

1 **Co-fermentation of onion and whey: a promising synbiotic combination**

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17 **ABSTRACT**

18 Juice from three different onion varieties was mixed with sweet whey and used as growth
19 substrate for four lactic acid bacteria strains, isolated from agri-food by-products, to
20 evaluate the possibility to exploit such substrates, known to be rich in bioactive
21 molecules, as fermented drinks for human consumption. Results show good growth
22 performance for *Lactobacillus fabifermentans*, *L. plantarum* and *Streptococcus*
23 *macedonicus*. On the contrary *S. thermophilus* did not grow in the mixture while *S.*
24 *macedonicus* did not develop in pure onion juice. After 48 h the overall sugar content
25 decreased significantly. In particular, glucose was not utilized while inulin was completely
26 preserved. Moreover, MS/MS analysis revealed the presence of the rare trisaccharide
27 lactosucrose.

28 In the light of these considerations, the formulation obtained may be considered a potential
29 synbiotic product with pleasant taste and beneficial effects for consumers and also an eco-
30 friendly solution to convert an agro-food by-product into value added products.

31
32 *Keywords:* prebiotic, lactosucrose, *Lactobacillus*, *Streptococcus*, functional food

33
34 **Highlights**

- 35 • Onion juice and whey were used for growth of *Lactobacillus* and *Streptococcus*
36 strains
- 37 • Lactosucrose was produced by bacteria during growth in onion juice and whey
- 38 • Inulin present in onion juice remained in the medium after bacterial growth

39

40 **1. Introduction**

41

42 Currently, the research of new functional foods is focused on synbiotic obtained by
43 combining probiotics and prebiotics in order to further improve human health benefits
44 (Pandey, Naik, & Vakil, 2015; Krumbeck, Maldonado-Gomez, Ramer-Tait, & Hutkins,
45 2016).

46 Lactic acid bacteria (LAB) are the most widely used microorganisms for production
47 of fermented foods (Kumar, Vijayendra, & Reddy, 2015) for their capability to enhance
48 shelf-life, safety, organoleptic and nutritional properties (Lante, Nardi, Zocca, Giacomini,
49 & Corich, 2011). They are widely recognized for their beneficial effects towards
50 gastrointestinal diseases, human immune system, lactose intolerance, allergies and fungal
51 infections (Masood, Qadir, Shirazi, & Khan, 2011) as a consequence of their metabolic
52 activities, immunomodulation, and interaction with the intestinal microbiota (Parvez,
53 Malik, Ah Kang, & Kim, 2006; De Almada, De Almada, Martinez, & De Souza Sant'Ana,
54 2015). The key role of LAB in vegetable fermentation has been definitely established
55 (Tamang, Watanabe, & Holzapfel, 2016). During growth, microbial metabolism transforms
56 plant material leading to the production of bioactive compounds and/or improve the final
57 pleasantness of the foods (Di Cagno, Coda, De Angelis, & Gobbetti, 2013). Spontaneously
58 fermented foods (Pogačić et al., 2010; Paramithiotis, Doulgeraki, Karahasani, & Drosinos,
59 2014; Paramithiotis, Kouretas, & Drosinos, 2014; Lucena-Adrós, & Ruiz-Barba, 2016) or
60 even by-products from food production (Bovo et al. 2012; Favaro, Corich, Giacomini,
61 Basaglia, & Casella, 2013) contain autochthonous microbial communities that constitute
62 the source for the isolation of new potentially interesting bacteria and yeasts.

63 Onion (*Allium cepa* L.), which is taxonomically included in the *Liliaceae* family, is
64 a vegetable whose cultivation is widespread worldwide and accounts for more than 88
65 million t produced in 2014 (FAO, 2017). Compounds from onion have been reported to
66 have a range of health benefits including anticarcinogenic, antiplatelet, antithrombotic,
67 antiasthmatic and antibiotic activities (Griffiths, Trueman, Crowther, Thomas, & Smith,
68 2002). Onions contain also prebiotic substances such as fructooligosaccharides (FOS) and
69 inulin (Sangeetha, Ramesh, & Prapulla, 2005) which are indigestible to humans enzymes
70 but promote growth and activity of bacterial strains and simultaneously enhance health
71 benefits in the large intestine (Al-Sheraji et al., 2013). Moreover, lactic acid fermentation
72 can reduce the typical pungency flavor of raw onions leading to a more favourable product
73 for the consumers (Roberts & Kidd, 2005).

74 Sweet whey is the main by-product of cheese production, a highly polluting material
75 produced in high amounts, since only about 10% of the milk is transformed in cheese
76 (Ryan & Walsh, 2016). It is rich in fermentable sugars (ca. 5% lactose) and contains
77 numerous bioactive compounds (Madadlou & Abbaspourrad, 2016).

78 The present work is aimed at developing a new functional food enriched in
79 prebiotics and lactic acid bacteria by co-fermenting onion juices and sweet milk whey with
80 *Lactobacillus* and *Streptococcus* strains. After evaluating microbial growth performance in
81 juices of different onion varieties, the characteristics of the fermented products have been
82 investigated in order to formulate a potential synbiotic product that, besides being
83 beneficial for consumers, can convert an agro-food by-product into value added products.

84

85 **2. Materials and methods**

86

87 **2.1 Strain selection and culture conditions**

88

89 The lactic acid bacteria (LAB) used in this study were from our laboratory collection,
90 namely *Lactobacillus fabifermentans* T30PCM01 (DSM 28391) (Treu, Vendramin, Bovo,
91 Giacomini, & Corich, 2014) and *L. plantarum* T30PCM38 (DSM 28393) (Campanaro et
92 al., 2014) isolated from grape marcs (Maragkoudakis et al., 2013), *Streptococcus*
93 *macedonicus* 33MO (Vendramin et al., 2014) and *S. thermophilus* TH1436 (Treu et al.,
94 2014) isolated from dairy products.

95 Lactobacilli were grown in de Man, Rogosa and Sharpe (MRS) broth (Oxoid) at 30°C
96 while streptococci were cultivated in M17 medium (Oxoid) supplemented with 0.5%
97 lactose at 37°C. Growth in liquid media was measured by optical density at 600 nm
98 (OD₆₀₀).

99 In order to use the same amount of bacteria for each experiment, Cryobeads, porous beads
100 intended as carriers to support the viability of microorganisms during storage (Thermo
101 Fisher Scientific, Rodano, IT) were prepared according to manufacturer's instruction using
102 stationary phase cultures and stored at -80° C. The number of bacterial cells adherent to
103 each Cryobead was verified cells by plate count to be 10⁷. One bead was used as inoculum
104 for each experiment by transferring it into the growth substrate.

105

106 **2.2 Preparation of onion juices**

107

108 Onions from red, white and yellow varieties (RO, WO and YO, respectively) were

109 purchased in a local market and stored at 4 °C in the dark until required. Onions were
110 peeled of their dry outer skin, de-stemmed and finally processed in a centrifugal juicer
111 (Moulinex JU655, Groupe SEB, Ecully, France) to obtain juice (Tinello & Lante, 2017).
112 After pH measurement, juices were centrifuged at 3,864 g for 15 min at 4 °C, filtered
113 through a Millipore 0.45 µm filter membrane (Merck Millipore, Billerica, MA, USA) and
114 stored at -20 °C in the dark.

115

116 ***2.3 Fermentation of onion juice***

117

118 Microbial cultures were grown in 15-ml sterile falcon tubes without agitation. Each strain
119 was inoculated into 10 mL of raw onion juice at the final concentration of 10⁶ cells/mL by
120 adding one Cryobead to each tube. Samples of raw onion juice without inoculum were used
121 as control.

122 Tubes were incubated at 30 °C for lactobacilli and 37 °C for streptococci. Each experiment
123 was repeated three times. After 96 h samples were collected for microbiological and
124 chemical analyses.

125

126 ***2.3 Co-fermentation of onion and whey***

127

128 Milk whey powder (Lactalis, Laval, France) was dissolved into deionized water to 20% w/v
129 in 250-mL flasks and pasteurized for 15 min at 72 °C (Maragkoudakis et al., 2016).

130 Fermentation media were prepared by mixing sweet whey with onion juice at 1:1 ratio.

131 Each strain was inoculated into the mixture at a concentration of 10⁶ cells/mL and incubated

132 for 48 hours at 30 °C or 37 °C, depending on the species. One sample of medium without
133 inoculum was used as negative control. Each experiment was repeated three times.

134

135 ***2.4 Microbiological analyses***

136

137 Viable cell counts were determined by the spread plate method. Samples were diluted in
138 sterile saline solution (0.9% NaCl w/v) and spread on plates. Plate Count Agar (Oxoid) was
139 used for total bacterial counts, incubated at 30 °C for 24 h.

140

141 ***2.5 High performance liquid chromatography (HPLC) analysis of sugars***

142

143 Sugars (fructose, galactose, glucose, inulin, and sucrose) contents were determined by
144 HPLC analysis using a SpectraSYSTEM™ UV6000LP HPLC system (Thermo Finnigan,
145 San Jose, CA, USA) with diode-array detection and an Aminex HPX-87C anion exchange
146 column (Bio-Rad, Hercules, CA) in accordance with the method proposed by Kagkli,
147 Corich, Bovo, Lante, & Giacomini (2016). Before injection into the column, samples were
148 filtered through Millipore 0.22 µm filter membranes (Merck Millipore, Billerica, MA,
149 USA). The mobile phase was deionized water, the temperature of analysis was 85°C and
150 the flow rate was 0.6 mL/min. Sugars were identified by comparing their retention times
151 with those of commercial standards.

152

153 ***2.6 Tandem mass spectrometry (MS/MS) analysis***

154

155 Further analysis of the peaks collected from the HPLC elution of the fermented mixtures of
156 sweet whey and yellow onion juice (YOW) with *L. fabifermentans*, *L. plantarum* and *S.*
157 *macedonicus* (retention time of 9.02 min) was made by MS/MS analysis using a Thermo
158 Scientific™ LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap Mass Spectrometer system
159 (Thermo Fisher Scientific, Waltham, MA, USA) with Fourier Transform (FT) detector.
160 Prior to analysis the sample was dissolved in methanol/0.1% v/v formic acid (1:1 v/v). The
161 flow rate of the constant infusion was 3 µL/min. The capillary voltage and temperature
162 were 4 kV and 200 °C, respectively. Identification of the compounds composing the peak
163 was performed according to the mass-to-charge ratio (m/z) of its corresponding fragment
164 ions.

165

166 **2.7 Statistical analysis**

167

168 Data, which were presented as means ± standard deviation (SD) of three replicates, were
169 subjected to one-way analysis of variance (ANOVA), after verifying the normal distribution
170 and homogeneity of variance, using the PROC GLM of SAS (Statistical Analysis System,
171 2013). The model included the onion cultivars and LAB strains as fixed effects. Differences
172 among means with $P \leq 0.05$ were accepted as representing statistically significant
173 differences in accordance with the Tukey's multiple range test.

174

175

176 **3. Results and discussion**

177

178 **3.1 Evaluation of onion juices**

179

180 Firstly, WO, RO and YO juices were assessed for their suitability as substrate for LAB
181 growth, since it is well known that these microbes have complex nutritional requirements
182 (Ozcelik et al. 2016).

183 Four strains were chosen: *L. fabifermentans* T30PCM01 and *L. plantarum* T30PCM38
184 isolated from grape marcs; *S. macedonicus* 33MO and *S. thermophilus* TH1436 coming
185 from dairy products. LAB belonging to the genera *Lactobacillus* and *Streptococcus* are
186 widely recognized for their health benefits as probiotics (Parvez, Malik, Ah Kang, & Kim,
187 2006; Masood, Qadir, Shirazi, & Khan, 2011. At species level *L. plantarum* (Seddik et al.,
188 2017, Fijan, 2014) and *S. thermophilus* (Uriot et al., 2017, De Vries, Vaughan, Kleerebezem
189 & de Vos, 2006) are known to include strains with probiotic properties. *L. fabifermentans* is
190 a recently introduced species, but it is closely related to *L. plantarum* (De Bruyne et al.,
191 2009). Several data are also available on probiotic characteristics of *S. macedonicus* strains
192 (Zoumpopoulou et al., 2008). We wanted to check primarily if these strains could act as
193 starters in the mixture and, in case of positive response, further work will be devoted to the
194 study of their probiotic potential.

195 Regarding pH (Table 1), all onion juices showed similar, slightly acidic values. The
196 juices were also rich in sugars but with significantly different ($P \leq 0.001$) contents (Table
197 1). In details, the juices from RO showed about twice the sugar content of WO, while YO
198 had intermediate values. Moreover, RO juices showed the highest content of fermentable
199 monosaccharides, especially fructose and glucose, which represented 39% and 47%
200 respectively of total sugars determined by HPLC analysis, while YO juice had the highest

201 content of sucrose. Interestingly, inulin, which is a prebiotic used in human diets for its
202 known health benefits (Peshev, & Van den Ende, 2014, Al-Sheraji et al., 2013) was present
203 in YO sample at double concentration with respect to RO and WO. Galactose content was
204 negligible in all juices.

205

206 **3.1 Fermentation of onion juices**

207

208 In order to verify the capability of onion juice to support the growth of the selected strains,
209 they were inoculated in RO, WO e YO juices and incubated for 96 h at 30 °C for
210 lactobacilli and 37 °C for streptococci. Streptococci did not grow on any of the onion
211 juices. This could be ascribed to their inability to use the sugars present, but it has also been
212 reported the possible toxic activity of onion for some species of *Streptococcus* (Kim, 1997).
213 Both lactobacilli grew very well in all the juices increasing their population to more than
214 10^8 cfu/ml (Table 2). *L. fabifermentans* did not show significant differences among the
215 different onion types while *L. plantarum* grew better in WO and YO.

216 Both lactobacilli lowered the pH between 3.41 and 3.72 (Table 2), a value that very
217 well hampers the growth of bacterial pathogens.

218 Regarding total sugars, their content significantly decreased ($P \leq 0.001$) after
219 bacterial growth in all onion juices (Table 2). In details, lactobacilli consumed the sugars in
220 a similar manner by significantly decreasing ($P \leq 0.001$) the content of monosaccharides
221 whose averaged percentage reductions of fructose, galactose and glucose were higher in
222 YO (25%, 39%, 39%, respectively) compared with RO (18%, 19%, 28%, respectively) and
223 WO (26%, 27%, 21%, respectively) (Table 2). This could be the consequence of the more

224 efficient growth of *L. plantarum* in YO with respect to RO and WO (almost 3X higher cell
225 counts).

226 Instead, lactobacilli did not use sucrose, since its values remained always unchanged
227 (Table 2). Even if *L. plantarum* is generally able to use this sugar (Gänzle & Follador,
228 2012), in the tested onion juices lactobacilli preferred to use other sugars. Hence they did
229 not need to activate sucrose usage because of the good availability of its constituting
230 monosaccharides, glucose and fructose.

231 Interestingly, inulin was completely preserved in all fermented onion juices (Table
232 2).

233

234 ***3.1 Co-fermentation of onion juice and whey***

235

236 Sweet whey was mixed with onion juices and used as growth substrate for LAB. Among
237 those tested, YO juice was chosen for this study, since it contained the highest amounts of
238 inulin and allowed the growth of the highest number of bacteria (Table 1, 2).

239 Whey provides mainly lactose, organic nitrogen from whey proteins and mineral salts.
240 Therefore streptococci, that did not grow in onion juices alone, could have a chance to
241 develop in the mixture, hence they were tested again. *L. fabifermentans*, *L. plantarum*, *S.*
242 *macedonicus* and *S. thermophilus* were added singularly to the YOW mixture and
243 incubated for 48 h at 30 °C for streptococci and 37 °C for lactobacilli.

244 Among streptococci, *S. macedonicus*, that did not grow in pure onion juice, developed very
245 well in the mixture (Table 3), evidently taking advantage from lactose as energy source and
246 probably also from organic nitrogen. Instead, *S. thermophilus* did not grow in the mixture.

247 Considered that this bacterium was isolated from dairy environment, the absence of growth
248 should evidently be attributed to some substances present in the onion juice that ~~resulted~~
249 were toxic for the strain.

250 Lactobacilli grew on the mixture (Table 3) at levels comparable with those obtained
251 in the sole onion juice (Table 2). All three strains lowered the pH to 3.9 (Table 3), a value
252 on average only slightly higher than that reached in onion juices alone (Table 2), but still
253 effective for pathogens control.

254 Regarding sugars, their overall content decreased significantly ($P \leq 0.01$), but not
255 dramatically, after fermentation (Table 3). In details, fructose was consumed mainly by *L.*
256 *plantarum* (36%) and only weakly by *S. macedonicus* (16%). In RO juice alone, lactobacilli
257 consumed the same amount of fructose (Table 2) while in the mixture *L. fabifermentans* did
258 not use it at all (Table 3).

259 It is well know that the species used here are able to utilize lactose. Galactose
260 content significantly increased ($P \leq 0.001$) after fermentation, evidently as consequence of
261 lactose degradation inside the cells (Table 3) while the presence of galactose in the medium
262 indicates that the strains are not able to metabolize this monosaccharide.

263 Interestingly, glucose, that was used during growth in onion juices (Table 2), was
264 not utilized by any of the bacteria in the mixture (Table 3), since it was found completely in
265 the liquid after fermentation.

266 Also inulin was completely preserved (Table 3). This in an important result since, in
267 view of a functional food preparation, it is very important that this prebiotic material could
268 remain intact.

269 Sucrose was not used by bacteria in sole juice fermentation (Table 2). In the mixture

270 this molecule has been eluted at the same retention time (9.02 min) of lactose, as confirmed
271 by HPLC and MS/MS analysis (data not shown), therefore it was not possible to establish
272 the level of utilization of these two sugars. In this regard, the percentage reduction of the
273 chromatographic peak containing lactose and sucrose was around 16% for all the strains
274 (Table 3). Moreover, the MS/MS analysis of the HPLC peak corresponding to YOW with *L.*
275 *fabifermentans*, *L. plantarum* and *S. macedonicus* at the retention time of 9.02 min revealed
276 in the m/z range of 400-600 the presence of lactosucrose (O- β -D-galactopyranosyl-(1,4)-O-
277 α -D-glucopyranosyl-(1,2)- β -D-fructofuranoside) (Fig.1S). This rare trisaccharide is a kind
278 of indigestible carbohydrate with a high quality taste similar to sucrose, it has good
279 prebiotic effects, promotes intestinal mineral absorption and it is selectively utilized by
280 intestinal *Bifidobacterium* (Al-Sheraji et al. 2013). This molecule is formed inside the cell
281 from lactose and sucrose by enzymatic transglycosylation (Kawase, Pilgrim, Araki, &
282 Hashimoto, 2001). A number of bacteria have been reported to produce this molecule, but
283 to date no lactic acid bacteria, although the presence of levansucrase has been reported in
284 several lactobacilli (Teixeira, McNeill, & Gänzle, 2012).

285 The enzymatic reaction that produces lactosucrose generates also a glucose
286 molecule from the cleavage of sucrose. Our data show that glucose levels did not decrease
287 at all in the mixtures after fermentation (Table 3), while in the sole juice glucose was used
288 by the bacteria (Table 2). This different behaviour could be determined by the generation of
289 internal glucose from production of lactosucrose that blocks that need to import external
290 glucose.

291 The new synbiotic combination, which could be designed for human consumption as soft
292 drink, could not only have beneficial effects on human health by combining potential

293 probiotics and prebiotics, but could also meet consumer acceptability. In this regard, no
294 off-flavors and off-odors have been detected (data not shown) in the co-fermented product
295 which was also tasted in our laboratory selecting odor and flavor as the most important
296 quality characteristics.

297

298 **4. Conclusions**

299

300 In this study we demonstrate that onion juice and whey together allow abundant growth of
301 *L. fabifermentans*, *L. plantarum* and *S. macedonicus* with interesting effects on the mixture
302 composition. Further studies will be necessary to study the probiotic potential of the strains
303 used but, even in case of negative results, they could anyway be used as starters and the
304 addition of a probiotic strain could be done separately.

305 ~~First of all,~~ The prebiotic inulin was not used by bacteria and this is an important result in
306 view of a functional food preparation. In addition, lactosucrose, another indigestible
307 carbohydrate with good prebiotic performance was produced by these bacteria. In brief,
308 there are all the ingredients for a cheap and eco-friendly functional food based on agro-food
309 by-products. However, a deeper sensory evaluation will be performed to correlate the
310 organoleptic attributes with technological analysis, and consumer acceptability of this novel
311 synbiotic product.

312

313 **Acknowledgements**

314

315 The authors wish to thank Stefania Zannoni and Giorgio Arrigoni for their technical

316 support.), MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, DOR) project
317 numbers 60A08-5771/11 and 60A08-0032/11.

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319 **References**

320 Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., & Hassan, F. A.
321 (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods*, 5(4),
322 1542–1553.

323 Bovo, B., Nardi, T., Fontana, F., Carlot, M., Giacomini, A., & Corich, V. (2012).
324 Acidification of grape marc for alcoholic beverage production: effects on indigenous
325 microflora and aroma profile after distillation. *International Journal of Food*
326 *Microbiology*, 152(3), 100–106.

327 Campanaro, S., Treu, L., Vendramin, V., Bovo, B., Giacomini, A., & Corich, V. (2014).
328 Metagenomic analysis of the microbial community in fermented grape marc reveals
329 that *Lactobacillus fabifermentans* is one of the dominant species: insights into its
330 genome structure. *Applied Microbiology and Biotechnology*, 98(13), 6015–6037.

331 De Almada, C. N., De Almada, C. N., Martinez, R. C. R., & De Souza Sant'Ana, A.
332 (2015). Characterization of the intestinal microbiota and its interaction with
333 probiotics and health impacts. *Applied Microbiology and Biotechnology*, 99(10),
334 4175–4199.

335 De Bruyne, K., Camu, N., De Vuyst, L., Vandamme, P. (2009). *Lactobacillus*
336 *fabifermentans* sp. nov. and *Lactobacillus cacaonum* sp. nov., isolated from
337 Ghanaian cocoa fermentations. *International Journal of Systematic and*
338 *Evolutionary Microbiology*, 59(1), 7–12.

- 339 De Vries, M. C., Vaughan, E. E., Kleerebezem, M., & de Vos, W. M. (2006).
340 *Lactobacillus plantarum* – survival, functional and potential probiotic properties in
341 the human intestinal tract. *International Dairy Journal*, 16(9), 1018–1028.
- 342 Di Cagno, R., Coda, R., De Angelis, M., & Gobbetti, M. (2013). Exploitation of
343 vegetables and fruits through lactic acid fermentation. *Food Microbiology*, 33(1), 1–
344 10.
- 345 FAO (Food and Agricultural Organization). FAOSTAT (Food and Agricultural
346 Organization of the United Nations Statistic Division: Rome, Italy). (2017)
347 <http://faostat3.fao.org/browse/Q/QC/E/> Accessed 20.04.17.
- 348 Favaro, L., Corich, V., Giacomini, A., Basaglia, M., Casella, S. (2013). Grape marcs as
349 unexplored source of new yeasts for future biotechnological applications. *World*
350 *Journal of Microbiology and Biotechnology*, 29(9), 1551–1562.
- 351 Fijan, S. (2014). Microorganisms with claimed probiotic properties: an overview of
352 recent literature. *International Journal of Environmental Research and Public*
353 *Health*, 11(5), 4745–4767.
- 354 Gänzle, M., & Follador, R. (2012). Metabolism of oligosaccharides and starch in
355 lactobacilli: a review. *Frontiers in Microbiology*, 3(340), 1–15.
- 356 Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. (2002). Onion – A
357 global benefit to health. *Phytotherapy Research*, 16(7), 603–615.
- 358 Kagkli, D. M., Corich, V., Bovo, B., Lante, A., & Giacomini, A. (2016). Antiradical and
359 antimicrobial properties of fermented red chicory (*Cichorium intybus* L.) by-
360 products. *Annals of Microbiology*, 66(4), 1377–1386.
- 361 Kawase, M., Pilgrim, A., Araki, T., & Hashimoto, K. (2001). Lactosucrose production

362 using a simulated moving bed reactor. *Chemical Engineering Science*, 56(2), 453–
363 458.

364 Kim, J. H. (1997). Anti-bacterial action of onion (*Allium cepa* L.) extracts against oral
365 pathogenic bacteria. *The Journal of Nihon University School of Dentistry*, 39(3),
366 136–141.

367 Krumbeck, J. A., Maldonado-Gomez, M. X., Ramer-Tait, A. E., & Hutkins, R. W.
368 (2016). Prebiotics and synbiotics: dietary strategies for improving gut health.
369 *Current Opinion in Gastroenterology*, 32(2), 110–119.

370 Kumar, B. V., Vijayendra, S. V. N., & Reddy, O. V. S. (2015). Trends in dairy and non-
371 dairy probiotic products-a review. *Journal of Food Science and Technology*, 52(10),
372 6112–6124.

373 Lante, A., Nardi, T., Zocca, F., Giacomini, A., & Corich, V. (2011). Evaluation of red
374 chicory extract as a natural antioxidant by pure lipid oxidation and yeast oxidative
375 stress response as model systems. *Journal of Agricultural and Food Chemistry*,
376 59(10), 5318–5324.

377 Lucena-Padrós, H., & Ruiz-Barba, J. L. (2016). Diversity and enumeration of
378 halophilic and alkaliphilic bacteria in Spanish-style green table-olive fermentations.
379 *Food Microbiology*, 53, 53–62.

380 Madadlou, A., & Abbaspourrad, A. (2016). Bioactive whey peptide particles: An
381 emerging class of nutraceutical carriers. *Critical Reviews in Food Science and*
382 *Nutrition*, <http://dx.doi.org/10.1080/10408398.2016.1264064>.

383 Maragkoudakis, P. A., Nardi, T., Bovo, B., D'Andrea, M., Howell, K. S., Giacomini,
384 A., & Corich, V. (2013). Biodiversity, dynamics and ecology of bacterial

385 community during grape marc storage for the production of grappa. *International*
386 *Journal of Food Microbiology*, 162(2), 143–151.

387 Maragkoudakis, P., Vendramin, V., Bovo, B., Treu, L., Corich, V., & Giacomini, A.
388 (2016). Potential use of scotta, the by-product of the ricotta cheese manufacturing
389 process, for the production of fermented drinks. *Journal of Dairy Research*, 83(01),
390 104–108.

391 Masood, M. I., Qadir, M. I., Shirazi, J. H., & Khan, I. U. (2011). Beneficial effects of
392 lactic acid bacteria on human beings. *Critical Reviews in Microbiology*, 37(1), 91–
393 98.

394 Özcelik, S., Kuley, E., & Özogul, F. (2016). Formation of lactic, acetic, succinic,
395 propionic, formic and butyric acid by lactic acid bacteria. *LWT-Food Science and*
396 *Technology*, 73, 536–542.

397 Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics-
398 a review. *Journal of Food Science and Technology*, 52(12), 7577–7587.

399 Paramithiotis, S., Doulgeraki, A. I., Karahasani, A., & Drosinos, E. H. (2014).
400 Microbial population dynamics during spontaneous fermentation of *Asparagus*
401 *officinalis* L. young sprouts. *European Food Research and Technology*, 239(2), 297–
402 304.

403 Paramithiotis, S., Kouretas, K., & Drosinos, E. H. (2014). Effect of ripening stage on
404 the development of the microbial community during spontaneous fermentation of
405 green tomatoes. *Journal of the Science of Food and Agriculture*, 94(8), 1600–1606.

406 Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. Y. (2006). Probiotics and their
407 fermented food products are beneficial for health. *Journal of Applied Microbiology*,

408 100(6), 1171–1185.

409 Peshev, D., & Van den Ende, W. (2014). Fructans: prebiotics and immunomodulators.

410 *Journal of Functional Foods*, 8, 348–357.

411 Pogačić, T., Samaržija, D., Corich, V., D’Andrea, M., Kagkli, D. M., Giacomini, A.,

412 Majhenič, A.C., & Rogelj, I. (2010). Microbiota of Karakačanski skakutanac, an

413 artisanal fresh sheep cheese studied by culture-independent PCR-ARDRA and PCR-

414 DGGE. *Dairy Science & Technology*, 90(4), 461–468.

415 Roberts, J. S., & Kidd, D. R. (2005). Lactic acid fermentation of onions. *LWT-Food*

416 *Science and Technology*, 38(2), 185–190.

417 Ryan, M. P., & Walsh, G. (2016). The biotechnological potential of whey. *Reviews in*

418 *Environmental Science and Bio/Technology*, 15(3), 479–498.

419 Sangeetha, P. T., Ramesh, M. N., & Prapulla, S. G. (2005). Recent trends in the

420 microbial production, analysis and application of fructooligosaccharides. *Trends in*

421 *Food Science & Technology*, 16(10), 442–457.

422 SAS (Statistical Analysis System). SAS/STAT(R) 9.2 User’s Guide, 2nd Edition, SAS

423 Institute Inc.: Cary, NC, USA. (2013).

424 [http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.ht](http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#glm_toc.htm)

425 [m#glm_toc.htm](http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#glm_toc.htm)/ Accessed 20.04.17.

426 Seddik, H. A., Bendali, F., Gancel, F., Fliss, I., Spano, G., Drider, D. (2017).

427 *Lactobacillus plantarum* and its probiotic and food potentialities. *Probiotics and*

428 *Antimicrobial Proteins*, 9(2), 111–122.

429 Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2016). Review: diversity of

430 microorganisms in global fermented foods and beverages. *Frontiers in*

431 *Microbiology*, 7(377), 1–28.

432 Teixeira, J. S., McNeill, V., & Gänzle, M. G. (2012). Levansucrase and sucrose
433 phosphorylase contribute to raffinose, stachyose, and verbascose metabolism by
434 lactobacilli. *Food Microbiology*, 31(2), 278–284.

435 Tinello, F., & Lante, A. (2017). Evaluation of antibrowning and antioxidant activities in
436 unripe grapes recovered during bunch thinning. *Australian Journal of Grape and*
437 *Wine Research*, 23(1), 33–41.

438 Treu, L., Vendramin, V., Bovo, B., Campanaro, S., Corich, V., & Giacomini, A. (2014).
439 Whole-genome sequences of *Streptococcus thermophilus* strains TH1435 and
440 TH1436, Isolated from Raw Goat Milk. *Genome Announcements*, 2(1), e01129–13.

441 Treu, L., Vendramin, V., Bovo, B., Giacomini, A., Corich, V., & Campanaro, S. (2014).
442 Genome sequence of *Lactobacillus fabifermentans* strain T30PCM01, isolated from
443 fermenting grape marc. *Genome Announcements*, 2(1), e00060–14.

444 Uriot, O., Denis, S., Junjua, M., Roussel, Y., Dary-Mourot, A., Blanquet-Diot, S.
445 (2017). *Streptococcus thermophilus*: From yogurt starter to a new promising
446 probiotic candidate? *Journal of Functional Foods*, 37, 74–89.

447 Vendramin, V., Treu, L., Bovo, B., Campanaro, S., Corich, V., & Giacomini, A. (2014).
448 Whole-genome sequence of *Streptococcus macedonicus* strain 33MO, isolated from
449 the curd of Morlacco cheese in the Veneto region (Italy). *Genome Announcements*,
450 2(4), e00746–14.

451 Zoumpopoulou, G., Foligne, B., Christodoulou, K., Grangette, C., Pot, B., Tsakalidou,
452 E. (2008). *Lactobacillus fermentum* ACA-DC 179 displays probiotic potential in
453 vitro and protects against trinitrobenzene sulfonic acid (TNBS)-induced colitis and

454 *Salmonella* infection in murine models. *International Journal of Food Microbiology*,
455 *121*(1), 18–26.
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Table 1 – Chemical parameters of onion juices from red, white, and yellow cultivars (RO, WO, and YO, respectively).

| Parameters | RO | WO | YO | Probability |
|---------------------|---------------------------|---------------------------|---------------------------|-------------|
| pH | 5.54 ± 0.02 | 5.60 ± 0.02 | 5.70 ± 0.01 | NS |
| <i>Sugars (g/L)</i> | | | | |
| Fructose | 38.66 ± 2.64 ^a | 24.58 ± 0.88 ^b | 22.47 ± 0.00 ^b | *** |
| Galactose | 0.55 ± 0.26 ^a | 0.19 ± 0.00 ^b | 0.44 ± 0.00 ^c | *** |
| Glucose | 46.56 ± 2.24 ^a | 25.44 ± 0.71 ^b | 29.90 ± 0.00 ^c | *** |
| Inulin | 2.89 ± 0.57 ^a | 2.97 ± 0.49 ^a | 6.33 ± 0.00 ^b | *** |
| Saccharose | 10.32 ± 0.41 ^a | 2.68 ± 0.07 ^b | 11.28 ± 0.00 ^c | *** |
| Total sugars | 98.99 ± 5.28 ^a | 55.86 ± 1.17 ^c | 70.40 ± 0.00 ^b | *** |

‘NS’ $P > 0.05$, ‘***’ $P \leq 0.001$.

^{a, b, c} Each value is expressed as mean ± SD (n = 3). Means within each row and with different lowercase letters are statistically different ($P \leq 0.05$).

Table 2 – Microbiological and chemical parameters of unfermented (control) and fermented onion juices from red, white and yellow cultivars (RO, WO, YO, respectively) after 96 hours at 37 °C.

| Parameters | Control | <i>L. fabifermentans</i> | <i>L. plantarum</i> | Probability |
|--------------------------|---------------------|--|--|-------------|
| Red onion (RO) | | | | |
| Viable cells (CFU/ml) | – | $3.11 \cdot 10^8 \pm 1.41 \cdot 10^8$ | $3.56 \cdot 10^8 \pm 2.45 \cdot 10^7$ | NS |
| pH | 5.54 ± 0.02^a | 3.49 ± 0.01^b | 3.49 ± 0.03^b | *** |
| <i>Sugars (g/L)</i> | | | | |
| Fructose | 40.73 ± 0.44^a | 33.51 ± 2.26^b | 33.03 ± 0.68^b | *** |
| Galactose | 1.77 ± 0.26^a | 1.37 ± 0.03^b | 1.50 ± 0.07^{ab} | * |
| Glucose | 48.35 ± 1.00^a | 33.23 ± 2.68^b | 36.10 ± 0.54^b | *** |
| Inulin | 2.65 ± 0.40 | 2.60 ± 0.47 | 2.75 ± 0.27 | NS |
| Sucrose | 9.82 ± 0.14 | 9.32 ± 0.30 | 9.62 ± 0.15 | NS |
| Total sugars | 103.32 ± 1.07^a | 80.03 ± 4.81^b | 82.99 ± 0.94^b | *** |
| White onion (WO) | | | | |
| Viable cells (CFU/ml) | – | $3.71 \cdot 10^8 \pm 2.10 \cdot 10^{7b}$ | $7.06 \cdot 10^8 \pm 1.81 \cdot 10^{8a}$ | * |
| pH | 5.57 ± 0.03^a | 3.41 ± 0.02^b | 3.50 ± 0.06^b | *** |
| <i>Sugars (g/L)</i> | | | | |
| Fructose | 33.14 ± 1.16^a | 23.51 ± 0.08^b | 25.01 ± 0.05^b | *** |
| Galactose | 1.67 ± 0.10^c | 1.17 ± 0.01^b | 1.27 ± 0.10^b | *** |
| Glucose | 32.33 ± 1.94^a | 24.43 ± 0.14^b | 26.43 ± 0.10^b | *** |
| Inulin | 2.40 ± 0.14 | 2.17 ± 0.40 | 2.27 ± 0.33 | NS |
| Sucrose | 2.85 ± 0.12 | 2.75 ± 0.50 | 2.80 ± 0.47 | NS |
| Total sugars | 72.40 ± 3.15^a | 54.04 ± 1.13^b | 57.78 ± 0.24^b | *** |
| Yellow onion (YO) | | | | |
| Viable cells (CFU/ml) | – | $3.85 \cdot 10^8 \pm 2.10 \cdot 10^{7b}$ | $9.23 \cdot 10^8 \pm 8.20 \cdot 10^7^a$ | *** |
| pH | 5.52 ± 0.00^a | 3.72 ± 0.02^b | 3.63 ± 0.04^b | *** |
| <i>Sugars (g/L)</i> | | | | |
| Fructose | 33.55 ± 2.41^a | 24.55 ± 0.08^b | 25.55 ± 1.32^b | *** |
| Galactose | 2.30 ± 0.27^a | 1.33 ± 0.01^b | 1.44 ± 0.03^b | *** |
| Glucose | 35.58 ± 1.33^a | 20.58 ± 0.14^b | 22.58 ± 1.40^b | *** |
| Inulin | 5.77 ± 0.99 | 5.53 ± 0.40 | 5.53 ± 0.27 | NS |
| Sucrose | 12.13 ± 0.78 | 11.03 ± 0.47 | 11.08 ± 0.38 | NS |
| Total sugars | 89.33 ± 5.19^a | 63.02 ± 1.65^b | 66.18 ± 2.47^b | *** |

‘NS’ $P > 0.05$, ‘*’ $P \leq 0.05$, ‘***’ $P \leq 0.01$, ‘****’ $P \leq 0.001$.

^{a, b, c} Each value is expressed as mean \pm SD (n = 3). Means within each row and with different lowercase letters are statistically different ($P \leq 0.05$).

Table 3 - Microbiological and chemical parameters of unfermented (control) and fermented YOW samples with lactobacilli and streptococci after 48 hours at 30 °C and 37 °C, respectively.

| Parameters | Control | <i>L. fabifermentans</i> | <i>L. plantarum</i> | <i>S. macedonicus</i> | Probability |
|------------------------------|---------------------|---------------------------------------|---------------------------------------|---------------------------------------|-------------|
| Viable cells (CFU/ml) | – | $3.20 \cdot 10^8 \pm 2.62 \cdot 10^8$ | $4.26 \cdot 10^8 \pm 2.52 \cdot 10^8$ | $5.45 \cdot 10^8 \pm 8.18 \cdot 10^7$ | NS |
| pH | 5.92 ± 0.03^a | 3.81 ± 0.01^c | 3.91 ± 0.01^b | 3.90 ± 0.01^b | *** |
| <i>Sugars (g/L)</i> | | | | | |
| Fructose | 12.00 ± 0.08^a | 11.72 ± 0.83^{ab} | 7.68 ± 0.48^c | 10.02 ± 0.81^b | *** |
| Galactose | 0.69 ± 0.06^b | 4.95 ± 0.29^a | 4.69 ± 0.21^a | 5.12 ± 0.46^a | *** |
| Glucose | 17.82 ± 2.37 | 16.79 ± 0.30 | 17.81 ± 0.12 | 17.05 ± 1.52 | NS |
| Inulin | 7.97 ± 0.30 | 7.71 ± 0.60 | 7.69 ± 0.48 | 7.50 ± 0.69 | NS |
| Lactose+Sucrose [†] | 82.26 ± 1.67^a | $69.44 \pm 0.91^{b††}$ | 68.80 ± 2.21^b | 68.72 ± 3.09^b | *** |
| Total sugars | 120.74 ± 4.33^a | 110.62 ± 0.85^b | 106.68 ± 1.25^b | 108.40 ± 6.43^b | ** |

[†]Sugars eluted at the same retention time (9.02 min) in the HPLC analysis

^{††} Presence of lactosucrose based on MS/MS analysis.

‘NS’ P > 0.05, ‘***’ P ≤ 0.01, ‘****’ P ≤ 0.001.

^{a, b} Each value is expressed as mean ± SD (n = 3). Means within each row and with different lowercase letters are statistically different (P ≤ 0.05).

SUPPLEMENTARY MATERIAL

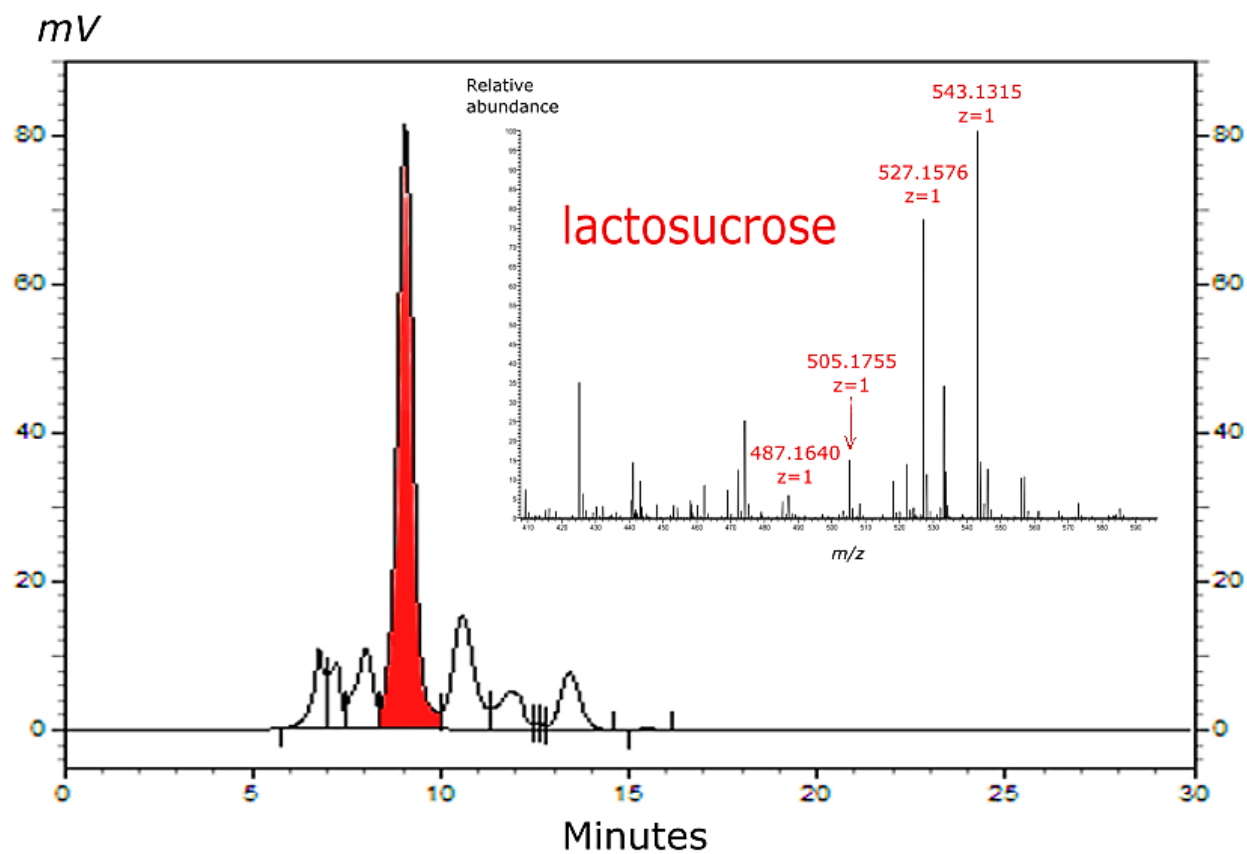


Fig. 1S – HPLC chromatogram of *L. fabifermentans* fermented YOW sample and MS/MS identification of lactosucrose corresponding to the chromatographic peak with retention time of 9.02 min.