard deviation.

Strains	Origin	T_0	48 h	12d
L. plantarum POM1	plant	7.03 ± 0.03	9.48 ± 0.05	9.39 ± 0.04
L. plantarum C1	plant	7.14 ± 0.01	9.38 ± 0.05	9.32 ± 0.06
L. plantarum 1LE1	plant	7.11 ± 0.03	9.21 ± 0.07	9.14 ± 0.02
L. plantarum 4186	dairy	7.29 ± 0.02	9.21 ± 0.12	9.38 ± 0.05
L. plantarum 285	dairy	7.53 ± 0.03	9.46 ± 0.08	9.43 ± 0.02
L. rhamnosus 2178	dairy	7.31 ± 0.08	9.29 ± 0.02	9.21 ± 0.07
L. rhamnosus 2140	dairy	6.89 ± 0.02	9.11 ± 0.03	9.02 ± 0.15
L. rhamnosus 2360	dairy	7.37 ± 0.01	8.80 ± 0.10	8.38 ± 0.05
L. rhamnosus 1473	dairy	7.03 ± 0.05	9.37 ± 0.05	9.01 ± 0.04
L. rhamnosus 1019	dairy	7.16 ± 0.03	9.12 ± 0.15	9.03 ± 0.14
L. casei 2246	dairy	7.00 ± 0.08	8.93 ± 0.5	8.95 ± 0.11
L. casei 2306	dairy	7.06 ± 0.18	7.24 ± 0.11	5.00 ± 0.00
L. casei 2057	dairy	7.19 ± 0.08	7.05 ± 0.11	6.38 ± 0.14
L. casei 2107	dairy	7.31 ± 0.01	9.42 ± 0.07	9.55 ± 0.03
L. casei 2240	dairy	7.40 ± 0.09	9.50 ± 0.10	9.46 ± 0.08

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 0.0 Log CFU/ml after 12 days at 4 °C (Table 1). 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 (48 h) 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.

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 2. 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

showing the capacity to grow in EJ, after 48 h from the beginning of the fermentation process and after storage at 4 °C.

Table 2. Assignment of GC–MS signals and their relatives flavour notes.

Peak number	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	etherea,l 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-cyclopenten-1-one		1350	MS	
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	N.I.		1644	MS	
50	Hydrocarbon not specified		1651	MS	
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, herbal	1981	MS + LRI	flavornet.org

76	N.I.		1991	MS	
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

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%) (Table S1 and Table S2, Fig.1).

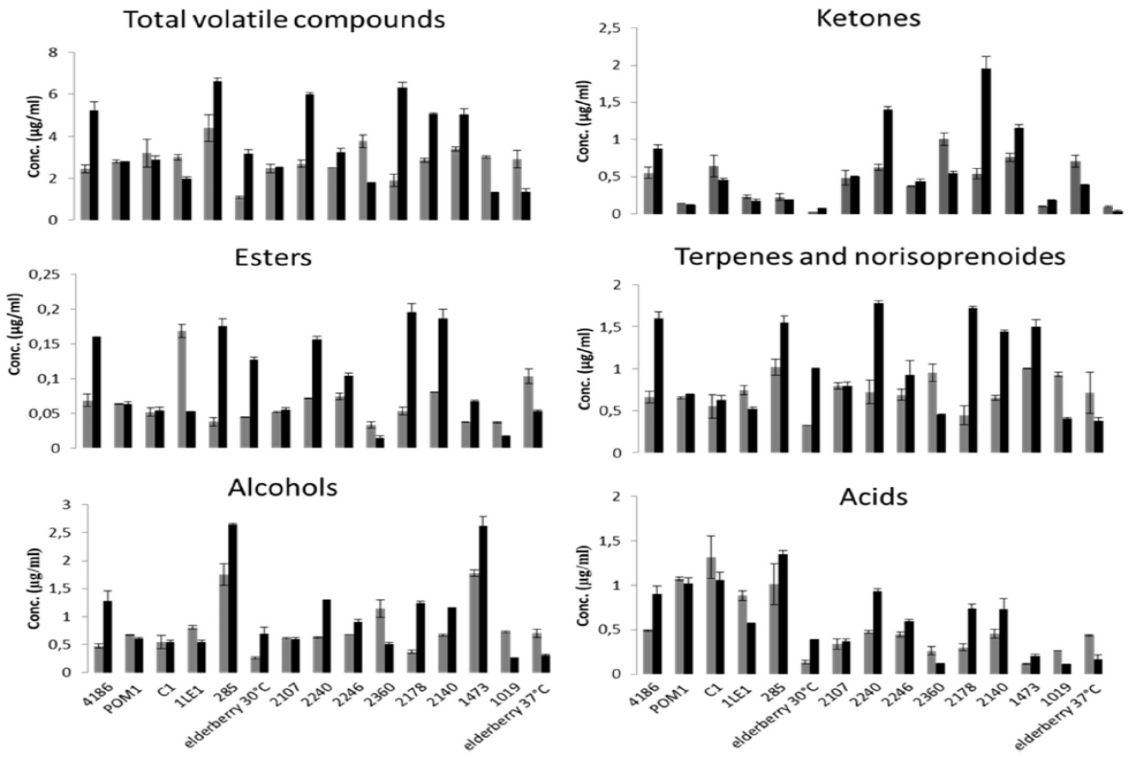

Figure 1. Total volatile compounds and volatile profile, express in µg/ml, (alcohols, ketones, terpenes and norisoprenoids, acids and esters) of elderberry juices inoculated with *L. plantarum* (4186, POM1, C1, 1LE1, 285), *L. casei* (2107, 2240, 2246), *L. rhamnosus* (2360, 2178, 2140, 1473,1019) and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

Fig. 1, 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 flavour 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 h (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.1). 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.1, 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; Jyoti et al., 2004; Branen & Keenan, 1971) present in many substrates such as fruits, even if it is usually associated with creamy and buttery note in dairy products (Curioni & Bosset, 2002). Acetoin could derive from the same pathway and small amounts of this compound have been also found in not fermented elderberry juice (Kaack et al., 2005). Overall, an increase in organic acids concentration was observed after 48 h 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.1, 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 (Künsch & 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. 1, 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 et 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 were also reported during the fermentation with *L. plantarum* strains of carrot and tomato juices (Filannino 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.1, 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.1, 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, Di Cagno et al., 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; Yamadaa 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 non-dairy isolates, data collected from five *L. plantarum* strains were further analysed (Fig. 2). Significant differences in volatile compounds, especially after storage, were observed by ANOVA test as showed in Fig. 2.

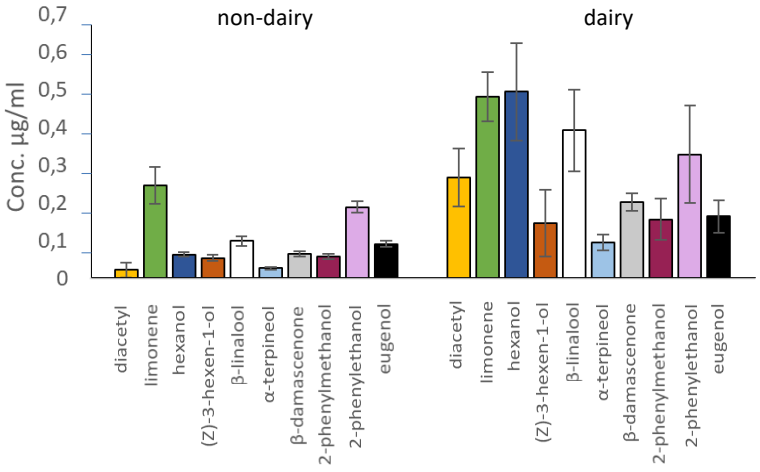

Figure 2. Statistically significant different concentrations (express as µg/mL) of variables with significant differences between elderberry juices started with *L. plantarum* strains isolated from plant (non-dairy) and from cheeses (dairy) after storage period. Statistical differences (p values < 0.05) between volatile compounds of elderberry juices started with dairy and non-dairy strains were determined with ANOVA test.

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 et al., 2005; Poll &

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.

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.3), 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 characterized 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 h of fermentation. Finally, we have a cluster of samples those after a prolonged storage present a reduction of most of the volatile compounds. Conversely, the same strains showed a high aromatic potential after 48 h of fermentation (clusters II and III). Since heat map analysis highlighted differences between fermented and stored samples, we decided to perform the following statistical analysis keeping the data separated for 48 h and 12 days samples. 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.

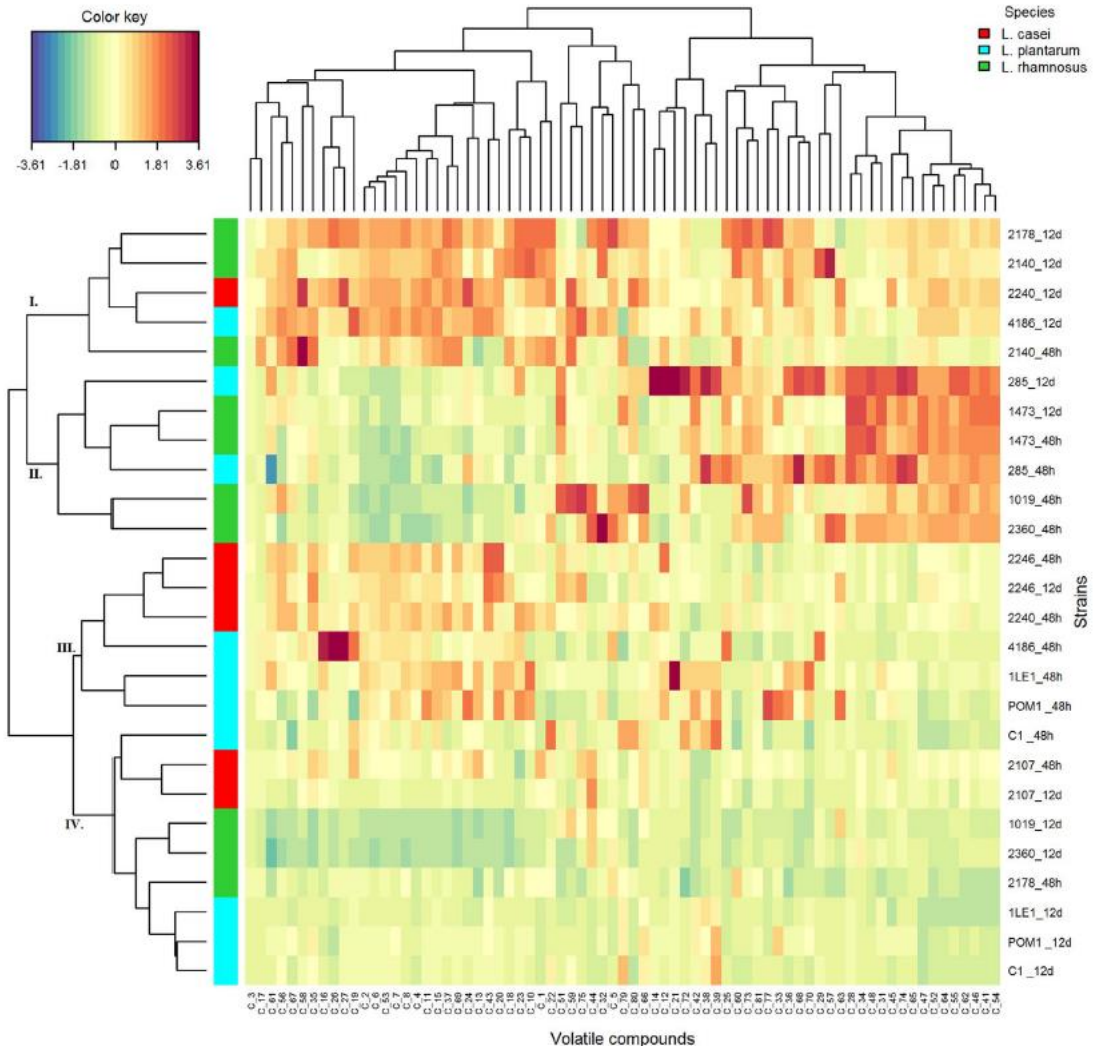

Figure 3. Hierarchical clustering analysis and heat-map visualization of the volatile profiles of the elderberry juice fermented with the 15 *Lactobacillus* strains based on the Euclidean distance of the amount of each measured compound. The color scale represents the scaled abundance of each variable, 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 (48 h), or the samples measured after further storage (12 d). 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Two separate analyses 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 3 and Table 4) and consequently two final PCA were performed using only the previously selected variables.

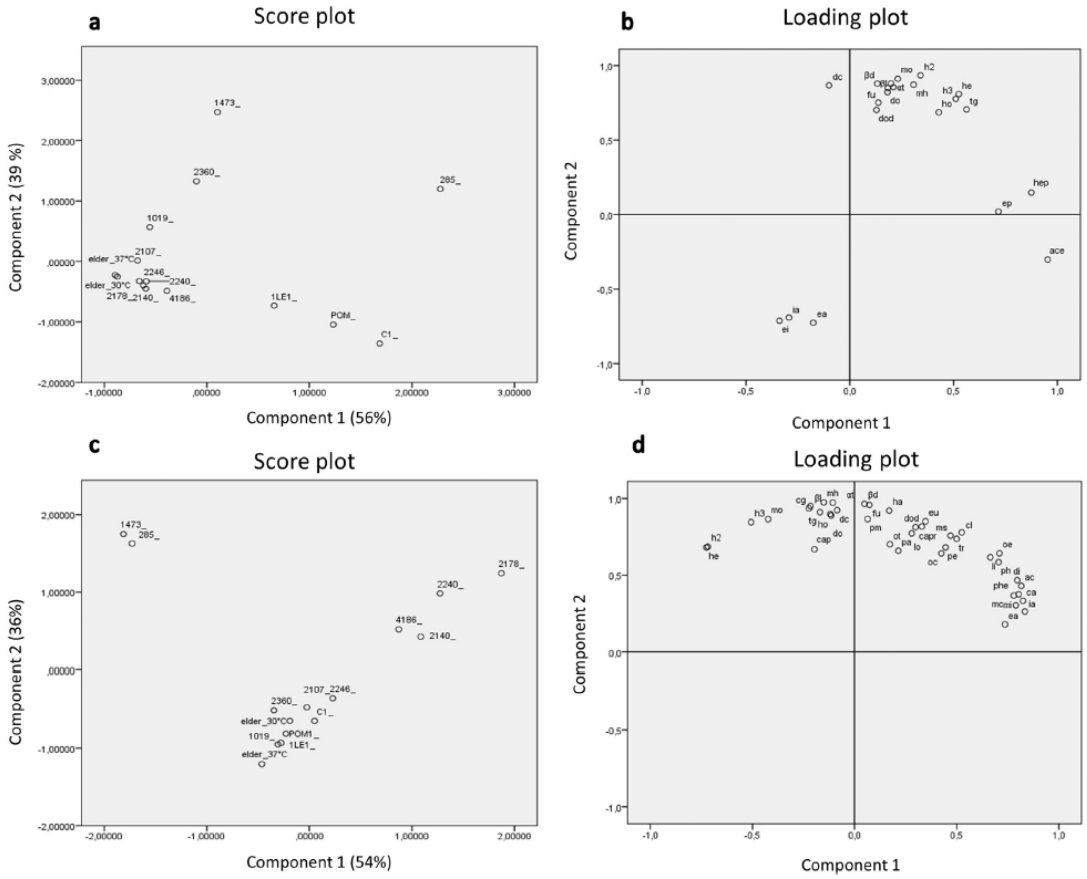

Figure 4. Principal component analysis (PCA), score plot and loading plot, based on volatile compounds with higher influence on elderberry juices characterization, identify after 48 h (a and b) and after storage (c and d) in *L. plantarum* (POM1, C1, 1LE1, 4186 and 285), *L. casei* (2246, 2240, 2107), *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 as reported in Tables 2 and 3.

In Fig. 4a and b the PCA score and loading plots related to samples after 48 h 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. 4a, b and Table 3). After storage an evolution in volatile profile was observed and the distribution of samples in the score plot was different from the 48 h of fermentation (Fig. 4c). 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. 4c, d and Table 4). 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 (48 h) 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.

Variable (48 h)	Variable code	Component	
		1	2
Ethyl acetate	(ea)	−0.089	−0.735
Ethyl isovalerate	(ei)	−0.250	−0.738
Hexanol	(he)	0.453	0.854
(Z)-3-hexen-1-ol	(h3)	0.244	0.827
(E)-2-hexen-1-ol	(h2)	0.243	0.962
Heptanol	(hep)	0.853	0.244
Acetic acid	(ace)	0.974	−0.204
β-linalool	(βl)	0.093	0.900
Hotrienol	(ho)	0.339	0.733
6-methyl-heptanol	(mh)	0.204	0.902
dihydro-citronellol	(dc)	−0.197	0.850
6-methyl-octanol	(mo)	0.135	0.925
2-furanmethanol	(fu)	0.047	0.755
Isovaleric acid	(ia)	−0.206	−0.725
α-Terpineol	(αt)	0.109	0.872
3,4 dimetil octene	(do)	0.074	0.843
Cis-geraniol	(cg)	0.082	0.861
β-damascenone	(βd)	0.026	0.887
Trans-geraniol	(tg)	0.484	0.749
Dodecanol	(dod)	0.042	0.714
4-ethyl-phenol	(ep)	0.712	0.098

Table 3. Selected variables, obtained after 48 h of fermentation, with higher loading on component 1 and component 2 either positive or negative (volatile compounds abbreviation reported on loading plot are indicated in brackets).

Variable (14d)	Variable code	Component	
		1	2
Ethyl acetate	(ea)	0.703	0.305
Diacetyl	(di)	0.748	0.539
Methyl isovalerate	(mi)	0.741	0.428
3-carene	(ca)	0.762	0.469
Limonene	(li)	0.558	0.727
M-cymene	(mc)	0.709	0.500
Acetoin	(ac)	0.714	0.568
2,3-octanedione	(oc)	0.269	0.759
2-penten-1-ol	(pe)	0.334	0.750
Hexanol	(he)	−0.792	0.536
(E)-2-hexenyl-acetate	(ha)	0.047	0.916
(Z)-3-hexen-1-ol	(h3)	−0.615	0.742
3-octanol	(ot)	0.067	0.713
(E)-2-hexen-1-ol	(h2)	−0.771	0.520
Cis-linalol oxide	(lo)	0.108	0.864
1-octen-3-ol	(oe)	0.611	0.741
β-linalool	(βl)	−0.307	0.857
Hotrienol	(ho)	−0.266	0.874
6-methyl-heptanol	(mh)	−0.257	0.900
Dihydro-citronellol	(dc)	−0.157	0.836
6-methyl-octanol	(mo)	−0.546	0.792
2-furanmethanol	(fu)	−0.043	0.932
Isovaleric acid	(ia)	0.773	0.414
α-Terpineol	(αt)	−0.204	0.899
3,4-dimetil octene	(do)	−0.250	0.874
Methyl salicylate	(ms)	0.340	0.836
Phenethyl acetate	(pa)	0.082	0.734
Cis-geraniol	(cg)	−0.340	0.866
β-damascenone	(βd)	−0.052	0.917
Trans-geraniol	(tg)	−0.335	0.902
1,2,4-trimethyl-1H-indene	(tr)	0.374	0.826
2- phenylmethanol	(pm)	−0.123	0.929
2-phenylethanol	(ph)	0.594	0.712
Caproic acid like	(cap)	−0.368	0.710
Dodecanol	(dod)	0.240	0.818
Cis-lanceol	(cl)	0.413	0.834
Phenol	(phe)	0.722	0.524
Caprylic acid	(capr)	0.210	0.813
Eugenol	(eu)	0.199	0.905

Table 4. Selected variables, obtained after 14 days of fermentation, with higher loading on component 1 and component 2 either positive and negative (volatile compounds abbreviation reported on loading plot are indicated in brackets).

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. Since the PCA analysis was mainly useful to understand the different aromatic potential of the strains, we decide to apply a classification model 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% (Fig. 5 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 (Fig. 5 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.

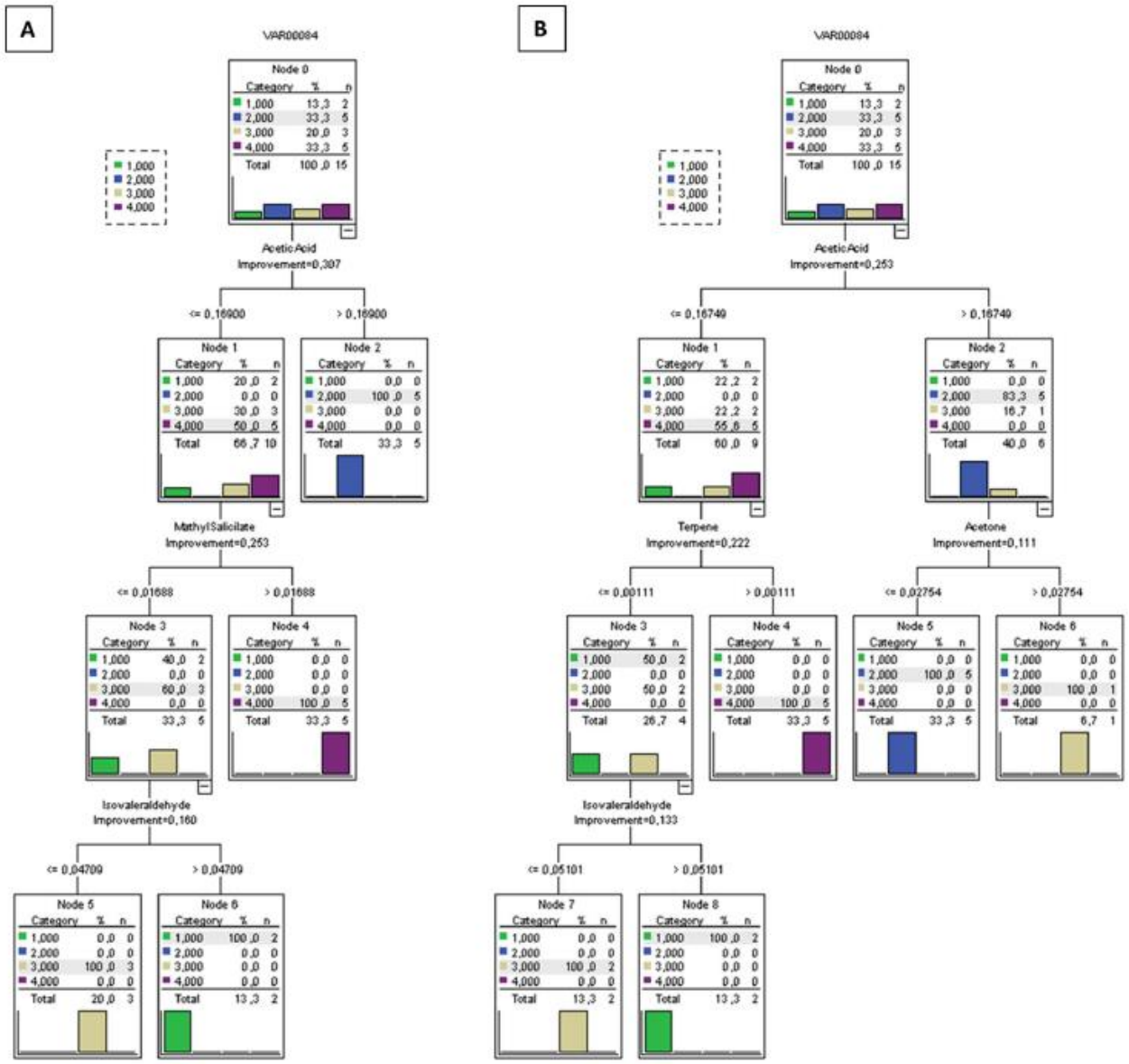

Figure 5. Classification tree analysis (CT) performed on total volatile compounds, obtained after 48 h (A) and further 12 days of refrigerate storage (B) of elderberry juice started with *L. plantarum* (POM1, C1, 1LE1, 4186 and 285), *L. casei* (2246, 2240,2107), *L. rhamnosus* (1019, 1473, 2360, 2140 and 2178) and unstarted juice. CT was carried out using CRT's method.

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 h, 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 h, 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. Different approaches were applied: heatmap indicated that fermentation and storage influence the production of volatile compounds, PCA well described the differences among the strains used and their source of isolation, while CT analysis permitted to relate the characteristic volatile profile of samples with the different species. To note, 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. 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.

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Chapter 2.1

Appendix

Table S1. Concentration (µg/ml) of volatile compounds identified in elderberry juices fermented with *L. plantarum* (4186, POM1, C1, 1LE1, 285), *L. casei* (2107, 2240, 2246), *L. rhamnosus* (2360, 2178, 2140, 1473, 1019) and in controls (juices treated at 30°C and 37°C) after 48 hours.

	4186	POM1	C1	1LE1	285	30°C
Ketones						
acetone	0.0102 ± 0.00050	0.0070 ± 0.00053	0.0074± 0.00121	0.0096 ± 0.00370	0.0079 ± 0.00024	0.0077 ± 0.00002
diacetyl	0.4007 ± 0.01811	0.0162 ± 0.00177	0.1283 ± 0.03349	0.0738 ± 0.01984	0.0421 ± 0.00416	0.0033 ± 0.00016
acetoin	0.0831 ± 0.00806	0.1077 ± 0.00711	0.4967 ± 0.10210	0.1348 ± 0.18738	0.1635 ± 0.04234	0.0014 ± 0.00118
2.3-octanedione	0.0024 ± 0.00165	0.0016 ± 0.00051	0.0014 ± 0.00068	0.0024 ± 0.00164	0.0016 ± 0.00016	0.0013 ± 0.00015
sulcatone	0.0202 ± 0.02284	0.0021 ± 0.00022	0.0046 ± 0.00132	0.0030 ± 0.00102	0.0013 ± 0.00136	0.0033 ± 0.00107
2,3,4,5-tetramethyl-2-cyclopenten-1-one	0.0327 ± 0.04153	0.0024 ± 0.00050	0.0030 ± 0.00084	0.0020 ± 0.00005	0.0020 ± 0.00004	0.0013 ± 0.00036
erbal ketone	0.0001 ± 0.00004	0.0001 ± 0.00006	0.0002 ± 0.00013	0.0006 ± 0.00024	0.0006 ± 0.00048	0.0001 ± 0.00005
2-undecanone	0.0001 ± 0.00002	0.0024 ± 0.00066	0.0008 ± 0.00024	0.0022 ± 0.00024	0.0030 ± 0.00062	0.0008 ± 0.00015
2-tridecanone	0.0010 ± 0.00031	0.0008 ± 0.00012	0.0009 ± 0.00058	0.0011 ± 0.00019	0.0025 ± 0.00044	0.0006 ± 0.00025
Esters						
ethyl acetate	0.0241 ± 0.00238	0.0252 ± 0.00136	0.0223 ± 0.00325	0.0267 ± 0.00436	0.0036 ± 0.00038	0.0157 ± 0.00030
methyl isovalerate	0.0068 ± 0.00057	0.0067 ± 0.00070	0.0058 ± 0.00087	0.0077 ± 0.00016	0.0007 ± 0.00009	0.0051 ± 0.00068
ethyl isovalerate	0.0115 ± 0.00114	0.0115 ± 0.00072	0.0072 ± 0.00157	0.0123 ± 0.00175	0.0013 ± 0.00059	0.0086 ± 0.00014
isopentyl acetate	0.0023 ± 0.00018	0.0047 ± 0.00079	0.0031 ± 0.00067	0.0053 ± 0.00095	0.0028 ± 0.00067	0.0012 ± 0.00033
isoamyl isovalerate	0.0037 ± 0.00206	0.0010 ± 0.00004	0.0025 ± 0.00071	0.0958 ± 0.13140	0.0007 ± 0.00055	0.0019 ± 0.00088
(E)-2-hexenyl-acetate	0.0043 ± 0.00352	0.0016 ± 0.00063	0.0016 ± 0.00086	0.0011 ± 0.00151	0.0041 ± 0.00025	0.0004 ± 0.00020
methyl salicylate	0.0101 ± 0.00136	0.0072 ± 0.00028	0.0054 ± 0.00009	0.0121 ± 0.00409	0.0245 ± 0.00374	0.0071 ± 0.00027
phenethyl acetate	0.0059 ± 0.00003	0.0056 ± 0.00002	0.0038 ±0.00136	0.0075 ± 0.00058	0.0002 ± 0.00017	0.0044 ± 0.00023
Aldehydes						
isovaleraldehyde	0.0189 ± 0.00048	0.0007 ± 0.00020	0.0020 ± 0.00116	0.0038 ± 0.00017	0.0005 ± 0.00035	0.0650 ± 0.01036
3-(2,3,6-trimetil-1-cicloesen-1-yl)-2-propenal	0.0009 ± 0.00008	0.0016 ± 0.00001	0.0005 ± 0.00008	0.0022 ± 0.00009	0.0017 ± 0.00024	0.0005 ± 0.00010
3-(2,3,6-trimetil-1-cicloesen-1-yl)-2-propenal “like”	0.0010 ± 0.00007	0.0016 ± 0.00008	0.0012 ± 0.00010	0.0018 ± 0.00008	0.0017 ± 0.00032	0.0002 ± 0.00001
Alcohols						
1-pentanol	0.0050 ± 0.00458	0.0033 ± 0.00244	0.0008 ± 0.00011	0.0050 ± 0.00552	0.0024 ± 0.00199	0.0002 ± 0.00007
ethanol	0.0643 ± 0.00784	0.0580 ± 0.00019	0.0650 ± 0.01639	0.0688 ± 0.01835	0.0476 ± 0.00402	0.0346 ± 0.00389
isoamyl alcohol	0.0685 ± 0.06574	0.1443 ± 0.00013	0.1171 ± 0.02277	0.1377 ± 0.05544	0.0382 ± 0.01587	0.0438 ± 0.00398
2-penten-1-ol	0.0030 ± 0.00124	0.0060 ± 0.00013	0.0027 ± 0.00149	0.0031 ± 0.00151	0.0046 ± 0.00011	0.0012 ± 0.00000
hexanol	0.0288 ± 0.03865	0.0892 ± 0.00193	0.0687 ± 0.01559	0.1084 ± 0.01174	0.5944 ± 0.05957	0.0309 ± 0.00174
(E)-3-hexen-1-ol	0.0195 ± 0.02479	0.0026 ± 0.00100	0.0014 ± 0.00004	0.0024 ± 0.00041	0.0199 ± 0.00123	0.0009 ±0.00008
(Z)-3-hexen-1-ol	0.0315 ± 0.00401	0.0643 ± 0.00429	0.0482 ± 0.01304	0.0962 ± 0.00899	0.1515 ± 0.01309	0.0183 ±0.00084
3-octanol	0.0009 ± 0.00004	0.0058 ± 0.00049	0.0017 ± 0.00016	0.0024 ± 0.00157	0.0044 ± 0.00023	0.0001 ± 0.00003
(E)-2-hexen-1-ol	0.0141 ± 0.01332	0.0185 ± 0.00018	0.0165 ± 0.00297	0.0218 ± 0.00443	0.5309 ± 0.04668	0.0071 ± 0.00011
1-octen-3-ol	0.0110 ± 0.00081	0.0135 ± 0.00089	0.0092 ± 0.00209	0.0144 ± 0.00083	0.0106 ± 0.00027	0.0056 ± 0.00034
heptanol	0.0017 ± 0.00046	0.0024 ± 0.00094	0.0038 ± 0.00004	0.0037 ± 0.00096	0.0067 ± 0.00022	0.0008 ± 0.00013
octanol	0.0026 ± 0.00024	0.0072 ± 0.00028	0.0054 ± 0.00016	0.0061 ± 0.00093	0.0065 ± 0.00033	0.0021 ± 0.00046
6-methyl-heptanol	0.0029 ± 0.00033	0.0026 ± 0.00075	0.0029 ± 0.00093	0.0040 ± 0.00073	0.0094 ± 0.00166	0.0015 ± 0.00014
6-methyl-octanol	0.0022 ± 0.00015	0.0019 ± 0.00009	0.0026 ± 0.00123	0.0030 ± 0.00129	0.0063 ± 0.00175	0.0014 ± 0.00027
nonanol	0.0007 ± 0.00026	0.0018 ± 0.00096	0.0031 ± 0.00194	0.0077 ± 0.00237	0.0120 ± 0.00443	0.0020 ± 0.00060
2-furanmethanol	0.0156 ± 0.00192	0.0144 ± 0.00053	0.0128 ± 0.00362	0.0176 ± 0.00645	0.0267 ± 0.00161	0.0080 ± 0.00085
2-phenylmethanol	0.0497 ± 0.00251	0.0596 ± 0.00291	0.0444 ± 0.00904	0.0771 ± 0.00989	0.1303 ± 0.02720	0.0231 ± 0.00176
2-phenylethanol	0.1581 ± 0.00535	0.1798 ± 0.00262	0.1449 ± 0.02603	0.2317 ± 0.02281	0.1555 ± 0.01829	0.0805 ± 0.01009
dodecanol	0.0006 ± 0.00004	0.0009 ± 0.00030	0.0008 ± 0.00060	0.0008 ± 0.00050	0.0019 ± 0.00009	0.0005 ± 0.00006

	4186	POM1	C1	1LE1	285	30°C
Terpenes and norisoprenoids						
Terpene not specified	0.0019 ± 0.00024	0.0023 ± 0.00010	0.0010 ± 0.00033	0.0017 ± 0.00014	0.0001 ± 0.00002	0.0008 ± 0.00018
3-carene	0.0048 ± 0.00020	0.0077 ± 0.00072	0.0025 ± 0.00075	0.0056 ± 0.00112	0.0015 ± 0.00001	0.0016 ± 0.00099
myrcene	0.0016 ± 0.00002	0.0030 ± 0.00038	0.0014 ± 0.00028	0.0028 ± 0.00233	0.0005 ± 0.00013	0.0004 ± 0.00015
Limonene	0.2230 ± 0.09688	0.2711 ± 0.00516	0.2065 ± 0.04711	0.2731 ± 0.01791	0.2257 ± 0.01889	0.1265 ± 0.00931
eucalyptol	0.0042 ± 0.00112	0.0042 ± 0.00049	0.0027 ± 0.00098	0.0041 ± 0.00070	0.0020 ± 0.00028	0.0021 ± 0.00018
γ-terpinene	0.0530 ± 0.05360	0.0149 ± 0.00103	0.0027 ± 0.00135	0.0216 ± 0.00526	0.0030 ± 0.00166	0.0049 ± 0.00487
m-cymene	0.0208 ± 0.01638	0.0010 ± 0.00063	0.0152 ± 0.00245	0.0095 ± 0.00005	0.0090 ± 0.00513	0.0082 ± 0.00345
terpinolene	0.0110 ± 0.00091	0.0176 ± 0.00026	0.0060 ± 0.00158	0.0149 ± 0.00657	0.0096 ± 0.00053	0.0073 ± 0.00409
α-ionene	0.0030 ± 0.00066	0.0006 ± 0.00000	0.0016 ± 0.00086	0.0017 ± 0.00130	0.0033 ± 0.00080	0.0012 ± 0.00024
cis-linalool oxide	0.0021 ± 0.00007	0.0058 ± 0.00047	0.0026 ± 0.00044	0.0049 ± 0.00238	0.0059 ± 0.00108	0.0021 ± 0.00144
β-linalool	0.1366 ± 0.00829	0.1207 ± 0.00359	0.1112 ± 0.02584	0.1437 ± 0.01335	0.3285 ± 0.02052	0.0793 ± 0.00272
β-Caryophyllene	0.0026 ± 0.00067	0.0021 ± 0.00017	0.0016 ± 0.00021	0.0020 ± 0.00195	0.0011 ± 0.00004	0.0008 ± 0.00087
hotrienol	0.0009 ± 0.00019	0.0032 ± 0.00120	0.0012 ± 0.00044	0.0028 ± 0.00142	0.0069 ± 0.00088	0.0011 ± 0.00105
dihydro-citronellol	0.0061 ± 0.00111	0.0041 ± 0.00006	0.0035 ± 0.00132	0.0052 ± 0.00108	0.0104 ± 0.00067	0.0031 ± 0.00061
α-Terpineol	0.0351 ± 0.00382	0.0338 ± 0.00006	0.0283 ± 0.00836	0.0408 ± 0.00601	0.0791 ± 0.00986	0.0191 ± 0.00051
Cadina-1(10)-4-diene	0.0034 ± 0.00092	0.0012 ± 0.00016	0.0025 ± 0.00128	0.0037 ± 0.00053	0.0012 ± 0.00105	0.0010 ± 0.00097
β-damascenone “like”	0.0038 ± 0.00061	0.0029 ± 0.00014	0.0031 ± 0.00060	0.0037 ± 0.00087	0.0070 ± 0.00071	0.0017 ± 0.00008
β-citronellol (R)	0.0107 ± 0.01274	0.0066 ± 0.00401	0.0052 ± 0.00151	0.0063 ± 0.00637	0.0039 ± 0.00035	0.0037 ± 0.00258
Terpene not identified	0.0025 ± 0.00237	0.0003 ± 0.00008	0.0080 ± 0.00147	0.0043 ± 0.00160	0.0032 ± 0.00039	0.0016 ± 0.00079
cis-geraniol	0.0033 ± 0.00034	0.0028 ± 0.00011	0.0024 ±0.00074	0.0039 ± 0.00023	0.0078 ± 0.00079	0.0012 ± 0.00019
β-damascenone	0.0822 ± 0.00390	0.0613 ± 0.00052	0.0534 ± 0.01507	0.0768 ± 0.01051	0.1566 ± 0.02193	0.0409 ± 0.00077
trans-geraniol	0.0086 ± 0.00027	0.0123 ± 0.00007	0.0111 ± 0.00328	0.0151 ± 0.00142	0.0343 ± 0.00502	0.0030 ± 0.00022
terpene not identified	0.0009 ± 0.00006	0.0011 ± 0.00032	0.0006 ± 0.00022	0.0014 ± 0.00027	0.0052 ± 0.00032	0.0004 ± 0.00046
cis-lanceol	0.0019 ± 0.00004	0.0042 ± 0.00004	0.0010 ± 0.00005	0.0017 ± 0.00036	0.0027 ± 0.00016	0.0009 ± 0.00012
trans-nerolidol	0.0000 ± 0.00000	0.0003 ± 0.00019	0.0012 ± 0.00127	0.0004 ± 0.00007	0.0001 ± 0.00016	0.0001 ± 0.00005
eugenol	0.0394 ± 0.01023	0.0698 ± 0.00439	0.0755 ± 0.03085	0.0945 ± 0.01788	0.1123 ± 0.01102	0.0143 ± 0.01015
Alkenes						
styrene	0.0114 ± 0.00576	0.0124 ± 0.00269	0.0038 ± 0.00183	0.0146 ± 0.00646	0.0020 ± 0.00128	0.0048 ± 0.00322
3,4-dimetil octene	0.0048 ± 0.00118	0.0052 ± 0.00054	0.0035 ± 0.00137	0.0059 ± 0.00001	0.0103 ± 0.00167	0.0024 ± 0.00045
1-2-4 trimetil-1H-indene	0.0016 ± 0.00012	0.0013 ± 0.00032	0.0008 ± 0.00051	0.0016 ± 0.00010	0.0018 ± 0.00030	0.0008 ± 0.00036
Acids						
acetic acid	0.2059 ± 0.02442	0.8153 ± 0.01302	1.0123 ± 0.16815	0.5865 ± 0.10306	0.9270 ± 0.22201	0.0003 ± 0.00020
isovaleric acid	0.2467 ± 0.01154	0.1968 ± 0.00980	0.2244 ± 0.05118	0.2348 ± 0.04180	0.0406 ± 0.00385	0.1226 ± 0.01384
caproic acid	0.0250 ± 0.00063	0.0510 ± 0.00169	0.0650 ± 0.01431	0.0467 ± 0.00424	0.0268 ± 0.00009	0.0052 ± 0.00733
caproic acid like	0.0004 ± 0.00042	0.0008 ± 0.00036	0.0012 ± 0.00005	0.0010 ± 0.00028	0.0006 ± 0.00045	0.0003 ± 0.00005
2-hexenoic acid (E)	0.0076 ± 0.00342	0.0029 ± 0.00129	0.0032 ± 0.00116	0.0076 ± 0.00047	0.0102 ± 0.00225	0.0001 ± 0.00004
caprylic acid	0.0057 ± 0.00047	0.0053 ± 0.00019	0.0106 ± 0.00435	0.0057 ± 0.00094	0.0066 ±0.00079	0.0050 ± 0.00001
Others						
dimethyl disulfide	0.0063 ± 0.00529	0.0020 ± 0.00027	0.0028 ± 0.00364	0.0025 ± 0.00084	0.0012 ± 0.00027	0.0032 ± 0.00164
dimethyl trisulfide	0.0057 ± 0.00360	0.0005 ± 0.00005	0.0020 ± 0.00218	0.0015 ± 0.00109	0.0007 ± 0.00024	0.0045 ± 0.00323
ionone e benzaldeide (co-elution)	0.0515 ± 0.00318	0.0112 ± 0.00922	0.0152 ± 0.00197	0.0404 ±0.01583	0.0989 ± 0.02252	0.1101 ± 0.00018
unknown	0.0292 ± 0.00082	0.0182 ± 0.00104	0.0171 ± 0.00509	0.0253 ± 0.00181	0.0374 ± 0.00643	0.0733 ± 0.00455
unknown	0.0036 ± 0.00038	0.0034 ± 0.00042	0.0031 ± 0.00091	0.0059 ± 0.00056	0.0159 ± 0.00150	0.0048 ± 0.00558
phenol	0.0046 ± 0.00062	0.0045 ± 0.00011	0.0034 ± 0.00054	0.0059 ± 0.00083	0.0028 ± 0.00015	0.0017 ± 0.00151
4-ethyl-phenol	0.0177 ± 0.00482	0.0941 ± 0.01009	0.0018 ± 0.00012	0.0182 ± 0.00746	0.1132 ± 0.02439	0.0059 ± 0.00336

	2107	2240	2246	2360	2178	2140	1473	1019	37°C
Ketones									
acetone	0.0214 ± 0.00137	0.0182 ± 0.00197	0.0150 ± 0.00002	0.0129 ± 0.00126	0.0128 ± 0.00161	0.0223 ± 0.00666	0.0100 ± 0.00035	0.0122 ± 0.00098	0.0296 ± 0.00145
diacetyl	0.2219 ± 0.02150	0.2766 ± 0.00597	0.2529 ± 0.00755	0.5868 ± 0.10469	0.3281 ± 0.05112	0.2620 ± 0.01192	0.0370 ± 0.00096	0.4573 ± 0.07724	0.0122 ± 0.00177
acetoin	0.2222 ± 0.07431	0.3145 ± 0.03430	0.0907 ± 0.00222	0.3642 ± 0.00650	0.1884 ± 0.02114	0.4562 ± 0.02656	0.0414 ± 0.00057	0.2151 ± 0.00063	0.0221 ± 0.00613
2.3-octanedione	0.0007 ± 0.00025	0.0031 ± 0.00031	0.0013 ± 0.00018	0.0020 ± 0.00222	0.0014 ± 0.00043	0.0024 ± 0.00237	0.0010 ± 0.00023	0.0008 ± 0.00030	0.0025 ± 0.00182
sulcatone	0.0033 ± 0.00017	0.0055 ± 0.00131	0.0045 ± 0.00056	0.0029 ± 0.00083	0.0038 ± 0.00078	0.0033 ± 0.00170	0.0021± 0.00088	0.0019 ± 0.00024	0.0059 ± 0.00137
2,3,4,5-tetramethyl-2-cyclopenten-1-one	0.0022 ± 0.00024	0.0036 ± 0.00016	0.0028 ± 0.00094	0.0017 ± 0.00025	0.0019 ± 0.00044	0.0037 ± 0.00233	0.0020 ± 0.00062	0.0023 ± 0.00024	0.0030 ± 0.00007
erbal ketone	0.0008 ± 0.00094	0.0002 ± 0.00002	0.0002 ± 0.00004	0.0135 ± 0.01775	0.0001 ± 0.00006	0.0003 ± 0.00017	0.0008 ± 0.00074	0.0027 ± 0.00222	0.0002 ± 0.00016
2-undecanone	0.0107 ± 0.00140	0.0016 ± 0.00037	0.0020 ± 0.00005	0.0187 ±0.00404	0.0011 ± 0.00002	0.0015 ± 0.00041	0.0059 ± 0.00390	0.0165 ± 0.00176	0.0018 ± 0.00092
2-tridecanone	0.0013 ± 0.00053	0.0017 ± 0.00017	0.0015 ± 0.00047	0.0034 ± 0.00109	0.0004 ± 0.00004	0.0017 ± 0.00140	0.0009 ± 0.00046	0.0018 ± 0.00193	0.0006 ± 0.00011
Esters									
ethyl acetate	0.0137 ± 0.00182	0.0197 ± 0.00013	0.0271 ± 0.00265	0.0031 ± 0.00009	0.0139 ± 0.00131	0.0227 ± 0.00065	0.0030 ± 0.00039	0.0025 ± 0.00065	0.0419 ± 0.00586
methyl isovalerate	0.0052 ± 0.00099	0.0071 ± 0.00106	0.0085 ± 0.00146	0.0002 ± 0.00010	0.0029 ± 0.00087	0.0064 ± 0.00058	0.0015 ± 0.00010	0.0016 ± 0.00049	0.0138 ± 0.00044
ethyl isovalerate	0.0074 ± 0.00013	0.0126 ± 0.00040	0.0122 ± 0.00055	0.0006 ± 0.00031	0.0081 ± 0.00079	0.0101 ± 0.00006	0.0012 ± 0.00031	0.0031 ± 0.00218	0.0190 ± 0.00118
isopentyl acetate	0.0024 ± 0.00003	0.0045 ± 0.00023	0.0025 ± 0.00001	0.0028 ± 0.00051	0.0031 ± 0.00025	0.0043 ± 0.00004	0.0032 ± 0.00067	0.0027 ± 0.00090	0.0023 ± 0.00028
isoamyl isovalerate	0.0025 ± 0.00099	0.0029 ± 0.00098	0.0033 ± 0.00147	0.0004 ± 0.00024	0.0011 ± 0.00100	0.0040 ± 0.00038	0.0006 ± 0.00052	0.0015 ± 0.00121	0.0031 ± 0.00104
(E)-2-hexenyl-acetate	0.0016 ± 0.00078	0.0023 ± 0.00051	0.0008 ± 0.00031	0.0010 ± 0.00110	0.0009 ± 0.00040	0.0023 ± 0.00043	0.0029 ± 0.00238	0.0012 ± 0.00111	0.0014 ± 0.00035
methyl salicylate	0.0142 ± 0.00196	0.0159 ± 0.00079	0.0128 ± 0.00033	0.0202 ± 0.00062	0.0202 ± 0.00147	0.0247 ± 0.00258	0.0185 ± 0.00061	0.0179 ± 0.00192	0.0140 ± 0.00331
phenethyl acetate	0.0050 ± 0.00007	0.0065 ±0.00022	0.0070 ± 0.00073	0.0052 ± 0.00485	0.0032 ± 0.00098	0.0059 ± 0.00216	0.0065 ± 0.00113	0.0066 ± 0.00062	0.0081 ± 0.00111
Aldehydes									
isovaleraldehyde	0.0125 ± 0.00909	0.0109 ± 0.00528	0.0292 ± 0.00194	0.0041 ± 0.00008	0.0064 ± 0.00163	0.0171 ± 0.00151	0.0107 ± 0.00016	0.0056 ± 0.00061	0.2250 ± 0.01957
3-(2,3,6-trimetil-1-cicloesen-1-yl)-2-propenal	0.0007 ± 0.00052	0.0008 ± 0.00007	0.0009 ± 0.00029	0.0012 ± 0.00030	0.0007 ± 0.00018	0.0015 ± 0.00012	0.0015 ± 0.00066	0.0015 ± 0.00011	0.0011 ± 0.00014
3-(2,3,6-trimetil-1-cicloesen-1-yl)-2-propenal “like”	0.0005 ± 0.00017	0.0007 ± 0.00031	0.0006 ± 0.00014	0.0008 ± 0.00018	0.0005 ± 0.00001	0.0012 ± 0.00070	0.0007 ± 0.00013	0.0007 ± 0.00001	0.0004 ± 0.00011
Alcohols									
1-pentanol	0.0017 ± 0.00038	0.0026 ± 0.00031	0.0007 ± 0.00030	0.0006 ± 0.00020	0.0029 ± 0.00344	0.0096 ± 0.00132	0.0012 ± 0.00025	0.0007 ± 0.00045	0.0171 ± 0.00592
ethanol	0.0530 ± 0.00708	0.0756 ± 0.00334	0.0763 ± 0.01274	0.0310 ± 0.00168	0.0403 ± 0.00070	0.0665 ± 0.00205	0.0420 ± 0.00143	0.0362 ± 0.00195	0.0921 ± 0.01009
isoamyl alcohol	0.0896 ± 0.01659	0.1254 ± 0.00346	0.1417 ± 0.02771	0.0429 ± 0.01289	0.0686 ± 0.01098	0.1539 ± 0.00585	0.0705 ± 0.01086	0.0537 ± 0.00359	0.1255 ± 0.03533
2-penten-1-ol	0.0037 ± 0.00023	0.0045 ± 0.00043	0.0033 ± 0.00033	0.0014 ± 0.00056	0.0023 ± 0.00078	0.0021 ± 0.00266	0.0011 ± 0.00003	0.0008 ± 0.00056	0.0043 ± 0.00168
hexanol	0.0168 ± 0.00330	0.0263 ± 0.00023	0.0599 ± 0.00151	0.2482 ±0.03665	0.0115 ± 0.00229	0.0189 ± 0.00188	0.6241 ± 0.01997	0.0562 ± 0.00298	0.0727 ± 0.00666
(E)-3-hexen-1-ol	0.0021 ± 0.00086	0.0033 ± 0.00056	0.0019 ± 0.00033	0.0115 ±0.00263	0.0005 ± 0.00013	0.0038 ± 0.00085	0.0021 ± 0.00078	0.0026 ± 0.00043	0.0145 ± 0.01629
(Z)-3-hexen-1-ol	0.0611 ± 0.00290	0.0359 ± 0.00280	0.0418 ± 0.00184	0.1301 ± 0.00730	0.0238 ± 0.00335	0.0378 ± 0.00014	0.1454 ± 0.00901	0.0971 ± 0.02516	0.0492 ± 0.00767
3-octanol	0.0019 ± 0.00025	0.0017 ± 0.00006	0.0012 ± 0.00004	0.0044 ± 0.00133	0.0018 ± 0.00025	0.0030 ± 0.00073	0.0023 ± 0.00032	0.0046 ± 0.00426	0.0005 ± 0.00008
(E)-2-hexen-1-ol	0.1260 ± 0.01174	0.0498 ± 0.00093	0.0172 ± 0.00068	0.4404 ± 0.06263	0.0073 ±0.00032	0.0140 ± 0.00367	0.6162 ± 0.01161	0.2130 ± 0.01239	0.0198 ± 0.00256
1-octen-3-ol	0.0119 ± 0.00054	0.0155 ± 0.00087	0.0112 ± 0.00003	0.0084 ± 0.00090	0.0107 ± 0.00102	0.0165 ± 0.00008	0.0092 ± 0.00111	0.0088 ± 0.00013	0.0137 ± 0.00171
heptanol	0.0002 ± 0.00014	0.0011 ± 0.00027	0.0013 ± 0.00027	0.0017 ± 0.00011	0.0005 ± 0.00001	0.0006 ± 0.00019	0.0023 ± 0.00061	0.0012 ± 0.00026	0.0024 ± 0.00026
octanol	0.0010 ± 0.00003	0.0010 ± 0.00006	0.0025 ± 0.00025	0.0011 ± 0.00026	0.0007 ± 0.00015	0.0013 ± 0.00014	0.0063 ± 0.00068	0.0034 ± 0.00039	0.0073 ± 0.00255
6-methyl-heptanol	0.0036 ± 0.00003	0.0033 ± 0.00089	0.0046 ± 0.00046	0.0085 ± 0.00113	0.0019 ± 0.00108	0.0029 ± 0.00105	0.0092 ± 0.00053	0.0069 ± 0.00019	0.0048 ± 0.00023
6-methyl-octanol	0.0026 ± 0.00038	0.0031 ± 0.00106	0.0040 ± 0.00154	0.0073 ± 0.00252	0.0014 ± 0.00025	0.0023 ± 0.00120	0.0097 ± 0.00095	0.0048 ± 0.00092	0.0028 ± 0.00035
nonanol	0.0047 ± 0.00026	0.0082 ± 0.00548	0.0126 ± 0.00298	0.0009 ± 0.00034	0.0003 ± 0.00004	0.0108 ± 0.00289	0.0167 ± 0.00075	0.0224 ± 0.00184	0.0068 ± 0.00019
2-furanmethanol	0.0170 ± 0.00051	0.0186 ± 0.00060	0.0195 ± 0.00193	0.0266 ± 0.00465	0.0114 ± 0.00029	0.0224 ± 0.00055	0.0271 ± 0.00315	0.0246 ± 0.00297	0.0205 ± 0.00078
2-phenylmethanol	0.0491 ± 0.00287	0.0540 ± 0.00093	0.0519 ± 0.00199	0.0524 ± 0.00289	0.0365 ± 0.00049	0.0671 ± 0.00146	0.0589 ± 0.00111	0.0562 ± 0.00213	0.0584 ± 0.00501
2-phenylethanol	0.1676 ± 0.01383	0.1996 ± 0.00898	0.2273 ± 0.01405	0.1252 ± 0.02374	0.1473 ± 0.00878	0.2459 ± 0.00290	0.1342 ± 0.00347	0.1304 ± 0.00923	0.2096 ± 0.01585
dodecanol	0.0008 ± 0.00006	0.0009 ± 0.00006	0.0009 ± 0.00005	0.0015 ± 0.00027	0.0011 ± 0.00019	0.0014 ± 0.00005	0.0023 ± 0.00013	0.0029 ± 0.00018	0.0008 ± 0.00002

	2107	2240	2246	2360	2178	2140	1473	1019	37°C
Terpenes and norisoprenoids									
Terpene not specified	0.0015 ± 0.00044	0.0019 ± 0.00012	0.0025 ± 0.00006	0.0005 ± 0.00036	0.0008 ± 0.00018	0.0019 ± 0.00022	0.0003 ± 0.00024	0.0004 ± 0.00000	0.0008 ± 0.00018
3-carene	0.0029 ± 0.00024	0.0053 ± 0.00054	0.0045 ± 0.00043	0.0009 ± 0.00080	0.0031 ± 0.00032	0.0061 ± 0.00045	0.0023 ± 0.00011	0.0021 ± 0.00039	0.0016 ± 0.00099
myrcene	0.0011 ± 0.00022	0.0028 ± 0.00071	0.0040 ± 0.00011	0.0004 ± 0.00033	0.0007 ± 0.00006	0.0030 ± 0.00116	0.0014 ± 0.00039	0.0010 ± 0.00016	0.0004 ± 0.00015
Limonene	0.2717 ± 0.00822	0.2154 ± 0.10337	0.2348 ± 0.09655	0.1977 ± 0.00151	0.1359 ± 0.06371	0.1325 ± 0.00169	0.1898 ± 0.01039	0.1559 ± 0.01730	0.1265 ± 0.00931
eucalyptol	0.0034 ± 0.00091	0.0059 ± 0.00165	0.0034 ± 0.00014	0.0022 ± 0.00065	0.0023 ± 0.00031	0.0044 ± 0.00227	0.0024 ± 0.00065	0.0023 ± 0.00077	0.0021 ± 0.00018
γ-terpinene	0.0213 ± 0.00781	0.0143 ± 0.00056	0.0177 ± 0.01455	0.0025 ± 0.00235	0.0001 ± 0.00001	0.0062 ± 0.00283	0.0029 ± 0.00056	0.0027 ± 0.00007	0.0049 ± 0.00487
m-cymene	0.0158 ± 0.00418	0.0143 ± 0.00136	0.0168 ± 0.00052	0.0054 ± 0.00097	0.0091 ± 0.00450	0.0093 ± 0.00328	0.0056 ± 0.00047	0.0036 ± 0.00113	0.0082 ± 0.00345
terpinolene	0.0070 ± 0.00182	0.0107 ± 0.00677	0.0218 ± 0.00349	0.0049 ± 0.00088	0.0036 ± 0.00091	0.0054 ± 0.00190	0.0074 ± 0.00437	0.0044 ± 0.00041	0.0073 ± 0.00409
α-ionene	0.0040 ± 0.00166	0.0045 ± 0.00024	0.0042 ± 0.00084	0.0014 ± 0.00134	0.0017 ± 0.00025	0.0056 ± 0.00046	0.0030 ± 0.00271	0.0010 ± 0.00037	0.0012 ± 0.00024
cis-linalool oxide	0.0034 ± 0.00035	0.0038 ± 0.00228	0.0024 ± 0.00021	0.0023 ± 0.00011	0.0015 ± 0.00013	0.0027 ± 0.00018	0.0031 ± 0.00043	0.0047 ± 0.00195	0.0021 ± 0.00144
β-linalool	0.1891 ± 0.00383	0.1552 ± 0.00714	0.1503 ± 0.01026	0.3120 ± 0.04888	0.0946 ± 0.00980	0.1459 ± 0.00151	0.3397 ± 0.01263	0.3184 ± 0.00694	0.0793 ± 0.00272
β-Caryophyllene	0.0023 ± 0.00018	0.0045 ± 0.00065	0.0057 ± 0.00095	0.0009 ± 0.00028	0.0019 ± 0.00047	0.0010 ± 0.00113	0.0016 ± 0.00022	0.0008 ± 0.00009	0.0008 ± 0.00087
hotrienol	0.0037 ± 0.00018	0.0014 ± 0.00039	0.0022 ± 0.00168	0.0053 ± 0.00225	0.0009 ± 0.00001	0.0008 ± 0.00034	0.0042 ± 0.00039	0.0057 ± 0.00056	0.0011 ± 0.00105
dihydro-citronellol	0.0075 ± 0.00086	0.0073 ± 0.00006	0.0078 ± 0.00032	0.0116 ± 0.00260	0.0055 ± 0.00056	0.0090 ± 0.00031	0.0141 ± 0.00249	0.0120 ± 0.00059	0.0031 ± 0.00061
α-Terpineol	0.0483 ± 0.00295	0.0434 ± 0.00105	0.0425 ± 0.00401	0.0748 ± 0.01204	0.0234 ± 0.00727	0.0441 ± 0.00135	0.0796 ± 0.00013	0.0723 ± 0.00195	0.0191 ± 0.00051
Cadina-1(10)-4-diene	0.0032 ± 0.00070	0.0054 ± 0.00026	0.0050 ± 0.00024	0.0006 ± 0.00003	0.0028 ± 0.00017	0.0057 ± 0.00081	0.0010 ± 0.00032	0.0059 ± 0.00179	0.0010 ± 0.00097
β-damascenone "like"	0.0030 ± 0.00021	0.0038 ± 0.00017	0.0030 ± 0.00031	0.0071 ± 0.00360	0.0018 ± 0.00037	0.0035 ± 0.00039	0.0034 ± 0.00031	0.0041 ± 0.00022	0.0017 ± 0.00008
β-citronellol (R)	0.0053 ± 0.00000	0.0043 ± 0.00144	0.0036 ± 0.00050	0.0031 ± 0.00059	0.0010 ± 0.00013	0.0318 ± 0.01144	0.0056 ± 0.00291	0.0018 ± 0.00031	0.0037 ± 0.00258
Terpene not identified	0.0019 ± 0.00080	0.0074 ± 0.00111	0.0091 ± 0.00399	0.0048 ± 0.00175	0.0012 ± 0.00039	0.0217 ± 0.00591	0.0038 ± 0.00065	0.0259 ± 0.00576	0.0016 ± 0.00079
cis-geraniol	0.0046 ± 0.00044	0.0049 ± 0.00152	0.0033 ± 0.00011	0.0077 ± 0.00100	0.0020 ± 0.00021	0.0041 ± 0.00049	0.0089 ± 0.00034	0.0084 ± 0.00129	0.0012 ± 0.00019
β-damascenone	0.0951 ± 0.00546	0.0945 ± 0.00595	0.0943 ± 0.00383	0.1569 ± 0.01540	0.0576 ± 0.01148	0.0900 ± 0.00228	0.1613 ± 0.00230	0.1443 ± 0.00023	0.0409 ± 0.00077
trans-geraniol	0.0126 ± 0.00077	0.0141 ± 0.00239	0.0071 ± 0.00006	0.0203 ± 0.00191	0.0085 ± 0.00035	0.0157 ± 0.00124	0.0256 ± 0.00205	0.0196 ± 0.00009	0.0030 ± 0.00022
terpene not identified	0.0007 ± 0.00007	0.0008 ± 0.00030	0.0008 ± 0.00024	0.0028 ± 0.00042	0.0004 ± 0.00004	0.0010 ± 0.00031	0.0031 ± 0.00012	0.0023 ± 0.00010	0.0004 ± 0.00046
cis-lanceol	0.0020 ± 0.00021	0.0020 ± 0.00006	0.0022 ± 0.00015	0.0031 ± 0.00059	0.0013 ± 0.00009	0.0015 ± 0.00024	0.0023 ± 0.00029	0.0023 ± 0.00032	0.0009 ± 0.00012
trans-nerolidol	0.0002 ± 0.00000	0.0008 ± 0.00042	0.0004 ± 0.00026	0.0011 ± 0.00007	0.0006 ± 0.00015	0.0013 ± 0.00088	0.0007 ± 0.00009	0.0012 ± 0.00007	0.0001 ± 0.00005
eugenol	0.0831 ± 0.00937	0.0946 ± 0.00415	0.0403 ± 0.00037	0.1251 ± 0.01834	0.0821 ± 0.01431	0.0990 ±0.00800	0.1272 ± 0.00261	0.1278 ± 0.00255	0.0143 ± 0.01015
Alkenes									
styrene	0.0047 ± 0.00016	0.0063 ± 0.00053	0.0083 ± 0.00032	0.0052 ± 0.00308	0.0086 ± 0.00215	0.0166 ± 0.00262	0.0032 ± 0.00014	0.0049 ± 0.00350	0.0048 ± 0.00322
3,4-dimetil octene	0.0056 ± 0.00068	0.0053 ± 0.00046	0.0054 ± 0.00033	0.0112 ± 0.00450	0.0040 ± 0.00058	0.0055 ± 0.00020	0.0112 ± 0.00138	0.0122 ± 0.00219	0.0024 ± 0.00045
1-2-4 trimetil-1H-indene	0.0019 ± 0.00003	0.0024 ± 0.00005	0.0023 ± 0.00002	0.0014 ± 0.00089	0.0014 ± 0.00001	0.0029 ± 0.00008	0.0018 ± 0.00022	0.0021 ± 0.00004	0.0008 ± 0.00036
Acids									
acetic acid	0.0623 ± 0.02627	0.1221 ± 0.00071	0.0940 ± 0.00174	0.1221 ± 0.02263	0.1165 ± 0.01222	0.1321 ± 0.03745	0.0472 ± 0.00795	0.0529 ± 0.00810	0.0003 ± 0.00020
isovaleric acid	0.1878 ± 0.02805	0.2878 ± 0.01792	0.3032 ± 0.02499	0.0241 ± 0.00101	0.1549 ± 0.02705	0.3053 ± 0.01075	0.0340 ± 0.00476	0.0309 ± 0.00116	0.1226 ± 0.01384
caproic acid	0.0626 ± 0.00175	0.041 ± 0.00182	0.0323 ± 0.00371	0.0883 ± 0.01749	0.0222 ± 0.00189	0.0011 ± 0.00088	0.0141 ± 0.00253	0.1318 ± 0.00457	0.0052 ± 0.00733
caproic acid like	0.0008 ± 0.00022	0.0006 ± 0.00021	0.0007 ± 0.00034	0.0008 ± 0.00056	0.0003 ± 0.00016	0.0009 ± 0.00008	0.0010 ± 0.00059	0.0011 ± 0.00011	0.0003 ± 0.00005
2-hexenoic acid (E)	0.0175 ± 0.00224	0.0131 ± 0.00062	0.0120 ± 0.00010	0.0186 ± 0.00455	0.0002 ± 0.00000	0.0117 ± 0.00116	0.0108 ± 0.00175	0.0356 ± 0.00549	0.0001 ± 0.00004
caprylic acid	0.0064 ± 0.00064	0.0063 ± 0.00061	0.0078 ± 0.00061	0.0068 ± 0.00158	0.0056 ± 0.00238	0.0037 ± 0.00002	0.0087 ± 0.00026	0.0127 ± 0.00036	0.0050 ± 0.00001
Others									
dimethyl disulfide	0.0069 ± 0.00715	0.0024 ± 0.00042	0.0061 ± 0.00450	0.0066 ± 0.00049	0.0085 ± 0.00770	0.0019 ± 0.00017	0.0017 ± 0.00005	0.0039 ± 0.00258	0.0032 ± 0.00164
dimethyl trisulfide	0.0036 ± 0.00258	0.0028 ±0.00101	0.0185 ± 0.01516	0.0041 ± 0.00297	0.0057 ± 0.00552	0.0041 ± 0.00007	0.0015 ± 0.00020	0.0020 ± 0.00002	0.0045 ± 0.00323
ionone e benzaldeide (co-elution)	0.0732 ± 0.00416	0.0597 ± 0.00099	0.0791 ± 0.00093	0.2263 ± 0.04887	0.0856 ± 0.01724	0.0836 ± 0.00667	0.2348 ± 0.01170	0.2015 ± 0.00099	0.1101 ± 0.00018
unknown	0.0320 ± 0.00259	0.0282 ± 0.00478	0.0446 ± 0.00003	0.0505 ± 0.00312	0.0315 ± 0.00695	0.0490 ± 0.00058	0.0361 ± 0.02575	0.0521 ± 0.00123	0.0733 ± 0.00455
unknown	0.0021 ± 0.00007	0.0026 ± 0.00026	0.0028 ± 0.00022	0.003 ± 0.00050	0.0020 ± 0.00026	0.0064 ± 0.00013	0.0043 ± 0.00051	0.0056 ± 0.00120	0.0048 ± 0.00558
phenol	0.0046 ± 0.00020	0.0059 ± 0.00007	0.0067 ± 0.00009	0.0020 ± 0.00022	0.0043 ± 0.00040	0.0072 ± 0.00012	0.0020 ± 0.00007	0.0024 ± 0.00065	0.0017 ± 0.00151
4-ethyl-phenol	0.0058 ± 0.00069	0.0096 ± 0.00143	0.0076 ± 0.00059	0.0028 ± 0.00082	0.0042 ± 0.00031	0.0141 ± 0.00120	0.0028 ± 0.00019	0.0033 ± 0.00070	0.0059 ± 0.00336

Table S2. Concentration (µg/ml) of volatile compounds identified in elderberry juices fermented with *L. plantarum* (4186, POM, C1, 1LE1, 285), *L. casei* (2107, 2240, 2246), *L. rhamnosus* (2360, 2178, 2140, 1473, 1019) and in controls (juices treated at 30°C and 37°C) after storage (14 days).

	4186	POM1	C1	1LE1	285	30°C
Ketones						
acetone	0.0226 ± 0.00221	0.0060 ± 0.00159	0.0054 ± 0.00101	0.0064 ± 0.00067	0.0100 ± 0.00266	0.0253 ± 0.00332
diacetyl)	0.4970 ± 0.00908	0.0061 ± 0.00046	0.0461 ± 0.00535	0.0165 ± 0.00026	0.0386 ± 0.00001	0.0081 ± 0.00003
acetoin	0.3273 ± 0.05722	0.0971 ± 0.00940	0.3949 ± 0.01292	0.1455 ± 0.02197	0.1120 ± 0.13672	0.0099 ± 0.01251
2,3-octanedione	0.0023 ± 0.00037	0.0017 ± 0.00015	0.0016 ± 0.00080	0.0015 ± 0.00085	0.0054 ± 0.00125	0.0026 ± 0.00063
sulcatone	0.0078 ± 0.00266	0.0018 ± 0.00009	0.0026 ± 0.00062	0.0019 ± 0.00046	0.0050 ± 0.00525	0.0100 ± 0.00040
2,3,4,5-tetramethyl-2-cyclopenten-1-one	0.0061 ± 0.00149	0.0029 ± 0.00076	0.0030 ± 0.00041	0.0019 ± 0.00034	0.0015 ± 0.00118	0.0054 ± 0.00177
erbal ketone	0.0030 ± 0.00405	0.0009 ± 0.00110	0.0002 ± 0.00002	0.0006 ± 0.00054	0.0014 ± 0.00093	0.0003 ± 0.00021
2-undecanone	0.0082 ± 0.00758	0.0012 ± 0.00019	0.0013 ± 0.00022	0.0014 ± 0.00033	0.0039 ± 0.00097	0.0018 ± 0.00008
2-tridecanone	0.0030 ± 0.00202	0.0014 ± 0.00030	0.0013 ± 0.00021	0.0014 ± 0.00026	0.0034 ± 0.00027	0.0010 ± 0.00065
Esters						
ethyl acetate	0.0453 ± 0.00261	0.0163 ± 0.00147	0.0143 ± 0.00101	0.0122 ± 0.00008	0.0061 ± 0.00060	0.0441 ± 0.00014
methyl isovalerate	0.0169 ± 0.00227	0.0070 ± 0.00005	0.0068 ± 0.00089	0.0047 ± 0.00010	0.0020 ± 0.00008	0.0143 ± 0.00095
ethyl isovalerate	0.0257 ± 0.00049	0.0099 ± 0.00025	0.0081 ± 0.00028	0.0077 ± 0.00000	0.0074 ± 0.00328	0.0239 ± 0.00037
isopentyl acetate	0.0083 ± 0.00106	0.0048 ± 0.00027	0.0033 ± 0.00022	0.0035 ± 0.00038	0.0052 ± 0.00018	0.0030 ± 0.00029
isoamyl isovalerate	0.0071 ± 0.00326	0.0026 ± 0.00094	0.0032 ± 0.00089	0.0020 ± 0.00031	0.0864 ± 0.11571	0.0056 ± 0.00015
(E)-2-hexenyl-acetate	0.0047 ± 0.00449	0.0013 ± 0.00063	0.0017 ± 0.00037	0.0008 ± 0.00020	0.0046 ± 0.00482	0.0017 ± 0.00067
methyl salicylate	0.0395 ± 0.00090	0.0142 ± 0.00006	0.0105 ± 0.00115	0.0156 ± 0.00140	0.0505 ± 0.00709	0.0226 ± 0.00029
phenethyl acetate	0.0119 ± 0.00020	0.0068 ± 0.00034	0.0063 ± 0.00109	0.0054 ± 0.00003	0.0135 ± 0.00032	0.0118 ± 0.00216
Aldehydes						
isovaleraldehyde	0.0235 ± 0.00033	0.0032 ± 0.00015	0.0045 ± 0.00149	0.0028 ± 0.00022	0.0110 ± 0.00453	0.1614 ± 0.01654
3-(2,6,6-trimetil-1-cicloesen-1-yl)-2-propenal	0.0027 ± 0.00048	0.0017 ± 0.00017	0.0008 ± 0.00031	0.0016 ± 0.00020	0.0045 ± 0.00054	0.0014 ± 0.00015
3-(2,3,6-trimetil-1-cicloesen-1-yl)-2-propenal "like"	0.0018 ± 0.00034	0.0015 ± 0.00007	0.0014 ± 0.00007	0.0013 ± 0.00016	0.0029 ± 0.00035	0.0006 ± 0.00003
Alcohols						
1-pentanol	0.0045 ± 0.00108	0.0014 ± 0.00008	0.0010 ± 0.00011	0.0011 ± 0.00001	0.0013 ± 0.00054	0.0116 ± 0.01538
ethanol	0.1259 ± 0.02936	0.0394 ± 0.00223	0.0346 ± 0.00544	0.0294 ± 0.00281	0.0394 ± 0.00389	0.0913 ± 0.01627
isoamyl alcohol	0.2843 ± 0.02418	0.1029 ± 0.02374	0.1051 ± 0.00723	0.1063 ± 0.00748	0.0780 ± 0.00086	0.0691 ± 0.08841
2-penten-1-ol	0.0082 ± 0.00254	0.0038 ± 0.00158	0.0027 ± 0.00122	0.0021 ± 0.00009	0.0055 ± 0.00242	0.0049 ± 0.00111
hexanol	0.1143 ± 0.00744	0.0714 ± 0.00050	0.0603 ± 0.00667	0.0615 ± 0.00322	0.8754 ± 0.00432	0.0869 ± 0.00499
(E)-3-hexen-1-ol	0.0030 ± 0.00075	0.0016 ± 0.00023	0.0020 ± 0.00028	0.0015 ± 0.00028	0.0305 ± 0.00256	0.0021 ± 0.00053
(Z)-3-hexen-1-ol	0.0715 ± 0.00929	0.0594 ± 0.00317	0.0443 ± 0.00297	0.0608 ± 0.00171	0.2234 ± 0.00954	0.0593 ± 0.00342
3-octanol	0.0029 ± 0.00005	0.0043 ± 0.00005	0.0019 ± 0.00020	0.0019 ± 0.00063	0.0033 ± 0.00121	0.0015 ± 0.00008
(E)-2-hexen-1-ol	0.0294 ± 0.00062	0.0176 ± 0.00084	0.0137 ± 0.00189	0.0136 ± 0.00123	0.8375 ± 0.02692	0.0231 ± 0.00126
1-octen-3-ol	0.0252 ± 0.00037	0.0118 ± 0.00016	0.0090 ± 0.00160	0.0090 ± 0.00136	0.0138 ± 0.00123	0.0161 ± 0.00250
heptanol	0.0030 ± 0.00065	0.0029 ± 0.00039	0.0030 ± 0.00064	0.0041 ± 0.00158	0.0116 ± 0.00020	0.0021 ± 0.00015
octanol	0.0073 ± 0.00169	0.0048 ± 0.00096	0.0037 ± 0.00034	0.0049 ± 0.00152	0.0101 ± 0.00183	0.0047 ± 0.00099
6-methyl-heptanol	0.0083 ± 0.00018	0.0040 ± 0.00040	0.0032 ± 0.00056	0.0017 ± 0.00009	0.0123 ± 0.00079	0.0041 ± 0.00020
6-methyl-octanol	0.0065 ±0.00000	0.0031 ± 0.00031	0.0026 ± 0.00002	0.0017 ± 0.00031	0.0159 ± 0.00054	0.0038 ± 0.00010
nonanol	0.0149 ± 0.01593	0.0034 ± 0.00237	0.0045 ± 0.00137	0.0026 ± 0.00100	0.0195 ± 0.00191	0.0021 ± 0.00030
2-furanmethanol	0.0313 ± 0.01376	0.0134 ± 0.00002	0.0132 ± 0.00053	0.0104 ± 0.00150	0.0405 ± 0.00131	0.0223 ± 0.00114
2-phenylmethanol	0.1108 ± 0.01823	0.0665 ± 0.00014	0.0548 ± 0.00337	0.0555 ± 0.00336	0.2040 ± 0.00181	0.0701 ± 0.00000
2-phenylethanol	0.4341 ± 0.06811	0.2029 ± 0.00513	0.1910 ±0.01281	0.1746 ± 0.01113	0.2237 ± 0.02797	0.2266 ± 0.01139
dodecanol	0.0022 ± 0.00001	0.0011 ± 0.00026	0.0010 ± 0.00002	0.0011 ± 0.00002	0.0025 ± 0.00050	0.0013 ± 0.00062

	4186	POM1	C1	1LE1	285	30°C
Terpenes and norisoprenoids						
terpene not identified	0.0056 ± 0.00017	0.0025 ± 0.00001	0.0022 ± 0.00027	0.0015 ± 0.00025	0.0005 ± 0.00017	0.0035 ± 0.00074
3-carene	0.0125 ± 0.00052	0.0047 ± 0.00053	0.0044 ± 0.00089	0.0035 ± 0.00074	0.0016 ± 0.00085	0.0083 ± 0.00220
myrcene	0.0045 ± 0.00062	0.0024 ± 0.00087	0.0017 ± 0.00010	0.0012 ± 0.00015	0.0235 ± 0.03210	0.0025 ± 0.00112
Limonene	0.6668 ± 0.01652	0.2917 ± 0.00292	0.2600 ± 0.02862	0.1902 ± 0.01172	0.2956 ± 0.08391	0.3821 ± 0.05453
eucalyptol	0.0068 ± 0.00000	0.0037 ± 0.00092	0.0025 ± 0.00002	0.0021 ± 0.00007	0.0467 ± 0.06079	0.0088 ± 0.00244
γ-terpinene	0.0254 ± 0.01162	0.0021 ± 0.00092	0.0047 ± 0.00047	0.0094 ± 0.00649	0.0096 ± 0.00141	0.0868 ± 0.08574
m-cymene	0.0516 ± 0.02358	0.0156 ± 0.00112	0.0135 ± 0.00214	0.0133 ± 0.00392	0.0055 ± 0.00406	0.0247 ± 0.01007
terpinolene	0.0243 ± 0.01019	0.0088 ± 0.00078	0.0093 ± 0.00418	0.0080 ± 0.00419	0.0091 ± 0.00472	0.0078 ± 0.00978
α-ionene	0.0049 ± 0.00288	0.0030 ± 0.00210	0.0029 ± 0.00070	0.0021 ± 0.00135	0.0016 ± 0.00030	0.0026 ± 0.00305
cis-linalol oxide	0.0060 ± 0.00238	0.0031 ± 0.00091	0.0017 ± 0.00026	0.0018 ± 0.00005	0.0118 ± 0.00529	0.0032 ± 0.00012
β-linalool	0.3002 ± 0.00925	0.1109 ± 0.00290	0.1020 ± 0.01052	0.0858 ± 0.00310	0.4844 ± 0.02064	0.2217 ± 0.01053
β-Caryophyllene	0.0075 ± 0.00255	0.0029 ± 0.00004	0.0026 ± 0.00057	0.0030 ± 0.00146	0.0011 ± 0.00007	0.0030 ± 0.00008
hotrienol	0.0017 ± 0.00007	0.0023 ± 0.00155	0.0011 ± 0.00013	0.0004 ± 0.00003	0.0084 ± 0.00305	0.0008 ± 0.00007
dihydro-citronellol	0.0138 ± 0.00489	0.0051 ± 0.00131	0.0052 ± 0.00032	0.0045 ± 0.00037	0.0169 ± 0.00152	0.0111 ± 0.00205
α-Terpineol	0.0800 ± 0.01432	0.0308 ± 0.00024	0.0272 ± 0.00066	0.0250 ± 0.00326	0.1128 ± 0.00464	0.0541 ± 0.00117
Cadina-1(10)-4-diene	0.0096 ± 0.00613	0.0039 ± 0.00078	0.0046 ± 0.00047	0.0029 ± 0.00095	0.0036 ± 0.00192	0.0042 ± 0.00070
β-damascenone “like”	0.0071 ± 0.00184	0.0029 ± 0.00085	0.0030 ± 0.00058	0.0021 ± 0.00062	0.0110 ± 0.00546	0.0036 ± 0.00023
β-citronellol (R)	0.0130 ± 0.00510	0.0037 ± 0.00137	0.0047 ± 0.00050	0.0040 ± 0.00010	0.0060 ± 0.00076	0.0089 ± 0.00185
Terpene not identified	0.0138 ± 0.00338	0.0029 ± 0.00072	0.0057 ± 0.00087	0.0020 ± 0.00048	0.0062 ± 0.00119	0.0027 ± 0.00027
cis-geraniol	0.0062 ± 0.00028	0.0031 ± 0.00066	0.0025± 0.00008	0.0020 ± 0.00014	0.0133 ± 0.00214	0.0032 ± 0.00013
β-damascenone	0.1852 ± 0.01997	0.0728 ± 0.00261	0.0644 ± 0.00360	0.0576 ± 0.00381	0.2208 ± 0.00135	0.1241 ± 0.00260
trans-geraniol	0.0162 ± 0.00382	0.0136 ± 0.00057	0.0129 ± 0.00063	0.0109 ± 0.00114	0.0526 ± 0.00174	0.0078 ± 0.00007
terpene not identified	0.0012 ± 0.00019	0.0011 ± 0.00018	0.0011 ± 0.00007	0.0009 ± 0.00001	0.0063 ± 0.00050	0.0007 ± 0.00035
cis-lanceol	0.0042 ± 0.00113	0.0014 ± 0.00008	0.0016 ± 0.00015	0.0011 ± 0.00029	0.0037 ± 0.00033	0.0022 ± 0.00035
trans-nerolidolo	0.0001 ± 0.00008	0.0011 ± 0.00001	0.0017 ± 0.00013	0.0012 ± 0.00040	0.0013 ± 0.00124	0.0005 ± 0.00024
eugenol	0.1293 ± 0.02642	0.1005 ± 0.00085	0.0879 ± 0.00755	0.0857 ± 0.00404	0.1999 ± 0.00420	0.0240 ± 0.00147
Alkenes						
styrene	0.0138 ± 0.00107	0.0057 ± 0.00001	0.0058 ± 0.00068	0.0061 ± 0.00133	0.0129 ± 0.00375	0.0218 ± 0.00283
3,4 dimetil octene	0.0119 ± 0.00183	0.0050 ± 0.00037	0.0048 ± 0.00033	0.0033 ± 0.00051	0.0173 ± 0.00025	0.0083 ± 0.00231
1-2-3 trimetil-1H-indene	0.0055 ± 0.00021	0.0028 ± 0.00053	0.0017 ± 0.00002	0.0017 ± 0.00009	0.0039 ± 0.00017	0.0022 ± 0.00086
Acids						
acetic acid	0.1924 ± 0.00721	0.6948 ± 0.07227	0.7762 ± 0.04981	0.3479 ± 0.01179	1.1847 ± 0.01342	0.0004 ± 0.00014
isovaleric acid	0.5774 ± 0.07449	0.2321 ± 0.00001	0.2006 ± 0.03540	0.1579 ± 0.00809	0.0589 ± 0.01925	0.3769 ± 0.01020
caproic acid	0.0645 ± 0.01098	0.0753 ± 0.00222	0.0667 ± 0.00626	0.0547 ± 0.00315	0.0799 ± 0.01271	0.0010 ± 0.00076
caproic acid “like”	0.0014 ± 0.00006	0.0005 ± 0.00011	0.0006 ± 0.00003	0.0005 ± 0.00017	0.0049 ± 0.00047	0.0009 ± 0.00001
2-hexenoic acid (E)	0.0512 ± 0.00978	0.0089 ± 0.00296	0.0082 ± 0.00037	0.0078 ± 0.00092	0.0055 ± 0.00081	0.0001 ± 0.00006
caprylic acid	0.0116 ± 0.00119	0.0043 ± 0.00053	0.0035 ± 0.00088	0.0046 ± 0.00010	0.0113 ± 0.00311	0.0036 ± 0.00481
Others						
dimethyl disulfide	0.0252 ± 0.00331	0.0071 ± 0.00538	0.0084 ± 0.00104	0.0040 ± 0.00243	0.0058 ± 0.00227	0.0035 ± 0.00014
dimethyl trisulfide	0.0161 ± 0.01229	0.0021 ± 0.00066	0.0040 ± 0.00036	0.0018 ± 0.00114	0.0022 ± 0.00004	0.0047 ± 0.00003
ionone e benzaldeide (co-elution)	0.1462 ± 0.01802	0.0500 ± 0.01227	0.0218 ± 0.00854	0.0313 ± 0.00648	0.2690 ± 0.01205	0.4153 ± 0.02792
unknown	0.0788 ± 0.00022	0.0287 ± 0.00302	0.0264 ± 0.00261	0.0220 ± 0.00008	0.1002 ± 0.02172	0.1772 ± 0.00535
unknown	0.0110 ± 0.00619	0.0042 ± 0.00016	0.0040 ± 0.00083	0.0040 ± 0.00025	0.0177 ± 0.00130	0.0123 ± 0.00021
phenol	0.0136 ± 0.00202	0.0055 ± 0.00013	0.0049 ± 0.00048	0.0041 ± 0.00022	0.0033 ± 0.00050	0.0082 ± 0.00046
4-ethyl-phenol	0.0094 ± 0.00180	0.1155 ± 0.00799	0.0161 ± 0.00409	0.0033 ± 0.00036	0.1904 ± 0.00114	0.0169 ± 0.00330

	2107	2240	2246	2360	2178	2140	1473	1019	37°C
Ketones									
acetone	0.0190 ± 0.00141	0.0325 ± 0.00168	0.0191 ± 0.00424	0.0064 ± 0.00088	0.0456 ± 0.00648	0.0390 ± 0.00098	0.0150 ± 0.00230	0.0051 ± 0.00115	0.0121 ± 0.00138
diacetyl)	0.1630 ± 0.01345	0.5594 ± 0.08828	0.2490 ± 0.03061	0.2904 ± 0.03796	0.9775 ± 0.02112	0.3894 ± 0.02770	0.0787 ± 0.00076	0.2231 ± 0.03355	0.0037 ± 0.00032
acetoin	0.2951 ± 0.00771	0.7701 ± 0.05212	0.1512 ± 0.01240	0.2458 ± 0.02054	0.8867 ± 0.16329	0.6989 ± 0.07557	0.0750 ± 0.00225	0.1521 ± 0.02904	0.0065 ± 0.00058
2,3-octanedione	0.0003 ± 0.00003	0.0037 ± 0.00136	0.0017 ± 0.00013	0.0001 ± 0.00001	0.0066 ± 0.00221	0.0065 ± 0.00550	0.0015 ± 0.00033	0.0008 ± 0.00027	0.0009 ± 0.00052
sulcatone	0.0047 ± 0.00043	0.0102 ± 0.00096	0.0062 ± 0.00052	0.0016 ± 0.00006	0.0125 ± 0.00056	0.0064 ± 0.00404	0.0045 ± 0.00032	0.0012 ± 0.00062	0.0048 ± 0.00068
2,3,4,5-tetramethyl-2-cyclopenten-1-one	0.0024 ± 0.00080	0.0117 ± 0.00312	0.0031 ± 0.00083	0.0010 ± 0.00011	0.0090 ± 0.00082	0.0055 ± 0.00010	0.0041 ± 0.00047	0.0010 ± 0.00008	0.0017 ± 0.00005
erbal ketone	0.0005 ± 0.00023	0.0010 ± 0.00055	0.0003 ± 0.00002	0.0004 ± 0.00048	0.0035 ± 0.00352	0.0036 ± 0.00151	0.0003 ± 0.00008	0.0012 ± 0.00087	0.0001 ± 0.00003
2-undecanone	0.0115 ± 0.00188	0.0060 ± 0.00245	0.0018 ± 0.00023	0.0080 ± 0.00114	0.0111 ± 0.00900	0.0041 ± 0.00029	0.0042 ± 0.00064	0.0083 ± 0.00134	0.0006 ± 0.00003
2-tridecanone	0.0012 ± 0.00003	0.0036 ± 0.00093	0.0031 ± 0.00196	0.0011 ± 0.00000	0.0008 ± 0.00042	0.0023 ± 0.00017	0.0019 ± 0.00030	0.0027 ± 0.00078	0.0004 ± 0.00002
Esters									
ethyl acetate	0.0117 ± 0.00027	0.0380 ± 0.00194	0.0295 ± 0.00076	0.0015 ± 0.00014	0.0390 ± 0.00105	0.0363 ± 0.00195	0.0043 ± 0.00033	0.0010 ± 0.00004	0.0182 ± 0.00112
methyl isovalerate	0.0052 ± 0.00057	0.0177 ± 0.00024	0.0121 ± 0.00111	0.0006 ± 0.00067	0.0173 ± 0.00469	0.0161 ± 0.00098	0.0047 ± 0.00044	0.0009 ± 0.00035	0.0064 ± 0.00027
ethyl isovalerate	0.0078 ± 0.00117	0.0230 ± 0.00190	0.0188 ± 0.00135	0.0003 ± 0.00013	0.0295 ± 0.00818	0.0228 ± 0.00321	0.0101 ± 0.00055	0.0028 ± 0.00036	0.0106 ± 0.00055
isopentyl acetate	0.0041 ± 0.00081	0.0125 ± 0.00356	0.0056 ± 0.00059	0.0016 ± 0.00026	0.0171 ± 0.00180	0.0180 ± 0.00105	0.0045 ± 0.00076	0.0017 ± 0.00005	0.0014 ± 0.00017
isoamyl isovalerate	0.0018 ± 0.00022	0.0070 ± 0.00446	0.0038 ± 0.00022	0.0003 ± 0.00010	0.0087 ± 0.00471	0.0114 ± 0.00982	0.0027 ± 0.00042	0.0004 ± 0.00040	0.0022 ± 0.00047
(E)-2-hexenyl-acetate	0.0026 ± 0.00194	0.0035 ± 0.00111	0.0015 ± 0.00038	0.0006 ± 0.00049	0.0056 ± 0.00232	0.0028 ± 0.00015	0.0048 ± 0.00015	0.0007 ± 0.00033	0.0008 ± 0.00002
methyl salicylate	0.0173 ± 0.00104	0.0432 ± 0.00047	0.0224 ± 0.00075	0.0089 ± 0.00198	0.0678 ± 0.00950	0.0681 ± 0.00073	0.0277 ± 0.00195	0.0073 ± 0.00054	0.0093 ± 0.00114
phenethyl acetate	0.0054 ± 0.00025	0.0114 ± 0.00010	0.0104 ± 0.00092	0.0006 ± 0.00068	0.0108 ± 0.00245	0.0106 ± 0.00050	0.0087 ± 0.00167	0.0026 ± 0.00122	0.0049 ± 0.00075
Aldehydes									
isovaleraldehyde	0.0092 ± 0.00330	0.0261 ± 0.00922	0.0110 ± 0.00009	0.0011 ± 0.00020	0.0145 ± 0.00340	0.0127 ± 0.00065	0.0135 ± 0.00680	0.0011 ± 0.00007	0.0910 ± 0.00059
3-(2,6,6-trimetil-1-cicloesen-1-yl)-2-propenal	0.0003 ± 0.00015	0.0026 ± 0.00069	0.0019 ± 0.00024	0.0006 ± 0.00009	0.0029 ± 0.00036	0.0027 ± 0.00021	0.0019 ± 0.00087	0.0005 ± 0.00001	0.0004 ± 0.00001
3-(2,3,6-trimetil-1-cicloesen-1-yl)-2-propenal "like"	0.0004 ± 0.00008	0.0012 ± 0.00031	0.0009 ± 0.00016	0.0004 ± 0.00009	0.0016 ± 0.00018	0.0015 ± 0.00025	0.0017 ± 0.00010	0.0004 ± 0.00008	0.0003 ± 0.00002
Alcohols									
1-pentanol	0.0017 ± 0.00068	0.0032 ± 0.00386	0.0014 ± 0.00045	0.0001 ± 0.00002	0.0031 ± 0.00054	0.0042 ± 0.00113	0.0014 ± 0.00001	0.0003 ± 0.00001	0.0058 ± 0.00760
ethanol	0.0420 ± 0.00168	0.1136 ± 0.00034	0.0767 ± 0.00171	0.0160 ± 0.00047	0.1040 ± 0.00379	0.0968 ± 0.00975	0.0557 ± 0.00901	0.0167 ± 0.00092	0.0377 ± 0.00604
isoamyl alcohol	0.0760 ± 0.00079	0.2389 ± 0.00689	0.1899 ± 0.03512	0.0171 ± 0.00226	0.2546 ± 0.03252	0.2944 ± 0.02855	0.0922 ± 0.00374	0.0299 ± 0.00067	0.0521 ± 0.00557
2-penten-1-ol	0.0034 ± 0.00073	0.0128 ±0.00718	0.0036 ± 0.00028	0.0010 ± 0.00121	0.0074 ± 0.00218	0.0044 ± 0.00396	0.0056 ± 0.00128	0.0012 ± 0.00025	0.0021 ± 0.00049
hexanol	0.0145 ± 0.00135	0.0543 ± 0.00375	0.0703 ± 0.00056	0.1002 ± 0.00905	0.0288 ± 0.00332	0.0173 ± 0.00590	0.9174 ± 0.05759	0.0099 ± 0.00339	0.0344 ± 0.00306
(E)-3-hexen-1-ol	0.0015 ± 0.00046	0.0035 ± 0.00084	0.0024 ± 0.00048	0.0043 ± 0.00027	0.0030 ± 0.00181	0.0286 ± 0.03594	0.0032 ± 0.00002	0.0023 ± 0.00182	0.0008 ± 0.00010
(Z)-3-hexen-1-ol	0.0616 ± 0.00641	0.0875 ± 0.00939	0.0475 ± 0.00019	0.0555 ± 0.01301	0.0942 ± 0.01004	0.0620 ± 0.00523	0.2129 ± 0.01521	0.0263 ± 0.02133	0.0219 ± 0.00242
3-octanol	0.0011 ± 0.00070	0.0021 ± 0.00181	0.0015 ± 0.00011	0.0018 ± 0.00028	0.0067 ± 0.00045	0.0039 ± 0.00153	0.0053 ± 0.00100	0.0014 ± 0.00040	0.0004 ± 0.00037
(E)-2-hexen-1-ol	0.1291 ± 0.00049	0.1200 ± 0.01695	0.0183 ± 0.00047	0.2047 ± 0.00313	0.0273 ± 0.00462	0.0138 ± 0.00045	0.9015 ± 0.06138	0.0676 ± 0.00165	0.0097 ± 0.00074
1-octen-3-ol	0.0123 ± 0.00107	0.0339 ± 0.00051	0.0156 ± 0.00105	0.0042 ± 0.00023	0.0365 ± 0.00038	0.0307 ± 0.00171	0.0156 ± 0.00145	0.0037 ± 0.00004	0.0072 ± 0.00018
heptanol	0.0004 ± 0.00027	0.0015 ± 0.00048	0.0018 ± 0.00019	0.0007 ± 0.00002	0.0010 ± 0.00114	0.0018 ± 0.00014	0.0030 ± 0.00079	0.0006 ± 0.00003	0.0009 ± 0.00001
octanol	0.0008 ± 0.00029	0.0040 ± 0.00031	0.0039 ± 0.00055	0.0006 ± 0.00015	0.0024 ± 0.00102	0.0026 ± 0.00052	0.0088 ± 0.00008	0.0005 ± 0.00039	0.0027 ± 0.00131
6-methyl-heptanol	0.0042 ± 0.00073	0.0080 ± 0.00021	0.0056 ± 0.00128	0.0043 ± 0.00060	0.0092 ± 0.00435	0.0069 ± 0.00001	0.0135 ± 0.00071	0.0031 ± 0.00022	0.0016 ± 0.00046
6-methyl-octanol	0.0022 ± 0.00027	0.0055 ± 0.00130	0.0050 ± 0.00029	0.0023 ± 0.00094	0.0056 ± 0.00058	0.0047 ± 0.00210	0.0127 ± 0.00048	0.0013 ± 0.00004	0.0013 ± 0.00031
nonanol	0.0032 ± 0.00075	0.0064 ± 0.00158	0.0166 ± 0.00216	0.0006 ± 0.00035	0.0047 ± 0.00064	0.0109 ± 0.00470	0.0221 ± 0.00064	0.0109 ± 0.00191	0.0018 ± 0.00044
2-furanmethanol	0.0156 ± 0.00254	0.0372 ± 0.00032	0.0247 ± 0.00032	0.0130 ± 0.00015	0.0333 ± 0.00297	0.0292 ± 0.00065	0.0390 ± 0.00098	0.0116 ± 0.00210	0.0083 ± 0.00090
2-phenylmethanol	0.0560 ± 0.00357	0.1224 ± 0.00067	0.0714 ± 0.00327	0.0283 ± 0.00043	0.1354 ± 0.01258	0.1203 ± 0.00002	0.1009 ± 0.00943	0.0272 ± 0.00160	0.0283 ± 0.00372
2-phenylethanol	0.1759 ± 0.00720	0.4449 ± 0.00215	0.3468 ± 0.00468	0.0591 ± 0.00193	0.4784 ± 0.04158	0.4328 ± 0.00972	0.2089 ± 0.01584	0.0533 ± 0.00400	0.0922 ± 0.01377
dodecanol	0.0007 ± 0.00008	0.0020 ± 0.00004	0.0023 ± 0.00014	0.0012 ± 0.00008	0.0042 ± 0.00031	0.0030 ± 0.00025	0.0029 ± 0.00053	0.0012 ± 0.00003	0.0007 ± 0.00024

	2107	2240	2246	2360	2178	2140	1473	1019	37°C
Terpenes and norisoprenoids									
terpene not identified	0.0018 ± 0.00031	0.0055 ± 0.00047	0.0040 ± 0.00020	0.0004 ± 0.00010	0.0051 ± 0.00049	0.0040 ± 0.00026	0.0005 ± 0.00021	0.0004 ± 0.00037	0.0011 ± 0.00006
3-carene	0.0035 ± 0.00033	0.0138 ± 0.00377	0.0084 ± 0.00076	0.0008 ± 0.00002	0.0123 ± 0.00133	0.0107 ± 0.00206	0.0024 ± 0.00045	0.0006 ± 0.00007	0.0040 ± 0.00020
myrcene	0.0015 ± 0.00035	0.0069 ± 0.00094	0.0043 ± 0.00127	0.0003 ± 0.00015	0.0043 ± 0.00059	0.0066 ± 0.00020	0.0015 ± 0.00012	0.0003 ± 0.00005	0.0034 ± 0.00089
Limonene	0.2437 ± 0.00675	0.6392 ± 0.01783	0.3379 ± 0.13201	0.0983 ± 0.01233	0.6332 ± 0.01428	0.5116 ± 0.03579	0.3261 ± 0.01864	0.0708 ± 0.02866	0.1417 ± 0.00211
eucalyptol	0.0026 ± 0.00047	0.0100 ± 0.00160	0.0051 ± 0.00095	0.0010 ± 0.00019	0.0081 ± 0.00306	0.0060 ± 0.00172	0.0031 ± 0.00011	0.0009 ± 0.00002	0.0030 ± 0.00067
γ-terpinene	0.0219 ± 0.00390	0.0473 ± 0.01262	0.0079 ± 0.00093	0.0012 ± 0.00150	0.0576 ± 0.00351	0.0172 ± 0.01796	0.0023 ± 0.00042	0.0006 ± 0.00038	0.0096 ± 0.00416
m-cymene	0.0115 ± 0.00066	0.0330 ± 0.00252	0.0223 ± 0.00260	0.0027 ± 0.00003	0.0436 ± 0.01096	0.0279 ± 0.00521	0.0083 ± 0.00146	0.0039 ± 0.00212	0.0021 ± 0.00113
terpinolene	0.0062 ± 0.00201	0.0269 ± 0.01323	0.0306 ± 0.00245	0.0016 ± 0.00081	0.0168 ± 0.00026	0.0261 ± 0.01568	0.0072 ± 0.00092	0.0021 ± 0.00102	0.0068 ± 0.00229
α-ionene	0.0031 ± 0.00084	0.0039 ± 0.00114	0.0054 ± 0.00141	0.0008 ± 0.00005	0.0050 ± 0.00308	0.0022 ± 0.00101	0.0024 ± 0.00025	0.0004 ± 0.00011	0.0014 ± 0.00002
cis-linalol oxide	0.0025 ± 0.00051	0.0115 ± 0.00878	0.0035 ± 0.00008	0.0011 ± 0.00008	0.0074 ± 0.00362	0.0096 ± 0.00817	0.0037 ± 0.00084	0.0010 ± 0.00021	0.0013 ± 0.00037
β-linalool	0.1844 ± 0.00838	0.3348 ± 0.00599	0.1915 ± 0.00737	0.1452 ± 0.00629	0.2934 ± 0.00546	0.2551 ± 0.00437	0.4932 ± 0.02761	0.1312 ± 0.00687	0.0946 ± 0.00636
β-Caryophyllene	0.0017 ± 0.00039	0.0060 ± 0.00015	0.0081 ± 0.00074	0.0003 ± 0.00015	0.0061 ± 0.00106	0.0035 ± 0.00086	0.0020 ± 0.00039	0.0004 ± 0.00020	0.0006 ± 0.00011
hotrienol	0.0026 ± 0.00036	0.0062 ± 0.00164	0.0027 ± 0.00274	0.0018 ± 0.00067	0.0046 ± 0.00111	0.0037 ± 0.00279	0.0055 ± 0.00027	0.0013 ± 0.00030	0.0003 ± 0.00004
dihydro-citronellol	0.0085 ± 0.00062	0.0132 ± 0.00604	0.0113 ± 0.00062	0.0069 ± 0.00042	0.0127 ± 0.00075	0.0122 ± 0.00176	0.0188 ± 0.00048	0.0059 ± 0.00046	0.0043 ± 0.00110
α-Terpineol	0.0459 ± 0.00022	0.0883 ± 0.00195	0.0486 ± 0.00085	0.0354 ± 0.00009	0.0832 ± 0.00640	0.0646 ± 0.00101	0.1194 ± 0.00661	0.0307 ± 0.00011	0.0212 ± 0.00246
Cadina-1(10)-4-diene	0.0036 ± 0.00006	0.0083 ± 0.00218	0.0078 ± 0.00030	0.0004 ± 0.00019	0.0066 ± 0.00189	0.0080 ± 0.00420	0.0064 ± 0.00051	0.0017 ± 0.00007	0.0012 ± 0.00072
β-damascenone "like"	0.0025 ± 0.00059	0.0070 ± 0.00068	0.0051 ± 0.00175	0.0021 ± 0.00012	0.0069 ± 0.00164	0.0241 ± 0.00606	0.0061 ± 0.00018	0.0009 ± 0.00013	0.0013 ± 0.00026
β-citronellol (R)	0.0068 ± 0.00209	0.0218 ± 0.00933	0.0063 ± 0.00026	0.0013 ± 0.00008	0.0107 ± 0.00383	0.0051 ± 0.00311	0.0051 ± 0.00098	0.0027 ± 0.00069	0.0034 ± 0.00085
Terpene not identified	0.0025 ± 0.00013	0.0159 ± 0.00686	0.0095 ± 0.01109	0.0001 ± 0.00001	0.0054 ± 0.00165	0.0062 ± 0.00221	0.0054 ± 0.00047	0.0093 ± 0.00004	0.0002 ± 0.00005
cis-geraniol	0.0055 ± 0.00062	0.0083 ± 0.00039	0.0049 ± 0.00180	0.0035 ± 0.00010	0.0068 ± 0.00032	0.0072 ± 0.00133	0.0113 ± 0.00137	0.0038 ± 0.00008	0.0015 ± 0.00043
β-damascenone	0.1045 ± 0.00998	0.1973 ± 0.01162	0.1296 ± 0.01669	0.0724 ± 0.00270	0.1931 ± 0.00428	0.1553 ± 0.00442	0.2337 ± 0.01316	0.0675 ± 0.00370	0.0473 ± 0.00469
trans-geraniol	0.0144 ± 0.00173	0.0280 ± 0.00167	0.0092 ± 0.00089	0.0105 ± 0.00049	0.0299 ± 0.00158	0.0289 ± 0.00140	0.0357 ± 0.00149	0.0090 ± 0.00081	0.0033 ± 0.00072
terpene not identified	0.0009 ± 0.00030	0.0020 ± 0.00002	0.0011 ± 0.00003	0.0012 ± 0.00013	0.0028 ± 0.00131	0.0023 ± 0.00024	0.0045 ± 0.00059	0.0012 ± 0.00000	0.0003 ± 0.00003
cis-lanceol	0.0021 ± 0.00002	0.0040 ± 0.00022	0.0030 ± 0.00035	0.0018 ± 0.00019	0.0068 ± 0.00032	0.0031 ± 0.00080	0.0029 ± 0.00066	0.0011 ± 0.00047	0.0008 ± 0.00016
trans-nerolidolo	0.0005 ± 0.00062	0.0011 ± 0.00072	0.0005 ± 0.00048	0.0006 ± 0.00033	0.0018 ± 0.00005	0.0011 ± 0.00002	0.0019 ± 0.00169	0.0005 ± 0.00000	0.0002 ± 0.00010
eugenol	0.1101 ± 0.01383	0.2444 ± 0.00134	0.0608 ± 0.01586	0.0610 ± 0.00751	0.2598 ± 0.01017	0.2389 ± 0.00637	0.1898 ± 0.01400	0.0548 ± 0.00015	0.0251 ± 0.02184
Alkenes									
styrene	0.0070 ± 0.00047	0.0090 ± 0.00365	0.0174 ± 0.00385	0.0012 ± 0.00023	0.0216 ± 0.00356	0.0235 ± 0.00167	0.0066 ± 0.00122	0.0010 ± 0.00011	0.0083 ± 0.00092
3,4 dimetil octene	0.0047 ± 0.00066	0.0097 ± 0.00168	0.0075 ± 0.00048	0.0041 ± 0.00100	0.0097 ±0.00042	0.0107 ± 0.00271	0.0129 ± 0.00032	0.0049 ± 0.00081	0.0029 ± 0.00066
1-2-3 trimetil-1H-indene	0.0017 ± 0.00062	0.0058 ± 0.00064	0.0038 ± 0.00001	0.0007 ± 0.00011	0.0050 ± 0.00002	0.0057 ± 0.00026	0.0036 ± 0.00133	0.0007 ± 0.00014	0.0011 ± 0.00024
Acids									
acetic acid	0.0818 ± 0.02495	0.2567 ± 0.03527	0.1017 ± 0.01756	0.0623 ± 0.00260	0.0980 ± 0.01606	0.1426 ± 0.05268	0.1065 ± 0.00196	0.0221 ± 0.00238	0.0001 ± 0.00004
isovaleric acid	0.1944 ± 0.00434	0.5323 ± 0.02658	0.4122 ± 0.03842	0.0084 ± 0.00929	0.5412 ± 0.02656	0.4777 ± 0.06568	0.0541 ± 0.00759	0.0140 ± 0.00203	0.1410 ± 0.02680
caproic acid	0.0683 ± 0.00179	0.0950 ± 0.01125	0.0345 ± 0.00337	0.0367 ± 0.00454	0.0861 ± 0.00162	0.0762 ± 0.00931	0.0184 ± 0.00176	0.0552 ± 0.00197	0.0101 ± 0.01348
caproic acid "like"	0.0004 ± 0.00003	0.0013 ± 0.00050	0.0011 ± 0.00006	0.0002 ± 0.00013	0.0018 ± 0.00005	0.0016 ± 0.00016	0.0010 ± 0.00066	0.0004 ± 0.00000	0.0009 ± 0.00079
2-hexenoic acid (E)	0.0197 ± 0.00401	0.0301 ± 0.00269	0.0389 ± 0.00241	0.0051 ± 0.00705	0.0005 ± 0.00047	0.0242 ± 0.01381	0.0154 ± 0.00097	0.0179 ± 0.00125	0.0021 ± 0.00294
caprylic acid	0.0077 ± 0.00199	0.0159 ± 0.00149	0.0091 ± 0.00042	0.0038 ± 0.00087	0.0122 ± 0.00031	0.0099 ± 0.00098	0.0115 ± 0.00276	0.0032 ± 0.00049	0.0078 ± 0.00725
Others									
dimethyl disulfide	0.0085 ± 0.00799	0.0239 ± 0.02162	0.0094 ± 0.00744	0.0020 ± 0.00081	0.0089 ± 0.00033	0.0124 ± 0.01046	0.0102 ± 0.00025	0.0028 ± 0.00026	0.0040 ± 0.00283
dimethyl trisulfide	0.0055 ± 0.00337	0.0168 ± 0.01286	0.0193 ± 0.01888	0.0023 ± 0.00156	0.0071 ± 0.00040	0.0283 ± 0.03351	0.0037 ± 0.00035	0.0031 ± 0.00012	0.0083 ± 0.00889
ionone e benzaldeide (co-elution)	0.0629 ± 0.00578	0.1303 ± 0.03149	0.0810 ± 0.00886	0.0631 ± 0.02642	0.1734 ± 0.01566	0.1184 ± 0.03553	0.2450 ± 0.01560	0.0617 ± 0.00102	0.1991 ± 0.00756
unknown	0.0423 ± 0.01574	0.0936 ± 0.00445	0.0542 ± 0.00364	0.0213 ± 0.00489	0.0790 ± 0.01131	0.0749 ± 0.00417	0.0633 ± 0.00101	0.0194 ± 0.00165	0.0748 ± 0.00330
unknown	0.0028 ± 0.00066	0.0060 ± 0.00109	0.0027 ± 0.00082	0.0029 ± 0.00044	0.0531 ± 0.01299	0.0133 ± 0.00510	0.0072 ± 0.00001	0.0022 ± 0.00018	0.0072 ± 0.00454
phenol	0.0040 ± 0.00015	0.0126 ± 0.00095	0.0088 ± 0.00007	0.0008 ± 0.00021	0.0129 ± 0.00138	0.0121 ± 0.00063	0.0027 ± 0.00018	0.0007 ± 0.00006	0.0034 ± 0.00046
4-ethyl-phenol	0.0028 ± 0.00019	0.0074 ± 0.00008	0.0060 ± 0.00082	0.0028 ± 0.00018	0.0127 ± 0.00057	0.0120 ± 0.00037	0.0034 ± 0.00090	0.0010 ± 0.00027	0.0074 ± 0.00054

Chapter 2.2

In vitro metabolism of elderberry juice polyphenols by lactic acid bacteria

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Abstract

In this study, ten strains of *Lactobacillus* were used to assess the in vitro metabolism of elderberry juice polyphenols. Total polyphenolic compounds increased after starter addition, especially with three *L. rhamnosus* and one *L. plantarum* strains, of dairy origin: quercetin-3-*O*-rutinoside was the most abundant compound (from 39.02 ± 5.28 to 127.56 ± 11.34 μg/mL) and hydroxycinnamic acids, flavonols and antocyanins reached the highest amounts. When *L. plantarum* were used phenyllactic acids presented a value of 7.05 ± 2.38 μg/mL, while in the other samples it was around 5.56 ± 1.65 μg/mL. Hydroxycinnamic and hydroxybenzoic acids were subjected to lactic acid bacteria metabolism: caffeic and protocatechuic acids were consumed during fermentation while dihydrocaffeic acid and catechol were produced. Anthocyanins increased in a strain-specific way. So, by this study we highlighted that dairy strains can produced (phenyllactic acids), modified (hydroxycinnamic acids) or increased (flavonols glycosides and anthocyanins) phenolic compounds.

Introduction

Elderberry (*Sambucus nigra L.*) is the most widespread shrub in Europe, Asia, North Africa and USA. Berries ripen in late summer and are used for concentrate or juice preparations. The juice contains sugar and organic acids, conferring a sweet and astringent taste, besides polyphenols, responsible of the typical black-purple colour of the berries (Sidor et al., 2015). Anthocyanins give this dark colour to the fruits and are considered an indicator of fruit quality (Veberic et al., 2009) Cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside are the most abundant cyaniding glycosides. Elderberries are also rich in flavonols and phenolic acids. The main flavonols are quercetin, kaempferol, and isorhamnetin-glycosides, with quercetin-3-*O*-rutinoside being among the principal components (Lee et al., 2007). The main acyl-quinic acid is 5-*O*-caffeoylquinic acid , with smaller amounts of 4-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid and other derivatives of caffeic and *p*-coumaric acid (Lee et al., 2007; Sidor et al., 2015). In the recent years, fermentation of fruit juice with lactic acid bacteria (LAB) has been used to produce functional beverages (Kwaw et al., 2018; Marsha et al., 2014). The use of fruit juices as vehicles for probiotic microorganisms has been increasing as an alternative for people that do not consume dairy products (Pereira and Rodrigues, 2018). Although there are many studies evaluating the efficacy of probiotics in fruit juices (Dimitrovski et al., 2015; Mousavi et al., 2011; Nagpal et al., 2012; Pereira et al., 2011), few commercially products are already on the market (Perricone et al., 2015). The widely known probiotic strains belong to the genus of *Lactobacillus*, especially to *Lactobacillus plantarum, L. casei* and *L. rhamnosus* species. Beyond the probiotic role of the starter used, which is strains specific and can be demonstrated by in vivo studies, different metabolites of interest are produced during fruit fermentation by LAB. LAB use sugars, organic acids (malic acid) and free amino acids present in fruits as energy sources to produce primary and secondary metabolites of interest (Filannino et al., 2014). Besides lactic acid and acetic acid production by homo- or

heterofermentative pathway, synthesis of B-group vitamins such as folates, functional fatty acids, anti-hypertensive peptides and exopolysaccharides is often described (Di Cagno et al., 2011; Espirito-Santo et al., 2015). It has been suggested that LABs improve the bioavailability and bioactivity of phytochemicals present in the juice and lead an increase of potentially functional microbial metabolites with putative benefits for human health (Filannino et al., 2018).

Polyphenols may negatively affect bacterial viability, only a few LABs are indeed able to grow in contact with substrates rich in these compounds. High concentrations of polyphenols negatively affect LAB wall and membrane integrity, dissipate the pH gradient, and delay the metabolism of carbohydrates (Filannino et al., 2014). Nevertheless, some species such as *Lactobacillus plantarum* are able to convert polyphenolics to phenolic acids and aromatic compounds which exhibit reduced antimicrobial activity and, as a consequence partially counteract these negative effects. Furthermore, it is of interest that some of these low molecular weight metabolites are potentially more bioactive, and have a more substantial impact on human health, that their parent compounds (Filannino et al., 2018). The present study investigated the ability of ten LAB strains, belonging to three different species (*L. plantarum*, *L. rhamnosus* and *L. casei*) and isolated from different niches, to metabolize polyphenolic compounds in elderberry juice.

Materials and methods

Chemicals

UHPLC phenolic standard cyanidin-3-*O*-rutinoside and cyanidin-3-*O*-glucoside were purchased from Extrasynthese Genay Cedex; 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, protocatechuic acid, quercetin-3-rutinoside hydrate, quercetin dihydrate, kaempferol,phenyllactic acid and catechol from Sigma-Aldrich, USA; dihydrocaffeic acid, *p*hydroxyphenyllactic acid from Santa Cruz Biotechnology, USA while luteolin from Extrasynthese, France.

Strains and cultures

Ten bacterial strains (Table 1) of three species (*L. plantarum*, *L. casei* and *L. rhamnosus*) were evaluated in this study, seven strains, of dairy origin, from the microbial collection of Food and Drug Department, University of Parma, Italy and three strains, of plant origin, from the microbial collection of the Department of Soil, Plant and Food Science, University of Bari, Italy.

Table 1. Strains used in elderberry juice fermentation

Strain	Species	Origin
POM1	*L. plantarum*	Tomato
1LE1	*L. plantarum*	Pineapple
C1	*L. plantarum*	Carrot
285	*L. plantarum*	Brazilian cheese
1473	*L. rhamnosus*	Parmigiano Reggiano cheese
1019	*L. rhamnosus*	Parmigiano Reggiano cheese
2360	*L. rhamnosus*	Grana Padano cheese
2140	*L. rhamnosus*	Grana Padano cheese
2178	*L. rhamnosus*	Grana Padano cheese
2107	*L. casei*	Grana Padano cheese

Bacterial cultures were stored at -80°C in Man Rogosa Sharpe (MRS) medium (Oxoid, Milan, Italy) containing 15% of glycerol (v/v). Cultures of *L. plantarum*, *L. rhamnosus* and *L. casei* were routinely grown in MRS broth (AnaeroGen, Oxoid). *L. plantarum* was incubated anaerobically at 30°C while *L. rhamnosus* and *L. casei* at 37°C, for 15 h. When use for fermentation, the strains were cultivated until the late exponential phase (ca. 15 h) and the cell were harvested by centrifugation (10,000 rpm for 10 min at 4°C), washed twice with Ringer's solution (Oxoid, Milan, Italy), and re-suspended in sterile distilled water to a final concentration of 9.0 Log CFU/mL.

Elderberry juice fermentation

A commercial elderberry juice, produced in Germany, obtained from berry grown spontaneously in organic agriculture, was purchased in a local organic food store. The information on the label revealed that the juice was non-filtrated and stable at room temperature. The amount of energy declared for 100 ml of juice was 38 kcal, the total fats 0.0 g, the total carbohydrates 6.5 g and the proteins 1.0 g. pasteurized non-filtered elderberry juice was used as substrate for the fermentation process. Each culture prepared as previously described was inoculated in order to reach 7 Log CFU/mL. The juice was fermented for 48 h, at 30°C with *L. plantarum*, and 37°C with *L. rhamnosus* and *L. casei* (optimal growth temperatures respectively of the different species), and then stored for 12 days at 4°C. Each fermentation was performed in triplicate. Samples were analysed at the end of fermentation process (48 h) and after the storage (14 days). Viable cell counts were carried out in triplicate by plate count on MRS agar (Oxoid, Milan, Italy), incubating anaerobically at the optimal temperature of the species (30°C or 37°C) for 48-72 h. Two types of controls were used: i) elderberry juice subjected to the same temperature treatments (30°C and 37°C), without the use of starters and ii) elderberry juice not subjected to temperature treatment and without starters. Microbial counts were carried out also in the controls, using Plate Count Agar medium (Oxoid, Milan, Italy) incubating at 30°C for 72 h.

Extraction of polyphenolic compounds

The extraction of polyphenolic compounds was performed according to the protocol of Sánchez-Salcedo et al. (2015) with slight modifications. In particular, 0.6 mL of a methanol-water solution (70:30 v/v) acidified with 0.2% of formic acid were added to 0.3 mL of each sample. All samples were stirred for 15 min at 200 rpm, then treated for 15 min in an ultrasonic bath and finally centrifuged for 10 min at 10,000 rpm. The pellet obtained was subjected to a second extraction and re-suspended in 0.3 mL of methanol-water solution and the second extraction was performed as described before.

Characterization of polyphenolic profile of started and unstarted elderberry juices

All samples, fermented and unfermented (i.e. not incubated and incubated at 30°C and 37°C), were analysed with an Accela UHPLC 1250 equipped with a linear ion trap-mass spectrometer (MS) (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization probe (H-ESI-II; Thermo Fisher Scientific Inc., San Jose, CA, USA). Separation was performed on an Acquity UPLC HSS T3 (2.1 x 100 mm) column coupled with a pre-column Acquity UPLC HSS T3 VanGuard (2.1x5 mm) (Waters, Milford, MA, USA). Volume injected was5 µL and oven temperature was set to 40 °C. For the determination of phenolic profile was followed the protocol reported by Zaupa et al. (2015) with some modification. Briefly the mobile phase was 0.1% (v/v) acetonitrile (phase A) and 0.1% (v/v) aqueous formic acid (phase B). Elution was performed at a flow rate of 0.3 mL/min. The gradient started with 95% B and 5% A, held until 0.5 min, in 9 min B decreased at 49% and A increased at 51%, then after 0.5 min, the column was flashed setting the eluent percentages at 20% B and 80% A until 11.50 min, and after that the initial conditions were restored (total run time = 17 min). The anthocyanin analysis was carried out in positive ionization mode, the MS worked with a capillary temperature set to 275°C, while the

source heater temperature was 300°C. The sheath gas (nitrogen) flow was 40 unit, while auxiliary and sweep gases (both nitrogen) were equal to 5 units, respectively.

Untargeted preliminary analyses were carried out in order to evaluate differences in the anthocyanin profile among the investigated samples. In detail, the mass spectrometer worked in full scan mode, data-dependent MS3 scanning from m/z 220–900, with Collision Induced Dissociation (CID) equal to 30. Once performed the identification, the anthocyanins have been quantified in Selective Reaction Monitoring (SRM) mode using a CID equal to 30. The other polyphenolic compounds were detected in negative ionisation mode, maintaining the same conditions described before, only the sheath gas flow was changed to 60 unit. Untargeted preliminary analyses were carried out in order to evaluate differences in the polyphenolic profile, as cited above for anthocyanin profile. In detail, the mass spectrometer worked in full scan mode, data-dependent MS3 scanning from m/z 100–2000, with CID equal to 35. Once performed the identification through untargeted analyses, the aglycone flavonoids have been quantified using the selected ion monitoring (SIM) mode, while the other negatively charged polyphenols were quantified in SRM mode using a CID equal to 35. Pure helium gas was used for CID. Data processing was performed using Xcalibur 2.2 software from Thermo Fisher Scientific Inc. (San Jose, CA, USA).

For the targeted analysis of catechol a different apparatus was used, since this molecule resulted of difficult ionization in an ion-trap mass spectrometer source. Catechol was monitored through a DIONEX Ultimate 3000 UHPLC coupled to a TSQ Vantage triple quadrupole (Thermo Fisher Scientific Inc., San Josè, CA, USA) fitted with a heated-electrospray ionisation (H-ESI) probe (Thermo Fisher Scientific Inc., San Josè, CA, USA). For UHPLC, mobile phase A was 0.2% aqueous formic acid and mobile phase B was acetonitrile containing 0.2% formic acid. Separations were performed using the same analytical column used for UHPLC-ion trap analyses. The gradient started with 5% B maintaining isocratic conditions for 1 min, and reached 50% B after 8 min. Then

B turned up to 80% keeping these conditions for 2 min before to re-establish the starting conditions maintaining it 8 min to re-equilibrate the column. The flow rate was 0.3 mL/min, the injection volume was 5 μL, the autosampler temperature was set at 10°C and the column oven was set at 40°C. The ESI source was operated in negative ionization mode. The vaporizer temperature was 300°C, while the capillary temperature was set to 270°C. The spray voltage was 3 kV and the sheath and auxiliary gases were set to 40 and 10 units respectively. The S-Lens value was 75 V. Catechol was monitored by SRM with the following characteristic transitions: 109.1 → 52.9 (CE 22 eV) and 109.1 → 80.9 (CE 18 eV). However, because of the poor sensitivity monitoring these product ions, the molecular ion at *m/z* 109.1 was monitored as base peak product ion (CE 15 eV). Pure argon gas was used for collision energy (CE). Data processing was performed using Xcalibur 2.1 software (Thermo Scientific Inc. San Josè, CA, USA). The quantification of phenolic compounds was performed using calibration curves with the standards reported in Table 2. All the analyses were performed in triplicate.

Statistical analyses

For each fermented sample and its control (elderberry juice incubated at 30°C for *L. plantarum* and 37°C for *L. casei* and *L. rhamnosus* strains) t-test, for independent samples, was applied in order to identify the differences between the juices. To evaluate the normally distribution for every group of independent samples Shapiro-Wilk test was used. Differences were considered statistically significant for p values < 0.05 and highly significant for p values < 0.001. In addition Pearson correlation analysis was performed to measures the strengths of association and the direction of the relationship between caffeic acid and dihydrocaffeic acid and between protocatechuic acid and catechol. Finally a Principal Component Analysis (PCA) was performed using correlation matrix. All the analyses were carried out using SPSS Statistics 23.0 software (SPSS Inc., Chicago, IL).

Table 2. Standard and acquisition mode used for the quantification of phenolic compounds in started and unstarted elderberry juices

Compounds identified	Standard used for quantification	Modality of acquisition
Cyanidin-3-*O*-sambubioside	Cyanidin-3-*O*-rutinoside	SRM
Cyanidin-3-*O*-glucoside	Cyanidin-3-*O*-glucoside	SRM
Cyanidin-3-*O*-sambubioside-glucoside	Cyanidin-3-*O*-rutinoside	SRM
Cyanidin-3-*O*-rutinoside	Cyanidin-3-*O*-rutinoside	SRM
3-*O*-caffeoylquinic acid	3-*O*-caffeoylquinic acid	SRM
5-*O*-caffeoylquinic acid	5-*O*-caffeoylquinic acid	SRM
4-*O*-caffeoylquinic acid	4-*O*-caffeoylquinic acid	SRM
Coumaroylquinic acid (1)	3-*O*-caffeoylquinic acid	SRM
Coumaroylquinic acid (2)	5-*O*-caffeoylquinic acid	SRM
Protocatechuic acid	Protocatechuic acid	SRM
Caffeic acid	Caffeic acid	SRM
Dihydrocaffeic acid	Dihydrocaffeic acid	SRM
Quercetin-3-*O*-rutinoside	Quercetin-3-rutinoside hydrate	SRM
Quercetin-3-*O*-glucoside	Quercetin-3-rutinoside hydrate	SRM
Kaempferol-*O*-rutinoside	Quercetin-3-rutinoside hydrate	SRM
Isorhamnetin-*O*-hexoside	Quercetin-3-rutinoside hydrate	SRM
Isorhamnetin-*O*-rutinoside	Quercetin-3-rutinoside hydrate	SRM
Quercetin-*O*-rutinoside-*O*-hexoside	Quercetin-3-rutinoside hydrate	SRM
Quercetin	Quercetin dihydrate	SIM
Luteolin	Luteolin	SIM
Kaempferol	Kaempferol	SIM
Isorhamnetin	Quercetin dihydrate	SIM
Phenyllactic acid	Phenyllactic acid	SRM
p-hydroxyphenyllactic acid	*p*-hydroxyphenyllactic acid	SRM
Catechol	Catechol	SRM

Results and discussion

Elderberry juice fermentation

A total of ten strains belonging to three different species of LAB (*L. plantarum*, *L. rhamnosus* and *L. casei*), isolated from dairy and plant matrices, were used individually for elderberry juice fermentation. Despite *L. plantarum* isolated from plant niches is considered the most well-adapted species and more exploitable as starter in fruit juices (Rodríguez et al., 2009), non-autochthonous strains belonging to *L. rhamnosus* and *L. casei* were also able to grow and steer the fermentation process, confirming our recent results (Ricci et al., 2018). Indeed, after 48 h of fermentation all strains were able to grow about two logarithmic cycles, from 7.20 ± 0.18 to 9.26 ± 0.20 Log CFU/mL (mean values), and cell viability did not decrease at the end of the storage period. The fermentation process was associated with a moderate shift of pH (3.9 before fermentation), that reached 3.61 ± 0.09 at the end of fermentation, and remained unchanged after the storage (3.55 ± 0.08).

Characterization of polyphenols

There is a lack of information on the effect of LAB fermentation on polyphenolic profiles in literature. On the other hand, few studies investigating this topic, report that LAB were able to increase the concentration of (poly)phenolic compounds, metabolize phenolic acids, and convert flavonoid glycosides and tannins (Kwaw et al., 2018; Curiel et al., 2015; Filannino et al., 2015; Filannino et al., 2013). For these reasons, this study investigates the impact of fermentation on the (poly)phenolic profile and concentration applying different strains of LABs to a matrix rich in polyphenols: elderberry juice. Using LC-MSn technique, 15 different compounds pertaining to polyphenols class were identified and quantified by comparison with reference authentic standards, while the remained 10 phenolic compounds, for which commercial standards were not available,

were tentatively identified based on the interpretation of their fragmentation patterns obtained from MS2 and MS3 spectra and by comparison with data in the literature. The retention times and mass spectral data along with peak assignments for the compounds identified are presented in Table 3. Flavonoids were the most predominant to be detected along with small amounts of phenolic acids. For the evaluation of the phenolic 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 respectively for *L. plantarum* and *L. casei, L. rhamnosus* fermented counterparts. The (poly)phenolic profile of fermented elderberry juice was mainly characterized by flavonols, flavonol glycosides, anthocyanins, hydroxycinnamic acids, phenyllactic acids and hydroxybenzoic acid (Figure 1).

All fermented elderberry juices showed concentrations of total (poly)phenolic compounds that were higher that the respective controls. The same trends observed after fermentation was maintained also at the end of storage. The different contribute of individual strains to the (poly)phenolic content can be observed in Figure 1. The strains that induced the highest increase in total (poly)phenolic compounds were *L. plantarum* 285, *L. rhamnosus* 1473, 1019 and 2360, after fermentation and storage, reaching respective concentrations of 338.2 and 302.5 μg/mL (mean values), resulting in an increase ranging between 65% and 80% compared to controls. Interestingly, all the strains that showed this capacity were isolated from dairy products. Regarding hydroxycinnamic acids, the highest and the lowest concentration, after fermentation and storage, were observed with two different strains of the species *L. rhamnosus*, revealing strain-specific ability to metabolise them (Figure 1).

Table 3. Mass spectral characteristics of (poly)phenolic compounds detected in started and unstarted elderberry juices. For each compound a specific variable, used in PCA analyses, was assigned.

Variable	Compounds	Rt (min)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)
Var1	3-*O*-caffeoylquinic acid	3.80	353	**191**,179,135,173	85,127,93,111,173
Var2	5-*O*-caffeoylquinic acid	4.45	353	**191**,179	173,127,85,93,111
Var3	4-*O*-caffeoylquinic acid	4.57	353	173,179,191	
Var4	Coumaroylquinic acid (1)	4.40	337	163,191,173	
Var5	Coumaroylquinic acid (2)	5.25	337	191,173,163	
Var6	Caffeic acid	4.90	179	135	
Var7	Dihydrocaffeic acid	5.29	181	109,119,**137**	
Var8	Protocatechuic acid	3.45	153	109	
Var9	Catechol	5.3	109	53,81,109	
Var10	Phenyllactic acid	6.03	165	147,119	
Var11	*p*-hydroxyphenyllactic acid	3.99	181	163, 135	
Var12	Luteolin	7.94	285	241,243,199,175,217,151	
Var13	Quercetin	7.92	301	179, 151, 273, 239,193	
Var14	Kaempferol	8.99	285	151,257,229,213,243,241 239,185,169	
Var15	Isorhamnetin	9.20	315	300,247	
Var16	Quercetin-3-*O*-glucoside	5.92	463	301	179,151,273,239,193
Var17	Kaempferol-*O*-rutinoside	6.17	593	**285**	257,267,241,239,229, 213,199,197,195,223, 163,151
Var18	Quercetin-*O*-rutinoside-*O*-hexoside	5.59	771	**301**	179,151
Var19	Isorhamnetin-*O*-hexoside	6.55	477	**314**,315,357,151,179	300,271,285,286,299, 275,243
Var20	Isorhamnetin-*O*-rutinoside	6.29	623	**315**,300,271,255	300,287,271
Var21	Quercetin-3-*O*-rutinoside	5.94	609	**301**,343	179,151,193,257
Var22	Cyanidin-3-*O*-sambubioside	3.43	581	**287**,449	287,231,241,259,213
Var23	Cyanidin-3-*O*-glucoside	4.38	449	287	
Var24	Cyanidin-3-*O*-rutinoside	4.47	595	**287**,449	
Var25	Cyanidin-3-*O*-sambubioside-hexoside	3.74	743	**287**,449,581	287,259,231,213,241

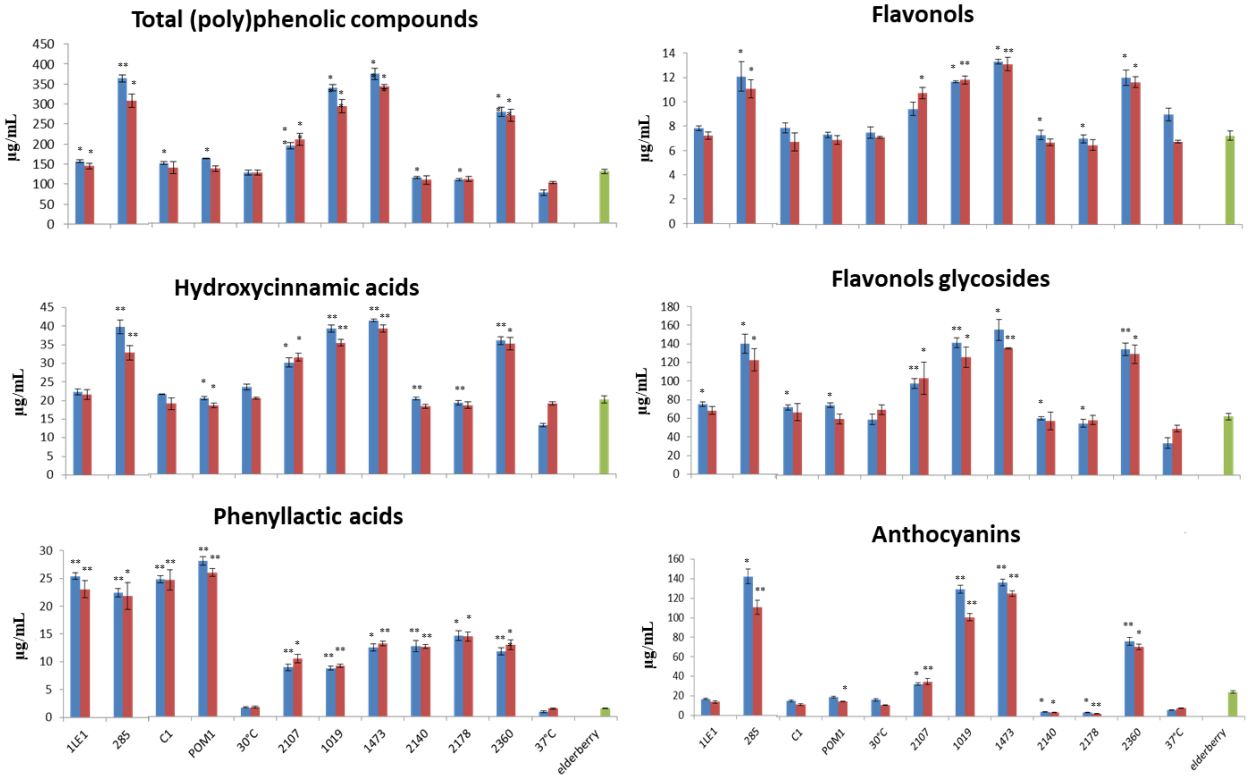

Figure 1. Total (poly)phenolic compounds and phenolic profile, express in µg/mL, (hydroxycinnamic acids, phenyllactic acids, flavonols, flavonols glycosides, antothocyanins) of elderberry juices inoculated with *L. plantarum* (1LE1, 285, C1, POM1), *L. casei* (2107), *L. rhamnosus* (1019, 1473, 2140, 2178, 2360), unstarted juice (incubated at 30°C and 37°C) and unstarted unincubated juice (elderberry, green bar). Blue bars refer to 48 h of fermentation while red bars to further 12 days of refrigerate storage. Significant differences ($p < 0.05$) between controls (30°C and 37°C) and samples are indicated by an asterisk, highly significant differences ($p < 0.001$) by two asterisks.

The increase in total hydroxycinnamic acids was principally due to the increase of 5-*O*-caffeoylquinic acid, mainly when dairy strains, i.e. *L. plantarum* 285, *L. rhamnosus* 1019, 1473, 2360 and *L. casei* 2107, were inoculated in the juice (Table S1 and S2). Interestingly, we also observed that *L. plantarum* strains were able to convert caffeic acid in its reduced metabolite

dihydrocaffeic acid, and this is true especially for *L. plantarum* 1LE1 (Table 4).The capacity of lactic acid bacteria (*L. plantarum* in particular) to catabolize caffeic acid has been previously reported (Filannino et al., 2015; Sánchez-Maldonado et al., 2011) and is in good agreement with results from the present study. It has been hypothesized that lactic acid bacteria may use hydroxycinnamic acids as external acceptors of electrons, using a phenolic acid reductase to convert caffeic acid in dihydrocaffeic acid (Filannino et al., 2014). Among hydroxybenzoic acids, protocatechuic acid was the only one identified. Most of the strains used in this study were able to metabolize it, by a decarboxylation step, into catechol (Table 5), a reaction that was previously reported for *L. plantarum* (Filannino et al., 2018; Filannino et al., 2015; Tabasco et al., 2011). Instead, to the best of our knowledge, this is the first time *L. rhamnosus* strains were observed able to carry out this specific reaction. However, not all protocatechuic acid was effectively converted into catechol, suggesting the existence of a different metabolic pathway (Ludwig et al., 2013). In order to measure the strengths of association and the direction of the relationship between caffeic acid/dihydrocaffeic acid and protocatechuic acid/catechol Pearson's correlation analysis was performed. A negative significant correlation was observed between caffeic acid/ dihydrocaffeic acid ($r = -0.700$; p value = 0.024) and protocatechuic acid/catechol ($r = -0.856$; p value <0.001) proving that caffeic acid and protocatechuic acid were consumed by lactic acid bacteria during fermentation while dihydrocaffeic acid and catechol were produced. These catabolic steps exerted by specific microorganisms may end up forming metabolites able to exert biological activities more relevant to human health in respect to their parent phenolic compounds. This is supported by a study conducted by Silva et al. in 2000 where it is reported that dihydrocaffeic acid is a more potent antioxidant than caffeic acid and that it can bioaccumulate inside the endothelial cells employing an intracellular antioxidant activities (Huang et al., 2004).

Table 4. Caffeic and dihydrocaffeic acid concentrations expressed as µg/ml for all the samples pertaining to *L. plantarum* strains, calculated after 48 h of fermentation (48 h) and 12 days of storage (14 d).

	48 h		14 d	
Sample	**Caffeic acid (µg/mL)**	**Dihydrocaffeic acid (µg/mL)**	**Caffeic acid (µg/mL)**	**Dihydrocaffeic acid (µg/mL)**
30°C	2.521 ± 0,036	ND	2.356 ± 0.054	ND
POM1	0.409 ± 0.007	0.708 ± 0.018	0.074 ± 0.005	1.364 ± 0.102
C1	0.664 ± 0.027	0.165 ± 0.013	0.254 ± 0.054	0.409 ± 0.017
285	0.054 ± 0.014	0.642 ± 0.036	0.039 ± 0.035	0.706 ± 0.047
1LE1	0.215 ± 0.030	1.518 ± 0.107	0.002 ± 0.004	1.713 ± 0.034

ND: not detected
The controls are reported in bold

Table 5. Protocatechuic acid and catechol concentrations expressed as µg/ml for all the samples considered in the study, calculated after 48 h of fermentation (48 h) and 12 days of storage (14 d).

	48 h		14 d	
Sample	**Protocatechuic acid (µg/mL)**	**Catechol (µg/mL)**	**Protocatechuic acid (µg/mL)**	**Catechol (µg/mL)**
Elderberry	11.704 ± 0.077	ND	ND	ND
37°C	10.660 ± 0.438	ND	15.440 ± 0.514	ND
2360	3.160 ± 0.075	3.036 ± 0.086	4.686 ± 0.427	3.258 ± 0.235
2178	1.863 ± 0.077	4.774 ± 0.511	2.461 ± 0.142	5.829 ± 0.387
2140	1.542 ± 0.439	5.716 ± 0.184	2.008 ± 0.181	5.714 ± 0.316
1019	2.997 ± 0.439	2.696 ± 0.225	3.747 ± 0.084	2.906 ± 0.271
30°C	15.556 ± 0.257	ND	14.878 ± 0.108	ND
POM1	2.139 ± 0.069	7.840 ± 0.450	2.409 ± 0.055	6.055 ± 0.385
C1	1.008 ± 0.143	5.221 ± 0.271	1.617 ± 0.122	5.431 ± 1.074
285	1.972 ± 0.155	4.413 ± 0.167	3.180 ± 0.939	4.833 ± 0.149
1LE1	1.848 ± 0.034	6.796 ± 0.639	2.104 ± 0.071	7.906 ± 0.218

ND: not detected
The controls are reported in bold

All fermented elderberry juices showed an increase in phenyllactic acids when compared to controls, confirming existing results (Valerio et al., 2004; Lavermicocca et al., 2003). After 48 h of incubation and further 12 days of cold storage, all *L. plantarum* strains generated the greatest increase. In particular, after the fermentation step an average value of 7.05 ± 2.38 μg/mL was reached. Minor levels were observed in the other species (5.56 ± 1.65 μg/mL). It is well known that phenyllactic acids may derive from amino acid metabolism, in particular as phenylalanine converted into phenylpyruvic acid by a reaction of transamination, and finally metabolized into phenyllactic acid by hydroxyl acid dehydrogenase, while *p*-hydroxyphenyllactic acid may originate from tyrosine metabolism (Lavermicocca et al., 2003). Since this is the first study fully focused on the polyphenolic fraction of fermented elderberry juice, we could only speculate that phenylalanine and tyrosine present in elderberry (Künsch & Temperli, 1978) can be used as precursors. The relevance of phenyllactic acids consist in their well-known antimicrobial ability against pathogenic strains such as *Listeria spp.*, *Staphylococcus aureus* and *Enterococcus faecalis*, their antifungal and anti mycotoxigenic (aflatoxins) properties (for example against *Aspergillus spp.* and *Penicillium spp*. especially those isolated from bakery products) (Valerio et al., 2004; Guimarães et al., 2018). Regarding flavonoids, the highest levels were principally observed in *L. plantarum* 285, *L. rhamnosus* 1019, 1473 and 2360 fermented juices. The capacity of *L. plantarum* to increase total polyphenols, flavonoids and anthocyanins after fermentation had already been documented (Curiel et al., 2015; Kwaw et al., 2018), but, to our knowledge, this is the first evidence for *L. rhamnosus*. After fermentation and storage, total flavonols reached the lowest concentration when *L. rhamnosus* 2178 was used as starter, whereas the highest level was obtained when *L. rhamnosus* 1473 was applied (Figure 1). Among flavonol glycosides, the most concentrated after fermentation was quercetin-3-*O*-rutinoside (from 39.02 ± 5.28 to 127.56 ± 11.34 μg/mL), followed by quercetin-3-*O*-glucoside (from 15.77 ± 1.01 to 27.39 ± 0.22 μg/mL) (Table S1 and S2). Anthocyanins were also observed in fermented elderberry juices, being relevant for both their recognised antioxidant

properties and for their colours, ranging from orange to blue, that makes them natural alternatives to replace synthetic colorants in food industry (Falcone Ferreyra et al., 2012). Notably, for four strains (*L. plantarum* 285, *L. rhamnosus* 1019,1473 and 2360), the same which have shown high content of flavonols, flavonol glycosides and total polyphenols, the increase in anthocyanins was observed after fermentation. Even if glycosylated flavonoids have been often described as systematically deconjugated by lactic acid bacteria thanks to β-glucosidase activity (Di Cagno et al., 2013; Svensson et al., 2010), giving rise to a stable decrease in their level during fermentation (Svensson et al., 2010; Filannino et al., 2015), in this work a net increase (in particular of quercetin-3-*O*-rutinoside, cyanidin-3-*O*-sambubioside, cyanidin-3-*O*-glucoside) was observed, especially when dairy strains were used. As a possible explanation, recently, bacteria have been reported able to express glycosyltransferases, known to be involved in the glycosylation of different compounds, such as glucans. Their ability to glycosylate phenolic structures has been hypothesised (Thai Huynh et al., 2014) as a way to enhance detoxification and solubilisation of phenolic substrates. Other reports describe the expression of glucansucrases in LABs, which have been described to be involved in luteolin, quercetin, myrcetin and catechol glycosylation (Bertrand et al., 2006; Te Poele et al., 2016; Devlamynck et al., 2016). In addition, Hur et al. (2014) reported that, during fermentation, enzymes such as glucosidase, amylase, cellulase, chitinase, inulinase, phytase, xylanase, tannase, and esterase could be produced, and they could be able to break down plant cell wall making specific compounds, such as flavonoids, more accessible.

Chemometrics analysis

To obtain more information on the parameters that could influence differences and similarities among the fermented elderberry juices, the concentrations of the (poly)phenolic compounds identified after fermentation and storage were used as variable vectors for Principal Component Analysis (PCA). Figure 2 reports score plot and loading plot obtained from PCA analysis. Applying

PCA on correlation matrix, two components were extracted representing together the 75% of total variance. In particular, component 1 explained the 55% of variance while the last 20% was explained by component 2. Observing the score plot, it is possible to highlight that the characteristics found in the samples after fermentation were maintained after storage. The main differences observed could be related to the strains applied. Samples analysed can be gathered in four groups according to the score plot.

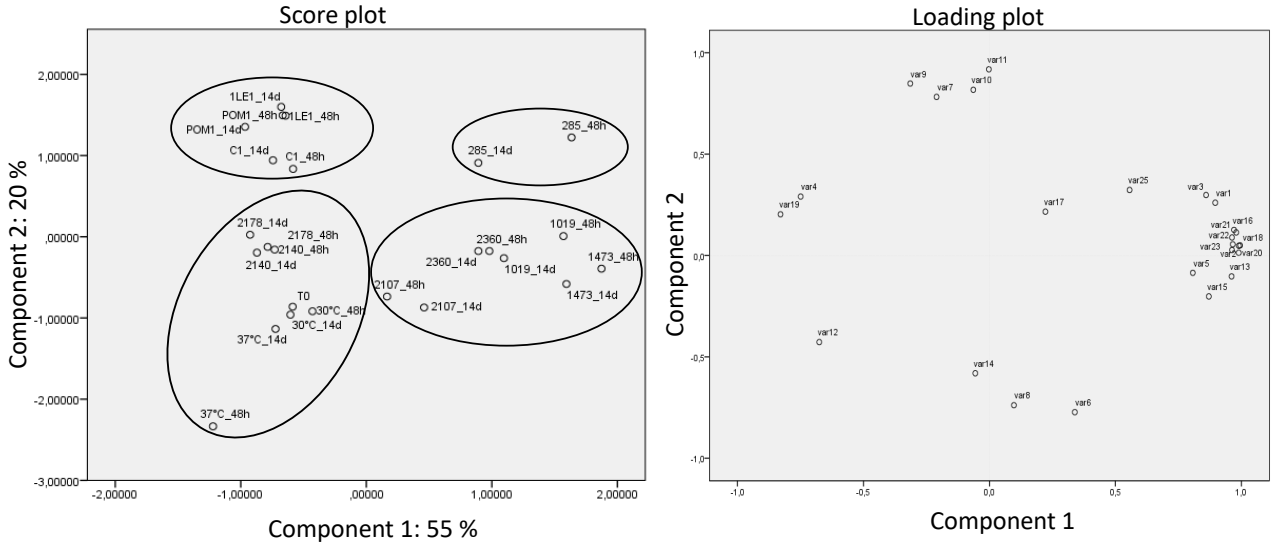

Figure 2. Principal component analysis (PCA). Score plot and loading plot based on (poly)phenolic compounds identify in started (1LE1, POM1, C1, 4186, 285, 2178, 2140, 1019, 1473, 2360 and 2107) and unstarted elderberry juices (30°C, 37°C and T0) after fermentation (48 h) and storage (14 d). The compounds used as variables were detailed in Table 2.

The first group is formed by elderberry juices fermented with *L. plantarum* 1LE1, POM1 and C1, and is characterized by those variables with negative values on component 1 and positive values on component 2, such as catechol, dihydrocaffeic acid, phenyllactic acid and *p*-hydroxyphenyllactic acid. The variables with negative values on component 1 and around zero, or weakly negative, on component 2, such as luteolin, isorhamnetin-*O*-hexoside and coumaroylquinic acid characterized instead the second group of samples, represented by juices fermented with *L. rhamnosus* 2178,

2140, and the unfermented samples. The third group was characterized by those variables positive on component 1 and around zero or weakly negative on component 2, like 5-*O*-caffeoylquinic acid, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, quercetin-*O*-rutinoside-*O*-hexoside, isorhamnetin-*O*-hexoside,cyanidin-3-*O*-glucoside, cyanidin-3-*O*-sambubioside. The last group is formed solely by the juice inoculated with *L. plantarum* 285. This sample was separated from the other groups and characterized by negative variables on component 1, such as quercetin-3-*O*-rutinoside, caffeoylquinic acids, cyanidin-3-*O*-sambubioside, cyanidin-3-*O*-glucoside, and positive on component 2, like phenyllactic acid and *p*-hydroxyphenyllactic acid. Interestingly, the score plot shows a clear separation among the considered species, as all elderberry juices fermented with *L. plantarum* strains were characterized by positive variables on component 2, whereas the juices inoculated with *L. casei* and *L. rhamnosus* strains were characterized by around zero or weak negative variables. On the base of component 1, microorganisms (with positive variables) able to increase more the total (poly)phenolic compound quantities, were separated from the others, characterized by negative variables. In particular these microorganisms are *L. plantarum* 285, *L. rhamnosus* 1019, 1473, 2360 and *L. casei* 2107.

Conclusions

Scientific research is being increasingly directed toward the study of bioactive compounds found in plant to develop healthy promoting food that can respond to new consumer demands. During fermentation, LAB act by modifying the poliphenolic profile. In this study we considered for the first time the effects of *L. rhamnosus* on poliphenols metabolism with particular reference to the different behaviours of plant and dairy isolates. We especially highlighted how the studied strains were able to produce (e.g. phenyllactic acids), modify (e.g. hydroxycinnamic acids) or increase (e.g. flavonoid glycosides) phenolic compounds. Thanks to statistical analysis (PCA), a clear clusterization of strains was observed, showing how the metabolism of phenolics is species and

strain dependent as how the features of each strain may be influenced by the isolation source. Although studies in situ are still to be evaluated, the increase in bioactive compounds pointed out after fermentation can open up the possibility for developing new food products such as fermented fruit juices, actually not widespread on the western world markets. Even more, the ability of non16 plant origin LAB strains to increase bioactive compounds and, as we previously observed also aromatic ones (Ricci et al., 2018), offers new perspectives for starter industries in the selection of a new generation of novel starter cultures.

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Chapter 2.2

Appendix

Table S1. Concentration (µg/mL) of (poly)phenolic compounds identified in elderberry juices fermented with *L. casei* 2107, *L. plantarum* (POM1, C1, 1LE1, 285), *L. rhamnosus* (2360, 2178, 2140, 1473, 1019), in controls (juices treated at 30°C and 37°C) and in unstarted and uninoculate juice after 48 hours. ND compounds not detected.

	2107	1LE1	285	C1	POM1	1019	1473	2140	2178	2360	30°C	37°C	Elderberry
Phenolic acids													
3-*O*-caffeoylquinic acid	7.7493±0.4224	7.2958±0.0714	9.3423±1.0804	7.0813±0.3275	6.9210±0.3851	8.8905±0.4936	9.4617±0.2996	6.4991±0.2434	6.0812±0.2549	8.0684±0.2980	7.2367±0.3724	3.4522±0.2608	6.5193±0.4229
5-*O*-caffeoylquinic acid	15.9966±0.6995	9.5581±0.2818	24.9427±0.9435	9.7814±0.3101	8.9216±0.1484	22.8425±0.4355	23.9626±0.0992	8.2074±0.2614	7.4784±0.3534	21.0677±0.3488	9.8756±0.7104	5.8469±0.1240	8.0243±0.4848
4-*O*-caffeoylquinic acid	3.4748±0.1629	3.6777±0.3824	4.7222±0.2243	3.5958±0.0954	3.3778±0.1628	4.2592±0.0454	4.5436±0.1168	3.0431±0.1269	3.1208±0.1745	3.8547±0.4250	3.7009±0.1957	1.9807±0.1104	3.1123±0.0889
Coumaroylquinic acid(1)	0.0193±0.0019	0.0281±0.0036	0.0111±0.0004	0.0287±0.0011	0.0294±0.0022	0.0114±0.0018	0.0141±0.0024	0.0207±0.0041	0.0210±0.0016	0.0102±0.0007	0.0276±0.0017	0.0123±0.0005	0.0250±0.0042
Coumaroylquinic acid(2)	0.0435±0.0028	0.0406±0.0021	0.0544±0.0024	0.0418±0.0018	0.0279±0.0025	0.0506±0.0021	0.0553±0.0047	0.0349±0.0032	0.0355±0.0015	0.0437±0.0027	0.0454±0.0065	0.0315±0.0045	0.0379±0.0020
Caffeic acid	2.6678±0.1697	0.2154±0.0296	0.0539±0.0142	0.6641±0.0271	0.4089±0.0069	3.0087±0.0461	3.2679±0.0968	2.4243±0.1267	2.3531±0.1289	2.8208±0.0592	2.5214±0.0362	1.8501±0.0650	2.3397±0.0531
Dihydrocaffeic acid	ND	1.5184±0.1068	0.6417±0.0357	0.1653±0.0129	0.7081±0.0180	ND	ND	ND	ND	ND	ND	ND	ND
Protocatechuic acid	13.1748±0.0708	1.8481±0.0342	1.9716±0.1545	1.0076±0.1430	2.1391±0.0687	2.9969±0.4390	11.8079±0.6262	1.5423±0.1137	1.8627±0.0768	3.1603±0.0751	15.5565±0.2571	10.6596±0.4377	11.7036±0.0767
Catechol	ND	6.7956±0.6393	4.4126±0.1671	5.2205±0.2711	7.8403±0.4498	2.6961±0.1588	ND	5.7161±0.1302	4.7740±0.3616	3.0363±0.0609	ND	ND	ND
Phenyllactic acid	3.9582±0.1892	9.3117±0.2143	5.6713±0.7094	6.7910±0.2133	9.4942±0.2945	3.1888±0.0751	4.6832±0.2331	5.8736±0.5734	6.9905±0.5998	7.0752±0.5379	1.0716±0.0903	0.5823±0.1217	0.9775±0.0215
p-Hydroxyphenyllactic acid	4.8915±0.4223	16.1017±0.7140	16.7839±0.4925	17.8587±0.4405	18.4183±0.5012	5.5038±0.4078	7.7285±0.4647	6.7905±0.4354	7.5565±0.2992	4.6528±0.2397	0.5455±0.0432	0.2691±0.0254	0.4764±0.0998
Flavonoids													
Luteolin	0.5828±0.0639	0.7174±0.0422	0.4235±0.0677	0.7239±0.0528	0.6263±0.0248	0.4451±0.0376	0.4808±0.0301	0.7012±0.0201	0.6999±0.0218	0.4421±0.0344	0.6915±0.0755	1.3577±0.0601	0.5921±0.0800
Quercetin	9.1527±0.5158	7.4946±0.1841	11.7774±1.1519	7.6011±0.4131	7.1178±0.2275	11.4426±0.1320	12.9717±0.1802	7.0139±0.3634	6.7168±0.3108	11.7015±0.5955	7.2405±0.4111	8.3504±0.4458	7.0341±0.3512
Kaempferol	0.2703±0.0222	0.2662±0.0144	0.2216±0.0509	0.2756±0.0164	0.2195±0.0290	0.2217±0.0465	0.2968±0.0104	0.2702±0.0231	0.2666±0.0375	0.2551±0.0359	0.2498±0.0491	0.6347±0.0951	0.2088±0.0371
Isorhamnetin	0.0633±0.0062	0.0466±0.0037	0.0666±0.0061	0.0466±0.0036	0.0433±0.0030	0.0689±0.0015	0.0872±0.0055	0.0553±0.0052	0.0517±0.0011	0.0773±0.0033	0.0453±0.0061	0.0520±0.0026	0.0416±0.0066
Quercetin-3-*O*-glucoside	21.5746±0.6422	19.3204±0.7892	25.6283±2.7612	18.8847±1.3228	18.4317±0.6067	26.5415±1.0778	27.3949±0.2193	15.7698±1.0075	15.7893±1.0653	23.8292±1.4701	17.2361±0.7093	12.6589±0.1137	16.0798±1.3472
Kaempferol-*O*-rutinoside	0.8799±0.0246	0.9450±0.0572	0.9307±0.0879	0.9904±0.0984	0.9209±0.0487	0.9200±0.0421	1.0223±0.0798	0.8157±0.0105	0.7822±0.0974	0.7603±0.0898	0.8999±0.0330	0.6367±0.0478	0.8022±0.0274
rcetin-*O*-rutinoside-*O*-hexoside	0.0106±0.0020	0.0062±0.0007	0.0241±0.0044	0.0057±0.0016	0.0062±0.0023	0.0222±0.0019	0.0243±0.0027	0.0048±0.0008	0.0043±0.0005	0.0176±0.0026	0.0058±0.0021	0.0020±0.0003	0.0049±0.0012
Isorhamnetin-*O*-hexoside	0.0213±0.0019	0.0287±0.0039	0.0118±0.0010	0.0273±0.0045	0.0220±0.0038	0.0103±0.0020	0.0113±0.0009	0.0197±0.0024	0.0196±0.0004	0.0114±0.0005	0.0260±0.0012	0.0187±0.0027	0.0231±0.0018
Isorhamnetin-*O*-rutinoside	0.1212±0.0138	0.0783±0.0036	0.1812±0.0106	0.0832±0.0053	0.0789±0.0058	0.1728±0.0054	0.1852±0.0125	0.0744±0.0041	0.0727±0.0052	0.1560±0.0042	0.0843±0.0070	0.0654±0.0050	0.0725±0.0049
Quercetin-3-*O*-rutinoside	75.6370±5.5247	54.7778±2.5879	113.4421±7.8415	52.7841±3.2363	55.7783±2.3301	114.5868±6.1176	127.5624±11.3403	44.4122±1.2314	39.0188±5.2825	110.7326±6.9635	41.6745±4.9104	21.1334±5.7346	45.9478±2.0262
Cyanidin-3-*O*-sambubioside	19.4998±0.7877	11.4346±0.6705	80.0761±4.4383	11.0064±0.8434	14.0277±0.5750	76.8593±3.1152	75.0179±3.3188	2.2494±0.2665	1.9296±0.5231	43.4081±2.4922	12.2045±1.0816	3.7214±0.4443	18.0691±0.7604
Cyanidin-3-*O*-glucoside	12.6157±0.3456	4.6063±0.1238	59.6003±2.7670	4.3655±0.1867	4.6037±0.2488	50.4934±1.4581	59.3546±1.5596	1.8318±0.0358	1.6815±0.0515	31.8610±1.5437	3.9913±0.2312	2.1900±0.1988	6.0381±0.3555
Cyanidin-3-*O*-rutinoside	0.3627±0.0120	0.0600±0.0035	1.7354±0.0936	0.0589±0.0061	0.0664±0.0035	1.4974±0.0070	1.7546±0.0263	0.0263±0.0035	0.0244±0.0013	0.9371±0.0211	0.0610±0.0008	0.1771±0.1404	0.0837±0.0004
idin-3-*O*-sambubioside- hexoside	0.5396±0.0375	0.6413±0.0132	1.0451±0.1398	0.6343±0.0385	0.9742±0.0155	1.2045±0.0834	0.8645±0.0550	0.3202±0.0272	0.3573±0.0119	0.5037±0.0104	0.8765±0.0553	0.2570±0.0251	0.7989±0.0450

Table S2. Concentration (µg/mL) of (poly)phenolic compounds identified in elderberry juices fermented with *L. casei* 2107, *L. plantarum* (POM1, C1, 1LE1, 285), *L. rhamnosus* (2360, 2178, 2140, 1473, 1019) and in controls (juices treated at 30°C and 37°C) after 14 days. ND compounds not detected.

	2107	1LE1	285	C1	POM1	1019	1473	2140	2178	2360	30°C	37°C
Phenolic acids												
3-*O*-caffeoylquinic acid	7.9470±0.3665	6.9598±0.5634	7.7791±0.7977	6.6947±0.6822	6.5557±0.2722	8.5245±0.3306	9.4208±0.6222	5.9749±0.2505	5.8293±0.1549	7.9887±0.5414	6.6681±0.2714	6.3807±0.4234
5-*O*-caffeoylquinic acid	16.3704±0.4999	9.3064±0.7118	20.5586±1.0019	8.2358±0.9069	7.4587±0.1633	20.0861±0.3604	22.3218±1.4277	7.1060±0.2437	7.4524±0.7949	20.4259±0.7895	8.1339±0.1135	7.3393±0.5353
4-*O*-caffeoylquinic acid	4.1087±0.5426	3.5279±0.0963	3.7513±0.4274	3.2332±0.0391	2.9073±0.1706	3.7520±0.2547	4.2127±0.1349	2.7156±0.1512	2.9271±0.1756	3.6383±0.3069	3.1323±0.1610	2.8763±0.3420
Coumaroylquinic acid(1)	0.0200±0.0016	0.0256±0.0030	0.0097±0.0014	0.0232±0.0038	0.0242±0.0005	0.0115±0.0008	0.0103±0.0011	0.0201±0.0019	0.0217±0.0014	0.0117±0.0019	0.0237±0.0013	0.0211±0.0002
Coumaroylquinic acid(2)	0.0478±0.0044	0.0432±0.0017	0.0443±0.0040	0.0406±0.0059	0.0201±0.0027	0.0421±0.0032	0.0532±0.0022	0.0350±0.0029	0.0362±0.0035	0.0432±0.0031	0.0380±0.0012	0.0377±0.0022
Caffeic acid	2.8785±0.0841	0.0021±0.0036	0.0392±0.0346	0.2541±0.0543	0.0738±0.0054	2.8904±0.0729	3.1143±0.2140	2.3233±0.0195	2.2481±0.0770	2.9257±0.0954	2.3562±0.0540	2.3149±0.0421
Dihydrocaffeic acid	ND	1.7127±0.0344	0.7065±0.0473	0.4088±0.0168	1.3642±0.1025	ND	ND	ND	ND	ND	ND	ND
Protocatechuic acid	15.9264±0.4488	2.1042±0.0714	3.1796±0.9385	1.6172±0.1121	2.4095±0.0551	3.7467±0.0843	13.0737±0.6996	2.0081±0.1805	2.4608±0.1421	4.6859±0.4269	14.8783±0.1077	15.4403±0.5139
Catechol	ND	7.9059±0.2180	4.8328±0.1492	5.4308±1.0741	6.0550±0.3584	2.9056±0.2713	ND	5.7141±0.3164	5.8289±0.3871	3.2580±0.2350	ND	ND
Phenyllactic acid	4.6369±0.4585	8.2874±0.7155	4.7484±0.6736	7.2468±0.6952	9.0476±0.1895	3.2983±0.0737	4.9804±0.3707	5.4845±0.0899	6.4517±0.4679	7.7613±0.5704	1.2333±0.1094	0.9546±0.1101
p-Hydroxyphenyllactic acid	5.7447±0.4062	14.7447±0.8274	17.1000±1.7405	17.2999±1.1082	16.8074±0.5333	5.7968±0.2526	8.1122±0.1581	7.0897±0.3223	7.9161±0.3889	5.0809±0.3855	0.4720±0.0941	0.4697±0.0937
Flavonoids												
Luteolin	0.7150±0.0266	0.6347±0.0320	0.3883±0.0401	0.6446±0.1069	0.6224±0.0292	0.4344±0.0402	0.5313±0.0546	0.6864±0.0540	0.6877±0.0517	0.5068±0.0122	0.6990±0.0070	0.6482±0.0333
Quercetin	10.3677±0.4689	6.9675±0.2906	10.8343±0.6649	6.5826±0.6664	6.7261±0.3167	11.5800±0.3002	12.7713±0.5297	6.4579±0.2577	6.2998±0.4102	11.3824±0.4071	6.9043±0.0991	6.5725±0.0918
Kaempferol	0.3580±0.0254	0.2137±0.0299	0.1918±0.0626	0.1572±0.0707	0.1878±0.0556	0.2316±0.0207	0.3147±0.0307	0.2410±0.0378	0.1809±0.0328	0.2423±0.0245	0.2359±0.0250	0.1908±0.0240
Isorhamnetin	0.0745±0.0022	0.0393±0.0013	0.0569±0.0097	0.0387±0.0090	0.0398±0.0048	0.0683±0.0036	0.0835±0.0049	0.0495±0.0047	0.0422±0.0024	0.0712±0.0025	0.0362±0.0006	0.0352±0.0003
Quercetin-3-*O*-glucoside	22.9663±1.6697	17.9625±0.5484	23.0412±2.3982	16.8004±1.6858	16.5877±1.5980	23.9089±1.1155	25.6954±1.1197	16.2807±1.2810	15.9253±0.5861	24.3682±1.7599	18.0239±0.4934	16.6202±0.2928
Kaempferol-*O*-rutinoside	1.0137±0.0956	1.0460±0.1255	0.6709±0.2427	0.8384±0.1547	0.8074±0.1906	0.8230±0.0416	0.9154±0.0460	0.7129±0.0785	0.7132±0.0794	0.8333±0.1071	1.1274±0.1917	0.7911±0.0608
Quercetin-*O*-rutinoside-*O*-hexoside	0.0136±0.0010	0.0054±0.0009	0.0171±0.0016	0.0060±0.0006	0.0047±0.0007	0.0183±0.0026	0.0222±0.0020	0.0046±0.0008	0.0040±0.0007	0.0179±0.0012	0.0056±0.0014	0.0049±0.0008
Isorhamnetin-*O*-hexoside	0.0178±0.0021	0.0258±0.0050	0.0121±0.0035	0.0235±0.0019	0.0217±0.0072	0.0093±0.0015	0.0125±0.0022	0.0211±0.0021	0.0236±0.0024	0.0102±0.0023	0.0226±0.0009	0.0178±0.0005
Isorhamnetin-*O*-rutinoside	0.1290±0.0103	0.0839±0.0027	0.1436±0.0208	0.0776±0.0064	0.0680±0.0010	0.1631±0.0141	0.1754±0.0198	0.0669±0.0036	0.0708±0.0066	0.1648±0.0041	0.0726±0.0008	0.0639±0.0063
Quercetin-3-*O*-rutinoside	80.1157±17.9556	49.2608±3.7910	98.8521±9.1655	49.8623±7.1701	43.1000±3.6408	101.8069±11.7285	109.6206±1.6601	41.2567±8.8108	42.4746±5.0298	104.5938±7.9108	50.9598±5.1287	32.3422±3.7962
Cyanidin-3-*O*-sambubioside	20.8337±1.9292	9.2596±1.1360	59.6824±5.0291	8.0308±0.6352	10.4958±0.2893	54.1609±1.7939	67.3514±3.0497	1.7173±0.1236	0.8028±0.2051	38.0905±2.1992	7.6089±0.1566	5.1090±0.4493
Cyanidin-3-*O*-glucoside	13.8691±1.0718	3.9219±0.0744	48.8454±2.2985	3.6836±0.2375	4.1461±0.2021	45.4227±2.0562	56.2297±1.6132	1.8176±0.0641	1.5713±0.0955	31.8000±1.5513	3.4286±0.0080	2.7346±0.0824
Cyanidin-3-*O*-rutinoside	0.4253±0.0487	0.1171±0.0279	1.4467±0.0450	0.0458±0.0037	0.0589±0.0038	1.3415±0.0794	1.5636±0.0347	0.0325±0.0033	0.0461±0.0084	0.9621±0.0046	0.0503±0.0033	0.0454±0.0029
Cyanidin-3-*O*-sambubioside- hexoside	0.5507±0.0698	0.5958±0.0365	0.7741±0.0351	0.4512±0.0254	0.6254±0.0249	0.6337±0.0360	0.7078±0.0485	0.3159±0.0131	0.3034±0.0096	0.4223±0.0396	0.5084±0.0121	0.5051±0.0106

Chapter 2.3

The influence of viable cells and cell-free extracts of Lactobacillus casei on volatile compounds and polyphenolic profile of elderberry juice

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Abstract

In this study four strains of *Lactobacillus casei*, as viable cells or cell-free extracts (CFE), were added to elderberry juice in order to evaluate their effect on phenolic and aromatic profile. Two of them were able to grow in juice while the others showed zero-growth. The same strains were lysed and added as extracts in elderberry juice. Multivariate statistical analysis show a separation among samples containing growing cells, non-growing cells, cell-free extracts, highlighting the particularities of specific strains. Juices added with CFE presented the highest amount of esters. The strains showing growth phenotype cause an increase of phenyllactic acids. The highest concentration of volatile compounds, particularly of alcohols, terpenes and norisoprenoids (responsible for typical elderberry notes) was observed in samples with strains showing zero-growth. Moreover, a significant increase in anthocyanin content was observed in these samples, suggesting the possible use of *Lactobacillus* for increasing specific molecules, even for non-multiplying bacterial cell. Considering that this is the first study concerning the use of non-growing cells in fruit juice, the potential of strains is still to be explored and it may have a significant technological application in the development of a microbial collection useful for fruit juice industry.

Introduction

Lactic acid bacteria (LAB) fermentation is an ancient technology of bio-preservation that in the last decade has been extensively applied to vegetable and fruit to increase their consumption. Moreover, following the fermentation, the nutritional value and different organoleptic traits, which are generally compromised during other manufacturing processes, can be improved (Ricci et al., 2018). As for other fermented food products, the selection of strains able to perform the conversion of substrate is essential to tailor specific functional or sensorial properties. Generally, selection of LAB strains for these applications implies: i) the ability of growth, ii) the synthesis of aroma compounds or precursor that can improve the sensorial features, iii) the production of metabolic end-products with nutritional value (Di Cagno et al., 2013). Cell adaptation and replication may depend upon polyphenols, which are abundant in fruits, and exert an inhibitory activity by damaging cytoplasmic membranes and cell walls, but could be converted by microbial enzymes into different metabolites, less toxic to cells and perhaps endorsed with other bioactivities (Huynh et al., 2014; Filannino et al., 2018). New metabolites could be produced through different bioconversion pathways, such as glycosylation, deglycosylation, ring cleavage, methylation, glucuronidation and sulfate conjugation (Huynh et al., 2014). To date, the metabolism of phenolics has been investigated, among LAB, mainly for *Lactobacillus plantarum* (Rodriguez et al., 2009; Filannino 2018). Even if *L. plantarum* is the LAB species most used to ferment fruits (Rodriguez et al., 2009), a recent study reported that also other species, as *Lactobacillus casei* and *Lactobacillus rhamnosus*, may be used in fermentation processes (Ricci et al., 2018). These closely related species belonging to the *Lactobacillus casei* group and representing the most widely researched and applied probiotic species of lactobacilli (Hill et al. 2018). In particular, fermentation of elderberry juice with dairy strains was found to be useful to enhance the typical aroma of elderberry (mainly linked to terpenes and alcohols), thus opening new opportunities in the selection of LAB for fruit fermentation (Ricci

et al., 2018). In other fermented foods the biochemical change of substrate relies not only on the action of metabolically active microbial cells but also on the activity of cytoplasmatic enzymes released after cell lysis (Smid and Kleerebezem, 2014). Indeed, the action of intracellular enzymes has a great relevance in aroma formation (Lazzi et al., 2016). The effect of microbial cell lysis (in LAB, yeasts and molds) or the addition of cell-free extracts was described by different authors in various fermented foods, such as dairy products, sausages and wine. Thanks to specific release of enzymes from the broken cells, the acceleration of ripening and/or the generation of volatile compounds occur (Lortal et al., 2005; Bolumar et al., 2006; Styger et al., 2011), but to our knowledge this topic has never been explored in fruits matrices. Based on these premises, this study focused on the effect on viable cells and cell-free extracts (CFE) added to elderberry juice. To this aim four strains of *L. casei*, isolated from dairy products, considered both as viable cells and CFE, were individually added to commercial elderberry juice in order to evaluate the differences among samples based on phenolic fraction and aromatic profile.

Materials and methods

Chemicals

Cyanidin-3-*O*-rutinoside (aka keracyanin), cyanidin-3-*O*-glucoside (aka kuromanin) and luteolin were purchased from Extrasynthese (Genay Cedex, France). 3-*O*-caffeoylquinic acid (aka neochlorogenic acid), 4-*O*-caffeoylquinic acid (aka cryptochlorogenic acid), 5-*O*-caffeoylquinic acid (aka chlorogenic acid), kaempferol, quercetin-3-*O*-rutinoside hydrate (aka rutin hydrate), quercetin dihydrate, DL-3-phenyllactic acid, 3,4-dihydroxybenzoic acid (aka protocatechuic acid), caffeic acid and toluene were purchased from Sigma-Aldrich (St. Louis, MO, USA). DL-*p*-hydroxyphenyllactic acid was from Santa Cruz Biotechnology (Dallas, TX, USA). HPLC-grade acetonitrile was from Sigma-Aldrich, while HPLC-grade water and LC-MS grade formic acid were purchased from VWR International (Milan, Italy).

Bacterial strains

Four strains of *L. casei* belonging to the Food and Drug Department, University of Parma (Italy) were used as adjunct in elderberry juice. *L. casei* 2057, isolated from Grana Padano cheese and *L. casei* 2306, 2240 and 2246 from Parmigiano Reggiano cheese were maintained as frozen stocks (-80°C) in de Man Rogosa Sharpe (MRS) medium (Oxoid, Milan, Italy) supplemented with 15% glycerol (v/v). The cultures were propagated three times with about 3% (v/v) inoculum in MRS and incubated in anaerobiosis (AnaeroGen, Oxoid) at 37 °C for 15 h.

Preparation of viable cells and CFE

Viable cells to be added to elderberry juices were 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, Milan, Italy), and finally re-suspending in sterile distilled water to a final concentration of 9.0 Log CFU/mL. CFE from the same strains were

prepared by sonication of microbial cultures using SONOPULS Ultrasonic homogenizers (Bandelin, Germany) In brief, three cycles of sonication at 12W for 30 sec, with 60 sec of pause between the cycles, were carried out. MRS plate agar count was performed in duplicate to verify the consistency of sonication. The efficacy of sonication was calculated as follow: E= 100-(n_t/n_0)*100 where n_0 = CFU/mL of *L. casei* cultures and n_t = CFU/mL of *L. casei* cultures after sonication treatment. The CFE were separated from the membrane residues by subsequent centrifugation at 10,000 x g for 15 min at 4°C (Centrifuge 5810 R, Eppendorf). The supernatant was sterilized by filtration and the absence of growth was evaluated in TSB 0.6% YE (tryptone soya broth, yeast extract) after incubation at 37°C for 48 h. All the samples were conserved at -80°C. Moreover, the protein content of CFE were determined by fluorometric assay, using Qubit protein assay kit and the measurements were carried out with Qubit Fluorometer (Invitrogen, Thermo Fisher Scientific, Massachusetts, USA).

Elderberry juice added with L. casei

A commercial pasteurized non-filtered elderberry juice was added with viable cells and CFE of *L. casei*. Viable cells of *L. casei* 2057, 2306, 2240, 2246, prepared as described previously, were inoculated into elderberry juice in order to reach 7 Log CFU/mL. The juice was fermented at 37°C for 48 hours. Microbial counts before and after fermentation were performed in triplicate using MRS Agar medium (Oxoid, Italy) and incubating at 37°C for 48-72 h, under anaerobiosis. Elderberry juice was also added with CFE from the same strains, incubated at 37°C for 48 hours and after this period the absence of cultivable cells was evaluated using MRS plate count agar. The eight experiments were carried out in triplicate and elderberry juice not added of *L. casei* was incubated at 37°C for 48 h and used as control. All the samples were maintained at -80°C until the analysis.

Characterization of volatile profile of elderberry juices with viable cells and CFE of L. casei

The volatile profile of elderberry juice samples added with viable inocula and CFE of *L. casei*, incubated for 48 h at 37°C was fully characterized by means of HS-SPME/GC-MS technique following the protocol reported by Ricci et al. (2018). Briefly, 2 mL of elderberry juice were placed in a 20 mL glass vial adding 5 µL of an aqueous toluene standard solution (100 µg/mL in 10 mL). Head space solid phase 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. 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).

Phenolic compounds analysis

All the juices added with *L. casei* and the control were analysed with an Accela UHPLC 1250 equipped with a linear ion trap-mass spectrometer (MS) (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization probe (H-ESI-II; Thermo Fisher Scientific Inc., San Jose, CA, USA). Analyte separation was performed using an Acquity UPLC HSS T3 (2.1 x 100 mm) column coupled with a pre-column Acquity UPLC HSS T3 VanGuard (2.1x5 mm) (Waters, Milford, MA, USA). The injection volume was 5 μL, the autosampler temperature was set at 10°C and column compartment temperature was set at 40°C. For the determination of phenolic profile was followed the protocol reported by Zaupa et al. (2015) with some modification. Briefly, acetonitrile (phase A) and water (phase B) both acidified with formic acid (0.1% v/v) were used as eluents applying a gradient of elution. In particular, the initial conditions were set at 95% B and 5% A and maintained for 0.5 min, then the phase A turned up at 51% in 9 min. After this step, at 9.5 min phase A was increased at 80%, the column was flashed under these conditions for 2 min and then, at 12 min, initial proportions were re-established. The total run time was 17 min at a flow rate of 0.3 mL/min.

The anthocyanin detection was carried out in positive ionization mode, with a capillary temperature of 275°C, and a source heater temperature of 300°C. The sheath gas (nitrogen) flow was 40 units, while auxiliary gas (nitrogen) was equal to 5 units. The source voltage was 4.5 kV, while the capillary and tube lens voltages were 20 V and 95 V, respectively. Untargeted preliminary analyses were carried out in order to evaluate differences in the anthocyanin profile among the investigated samples. In detail, the mass spectrometer worked in full scan mode, data-dependent MS^3 scanning from m/z 220–900, with CID equal to 30. Once performed the identification, the anthocyanins have been quantified in Selective Reaction Monitoring (SRM) mode using a Collision Induced Dissociation (CID) equal to 30. Cyanidin-3-*O*-glucoside was quantified through its authentic

standard compound, while cyanidin-*O*-sambubioside, cyanidin-*O*-sambubioside-*O*-hexoside and cyanidin-3-*O*-rutinoside were quantified by using authentic standard of cyanidin-3-*O*-rutinoside.

The other polyphenolic compounds were analysed in negative mode, applying the same capillary and source temperatures used before. The sheath gas (nitrogen) flow was 40 units, while auxiliary gas (nitrogen) was equal to 5 units. The source voltage was 4 kV, while the capillary and tube lens voltages were -33 V and -98 V, respectively. Untargeted preliminary analyses were carried out in order to evaluate differences in the polyphenolic profile, as cited above for anthocyanin profile. In detail, the mass spectrometer worked in full scan mode, data-dependent MS^3 scanning from m/z 100–2000, with CID equal to 35. Once performed the identification through untargeted analyses, the aglycone flavonoids have been quantified in SIM mode, while the other negatively charged polyphenols were quantified in SRM mode using a CID equal to 35. Pure helium gas was used for CID. Data processing was performed using Xcalibur 2.2 software from Thermo Fisher Scientific Inc. (San Jose, CA, USA). Where possible, polyphenols were quantified trough external calibration curves by using the reference standard compound. When such standards were not available, polyphenols were quantified using a structurally related compound. In detail, isorhamnetin has been quantified as quercetin dihydrate equivalents, coumaroylquinic acid I and coumaroylquinic acid II were quantified as 3-*O*-Caffeoylquinic acid and 5-*O*-Caffeoylquinic acid, respectively. Quercetin-3-*O*-glucoside, isorhamnetin-*O*-hexoside, kaempferol-*O*-rutinoside, isorhamnetin-*O*-rutinoside and quercetin-*O*-rutinoside-*O*-hexoside were quantified as rutin hydrate equivalents.

Chemometrics analysis

One way ANOVA was used to compare the different results obtained in juices added with viable and CFE *L. casei* strains, performed with IBM SPSS statistics package v. 21 (IBM Corp., Armonk, NY, USA). To evaluate the normal distribution for every group of independent samples Shapiro-Wilk test was used; multiple comparisons were performed with Tukey's test, differences were

considered statistically significant when $p < 0.05$. Data obtained from the analyses, such as volatile compounds amounts and polyphenols concentration were analysed by Principal Component Analysis (PCA) using the packages *ade4* and *FactoMineR* on R environment (http://www.r-project.org). The principal coordinates were selected depending on their weight on each of the principal components, and a threshold value was set at ± 0.75 (Table S1). Volatile compounds and polyphenols concentrations were Z-transformed and used to build heatmaps, by using the *cim* function in *mixOmics* package; radarplot graphs were built using package *fmsb*, both running on R software v. 3.4.1.

Results

Growth of L. casei in elderberry juice

The elderberry juice was added, singularly, of four cultures containing viable cells of *L. casei* (2057, 2306, 2240, 2246) and of the respective CFE. The growth ability of the four viable strains was evaluated after 48 h of incubation by plate count in MRS agar, revealing two different behaviours. Strains 2057 and 2306 shown zero-growth, meaning that cell densities remained unchanged from the original inocula (about 7 Log CFU/mL). On the other hand, strains 2240 and 2246 grew of about 2 Log CFU/mL. During the incubation of the started juices the pH only moderately decreased from an initial value of 3.84 ± 0.07 to 3.73 ± 0.05 (mean value). Unstarted juice (control sample) didn't show microbial growth after 48 h.

L. casei lysis

The efficacy of sonication in *L. casei* cellsresulted equal to 99%. To obtain a sterile sample the absence of bacterial growth was evaluated in TSB added with 0.6% of yeast extract after filtration step. In order to analyse the protein content of CFE, fluorometric assay was used. The protein content obtained was 0.81 ± 0.10 mg/mL (zero-growth strains) and 2.59 ± 0.05 mg/mL (grow

strains) according with cellular concentration. CFE was added in elderberry juice to obtain comparable concentrations with viable cells (9 Log CFU/mL, grow strains, 2240 and 2246; 7 Log CFU/mL, zero-growth strains, 2057 and 2306) and the absence of bacterial growth was tested after 48 h of incubation.

Global view: PCA and heatmap of volatile compounds and polyphenols

Alcohols, terpenes and norisoprenoids, acids, ketones and esters (67 compounds) were the main volatiles detected and semi-quantified in elderberry juices added with viable cells, CFE and control (Table 1, Table 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	Ethanol	strong, alcoholic	903	MS + LRI	Goodner 2008
4	Diacetyl	strong, butter	973	MS + LRI	Ferreira et al. 2001
5	Methyl isovalerate	strong, apple, pineapple	1020	MS + LRI	Sanz et al. 2001
6	Terpene not specified		1059	MS	
7	Ethyl isovalerate	fruity, sweet, apple	1065	MS + LRI	Ferreira et al. 2001
8	Isopentyl acetate	sweet, fruity, banana	1117	MS + LRI	Ferreira et al. 2001
9	3-Carene	sweet, citrus, terpenic	1128	MS + LRI	Chung et al. 1993
10	Myrcene	peppery, spicy	1143	MS + LRI	Kaack et al. 2005
11	Limonene	citrus	1175	MS + LRI	Cirlini et al. 2012
12	Eucalyptol	eucalyptus	1197	MS + LRI	Chisholm et al. 2003
13	Isoamyl alcohol	alcoholic, whiskey	1220	MS + LRI	Dall'Asta et al. 2011
14	*γ*-terpinene	oily, woody, terpenic, tropical	1227	MS + LRI	Cirlini et al. 2012
15	*m*-cymene		1258	MS + LRI	Cavalli et al. 2003
16	Terpinolene	fresh, woody, sweet, citrus	1264	MS + LRI	Choi 2003
17	Isoamyl isovalerate	sweet, fruity, green	1285	MS	

18	Acetoin	sweet, buttery, creamy, dairy	1300	MS + LRI	Bianchi et al. 2007
19	2,3-Octanedione	dill, cooked, broccoli	1322	MS + LRI	Bianchi et al. 2007
20	2-Penten-1-ol	green	1325	MS + LRI	Pennarum et al. 2002
21	(*E*)-2-Hexenyl-acetate	green, fruity	1329	MS + LRI	Jorgensen et al. 2000
22	Sulcatone	citrus	1336	MS + LRI	Chung et al. 1993
23	2,3,4,5-Tetramethyl-2-cyclopenten-1-one		1350	MS	
24	Hexanol	herbal	1354	MS + LRI	Cirlini et al. 2012
25	(*E*)-3-Hexen-1-ol	green, leafy	1365	MS + LRI	Bianchi et al. 2007
26	(*Z*)-3-Hexen-1-ol	green, leafy	1386	MS + LRI	Bianchi et al. 2007
27	Herbal ketone	fresh, sweet, green, weedy	1388	MS	
28	3-Octanol	earthy, mushroom	1392	MS	
29	(*E*)-2-Hexen-1-ol	fruity, green, leafy	1407	MS + LRI	Bianchi et al. 2007
30	*α*-Ionene		1437	MS	
31	*cis*-Linalool oxide		1442	MS + LRI	Cirlini et al. 2012
32	1-Octen-3-ol	earthy	1449	MS + LRI	Cirlini et al. 2012
33	Heptanol	green	1455	MS + LRI	Cirlini et al. 2012
34	Acetic acid	sharp, pungent, vinegar	1470	MS + LRI	Bianchi et al. 2007
35	*β*-Linalool	floral	1547	MS + LRI	Cirlini et al. 2012
36	Octanol	waxy, green, orange	1555	MS + LRI	Dall'Asta et al. 2011
37	*β*-Caryophyllene	sweet, woody	1588	MS + LRI	Bianchi et al. 2007
38	2-Undecanone	waxy, fruity, creamy	1598	MS + LRI	Bianchi et al. 2007
39	Hotrienol	sweet, tropical	1609	MS + LRI	Kaack et al. 2005
40	6-Methyl-heptanol		1612	MS	
41	Dihydro-citronellol	floral	1617	MS + LRI	Umano et al. 1999
42	6-Methyl-octanol		1626	MS	
43	Nonanol	fresh, fatty, floral	1657	MS + LRI	Dall'Asta et al. 2011
44	2-Furanmethanol	alcoholic, chemical, caramellic, bready	1665	MS + LRI	Dall'Asta et al. 2011
45	Isovaleric acid	stinky feet, cheese	1675	MS + LRI	Ferreira et al. 2001
46	*α*-Terpineol	pine, terpene, lilac	1695	MS + LRI	Cullere et al. 2004
47	Cadina-1(10)-4-diene		1751	MS	
48	*β*-damascenone "like"	woody, sweet, fruity, earthy	1758	MS	
49	*β*-citronellol (R)	floral	1763	MS +LRI	Ferreira et al. 2001
50	Terpene not specified		1773	MS	

51	Methyl salicylate	wintergreen mint	1778	MS + LRI	Kaack et al. 2005
52	Phenethyl acetate	floral, rose, sweet, honey	1787	MS + LRI	Ong and Acree 1999
53	*cis*-Geraniol	sweet, floral, fruity, rose	1798	MS + LRI	Nishimura 1995
54	2-Tridecanone	fatty, waxy, dairy, milky	1806	MS	
55	*β*-damascenone	woody, sweet, fruity, earthy	1820	MS + LRI	Valim et al. 2003
56	*trans*-Geraniol	sweet, floral, fruity, rose	1845	MS + LRI	Goodner 2008
57	Caproic acid	sour, fatty, sweaty, cheesy	1855	MS + LRI	Ferreira et al. 2001
58	2- Phenylmethanol	Floral	1879	MS + LRI	Chung et al. 1993
59	2-Phenylethanol	floral	1914	MS + LRI	Ferreira et al. 2001
60	Caproic acid like		1953	MS	
61	Dodecanol	earthy, soapy, waxy, fatty	1963	MS + LRI	flavornet.org
62	Terpene not specified		1971	MS	
63	2-Hexenoic acid (E)	fruity, sweet, warm, herbal	1981	MS + LRI	flavornet.org
64	*cis*-Lanceol		1995	MS	
65	*trans*-Nerolidol	floral, green, citrus, woody	2035	MS	
66	Caprylic acid	fatty, waxy, rancid, oily	2062	MS + LRI	Ferreira et al. 2001
67	Eugenol	spicy	2157	MS + LRI	Cirlini et al. 2016

Phenolic acids and flavonoids (Table 3, Table 4) were also identified and quantified resulting in a total of 23 compounds. PCA considering the identified volatiles and polyphenols was performed to highlight the differences among the samples analysed. Considering the 90 variables, the total variance explained by PCA accounted to 64.8% and a good clustering of the samples was obtained according to the growth profile of the viable cells, either growing or zero-growing, and of juices added with CFE (data not shown). Nevertheless, due to the high number of variables used for the classification, 34 of them belonging to volatile compounds (acids, alcohol, esters, ketones, terpenes) and polyphenols (anthocyanins, flavonol glycosides, hydroxycinnamic acids) were selected according to their contribution either to dimension 1 or dimension 2 of the PCA (Table S1). The new PCA analysis explains 77.3% of the variance, with PC1 and PC2 describing 48.3% and 29% of variance respectively (Figure 1a and 1b).

Table 2. Concentrations (µg/mL) of volatile compounds identify in elderberry juice (control) and elderberry juices added with *L. casei* 2057, 2306, 2240, 2246 and the respective cell-free extracts (CFE). For each compound a specific variable (VAR), used in statistical and graphical analyses, was assigned.

		Zero-growth						**CFE**						**Growth**			
Compounds	VAR	Control		2057		2306		2057		2240		2246		2306		2240		2246	
Ketones																			
Acetone	V-K1	0.047 ± 0.018	a,b	0.045 ± 0.034	a,b	0.032 ± 0.006	a,b	0.152 ± 0.038	c	0.072 ± 0.011	a,b	0.093 ± 0.000	b,c	0.053 ± 0.000	a,b	0.019 ± 0.002	a	0.016 ± 0.001	a,b
Diacetyl	V-K2	0.007 ± 0.006	a	0.443 ± 0.047	d	0.37 ± 0.016	c	0.011 ± 0.005	a	0.004 ± 0.003	a	0.003 ± 0.001	a	0.003 ± 0.000	a	0.279 ± 0.007	b	0.255 ± 0.007	b
Acetoin	V-K3	0.021 ± 0.001	a	0.168 ± 0.103	a,b	0.116 ± 0.037	a	0.057 ± 0.018	a	0.026 ± 0.009	a	0.026 ± 0.000	a	0.015 ± 0.000	a	0.316 ± 0.035	b	0.092 ± 0.002	a
2,3-Octanedione	V-K4	0.003 ± 0.000	a,b,c	0.001 ± 0.001	a	0.001 ± 0.001	a,b	0.006 ± 0.002	c	0.005 ± 0.001	b,c	0.004 ± 0.002	a,b,c	0.004 ± 0.000	a,b,c	0.004 ± 0.000	a,b,c	0.001 ± 0.000	a,b
Sulcatone	V-K5	0.003 ± 0.003	a	0.002 ± 0.003	a	0.003 ± 0.001	a	n.d.		n.d.		0.001 ± 0.001	a	n.d.		0.005 ± 0.001	a	0.004 ± 0.001	a
Herbal ketone	V-K6	n.d.		0.001 ± 0.001	a	0.01 ± 0.011	a	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
2-Undecanone	V-K7	0.001 ± 0.001	a	0.003 ± 0.004	a	0.002 ± 0.002	a	n.d.		n.d.		0.001 ± 0.001	a	n.d.		0.002 ± 0.000	a	0.002 ± 0.000	a
2-Tridecanone	V-K8	n.d.		0.005 ± 0.005	a	0.005 ± 0.006	a	n.d.		0.001 ± 0.000	a	0.001 ± 0.000	a	n.d.		0.003 ± 0.001	a	0.001 ± 0.000	a
2,3,4,5-Tetramethyl-2-cyclopenten-1-one	V-K9	0.002 ± 0.001	a	0.002 ± 0.002	a	0.003 ± 0.003	a	0.001 ± 0.000	a	n.d.		0.001 ± 0.001	a	n.d.		0.004 ± 0.000	a	0.003 ± 0.001	a
Esters																			
Ethyl acetate	V-E1	0.054 ± 0.012	b,c	0.013 ± 0.01	a	0.013 ± 0.011	a	0.098 ± 0.001	d	0.077 ± 0.019	c,d	0.073 ± 0.002	c,d	0.053 ± 0.007	a,b,c	0.02 ± 0.000	a,b	0.028 ± 0.003	a,b
Methyl isovalerate	V-E2	0.012 ± 0.002	c,d	0.001 ± 0.001	a	0.002 ± 0.001	a,b	0.014 ± 0.000	d	0.01 ± 0.002	c,d	0.01 ± 0.003	c,d	0.011 ± 0.001	c,d	0.008 ± 0.002	a,b,c	0.008 ± 0.001	b,c,d
Ethyl isovalerate	V-E3	0.014 ± 0.005	a	0.002 ± 0.002	a	0.002 ± 0.000	a	0.021 ± 0.014	a	0.007 ± 0.003	a	0.007 ± 0.000	a	0.006 ± 0.000	a	0.014 ± 0.000	a	0.011 ± 0.002	a
Isopentyl acetate	V-E4	0.001 ± 0.001	a,b	0.002 ± 0.001	a,b	0.003 ± 0.001	a,b	0.002 ± 0.002	a,b	0.001 ± 0.001	a	0.001 ± 0.000	a,b	0.001 ± 0.000	a,b	0.004 ± 0.001	b	0.003 ± 0.000	a,b
Isoamyl isovalerate	V-E5	0.002 ± 0.001	a	0.001 ± 0.000	a	0.001 ± 0	a	n.d.		n.d.		0.001 ± 0.000	a	n.d.		0.003 ± 0.001	a	0.003 ± 0.001	a
(E)-2-Hexenyl-acetate	V-E6	0.001 ± 0.001	a	n.d.		0.002 ± 0.001	a	n.d.		n.d.		n.d.		0.001 ± 0.000	a	0.003 ± 0.001	a	0.001 ± 0.000	a
Methyl salicylate	V-E7	0.009 ± 0.005	a	0.022 ± 0.012	a	0.006 ± 0.000	a	0.013 ± 0.005	a	0.01 ± 0.004	a	0.008 ± 0.001	a	0.007 ± 0.001	a	0.017 ± 0.001	a	0.014 ± 0.000	a
Phenethyl acetate	V-E8	0.004 ± 0.004	a	0.005 ± 0.004	a	0.006 ± 0.008	a	0.001 ± 0.002	a	0.002 ± 0.000	a	0.001 ± 0.000	a	0.001 ± 0.001	a	0.007 ± 0.000	a	0.007 ± 0.001	a
Alcohols																			

Ethanol	V-AL1	0.103 ± 0.011	a	0.098 ± 0.047	a	0.081 ± 0.03	a	0.291 ± 0.11	b	0.141 ± 0.031	a,b	0.161 ± 0.009	a,b	0.093 ± 0.009	a	0.077 ± 0.003	a	0.075 ± 0.012	a
Isoamyl alcohol	V-AL2	0.112 ± 0.013	a	0.158 ± 0.135	a	0.153 ± 0.1	a	0.306 ± 0.114	a	0.146 ± 0.048	a	0.138 ± 0.000	a	0.091 ± 0.003	a	0.126 ± 0.004	a	0.143 ± 0.027	a
2-Penten-1-ol	V-AL3	0.004 ± 0.000	a	0.007 ± 0.004	a	0.005 ± 0.003	a	0.01 ± 0.003	a	0.005 ± 0.002	a	0.005 ± 0.001	a	0.003 ± 0.000	a	0.005 ± 0.000	a	0.003 ± 0.000	a
Hexanol	V-AL4	0.069 ± 0.004	a	1.968 ± 1.072	b	1.47 ± 0.474	a,b	0.176 ± 0.067	a	0.094 ± 0.027	a	0.092 ± 0.000	a	0.061 ± 0.002	a	0.027 ± 0.001	a	0.062 ± 0.002	a
(*E*)-3-Hexen-1-ol	V-AL5	0.008 ± 0.007	a,b	0.067 ± 0.033	c	0.049 ± 0.005	b,c	0.002 ± 0.001	a	0.001 ± 0.000	a	0.001 ± 0.001	a	0.001 ± 0.000	a	0.003 ± 0.001	a	0.002 ± 0.000	a
(*Z*)-3-Hexen-1-ol	V-AL6	0.043 ± 0.006	a	0.422 ± 0.237	b	0.294 ± 0.044	a,b	0.1 ± 0.025	a	0.057 ± 0.017	a	0.059 ± 0.002	a	0.036 ± 0.001	a	0.038 ± 0.004	a	0.042 ± 0.002	a
3-Octanol	V-AL7	n.d.		0.002 ± 0.001	c	0.001 ± 0.000	a,b	n.d.	a	0.001 ± 0.001	a	n.d.		n.d.		0.002 ± 0.000	b,c	0.001 ± 0.000	a,b,c
(*E*)-2-Hexen-1-ol	V-AL8	0.012 ± 0.008	a	1.763 ± 0.965	b	1.361 ± 0.399	a,b	0.01 ± 0.003	a	0.006 ± 0.002	a	0.006 ± 0.000	a	0.004 ± 0.000	a	0.05 ± 0.001	a	0.017 ± 0.001	a
1-Octen-3-ol	V-AL9	0.011 ± 0.003	a	0.02 ± 0.01	a	0.015 ± 0.001	a	0.021 ± 0.007	a	0.012 ± 0.004	a	0.011 ± 0.000	a	0.007 ± 0.000	a	0.018 ± 0.001	a	0.011 ± 0	a
Heptanol	V-AL10	0.002 ± 0.000	a	0.015 ± 0.005	c	0.009 ± 0.000	b,c	0.005 ± 0.001	a,b	0.002 ± 0.000	a	0.003 ± 0.000	a	0.002 ± 0.001	a	0.001 ± 0.000	a	0.001 ± 0.000	a
Octanol	V-AL11	0.005 ± 0.002	a	0.013 ± 0.003	b	0.007 ± 0.002	a,b	0.006 ± 0.002	a,b	0.004 ± 0.001	a	0.004 ± 0.001	a	0.003 ± 0.000	a	0.001 ± 0.000	a	0.003 ± 0.000	a
6-Methyl-heptanol	V-AL12	0.005 ± 0.000	a	0.025 ± 0.007	c	0.016 ± 0.001	b,c	0.011 ± 0.003	a,b	n.d.		0.005 ± 0.000	a,b	0.004 ± 0.000	a	0.003 ± 0.001	a	0.005 ± 0.000	a
Nonanol	V-AL13	0.004 ± 0.003	a	0.003 ± 0.001	a	0.02 ± 0.027	a	0.002 ± 0.001	a	0.002 ± 0.001	a	0.002 ± 0.000	a	0.001 ± 0.000	a	0.007 ± 0.004	a	0.013 ± 0.003	a
2-Furanmethanol	V-AL14	0.019 ± 0.001	a	0.079 ± 0.031	b	0.05 ± 0.008	a,b	0.049 ± 0.016	a,b	0.025 ± 0.007	a	0.023 ± 0.000	a	0.018 ± 0.002	a	0.019 ± 0.001	a	0.019 ± 0.002	a
2- Phenylmethanol	V-AL15	0.037 ± 0.021	a	0.067 ± 0.027	a	0.047 ± 0.024	a	0.048 ± 0.014	a	0.029 ± 0.008	a	0.028 ± 0.001	a	0.022 ± 0.004	a	0.054 ± 0.001	a	0.052 ± 0.002	a
2-Phenylethanol	V-AL16	0.124 ± 0.085	a	0.196 ± 0.062	a	0.194 ± 0.031	a	0.114 ± 0.031	a	0.066 ± 0.022	a	0.064 ± 0.003	a	0.045 ± 0.005	a	0.202 ± 0.008	a	0.227 ± 0.014	a
Dodecanol	V-AL17	n.d.		0.003 ± 0.004	a	0.001 ± 0.001	a	n.d.		0.001 ± 0.001	a	n.d.		n.d.		0.001 ± 0.000	a	0.001 ± 0.000	a
6-Methyl-octanol	V-AL18	0.004 ± 0.001	a	0.025 ± 0.01	b	0.024 ± 0.003	b	0.017 ± 0.006	a,b	0.009 ± 0.003	a,b	0.008 ± 0	a,b	0.007 ± 0.001	a	0.003 ± 0.001	a	0.004 ± 0.002	a
Terpenes and norisoprenoids																			
Terpene not specified	V-T1	0.002 ± 0.001	a	0.001 ± 0.001	a	0.001 ± 0.001	a	0.002 ± 0.001	a	n.d.		0.001 ± 0.000	a	n.d.		0.002 ± 0.000	a	0.002 ± 0.000	a
3-Carene	V-T2	0.004 ± 0.002	a	0.002 ± 0.001	a	0.002 ± 0.002	a	0.005 ± 0.002	a	0.002 ± 0.000	a	0.002 ± 0.000	a	0.002 ± 0.001	a	0.004 ± 0.001	a	0.004 ± 0.000	a
Myrcene	V-T3	0.001 ± 0.001	a,b	0.001 ± 0.000	a,b	0.001 ± 0.000	a,b	0.001 ± 0.000	a,b	n.d.		0.001 ± 0.000	a	n.d.		0.003 ± 0.001	b,c	0.004 ± 0.000	c
Limonene	V-T4	0.215 ± 0.061	a	0.28 ± 0.242	a	0.501 ± 0.062	a	0.323 ± 0.164	a	0.145 ± 0.034	a	0.138 ± 0.001	a	0.123 ± 0.009	a	0.216 ± 0.103	a	0.235 ± 0.097	a
Eucalyptol	V-T5	0.005 ± 0.005	a	0.005 ± 0.003	a	0.003 ± 0.002	a	0.002 ± 0.001	a	0.001 ± 0.000	a	0.001 ± 0.000	a	0.001 ± 0.000	a	0.007 ± 0.002	a	0.003 ± 0.000	a
γ-terpinene	V-T6	0.006 ± 0.001	a	0.004 ± 0.005	a	0.004 ± 0.004	a	0.01 ± 0.007	a	0.006 ± 0.002	a	0.005 ± 0.001	a	0.005 ± 0.001	a	0.014 ± 0.001	a	0.018 ± 0.015	a
m-cymene	V-T7	0.011 ± 0.001	a	0.013 ± 0.003	a	0.012 ± 0.003	a	0.02 ± 0.016	a	0.006 ± 0.001	a	0.006 ± 0.000	a	0.005 ± 0.001	a	0.014 ± 0.001	a	0.016 ± 0.001	a
Terpinolene	V-T8	0.005 ± 0.003	a	0.007 ± 0.001	a	0.006 ± 0.003	a	0.003 ± 0.000	a	0.002 ± 0.001	a	0.002 ± 0.000	a	0.002 ± 0.000	a	0.012 ± 0.007	a,b	0.021 ± 0.003	b
α-Ionene	V-T9	0.002 ± 0.001	a	0.007 ± 0.001	a	0.004 ± 0.005	a	0.002 ± 0.001	a	0.001 ± 0.000	a	0.002 ± 0.000	a	0.001 ± 0.000	a	0.004 ± 0.001	a	0.004 ± 0.001	a
cis-Linalool oxide	V-T10	0.003 ± 0.001	a	0.007 ± 0.003	a	0.004 ± 0.002	a	0.004 ± 0.001	a	0.003 ± 0.001	a	0.006 ± 0.002	a	0.003 ± 0.002	a	0.004 ± 0.002	a	0.002 ± 0.000	a

β-Linalool	V-T11	0.198 ± 0.009	a,b	0.941 ± 0.347	c	0.713 ± 0.049	b,c	0.561 ± 0.216	a,b,c	0.301 ± 0.075	a,b	0.291 ± 0.002	a,b	0.202 ± 0.013	a,b	0.156 ± 0.007	a	0.151 ± 0.011	a
β-Caryophyllene	V-T12	0.003 ± 0.002	a	0.002 ± 0.002	a	0.001 ± 0.000	a	0.001 ± 0.000	a	n.d.	a	0.001 ± 0.000	a	0.001 ± 0.000	a	0.005 ± 0.001	a	0.006 ± 0.001	a
α-Terpineol	V-T13	0.039 ± 0.007	a,b	0.149 ± 0.003	c	0.138 ± 0.002	c	0.069 ± 0.02	b	0.041 ± 0.004	a,b	0.037 ± 0.001	a,b	0.029 ± 0.011	a	0.046 ± 0.001	a,b	0.044 ± 0.004	a,b
Cadina-1(10)-4-diene	V-T14	0.003 ± 0.002	a	0.004 ± 0.003	a	0.002 ± 0.002	a	0.001 ± 0.000	a	0.001 ± 0.000	a	0.001 ± 0.000	a	0.001 ± 0.000	a	0.005 ± 0.000	a	0.004 ± 0.000	a
β-damascenone "like"	V-T15	0.003 ± 0.000	a	0.015 ± 0.002	b	0.013 ± 0.002	b	0.007 ± 0.002	a	0.005 ± 0.000	a	0.004 ± 0.001	a	0.003 ± 0.001	a	0.004 ± 0.000	a	0.003 ± 0.000	a
β-citronellol *(R)*	V-T16	0.003 ± 0.003	a	0.015 ± 0.016	a	0.001 ± 0.001	a	0.001 ± 0.001	a	0.001 ± 0.000	a	0.001 ± 0.000	a	0.001 ± 0.001	a	0.004 ± 0.001	a	0.003 ± 0.000	a
Terpene not specified	V-T17	0.002 ± 0.002	a,b	0.002 ± 0.001	a,b	0.001 ± 0.000	a	n.d.	a	0.001 ± 0.000	a	0.001 ± 0.000	a	n.d.	a	0.008 ± 0.001	b,c	0.009 ± 0.004	c
cis-Geraniol	V-T18	0.002 ± 0.001	a	0.015 ± 0.004	c	0.012 ± 0.004	b,c	0.005 ± 0.001	a,b	0.004 ± 0.000	a,b	0.003 ± 0.001	a	0.002 ± 0.000	a	0.006 ± 0.002	a	0.003 ± 0.000	a
β-damascenone	V-T19	0.066 ± 0.024	a	0.351 ± 0.036	b	0.268 ± 0.004	b	0.11 ± 0.034	a	0.067 ± 0.016	a	0.062 ± 0.002	a	0.047 ± 0.006	a	0.095 ± 0.007	a	0.097 ± 0.003	a
trans-Geraniol	V-T20	0.005 ± 0.001	a	0.035 ± 0.001	d	0.026 ± 0.003	c	0.01 ± 0.004	a,b	0.006 ± 0.001	a,b	0.006 ± 0.001	a,b	0.005 ± 0.002	a	0.014 ± 0.002	b	0.007 ± 0.000	a,b
Terpene not specified	V-T21	n.d.		0.002 ± 0.001	a	0.001 ± 0.001		n.d.		n.d.		n.d.		n.d.		0.001 ± 0.000	a	0.001 ± 0.000	a
cis-Lanceol	V-T22	0.001 ± 0.001	a	0.003 ± 0.004	a	0.002 ± 0.000	a	n.d.		0.001 ± 0.000	a	0.001 ± 0.000	a	n.d.		0.002 ± 0.001	a	0.002 ± 0.000	a
trans-Nerolidol	V-T23	n.d.		0.002 ± 0.002	a	0.001 ± 0.001	a	n.d.		0.001 ± 0.000	a	0.001 ± 0.001	a	0.001 ± 0.000	a	0.001 ± 0.000	a	n.d.	
Eugenol	V-T24	0.008 ± 0.008	a	0.014 ± 0.006	a	0.008 ± 0.003	a	0.002 ± 0.001	a	0.001 ± 0.000	a	0.001 ± 0.001	a	0.001 ± 0.000	a	0.094 ± 0.004	c	0.042 ± 0.001	b
Hotrienol	V-T25	0.001 ± 0.000	a	0.004 ± 0.001	a	0.002 ± 0.002	a	0.001 ± 0.002	a	n.d.		0.001 ± 0.000	a	n.d.		0.001 ± 0.000	a	0.002 ± 0.002	a
Dihydro-citronellol	V-T26	0.008 ± 0.001	a	0.042 ± 0.018	b	0.025 ± 0.002	a,b	0.022 ± 0.009	a,b	0.011 ± 0.004	a	0.009 ± 0.001	a	0.007 ± 0.001	a	0.007 ± 0.000	a	0.006 ± 0.000	a
Acids																			
Acetic acid	V-AC1	0.007 ± 0.005	a	0.001 ± 0.000	a	0.002 ± 0.002	a	0.02 ± 0.001	b	n.d.	a	n.d.	a	n.d.	a	0.123 ± 0.001	d	0.096 ± 0.002	c
Isovaleric acid	V-AC2	0.307 ± 0.095	a	0.007 ± 0.004	a	0.067 ± 0.023	a	0.676 ± 0.153	b	0.275 ± 0.124	a	0.267 ± 0.017	a	0.159 ± 0.013	a	0.289 ± 0.018	a	0.308 ± 0.028	a
Caproic acid	V-AC3	0.009 ± 0.009	a	0.001 ± 0.000	a	0.001 ± 0.000	a	n.d.		n.d.		n.d.		n.d.		0.043 ± 0.003	b	0.032 ± 0.004	b
Caproic acid like	V-AC4	n.d.		0.001 ± 0.000	a	n.d.		n.d.		0.001 ± 0.001	a	n.d.		n.d.		0.001 ± 0.000	a	0.001 ± 0.000	a
2-Hexenoic acid *(E)*	V-AC5	n.d.		0.002 ± 0.001	b	n.d.		n.d.		0.001 ± 0.000	a,b	n.d.		n.d.		0.012 ± 0.001	c	0.012 ± 0.000	c
Caprylic acid	V-AC6	0.001 ± 0.001	a	0.001 ± 0.001	a	n.d.		n.d.		n.d.		0.001 ± 0.001	a	n.d.		0.006 ± 0.001	b	0.008 ± 0.001	b

Table 3. Mass spectral characteristics of (poly)phenolic compounds detected in elderberry juices added with viable cells and the respective cell-free extracts.

Compounds	Rt (min)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)
3-*O*-caffeoylquinic acid	3.80	353	**191**,179,135,173	85,127,93,111,173
5-*O*-caffeoylquinic acid	4.45	353	**191**,179	173,127,85,93,111
4-*O*-caffeoylquinic acid	4.57	353	173,179,191	
Coumaroylquinic acid (1)	4.40	337	163,191,173	
Coumaroylquinic acid (2)	5.25	337	191,173,163	
Caffeic acid	4.90	179	135	
Protocatechuic acid	3.45	153	109	
Phenyllactic acid	6.03	165	147,119	
p-hydroxyphenyllactic acid	3.99	181	163, 135	
Luteolin	7.94	285	241,243,199,175,217,151	
Quercetin	7.92	301	179, 151, 273, 239,193	
Kaempferol	8.99	285	151,257,229,213,243,241 239,185,169	
Isorhamnetin	9.20	315	300,247	
Quercetin-3-*O*-glucoside	5.92	463	301	179,151,273,239,193
Kaempferol-*O*-rutinoside	6.17	593	**285**	257,267,241,239,229, 213,199,197,195,223, 163,151
Quercetin-*O*-rutinoside-*O*-hexoside	5.59	771	**301**	179,151
Isorhamnetin-*O*-hexoside	6.55	477	**314**,315,357,151,179	300,271,285,286,299, 275,243
Isorhamnetin-*O*-rutinoside	6.29	623	**315**,300,271,255	300,287,271
Quercetin-3-*O*-rutinoside	5.94	609	**301**,343	179,151,193,257
Cyanidin-3-*O*-sambubioside	3.43	581	**287**,449	287,231,241,259,213
Cyanidin-3-*O*-glucoside	4.38	449	287	
Cyanidin-3-*O*-rutinoside	4.47	595	**287**,449	
Cyanidin-3-*O*-sambubioside- hexoside	3.74	743	**287**,449,581	287,259,231,213,241

Elderberry juices added with viable cells and CFE were grouped in three distinct cluster, separated from the control (Figure 1a). Samples where bacterial growth occurred were separated from the control in the upper right quadrant of PCA, strongly positively associated with all the acids (acetic acid, caproic acid, 2-hexenoic acid (E) acid, caprilic acid), isoamyl isovalerate and two terpenes.

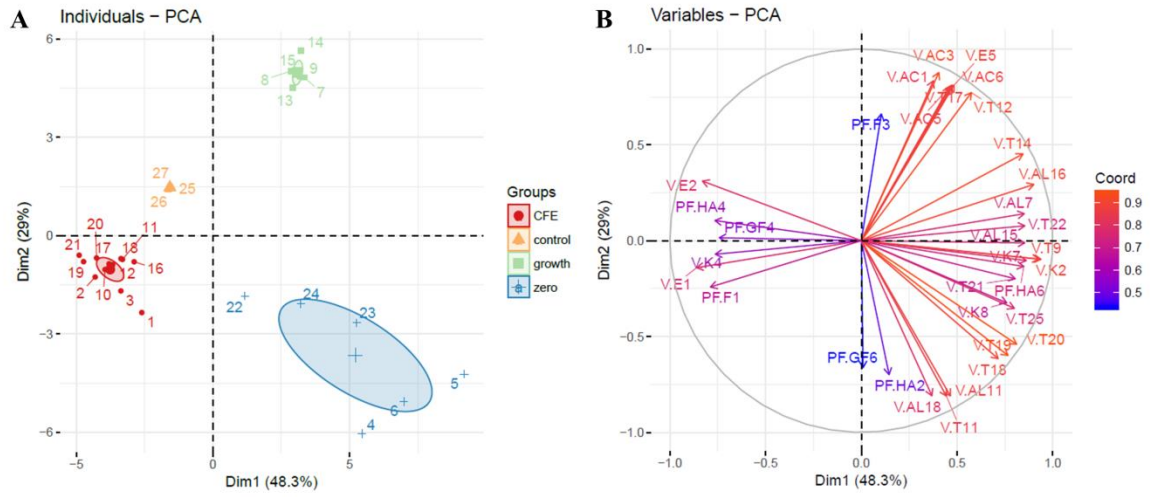

Figure 1. Principal component analysis (PCA). **(A)** Individuals plot, samples are represented as follows: 1-3, *L. casei* 2057 CFE; 4-6, *L. casei* 2057 zero growth; 7-9, *L. casei* 2240 growth; 10-12, *L. casei* 2240 CFE; 13-15, *L. casei* 2246 growth; 16-18, *L. casei* 2246 CFE; 19-21, *L. casei* 2306 CFE; 22-24, *L. casei* 2306 zero growth. Ellipses are drawn at a confidence level of 0.95. **(B)** Variables plot based on volatile compounds and polyphenols with higher influence on elderberry juices characterization, variables are coloured according to their weight on the PCA dimensions. Abbreviations are the same as in Table 2 for volatile compounds (V.) and Table 4 for polyphenols (PF.).

On the other hand, the variables with strongly negative values on the first component (quercetin, ethyl acetate, methyl isovalerate) were determinant to group the elderberry juices added with CFE.

Interestingly, elderberry juice added with *L. casei* showing zero-growth appeared well separated from the control in lower right quadrant of PCA, associated with variables with positive values on component 1 and negative value on component 2, as many terpenes and alcohols.

In accordance with the PCA analysis, the heatmap describing the volatile compounds showed a separation among the three type of samples highlighting the peculiarities of specific strains (Figure 2). Indeed, elderberry juice added with CFE of *L. casei* 2057, differently from the other CFE samples and the control, has a profile characterized by a higher production of volatile compounds, especially esters and alcohols. Despite esters were overall less abundant among the samples (Table 2) they were associated to fruit notes contributing positively to the aromatic profile. The heatmap also clearly displays that samples showing either a growth or a zero-growth phenotype were marked by different volatiles, that were mainly acid, esters and ketones for the former, and alcohols and terpenes for the latter. Most of the acids, acetic in particular, increased in juice fermented with *L. casei* growing strains 2240 and 2246 (Table 2), whereas isovaleric acid resulted higher in the juice added with CFE of *L. casei* 2057 compared to the control ($p<0.05$), where it reached 0.68±0.15 µg/mL.

Moreover, after 48 h of incubation the alcohol content increased, especially in samples with zero-growth, with the highest production found in elderberry juice with *L. casei* 2057 (4.93±0.73 µg/mL) (Table 3). Notably, hexanol (V-AL4) and (*E*) 2- hexen-1-ol (V-AL8), associated with green aromatic notes, were the most abundant and their concentration significantly differs from control ($p<0.05$). Similarly, terpenes and norisoprenoids, such as *β*-damascenone, *β*-linalool and *α*-terpineol, increased mainly in juice added with zero-growth strains *L. casei* 2057 and *L. casei* 2306 (Table 2).

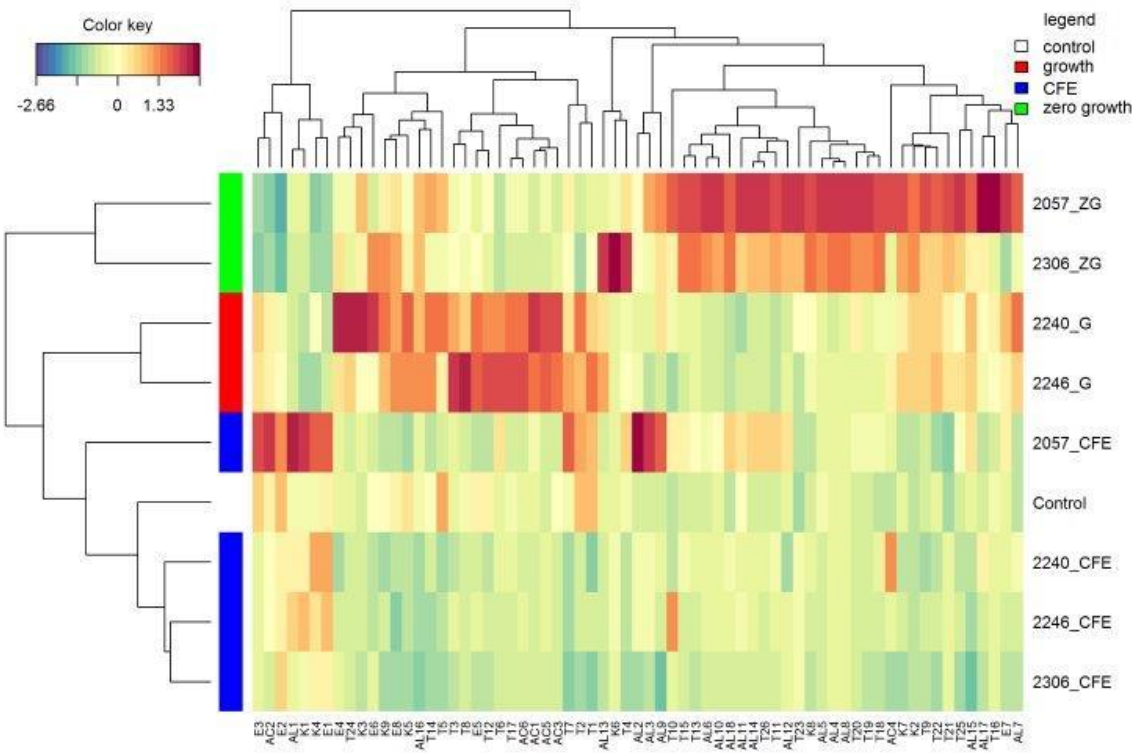

Figure 2. Hierarchical clustering analysis and heatmap visualization of the volatile profiles of the elderberry added with zero growth (ZG) strains, growing strains (G), or cell-free extracts (CFE), and the unstarted juice (control), based on the Euclidean distance calculated on the amount of each volatile compound. The leftmost column bar is coloured according to the growth condition used for the fermentation, as reported in the legend. The colour scale represents the scaled abundance of each variable, denoted as d^2 (squared Euclidean distance), with red indicating high abundance and blue indicating low abundance. The compounds represented in the heat-map are named as in Table 2.

The polyphenolic profiles drive a different clusterization of samples in comparison with volatile compounds (Figure 3). CFE and growth strains grouped separately, instead samples with zero-growth showed a different potential. To note, despite the absence of growth, *L. casei* 2057 was able to significantly increase many polyphenolic compounds, especially anthocyanins (Table 4).

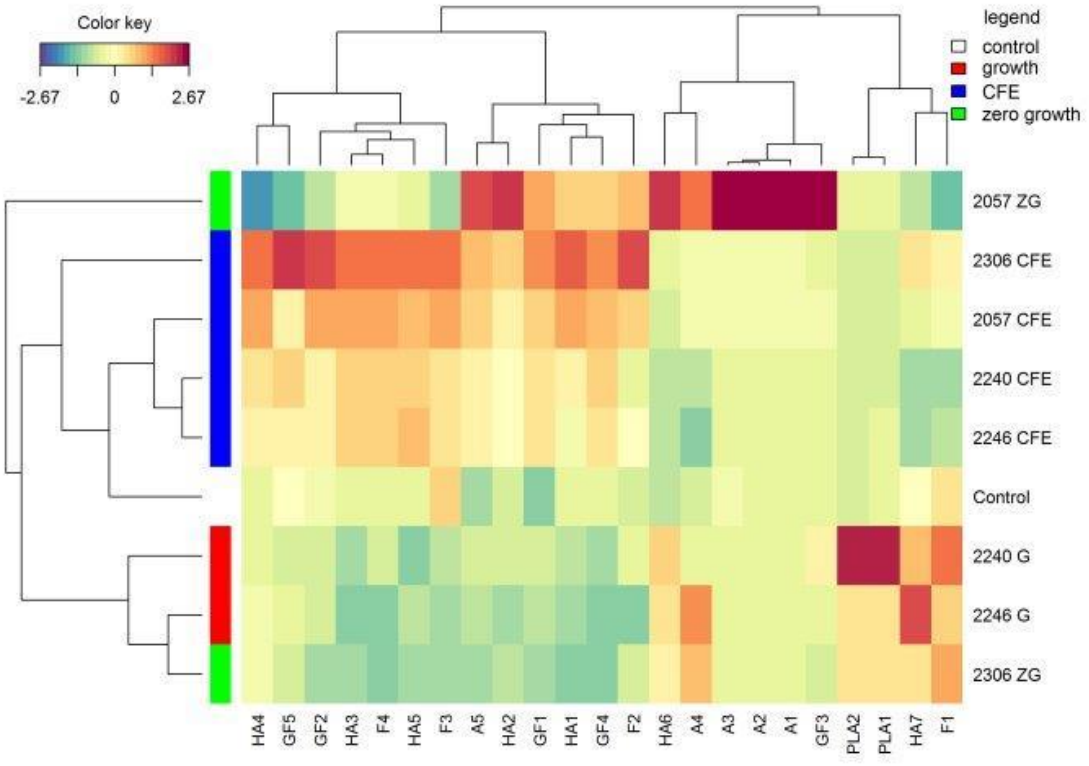

Figure 3. Hierarchical clustering analysis and heatmap visualization of the polyphenolic compounds identified in the elderberry juices added with zero growth (ZG) strains, growing strains (G), or cell-free extracts (CFE), and the unstarted juice (control), based on the Euclidean distance calculated on the amount of each identified polyphenol. The leftmost column bar is coloured according to the growth condition used for the fermentation, as reported in the legend. The colour scale represents the scaled abundance of each variable, denoted as d^2 (squared Euclidean distance), with red indicating high abundance and blue indicating low abundance. The compounds represented in the heat-map are named as in Table 4.

Table 4. Concentration (µg/mL) of (poly)phenolic compounds identified in elderberry juices added with *L. casei* 2057, 2306, 2240, 2246 and the respective cell-free extracts (CFE). For each compound a specific variable (VAR), used in statistical and graphical analyses, was assigned.

		Zero-growth						**CFE**								**Growth**			
Compounds	VAR	Control		2057		2306		2057		2240		2246		2306		2240		2246	
Hydroxycinnamic acids																			
3-*O*-caffeoylquinic acid	PF-HA1	6.472 ± 0.460	a	8.614 ± 0.648	c,d	6.449 ± 0.326	a	8.994 ± 0.257	c,d	7.987 ± 0.315	b,c	7.649 ± 0.619	a,b,c	9.931 ± 0.957	d	6.96 ± 0.109	a,b	6.64 ± 0.402	a,b
5-*O*-caffeoylquinic acid	PF-HA2	5.605 ± 0.105	a	16.116 ± 0.429	f	6.439 ± 0.411	a	10.23 ± 0.129	d	9.659 ± 0.454	c,d	9.263 ± 0.467	c	11.569 ± 0.242	e	7.514 ± 0.137	b	6.227 ± 0.121	a
4-*O*-caffeoylquinic acid	PF-HA3	2.808 ± 0.153	a	3.92 ± 0.277	b,c	3.108 ± 0.087	a,b	4.913 ± 0.262	d	4.62 ± 0.152	c,d	4.559 ± 0.351	c,d	5.326 ± 0.645	d	3.239 ± 0.198	a,b	3.048 ± 0.311	a,b
Coumaroylquinic acid (1)	PF-HA4	0.008 ± 0	b	0.004 ± 0.001	a	0.01 ± 0.001	b,c	0.013 ± 0.001	d,e	0.012 ± 0.001	b,c	0.01 ± 0.002	b,c	0.015 ± 0.002	e	0.009 ± 0	b,c	0.01 ± 0	b,c
Coumaroylquinic acid (2)	PF-HA5	0.032 ± 0.005	a	0.037 ± 0.005	a,b	0.032 ± 0.002	a	0.047 ± 0.005	b,c	0.046 ± 0.001	b,c	0.046 ± 0.001	b,c	0.053 ± 0.005	c	0.031 ± 0.002	a	0.034 ± 0.003	a
Caffeic acid	PF-HA6	1.692 ± 0.056	b	2.21 ± 0.045	c	1.656 ± 0.086	b	1.41 ± 0.028	a	1.326 ± 0.064	a	1.316 ± 0.081	a	1.475 ± 0.037	a	1.745 ± 0.049	b	1.688 ± 0.01	b
Total		16.617 ± 0.613	a	30.889 ± 1.202	e	17.684 ± 0.734	a,b	25.599 ± 0.389	c,d	23.643 ± 0.829	c	22.838 ± 1.426	c	28.36 ± 1.873	d,e	19.488 ± 0.181	b	17.637 ± 0.573	a,b
Hydroxybenzoic acid																			
Protocatechuic acid	PF-HA7	9.077 ± 0.367	b,c	7.847 ± 0.183	a,b	9.212 ± 0.157	c	8.234 ± 0.084	a,b,c	7.764 ± 0.424	a	7.608 ± 0.178	a	9.146 ± 1.031	c	9.463 ± 0.09	c,d	10.509 ± 0.007	d
Phenyllactic acids																			
Phenyllactic acid	PF-PLA1	1.452 ± 0.126	b	0.881 ± 0.033	a	2.453 ± 0.105	c	0.643 ± 0.017	a	0.62 ± 0.007	a	0.886 ± 0.077	a	0.73 ± 0.063	a	6.082 ± 0.353	d	2.67 ± 0.05	c
p-hydroxyphenyllactic acid	PF-PLA2	1.093 ± 0.034	b,c	1.361 ± 0.079	c	3.735 ± 0.177	d	0.433 ± 0.039	a	0.44 ± 0.116	a	0.768 ± 0.069	a,b	0.496 ± 0.112	a	9.101 ± 0.392	e	3.809 ± 0.223	d
Total		2.544 ± 0.144	c	2.236 ± 0.111	c	6.173 ± 0.256	d	1.072 ± 0.041	a,b	1.056 ± 0.12	a	1.648 ± 0.146	a,b	1.222 ± 0.168	a,b	15.146 ± 0.378	e	6.463 ± 0.263	d
Flavone																			

Luteolin	PF-FF	1.359 ± 0.058	d,e	0.919 ± 0.055	a	1.374 ± 0.066	d,e	1.135 ± 0.036	b,c	1.016 ± 0.053	a,b	1.029 ± 0.02	a,b	1.2250.085	c,d	1.4480.014	e	1.2940.085	c,d,e
Flavonols																			
Quercetin	PF-F1	7.034 ± 2.428	a	14.424 ± 2.127	a	9.148 ± 1.039	a	13.184 ± 7.115	a	9.292 ± 8.143	a	11.375 ± 4.786	a	18.473 ± 2.276	a	9.221 ± 1.621	a	6.506 ± 2.742	a
Kaempferol	PF-F2	0.466 ± 0.101	a	0.516 ± 0.053	a	0.458 ± 0.044	a	1.441 ± 0.06	c	1.201 ± 0.06	b	1.138 ± 0.092	b	1.638 ± 0.115	c	0.624 ± 0.029	a	0.48 ± 0.059	a
Isorhamnetin	PF-F3	0.078 ± 0.003	a	0.116 ± 0.01	c	0.082 ± 0.003	a,b	0.163 ± 0.003	d,e	0.15 ± 0.008	d	0.144 ± 0.008	d	0.177 ± 0.015	e	0.1 ± 0.002	b,c	0.078 ± 0.002	a
Total		7.578 ± 2.325	a	15.057+2.135	a,b	9.688 ± 0.996	a,b	14.787 ± 7.132	a,b	10.644 ± 8.147	a,b	12.656 ± 4.885	a,b	20.288 ± 2.405	b	9.945 ± 1.599	a,b	7.064 ± 2.747	a
Flavonol glycosides																			
Quercetin-3-*O*-glucoside	PF-GF1	13.276 ± 1.223	a	23.811 ± 1.819	d	13.395 ± 1.496	a	21.477 ± 0.804	c,d	20.884 ± 0.394	c	20.845 ± 1.66	c	24.762 ± 1.302	b	15.595 ± 0.567	b	14.58 ± 0.455	a,b
Kaempferol-*O*-rutinoside	PF-GF2	0.434 ± 0.049	a	0.447 ± 0.031	a,b	0.423 ± 0.024	a	0.614 ± 0.02	c,d	0.546 ± 0.044	b,c	0.544 ± 0.028	b,c	0.68 ± 0.049	d	0.472 ± 0.022	a,b	0.457 ± 0.031	a,b
Quercetin-*O*-rutinoside-*O*-hexoside	PF-GF3	0.004 ± 0.001	a	0.016 ± 0.001	b	0.005 ± 0	a	0.006 ± 0.001	a	0.006 ± 0.001	a	0.006 ± 0.001	a	0.006 ± 0.002	a	0.008 ± 0.001	a	0.005 ± 0.001	a
Isorhamnetin-*O*-hexoside	PF-GF4	0.023 ± 0.003	a,b	0.017 ± 0.005	a	0.024 ± 0.001	a,b	0.031 ± 0	b	0.033 ± 0.005	b	0.03 ± 0.001	b	0.045 ± 0.006	c	0.024 ± 0.002	a,b	0.025 ± 0.003	a,b
Isorhamnetin-*O*-rutinoside	PF-GF5	0.067 ± 0.005	a	0.142 ± 0.02	b,c	0.069 ± 0.003	a	0.147 ± 0.005	b,c	0.141 ± 0.008	b,c	0.129 ± 0.017	b	0.166 ± 0.019	c	0.079 ± 0.002	a	0.071 ± 0.006	a
Quercetin-3-*O*-rutinoside	PF-GF6	29.98 ± 0.654	a	63.319 ± 3.345	d	31.389 ± 2.938	a,b	48.582 ± 1.955	c	43.937 ± 2.662	b,c	45.226 ± 4.339	c	52.34 ± 3.513	a,b	35.664 ± 3.999	a,b	32.237 ± 3.297	a,b
Total		35.737 ± 8.274	a	87.752 ± 5.18	d	45.305 ± 4.008	a,b	70.858 ± 1.439	c	65.547 ± 2.245	b,c	66.779 ± 3.786	b,c	77.998 ± 2.175	c,d	51.842 ± 4.143	b	47.375+3.709	a,b
Anthocyanins																			
Cyanidin-3-*O*-sambubioside	PF-A1	6.318 ± 3.097	a	69.114 ± 7.223	b	6.537 ± 0.666	a	7.656 ± 0.652	a	6.042 ± 0.218	a	5.762 ± 0.726	a	8.019 ± 0.666	a	5.17 ± 0.271	a	6.263 ± 0.502	a
Cyanidin-3-*O*-glucoside	PF-A2	2.772 ± 0.543	a	44.524 ± 1.833	b	2.257 ± 0.09	a	3.319 ± 0.158	a	2.842 ± 0.097	a	2.809 ± 0.31	a	3.46 ± 0.132	a	2.927 ± 0.121	a	2.122 ± 0.065	a
Cyanidin-3-*O*-rutinoside	PF-A3	0.096 ± 0.007	a	1.146 ± 0.075	b	0.042 ± 0.004	a	0.08 ± 0.006	a	0.072 ± 0.005	a	0.074 ± 0.006	a	0.087 ± 0.004	a	0.071 ± 0.004	a	0.036 ± 0.002	a
Cyanidin-3-*O*-sambubioside- hexoside	PF-A4	0.436 ± 0.308	a	0.785 ± 0.324	c	0.675 ± 0.072	b,c	0.483 ± 0.037	a,b,c	0.377 ± 0.03	a,b	0.339 ± 0.053	a,b	0.501 ± 0.034	a,b,c	0.447 ± 0.014	a,b,c	0.744 ± 0.04	c
Total		9.516 ± 4.316	a	115.57 ± 8.584	b	9.51 ± 0.827	a	11.538 ± 0.816	a	9.333 ± 0.221	a	8.983 ± 1.072	a	12.068 ± 0.791	a	8.615 ± 0.398	a	9.166 ± 0.589	a

Effect of viable cells and cell-free extracts of Lactobacillus casei on polyphenolic profile

The (poly)phenolic profile of samples analysed was mainly characterized by hydroxycinnamic acids, phenyllactic acids, flavone, flavonols, flavonol glycosides, anthocyanins and hydroxybenzoic acid. Samples shown a different trend of hydroxycinnamic acids. An increase, in comparison with the control, was observed for all the juice added with CFE, but especially in juice added with *L. casei* 2057 zero-growth (Table 4). Conversely, juice fermented with growing *L. casei* showed a reduction in these compounds after 48 h or no differences with unfermented juice (Table 4). About phenyllactic acids only 3 samples have shown differences in comparison to the control and among these, fermentation with 2240 growing strain, lead to the highest concentration of phenyllatic acid (6.08±0.35 µg/mL) and *p*-hydroxyphenyllactic acid (9.10±0.39 µg/mL) (Table 4). Flavonoids identified comprise flavone, flavonols (mainly found as glycosides) and anthocyanins. Total flavonols (not glycosylated), as quercetin, kaempferol and isorhamnetin, resulted highest in the juice added with CFE of *L. casei* 2306 (20.29±2.40 µg/mL) (Table 4). However, a higher concentration of these compounds was observed in their glycosylated forms: quercetin as quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol as kaempferol-*O*-rutinoside and isorhamnetin as isorhamnetin-*O*-hexoside or isorhamnetin-*O*-rutinoside. Quercetin-3-*O*-rutinoside resulted the highest especially in juice added with *L. casei* 2057 zero-growth and quercetin-3-*O*-glucoside in CFE of 2306 (both significantly different when compared to the control, $p<0.01$). The last and the most interesting results were about the anthocyanins (Table 4). Surprisingly, we observed a huge increase of these compounds in juice added with *L. casei* 2057 zero-growth, where their concentration reached 115.57±8.58µg/mL, in comparison of 9.62±0.54µg/mL of the control. In particular, the most abundant anthocyanins were cyanidin-3-*O*-sambubioside and cyanidin-3-*O*-glucoside, while cyanidin-3-*O*-rutinoside and cyanidin-*O*-sambubioside-*O*-hexoside were almost negligible.

Comparison of juices added with viable cells and CFE of L. casei

In order to better highlight the differences among juices added with viable cells and CFE we focused on variables that significantly differ among the samples, belonging to the most abundant classes of detected compounds which are alcohols and terpenes for the volatiles and anthocyanins and flavonol glycosides for polyphenols. Moreover, we have considered phenyllactic acids because they are often found in fermented food as a consequence of microbial metabolism and exert an interesting role as antimicrobials.

Figure 4. Radarplot of the scaled quantity of the compounds that significantly differ among samples. Radars show the scaled amount of compounds produced by the four strains added to elderberry juice as viable cells (pink) or as cell-free extract (CFE, blue). The abbreviations for volatile compounds (V) and polyphenols (PF) are the following: V.AL4, Hexanol; V.AL8, (E)-2-Hexen-1-ol; V.T19, β-damascenone; V.T11, β-Linalool; V-T13, α-Terpineol; PF.PLA1, DL-phenyllactic acid; PF.PLA2, p-Hydroxyphenyllactic acid; PF-GF6, Rutin; PF.GF1, Quercetin-*O*-hexoside; PF.A1, Cianidina-*O*-sambubioside; PF.A2, Cianidina-*O*-esoside

The radar plot (Figure 4) shows how viable cells and CFE from the same strain differently impact on the variables considered. Firstly, the viable cells lead to increase the relevant polyphenols and volatiles more than CFE, except for strain 2246. It can also be observed that viable cells of growing strains, in particular the strain 2240, were able to especially increase the content of phenyllactic acids while viable cells of non-growing strains strongly impact on more variables, especially alcohol and terpenes. Finally, the strain 2057, even in absence of growth, shows the greatest impact to increase the polyphenolic content (especially anthocyanins) and the volatile compounds associated with typical aroma of elderberry juice (hexanol, β-linalool, α-terpineol, β-damascenone and (*E*)-2-hexen-1-ol).

Discussion

The aim of this study was to investigate the changes in volatile compounds and polyphenols in elderberry juice added with viable and CFE of 4 *L. casei* strains.

Beyond the active metabolism of growing cells, it was demonstrated that flavour production occurs when LAB reach a non-growing state (Van de Bunt et al., 2014). Moreover, during the ripening of fermented food, the contribution of microbial intracellular components, released after cells lysis, have a fundamental role in the modulation of aromatic profile (Lortal et al., 2005; Bolumar et al.,2006; Styger et al., 2011). So far, no previous work had focused on the addition of cell extracts or viable but non-multiplying bacterial cells into fruit juices and, to the best of our knowledge, only our group did considered the use of LAB specifically in elderberry juice (Ricci et al., 2018). To better describe the different contribution of viable cells and CFE, a commercial pasteurized elderberry juice was used because, even if heat treatments could in part change the chemical

composition of the raw material, sterilized/pasteurized juices are the best model systems to carry out a steered fermentation (Gardner et al., 2001).

Juices added with CFE, especially *L. casei* 2057, showed the highest amount of esters, which are formed by the esterification of acids and alcohols (McSweeney 2004; Smid and Kleerebezem, 2014). They exhibit fruity notes with low threshold concentrations, many times lower than their precursors, (Di Cagno et al., 2009), and for this reason are considered influential even if detected at low levels. The main compounds identified in juice added with CFE, namely ethyl acetate and methyl salicylate, probably derived from the esterification of ethanol with acetic acid and shikimic acid, a metabolite present in elderberry, respectively (Kaack et al., 2005).

Viable cells of *L. casei* strains added to elderberry juice showed two distinct effects, described as bacterial growth, associated to fermentation, and absence of growth, or zero-growth.

The strains showing a growth phenotype (2240 and 2246) elicited an increase in acids production, such as acetic acid, as a result of heterofermentative pathways, in line with other works on lactic acid fermentation of plant matrix (Filannino et al., 2013; Filannino et al., 2017). Furthermore, these strains significantly increased the amounts of phenyllactic acids (phenyllactic acid and *p*-hydroxyphenyllactic acid). Phenyllactic acids are naturally found in honey and LAB fermented food (Mu et al., 2012) and can be produced by different species of LAB in a strain-dependent way (Valerio et al., 2004), but, to the best of our knowledge, this is the first time that has been reported for *L. casei*. Originating from the metabolism of aromatic amino acids, phenyllactic acid is produced from degradation of phenylalanine, while *p*-hydroxyphenyllactic acid is produced from degradation of tyrosine (Valerio et al., 2004). Thanks to their antimicrobial activity, in particular against fungi, but also against different species of bacteria (Lavermicocca et al., 2000; Valerio et al., 2009; Cortès-Zavaleta et al., 2014), their use as natural preservative in food has been proposed (Lavermicocca et al., 2000).

Regarding volatile compounds, the strains exhibiting zero-growth lead to the highest increase in their total amount, in particular in those alcohols, terpenes and norisoprenoids which exhibit typical elderberry scent (Kaack et al., 2005; Kaack, 2008).

Among alcohols, 2-hexen-1-ol and hexanol, related with the green elderberry note, were produced in the highest amounts. These compounds are degradation products of linoleic and linolenic acid, found in elderberry seeds (Dulf et al., 2013), which might be formed via the action of lipoxygenase (Sabatini et al., 2008). Their increase had also been previously reported in elderberry juice fermented with *L. plantarum* and *L. rhamnosus* strains (Ricci et al., 2018) and in olives after lactic acid fermentation (Sabatini et al., 2008), but no study reported this ability in non-growing bacteria. Compared with our previous results (Ricci et al. 2018), where growing strains were employed in elderberry juice, non-growing strains highly increase the concentration of alcohols mentioned above (about 1.4-1.6 µg/mL).

Zero-growth strains had also the capacity to significantly increase the concentration of *β*-damascenone, an important norisoprenoid strongly associated with elderberry scent. The terpenes *β*-linalool and *α*-terpineol impart a floral note to elderberry (Kaack et al., 2005) and are positively affected by zero growth strains used in this study. Different works highlighted the increase of these compounds after fermentation with LAB and their relevance as odorous molecules (Ugliano and Moio, 2006; Cañas et al., 2008). Terpenes and norisoprenoids are usually found in nature as glycosides, but some studies reported the ability of microorganisms to produce glycosylases resulting in the release of aglyconic forms (Ugliano and Moio, 2006;; Cañas et al., 2008; Di Cagno et al., 2013). Moreover, Belviso et al. (2011) suggested the possibility for some lactic acid bacteria to synthetize *ex novo* specific terpenes as secondary metabolites.

Another interesting potentiality of zero-growth strains was the ability to increase anthocyanins, particularly cyanidin-3-*O*-sambubioside and cyanidin-3-*O*-glucoside, and glycosylated flavonols,

especially quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside. An increase of cyanidin-3-*O*-glucoside and quercetin-3-*O*-rutinoside during mulberry fermentation was also reported by Kwaw et al. (2018) especially when *L. paracasei* and *L. acidophilus* were used.

Overall, the most interesting sample, considering all the variables, was the elderberry juice added with *L. casei* 2057. Despite the absence of growth, an increase of volatile compounds, especially alcohols, terpenes and norisoprenoids, and polyphenols, in particular anthocyanins, was observed. Even if the literature is not completely conclusive on the topic, mounting evidence from prospective studies, also supported by recent intervention trials, suggests that an increased intake of anthocyanins could result in health benefits in humans, particularly in the cardiovascular framework. For this reason, every strategy that results in increased content of anthocyanins in food, including fermentation, could be considered as advantageous.

The different effects observed with regards to the growth phenotype exhibited by the *L. casei* strain suggests that, in the selection of starters for fruit juice fermentation, the growth capability of certain strains in the substrate should not be the sole selection criterion. This observation is particularly relevant for bacteria used in industrial food fermentation, where bacterial cells persisting in a non-replicating state have an active metabolism with a detectable transcriptional activity and maintain the capability to produce volatile compounds (Ercan et al., 2015; Van Mastrigt et al., 2018). Further studies will be directed to assess if mechanisms involved in the entrance into non-growing state such as toxin-antitoxin systems, recently described in *Lactobacillus casei* group (Klimina et al., 2013; Folli et al., 2017), could be related with changes in the metabolism of polyphenols and aromatic compounds.

Conclusion

The selection of microbial culture used in food relies on the ability of growth, on the production of aromatic compounds, and on the increase of nutritional value. Nevertheless, some authors are actively debating about zero-growth state as a physiological condition useful to enhance the aromatic features of a product. In this study, the first carried out using non-growing LAB in fruit juices, we have demonstrated that the polyphenolic profile (i.e. anthocyanins) and the typical elderberry aroma (alcohols, terpenes and norisoprenoids) is strongly modulated in absence of growth.

These results may have a significant technological application, since the potential of non-growing *Lactobacillus* cells, able to boost the presence of specific molecules, could lead to develop a microbial collection exploitable in the fruit juice industry.

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Chapter 2.3

Appendix

Table S1. Variables loading on dimensions 1 and 2 of the principal components analysis (PCA), bold characters highlight the ones selected for further PCA analysis, variables abbreviation are as reported in Tables 2 and 4**.**

Variable	Dim.1	Dim.2
V.K1	0.563447	-0.48795
V.K2	-0.9548	-0.04539
V.K3	-0.66065	0.27568
V.K4	0.761758	-0.13358
V.K5	-0.65425	0.702856
V.K6	-0.24407	-0.15978
V.K7	-0.83106	-0.02587
V.K8	-0.75184	-0.21257
V.K9	-0.73453	0.522276
V.E1	0.854216	-0.20771
V.E2	0.852292	0.242426
V.E3	0.290788	0.386463
V.E4	-0.56283	0.566947
V.E5	-0.43298	0.817155
V.E6	-0.47942	0.558809
V.E7	-0.62416	-0.10316
V.E8	-0.71134	0.484272
V.AL1	0.458011	-0.36259
V.AL2	0.08589	-0.30835
V.AL3	-0.03394	-0.55056
V.AL4	-0.68335	-0.61529
V.AL5	-0.73578	-0.56472
V.AL6	-0.66456	-0.6509
V.AL7	-0.81457	0.117299
V.AL8	-0.71506	-0.57077
V.AL9	-0.4269	-0.35512
V.AL10	-0.64823	-0.72542
V.AL11	-0.52808	-0.76548
V.AL12	-0.67141	-0.65335
V.AL13	-0.48156	0.330624
V.AL14	-0.55069	-0.72782
V.AL15	-0.83171	0.04752
V.AL16	-0.88148	0.362635
V.AL17	-0.67078	-0.30039
V.AL18	-0.45071	-0.75148
V.T1	-0.2744	0.534271
V.T2	-0.11052	0.640279
V.T3	-0.50403	0.722857
V.T4	-0.51599	-0.10296
V.T5	-0.67765	0.372237
V.T6	-0.12675	0.63994
V.T7	-0.39467	0.163035
V.T8	-0.51298	0.628075
V.T9	-0.91543	-0.02757
V.T10	-0.30514	-0.58717
V.T11	-0.52139	-0.76421
V.T12	-0.50539	0.771227
V.T13	-0.74935	-0.5617
V.T14	-0.79226	0.475603
V.T15	-0.67976	-0.65671
V.T16	-0.64934	-0.25898
V.T17	-0.40526	0.772014
V.T18	-0.77309	-0.53307
V.T19	-0.81999	-0.53332
V.T20	-0.85574	-0.47514
V.T21	-0.7732	-0.14372
V.T22	-0.81149	0.118434
V.T23	-0.56966	-0.33173
V.T24	-0.44662	0.703271
V.T25	-0.8161	-0.29213
V.T26	-0.58015	-0.71811
V.AC1	-0.31562	0.806214
V.AC2	0.509637	0.238763
V.AC3	-0.34639	0.860916
V.AC4	-0.45909	-0.37886
V.AC5	-0.39421	0.763151
V.AC6	-0.39567	0.769655
PF.A1	-0.63245	-0.62528
PF.A2	-0.62784	-0.62404
PF.A3	-0.61647	-0.61961
PF.A4	-0.64413	-0.16958
PF.FF	0.187578	-0.45213
PF.F1	0.799588	-0.33818
PF.F2	0.651078	-0.5119
PF.F3	-0.10697	0.753484
PF.GF1	0.322107	-0.69819
PF.GF2	0.727809	-0.2629
PF.GF3	-0.61536	-0.52017
PF.GF4	0.766333	-0.05092
PF.GF5	0.432861	-0.68325
PF.GF6	-0.00901	-0.7696
PF.HA1	0.377784	-0.59009
PF.HA2	-0.15049	-0.79229
PF.HA3	0.610601	-0.54231
PF.HA4	0.778952	0.073227
PF.HA5	0.678949	-0.46884
PF.HA6	-0.85903	-0.12632

Chapter 3

Use of dairy and plant-derived lactobacilli as starters for cherry juice fermentation

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Abstract

Background: Lactic acid bacteria (LAB) exhibit a great biodiversity that can be exploited for different purposes, such as to enhance flavours or metabolize phenolic compounds. In the present study, the use of dairy and plant-derived LAB strains to perform cherry juice fermentation was reported. Methods: The growth ability of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* was studied in cherry juice. Profiling of sugars, organic acids and volatile compounds was performed by GC-MS, while the phenolic fraction was characterized using UHPLC equipped with a linear ion trap-mass spectrometer. Results: Sucrose significantly decreased in all fermented samples as well as malic acid, converted to lactic acid by malolactic fermentation. The total amount of volatile compounds increased. Specifically, propyl acetate, an ester with fruit notes, reached the highest concentration in *L. rhamnosus* and *L. paracasei* (dairy strains) fermented juices. Phenolics were extensively metabolized: caffeic acid was converted into dihydrocaffeic acid, *p*-coumaric acid into 4-ethylphenol and phenyllactic acid was produced. Conclusion: Lactic acid fermentation confer fruit notes to the juice and enhance phenyllactic acids, especially employing dairy strains (*L. rhamnosus* and *L. paracasei*). The level of dihydrocaffeic acid, a compound with putative biological activity was also increased (in particular with *L. plantarum*).

Introduction

Sweet cherry, *Prunus avium L.*, is a native tree of Europe and western Asia and grows wildly around the world (Chockchaisawasdee et al., 2016). Its fruit is consumed fresh, but also after processing, as canned, dried, frozen, and in syrups and juices. The main producers of sweet cherry are Turkey, United States, Iran, Spain, Italy and Chile. In Italy the annual production is about 100,000 tonnes, and the Apulia region is the most relevant area of production (Di Cagno et al., 2011). Sweet cherry is characterized by a high content of micronutrients and bioactive compounds, even if these attributes strongly depend on cultivar, ripening, growth condition, pre and post-harvest treatments (Chockchaisawasdee et al., 2016). Sugars and organic acids in fruit have been reported in the 125-265 g/kg and 3.67 to 8.66 g/kg ranges on fresh weight basis, respectively (Usenik et al., 2008). The balance between sweetness (sugars, mainly glucose and fructose) and sourness (acids, mainly malic acid) is paramount for the acceptance of the product by consumers (Serrano et al., 2005) as well as the fruit aroma, despite the fact that aromatic compounds represent only the 0.001 to 0.01% of the total fruit weight (Serradilla et al., 2012). The aroma of cherry is related to a wide number of organic compounds including aldehydes, alcohols, esters, acids and terpenes (Sun et al., 2010). Aldehydes and alcohols represent more than the 80% of total volatile compounds, followed by acids, esters and terpenes. In particular, the most represented compounds are hexanal, (E)-2-hexenal (green note and fresh green odours), 1-hexanol (floral and grape notes), (E)-2-hexen-1-ol (vegetable note), benzyl alcohol (floral note), and benzaldehyde as the most important contributor of the typical cherry note. Moreover, the aroma of sweet cherry fruits is also influenced by non-volatile glycosidically bound precursors that, in sweet cherry, are more concentrated than the free forms. Glycosylated forms of alcohols, terpenes, norisoprenoids and organic acids are well known, and it is now accepted that the release of these compounds could strongly modulate fruit flavour (Wen et al., 2014; Ubeda et al., 2012; Garcia et al., 2011).

Cherries are also rich in polyphenols, compounds derived from secondary plant metabolism and characterized by one or more hydroxylated aromatic rings: anthocyanins, phenolic acids, and flavonoids are the main phenolics observed in cherries.

Lactic acid bacteria (LAB) are the most widespread microorganisms involved in food fermentation and their ability to convert phenolic compounds has been reported in literature (Ricci et al., 2019; Filannino et al., 2018; Zhao et al., 2016; Rodríguez et al., 2009; Selma et al., 2009). The field of fruit juice fermentation represents a new interesting ever increasing line of research for product innovation (Mantzourani et al., 2018; Corbo et al., 2014), although the number of fermented commercial products is still limited. The starter strains generally used for fruit fermentation belong to *Lactobacillus plantarum* species, recognized to be the most adapted for these types of substrate, and often autochthonous strains have been used for the same reason. Lactic acid bacteria exhibit a great biodiversity, also derived from their ability to adapt to different environments, and this biodiversity can be exploited for different purposes, such as to enhance flavours (Ricci et al., 2018; Dongmo et al., 2017). Based on these assumptions, in the present work, the contribution of different LAB species, isolated from dairy and plant products, were evaluated in the framework of cherry juice fermentation. Although *L. plantarum* was already used to ferment cherry juice (Filannino et al., 2015), but the novelty of this study was to evaluate the contribution of different LAB species, isolated from dairy and plant products. The ability to adapt to this specific matrix, the metabolism of sugars and organic acids, and the effect on the fruit volatile and phenolic profiles were investigated considering a large set of dairy strains as starters.

Material and methods

Chemicals

Analytical standards of 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, protocatechuic acid, quercetin-3-rutinoside hydrate, quercetin dihydrate, kaempferol, phenyllactic acid, caffeic acid, naringenin, (+)-catechin, (-)-epicatechin, p-coumaric acid and toluene were from Sigma-Aldrich, USA. Dihydrocaffeic acid, p-hydroxyphenyllactic acid were from Santa Cruz Biotechnology, USA, while luteolin was from Extrasynthese, France. HPLC-grade acetonitrile was from Sigma-Aldrich, while HPLC-grade water and LC-MS grade formic acid were purchased from VWR International (Milan, Italy).

Bacterial strains

Fourteen strains belonging to different species of lactic acid bacteria (*L. plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus casei* and *Lactobacillus paracasei*) were singly used for the fermentation of a commercial cherry juice (Table 1). All bacterial strains were stored at -80 °C in de Man Rogosa and Sharpe (MRS) medium (Oxoid, Milan, Italy) supplemented with 25% glycerol (v/v). The cultures were propagated three times with about 3% (v/v) of inoculum in MRS and incubated in anaerobiosis (AnaeroGen, Oxoid) overnight at 30°C for L. plantarum and 37°C for *L. rhamnosus, L. casei* and *L. paracasei*.

Table 1. Microbial strains used in the study and their origin.

Species	Strain	Origin
L. plantarum	POM1*	Tomato (plant)
	C1*	Carrot (plant)
	1LE1*	Pineapple (plant)
	285**	Minas cheese (dairy)
L. rhamnosus	2178**	Parmigiano Reggiano cheese (dairy)
	2140**	Parmigiano Reggiano cheese (dairy)
	2360**	Parmigiano Reggiano cheese (dairy)
	1473**	Parmigiano Reggiano cheese (dairy)
	1019**	Parmigiano Reggiano cheese (dairy)
L. casei	2246**	Parmigiano Reggiano cheese (dairy)
	2306**	Parmigiano Reggiano cheese (dairy)
	2057**	Parmigiano Reggiano cheese (dairy)
	2107**	Parmigiano Reggiano cheese (dairy)
L. paracasei	4186**	Pecorino cheese (dairy)

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Fermentation process and storage

A commercial pasteurized cherry juice (Bionaturae) was used for fermentation. The absence of microbial contamination in the juice was evaluated on Plate Count Agar at 30°C and 37°C. LAB strains were cultivated in MRS broth for 15h at 30°C for *L. plantarum* and at 37°C for *L. rhamnosus*, *L. casei* and *L. paracasei*, to reach the late exponential growth phase, centrifuged at 10,000 g for 10 min at 4°C, washed twice with Ringer's solution (Oxoid, Milan, Italy) and re-suspended in sterile distilled water. These cultures were singly inoculated into cherry juice to reach the final concentration of ca. 7 Log CFU/mL. The juices were incubated at 30°C for *L. plantarum* and at 37°C for *L. rhamnosus*, *L. casei* and *L. paracasei* for 48 hours and then stored for 12 days at

4°C. Unfermented cherry juices (not added of starter cultures) were incubated at 30°C and at 37°C for 48 hours, then stored for 12 days at 4°C and used as controls. All the fermentation were carried out in triplicate.

Evolution of bacterial growth and acidification of cherry juice

Cherry juice, inoculated with starters, was analysed before and after fermentation (48 hours) and after the storage period (12 days). Cultivable cells were determined using the standard plate count agar method as follows: decimal dilutions of samples were carried out in Ringer solution (Oxoid, Milan, Italy) and plated on MRS agar, then incubated at 30°C (*L. plantarum*) and 37°C (*L. rhamnosus, L. casei* and *L. paracasei*) for 48 hours under anaerobic condition. The pH of samples was measured using a pH metre (Mettler Toledo, Switzerland). Plate count and pH measurement was carried out in triplicate.

Sugars and organic acid analysis

Sugars and organic acids were analysed after fermentation and storage, both for fermented juices and for controls. The method reported by Cirlini et al. 2009, with slight modifications, was applied. Briefly, 10 µL of samples were added to 1 ml of a solution containing two internal standards (turanose and glutaric acid 500 µg/mL each), dried under vacuum and dissolved with 500 µL of dimethylformamide. Subsequently, silylation was carried out adding 400 µL of hexamethyldisilazane and 200 µL of trimethylchlorosilane to the samples and heating for 30 minutes at 70°C. Samples were analysed by a Thermo Scientific Trace 1300 gas chromatograph coupled to a Thermo Scientific ISQ single quadrupole mass spectrometer equipped with an electronic impact (EI) source on a BP5MS capillary column (30 m x 0.25 mm, with 0.25 µm film thickness, SGE Analytical Science, Milan, Italy). Chromatographic conditions were the following: initial oven temperature, 60°C, then increase of 20°C/min up to 280°C; carrier gas, (flow rate, 1

mL/min). Temperature of the transfer line was maintained at 280°C, while the ion source was set at 230°C. The acquisition mode was full scan (m/z: 40-550). Signals were identified on the basis of their mass spectra compared with those present in the instrument library (NIST 14). In addition, once the glucidic and organic acid fractions were recognized, proper analytical standards were used in order to confirm the identifications. The semi quantification of all detected gas-chromatographic signals was performed on the basis of the use of two internal standards: turanose for sugar quantification and glutaric acid for organic acid quantification. For each identified compound, the Response Factor (RF) was calculated and the values range between 0.8 and 1.2.

Characterization of the volatile profile

The volatile profile of fermented and unfermented samples was analysed after 48 hours of incubation and after 12 days of storage. Volatiles were characterized by HS-SPME/GC-MS technique following the protocol reported by Ricci et al. 2018. In brief, 2 mL of cherry juice was placed in a glass vial and added with an aqueous toluene standard solution (0.25 µg/mL). Head space micro-extraction was performed for 30 min at 40°C after 15 min of equilibration time. A SPME fiber coated with 50/30 µm of Divinylbenzene–Carboxen–Polydimethylsiloxane (DVB/Carboxen/PDMS) was used (Supelco, Bellefonte, PA, USA). The desorption of volatiles was accomplished exposing the fiber into the GC injector for 2 min at 250°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. 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) with the following program gradient: initial temperature, 50 °C for 3 min, linear increase by 5 °C per minute to 200 °C, then maintained 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 cherry juices were identified both on the basis of their mass spectra compared with the library NIST 14 mass spectra, as by calculation of linear retention indices (LRI). The semi-quantification of all detected gas-chromatographic signals was performed on the basis of the use of an internal standard (toluene).

Characterization of polyphenolic profile of fermented and unfermented cherry juices

All fermented and unfermented samples were analysed by an Accela UHPLC 1250 equipped with a linear ion trap-mass spectrometer (MS) (LTQ XL, Thermo Fisher Scientific Inc, San Jose, CA, USA) fitted with a heated-electrospray ionization probe (H-ESI-II, Thermo Fisher Scientific Inc, San Jose, CA, USA). Separation was performed on an Acquity UPLC HSS T3 (2.1 x 100 mm) column coupled with a pre-column Acquity UPLC HSS T3 VanGuard (2.1x5 mm) (Waters, Milford, MA, USA). Volume injected was 5 μL and oven temperature was set to 40 °C. Phenolic profiling was performed following the protocol reported by Ricci et al., 2019. Briefly, the mobile phase was 0.1% (v/v) acetonitrile (phase A) and 0.1% (v/v) aqueous formic acid (phase B). Elution was performed at a flow rate of 0.3 mL/min. The gradient started with 95% B and 5% A for 0.5 min, then eluent B decreased at 49% and A increased at 51% in 9 min. After 0.5 min, the column was flushed setting the eluent percentages at 20% B and 80% A for 11.00 min. Finally, the initial conditions were restored (total run time = 17 min). Data processing was performed using Xcalibur 2.2 software from Thermo Fisher Scientific Inc, (San Jose, CA, USA).

Statistical analysis

To evaluate the normal distribution for each group of independent samples Shapiro-Wilk test was used. One-way ANOVA was applied to discriminate the significant differences among the samples, applying Bonferroni post hoc test and the results were considered different for values of $p < 0.05$. All the detected compounds (volatiles, phenolics, organic acids and sugars) were used as variables

for Principal Component Analyses (PCA) which was performed applying correlation matrix. All the mentioned analyses were performed on SPSS Statistics 21.0 software (SPSS Inc., Chicago, IL), whereas hierarchical clustering and heat map were carried out using Heatmapper (Babicki et al., 2016).

Results

Fermentation and acidification of cherry juice

Cherry juice microflora was checked before fermentation and no cell viability was observed by plate count. The fermentation was carried out inoculating four strains of *L. plantarum*, five of *L. rhamnosus*, four of *L. casei* and one of *L. paracasei*. After 48 hours of incubation all *L. plantarum* strains were able to grow in commercial cherry juice (> 1 Log cycle). In addition, also *L. rhamnosus* 2360 and *L. paracasei* 4186 strains were able to grow significantly (about 1 Log cycles). However, for most of the tested strains an increase lower than one half Log cycles or even a decrease was observed. Cells viability remained almost unchanged after 12 days of storage at 4°C and only in some cases a slight decrease was observed (Table 2). Lactic acid fermentation and storage did not affect the initial pH = 3.61 ± 0.04 (mean value ± standard deviation). All further analyses were performed considering only the strains that have shown a growth around 1 Log CFU/mL or higher, namely *L. plantarum* 1LE1, POM1, C1, 285, *L. rhamnosus* 2360 and *L. paracasei* 4186 (Table 2).

Table 2. Lactic acid bacteria growth. Difference in Log CFU/mL detected between cells number after 48 h of incubation and the initial value, $\Delta(T_{48\ h}\text{-}T_0)$, and between cells number after storage (14 days) and the initial value, $\Delta(T_{14\ d}\text{-}T_0)$. The origin of the isolated strain (plant/dairy products) is reported.

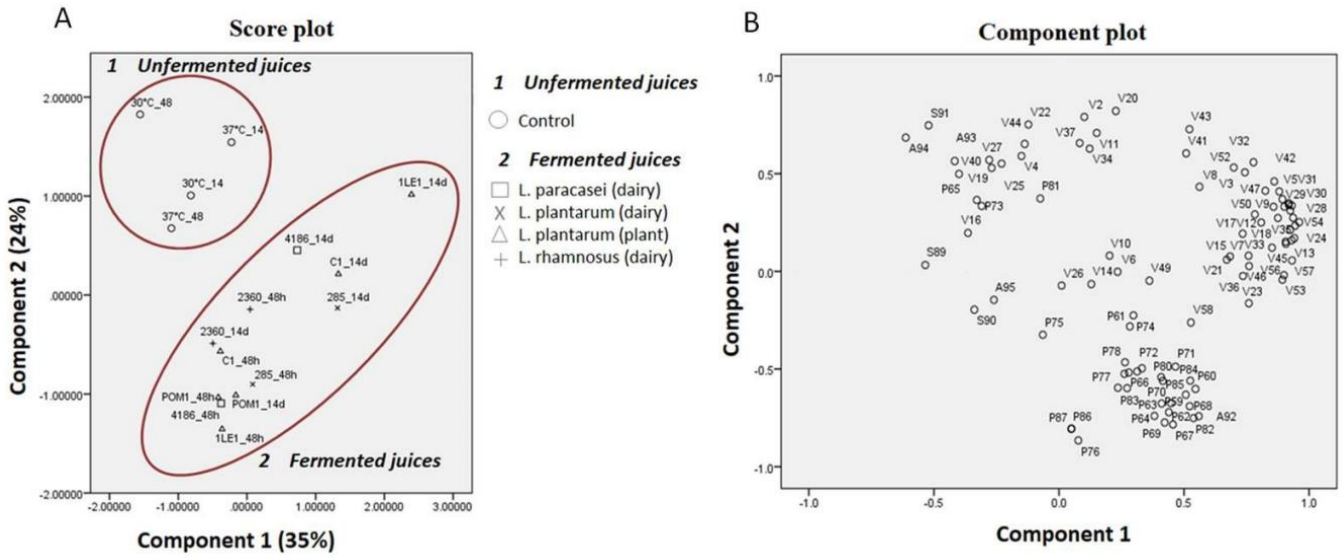
Species	Strain	Origin	$\Delta(T_{48\,h}-T_0)$	$\Delta(T_{14\,d}-T_0)$
L. plantarum	POM1	Plant	1.82 ± 0.05	1.69 ± 0.03
	C1	Plant	1.22 ± 0.10	1.11 ± 0.04
	1LE1	Plant	1.32 ± 0.07	1.39 ± 0.17
	285	Dairy	1.44 ± 0.07	1.46 ± 0.03
L. rhamnosus	2360	Dairy	1.02 ± 0.10	0.97 ± 0.03
L. paracasei	4186	Dairy	0.96 ± 0.13	0.80 ± 0.14

Principal component analyses: overview on volatiles, phenolic compounds, sugars and organic acids.

The main organic acids (lactic, malic, tartaric and citric) and sugars (fructose, glucose and sucrose) (Table S1) were identified and semi-quantified in fermented and unfermented cherry juice. Among volatile components, alcohols, acids, ketones, esters, terpenes and norisoprenoids were detected and semi-quantified (57 compounds) (Table S2, Table S3A and S3B). Also several phenolic acids and flavonoids (Table S4, Table S5) were identified (34 compounds) and quantified (30 compounds). PCA was performed considering the semi-quantified organic acids, sugars and volatiles and the quantified polyphenols, to highlight differences among the analysed samples. Considering a total of 94 variables, the total variance explained by PCA reached 59%, with component 1 and component 2 describing the 35% and 24%, respectively. A defined clustering among fermented and unfermented (control) samples was observed (Figure 1A and 1B). Unfermented samples were characterized by those variables showing negative value on component 1 and positive value on component 2 such as sucrose, fructose and caffeic acid, at their highest concentration. On the other hand, fermented juices were all grouped together, even if some differences could be observed. Indeed, after storage, the juices inoculated with 4186, C1, 285 were found to be correlated with slightly positive variables for both components (volatile compounds as propyl acetate) and were clustered separately the main

group. The most different sample was the juice containing *L. plantarum* 1LE1, which was characterized by more positive variables, especially volatiles compounds (such as 4-hydroxybutanoic acid, 1-heptanol and trans-geraniol) (Figure 1A and 1B).

Figure 1. Principal component analyses. (A). Score plot obtained by the analyses of unfermented (control) and fermented (*L. plantarum, L. rhamnosus,L. paracasei* and *L. casei*) cherry juice. Each sample is identified by the strain number and the time of incubation. For example, 1LE1_48 h is used for strain *L. plantarum* 1LE1 after fermentation. (B). Loading plot are based on sugars, organic acids, volatile and phenolic compounds. Abbreviations are the same as reported in Table S1 for organic acids (A) and sugars (S), Table S2 for volatile compounds (V), Table S4 for polyphenols (P).

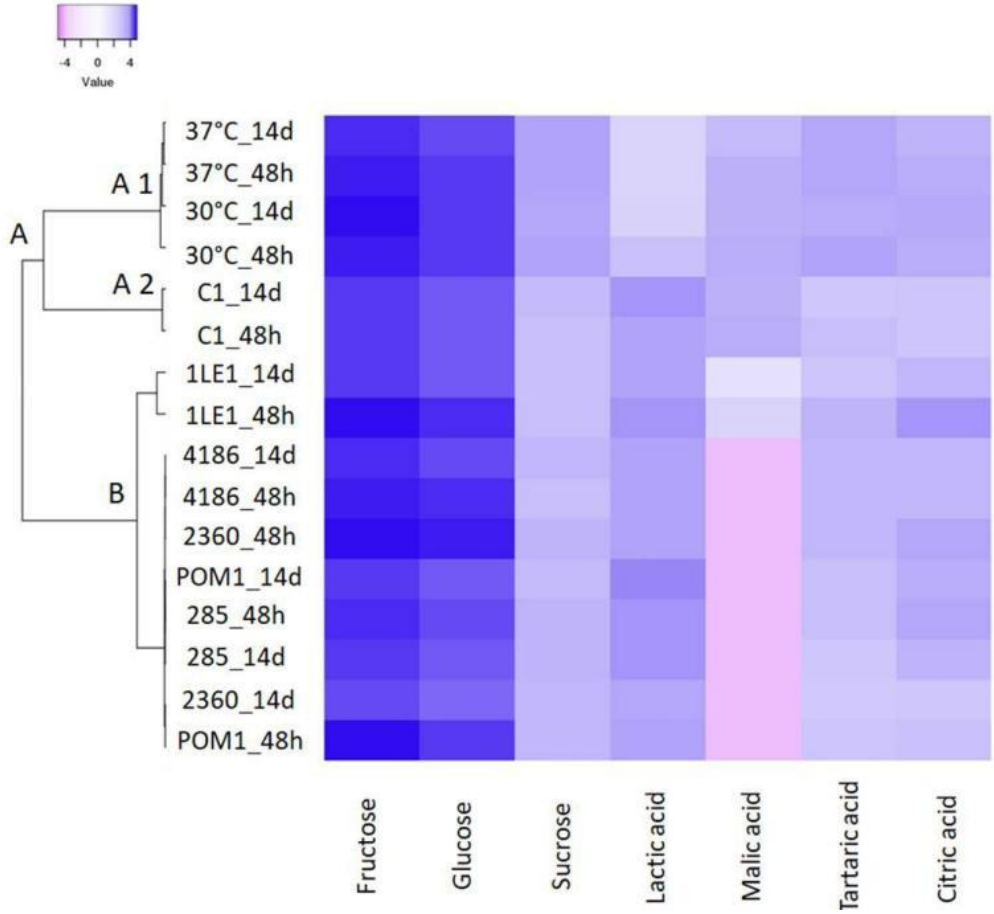


Sugars and organic acid metabolism

Sugar in cherry juice was mainly fructose, glucose and sucrose. Generally, their microbial metabolism was limited (Figure 2, Table S1): as an example, fructose, the most concentrated sugar, was only partially metabolized especially after storage by *L. plantarum* strains ($p < 0.05$). Microbial

fermentation did not change glucose concentration, but only during storage was metabolized, whereas sucrose significantly decreased in all fermented samples. Lactic acid was the main end product in all fermentations, reaching the highest amount when *L. plantarum* POM1 was used as starter (9.47 ± 0.32 mg/mL). Malolactic fermentation occurred, leading to total degradation of malic acid, in particular when strains of dairy origin were used. Only one strain, *L. plantarum* C1, did not converted malic acid (Figure 2 and Table S1).

Figure 2. Sugars and organic acid metabolism. Hierarchical clustering and heat map performed on sugars and organic acids detected in fermented juice with *L. plantarum* (C1, 1LE1, POM1 and 285), *L. rhamnosus* (2360) and *L. paracasei* (4186) and unfermented juice (30°C and 37°C) after fermentation (48 h) and storage (14 d). A scale ranging from a maximum of 4 (blue) and a minimum of −4 (pink) was used.



Tartaric acid metabolism was observed after fermentation and storage, especially by *L. plantarum* strains ($p < 0.05$, reduction of 92%), but was also observed in *L. rhamnosus* and *L. paracasei* (Figure 2, group A2 and B Table S1). No significant differences were observed for citric acid among fermented and unfermented samples (Figure 2 and Table S1).

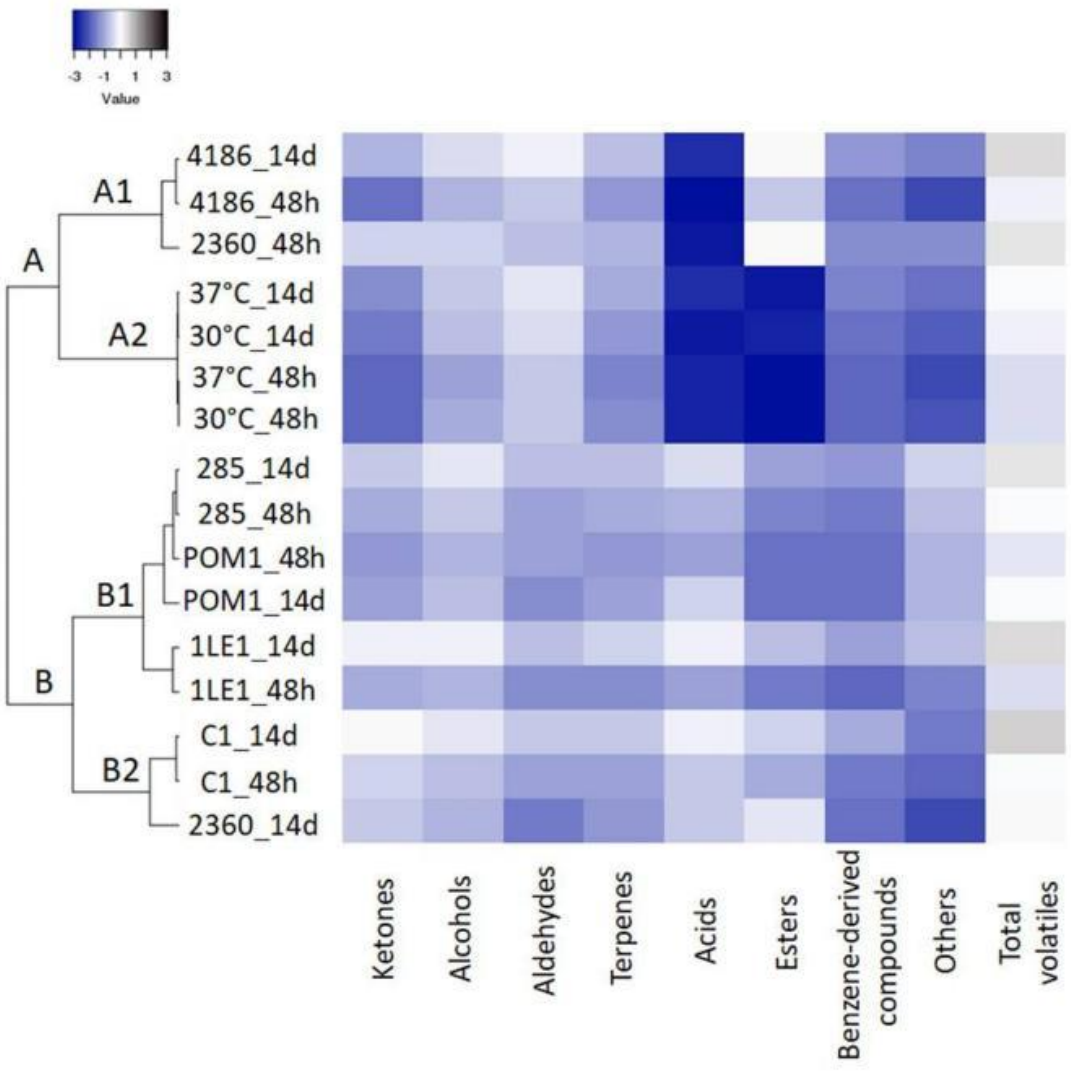
Characterization of volatile profile

During the characterization of unfermented and fermented cherry juices, carried out using HS-SPME/GC-MS analysis, 57 different compounds were detected in the headspace of every sample. For their identification, the mass spectrum was compared with those present in the instrument library NIST 14. Furthermore, linear retention indices (LRIs) were calculated for every signal using the retention time of a linear alkane solution analysed in the same condition, and the LRIs were compared with those reported in literature. The identification of all detected volatile compounds was reported in Table S2.

Overall, after fermentation, an increase in the concentration of total volatile compounds was observed. The main identified chemical classes were ketones, alcohols, aldehydes, terpenes, terpenic derivatives and norisoprenoids, acids and esters (Figure 3 and Table S3A and S3B). In fermented samples an increased content of ketones was found: among them, acetoin was the most concentrated, especially when strains of plant origin were employed. This compound showed a further increase after storage, reaching the highest concentration when *L. plantarum* C1 was used (Table 3). Benzene methanol was the most abundant alcohol (maximum amount detected with L. plantarum 1LE1 after storage, Table 3). Aldehydes were generally not affected by fermentation and storage. Acids were present at low concentrations both in controls and in fermented juice: an increase after fermentation and furthermore at the end of refrigerated storage was clearly observed especially when *L. plantarum* strains were employed (Figure 3, group B1 and B2). Acetic acid was the most concentrated compound, especially produced by *L. plantarum*, in particular by the C1

strain (Table 3) but this compound was also produced and converted to the corresponding ester by *L. rhamnosus* and *L. paracasei*: propyl acetate increased after fermentation with *L. rhamnosus* and *L. paracasei* (Table 3). Overall, after fermentation and refrigerated storage, the concentration of terpenes, terpenic derivatives and norisoprenoids increased in fermented samples, with β-linalool as the most abundant one.

Figure 3. Volatile profile. Hierarchical clustering and heat map performed on sugars and organic acids detected in fermented cherry juice with *L. plantarum* (C1, 1LE1, POM1 and 285), *L. rhamnosus* (2360) and *L. paracasei* (4186) and in unfermented juice (30°C and 37°C) after 48 h and 14 d (storage). A scale ranging from a maximum of 3 (black) and a minimum of −3 (blue) was used.



At the end of storage, when *L. plantarum* (1LE1, 285, C1) and *L. paracasei* 4186 were used as starters, the content of *β*-linalool significantly increased in comparison to controls. Other volatile compounds derived from the bacterial metabolism were found in fermented cherry juices and, among them, 4-ethylphenol reached its higher concentration when the fermentation was carried out by *L. plantarum* 285, and after storage (Table 3, Figure 4B).

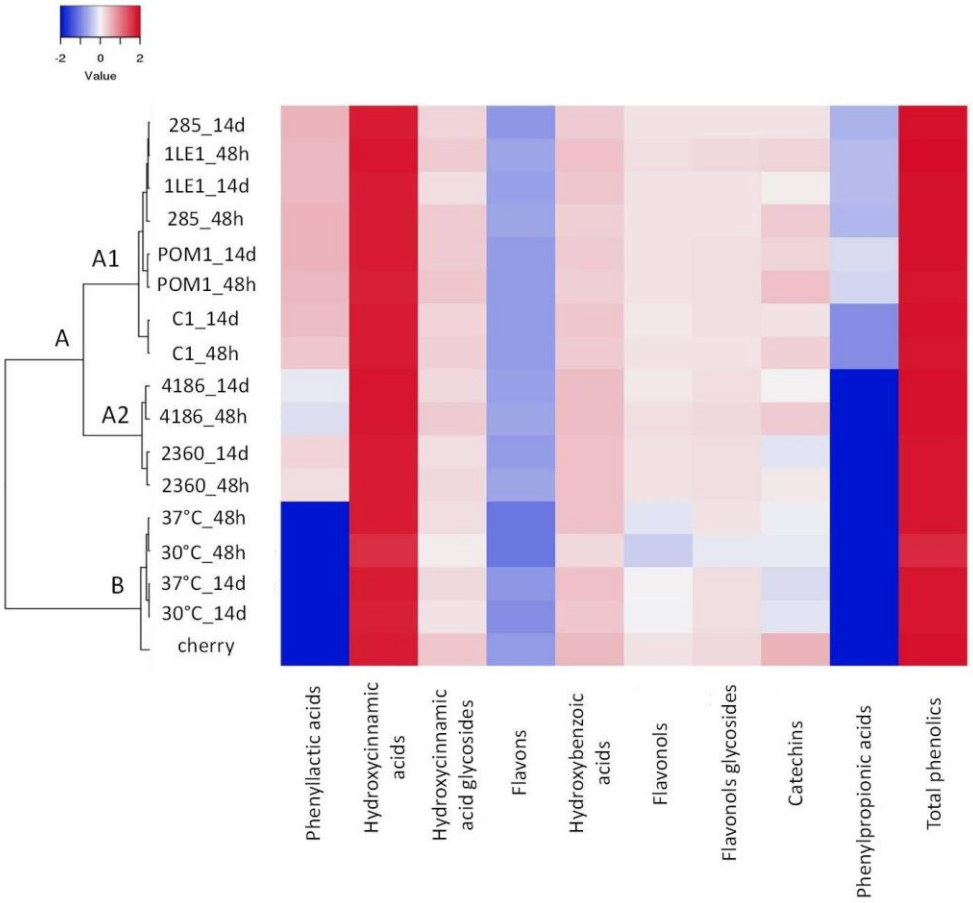
Characterization of polyphenolic profile

Using LC-MS^n, 34 polyphenolic compounds were identified, 16 by comparison with authentic standards while for the others, for which reference compounds were not available, tentative identification was based on the interpretation of their fragmentation patterns obtained from MS^2 and MS^3 spectra, and by comparison with data reported in literature. Retention times and mass spectral data, along with peak assignments for the identified compounds, are reported in Table S4. Quantified compounds were reported in Table S5. The total polyphenolic concentration didn't change upon treatment, but differences inside individual polyphenolic subclasses emerged, suggesting that specific compounds were metabolized by lactic acid bacteria.

Among hydroxycinnamic acids, caffeic acid and p-coumaric acid were mainly affected by fermentation. The conversion of caffeic acid was observed in L. plantarum strains, with a complete degradation of the compound by L. plantarum 285. The metabolism of caffeic acid led to the accumulation of dihydrocaffeic acid, the only phenylpropionic acid detected (Figure 4A, group A2, Figure 4B, Table3). p-Coumaric acid was totally metabolized by L. plantarum 285 and POM1, whereas for the C1 strain a partial conversion was observed after fermentation, and the compound was totally metabolised upon storage (Figure 4B). Protocatechuic acid was also metabolised by LAB, mainly by L. plantarum (Table4). In addition, all the strains were able to produce p-hydroxyphenyllactic acid and phenyllactic acid: the highest levels of these compounds were

measured after L. plantarum 285 fermentation (1.47 ± 0.16 µg/mL and 2.15 ± 0.15 µg/mL respectively).

Figure 4. Phenolic compounds. Hierarchical clustering and heat map performed on phenolic compounds detected in fermented juice with *L. plantarum* (C1, 1LE1, POM1 and 285), *L. rhamnosus* (2360) and *L. paracasei* (4186) and unfermented juice (30°C and 37°C) after 48 h and 14 d (storage). A scale ranging from a maximum of 2 (red) and a minimum of −2 (blue) was used.



Discussion

L. plantarum is the most employed species for lactic acid fermentation of fruit juices, as these strains isolated from plant environments are better adapted to these matrices (Rodríguez et al., 2009). Indeed, chemical characteristics of fruits, such as pH and the concentration of sugars and

organic acids, make them a hostile environment for microorganisms (Perricone et al., 2015; Filannino et al., 2014; Nualkaekul et al., 2011). However a great biodiversity among LAB, species and strain dependent, was observed that allow them to adopt specific alternative metabolic pathways in adverse conditions, by using non-conventional carbon sources for the exploitation of alternative substrates or in a global stress response. This adaptation may result in formation of volatile compounds (Ricci et al., 2018; Dongmo et al., 2017; Filannino et al., 2014), metabolism of phenolic compounds (Filannino et al., 2018; Fritsch et al., 2016), and production of new molecules. Starting from these premises, a large number of dairy strains were used for cherry juice fermentation and a global view on the metabolism of both dairy and plant-derived microorganisms was proposed.

Cherry juice is recognised to be a stressful substrate for microorganisms, due to its low pH, its high sugar and phenolic content and the presence of malic acid (Filannino et al., 2014). This unfavourable environment affects the growth and cell viability of most tested strains, with a particular negative effect on *L. casei* and *L. rhamnosus*, which were not able to grow satisfactorily in this medium. On the contrary, all *L. plantarum* strains, both of dairy and plant origin, showed a good adaptability. Moreover, also *L. rhamnosus* 2360 and *L. paracasei* 4186 were able to ferment cherry juice components and to survive during refrigerated storage, showing the possibility to convey viable cells also of non-plant origin in this fruit juice. PCA analysis showed a clear separation between unfermented and fermented juices, on account of the significant modulation of the composition of the juice matrix by lactic acid fermentation.

The adaptation to the acid environment of cherry juice is based on the ability of LAB to metabolize malic acid (Perricone et al., 2015), resulting in its almost complete degradation. Actually, all tested dairy and plant strains, independently from the species and with the exception of *L. plantarum* C1, completely converted malic acid into lactic acid. Indeed, most LAB can convert malic acid into

lactic acid thanks to the malolactic enzyme, decarboxylating malate to lactate by a NAD^+ and Mn^{2+}-dependent malolactic enzyme (Filannino et al., 2014; Landete et al., 2013; Jyoti et al., 2004). At low pH, the choice of malic acid as preferred energy source over glucose had been already reported in literature and linked to the increase of intracellular pH and the increase of reducing power (Mozzi et al., 2015), with associated modification of cellular permeability (Filannino et al., 2014). In the present work, LAB did not consume glucose and fructose during fermentation, whereas sucrose showed a marked decrease. The metabolic activity of the tested strains, especially in *L. rhamnosus* and *L. paracasei*, is mainly based on malolactic fermentation, aimed at the maintenance of vitality, than to sugars fermentation, which is mainly correlated to growth (Filannino et al., 2014). The exploitation of an organic carbon source by a microorganism strongly depends on the type of substrate. Two different works on cherry juice fermentation reported the absence of sugars metabolism in one case (Filannino et al., 2014) and a slight consumption after fermentation in the other (Di Cagno et al., 2011), the latter being in very good agreement with our results. Observed differences may be related to cultivar, to ripening time and applied processing condition which could affect the concentration of nutrients and/or acids (Sinha et al., 2006; Chockchaisawasdee et al., 2016). In the present work, also tartaric acid was consumed by LAB, especially by *L. plantarum*, in agreement with literature where *Lactobacillus spp*. were reported to be able to convert it into oxaloacetic acid (and then into lactic acid, acetic acid, and CO_2), thanks to the tartrate dehydratase enzyme (Bevilacqua et al., 2017; Todorov et al., 2010). Nevertheless the degradation of tartaric acid is not widespread in LAB and it was studied especially in wine. Moreover not all LAB were able to metabolize tartaric acid in the same way: for instance, differently from *L. plantarum*, in *Lactobacillus brevis* succinic acid can be produced instead of lactic acid (Toit et al., 2000). The acetic acid found in several fermented fruit juices (Ricci et al., 2018; Filannino et al., 2014; Filannino et al., 2013 is characterized by sharp, pungent and vinegar notes. However, thanks to specific bacterial metabolic traits, acetic acid can be converted to the corresponding esters (Di

Cagno et al., 2017; Di Cagno et al., 2009) such as propyl acetate: this was especially observed after fermentation in *L. rhamnosus* 2360 and at the end of storage using *L. paracasei* 4186. The increase of *β*-linalool, observed in different fermented samples, could be ascribed to glycosylases produced by LAB, involved in the release of aglycones from glycosylated terpenes (Ricci et al., 2018). Acetoin was also affected by fermentation and its bio-synthesis can be derived from citrate metabolism (Ricci et al., 2018; Jyoti et al., 2004).

Phenolic compounds have been reported to exert health benefits in humans (Sabokbar et al., 2016), to exhibit antimicrobial activity and to impact on flavour, taste and colour (Rodríguez et al., 2009) of plant products (Naczk et al., 2003). Their beneficial activities have been partially correlated to microbial metabolism that can occur during fermentation processes (Valero-Cases et al., 2017). Moreover, the metabolism of phenolics may contribute to bacterial stress response when microorganisms are in hostile conditions (Filannino et al., 2015; Sanchez-Maldonado et al., 2011).

In the present study, hydroxycinnamic acids were transformed by LAB, ideally through phenolic acid decarboxylases or phenolic acid reductases (Filannino et al., 2015). Caffeic acid was effectively converted into dihydrocaffeic acid, as already reported in literature also for *L. plantarum* POM1 (Ricci et al., 2019; Filannino et al., 2018; Filannino et al., 2015), with a potential impact on health, due to its more effective capacity to inhibit platelet activation than its phenolic precursor (Baeza et al., 2017) and to its antioxidant effect on endothelial cells (Huang et al., 2004). Besides putative effects on the health promoting activity of fermentation metabolites, the microbial transformation exerted by LAB may be relevant for other purposes. For example, *p*-coumaric acid was decarboxylated into *p*-vinylphenol and subsequently reduced, possibly by a phenolic acid reductase, to the phenolic volatile 4-ethylphenol, which contributes to aroma in fermented food (Filannino et al., 2015). The reduction of *p*-vinylphenol was favoured under anaerobic conditions or in absence of electron acceptors, i.e. when fructose is found at high concentration, to increase

NAD^+ quantity (Filannino et al., 2015; Silva et al., 2011). Protocatechuic acid was also metabolized (Ricci et al., 2019; Filannino et al., 2018), in particular by *L. plantarum* 285 and POM1, reaching complete depletion, but catechol was not detected. Finally, worthy of note is that phenyllactic acids were produced ex-novo by all the tested strains, probably deriving from amino acid metabolism. Phenylalanine can be converted into phenylpyruvic acid by a transamination reaction, and finally metabolized into phenyllactic acid by hydroxyacid dehydrogenase, while p-hydroxyphenyllactic acid may originate from tyrosine metabolism (Lavermicocca et al., 2003). Their antimicrobial activity against pathogenic strains and moulds has been well documented (Valerio et al., 2004; Lavermicocca et al., 2003) even if the concentrations found in the present study were lower compared to those explain antimicrobial activity.

Conclusion

In the present study, an overview on the metabolism of LAB strains, of plant but especially dairy origin, during cherry juice fermentation and further storage, was reported. *L. plantarum* 285, of dairy origin, was the most interesting strain for its ability to modulate the juice phenolic profile, while *L. rhamnosus* 2360 and *L. paracasei* 4186 were useful to modify and increase the presence of aromatic compounds. Based on these results, a selection of strains exploitable as starters in cherry juice fermentation can be performed based on sound analytical data. *L. rhamnosus* and *L. paracasei* conferred fruit notes to the juice, on the other hand, *L. plantarum* increased the levels of dihydrocaffeic acid, with putative biological activity, and of phenyllactic acids.

Table 3. Volatile compounds. Volatile compound concentrations (ng/mL) in fermented and unfermented cherry juice.

48 Hours								
Compound	**37 °C**	**2360**	**4186**	**30 °C**	**1LE1**	**285**	**C1**	**POM1**
acetoin	0.001 ± 0.000	260.679 ± 34.426*	5.909 ± 0.480	0.002 ± 0.000	71.373 ± 4.486*	76.300 ± 18.884*	287.902 ± 14.971*	44.022 ± 1.515*
benzene methanol	51.130 ± 13.431	164.411 ± 4.744*	70.560 ± 13.868	64.548 ± 0.700	70.513 ± 1.502	158.829 ± 3.660*	95.692 ± 15.202	90.078 ± 11.908*
β-linalool	13.742 ± 3.515	39.630 ± 2.624*	21.464 ± 2.627*	15.670 ± 0.326	15.305 ± 0.181	28.975 ± 0.424*	19.964 ± 2.326*	19.593 ± 1.604*
acetic acid	0.087 ± 0.030	0.017 ± 0.017*	0.014 ± 0.002*	0.013 ± 0.011	54.831 ± 18.642*	125.405 ± 10.324*	184.836 ± 21.933*	69.201 ± 9.371*
propyl acetate	0.009 ± 0.005	1186.731 ± 460.827*	201.607 ± 26.688	0.008 ± 0.005	24.680 ± 0.422*	27.876 ± 5.804*	83.370 ± 6.871*	18.539 ± 0.273*
4-ethylphenol	0.341 ± 0.016	29.025 ± 12.443*	1.828 ± 0.232	0.503 ± 0.144	23.832 ± 4.294*	150.460 ± 3.007*	8.997 ± 3.734*	106.967 ± 6.068*

14 Days								
Compound	**37 °C**	**2360**	**4186**	**30 °C**	**1LE1**	**285**	**C1**	**POM1**
acetoin	0.004 ± 0.005	180.078 ± 22.938*	65.013 ± 1.293*	0.004 ± 0.000	580.569 ± 61.646*	161.905 ± 50.669	1058.883 ± 222.855*	63.563 ± 22.691
benzene methanol	146.075 ± 6.008	79.259 ± 21.526*	250.467 ± 32.932*	99.225 ± 1.022	462.867 ± 76.120*	311.721 ± 47.740*	327.123 ± 54.469*	116.204 ± 42.234
β-linalool	41.464 ± 3.599	19.467 ± 2.875*	70.918 ± 15.120*	27.205 ± 2.210	92.675 ± 20.477*	57.517 ± 9.385*	68.307 ± 4.195*	22.431 ± 8.860
acetic acid	0.024 ± 0.009	228.633 ± 71.471*	0.354 ± 0.192	0.013 ± 0.009	535.465 ± 92.920*	305.391 ± 5.248*	737.615 ± 193.995*	257.568 ± 75.381*
propyl acetate	0.026 ± 0.004	509.194 ± 12.345*	1230.067 ± 222.171*	0.015 ± 0.014	160.882 ± 2.895*	61.035 ± 14.937*	281.546 ± 28.411*	16.706 ± 5.548
4-ethylphenol	0.848 ± 0.141	3.210 ± 0.751	21.854 ± 5.943*	0.608 ± 0.144	129.556 ± 2.648*	281.248 ± 21.113*	12.287 ± 0.017*	136.453 ± 43.612*

* significant differences between the concentrations of each compound observed in fermented cherry juices and in the respective control, 37°C for *L. rhamnosus* 2360 and *L. paracasei* 4186, 30°C for *L. plantarum* 1LE1, 285, C1 and POM1.

Table 4. Phenolic compounds. Phenolic compounds (µg/mL) metabolized by lactic acid bacteria during cherry juice fermentation and further storage.

48 hours								
Compounds	**37°C**	**2360**	**4186**	**30°C**	**1LE1**	**285**	**C1**	**POM1**
p-Hydroxyphenyllactic acid	ND	0.986 ± 0.121*	0.209 ± 0.047*	ND	1.229 ± 0.187*	1.462 ± 0.089*	1.241 ± 0.148*	1.134 ± 0.125*
Phenyllactic acid	ND	0.483 ± 0.062*	0.449 ± 0.024*	ND	2.098 ± 0.187*	1.905 ± 0.150*	1.219 ± 0.076*	1.930 ± 0.026*
Caffeic acid	0.633 ± 0.025	0.743 ± 0.038*	0.722 ± 0.030*	0.586 ± 0.033	0.406 ± 0.270	ND	0.305 ± 0.040	0.062 ± 0.014*
p-Coumaric acid	0.609 ± 0.274	0.594 ± 0.061	0.486 ± 0.105	0.288 ± 0.124	0.484 ± 0.134	ND	0.354 ± 0.207	ND
Protocatechuic acid	0.457 ± 0.197	0.508 ± 0.023	0.382 ± 0.103	0.253 ± 0.114	0.380 ± 0.131	ND	0.308 ± 0.273	ND
Dihydrocaffeic acid	ND	ND	ND	ND	0.307 ± 0.222	0.284 ± 0.043	0.131 ± 0.062	0.568 ± 0.070*
14 Days								
Compounds	**37°C**	**2360**	**4186**	**30°C**	**1LE1**	**285**	**C1**	**POM1**
p-Hydroxyphenyllactic acid	ND	1.272 ± 0.159*	0.259 ± 0.030*	ND	1.225 ± 0.025*	1.473 ± 0.158*	1.271 ± 0.115*	1.295 ± 0.131*
Phenyllactic acid	ND	0.559 ± 0.034*	0.513 ± 0.006*	ND	1.986 ± 0.087*	2.150 ± 0.153*	1.494 ± 0.141*	2.208 ± 0.095*
Caffeic acid	0.685 ± 0.062	0.789 ± 0.050	0.668 ± 0.038	0.702 ± 0.037	0.345 ± 0.241*	ND	0.259 ± 0.024*	0.092 ± 0.020*
p-Coumaric acid	0.670 ± 0.294	0.472 ± 0.050	0.485 ± 0.126	0.412 ± 0.103	0.337 ± 0.019	ND	ND	ND
Protocatechuic acid	0.560 ± 0.290	0.484 ± 0.170	0.497 ± 0.217	0.357 ± 0.148	0.285 ± 0.052	ND	0.459 ± 0.247	ND
Dihydrocaffeic acid	ND	ND	ND	ND	0.317 ± 0.314	0.278 ± 0.022	0.124 ± 0.040	0.609 ± 0.041*

* significant differences between the concentrations of each compound observed in fermented cherry juices and in the respective control, 37°C for *L. rhamnosus* 2360 and *L. paracasei* 4186, 30°C for *L. plantarum* 1LE1, 285, C1 and POM1.

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Chapter 3

Appendix

Table S1. Carbohydrates and organic acids of fermented and unfermented cherry juice. Concentration (µg/mL) of carbohydrates (fructose, glucose and sucrose) and organic acids (lactic acid, malic acid, tartaric acid, citric acid) detected in fermented cherry juices (1LE1, 285, C1, POM1, 4186 and 2360) and in unfermented cherry juices used as controls (30°C and 37°C). All the samples were treated in the same conditions and the analyses were carried out after 48 hours of incubation and after storage (14 days). For each compound a specific variable, used in PCA analyses, was assigned.

48 Hours

		30°C	1LE1	285	C1	POM1	37°C	4186	2360
S89	Fructose	45.74 ± 4.34	54.32 ± 26.55	38.87 ± 0.67	28.37 ± 5.19	54.20 ± 5.06	49.56 ± 0.58	44.34 ± 7.09	54.84 ± 1.21
S90	Glucose	27.02 ± 2.70	39.50 ± 2.54	23.32 ± 1.11	18.87 ± 3.069	31.22 ± 3.36	27.82 ± 4.53	33.47 ± 2.91	40.57 ± 2.89
S91	Sucrose	5.84 ± 4.22	0.99 ± 0.14*	2.06 ± 0.90*	1.13 ± 0.18*	1.51 ± 0.16*	6.18 ± 0.57	1.17 ± 0.20*	1.98 ± 0.13*
A92	Lactic acid	0.81 ± 0.09	7.75 ± 2.54*	8.44 ± 0.16*	5.75 ± 1.98*	6.58 ± 2.45*	0.15 ± 0.04	5.65 ± 0.53*	5.99 ± 0.59*
A93	Malic acid	3.23 ± 0.91	0.13 ± 0.14	ND	2.95 ± 0.69	ND	2.59 ± 0.14	ND	ND
A94	Tartaric acid	6.67 ± 0.66	2.19 ± 0.93	1.09 ± 0.86*	0.95 ± 0.17*	0.61 ± 0.04*	4.51 ± 0.67	1.54 ± 0.64	1.61 ± 0.26*
A95	Citric acid	3.37 ± 1.08	8.28 ± 0.93	5.42 ± 0.22	0.63 ± 0.36	0.81 ± 0.44	3.23 ± 1.62	1.48 ± 0.06	4.99 ± 2.53

14 Days

		30°C	1LE1	285	C1	POM1	37°C	4186	2360
S89	Fructose	62.47 ± 10.34	27.42 ± 3.02*	29.87 ± 5.70*	28.92 ± 3.93*	30.68 ± 2.74*	38.84 ± 8.05	34.43 ± 2.03	22.71 ± 2.33*
S90	Glucose	30.35 ± 7.39	20.48 ± 1.40*	19.97 ± 5.05*	20.40 ± 0.53*	19.47 ± 2.61*	22.10 ± 0.21	22.31 ± 2.68	15.50 ± 2.44*
S91	Sucrose	4.59 ± 1.44	1.15 ± 0.05*	2.19 ± 0.38*	1.43 ± 0.08*	1.42 ± 0.00*	6.18 ± 0.57	1.51 ± 0.73*	1.72 ± 0.47*
A92	Lactic acid	0.19 ± 0.09	6.73 ± 0.17*	7.92 ± 1.78*	7.90 ± 2.04*	9.47 ± 0.31*	0.15 ± 0.36	6.04 ± 0.68*	5.54 ± 0.42*
A93	Malic acid	2.36 ± 0.14	0.04 ± 0.03	ND	2.38 ± 0.66	ND	1.43 ± 0.12	ND	ND
A94	Tartaric acid	3.18 ± 0.04	0.73 ± 0.03*	0.56 ± 0.01*	0.61 ± 0.04*	0.94 ± 0.05*	4.52 ± 0.67	1.54 ± 0.64*	0.40 ± 0.07*
A95	Citric acid	4.28 ± 0.09	1.52 ± 0.07	2.12 ± 0.34	0.76 ± 0.12	3.01 ± 0.04	2.24 ± 0.82	1.78 ± 0.46	0.55 ± 0.10

* significant differences between the concentrations of each compound observed in fermented cherry juices and in the respective control, 37°C for *L. rhamnosus* 2360 and *L. paracasei* 4186, 30°C for *L. plantarum* 1LE1, 285, C1 and POM1.

Table S2. Identification of volatile compounds found in sweet cherry juices fermented with L. plantarum (POM1, C1, 1LE1, 285), L. rhamnosus (2360), L. paracasei (4186) and in controls (juices treated at 30°C and 37°C). MS: mass spectrometer; LRI: linear retention index. A specific variable, used in PCA analyses, was assigned for each compound.

Peak number	Identification	Aromatic note	LRI	Identification method	References
V1	2-Ethoxy-2-methyl-propane		717	MS	
V2	Acetone	Ethereal, apple, pear	810	MS	
V3	Isovaleraldehyde	Ethereal, aldehydic	888	MS + LRI	Qian & Reineccius, 2003
V4	Ethanol	Strong, alcoholic	902	MS + LRI	Goodner, 2008
V5	Propyl acetate	Estery, fruity, ethereal, tutti frutti, banana, honey	977	MS + LRI	Kevei & Kozma, 1976
V6	Ethyl benzene		1117	MS + LRI	Alasalvar et al., 2005
V7	Unidentified terpene		1133	MS	
V8	Limonene	Citrus	1175	MS + LRI	Cirlini et al., 2012
V9	2-Heptanone	Cheesy, fruity, coconut, waxy, green	1180	MS + LRI	Tatsuka et al., 1990
V10	4-Methyl-2-heptanone		1206	MS + LRI	Canuti et al., 2009
V11	2-Pentylfuran	Green, waxy, cooked, caramellic	1218	MS + LRI	Mahadevan & Farmer, 2006
V12	Isopentyl alcohol	Fermented, fruity, banana, ethereal, cognac	1222	MS + LRI	Dall'Asta et al., 2011
V13	Ethyl isoamyl ketone		1249	MS	
V14	Styrene	Sweet, balsamic, floral	1252	MS + LRI	Bianchi et al., 2007
V15	3-Pentenol		1256	MS	
V16	m-Cymene		1257	MS + LRI	Cavalli et al., 2003b
V17	Isobutenylcarbinol		1261	MS + LRI	Wirth et al., 2001

V18	Octanal	Aldehydic, green, citrus	1285	MS + LRI	Bianchi et al., 2007
V19	1-Decen-3-one		1298	MS	
V20	Acetoin	Sweet, buttery, creamy, dairy	1302	MS + LRI	Bianchi et al., 2007
V21	2-Methyl-2-octanal	Floral, rose, lily, muguet	1319	MS	
V22	Prenol	Green, fruity	1325	MS + LRI	Lee et al., 2005
V23	Hexanol	Green, fruity, apple skin, oily	1353	MS + LRI	Cirlini et al., 2012
V24	Dimethyl trisulfide	Sulfureous, alliaceous, savory, meaty, fresh, vegetable	1376	MS + LRI	Pozo-Bayon et al., 2007
V25	2-Nonanone	Green, fruity, cheesy, buttery, dairy	1385	MS + LRI	Fernandez-Segovia et al., 2006
V26	Nonanal	Aldehydic, citrus, cucumber, melon, raw potato, oily, coconut, nutty	1390	MS + LRI	Mahmood et al., 2004
V27	(E)-2-Hexen-1-ol	Fruity, green, leafy	1406	MS + LRI	Bianchi et al. 2007
V28	m-Di-tert-butylbenzene		1421	MS	
V29	α-Ionene		1440	MS	
V30	trans-Linalool dioxide		1441	MS	
V31	Amyl vinyl carbinol	Mushroom, earthy, green, oily, vegetable, umami, savory, brothy	1449	MS + LRI	Pinto et al., 2006
V32	1-Heptanol	Fermented, oily, nutty, fatty, green, aldehydic	1454	MS + LRI	Cirlini et al., 2012
V33	Furfural	Sweet, woody bready, nutty, caramellic, astringent	1469	MS + LRI	Pozo-Bayon et al., 2007
V34	2-Ethyl-1-hexanol	Sweet, fatty, fruity	1488	MS + LRI	Mahattanatawee et al., 2007
V35	Acetic acid	Pungent, sharp,	1488	MS + LRI	Bianchi et al., 2007

		vinegar			
V36	Benzaldehyde	Sweet, oily, almond cherry, nutty, woody	1526	MS + LRI	Bianchi et al., 2007
V37	β-Linalool	Floral	1547	MS + LRI	Cirlini et al., 2012
V38	Octanol	Waxy, green, orange	1555	MS + LRI	Dall'Asta et al., 2011
V39	Pivalic acid		1584	MS + LRI	Johanningsmeier & McFeeters , 2011
V40	4-Methyldihydro-2(3H)-furanone		1621	MS + LRI	Cho et al., 2006
V41	4-Hydroxybutanoic acid		1635	MS	
V42	Safranal	Woody, phenolic spicy, fruity, herbal	1646	MS + LRI	Kaypak & Avsar, 2008
V43	p-Tolualdehyde	Fruity, cherry, phenolic	1651	MS + LRI	Miles et al., 1965
V44	1-Nonanol	Fresh, fatty, floral	1657	MS + LRI	Dall'Asta et al., 2011
V45	2-Furanmethanol	Sweet, caramellic	1664	MS + LRI	Dall'Asta et al., 2011
V46	α,4-Dimethyl-3-cyclohexene-1-acetaldehyde	Spicy, herbal	1687	MS + LRI	Garneau et al., 1994
V47	p-Menthen-8-ol		1696	MS	
V48	Naphthalene	Pungent, dry	1740	MS + LRI	Alasalvar et al., 2005
V49	1-1-6-Trimethyl-1,2-dihydronaphthalene (TDN)		1742	MS + LRI	López et al., 2004
V50	Decanol	Aldehydic, waxy, green, fatty, tart, floral	1759	MS + LRI	Riu-Aumatell et al., 2005
V51	Methyl salicylate	Wintergreen, mint	1779	MS + LRI	Kaack, 2005
V52	trans-Geraniol	Sweet, floral, fruity, rose	1846	MS + LRI	Goodner, 2008
V53	Benzene methanol	Fruity, balsamic	1879	MS + LRI	Nagarajan et al., 2001
V54	Benzene ethanol	Floral, sweet, rose, bready	1914	MS + LRI	Botelho et al., 2007
V55	p-Mentha-1(7), 8(10)-dien-9-ol		1996	MS	

V56	Eugenol	Sweet, warm, spicy, clove, woody	2157	MS + LRI	Goodner, 2008
V57	4-Ethylphenol	Phenolic	2165	MS + LRI	Ferreira et al., 2001

Table S3A. Concentration (ng/mL) of volatile compounds identified in sweet cherry juices fermented with *L. plantarum* (POM1, C1, 1LE1, 285), *L. rhamnosus* (2360), *L. paracasei* (4186) and in controls (juices treated at 30°C and 37°C) after 48 hours.

Compound	37 °C	2360	4186	30 °C	1LE1	285	C1	POM1
Ketones								
acetone	5.340 ± 0.256	13.997 ± 1.524	4.883 ± 0.137	4.819 ± 0.034	3.500 ± 0.042	7.026 ± 0.908	3.254 ± 0.339	4.029 ± 0.084
2-heptanone	0.331 ± 0.467	2.598 ± 0.295	0.183 ± 0.223	0.461 ± 0.436	0.015 ± 0.012	1.172 ± 0.129	0.509 ± 0.070	0.174 ± 0.243
4-methyl-2-heptanone	1.216 ± 0.318	0.014 ± 0.008	0.007 ± 0.006	1.469 ± 0.127	0.408 ± 0.125	0.746 ± 0.042	0.492 ± 0.034	0.007 ± 0.002
ethyl isoamyl ketone	0.003 ± 0.003	1.215 ± 0.172	0.193 ± 0.081	0.004 ± 0.003	0.069 ± 0.007	0.221 ± 0.002	0.137 ± 0.023	0.017 ± 0.013
1-decen-3-one	0.263 ± 0.049	0.003 ± 0.001	0.077 ± 0.107	0.164 ± 0.127	0.000 ± 0.001	0.014 ± 0.015	0.041 ± 0.041	0.008 ± 0.004
acetoin	0.001 ± 0.000	260.679 ± 34.426	5.909 ± 0.480	0.002 ± 0.000	71.373 ± 4.486	76.300 ± 18.884	287.902 ± 14.971	44.022 ± 1.515
2-nonanone	0.004 ± 0.003	6.596 ± 0.274	0.013 ± 0.013	0.003 ± 0.001	0.015 ± 0.003	0.225 ± 0.081	0.018 ± 0.006	0.014 ± 0.001
Total	7.159 ± 1.096	285.103 ± 36.134	11.264 ± 0.361	6.922 ± 0.720	75.380 ± 4.316	85.704 ± 19.803	292.353 ± 15.438	48.271 ± 1.859
Alcohols								
ethanol	3.156 ± 0.391	8.538 ± 1.349	4.133 ± 0.325	3.723 ± 0.119	3.029 ± 0.083	5.866 ± 0.619	4.071 ± 0.326	4.484 ± 0.508
isopentyl alcohol	0.179 ± 0.070	1.681 ± 0.508	0.170 ± 0.075	0.003 ± 0.000	0.821 ± 0.213	1.153 ± 0.011	0.586 ± 0.145	0.664 ± 0.554
3-pentenol	0.092 ± 0.128	0.006 ± 0.003	0.004 ± 0.001	0.356 ± 0.502	0.005 ± 0.001	0.002 ± 0.002	0.002 ± 0.001	0.827 ± 1.167
isobutenylcarbinol	0.359 ± 0.185	3.148 ± 0.453	0.506 ± 0.712	0.004 ± 0.000	1.317 ± 0.738	2.579 ± 0.487	0.005 ± 0.003	2.094 ± 0.622
hexanol	0.704 ± 0.127	2.098 ± 0.424	1.120 ± 0.056	0.816 ± 0.084	1.875 ± 0.168	3.497 ± 0.174	2.235 ± 0.248	2.309 ± 0.208
(E)-2-hexen-1-ol	10.468 ± 1.595	25.830 ± 2.156	12.885 ± 0.371	12.479 ± 0.040	11.673 ± 0.282	22.592 ± 0.628	13.236 ± 2.822	17.685 ± 1.788
amyl vinyl carbinol	0.496 ± 0.189	0.633 ± 0.162	0.187 ± 0.169	0.495 ± 0.180	0.446 ± 0.340	0.705 ± 0.031	0.358 ± 0.069	0.239 ± 0.006
1-heptanol	0.068 ± 0.022	0.067 ± 0.040	0.119 ± 0.126	0.089 ± 0.021	0.528 ± 0.032	0.882 ± 0.122	0.658 ± 0.007	0.375 ± 0.254
2-ethyl-1-hexanol	1.629 ± 0.462	5.359 ± 1.204	2.872 ± 0.480	2.056 ± 0.133	1.873 ± 0.193	3.917 ± 0.433	7.281 ± 3.477	2.910 ± 0.777
octanol	3.364 ± 0.791	8.419 ± 0.975	5.200 ± 1.363	3.596 ± 0.463	4.465 ± 0.210	7.831 ± 0.570	5.716 ± 0.462	4.813 ± 1.201
1-nonanol	0.037 ± 0.036	0.691 ± 0.071	2.170 ± 0.880	0.039 ± 0.014	2.828 ± 0.215	4.945 ± 0.439	4.492 ± 0.249	1.237 ± 0.467
2-furanmethanol	0.454 ± 0.063	11.002 ± 0.123	1.011 ± 0.197	0.625 ± 0.091	1.841 ± 0.274	5.907 ± 0.357	7.694 ± 0.660	2.410 ± 0.503
decanol	1.503 ± 0.545	5.067 ± 0.220	2.938 ± 1.097	1.873 ± 0.066	3.231 ± 0.078	7.269 ± 0.351	5.855 ± 0.340	2.755 ± 0.726
benzene methanol	51.130 ± 13.431	164.411 ± 4.744	70.560 ± 13.868	64.548 ± 0.700	70.513 ± 1.502	158.829 ± 3.660	95.692 ± 15.202	90.078 ± 11.908
benzene ethanol	2.911 ± 0.604	8.214 ± 0.853	2.757 ± 0.699	4.529 ± 0.055	4.598 ± 0.919	11.132 ± 0.126	6.623 ± 1.644	5.263 ± 0.190
Total	76.551 ± 18.011	245.163 ± 10.997	106.632 ± 19.655	95.230 ± 0.480	109.044 ± 4.535	237.105 ± 2.060	154.504 ± 25.003	138.144 ± 16.181
Aldehydes								
isovaleraldehyde	0.934 ± 0.149	0.045 ± 0.017	0.262 ± 0.047	0.647 ± 0.107	0.020 ± 0.012	0.024 ± 0.011	0.018 ± 0.007	0.029 ± 0.002
octanal	0.362 ± 0.080	0.028 ± 0.015	0.104 ± 0.011	0.117 ± 0.157	0.042 ± 0.055	0.009 ± 0.011	0.003 ± 0.001	0.008 ± 0.008
2-methyl-2-octanal	0.477 ± 0.069	0.021 ± 0.021	0.002 ± 0.001	0.766 ± 0.010	0.047 ± 0.015	0.267 ± 0.083	0.079 ± 0.081	0.009 ± 0.010

nonanal	1.515 ± 0.515	0.018 ± 0.005	0.751 ± 0.261	1.644 ± 0.457	0.091 ± 0.121	0.011 ± 0.006	0.013 ± 0.004	0.143 ± 0.020
furfural	91.014 ± 17.720	90.124 ± 13.702	97.692 ± 17.260	101.720 ± 0.611	26.133 ± 0.773	36.823 ± 3.768	51.550 ± 4.172	43.482 ± 11.129
benzaldehyde	90.499 ± 18.250	75.997 ± 11.340	94.198 ± 12.843	100.453 ± 3.509	15.289 ± 1.129	21.859 ± 1.246	20.195 ± 1.784	33.364 ± 10.877
safranal	1.735 ± 0.190	1.398 ± 0.279	1.403 ± 0.063	1.807 ± 0.047	0.837 ± 0.071	1.292 ± 0.003	0.972 ± 0.187	0.789 ± 0.162
p-tolualdehyde	0.841 ± 0.162	0.017 ± 0.017	0.002 ± 0.003	0.661 ± 0.000	0.308 ± 0.031	0.005 ± 0.004	0.042 ± 0.012	0.003 ± 0.002
α,4-dimethyl-3-cyclohexene-1-acetaldehyde	1.786 ± 0.487	0.899 ± 0.009	1.406 ± 0.408	0.652 ± 0.043	0.713 ± 0.247	1.386 ± 0.110	1.422 ± 0.584	0.416 ± 0.041
Total	189.163 ± 37.324	168.546 ± 25.300	195.820 ± 30.771	208.467 ± 3.620	43.480 ± 0.197	61.676 ± 4.987	74.292 ± 5.499	78.243 ± 21.887
Terpenes, terpenic derivatives and norisoprenoids								
unidentified terpene	0.166 ± 0.118	0.943 ± 0.358	0.372 ± 0.063	0.243 ± 0.265	0.215 ± 0.044	0.217 ± 0.187	0.285 ± 0.022	0.160 ± 0.016
limonene	2.789 ± 0.030	7.857 ± 1.153	2.988 ± 1.150	2.331 ± 0.005	1.756 ± 0.734	4.032 ± 0.759	1.892 ± 0.231	3.054 ± 0.134
m-cymene	0.675 ± 0.560	1.664 ± 0.308	0.676 ± 0.269	0.847 ± 0.375	0.480 ± 0.032	0.754 ± 0.177	0.586 ± 0.120	1.091 ± 0.749
prenol	0.015 ± 0.015	0.965 ± 0.339	0.305 ± 0.215	0.042 ± 0.037	0.712 ± 0.255	2.364 ± 0.842	1.094 ± 0.293	1.816 ± 0.074
α-ionene	2.130 ± 0.942	5.433 ± 0.981	3.356 ± 1.741	2.664 ± 0.086	2.710 ± 0.198	4.201 ± 0.987	3.830 ± 0.962	3.790 ± 0.490
trans-linalool dioxide	0.790 ± 0.013	1.830 ± 0.275	0.666 ± 0.155	0.790 ± 0.376	0.725 ± 0.184	1.217 ± 0.073	0.827 ± 0.141	0.655 ± 0.922
β-linalool	13.742 ± 3.515	39.630 ± 2.624	21.464 ± 2.627	15.670 ± 0.326	15.305 ± 0.181	28.975 ± 0.424	19.964 ± 2.326	19.593 ± 1.604
p-menthen-8-ol	3.749 ± 1.066	10.101 ± 0.669	5.436 ± 1.601	4.413 ± 0.130	3.867 ± 0.100	7.823 ± 0.394	5.297 ± 0.586	4.959 ± 0.953
trans-geraniol	0.291 ± 0.065	4.174 ± 0.365	0.781 ± 0.156	0.323 ± 0.082	2.547 ± 0.033	8.922 ± 0.406	4.593 ± 0.416	4.586 ± 0.507
p-mentha-1(7), 8(10)-dien-9-ol	0.032 ± 0.030	1.615 ± 0.082	0.178 ± 0.012	0.096 ± 0.022	0.932 ± 0.005	2.048 ± 0.128	1.410 ± 0.107	1.114 ± 0.013
eugenol	2.425 ± 0.516	26.830 ± 0.111	3.910 ± 0.643	3.200 ± 0.124	11.051 ± 0.397	22.447 ± 0.111	14.671 ± 1.352	12.971 ± 1.513
Total	26.804 ± 6.754	101.042 ± 6.535	40.131 ± 8.180	30.617 ± 0.168	40.300 ± 1.304	83.000 ± 1.734	54.449 ± 3.930	53.791 ± 5.847
Acids								
acetic acid	0.087 ± 0.030	0.017 ± 0.017	0.014 ± 0.002	0.013 ± 0.011	54.831 ± 18.642	125.405 ± 10.324	184.836 ± 21.933	69.201 ± 9.371
pivalic acid	0.721 ± 0.102	0.055 ± 0.031	0.009 ± 0.010	0.678 ± 0.091	0.012 ± 0.014	0.012 ± 0.001	0.012 ± 0.005	0.011 ± 0.004
4-hydroxybutanoic acid	1.029 ± 0.192	1.304 ± 0.072	0.943 ± 0.195	0.982 ± 0.187	0.743 ± 0.037	1.422 ± 0.017	0.901 ± 0.154	0.370 ± 0.141
Total	1.837 ± 0.263	1.375 ± 0.086	0.966 ± 0.183	1.673 ± 0.107	55.585 ± 18.619	126.839 ± 10.340	185.748 ± 22.093	69.582 ± 9.508
Esters								
propyl acetate	0.009 ± 0.005	1186.731 ± 460.827	201.607 ± 26.688	0.008 ± 0.005	24.680 ± 0.422	27.876 ± 5.804	83.370 ± 6.871	18.539 ± 0.273
methyl salicylate	0.841 ± 0.175	1.928 ± 0.008	0.676 ± 0.143	0.958 ± 0.335	0.600 ± 0.155	0.641 ± 0.351	0.204 ± 0.199	0.164 ± 0.140
Total	0.850 ± 0.170	1188.660 ± 460.835	202.284 ± 26.544	0.966 ± 0.340	25.279 ± 0.266	28.517 ± 6.155	83.574 ± 6.673	18.703 ± 0.133
Derivatives of benzene								
ethyl benzene	0.143 ± 0.006	0.057 ± 0.032	0.313 ± 0.013	0.074 ± 0.099	0.112 ± 0.057	0.220 ± 0.138	0.197 ± 0.084	0.357 ± 0.087
styrene	0.125 ± 0.076	0.397 ± 0.026	0.497 ± 0.118	0.256 ± 0.094	0.066 ± 0.009	0.244 ± 0.159	0.237 ± 0.122	1.467 ± 1.278
m-di-tert-butylbenzene	9.004 ± 4.001	25.514 ± 5.636	12.572 ± 2.402	11.422 ± 0.409	8.313 ± 0.294	16.358 ± 3.493	12.119 ± 1.264	9.563 ± 1.992
naphthalene	0.115 ± 0.043	1.845 ± 0.336	0.659 ± 0.374	0.211 ± 0.039	1.791 ± 0.314	3.836 ± 0.787	8.589 ± 0.605	0.593 ± 0.331
1-1-6-trimethyl-1,2-dihydronaphthalene (TDN)	1.683 ± 0.588	7.825 ± 0.359	3.402 ± 1.785	1.910 ± 0.220	1.506 ± 0.102	3.981 ± 0.969	0.671 ± 0.090	3.046 ± 1.050

Total	11.070 ± 4.562	35.638 ± 6.326	17.442 ± 3.944	13.872 ± 0.861	11.788 ± 0.441	24.639 ± 3.972	21.813 ± 1.985	15.025 ± 1.519
Others								
2-ethoxy-2-methyl-propane	2.377 ± 0.073	0.248 ± 0.266	0.008 ± 0.003	2.582 ± 0.253	0.393 ± 0.028	0.568 ± 0.133	0.631 ± 0.061	0.024 ± 0.009
2-pentylfuran	1.580 ± 0.616	3.929 ± 1.013	1.307 ± 0.606	1.597 ± 0.509	1.230 ± 0.283	2.298 ± 0.187	1.676 ± 0.158	1.219 ± 0.043
dimethyl trisulfide	0.704 ± 0.140	1.270 ± 0.274	0.526 ± 0.210	0.697 ± 0.168	0.013 ± 0.009	0.003 ± 0.002	0.017 ± 0.000	0.012 ± 0.000
4-methyldihydro-2(3H)-furanone	0.701 ± 0.152	0.674 ± 0.137	1.551 ± 0.406	1.918 ± 0.154	0.917 ± 0.057	1.161 ± 0.011	0.463 ± 0.108	0.311 ± 0.200
4-ethyl-phenol	0.341 ± 0.016	29.025 ± 12.443	1.828 ± 0.232	0.503 ± 0.144	23.832 ± 4.294	150.460 ± 3.007	8.997 ± 3.734	106.967 ± 6.068
Total	5.703 ± 0.686	35.146 ± 11.029	5.219 ± 1.032	7.297 ± 0.892	26.384 ± 4.614	154.491 ± 2.675	11.784 ± 3.845	108.532 ± 5.902
Total	319.136 ± 68.866	2060.672 ± 535.185	579.758 ± 36.858	365.044 ± 3.750	387.242 ± 25.660	801.971 ± 34.964	878.517 ± 80.494	530.291 ± 43.821

Table S3B. Concentration (ng/mL) of volatile compounds identified in sweet cherry juices fermented with *L. plantarum* (POM1, C1, 1LE1, 285), *L. rhamnosus* (2360), *L. paracasei* (4186) and in controls (juices treated at 30°C and 37°C) after storage (14 days).

Compound	37 °C	2360	4186	30 °C	1LE1	285	C1	POM1
Ketones								
acetone	17.019 ± 0.334	7.165 ± 0.532	18.316 ± 3.144	11.018 ± 0.266	23.464 ± 0.394	18.394 ± 2.564	10.602 ± 0.392	4.140 ± 0.988
2-heptanone	0.412 ± 0.166	1.191 ± 0.484	0.744 ± 0.077	0.703 ± 0.240	0.491 ± 0.301	0.453 ± 0.276	1.662 ± 0.675	0.011 ± 0.003
4-methyl-2-heptanone	4.280 ± 1.064	0.008 ± 0.009	0.023 ± 0.005	1.116 ± 1.570	1.962 ± 0.107	0.990 ± 0.426	1.740 ± 0.004	0.010 ± 0.005
ethyl isoamyl ketone	0.005 ± 0.002	0.508 ± 0.007	1.256 ± 0.225	0.002 ± 0.002	0.001 ± 0.001	0.004 ± 0.001	0.168 ± 0.075	0.006 ± 0.001
1-decen-3-one	0.352 ± 0.175	0.003 ± 0.004	0.214 ± 0.037	0.330 ± 0.147	0.417 ± 0.179	0.057 ± 0.051	0.152 ± 0.144	0.004 ± 0.004
acetoin	0.004 ± 0.005	180.078 ± 22.938	65.013 ± 1.293	0.004 ± 0.000	580.569 ± 61.646	161.905 ± 50.669	1058.883 ± 222.855	63.563 ± 22.691
2-nonanone	0.003 ± 0.004	2.868 ± 1.433	1.264 ± 0.915	0.010 ± 0.010	0.137 ± 0.007	0.048 ± 0.000	0.079 ± 0.053	0.019 ± 0.005
Total	22.075 ± 1.396	191.821 ± 21.541	86.831 ± 2.956	13.184 ± 1.940	607.041 ± 61.244	181.852 ± 53.434	1073.285 ± 223.942	67.753 ± 23.683
Alcohols								
ethanol	10.279 ± 0.187	3.171 ± 0.818	13.702 ± 2.820	6.607 ± 0.829	17.334 ± 0.324	12.610 ± 1.060	12.145 ± 1.982	4.724 ± 1.259
isopentyl alcohol	1.236 ± 0.515	0.293 ± 0.400	1.380 ± 0.162	0.006 ± 0.004	3.879 ± 0.132	1.658 ± 0.743	2.324 ± 0.223	0.709 ± 0.078
3-pentenol	0.009 ± 0.004	0.004 ± 0.000	0.022 ± 0.018	1.104 ± 0.268	0.013 ± 0.010	0.029 ± 0.019	0.010 ± 0.001	0.005 ± 0.001
isobutenylcarbinol	2.038 ± 0.380	2.111 ± 1.091	6.378 ± 0.091	0.009 ± 0.008	11.220 ± 0.895	5.171 ± 1.246	3.794 ± 0.915	2.769 ± 0.097
hexanol	1.980 ± 0.143	1.048 ± 0.185	4.093 ± 0.652	1.488 ± 0.016	13.582 ± 1.441	8.096 ± 1.266	7.732 ± 0.582	2.438 ± 0.691
(E)-2-hexen-1-ol	30.927 ± 0.265	12.219 ± 1.310	43.517 ± 9.104	20.374 ± 0.288	73.581 ± 10.961	49.312 ± 4.692	44.018 ± 5.733	19.185 ± 6.566
amyl vinyl carbinol	1.074 ± 0.709	0.365 ± 0.020	1.198 ± 0.555	1.108 ± 0.618	2.037 ± 0.360	1.226 ± 0.190	1.224 ± 0.292	0.300 ± 0.016

1-heptanol	0.036 ± 0.008	0.074 ± 0.028	0.350 ± 0.023	0.070 ± 0.039	5.259 ± 0.861	1.880 ± 0.038	2.282 ± 0.612	1.145 ± 0.357
2-ethyl-1-hexanol	5.300 ± 0.280	3.843 ± 0.840	8.464 ± 0.821	3.854 ± 0.373	21.104 ± 7.591	11.307 ± 3.796	7.173 ± 0.514	3.125 ± 2.427
octanol	9.593 ± 0.892	3.233 ± 0.490	18.840 ± 4.930	6.970 ± 0.015	27.485 ± 5.391	14.897 ± 1.322	21.430 ± 2.702	5.780 ± 2.838
1-nonanol	0.087 ± 0.076	0.215 ± 0.129	12.439 ± 5.094	0.342 ± 0.254	22.386 ± 5.568	8.428 ± 0.357	16.863 ± 4.236	2.360 ± 1.378
2-furanmethanol	1.575 ± 0.281	6.113 ± 1.576	7.858 ± 0.346	0.613 ± 0.026	14.657 ± 4.815	10.287 ± 1.807	27.780 ± 2.299	4.149 ± 1.524
decanol	5.322 ± 0.595	2.025 ± 0.451	16.246 ± 3.482	3.158 ± 0.348	25.055 ± 3.666	13.155 ± 1.883	23.224 ± 4.829	5.609 ± 3.053
benzene methanol	146.075 ± 6.008	79.259 ± 21.526	250.467 ± 32.932	99.225 ± 1.022	462.867 ± 76.120	311.721 ± 47.740	327.123 ± 54.469	116.204 ± 42.234
benzene ethanol	7.855 ± 1.017	4.045 ± 0.824	13.254 ± 0.767	5.818 ± 0.339	31.874 ± 4.004	21.152 ± 3.347	23.079 ± 4.527	7.650 ± 2.463
Total	223.386 ± 5.742	118.019 ± 25.871	398.208 ± 60.553	150.746 ± 0.615	732.332 ± 120.329	470.929 ± 60.746	520.201 ± 81.637	176.153 ± 64.981
Aldehydes								
isovaleraldehyde	4.504 ± 0.127	0.008 ± 0.008	1.422 ± 0.492	0.995 ± 0.130	0.060 ± 0.033	0.046 ± 0.020	0.141 ± 0.112	0.020 ± 0.008
octanal	1.880 ± 0.964	0.011 ± 0.015	0.009 ± 0.009	2.020 ± 0.905	0.017 ± 0.019	0.017 ± 0.003	0.007 ± 0.000	0.011 ± 0.003
2-methyl-2-octanal	2.054 ± 0.898	0.033 ± 0.015	0.067 ± 0.059	0.889 ± 0.376	0.586 ± 0.438	0.171 ± 0.182	0.223 ± 0.240	0.019 ± 0.007
nonanal	7.013 ± 1.434	0.160 ± 0.212	0.590 ± 0.824	9.584 ± 0.786	0.070 ± 0.034	0.785 ± 0.988	0.677 ± 0.919	0.017 ± 0.008
furfural	265.690 ± 8.002	8.274 ± 2.030	290.648 ± 57.628	170.423 ± 3.198	78.144 ± 3.941	81.231 ± 36.107	139.988 ± 0.807	13.842 ± 6.288
benzaldehyde	267.490 ± 2.961	14.710 ± 3.707	279.968 ± 63.993	175.687 ± 5.154	81.874 ± 4.331	93.758 ± 34.784	80.357 ± 2.603	18.478 ± 7.338
safranal	4.789 ± 0.189	0.791 ± 0.197	3.700 ± 1.130	3.176 ± 0.025	4.299 ± 0.723	3.013 ± 0.605	2.833 ± 0.406	0.966 ± 0.440
p-tolualdehyde	3.402 ± 0.490	0.010 ± 0.006	0.093 ± 0.088	1.988 ± 0.668	0.588 ± 0.073	0.817 ± 0.582	0.018 ± 0.002	0.013 ± 0.004
α.4-dimethyl-3-cyclohexene-1-acetaldehyde	2.471 ± 0.377	0.661 ± 0.433	2.069 ± 0.054	1.496 ± 0.215	4.986 ± 1.873	2.650 ± 0.779	3.060 ± 0.577	1.386 ± 0.732
Total	559.293 ± 8.135	24.659 ± 5.260	578.568 ± 122.520	366.258 ± 6.051	170.624 ± 2.736	182.487 ± 72.034	227.304 ± 1.990	34.753 ± 14.790
Terpenes. terpenic derivatives and norisoprenoids								
unidentified terpene	1.083 ± 0.488	0.024 ± 0.004	0.670 ± 0.212	0.197 ± 0.046	1.026 ± 0.361	0.386 ± 0.024	0.521 ± 0.283	0.310 ± 0.059
limonene	5.535 ± 1.386	2.747 ± 0.054	9.462 ± 2.056	3.048 ± 0.906	10.710 ± 1.511	7.443 ± 1.183	8.309 ± 1.944	2.989 ± 0.383
m-cymene	1.128 ± 0.266	0.307 ± 0.417	2.064 ± 0.182	0.830 ± 0.021	2.410 ± 0.372	4.658 ± 3.293	2.198 ± 0.325	0.704 ± 0.316
prenol	0.044 ± 0.037	0.442 ± 0.091	0.699 ± 0.426	0.019 ± 0.003	3.679 ± 0.221	6.079 ± 0.513	2.406 ± 0.434	1.643 ± 0.235
α-ionene	4.719 ± 0.343	2.858 ± 0.133	9.821 ± 0.952	3.848 ± 0.951	14.112 ± 4.440	8.683 ± 1.158	11.341 ± 0.388	3.355 ± 1.964
trans-linalool dioxide	1.645 ± 0.056	0.845 ± 0.013	2.530 ± 0.650	0.793 ± 1.114	4.340 ± 0.907	1.897 ± 0.251	2.552 ± 0.367	0.924 ± 0.292
β-linalool	41.464 ± 3.599	19.467 ± 2.875	70.918 ± 15.120	27.205 ± 2.210	92.675 ± 20.477	57.517 ± 9.385	68.307 ± 4.195	22.431 ± 8.860
p-menthen-8-ol	11.039 ± 0.950	4.659 ± 1.011	18.832 ± 3.255	6.965 ± 0.034	25.450 ± 4.907	15.603 ± 1.759	19.160 ± 1.918	5.929 ± 2.607
trans-geraniol	1.472 ± 0.039	1.798 ± 0.348	3.871 ± 0.457	1.012 ± 0.054	17.614 ± 2.150	16.240 ± 0.618	16.176 ± 2.694	7.563 ± 3.181
p-mentha-1(7). 8(10)-dien-9-ol	0.143 ± 0.169	0.981 ± 0.071	1.057 ± 0.125	0.094 ± 0.070	5.324 ± 1.211	2.963 ± 0.206	4.736 ± 0.836	1.484 ± 0.798
eugenol	6.731 ± 0.296	11.209 ± 2.994	19.468 ± 0.240	4.680 ± 0.167	64.981 ± 10.397	36.587 ± 3.605	52.905 ± 11.791	17.755 ± 5.498
Total	75.003 ± 4.020	45.338 ± 7.737	139.391 ± 17.919	48.690 ± 3.125	242.321 ± 46.952	158.056 ± 13.972	188.611 ± 20.721	65.086 ± 24.193
Acids								
acetic acid	0.024 ± 0.009	228.633 ± 71.471	0.354 ± 0.192	0.013 ± 0.009	535.465 ± 92.920	305.391 ± 5.248	737.615 ± 193.995	257.568 ± 75.381
pivalic acid	0.009 ± 0.002	0.022 ± 0.019	0.042 ± 0.030	0.010 ± 0.002	0.058 ± 0.017	0.019 ± 0.006	0.232 ± 0.080	0.065 ± 0.066

4-hydroxybutanoic acid	1.925 ± 0.150	0.745 ± 0.368	2.162 ± 0.084	1.422 ± 0.098	3.665 ± 0.433	2.802 ± 0.191	2.784 ± 0.428	0.579 ± 0.084
Total	1.957 ± 0.143	229.400 ± 71.820	2.557 ± 0.138	1.445 ± 0.108	539.188 ± 93.370	308.211 ± 5.064	740.631 ± 194.502	258.211 ± 75.531
Esters								
propyl acetate	0.026 ± 0.004	509.194 ± 12.345	1230.067 ± 222.171	0.015 ± 0.014	160.882 ± 2.895	61.035 ± 14.937	281.546 ± 28.411	16.706 ± 5.548
methyl salicylate	1.221 ± 0.585	0.821 ± 0.678	1.261 ± 0.432	1.569 ± 0.166	3.305 ± 0.101	1.598 ± 1.776	1.668 ± 0.212	0.492 ± 0.025
Total	1.247 ± 0.581	510.015 ± 13.023	1231.328 ± 222.604	1.585 ± 0.180	164.187 ± 2.996	62.634 ± 16.714	283.214 ± 28.623	17.199 ± 5.573
Derivatives of benzene								
ethyl benzene	0.313 ± 0.131	0.276 ± 0.108	0.259 ± 0.202	0.066 ± 0.030	0.935 ± 0.534	0.979 ± 0.598	0.255 ± 0.195	0.123 ± 0.088
styrene	0.574 ± 0.092	0.792 ± 0.118	0.842 ± 0.147	0.257 ± 0.275	2.261 ± 2.241	3.107 ± 3.482	0.575 ± 0.138	0.161 ± 0.022
m-di-tert-butylbenzene	22.624 ± 1.371	12.075 ± 1.783	41.505 ± 7.869	12.545 ± 0.661	53.579 ± 13.912	32.527 ± 8.208	49.598 ± 7.273	13.282 ± 6.780
naphthalene	0.463 ± 0.003	0.471 ± 0.281	0.730 ± 0.098	0.314 ± 0.125	1.782 ± 0.318	0.728 ± 0.141	31.024 ± 7.704	2.946 ± 1.377
1-1-6-trimethyl-1.2-dihydronaphthalene (TDN)	5.129 ± 0.668	3.157 ± 0.772	13.343 ± 3.346	3.228 ± 0.637	15.379 ± 5.184	9.093 ± 1.748	3.737 ± 1.724	2.750 ± 1.848
Total	29.103 ± 2.258	16.772 ± 2.846	56.679 ± 11.466	16.409 ± 0.406	73.936 ± 21.555	46.434 ± 14.178	85.190 ± 13.196	19.263 ± 9.939
Others								
2-ethoxy-2-methyl-propane	7.172 ± 0.012	0.030 ± 0.001	0.025 ± 0.010	4.289 ± 0.099	4.147 ± 0.641	2.023 ± 0.159	1.993 ± 1.045	0.023 ± 0.005
2-pentylfuran	2.776 ± 1.974	0.891 ± 0.204	6.134 ± 0.909	1.610 ± 0.076	6.036 ± 1.548	8.781 ± 1.384	4.511 ± 1.086	1.559 ± 0.878
dimethyl trisulfide	1.777 ± 0.421	0.610 ± 0.114	1.821 ± 0.286	1.157 ± 0.226	0.009 ± 0.007	0.016 ± 0.012	0.041 ± 0.036	0.013 ± 0.000
4-methyldihydro-2(3H)-furanone	3.750 ± 1.128	0.516 ± 0.710	1.582 ± 1.008	1.688 ± 0.497	5.612 ± 0.747	1.646 ± 0.683	0.950 ± 0.945	0.287 ± 0.104
4-ethyl-phenol	0.848 ± 0.141	3.210 ± 0.751	21.854 ± 5.943	0.608 ± 0.144	129.556 ± 2.648	281.248 ± 21.113	12.287 ± 0.017	136.453 ± 43.612
Total	16.324 ± 1.396	5.257 ± 1.781	31.416 ± 3.729	9.352 ± 0.104	145.360 ± 1.001	293.713 ± 20.560	19.782 ± 1.133	138.335 ± 44.380
Total	928.388 ± 19.716	1141.280 ± 149.879	2524.976 ± 434.426	607.670 ± 3.670	2674.990 ± 348.181	1704.317 ± 246.572	3138.218 ± 565.745	776.754 ± 263.071

Table S4. Identification of phenolic compounds**.** Mass spectral characteristics of phenolic compounds detected in started and unstarted cherry juices. For quantified compound a specific variable, used in PCA analyses, was assigned

	Compound	RT	[M-H]- (m/z)	MS2 ion fragments (m/z)	Quantification	Standard
P59	*p*-Hydroxyphenyllactic acid	4.18	181	163, 135	SRM	*p*-Hydroxyphenyllactic acid
P60	Phenyllactic acid	6.21	165	147, 119	SRM	Phenyllactic acid
P61	3-*O*-caffeoylquinic acid	4	353	191, 173, 179	SRM	3-*O*-caffeoylquinic acid
P62	5-*O*-caffeoylquinic acid	4.7	353	191, 173, 179	SRM	5-*O*-caffeoylquinic acid
P63	4-*O*-caffeoylquinic acid	4.8	353	191, 173, 179	SRM	4-*O*-caffeoylquinic acid
P64	Caffeoylquinic acid	5.16	353	191, 173, 179	SRM	5-*O*-caffeoylquinic acid
P65	Caffeic acid	5.15	179	135	SIM	Caffeic acid
P66	Dicaffeoylquinic acid (1)	6.62	515	353, 191, 179	SRM	5-*O*-caffeoylquinic acid
P67	Dicaffeoylquinic acid (2)	6.6	515	179, 191, 353	SRM	5-*O*-caffeoylquinic acid
P68	Dicaffeoylquinic acid (3)	6.84	515	179, 191, 353	SRM	5-*O*-caffeoylquinic acid
P69	Coumaroylquinic acid (1)	4.58	337	191, 163, 173	SRM	3-*O*-caffeoylquinic acid
P70	Coumaroylquinic acid (2)	5.2	337	191, 163, 173	SRM	4-*O*-caffeoylquinic acid
P71	Coumaroylquinic acid (3)	5.33	337	191, 163, 173	SRM	4-*O*-caffeoylquinic acid
P72	Coumaroylquinic acid (4)	5.75	337	191, 163, 173	SRM	5-*O*-caffeoylquinic acid
P73	*p*-coumaric acid	6.12	163		SIM	*p*-coumaric acid

P74	Feruloylquinic acid	4.87	367	193, 191, 173	SRM	3-*O*-caffeoylquinic acid
P75	Coumaroylquinic acid lactone	6.58	319	145, 119, 163	SRM	3-*O*-caffeoylquinic acid
P76	Coumaric acid-*O*-hexoside	4.11	325	163, 119	SIM	p-coumaric acid
P77	Caffeic acid-*O*-hexoside	4.55	341	179, 135	SIM	Caffeic acid
P78	Caffeoylquinic acid-*O*-hexoside	3.77	515	341,179, 353, 173, 191	SRM	3-*O*-caffeoylquinic acid
P79	Luteolin	8.24	285	241, 175	SIM	Luteolin
P80	Dihdroxybenzoic acid-*O*-hexoside	3.36	315	153, 109	SIM	Protocatecuic acid
P81	Protocatecuic acid	3.8	153	109	SIM	Protocatecuic acid
P82	Quercetin	8.3	301	179, 151	SIM	Quercetin
P83	Rutin	5.97	609	301, 343	SRM	Rutin hydrate
P84	Quercetin-3-*O*-glucoside	6.23	463	301, 179	SRM	Rutin hydrate
P85	Kaempferol-*O*-rutinoside	6.41	593	285, 327	SRM	Rutin hydrate
P86	Catechin	4.71	289	245, 205, 179, 271, 137, 125	SIM	Catechin
P87	Epicatechin	5.25	289	245, 205, 179, 271, 137, 125	SIM	Epicatechin
P88	Dihydrocaffeic acid	5.29	181	109, 119, 137	SRM	Dihydrocaffeic acid
NQ	Naringenin	9.1	271	151, 177, 107, 201	SIM	Naringenin
NQ	Kaempferol	9.32	285	257, 151	SIM	Kaempferol
NQ	Feruloylquinic acid	5.59	367	193, 191, 173	SRM	4-*O*-caffeoylquinic acid
NQ	Coumaroylquinic acid-*O*-hexoside	3.5	499	325, 163	SRM	3-*O*-caffeoylquinic acid

Table S5. Phenolic profile. Concentration (µg/mL) of phenolic compounds detected in unfermented (37°C and 30°C) and fermented with *L. rhamnosus* 2360, *L. paracasei* 4186, *L. plantarum* 1LE1, 285, C1 and POM1 cherry juice after 48 hours of fermentation and further storage (14 days).

				48 hours				
Compounds	**37°C**	**2360**	**4186**	**30°C**	**1LE1**	**285**	**C1**	**POM1**
Phenyllactic acids								
p-hydroxyphenyllactic acid	ND	0.986 ± 0.121	0.209 ± 0.047	ND	1.229 ± 0.187	1.462 ± 0.089	1.241 ± 0.148	1.134 ± 0.125
Phenyllactic acid	ND	0.483 ± 0.062	0.449 ± 0.024	ND	2.098 ± 0.187	1.905 ± 0.150	1.219 ± 0.076	1.930 ± 0.026
Total	ND	1.469 ± 0.156	0.659 ± 0.034	ND	3.327 ± 0.365	3.367 ± 0.157	2.461 ± 0.166	3.064 ± 0.120
Hydroxycinnamic acids								
3-O-caffeoylquinic acid	19.407 ± 2.234	17.470 ± 1.020	19.323 ± 0.343	17.706 ± 0.563	20.469 ± 1.536	18.452 ± 0.693	18.305 ± 1.324	17.164 ± 0.776
5-O-caffeoylquinic acid	2.652 ± 0.183	2.964 ± 0.339	3.660 ± 0.233	2.446 ± 0.099	3.441 ± 0.321	3.361 ± 0.062	2.898 ± 0.180	2.968 ± 0.214
4-O-caffeoylquinic acid	8.418 ± 1.077	8.347 ± 0.295	10.304 ± 0.248	7.101 ± 0.001	9.778 ± 1.261	8.887 ± 0.580	9.028 ± 0.176	8.683 ± 0.817
caffeoylquinic acid	0.036 ± 0.002	0.040 ± 0.004	0.041 ± 0.003	0.039 ± 0.003	0.044 ± 0.009	0.044 ± 0.007	0.038 ± 0.003	0.039 ± 0.003
caffeic acid	0.633 ± 0.025	0.743 ± 0.038	0.722 ± 0.030	0.586 ± 0.033	0.406 ± 0.270	ND	0.305 ± 0.040	0.062 ± 0.014
dicaffeoylquinic acid (1)	0.004 ± 0.004	0.008 ± 0.001	0.011 ± 0.005	0.004 ± 0.000	0.009 ± 0.003	0.008 ± 0.003	0.008 ± 0.002	0.010 ± 0.002
dicaffeoylquinic acid (2)	0.010 ± 0.004	0.025 ± 0.002	0.028 ± 0.006	0.011 ± 0.001	0.035 ± 0.002	0.032 ± 0.007	0.030 ± 0.000	0.023 ± 0.005
dicaffeoylquinic acid (3)	0.025 ± 0.005	0.037 ± 0.006	0.043 ± 0.014	0.020 ± 0.003	0.049 ± 0.010	0.037 ± 0.001	0.039 ± 0.006	0.036 ± 0.002
coumaroylquinic acid (1)	21.714 ± 2.529	24.020 ± 1.087	24.680 ± 1.949	21.828 ± 0.354	24.564 ± 2.152	24.898 ± 1.265	22.903 ± 0.415	23.357 ± 0.853
coumaroylquinic acid (2)	6.537 ± 0.266	6.466 ± 0.481	7.647 ± 0.320	5.547 ± 0.502	7.478 ± 0.865	6.398 ± 0.473	6.245 ± 0.104	6.563 ± 0.106
coumaroylquinic acid (3)	3.746 ± 0.151	4.002 ± 0.597	4.584 ± 0.116	3.445 ± 0.308	4.220 ± 0.194	4.053 ± 0.250	3.811 ± 0.132	3.654 ± 0.100
coumaroylquinic acid (4)	0.011 ± 0.001	0.011 ± 0.002	0.012 ± 0.001	0.008 ± 0.001	0.012 ± 0.001	0.010 ± 0.000	0.010 ± 0.001	0.011 ± 0.001

p-coumaric acid	0.609 ± 0.274	0.594 ± 0.061	0.486 ± 0.105	0.288 ± 0.124	0.484 ± 0.134	ND	0.354 ± 0.207	ND
feruloylquinic acid (1)	0.338 ± 0.021	0.349 ± 0.031	0.377 ± 0.052	0.360 ± 0.024	0.385 ± 0.081	0.332 ± 0.021	0.302 ± 0.039	0.324 ± 0.000
coumaroylquinic acid lactone	0.155 ± 0.014	0.160 ± 0.024	0.168 ± 0.008	0.139 ± 0.024	0.150 ± 0.013	0.145 ± 0.021	0.148 ± 0.018	0.141 ± 0.001
Total	64.295 ± 5.736	65.234 ± 3.421	72.084 ± 2.807	59.530 ± 0.113	71.362 ± 5.679	66.658 ± 1.769	64.423 ± 2.044	63.035 ± 2.538
Hydroxycinnamic acid glycosides								
coumaric acid O-hexoside	0.408 ± 0.033	0.700 ± 0.132	1.077 ± 0.099	0.378 ± 0.011	0.885 ± 0.076	1.082 ± 0.123	0.892 ± 0.254	1.162 ± 0.085
caffeic acid O-hexoside	0.211 ± 0.017	0.206 ± 0.005	0.237 ± 0.033	0.197 ± 0.002	0.232 ± 0.013	0.229 ± 0.008	0.211 ± 0.014	0.229 ± 0.020
caffeoylquinic acid O-hexoside	0.951 ± 0.050	0.796 ± 0.078	0.943 ± 0.061	0.777 ± 0.013	0.977 ± 0.072	0.826 ± 0.045	0.881 ± 0.045	0.877 ± 0.006
Total	1.570 ± 0.036	1.702 ± 0.185	2.258 ± 0.188	1.353 ± 0.026	2.093 ± 0.082	2.136 ± 0.098	1.984 ± 0.288	2.268 ± 0.064
Flavon								
luteolin	0.080 ± 0.029	0.204 ± 0.022	0.209 ± 0.025	0.087 ± 0.016	0.194 ± 0.019	0.193 ± 0.005	0.173 ± 0.008	0.175 ± 0.029
Hydroxybenzoic acids								
Dihydroxybenzoic acid O-hexoside	2.085 ± 0.291	1.985 ± 0.185	2.393 ± 0.038	2.024 ± 0.177	2.453 ± 0.297	2.048 ± 0.281	1.943 ± 0.102	2.061 ± 0.163
Protocatechuic acid	0.457 ± 0.197	0.508 ± 0.023	0.382 ± 0.103	0.253 ± 0.114	0.380 ± 0.131	ND	0.308 ± 0.273	ND
Total	2.542 ± 0.327	2.493 ± 0.208	2.775 ± 0.107	2.277 ± 0.291	2.706 ± 0.535	2.048 ± 0.281	2.250 ± 0.367	2.061 ± 0.163
Flavonol								
quercetin	0.712 ± 0.058	1.335 ± 0.035	1.491 ± 0.121	0.657 ± 0.074	1.482 ± 0.105	1.339 ± 0.015	1.334 ± 0.066	1.375 ± 0.089
Flavonols glycosides								
quercetin-3-O-rutinoside	1.168 ± 0.029	1.269 ± 0.106	1.338 ± 0.065	0.938 ± 0.085	1.386 ± 0.127	1.206 ± 0.067	1.197 ± 0.095	1.241 ± 0.112

quercetin-3-O-glucoside	0.099 ± 0.012	0.118 ± 0.007	0.127 ± 0.009	0.086 ± 0.005	0.131 ± 0.015	0.116 ± 0.006	0.112 ± 0.007	0.107 ± 0.001
Kaempferol-O-rutinoside	0.124 ± 0.007	0.133 ± 0.003	0.139 ± 0.006	0.105 ± 0.005	0.145 ± 0.019	0.134 ± 0.003	0.117 ± 0.010	0.128 ± 0.006
Total	1.391 ± 0.039	1.521 ± 0.105	1.604 ± 0.052	1.129 ± 0.095	1.662 ± 0.153	1.456 ± 0.071	1.427 ± 0.094	1.477 ± 0.106
Catechins								
catechin	0.227 ± 0.009	0.338 ± 0.033	0.568 ± 0.025	0.289 ± 0.045	0.453 ± 0.083	0.516 ± 0.056	0.483 ± 0.103	0.629 ± 0.024
epicatechin	0.631 ± 0.046	0.956 ± 0.048	1.684 ± 0.294	0.801 ± 0.077	1.327 ± 0.089	1.576 ± 0.075	1.566 ± 0.204	1.992 ± 0.097
Total	0.858 ± 0.051	1.294 ± 0.060	2.252 ± 0.319	1.089 ± 0.122	1.780 ± 0.172	2.092 ± 0.100	2.049 ± 0.307	2.621 ± 0.118
Hydroxyphenylpropionic acids								
Dihydrocaffeic acid	ND	ND	ND	ND	0.307 ± 0.222	0.284 ± 0.043	0.131 ± 0.062	0.568 ± 0.070
Total phenolic compounds	71.449 ± 6.089	75.252 ± 3.974	83.331 ± 3.324	66.122 ± 0.161	84.913 ± 6.671	79.575 ± 1.593	76.230 ± 2.618	76.641 ± 2.709

	14 Days							
	37°C	**2360**	**4186**	**30°C**	**1LE1**	**285**	**C1**	**POM1**
Phenyllactic acids								
p-hydroxyphenyllactic acid	ND	1.272 ± 0.159	0.259 ± 0.030	ND	1.225 ± 0.025	1.473 ± 0.158	1.271 ± 0.115	1.295 ± 0.131
Phenyllactic acid	ND	0.559 ± 0.034	0.513 ± 0.006	ND	1.986 ± 0.087	2.150 ± 0.153	1.494 ± 0.141	2.208 ± 0.095
Total	ND	1.831 ± 0.190	0.772 ± 0.030	ND	3.211 ± 0.104	3.623 ± 0.264	2.765 ± 0.245	3.503 ± 0.222
Hydroxycinnamic acids								

3-O-caffeoylquinic acid	19.271 ± 0.407	17.498 ± 0.344	20.036 ± 1.511	18.976 ± 0.967	18.775 ± 2.710	17.786 ± 0.946	18.496 ± 1.410	18.544 ± 1.360
5-O-caffeoylquinic acid	2.719 ± 0.180	2.979 ± 0.077	3.298 ± 0.100	2.700 ± 0.341	2.941 ± 0.220	3.309 ± 0.243	3.027 ± 0.343	2.930 ± 0.233
4-O-caffeoylquinic acid	8.559 ± 0.784	8.395 ± 1.264	9.105 ± 0.907	7.859 ± 0.484	8.969 ± 0.828	8.933 ± 1.002	8.700 ± 0.489	8.984 ± 0.382
Caffeoylquinic acid	0.037 ± 0.006	0.042 ± 0.006	0.040 ± 0.005	0.036 ± 0.006	0.039 ± 0.008	0.041 ± 0.005	0.038 ± 0.001	0.039 ± 0.004
Caffeic acid	0.685 ± 0.062	0.789 ± 0.050	0.668 ± 0.038	0.702 ± 0.037	0.345 ± 0.241	ND	0.259 ± 0.024	0.092 ± 0.020
Dicaffeoylquinic acid (1)	0.010 ± 0.004	0.009 ± 0.001	0.010 ± 0.001	0.008 ± 0.001	0.007 ± 0.001	0.011 ± 0.001	0.008 ± 0.003	0.009 ± 0.003
Dicaffeoylquinic acid (2)	0.013 ± 0.005	0.022 ± 0.007	0.017 ± 0.006	0.011 ± 0.005	0.028 ± 0.005	0.025 ± 0.002	0.031 ± 0.005	0.033 ± 0.004
Dicaffeoylquinic acid (3)	0.029 ± 0.004	0.036 ± 0.001	0.032 ± 0.003	0.030 ± 0.004	0.038 ± 0.005	0.043 ± 0.007	0.041 ± 0.002	0.031 ± 0.005
Coumaroylquinic acid (1)	21.825 ± 0.576	23.674 ± 0.672	23.440 ± 1.247	22.012 ± 2.190	22.976 ± 0.759	22.844 ± 1.427	24.561 ± 1.478	24.533 ± 0.303
Coumaroylquinic acid (2)	6.936 ± 0.426	6.450 ± 0.212	6.768 ± 0.315	6.370 ± 1.109	6.816 ± 0.141	7.116 ± 0.461	6.765 ± 0.102	7.412 ± 0.704
Coumaroylquinic acid (3)	3.987 ± 0.214	4.000 ± 0.273	4.212 ± 0.152	3.803 ± 0.464	4.056 ± 0.139	4.073 ± 0.268	3.955 ± 0.266	3.735 ± 0.150
Coumaroylquinic acid (4)	0.012 ± 0.001	0.011 ± 0.001	0.011 ± 0.001	0.011 ± 0.001	0.010 ± 0.001	0.012 ± 0.000	0.011 ± 0.001	0.011 ± 0.000
p-coumaric acid	0.670 ± 0.294	0.472 ± 0.050	0.485 ± 0.126	0.412 ± 0.103	0.337 ± 0.019	ND	ND	ND
Feruloylquinic acid (1)	0.340 ± 0.040	0.302 ± 0.038	0.339 ± 0.042	0.364 ± 0.047	0.376 ± 0.047	0.325 ± 0.037	0.323 ± 0.046	0.345 ± 0.016
Coumaroylquinic acid lactone	0.144 ± 0.004	0.177 ± 0.031	0.166 ± 0.016	0.143 ± 0.005	0.131 ± 0.032	0.149 ± 0.023	0.140 ± 0.002	0.126 ± 0.009
Total	65.236 ± 0.952	64.858 ± 1.759	68.627 ± 2.251	63.437 ± 5.218	65.732 ± 2.909	64.668 ± 0.863	66.355 ± 2.513	66.826 ± 2.637
Hydroxycinnamic acid glycosides								
Coumaric acid O-hexoside	0.541 ± 0.026	0.516 ± 0.133	0.621 ± 0.136	0.446 ± 0.170	0.480 ± 0.014	0.790 ± 0.020	0.732 ± 0.174	1.145 ± 0.142
Caffeic acid O-hexoside	0.213 ± 0.005	0.198 ± 0.003	0.215 ± 0.003	0.211 ± 0.011	0.215 ± 0.009	0.213 ± 0.010	0.217 ± 0.007	0.204 ± 0.018
Caffeoylquinic acid O-hexoside	0.901 ± 0.086	0.785 ± 0.086	0.788 ± 0.056	0.781 ± 0.053	0.882 ± 0.044	0.882 ± 0.065	0.898 ± 0.056	0.868 ± 0.043
Total	1.655 ± 0.073	1.500 ± 0.056	1.623 ± 0.123	1.438 ± 0.224	1.576 ± 0.043	1.885 ± 0.095	1.847 ± 0.162	2.217 ± 0.188

Flavon							
Luteolin	0.154 ± 0.012	0.175 ± 0.030	0.185 ± 0.021	0.129 ± 0.008	0.176 ± 0.011	0.157 ± 0.024	0.165 ± 0.014	0.166 ± 0.011
Hydroxybenzoic acids								
Dihydroxybenzoic acid O-hexoside	2.116 ± 0.067	2.202 ± 0.179	2.336 ± 0.311	2.040 ± 0.426	2.223 ± 0.123	2.228 ± 0.046	1.894 ± 0.179	2.157 ± 0.104
Protocatechuic acid	0.560 ± 0.290	0.484 ± 0.170	0.497 ± 0.217	0.357 ± 0.148	0.285 ± 0.052	ND	0.459 ± 0.247	ND
total	2.676 ± 0.329	2.686 ± 0.315	2.833 ± 0.381	2.397 ± 0.568	2.413 ± 0.142	2.228 ± 0.046	2.353 ± 0.296	2.157 ± 0.104
Flavonol								
Quercetin	1.091 ± 0.036	1.353 ± 0.038	1.333 ± 0.072	0.957 ± 0.075	1.339 ± 0.020	1.353 ± 0.056	1.332 ± 0.036	1.348 ± 0.093
Flavonols glycosides								
Quercetin-3-O-rutinoside	1.270 ± 0.064	1.315 ± 0.127	1.285 ± 0.117	1.284 ± 0.168	1.146 ± 0.063	1.184 ± 0.267	1.250 ± 0.079	1.335 ± 0.026
Quercetin-3-O-glucoside	0.118 ± 0.005	0.126 ± 0.006	0.127 ± 0.015	0.109 ± 0.014	0.117 ± 0.009	0.125 ± 0.011	0.123 ± 0.007	0.119 ± 0.012
Kaempferol-O-rutinoside	0.136 ± 0.003	0.123 ± 0.005	0.139 ± 0.009	0.114 ± 0.009	0.125 ± 0.007	0.132 ± 0.008	0.127 ± 0.009	0.126 ± 0.007
Total	1.524 ± 0.071	1.564 ± 0.128	1.551 ± 0.141	1.508 ± 0.185	1.388 ± 0.068	1.441 ± 0.278	1.500 ± 0.078	1.580 ± 0.019
Catechins								
Catechin	0.195 ± 0.020	0.192 ± 0.028	0.291 ± 0.011	0.212 ± 0.047	0.291 ± 0.029	0.390 ± 0.035	0.332 ± 0.057	0.461 ± 0.014
Epicatechin	0.424 ± 0.107	0.532 ± 0.171	0.744 ± 0.043	0.534 ± 0.080	0.849 ± 0.120	0.984 ± 0.125	1.108 ± 0.135	1.360 ± 0.039
Total	0.619 ± 0.117	0.724 ± 0.188	1.035 ± 0.052	0.746 ± 0.127	1.140 ± 0.111	1.374 ± 0.158	1.440 ± 0.108	1.821 ± 0.052
Hydroxyphenylpropionic acids								
Dihydrocaffeic acid	ND	ND	ND	ND	0.317 ± 0.314	0.278 ± 0.022	0.124 ± 0.040	0.609 ± 0.041

Total phenolic compounds	72.955 ± 0.904	74.691 ± 2.298	77.960 ± 2.585	70.611 ± 6.185	77.293 ± 3.104	77.007 ± 0.879	77.881 ± 2.910	80.226 ± 2.548

Chapter 4

Orange peels: from by-product to resource through lactic acid fermentation

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Abstract

Orange peels, a frequently discarded by-product from citrus industries, can be used as the sole nutrient for solid state fermentation for lactic acid production. For this purpose, different species of lactic acid bacteria were employed, singly and in co-culture. After only one day of SSF, all the tested strains increased in cells number of about 2 Log cycles, showing that orange peels, without any previous pre-treatment or supplementation with extra nutrients, represent a suitable substrate for lactic acid bacteria growth. The consumption of reducing sugars was clearly related with lactic acid production. *L. casei* 2246 was the more efficient strain, reaching the highest concentration of lactic acid (209.65 mg/g) and yield (0.88 mg/mg). After four days of fermentation, the co-culture of *L. plantarum* and *L. paracasei,* produced an amount of this acid comparable to *L. casei* 2246, highlighting the interest to employing mix cultures in some cases.

Introduction

Lactic acid (LA) (2-hydroxypropionic acid) is the simplest hydroxycarboxylic acid. It appears as a yellow-colourless and odourless liquid (Komesu et al., 2017). The increasing interest on this acid production is due to the wide range of applications in food, cosmetic, pharmaceutical, and chemical industries. One the most recent applications is in polylactic acid (PLA) production (Jaramillo et al., 2018; Komesu et al., 2017; Wee, Kim, & Ryul, 2006). PLA is considered a biodegradable and biocompatible plastic, which represents a suitable and sustainable alternative to petroleum-derived plastics (Komesu et al., 2017; Dìaz et al., 2017; Tawakkal et al., 2014; Nampoothiri, Nair & John, 2010). It is primarily used for the manufacture of rigid containers, bags, shrink wrap and food packaging (Komesu et al., 2017; Tawakkal et al., 2014) with a particular focus on films and coating suitable for short shelf life and ready-to-eat food products.

Lactic acid is identify as a GRAS (generally recognize as safe) food additive by FDA (Food and Drug Administration) and for this reason it is largely applied to increase shelf life, enhance flavours, modulate the acidity of food and in the control of foodborne pathogens. It is also extensively used, with its derived salts, in cosmetics applications due to its antimicrobial activity, skin lightening and hydration properties. The pharmaceutical purpose is related to its utilization as an electrolyte in parenteral and mineral preparations. Another large scale application is visible in chemical industry, where it is employed as monomer for chemical conversions into potential useful compounds thanks to the presence of two reactive functional groups (carboxylic and hydroxyl groups) (Komesu et al., 2017; Wee, Kim, & Ryul, 2006). Two processes can be used for lactic acid production: lactic acid fermentation from renewable or non-renewable substrates (Tawakkal et al., 2014) or chemical synthesis from petrochemical resources (Ghaffar et al., 2014). The cost of raw material is one of the main barriers for the production of LA by bioprocesses. This obstacle can be avoided by using agro-industrial wastes/by-products as cheap substrates for the fermentation

process. In this way, not only the cost of the process can be reduced, but also it contributes to the complicate management of food waste (John, Nampoothiri, & Pandey, 2005). The production of food waste is a worldwide problem, in fact, only Europe is estimated to generate around 90 million tons of discarded products annually (Girotto et al., 2015). Fruits and vegetables industries are one of the major producers and, among fruits, given that citrus fruits have a small edible part, a wide portion, such as peels and seeds, is generated during juice production and processing (Mirabella, Castellani, & Sala, 2014). Although orange peels can be used to produce fibre, pectin, flavonoids, essential oils or animal feed (Aboagye et al., 2017), they are frequently discarded by industries.

Constituting ca. 70 % of the total citrus fruits, the sweet orange (*Citrus sinensis*) is the most produced and consumed (Sharma et al., 2017). In European Union orange production is concentrated in the Mediterranean area, especially in Spain, which is the main producer, and Italy, reaching together the 80 % of the total production (USDA Foreign Agricultural Service, 2017).

Agro-industrial substrates are the preferred substrates for solid state fermentation (SSF) technology. It is defined as the fermentation involving solids in the absence (or near absence) of free water. The substrate, however, must contain sufficient moisture to support the growth and metabolism of the microorganisms. SSF has turned out to be a promising technology for waste valorisation, which has been proved to be very efficient in terms of product yields and productivities, low energy consumption, and solving disposal problems (Botella et al., 2007; Díaz et al., 2012; Yazid et al., 2017). In most cases, filamentous fungi are preferred for SSF processes because of their unique capacity to colonize the interparticular spaces of solid matrices and to secrete various enzymes to hydrolyse the solid substrate (Gutiérrez Rojas et al., 1995). In fact, there are very few papers dealing with the production of LA by bacteria in SSF. Wheat bran, supplemented with nitrogen sources such as red lentil and yeast cells, has been successfully used as substrate and support for production of lactic acid in solid state fermentation by *Lactobacillus amylophilus* GV6 (Altaf et al.,

2006). Moreover, sugarcane bagasse, which was impregnated with the sugar solution obtained from another agro-residue (cassava bagasse) has been used as an inert support for the bacterial culture of *Lactobacillus delbrueckii* for producing lactic acid by SSF (John, Nampoothiri, & Pandey, 2006). In the same way, *Lactobacillus casei* subsp. *case*i CFTRI 2022 produced 0.0527 g of lactic acid/g of dry sugar-cane pressmud in an SSF system (Xavier & Lonsane, 1994).

Considering the large amounts of peels derived from orange processing, approximately 50% in weight of orange (Anjum et al., 2017; Sharma et al., 2017), and the high cost of non-renewable raw material for LA production, the aim of this article was to explore the feasibility of using orange peels as the sole nutrient source for LA production in SSF. For this purpose, different species of lactic acid bacteria (LAB) (*L. plantarum, L. casei, L. rhamnosus* and *L. paracasei*) were employed singly and in co-culture, in order to evaluate their ability to ferment orange peels and produce LA. To the best of our knowledge, there have been no reports regarding the production of lactic acid by *Lactobacillus* species under SSF using orange peels as sole nutrient source.

Material and methods

Microbial strains used for fermentation

Four strains of *L. plantarum* (285, isolated from Minas cheese), *L. rhamnosus* (1019, from Parmigiano Reggiano cheese), *L. casei* (2246, from Parmigiano Reggiano cheese) and *L. paracasei* (4186, from Pecorino cheese) were used singly for orange peels SSF. Moreover, *L. plantarum* 285 and *L. paracasei* 4186 were used in co-culture. All the species employed in this study were already applied, for the same purpose, in previous works giving good results in term of lactic acid production, productivity and yield (Wee, Kim, & Ryul, 2006). The bacterial cultures, maintained as frozen stocks (−80 °C) in Man Rogosa Sharpe (MRS) medium (Oxoid, Milan, Italy) supplemented with 15% glycerol (v/v), were propagated three times in MRS medium. Inoculum size was 3%

(v/v), temperature was fixed at 30 °C for *L. plantarum* and at 37 °C for *L. casei* and *L. rhamnosus* and the incubation time was 15 hours. All bacterial strains used belonging to the collection of the Department of Food and Drugs of the University of Parma.

Solid conditioning

Orange peels from Washington Navel variety were collected in the canteen of the University of Cadiz after juice extraction. They were cut into pieces, dried in an oven at 40 °C, until complete dehydration, and finally milled to reduce their particle size (62.8% of their weight was constituted by particles over 1 mm in diameter).

Solid state fermentation

SSF experiments were carried out in static conditions using 5 g of dried solid substrate. Initial moisture content of dried solid was adjusted to 75% with distilled water. Afterwards, it was sterilized in an autoclave (20 min, 120 ºC, 1.2 atm) and the pH was adjusted to 5 or 6.5 with Na(OH) 10%. In the samples with an initial pH of 6.5, also 17% w/w of $CaCO_3$ was added. A starter at the concentration of ca. 7 Log CFU/g was added to each plate. SSF was carried out for 5 days at the optimum growth temperature of the microorganisms employed, 37 °C for *L. rhamnosus, L. casei* and *L. paracasei* and 30 °C for *L. plantarum* and the co-culture.

Growth assay and pH evaluation

Bacterial growth in orange peels was analysed every day for five days. Decimal dilutions of started samples were made in quarter-strength Ringer solution (Oxoid, Milan, Italy), plated in MRS agar and incubated at the optimum temperature of each microorganism for 48-72 h.

The pH of the samples was measured by a Crison pH-meter (Crison Instruments, Barcelona, Spain, model Basic 20) as follow: decimal dilution of each sample was performed and the solution

obtained was used for the pH measurement (Adinarayana et al., 2004). Microbial counts and pH measurement were performed in triplicate.

Lactic acid extraction

For the determination of LA and reducing sugars (RS), an extraction of fermented solid with sulfuric acid was performed as suggested by John, Nampoothiri, & Pandey, 2005. Briefly, 50 mL of H_2SO_4 1 M were added to 5 g of fermented orange peels, mixed for 30 min at 150 rpm and 25 °C, then centrifuged for 15 min at 8000 rpm and 25 °C. Finally, the solid was discarded and the supernatant was collected and used to determine the concentration of LA and RS.

Reducing sugars and lactic acid quantification

The dinitrosalicylic acid method (DNS) adapted to microplate with some modifications was used for the determination of RS along fermentation (Gonçalves et al., 2010; Miller, 1959). For a proper operation of this method the pH should be alkaline so, 25% (w/v) NaOH was added to the samples for this purpose. The solutions obtained were centrifuged for 1 min at 10000 rpm and the supernatant was collected and used for the reaction with DNS. 25 µl of supernatant were added to 25 µl of DNS reagent, heated at 105 °C for 10 min and cold immediately. After cooling, 250 µl of distilled water were added and the absorbance was read at 540 nm. For the quantification, a glucose calibration curve was used.

LA concentration in all the samples was determined by an ion exchange chromatography (Metrohm, 930 Compact IC Flex, Suiza) coupled with a conductivity detector. The opportune dilutions were prepared using a solution of sulfuric acid (0.4 mmol/L)/acetone (12%) and the separation of compounds in the samples was carried out using Metrosep Organic Acids - 250/7.8 column. For the quantification, a calibration curve with external standard (lactic acid) was performed.

LA and RS concentrations were monitored for the five days of fermentation. All the results were expressed as the mean value of three independent experiments ± standard deviation. LA was reported as mg of lactic acid per g of dry orange peels (mg/g). The productivity and the yield were calculated and expressed as the concentration of LA per fermentation time and the amount of LA produced (mg) from one milligram of reducing sugar consumed, respectively.

Statistical analyses

To evaluate the normal distribution for every group of independent samples Shapiro-Wilk test was used. One way ANOVA was applied to discriminate the significant differences among the samples. The analyses were conducted with SPSS Statistics 21.0 software (SPSS Inc., Chicago, IL) using Tukey test and the results were considered different for values of $p < 0.05$.

Results and discussion

Effect of pH on LA production

The importance of the medium pH in SSF for LA production was evaluated with the strain *L. plantarum* 285. As, the initial pH of orange peels was around 4, which is very low for optimal LAB growth, it was adjusted to initial values of 5 and 6.5. Results obtained showed that after one day of fermentation a marked decreased was observed for both pH assayed reaching a value of 3.77 ± 0.1 and remaining stable until the end of the process (Fig. 1A). Maintenance of pH is important during lactic acid fermentation to provide the optimum pH for the microorganism to allow it to utilize maximum substrate. $CaCO_3$ has been found to be best buffering agent to control pH in submerged and solid state fermentations to produce organic acids (Hofvendahl & Hahn-Hagerdal, 2000). For this reason, and to avoid the problem of pH decrease as LA accumulation starts, 17% w/w of $CaCO_3$ was also added to the solid mass were the SSF was carried out at an initial pH of 6.5.

The bacterial growth, RS consumption and LA production were analysed in these three different conditions and results obtained are shown in Fig. 1 (B, C, D). *L. plantarum* 285 showed a good ability to grow in orange peels in all the conditions tested. The cells number increased about 2 Log cycles in just one day of fermentation, remaining unchanged during the following days; demonstrating that the decrease or maintenance of pH did not affect the growth and cells viability (Fig. 1B). However, the consumption of RS observed was markedly higher when $CaCO_3$ was added (Fig. 1C), reaching the highest reduction after 4 days of fermentation (maximum consumption of 161.46 mg/g). In the same conditions, LA production resulted in the highest amount, with a maximum value of 123.82 ± 1.47 mg/g (Fig. 1D), statistically different from the other two conditions tested ($p < 0.05$).

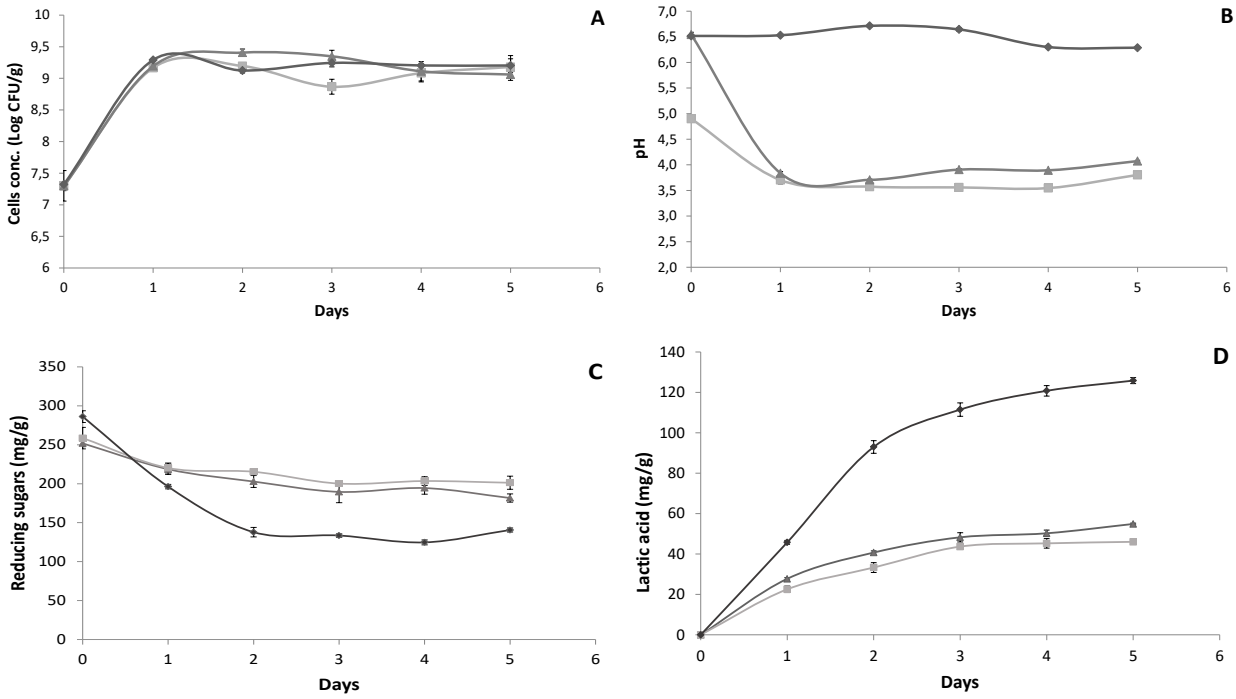

Figure 1. *L. plantarum* 285 orange peels fermentation. Initial pH of 5 (—■—), 6.5 (—▲—) and 6.5 added with $CaCO_3$ (—◆—) were used for fermentations. Bacterial growth (A), pH (B), reducing sugars (C) and lactic acid (D) were evaluated over five days of fermentation. All the data were obtained from three independent experiment and express as the mean value ± standard deviation.

About the productivity analysis, differences can be observed in the different situations tested. In particular, the highest LA productivities were found when the pH remained stable over time by adding $CaCO_3$ and the initial pH was adjusted to 6.5 (Table 1).

Table 1. *L. plantarum* productivity. Productivity (concentration of LA per gram of dried orange peels and fermentation time), expressed in mg/g*h, calculated for *L. plantarum* 285 in the three different conditions tested (initial pH 6.5 with 17% $CaCO_3$, initial pH 6.5 and initial pH 5).

Days	pH 6.5 + $CaCO_3$	pH 6.5	pH 5
0	0.00	0.00	0.00
1	1.90	1.15	0.94
2	1.93	0.84	0.69
3	1.54	0.67	0.61
4	1.25	0.52	0.47
5	1.04	0.46	0.38

In this situation, the LA formed is readily converted to calcium lactate. For this reason, these conditions were used for all the following fermentations. The used of $CaCO_3$, to increase the quantity of LA produced, is in accordance with literature, which reported the employment of calcium carbonate to buffer the fermentation matrices (Hofvendahl & Hahn-Hagerdal, 2000). The amount of $CaCO_3$ applied in this work is different from preview studies, which reported higher or lower concentrations, depending on the specific substrate of fermentation and the initial simple sugars concentration in the medium of fermentation (Cui, Li, & Wan, 2011; John et al., 2007, John, Nampoothiri, & Pandey, 2006; Naveena et al., 2004). Also in this work different concentration of $CaCO_3$ were tested, but the use of the 17% seems to be the lower concentration able to buffer the decrease of pH (data not shown).

All these observations demonstrate the suitability of orange peels, without addition of other nutrients, as substrate for *L. plantarum* SSF. In spite of the acid pH of this substrate, its composition rich in fibre, soluble and insoluble carbohydrates content (cellulose, hemicellulose and pectin)

makes it a very suitable natural matrix for the production of high added value products. To the best of our knowledge, this is the first evidence of LAB growth on this matrix, instead of other microorganisms such as fungi and yeasts, which have been mainly employed in SSF of orange peels fermentation for different purposes (Mantzouridou, Paraskevopoulou & Lalou, 2015; Díaz et al., 2013). Research regarding to the microbial production of organic acids using orange peels has been mainly focused on citric acid with the fungus *Aspergillus niger* (Torrado et al, 2011; Rivas et al, 2008). Therefore, there is a need to diversify the uses of this substrate for the production of other organic acids with a wide range of industrial applications, such as LA, and above results demonstrate the potentiality of orange peels SSF with lactic acid bacteria.

Evaluation of different LAB strains on orange peels

The growth ability, the consumption of RS, and the production of LA on orange peels was tested for four LAB strains belonging to different species (*L. plantarum*, *L. casei*, *L. rhamnosus* and *L. paracasei*) and one co-culture of *L. plantarum* 285 and *L. paracasei* 4186. In fermented food products, co-cultures are currently used, having been demonstrated the existence of symbiotic relationship among different species involved. Indeed, they are used in dairy industry, involved in the manufacturing of fermented milk and cheeses or for the production of LA starting from culture media, corn steep liquor or cassava bagasse (Lee, 2005; John et al, 2007). For this reason, in the present work the use of a co-culture as starter was performed in order to evaluate if sugars were consumed more efficiently and an increase of LA amount was observed.

After only one day of fermentation, all the strains tested increased in cell number of about 2 Log cycles, remaining almost unchanged in the following days. The same trend was also observed in the co-culture (Table 2). Therefore, orange peels can be considered a suitable substrate for the growth, not only for *L. plantarum*, but also for other different species of LAB. Since all the strains tested in the present work were isolated from dairy products, it can be confirmed what it was previously

reported, LAB strains isolated from different niches are able to be adapted to plant matrices (Ricci et al., 2018).

Table 2. Bacterial growth. Bacterial cells concentration (Log CFU/g of orange peels) during 5 days of fermentation. Microbial concentration is expressed as the mean value of three independent experiment ± standard deviation.

	Time (days)					
Strain	0	1	2	3	4	5
285	7.32 ± 0.04	9.29 ± 0.02	9.12 ± 0.04	9.24 ± 0.06	9.20 ± 0.03	9.15 ± 0.11
2246	7.50 ± 0.04	9.86 ± 0.05	9.94 ± 0.05	9.88 ± 0.12	9.46 ± 0.13	9.33 ± 0.05
1019	7.45 ± 0.06	9.67 ± 0.07	9.68 ± 0.06	9.34 ± 0.09	9.31 ± 0.11	8.86 ± 0.18
4186	7.51 ± 0.07	9.54 ± 0.01	9.66 ± 0.08	9.39 ± 0.04	9.21 ± 0.22	9.06 ± 0.02
285/4186	7.36 ± 0.12	9.34 ± 0.10	9.55 ± 0.11	9.58 ± 0.05	9.43 ± 0.04	9.56 ± 0.07

During orange peels SSF, not all strains consumed the same quantity of RS and the speed of their consumption was also different (Fig. 2A). So, after one day no significant differences between *L. casei* 2246 and *L. rhamnosus* 1019 ($p < 0.05$) were observed but, over time, only the first strain kept on metabolizing RS. Instead for *L. plantarum* 285 and *L. paracasei* 4186, in single culture, the RS consumption was lower than *L. casei* since the start of fermentation. The highest consumption was observed in *L. casei* 2246 after three days with a RS decrease of 83.8% (Fig. 2A). For the first three days of fermentation the co-culture didn't show a particularly interesting behaviour but, starting from the fourth day, the RS observed were comparable with those detected in *L. casei* 2246 fermentation ($p < 0.05$), demonstrating a better performance of the co-culture compared to the respective single strains (*L. plantarum* 285 and *L. paracasei* 4186) (Fig. 2A).

The consumption of RS is clearly and strongly related to the LA production, indeed, LA is usually produced during sugars metabolism in *Lactobacillus* (Wee et al., 2006). The highest amount of LA was detected in *L. casei* 2246 and in the co-culture of *L. plantarum* 285 and *L. paracasei* 4186 (Fig. 2B) . For *L. plantarum*, *L. paracasei* and *L. rhamnosus* the production of LA and the decrease of RS

was comparable after 48 hours (Fig. 2B). Productivity, which correlates LA production with fermentation time, was calculated for the different strains assayed. As it can be seen in Figure 2C, the productivity curve followed the same trend for all the strains assayed: I) an initial increase, exhibiting different slopes for each strain, II) a maximum and III) a decreased.

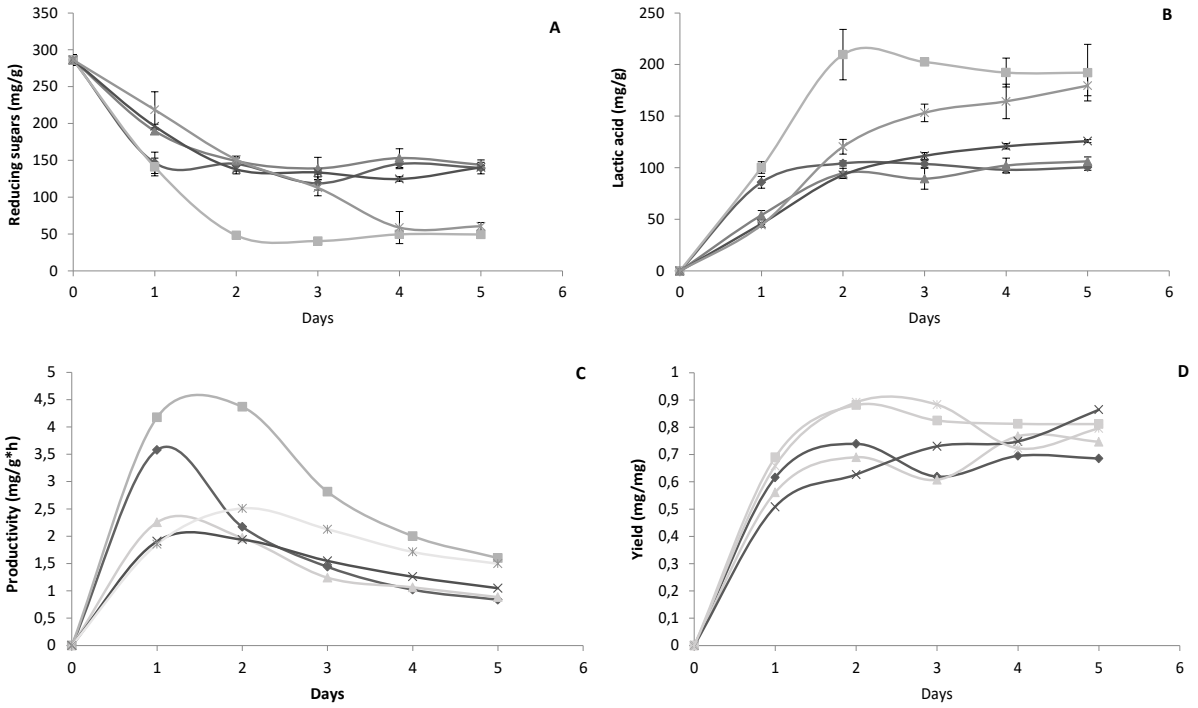

Figure 2. Reducing sugars consumption (A), lactic acid production (B), productivity (C) and yield (D) during five days of orange peels fermentation by *L. plantarum* 285 (—×—), *L. casei* 2246 (—■—), *L. rhamnosus* 1019 (—◆—), *L. paracasei* 4186 (—▲—) and *L. plantarum* 285 and *L. paracasei* 4186 co-culture (—✳—). Data were obtained from three independent experiment and express as the mean value ± standard deviation.

The highest values were reached for *L. casei* 2246 and *L. rhamnosus* 1019 after around one day of fermentation. However, the shape of the curve of yield, which relates LA production with RS consumption, was different, presenting an initial sharp increase and then flattening (Fig. 2D). In this case, the higher yields were observed for *L. casei* 2246 and the co-culture. When *L. plantarum* 285

and *L. paracasei* 4186 were used in co-culture, practically the same yield than for the single culture of *L. casei* 2246 was obtained. Therefore, the employment of co-culture could be useful for the production of LA from by-products using SSF. The benefit of mixing microbial culture, for the same objective, was also reported by some authors in submerged and in SSF using cassava bagasse as substrate (John et al., 2007; Lee, 2005). However, in the research of Lee (2005) the co-cultures lead to a clearly manifested higher amount of LA than the single strains after the fermentation of culture media. The existence of symbiotic relationship among various *Lactobacillus* (John et al., 2007) species/strains could explained these results. Different works have reported the production of LA through lactic acid fermentation. Nevertheless, only few of them reported the exploitation of solid state fermentation of agro-industrial wastes or by-products, such as cassava, sugarcane bagasse and corn stover, for this purpose (Díaz et al., 2017; Cui, Li, & Wan, 2011; John et al., 2007; Qi & Yao, 2007; John, Nampoothiri, & Pandey, 2006; Naveena et al., 2004) and none of them using orange peels. To our knowledge, this is the first study in which LAB have been used for orange peels SSF in order to produce LA.

In order to increase the available RS concentration for bacterial SSF, in different studies an enzymatic hydrolysis of the agro-waste with commercial enzymes prior to the fermentation was carried out. For example, cassava bagasse was enzymatically hydrolysed and the hydrolysate was used to moisten the inert sugarcane bagasse, which was used as solid support for SSF. This substrate was also supplemented with 0.5 g/5 g support NH_4Cl and yeast extract. SSF was carried out by *L. delbrueckii* and a maximum concentration of 249 mg/g of LA was measured (John, Nampoothiri, & Pandey, 2006). Also, pre-treated rice straw was enzymatically hydrolysed, and the hydrolyzate, supplemented with $(NH_4)_2SO_4$, $MnSO_4$, and yeast extract, was used as moistening agent to impregnate 5g of rice straw, which was used as the inert support for SSF. Maximum production obtained using *L. casei* was of 693 mg/gds of L-lactic acid (Qi &Yao, 2007). However, in this work, to avoid the increment of the process cost associated with the high cost of the

enzymes, the fermentation was conducted directly on the orange peels, without a previous hydrolysis and with no addition of extra nitrogen sources. The maximum concentration reached was obtained in the SSF of *L. casei* 2246, resulting in 209.65 mg/g of LA. As it can be seen, this concentration is comparable with those previously described. Also, the yield in this work, even greater than 0.8 mg/mg (fermentation with *L. casei* 2246 and co-culture), was equivalent or higher to those reported in the literature (Cui, Li, & Wan, 2011; John et al., 2007; Wee, Kim, & Ryul 2006).

Conclusion

In citrus fruits, a reduced part can be effectively consumed or processed, while a wide portion, such as, peels is frequently discarded. Orange peels, given their composition rich in fibre and soluble and insoluble carbohydrates, can be considered as cheap raw material for solid state fermentation processes. In this work, orange peels have demonstrated to be a suitable matrix for lactic acid fermentation, allowing the growth of the different species tested without needing to hydrolyse or to add nutritional supplements to the peels. Not all strains, even if they revealed the same growth ability, leaded to the same LA concentration, revealing different peculiarities in the LA production depending on the species/strains. *L. casei* 2246 exhibited the highest LA production and yield, demonstrating the best performance among the single cultures tested. LA concentrations produced by *L. plantarum* 285 and *L. paracasei* 4186, in singly fermentation, were significantly lower than the one obtained by *L. casei* 2246. However, when these two strains were used in co-culture, they produced around the same quantity detected in *L. casei* 2246 but at a higher fermentation time. From these observations, it can be deduced that the employment of a co-culture could give an advantage for a higher consumption of sugar and lactic acid production.

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Conclusions

The consumption of fruit and vegetable is nowadays encouraged as adjuvant for the prevention of chronic diseases. For this reason consumers were orientated to use ready-to-eat fruit and vegetables products. In order to offer to consumers safety products with a long shelf-life and with high nutritional and organoleptic features, lactic acid fermentation could be a suitable strategy which can be applied to reach these purposes. In addition a great quantity of residues were produced in all the food life cycle, especially from fruit and vegetable, leading problems in their environmental and economic management. Also in this case, a valid method to valorise residues and to produce interesting compounds could be the fermentation. In this way fermentation, especially lactic acid fermentation, could be exploited for two purposes: I) the production of new functional food and beverages and II) the recycle of food residues to obtain chemical molecules.

In the field of fruit fermentation the species most employ is *L. plantarum*, recognize to be the most adapted in these substrates, and often autochthonous strains were used for the same reason. The great biodiversity, among species and strains of lactic acid bacteria, derived from their ability to adapt in different environments and can be exploited for different purposes such as increase flavours, modulate polyphenolic compounds or produce specific metabolites.

Based on these assumptions the aim of the present thesis was to exploit lactic acid bacteria in order to valorise different plant matrices, from fruit juices to food by-products. An overview on the metabolism of LAB strains, of plant but especially dairy origin, during fermentation, was reported. Two types of fruit juices, elderberry and cherry, and one by-product, orange peels, were used for fermentations in order to study the metabolism of the different strains applied, to modulate/improve the organoleptic and nutritional properties of juices and produce lactic acid, a valuable chemical compound, from orange by-products.

Elderberry juices have demonstrated to be a suitable substrates for the growth and a good carrier of different LAB species, especially *L. plantarum* and *L. rhamnosus*. Thanks to fermentation, volatile compounds associated with typical elderberry aroma, the norisoprenoid *β*-damascenone and the

alcohols hexanol, 3-hexen-1-ol (*Z*) and 2-hexen-1-ol (*E*) were increased. Among all the tested strains the best performance about volatile profile was reached applying dairy strains, in particular *L. plantarum* 285. Also polyphenolic profile was affected by the action of microorganisms, especially by strains of dairy origin (*L. plantarum* 285 and *L. rhamnosus* 1473). Hydroxycinnamic and hydroxybenzoic acids were subjected to lactic acid bacteria metabolism: caffeic and protocatechuic acids were consumed during fermentation, while dihydrocaffeic acid and catechol were produced and anthocyanins increased in a strain-specific way. The conversion of protocatechuic acid into catechol, since this moment, was only reported for *L. plantarum*, instead this is the first evidence for *L. rhamnosus*. In addition LAB, especially *L. plantarum* strains, were able to produce phenyllactic acid. Overall, dairy strains have demonstrated to possess the metabolic traits most useful to improve the organoleptic and nutritional properties of elderberry juice. Differently from *L. plantarum* and *L. rhamnosus*, some *L. casei* dairy strains were negatively affected by elderberry juice, exhibiting zero-growth. Unexpectedly, this physiological state lead to an increase of the total volatiles amount, in particular in those alcohols, terpenes and norisoprenoids related with typical elderberry scent. To note, despite the absence of replication, *L. casei* 2057 was also able to significantly increase many polyphenolic compounds, especially anthocyanins. This observations suggested that strains growth capability should not be the only criteria for starter selection. In particular this data is relevant for *Lactobacillus* used in industrial food fermentation, where often bacterial cells persisting in a non-replicating state but remaining in a state of active metabolism.

Cherry juice, characterized by high concentration of malic acid, affect the vitality of some strains, while, in others, the growth was not compromised by the hostile substrate. Dairy strains (*L. rhamnosus* 2360 and *L. paracasei* 4186) exhibited the highest production of propyl acetate, interesting ester with fruit note. They also produce phenyllactic acids but the highest quantity was detected using *L. plantarum* 285, of dairy origin too. In this sample the metabolism of

hydroxycinnamic acids (caffeic acid and *p*-coumaric acid) and hydroxybenzoic acid (protocatecuic acid) was highlighted, revealing that also in this substrate stress conditions induce the production of compounds of interest.

The last topic of the present work was the exploitation of orange by-products through lactic acid fermentation for lactic acid production. Orange peels have demonstrated to be an appropriate matrix for lactic acid fermentation, allowing the growth of different species without needing to hydrolyse or to add nutritional supplements to the peels. *L. casei* 2246 exhibited the highest LA production and yield (0.88%), demonstrating the best performance among the single cultures tested. Co-culture was also tested, giving advantage in sugar consumption and lactic acid production.

From the results obtained with this Ph.D. project, can be highlighted how much LAB are versatile and can be exploited for different purposes. Their potentiality could be influenced by stress conditions and by different environments which can modulate the expression of specific genetic treats resulting in the metabolism of specific compounds. Beyond the knowledge acquired on the possibility to ferment different substrates, this study remarks the importance to explore the potential of strains isolated from non-plant niches in fruit lactic acid fermentation allowing also the possible use of non-growing strains. In addition this study lays the foundation to set up a microbial collection dedicated to fruit fermentation in which the information on the metabolic traits express by each strain, in a specific environment, can be conserved in order to steered the choose of starter strains basing on their expressed characteristics.

About the author

Annalisa Ricci was born on June 9th 1988 in La Spezia, Italy. She got the bachelor degree in Biology at the University of Genoa where she prosecuted her studies with the master degree programme in Molecular and Health Biology. She was volunteer in the Laboratory of Experimental and Epidemiological Microbiology, Univeristy of Genoa and then she move to the University of Parma to start the Doctoral School in Food Sciences, under the supervision of Prof. Camilla Lazzi and Prof. Gianni Galaverna. During the Ph.D. she has worked on fruit and vegetables lactic acid fermentation, carring out also a short-term visit, of six months, at the University of Cadiz, to deep her the knowledge on solid state fermentation, under the supervision of Prof. Ana Blandino. The main results, achieved during the Ph.D., were reported in this thesis.

Scientific activity

Original paper

Ricci, A., Cirlini, M., Calani, L., Bernini, V., Neviani, E., Del Rio, D., Galaverna, G., Lazzi, C. (2019). In vitro metabolism of elderberry juice polyphenols by lactic acid bacteria. Food Chemistry, 276, 692-699 (In press).

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[$] Both authors contributed equally to this manuscript

Poster presentation

Ricci, A., Cirlini, M., Bernini, V., Galaverna, G., Lazzi, M. Fermented fruit juices with dairy and plant isolates. 4th International Conference on Microbial Diversity. Bari October 24th-26th,2017.

Ricci, A. Fermented fruit juices: features influencing the antioxidant activity, the polyphenolic content and the volatile profile. 22nd Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology. Univeristy of Bolzan, Bolzan 20th-22nd, 2017.

Ricci, A. Exploiting lactic acid bacteria strains for novel fermented fruit juices. 21st Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology. University of Naples Federico II, Portici September 14th-16th, 2016.

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Oral presentation

Ricci A. Solid state fermentation a bioprocess for agri-food chain valorisation. Welcome meeting Missouri University "The University of Parma: where the food counts". Department of Food and Drug, University of Parma, Parma, Italy, 10th January, 2019.

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