Application of lactic acid fermentation to elderberry juice: Changes in acidic and glucidic fractions

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

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Abstract

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

Key words

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

Highlights

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

2.1 Chemicals

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

2.2 Elderberry juice fermentation

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

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

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

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

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

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

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

2.4 Data elaboration

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

3. Results and discussion

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

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

3.1 Characterization of the organic acid fraction

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

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

Among the other organic acids detected, malic acid was the most abundant in unfermented juice showing an amount of 1050 ± 52 mg/kg, in agreement with the data reported by Veberic et al. (2009), who observed a mean value of 1100 ± 30 mg/kg in black elderberries. After fermentation a significant decrease of malic acid was observed in all the fermented samples compared to control (Figure 1). In particular the three strains belonging to *L. casei* species completely converted malic acid. The same concentrations observed after fermentation, or slight reductions were observed in all 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 Mn^{2+}-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 CO_2 (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*

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

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

3.3 Statistical analyses

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

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

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

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

4. Conclusions

Thanks to this study we could highlighted different key points on the impact of lacto-fermentation as on the metabolic behaviour, related to sugars and organic acid fractions, of LAB in elderberry juice, a complex and stressful matrix. I) Lactic acid was produced; II) malic acid was metabolized by all the LAB used in this work reaching its complete depletion after the employment of *L. casei* strains; III) citric acid was another organic acid, preponderant in elderberry juice, which is consumed by microorganisms, in particular by *L. casei* strains, which depleted it completely; IV) sugars, such as glucose and fructose, really concentrated in fruits and juices, were not consumed by LAB which notoriously used them as first carbon source if they are available in the substrate. From these observations we can conclude that in this hostile substrate, LAB (twelve strains of different species were tested) shift their metabolism, consuming organic acids instead of free sugars. Indeed, some chemicals and physical parameters, such as pH, the concentration of sugars and organic acids, make elderberry juice an hostile environment for microorganisms. 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. For example the decarboxylation of malic acid, observed in high content in elderberry

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

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

Species	Strain number	Source
Lactobacillus plantarum	285[a]	Brazilian cheese
	1LE1[b]	Pineapple
	POM1[b]	Tomato
Lactobacillus paracasei	4186[a]	Pecorino cheese
Lactobacillus casei	2107 [a]	Grana Padano cheese
	2240 [a]	Parmigiano Reggiano cheese
	2246 [a]	Parmigiano Reggiano cheese
Lactobacillus rhamnosus	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 donned by University of Bari

Table 2

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
L. plantarum			
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}
L. paracasei			
4186	7.39 ± 0.02^{a}	9.31 ± 0.12^{b}	9.38 ± 0.05^{b}
L. casei			
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}
L. rhamnosus			
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 ( ).

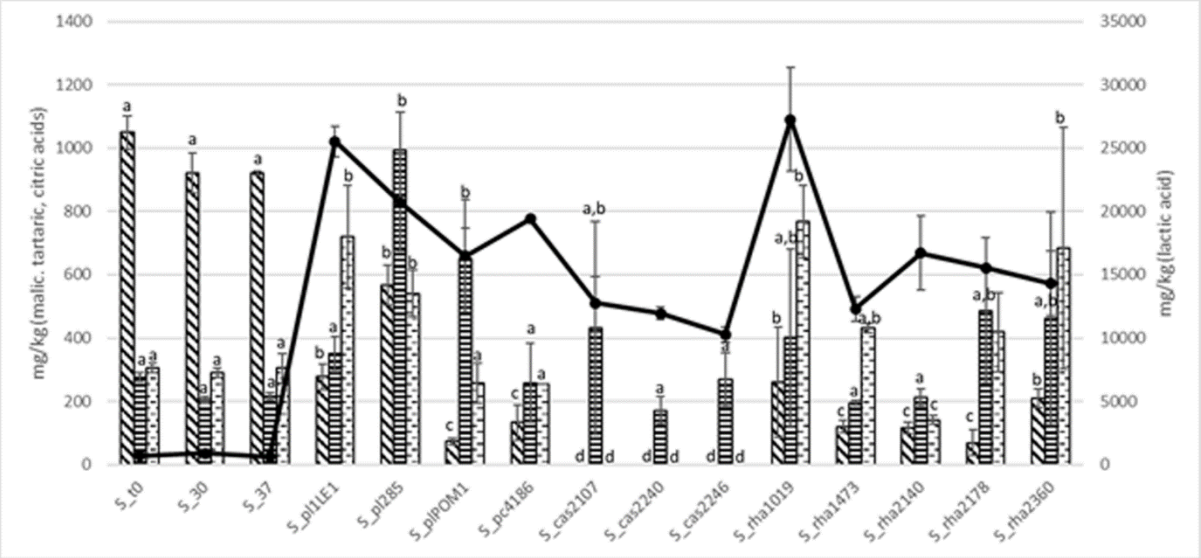


Figure 2

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 ( )

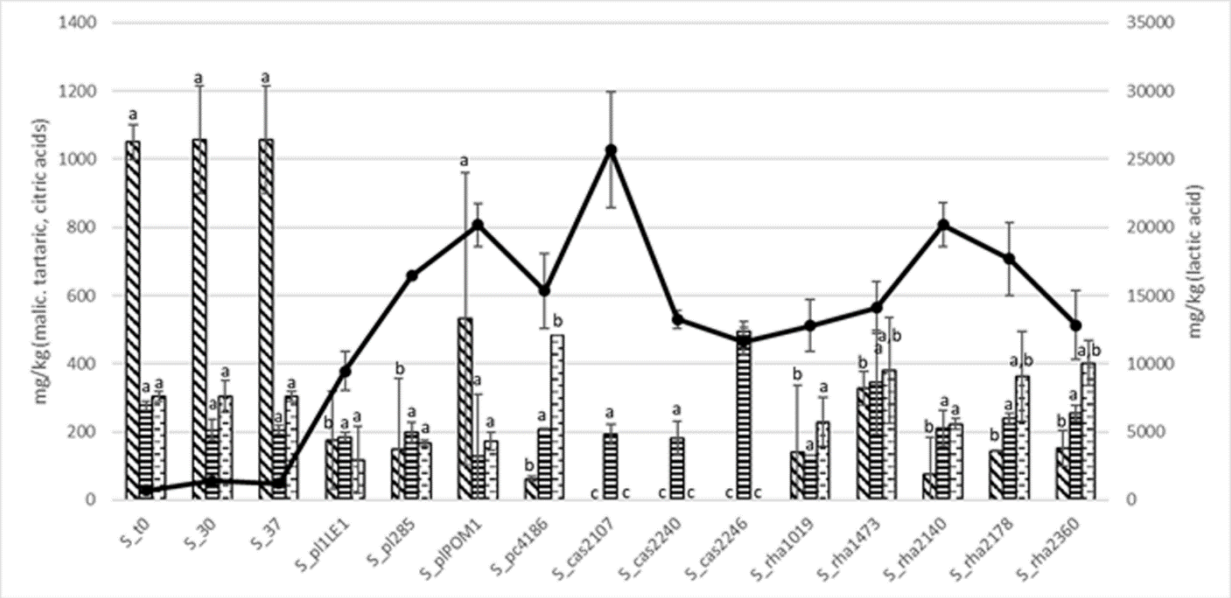


Figure 3

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).

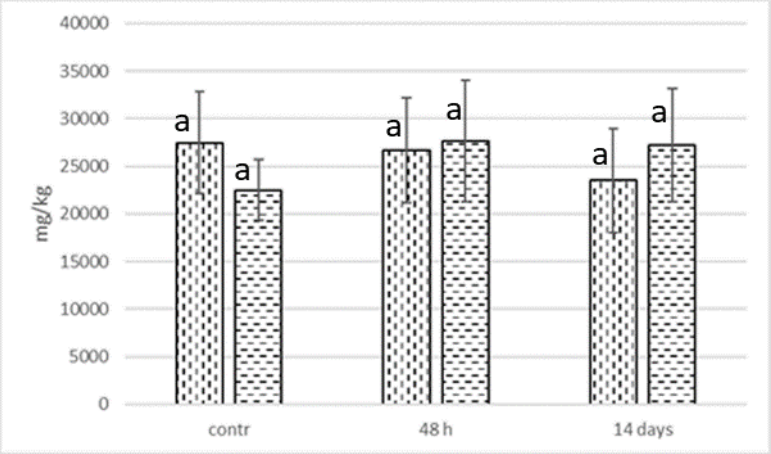
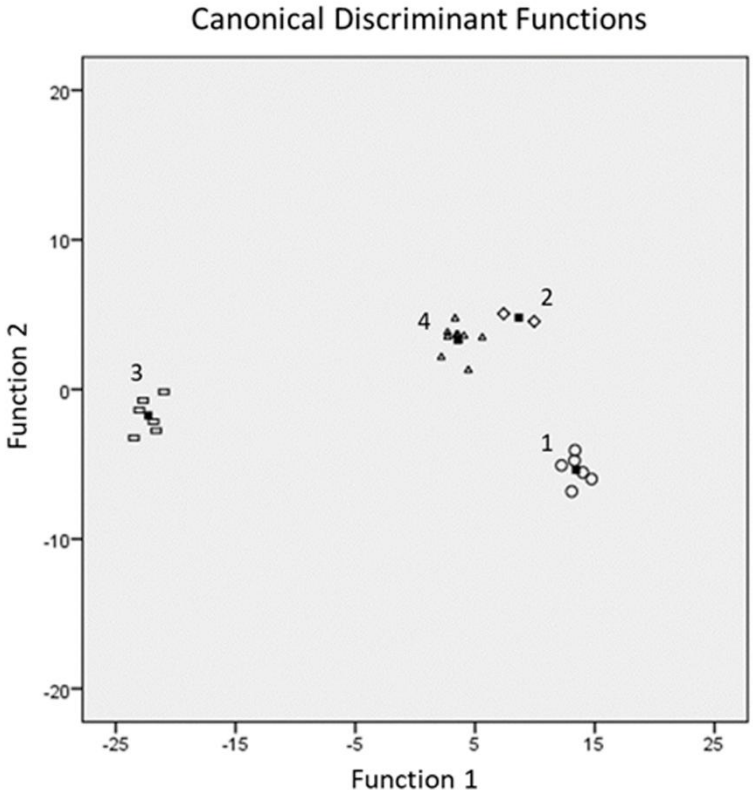


Figure 4

Figure 4: DA plot of Function1 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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- o 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.

- o This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

- o The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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