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1 **APPLICATION OF LACTIC ACID FERMENTATION TO ELDERBERRY JUICE:**
2 **CHANGES IN ACIDIC AND GLUCIDIC FRACTIONS**

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22

23 **Abstract**

24 In the present work, the changes in sugar and organic acid profiles of elderberry juice fermented by
25 lactic acid bacteria were reported for the first time. Twelve different LAB strains belonging to
26 *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus casei* and *Lactobacillus*
27 *rhamnosus* species were applied for fermentation. All the samples obtained were analysed by GC-
28 MS technique, after fermentation and cold storage. Lactic acid was the main end product: its
29 concentration ranged between 10287 ± 585 mg/kg (juice started with *L. casei* 2246) and $27261 \pm$
30 4089 mg/kg (juice fermented with *L. rhamnosus* 1019), increasing its amount of about 40 fold
31 compared to the unstarted product. Malic and citric acids were extensively metabolized by LAB,
32 while glucose and fructose (27080 ± 4062 mg/kg and 27338 ± 4101 mg/kg respectively) were
33 almost unaffected suggesting a switch in the microbial metabolism with the use of organic acids
34 instead of sugars, probably due to the hostile environment represented by elderberry juice. To better
35 highlight the differences among the samples, Discriminant Analysis was performed revealing
36 differences between *L. casei* and all the other species tested especially due to the organic acid
37 concentrations.

38

39 *Key words*

40 Elderberry juice, lactic acid bacteria (LAB), fermentation, sugars, organic acids.

41

42 *Highlights*

- 43 - Changes in glucidic/organic acid fraction of fermented elderberry juice were monitored by
- 44 GC-MS;
- 45 - Lactic acid was the main end-product in fermented elderberry juice;
- 46 - No significant decrease of sugar concentration was observed;
- 47 - Malic acid was totally consumed by *L. casei*;
- 48 - Different metabolism of citric acid among strains was observed.

49

50

51 **1. Introduction**

52 Lactic acid bacteria (LAB) are widespread microorganisms universally applied as starter cultures in
53 fermented foods. By the way, since LAB are “Generally Recognized as Safe” (GRAS), lactic acid
54 fermentation can be applied to extend product shelf life and/or to improve physical and organoleptic
55 properties of vegetable products (Kohajdová, Karovičová, & Greifová, 2006).

56 Often, LAB are used in dairy fermentations to obtain fermented milk, cheese, yogurt, etc., but
57 consumption of these products may be somehow limited on the base of health or ethical reasons.
58 Indeed, in the last decades, an enlargement was observed in lactic acid fermentation of fruit and
59 vegetables with the aim to increase/improve nutritional and sensorial characteristics, to prolong the
60 shelf life, but also to obtain new functional foods thanks to lactic acid bacteria features (Parvez,
61 Malik, Kang, & Kim, 2006).

62 Upon LAB fermentation, lactic acid is the main end-product and its production is usually linked to
63 the metabolism of the different occurring sugars (such as glucose, fructose, sucrose, maltose,
64 galactose) (Mousavi, Mousavi, Razavi, Emam-Djomeh, & Kiani, 2011; Filannino et al., 2014),
65 although also organic acids, such as malic acid, can be used as carbon source for its production.
66 Indeed, it has been reported that in fruit, when the acidity is high and the environment hostile to
67 bacterial growth, LAB prefer to metabolise acids instead of conventional sugars, as a defensive
68 mechanism of stress response (Filannino et al., 2014).

69 Elderberry fruits (*Sambucus nigra* L., Caprifoliaceae) are berries produced by a shrub or a small
70 tree native of the Northern Hemisphere, particularly rich of sugars and acids, but also of secondary
71 metabolites such as polyphenols (Veberic, Jakopic, Stampar, & Schmitzer, 2009). Among sugars
72 the most concentrated are fructose and glucose, whereas sucrose occurs only in small amounts,
73 depending on the cultivar. Citric acid is the most abundant organic acid, followed by malic acid and
74 small amounts of shikimic and fumaric acid (Veberic et al., 2009).

75 Due to their dark colour, elderberry berries are mainly used to extract food colorants but also as
76 components in pharmaceutical preparations (Schmitzer, Veberic, Slatnar, & Stampar, 2010). In fact,
77 elderberry fruits are known to contain considerable health-promoting bioactive compounds, such as
78 anthocyanins, quercetins, and hydroxycinnamic acids, with strong antioxidant properties (Veberic et
79 al., 2009; Olejnik et al., 2016). Generally, elderberry fruits are not consumed as fresh fruits but are
80 squeezed to obtain a juice very rich in sugars and organic acids, as well as in polyphenols and
81 aromatic volatile molecules, with a strong typical sweet taste, flavoured and astringent (Jensen,
82 Christensen, Hansen, Jørgensen, & Kaack, 2000). Recently, lactic acid fermentation has been
83 successfully applied to obtain a fermented elderberry juice with an enriched flavour and increased
84 polyphenol content. In particular, it was demonstrated that different *Lactobacillus plantarum*,

85 *Lactobacillus casei* and *Lactobacillus rhamnosus* strains were able to boost the concentration of
86 specific molecules such as polyphenols and alcohols, terpenes and norisoprenoids associated with
87 the typical aroma of elderberry (Ricci et al., 2018a; Ricci et al., 2018b; Ricci et al., 2019).

88 The aim of this research was to analyse the effect of lactic acid fermentation on the glucidic and
89 organic acid fractions of elderberry juice investigating the compounds consumed and produced
90 during fermentation and refrigerated storage.

91

92 **2. Materials and methods**

93 *2.1 Chemicals*

94 Analytical standard of sugars and polyalcohols (glucose, fructose, arabinose, xylose, sucrose,
95 trehalose, glycerol, myo-inositol, sorbitol, and turanose), and of organic acids (lactic, malic, citric,
96 tartaric and glutaric acid) were all purchased from Sigma-Aldrich (Milan, Italy). Moreover,
97 trimethylchlorosilane (TMCS), hexamethyldisilazane (HMDS), dimethylformamide (DMF) and n-
98 hexane used for derivatization and extraction were also obtained from Sigma-Aldrich (Milan, Italy).

99 *2.2 Elderberry juice fermentation*

100 Elderberry juice used for fermentation experiments was obtained in a local market. The juice,
101 derived from berries cultivated under organic agriculture, was industrially pasteurized. The
102 following information were reported in the label: energy 38 kcal/100 mL, 6.5 % of sugars, 1 % of
103 proteins and 0 % of fats. The fermentation was carried out using different bacterial strains
104 pertaining to four species: *L. plantarum*, *L. casei*, *L. paracasei* and *L. rhamnosus* (Table 1).

105 To prepare the inoculum, all the strains considered were propagated in Man Rogosa Sharpe (MRS)
106 broth (Oxoid, Milan, Italy) at their specific growth temperature (30 °C for *L. plantarum*, and 37 °C
107 for *L. casei*, *L. paracasei* and *L. rhamnosus* species) until the concentration of 9.0 Log CFU/mL.
108 The microbial cultures were washed in Ringer's solution (Oxoid, Milan, Italy), and finally re-
109 suspended in sterile distilled water and each culture was inoculated to reach 7 Log CFU/mL. Then,
110 samples started with *L. plantarum* strains were incubated at 30 °C, and samples added of *L. casei*,
111 *L. paracasei* and *L. rhamnosus* strains at 37 °C, for 48 h. After fermentation, all samples were
112 stored at 4 °C for 12 days. At the same time, different aliquots of unstarted elderberry juice were
113 submitted to the same procedure as control samples. All the fermentation experiments were
114 performed in triplicate. The number of cultivable cells were monitored using plate count method
115 after the initial inoculum, the fermentation and at the end of the storage period.

116 The GC-MS analyses were conducted on control samples, fermented and stored samples in
117 duplicate. Moreover, also the not incubated juice was analysed.

118

119 *2.3 Derivatization and analysis of fermented elderberry juice: determination of organic acids and* 120 *sugars by GC-MS technique*

121 For the determination and analysis of sugars, polyalcohols and organic acids of fermented
122 elderberry juices the protocol described by Cirlini, Caligiani, & Palla in 2009 was adopted, with
123 slight modifications. Briefly, 10 μ l of each sample were added with 1 ml of internal standard
124 solution (turanose and glutaric acid 500 mg/kg) and evaporated to dryness under vacuum at 40 °C.
125 The residue was then dissolved with 0.5 ml of DMF and transferred into a 1.5 ml vial. The samples
126 were derivatized adding 0.2 ml of TMCS and 0.4 ml of HMDS and heating the solution at about 70
127 °C for 30 minutes. In this way, all the analytes of interest were transformed in their corresponding
128 trimethyl-silyl-ethers. The derivatized compounds were then extracted using 1 ml of n-hexane at
129 room temperature and, after that, injected into a GC-MS apparatus. This application allowed a
130 rapid sample preparation, without an extraction step, and at the same time allowed the simultaneous
131 determination of sugars (mono- and di-saccharides), polyalcohols, and organic acids.

132 All the analyses were performed on a Thermo Scientific Trace 1300 gas-chromatograph coupled to
133 a Thermo Scientific ISQ mass spectrometer equipped with electronic impact (EI) source. The
134 separation of analytes was achieved using a BP5MS (30 m x 0.25 mm, with 0.25 μ m film thickness,
135 SGE Analytical Science, Milan, Italy) capillary column using helium as carrier gas. A temperature
136 gradient was applied to the column starting from 60 °C, maintaining this value for 3 min, and
137 increasing the oven temperature of 20 °C/min until 280 °C. The final temperature was kept for 10
138 min, with a total run time of 24 min. The injector and auxiliary temperatures were set at 280 °C.
139 The injection was performed in split mode with a split ration of 1/20, injecting 1 μ l of sample. Full
140 scan mode was selected as the acquisition mode considering as m/z range of 40 - 500.

141 All the samples were derivatized and analysed in duplicate. The quantification of the identified gas-
142 chromatographic signals was performed using two selected internal standards, glutaric acid for the
143 organic acid fraction and turanose for sugars and polyalcohols, also. The analytical standards of the
144 identified organic acids, sugars and polyalcohols were used for the determination of their respective
145 response factors (RF) for quantitative analyses.

146

147 *2.4 Data elaboration*

148 All the data obtained from GC-MS analyses were statistically elaborated using the statistical
149 software SPSS ver. 23 (SPSS Inc., Chicago, IL). In particular, one way ANOVA was used to
150 compare the concentrations of organic acids, sugars and polyalcohols of the different fermented and
151 stored samples (fermented = 48 h, or stored = 14 days) compared to the relative control samples.
152 Analyses were performed using Tukey test and the results were considered significantly different
153 for values of $p < 0.05$. Moreover, discriminant analysis (DA) was applied in order to better
154 understand and describe differences and/or similarities among the considered strains.

155

156 **3. Results and discussion**

157 After 48 h of fermentation at the optimal growth temperature, all the tested strains were able to
158 grow of about two Log cycles, showing significant differences from the initial inoculum. Overall,
159 their vitality was maintained also during the 12 days of refrigerated storage (Table 2), in accordance
160 with our previous observations (Ricci et al., 2018a). Both fermented and stored samples were
161 analysed by GC-MS to investigate changes in glucidic and organic acid fractions.

162 First of all, unstarted elderberry juice was analysed to identify all the occurring components: a total
163 of 24 different gas-chromatographic signals were observed (Tables S1 and S2). The use of
164 hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) to obtain trimethylsilyl ethers of
165 polyalcohols and sugars is one of the most common derivatization method applied for their
166 determination in GC (Ruiz-Matute, Hernández-Hernández, Rodríguez-Sánchez Sanz, & Martínez-
167 Castro, 2011), and it is also suitable for organic acids. This kind of derivatization reaction allows to
168 recognize anomeric forms of all the mono-saccharides present as furanic and pyranosidic forms
169 (Sweeley, Bentley, Marita, & Wells, 1963). The identification of all the signals was then performed
170 by comparing the mass spectra obtained from elderberry juice analysis with the reference mass
171 spectra library (NIST 14). In particular, a match quality of 95-98% minimum was used as
172 identification criterion. In addition, identifications were confirmed by using analytical standards of
173 the identified components. Finally, the proper internal standards were selected and the response
174 factors (RFs) were calculated for each detected analyte in order to obtain a more precise
175 quantification. All the calculated RFs ranged between 0.8 and 1.2. The same analytical procedure
176 was then applied to all the fermented and stored samples.

177

178 *3.1 Characterization of the organic acid fraction*

179 Among organic acids, four main components were identified both in fermented elderberry juices, as
180 well as in control samples: lactic, malic, tartaric and citric acids (Table S1).

181 Lactic acid, produced by LAB during fermentation, was the most representative organic acid
182 formed. Its amount increased a lot after fermentation. In all the control samples the amount of lactic
183 acid ranged between 611 ± 55 mg/kg and 896 ± 30 mg/kg (Figure 1) while in fermented samples the
184 concentration ranged between 10287 ± 585 mg/kg (sample started with *L. casei* 2246) and $27261 \pm$
185 4089 mg/kg (sample fermented with *L. rhamnosus* 1019) (Figure 1). After 48 h of fermentation the
186 amount of lactic acid in all the samples resulted statistically different from the controls. After cold
187 storage (4 °C) lactic acid values did not decrease significantly in comparison with fermented
188 samples; only in two cases a reduction of lactic acid occurred, in particular when *L. plantarum*
189 1LE1 and *L. rhamnosus* 1019 were used (Figure 2, Table S1). On the contrary, with *L. casei* 2107
190 an increase of lactic acid was observed after storage. Lactic acid was found to be the main
191 fermentation end product also in other papers, focused on pomegranate, pineapple, carrot, tomato,
192 cherry, broccoli and papaya lactic acid fermentation (Mousavi et al., 2011; Filannino et al., 2013;
193 Filannino et al., 2014; Filannino, Bai, Di Cagno, Gobbetti, & Gänzle, 2015; Chen, Chen, Chen,
194 Zhang, & Chen, 2018). Its concentration depends on the matrix used for fermentation but also on
195 the LAB species and strains applied. Lactic acid can be produced by LAB from sugars metabolism.
196 However, this is not the only way in which LAB produce this metabolite. Indeed, also polyalcohols,
197 such as glycerol, or acids, such as malic acid, can be metabolized leading to the release of lactic
198 acid (Lahtinen, Ouwehand, Salminen & Von Wright, 2012). It is worth noting that lactic acid, and
199 more broadly organic acids, may act as bio-preservative even in combination with other
200 components such as carbon dioxide, diacetyl, hydrogen peroxide and bacteriocins (De Vuyst, &
201 Vandamme, 1994). Indeed, lactic acid and generally low pH damages both the cell wall and the cell
202 membrane, altering the membrane potential and the active transport (Davidson, 1997), thus leading
203 to energy depletion and cell death. Therefore, thanks to the high content of lactic acid produced by
204 LAB, the preservation of fermented juices could be favoured.

205 Among the other organic acids detected, malic acid was the most abundant in unfermented juice
206 showing an amount of 1050 ± 52 mg/kg, in agreement with the data reported by Veberic et al.
207 (2009), who observed a mean value of 1100 ± 30 mg/kg in black elderberries. After fermentation a
208 significant decrease of malic acid was observed in all the fermented samples compared to control
209 (Figure 1). In particular the three strains belonging to *L. casei* species completely converted malic
210 acid. The same concentrations observed after fermentation, or slight reductions were observed in all
211 the samples after storage (Figure 2), in agreement with the data reported by Filannino et al. (2013).

212 Indeed, most LAB can metabolize malic acid thanks to the malolactic enzyme, via decarboxylation
213 to lactate by a NAD⁺ and Mn²⁺-dependent malolactic enzyme (Filannino et al., 2014; Landete,
214 Ferrer, Monedero, & Zúñiga, 2013; Jyoti, Suresha, & Venkatesha, 2004). However, *L. casei* can
215 also degrade malic acid into pyruvate with a malic enzyme enabling their growth on malate as a
216 carbon source (Landete et al., 2013; Landete et al. 2010). Different studies based on fermented plant
217 products showed that the consumption of malate is correlated with an increase of pH, this
218 implementation giving an advantage to microbial cells, protecting them from the stress associated
219 with low pH (Filannino et al., 2014; Landete et al., 2013; Takanami, Kuribayashi, Osawa, &
220 Yoshida, 1991, Papadimitriou et al., 2016).

221 Citric acid showed a different trend depending on the LAB species used for fermentation. Also this
222 acid, as the malic one, was used as a carbon source by all *L. casei* strains employed for elderberry
223 juice fermentation. When both acids were present in the fermentation matrix, their simultaneous use
224 can occur by LAB, even if the extent of their relative conversion may be different (Fugelsang &
225 Edwards, 2007). Usually, the metabolism of citrate is coupled with the consumption of sugars or
226 other energy sources (Mortera, Pudlik, Magni, Alarcón, & Lolkemaa, 2013). In LAB, citrate can be
227 converted into oxaloacetate and acetate by a citrate lyase while oxaloacetate can be converted into
228 pyruvate. Pyruvate can be converted by LAB in the flavour compound acetoin (Mortera et al.,
229 2013), which was found in fermented elderberry juice (Ricci et al., 2018). *L. casei* strains used in
230 this work seemed to be more prone to use citric acid than all the other LAB considered (after 48 h
231 of fermentation). Conversely, in some cases (*L. plantarum* 1LE and 285 and *L. rhamnosus* 1019 and
232 2360) citric acid showed an increase after fermentation, as already reported by Bergqvist, Sandberg,
233 Carlsson, & Andlid (2005). After storage this trend was maintained, indeed *L. plantarum*, *L.*
234 *rhamnosus* and *L. paracasei* presented higher citric acid concentrations than *L. casei*.

235 Tartaric acid was also detected in fermented elderberry juice and in control. The metabolism of
236 tartaric acid is quite uncommon in bacteria and have been poorly investigated especially in wine.
237 Only two *Lactobacillus* species (*L. plantarum* and *L. brevis*) have been demonstrated to metabolize
238 tartaric acid to lactic acid, acetic acid, succinic acid, and CO₂ (Lahtinen, Ouwehand, Salminen &
239 Von Wright, 2012). The consumption of tartaric acid was reported before in different matrices such
240 as wine (Lahtinen, Ouwehand, Salminen & Von Wright, 2012) and fermented cherry juice (Ricci et
241 al., 2019). However, in this work the concentrations of tartaric acid did not change significantly
242 after fermentation and storage compared to the control.

243

244 3.2 Characterization of the glucidic fraction

245 Different polyalcohols, mono- and di-saccharides have been identified, quantified and monitored in
246 all the fermented and stored samples, i.e. glycerol, arabitol, sorbitol and myo-inositol
247 (polyalcohols), arabinose, levoglucosan, ribose, fructose, glucose, and xylose (mono-saccharides)
248 and sucrose and trehalose (di-saccharides) (Table S2). Glucose and fructose were the most abundant
249 sugars, while sucrose occurred in small amount. Overall, glucose and fructose concentrations in
250 unstarted juice (27080 ± 4062 mg/kg and 27338 ± 4101 mg/kg respectively) were comparable to
251 those occurring in elderberry fruits (Veberic et al., 2009).

252 Not significant changes were found monitoring the concentrations of these two compounds during
253 the fermentation and storage periods (Figure 3). Similar results were obtained by Filannino et al. in
254 2014, who did not find a significant variation in glucose and fructose amounts in cherry and
255 pineapple fermented juices compared to the control. These results support the hypothesis of a
256 switch in the metabolism of LAB with the use of organic acids instead of sugars due to the hostile
257 environment represented by elderberry and similar juices, as hypothesized in previous studies
258 (Filannino et al., 2014). Similarly, no significant differences in sugars and polyalcohols profiles
259 among the different fermented juices were noted, even in comparison with control samples. A
260 similar behaviour was observed after storage, underlying the good stability of the products, even if a
261 slight decrease in sorbitol content was observed in the samples fermented with *L. plantarum*, *L.*
262 *paracasei* and *L. rhamnosus* strains in comparison with the untreated juices. The consumption of
263 sorbitol seems to be related with the ability of strains to uptake it using an inducible specific
264 phosphotransferase system also observed in *L. casei* (Viana et al., 2000).

265

266 3.3 Statistical analyses

267 To better analyse all the data resulting from the GC-MS analyses, and to discriminate samples on
268 the basis of the different species used for fermentation, a discriminant analysis (DA) was
269 performed. DA analysis is a supervised multivariate method that can be applied when information
270 on sample characteristics are available and useful to create groups of samples. For this analysis,
271 samples were divided in 4 groups on the basis of the strains used for fermentation, and 16 variables
272 were used: in particular, for those compounds presenting more than one gas-chromatographic
273 signal, the total amount deriving from the sum of the single concentrations was considered (Table
274 S3).

275 Samples actually were clustered in 4 groups (Figure 4) on the basis of the strains used for
276 fermentation: groups 1, 2 and 4 (juices started with *L. plantarum*, *L. paracasei* and *L. rhamnosus*
277 strains, respectively) were positioned closely in a restricted area of the plot, showing not significant
278 differences among them, while samples fermented with *L. casei* strains (group 3) were clearly

279 separated from the others, on function 1. This distinction was mainly ascribed to the different
280 content of some organic acids and sorbitol. In particular, malic acid was undetectable in all the
281 samples started with *L. casei* strains while in the other samples values between 134 ± 56 mg/kg
282 (juices fermented with *L. paracasei* strains) and 702 ± 648 mg/kg (juices added with *L. plantarum*)
283 were measured. A similar behaviour was observed for citric acid which occurred at average
284 concentrations ranging between 254 ± 0 mg/kg and 506 ± 255 mg/kg in *L. paracasei* started
285 samples and in *L. plantarum* fermented juices, respectively, while citric acid was completely
286 consumed in samples started with *L. casei*. In addition, the concentration of sorbitol was higher in
287 *L. casei* fermented samples (2133 ± 345 mg/kg), similar to control samples (1966 ± 1261 mg/kg), in
288 comparison with the other samples (ranging from 195 ± 28 mg/kg of *L. paracasei* added samples to
289 372 ± 179 mg/kg of *L. rhamnosus* fermented juices).

290 On the base of DA results, it is worthy of note that the main observed differences between
291 fermented juices can be species dependent. So, the application of a supervised multivariate
292 statistical method allowed to highlight the differences and as a consequence the analogies among
293 the strains used for the fermentation, helping in the choice of the strain that better maintains the
294 initial characteristic of elderberry juice in term of sugar content and, on the other hand, that
295 improves the value of the product in term of formation of organic acids (i.e. lactic acid).

296

297 **4. Conclusions**

298 Thanks to this study we could highlighted different key points on the impact of lacto-fermentation
299 as on the metabolic behaviour, related to sugars and organic acid fractions, of LAB in elderberry
300 juice, a complex and stressful matrix. I) Lactic acid was produced; II) malic acid was metabolized
301 by all the LAB used in this work reaching its complete depletion after the employment of *L. casei*
302 strains; III) citric acid was another organic acid, preponderant in elderberry juice, which is
303 consumed by microorganisms, in particular by *L. casei* strains, which depleted it completely; IV)
304 sugars, such as glucose and fructose, really concentrated in fruits and juices, were not consumed by
305 LAB which notoriously used them as first carbon source if they are available in the substrate. From
306 these observations we can conclude that in this hostile substrate, LAB (twelve strains of different
307 species were tested) shift their metabolism, consuming organic acids instead of free sugars. Indeed,
308 some chemicals and physical parameters, such as pH, the concentration of sugars and organic acids,
309 make elderberry juice an hostile environment for microorganisms. To adapt, in these adverse
310 conditions, bacteria should adopt specific metabolic pathways, involved in the use of non-
311 conventional carbon source, in the exploitation of alternative substrates or in a global stress
312 response. For example the decarboxylation of malic acid, observed in high content in elderberry

313 juice, lead to the increase of intracellular pH and to the reducing power synthesis giving advantages
314 to microbial cells.

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414

Table 1: Lactic acid bacteria used as starter for lactic acid fermentation.

Species	Strain number	Source
<i>Lactobacillus plantarum</i>	285 ^a	Brazilian cheese
	1LE1 ^b	Pineapple
	POM1 ^b	Tomato
<i>Lactobacillus paracasei</i>	4186 ^a	Pecorino cheese
<i>Lactobacillus casei</i>	2107 ^a	Grana Padano cheese
	2240 ^a	Parmigiano Reggiano cheese
	2246 ^a	Parmigiano Reggiano cheese
<i>Lactobacillus rhamnosus</i>	1019 ^a	Parmigiano Reggiano cheese
	1473 ^a	Parmigiano Reggiano cheese
	2140 ^a	Grana Padano cheese
	2178 ^a	Grana Padano cheese
	2360 ^a	Grana Padano cheese

^a Food Microbiology Unit, Department of Food and Drug, University of Parma

^b Gently donated by University of Bari

Table 2: LAB strains growth ability after 48 hours and vitality after storage. Letters (a-c) indicate significant difference ($p < 0.05$) among T₀ , 48 hours and 14 days within the same row.

	T ₀	48 Hours	14 Days
<i>L. plantarum</i>			
1LE1	7.21 ± 0.03 ^a	9.25 ± 0.07 ^b	9.24 ± 0.02 ^b
285	7.43 ± 0.03 ^a	9.46 ± 0.08 ^b	9.33 ± 0.02 ^b
POM1	7.13 ± 0.03 ^a	9.38 ± 0.05 ^b	9.29 ± 0.04 ^b
<i>L. paracasei</i>			
4186	7.39 ± 0.02 ^a	9.31 ± 0.12 ^b	9.38 ± 0.05 ^b
<i>L. casei</i>			
2107	7.41 ± 0.01 ^a	9.42 ± 0.07 ^b	9.55 ± 0.03 ^b
2240	7.40 ± 0.09 ^a	9.40 ± 0.10 ^b	9.36 ± 0.08 ^b
2246	7.10 ± 0.08 ^a	8.98 ± 0.05 ^b	8.85 ± 0.11 ^b
<i>L. rhamnosus</i>			
1019	7.26 ± 0.03 ^a	9.22 ± 0.15 ^b	9.13 ± 0.14 ^b
1473	7.03 ± 0.05 ^a	9.37 ± 0.05 ^b	9.01 ± 0.04 ^c
2140	6.99 ± 0.02 ^a	9.21 ± 0.03 ^b	9.12 ± 0.15 ^b
2178	7.21 ± 0.08 ^a	9.19 ± 0.02 ^b	9.11 ± 0.07 ^b
2360	7.47 ± 0.01 ^a	8.90 ± 0.10 ^b	8.48 ± 0.05 ^c

Figure 1

Figure 1: Trend of organic acids (mg/kg) in samples analysed after the fermentation step (48 h): malic acid (▨), tartaric acid (▩), citric acid (▧) and lactic acid (●).

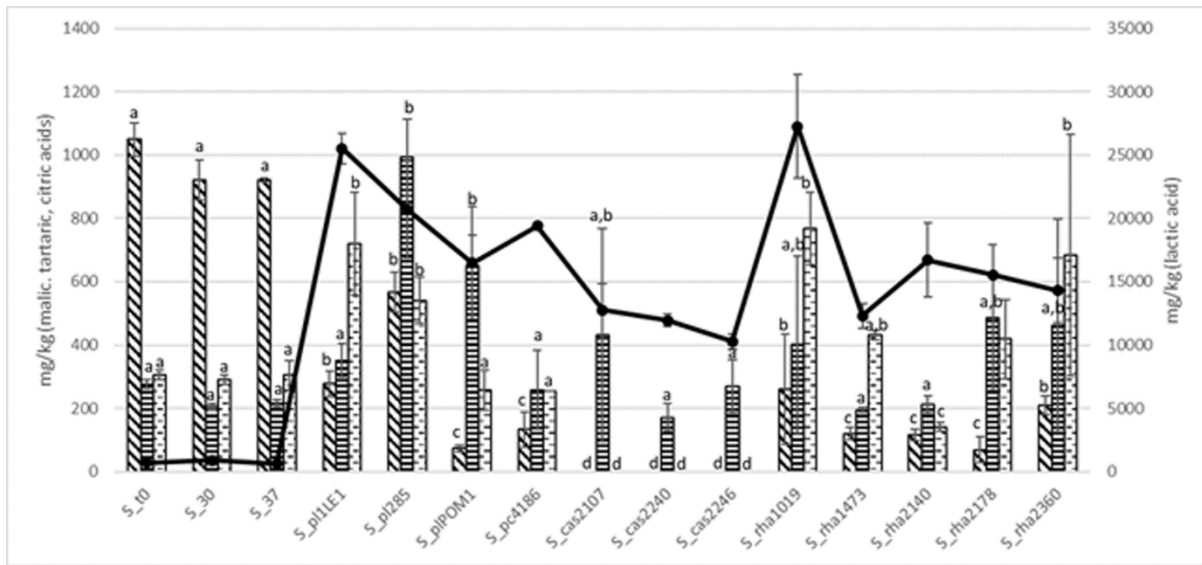


Figure 2

Figure 2: Trend of organic acids (mg/kg) in samples analysed after the cold storage (14 days at 4°C): malic acid (▨), tartaric acid (▩), citric acid (▧) and lactic acid (—)

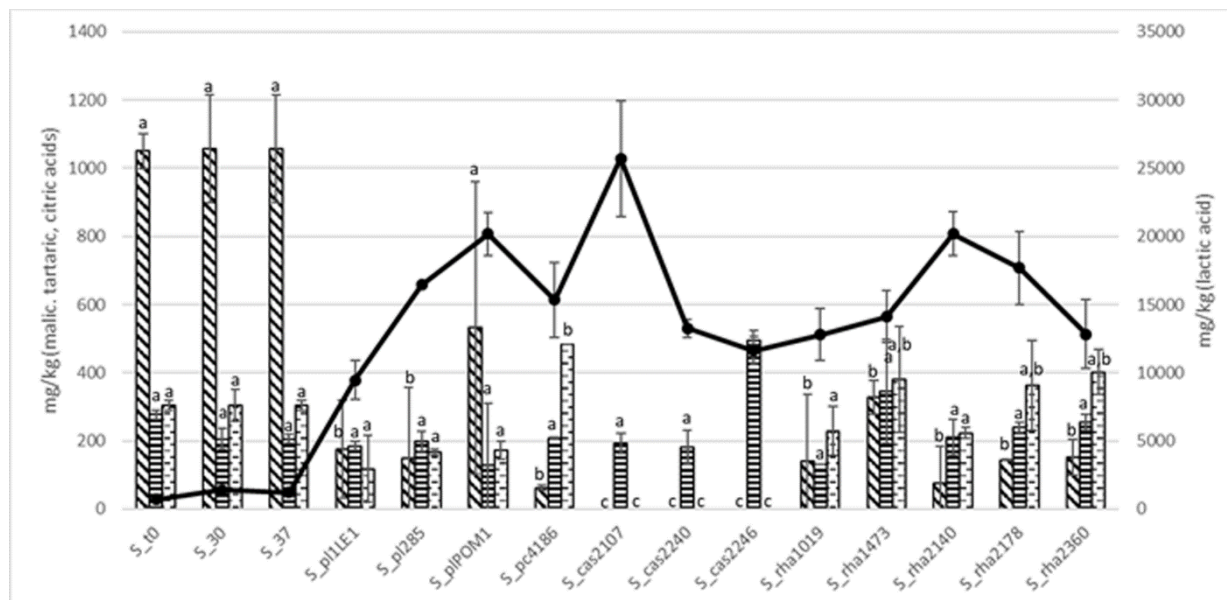


Figure 3: Average trend of main sugars, fructose (▣) and glucose (▢), in samples analysed before (contr) and after fermentation (48 h), as after the cold storage (14 days) (mg/kg).

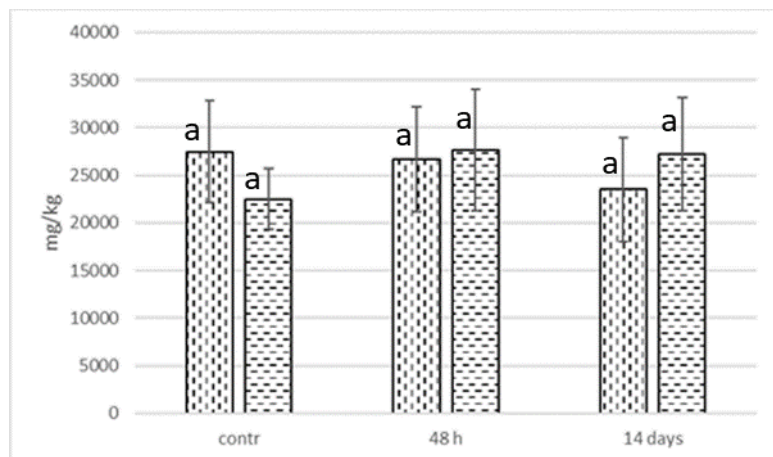
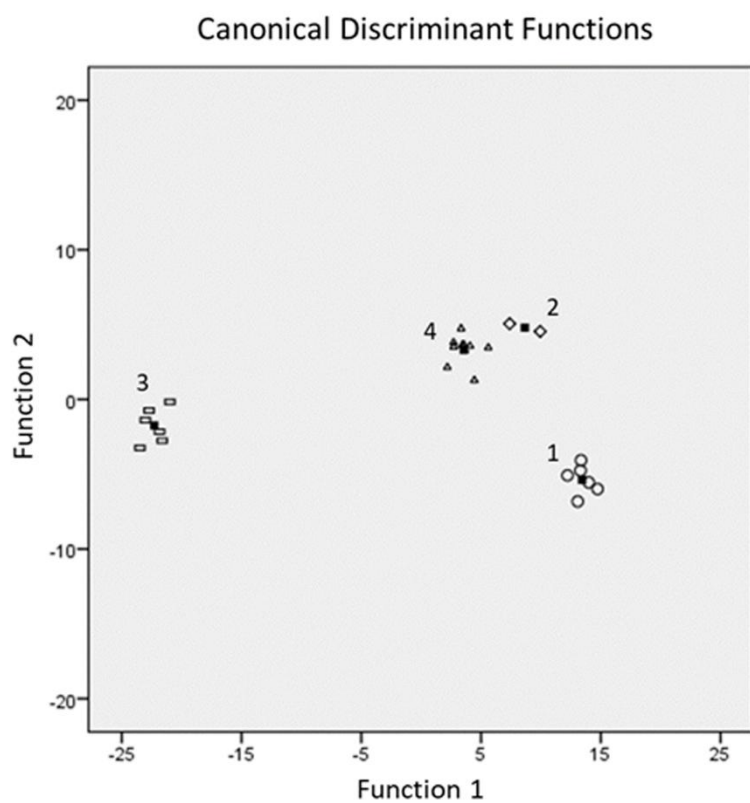


Figure 4

Figure 4: DA plot of Function 1 versus Function 2, obtained using the results of the GC-MS analyses for fermented samples: group 1 (juices started with *L. plantarum*, ○), group 2 (juices started with *L. paracasei*, ◇), group 3 (juices started with *L. casei*, ◻), and group 4 (juices started with *L. rhamnosus*, ▲).



Supplementary Material

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Please check the following as appropriate:

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
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