<https://helda.helsinki.fi>

Characterization of indigenous *Pediococcus pentosaceus*, *Leuconostoc kimchii*, *Weissella cibaria* and *Weissella confusa* for faba bean bioprocessing

Rizzello, Carlo G.

2019-08-02

Rizzello , C G , Coda , R , Wang , Y , Verni , M , Kajala , I , Katina , K & Laitila , A 2019 , ' Characterization of indigenous *Pediococcus pentosaceus*, *Leuconostoc kimchii*, *Weissella cibaria* and *Weissella confusa* for faba bean bioprocessing ' , International Journal of Food Microbiology , vol. 302 , pp. 24-34 . <https://doi.org/10.1016/j.ijfoodmicro.2018.08.014>

<http://hdl.handle.net/10138/315012>

<https://doi.org/10.1016/j.ijfoodmicro.2018.08.014>

cc_by_nc_nd

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

**Characterization of indigenous *Pediococcus pentosaceus*, *Leuconostoc kimchii*,
Weissella cibaria and *Weissella confusa* for faba bean bioprocessing**

Carlo G. Rizzello ^{*a†}, Rossana Coda ^{b†}, Yaqin Wang ^b, Michela Verni ^a, Ilkka Kajala ^c, Kati
Katina ^b, Arja Laitila ^c

^a *Department of Soil, Plant, and Food Sciences, University Aldo Moro of Bari, Via Amendola 165/a,
70125 Bari, Italy*

^b *Department of Food and Environmental Science, University of Helsinki, Helsinki, Agnes
Sjioberginkatu 2, Helsinki, Finland*

^c *VTT Technical Research Centre of Finland Ltd., P.O.Box 1000, FI-02044 VTT, Finland*

^{*}*Corresponding author. Email: carlogiuseppe.rizzello@uniba.it*

[†]*These authors contributed equally to this work and are joint first authors.*

18 **Abstract**

19 The interest towards legumes in food applications has risen over the past decades. However, the
20 presence of antinutritional factors (ANF) and the poor technological performances still restricts their
21 application in food fortification. In this study, four lactic acid bacteria (LAB) isolated from faba bean
22 were applied as starter cultures for faba bean bioprocessing. None of the strains employed produced
23 exopolysaccharides from raffinose, on the contrary, they did with sucrose as substrate. The fermented
24 doughs were characterized and the strains were compared for their adaptation capacity and metabolic
25 performance including the formation of dextrans, the degradation of ANF and the ability to improve
26 antioxidant activity and *in vitro* protein digestibility (IVPD). A contribution to the proteolysis was
27 given by the presence of endogenous enzymes, responsible for the increase of peptides and amino acids
28 in dough from irradiated flour. However, the LAB strains further enhanced proteolysis. *Weissella*
29 *cibaria* VTT E-153485 led to the highest peptide release and consequentially to the highest IVPD. In
30 doughs fermented with *Pediococcus pentosaceus* VTT E-153483 and *Leuconostoc kimchi* VTT E-
31 153484, phytic acid was reduced to more than half the initial concentration. Inoculated doughs had
32 significantly lower content of oligosaccharides after 24 h of incubation compared to the controls. The
33 most efficient raffinose consumption was found for *Leuc. kimchi* and *W. cibaria*. Doughs inoculated
34 with weissellas contained more than 1% of dextrans. *Weissella confusa* VTT E-143403 induced a
35 significant increment in viscosity (ca. 7 times higher than the controls). This study revealed that well-
36 characterized, indigenous LAB provided beneficial biotechnological features in faba bean dough
37 processing and contributed to its implementation in the food production.

38

39 **Keywords**

40 Lactic acid bacteria, dextrans, faba bean fermentation

41 **Abbreviations**

42 ANF: Antinutritional factors; E3403: Faba bean dough fermented with *Weissella confusa* VTT E-
43 143403; E3483: Faba bean dough fermented with *Pediococcus pentosaceus* VTT E-153483; E3484:
44 Faba bean dough fermented with *Leuconostoc kimchii* VTT E-153484; E3485: Faba bean dough
45 fermented with *Weissella cibaria* VTT E-153485; EPS: Exopolysaccharydes; FAA: Free amino acids;
46 FQ: Fermentation quotient; IF: Dough from irradiated faba bean flour; IVPD: *in vitro* protein
47 digestibility; LAB: lactic acid bacteria; L-DOPA: L-3,4-dihydroxyphenylalanine; ME: Methanolic
48 extract; NF: Dough from native faba bean flour; TTA: Total titratable acidity; WSE: Water/salt-soluble
49 extracts.

50
51
52
53
54
55

1. Introduction

In the last years, the studies on legumes as protein-sources in food applications have increased. The main reasons for this incremental interest are their worldwide cultivation and good nutritional profile including important micronutrients (Multari et al., 2015). Research has demonstrated that high-protein (predominantly animal-based) diets are likely to be harmful to gut health in longer term, because of a reduction in cancer-protective compounds and an increase in hazardous metabolites (Windey et al., 2012). A possible reason is the lack of adequate fiber and associated phytochemicals. Thus, replacement of animal proteins with plant proteins may affect the nutrient adsorption at gut level, allowing changes in amino acids and triacylglycerol plasma concentration and exerting hypoglycemic and hypotriacylglycemic activity (Fernández-Quintela et al., 1998).

Faba bean (*Vicia faba* L.) is the second most cultivated legume and has been widely utilised as feed and food in both raw and processed forms. However, in spite of its potential as protein source for human diet, faba bean still remains underutilized, particularly in Western countries (Tijhuis et al., 2012). In addition to proteins, faba bean is a rich source of fiber and functional secondary metabolites (Aune et al., 2011), such as L-3,4-dihydroxyphenylalanine (L-DOPA), the precursor to the neurotransmitter catecholamine (Ramya and Thaakur, 2007).

Besides healthy aspects, the regular intake of plant-based foods such as faba bean in substitution of animal proteins in the diet is encouraged due to sustainability reasons (Multari et al., 2015). Indeed, the intensive cereals food and feed production is a major cause of soil degradation and crops such as faba bean, which have the ability to fix nitrogen, can offer an effective strategy to tackle the environmental damages of monoculture practices (Chapagain and Riseman, 2015).

Faba bean, its fractions, and its processing products (grains, hulls, and flours) contain compounds potentially having negative impact on nutrition, defined antinutritional factors (ANF) such as saponins,

79 condensed tannins, protease inhibitors, α -galactosides, phytic acid and vicine and convicine (Multari et
80 al., 2015).

81 Different strategies have been proposed to decrease the ANF content in faba beans and other legumes,
82 ranging from crop genetic improvement to processing (for review see Multari et al., 2015). In particular,
83 bioprocessing and fermentation are successful strategies to decrease the content of ANF in faba bean
84 while at the same time enhancing its technological and nutritional properties and thereby opening new
85 application scenarios for the food industry (Adebisi et al., 2017; Chandra-Hioe et al., 2016; Coda et al.,
86 2015, 2017a; Rizzello et al., 2016a).

87 In the past decade, studies on innovative food products have explored the metabolic traits of starters
88 isolated from the matrix to be processed, most often displaying the best adaptation and most suitable
89 performance (Corbo et al., 2017; Di Cagno et al., 2013). Typically, these starters have been referred to
90 as indigenous or “autochthonous” intending strains isolated from and used to ferment the same raw-
91 matrix.

92 Based on the expected growth of the vegetarian product market within the next few years (Multari et al.,
93 2015) and the recognized potential of the lactic acid bacteria (LAB) fermentation to exploit more of
94 faba bean as food ingredient, the selection of proper starters for industrial processing is needed. In a
95 recent characterization of LAB species isolated from spontaneous faba bean fermentation, some
96 relevant features related to its transformation were assessed allowing the identification of
97 technologically relevant biotypes (Verni et al., 2017).

98 In this study, four LAB strains belonging to *Pediococcus pentosaceus*, *Leuconostoc kimchii*, *Weissella*
99 *cibaria* and *Weissella confusa* species, originating from faba bean were characterized and compared for
100 their adaptation capacity and metabolic performance including formation of exopolysaccharides (e.g.
101 dextrans), reduction of α -galactosides and phytic acid content, generation of antioxidant activity, and *in*
102 *vitro* protein digestibility.

2. Materials and Methods

2.1 Microorganisms and raw materials

The faba bean (*Vicia faba major*) flour used in this study (CerealVeneta, San Martino di Lupari, PD, Italy) had the following composition: moisture, $9.50 \pm 0.08\%$; protein, $24.21 \pm 0.09\%$ of dry matter (d.m.); fat, $1.33 \pm 0.05\%$ of d.m.; total carbohydrates, $57.45 \pm 0.80\%$ of d.m. of which starch, $44.90 \pm 0.20\%$ of d.m.; dietary fibers, $9.80 \pm 0.40\%$ of d.m. and ash, $3.60 \pm 0.04\%$ of d.m.

A batch of faba bean flour was irradiated with a gamma radiation dose of 11.8 kGy (Scandinavian Clinics Estonia OÜ, Tallinn, Estonia) in order to inactivate the indigenous microbiota and was used as control as described below.

Four LAB strains (*Pediococcus pentosaceus* VTT E-153483, *Leuconostoc kimchii* VTT E-153484, *Weissella cibaria* VTT E-153485, and *Weissella confusa* VTT E-143403) isolated from the faba bean flour were deposited to the VTT Culture Collection, Espoo Finland (<http://culturecollection.vtt.fi/>). LAB were routinely propagated in MRS broth (Oxoid Ltd., Basingstoke, Hampshire, UK) in anaerobic conditions at 30 °C and used as described below.

2.2 Faba bean fermentation

After propagation in MRS broth in anaerobic conditions at 30 °C for 24 h, LAB cells were harvested by centrifugation ($10,000 \times g$, 10 min), washed once with Ringer's solution (Oxoid) and re-suspended in sterile tap water at the cell density of ca. $8.0 \log \text{ cfu/mL}$. Semi-liquid doughs (300 g) were obtained by mixing faba bean flour and sterile tap water (ratio flour:water, 1:2), and were inoculated with the LAB cells suspensions at the initial cell density of ca. $6 \log \text{ cfu/g}$.

125 Doughs were fermented with *P. pentosaceus* VTT E-153483 (E3483), *Leuc. kimchii* VTT E-153484
126 (E3484), *W. cibaria* VTT E-153485 (E3485), and *W. confusa* VTT E-143403 (E3403) and incubated in
127 static conditions at 25 °C for 48h. Two spontaneously fermented (not inoculated) doughs were included
128 in this study: a) one obtained with native faba bean flour (NF) and one with irradiated flour (IF). These
129 doughs were incubated in the same conditions as the LAB started doughs and were used as control
130 samples in order to discriminate the potential effect of the autochthonous microbiota and of the
131 endogenous enzymes, respectively. The fermentation experiments were performed as triplicates.
132 Aliquots of each dough were withdrawn after 24 and 48 h of fermentation and used for chemical
133 characterization.

134

135 **2.3 Microbiological analyses**

136 Microbiological analyses of the doughs were performed by plate count at the beginning and end of
137 incubation time. LAB were determined on MRS agar (Oxoid) after incubation in anaerobic conditions
138 at 30 °C for 3 days. MRS was supplemented with 0.01% cycloheximide (Sigma Chemical, St. Louis,
139 MO, USA) and 0.2% 2-phenylethanol to suppress the growth of fungi and Gram-negative bacteria. EPS
140 production ability of LAB was assessed on MRS supplemented with 2% sucrose or 2% raffinose (w/v)
141 (Juvonen et al., 2015). Yeast and moulds were determined on YM agar (Difco Laboratories), after
142 incubation in aerobic conditions at 25 °C for 3-5 days. Chlortetracycline and chloramphenicol (both at
143 0.01%) were added to YM medium to prevent bacterial growth. In addition, 0.02% of Triton-X 100
144 (BDH) was used to limit the spreading of fungal colonies on YM-agar. Gram-negative coliforms were
145 determined on Chromocult® coliform agar (Merck Millipore) after incubation in aerobic conditions at
146 37 °C for 24 h.

147

148 **2.4 Kinetics of acidification**

149 Kinetics of acidification during LAB fermentation of faba bean doughs were determined and modelled
150 in agreement with the Gompertz equation as modified by Zwietering et al. (1990): $y = k + A \exp\{-\exp$
151 $[(V_{\max} e/A)(1 - t) + 1]\}$; where y is the acidification rate expressed as dpH/dt (units of pH/h) at the time t ;
152 k is the initial level of the dependent variable to be modelled (pH units); A is the pH (units) variation
153 (between inoculation and the stationary phase); $V_{\max}$ is the maximum acidification rate expressed as
154 dpH/h , respectively; λ is the length of the lag phase measured in hours. The experimental data were
155 modelled by the non-linear regression procedure of the Statistica 8.0 software (Statsoft, Tulsa, USA).

156

157 **2.5 Chemical characterization**

158 Total titratable acidity (TTA) was determined after homogenization of 10 g of samples with 90 mL of
159 distilled water, and expressed as the amount (mL) of 0.1 M NaOH required to neutralize the solution,
160 using phenolphthalein as indicator (official AACC method 02-31.01).

161 Water/salt-soluble extracts (WSE) of freeze dried sourdoughs were prepared according to Weiss et al.
162 (1993) and used to analyze organic acids, ethanol, peptides, and free amino acids (FAA). Organic acids
163 were determined by High Performance Liquid Chromatography (HPLC), using an ÄKTA Purifier
164 system (GE Healthcare, Buckinghamshire, UK) equipped with an Aminex HPX-87H column (ion
165 exclusion, Biorad, Richmond, CA), and an UV detector operating at 210 nm (Rizzello et al., 2010). The
166 fermentation quotient (FQ) was determined as the molar ratio between lactic and acetic acids.

167 The concentration of peptides and FAA was determined on the WSE, to evaluate the degree of
168 proteolysis of the native proteins of doughs. For the peptide analysis, WSE were treated with
169 trifluoroacetic acid (0.05% wt/vol) and subject to dialysis (cut-off 500 Da) to remove proteins and FAA,
170 respectively. Then peptides concentration was determined by the *o*-phthalaldehyde (OPA) method as
171 described by Church et al. (1983). FAA were analyzed by a Biochrom30 series Amino Acid Analyzer

172 (Biochrom Ltd, Cambridge Science Park, UK) with a Na-cation exchange column (20 cm x 0.46 cm
173 internal diameter) as reported in Rizzello et al. (2010).

174

175 **2.6 Sugars and mannitol**

176 One hundred milligrams of freeze-dried samples were mixed with 5.0 mL of Milli-Q water and
177 vortexed for 5 min to allow the complete dissolution of free sugars and mannitol. Then, the suspensions
178 were boiled for 5 min. After cooling, samples (400 μ L) were filtered using Amicon Ultra-0.5
179 centrifugal filter units (Millipore, Billerica, MA) at $12,000 \times g$ for 10 min to remove polymeric
180 molecules (above 10 kDa). Before further analysis, samples were diluted with Milli-Q water. Mono-,
181 di- and oligo-saccharides in faba bean doughs were analyzed by high performance anion exchange
182 chromatography with pulse amperometric detection (HPAEC-PAD) system. A CarboPac PA1 column
183 (250×4 mm i.d., Dionex, Sunnyvale, CA) and a Waters 2465 pulsed amperometric detector (Waters,
184 USA) were used (Xu et al., 2017a). Glucose, fructose, sucrose (Merck, Germany), galactose, melibiose,
185 raffinose, stachyose (Sigma-Aldrich) and verbascose (Megazyme, Ireland) were used as standards and
186 2-deoxy-D-galactose (Sigma-Aldrich) was used as the internal standard for quantification. Mannitol
187 was quantified by the HPAEC-PAD system equipped with a CarboPac MA-1 analytical column and a
188 DECADE detector (Antec Leyden, The Netherlands) as reported by Xu et al. (2017a). Mannitol
189 (Sigma-Aldrich) was used as the standard for quantification. Under the experimental conditions, a
190 detection limit of 0.02% (on dough dry weight) was determined for sugars and mannitol.

191

192 **2.6 Dextran production and viscosity**

193 The amount of dextran in freeze-dried and homogenized sourdough samples was determined by an
194 enzyme-assisted method using an enzyme mixture of dextranase (Sigma-Aldrich, Germany) and α -
195 transglucosidase (Megazyme, Ireland) as described by Katina et al. (2009). Commercial dextran from

196 *Leuconostoc* spp. was purchased from Sigma-Aldrich (Sweden) and was hydrolyzed in the same
197 conditions of the samples to verify the enzyme activity.

198 Viscosity of doughs was measured at 23 °C. Constant rate measurement of viscosity as a function of
199 shear rate was performed with a rotational rheometer (Rheolab QC, Anton Paar GmbH, Vienna,
200 Austria) using a ST22.02-4V probe in a beaker (100 mL). The measuring profile was set to increase
201 shear rates from 2 s⁻¹ to 100 s⁻¹ and return to 2 s⁻¹ at 23 °C. Two replicate measurements were
202 conducted, and the average value was reported. Only viscosities that were measured at 100 s⁻¹ were
203 presented to reveal viscosity variations in the studied sourdoughs.

204

205 **2.7 *In vitro* protein digestibility**

206 The *in vitro* protein digestibility (IVPD) of doughs was determined by the method originally described
207 by of Akeson and Stahmann (1964) with some modification (Coda et al., 2017a). The IVPD was
208 expressed as the percentage of the total protein, which was solubilized after the sequential enzymatic
209 hydrolysis with pepsin and pancreatin.

210

211 **2.8 Phytase activity and phytic acid**

212 Phytase activity of sourdough samples was determined according to the method of De Angelis et al.
213 (2003). The extract was prepared by suspending of 1 g of freeze-dried sourdough samples in 4 mL of
214 Tris-HCl buffer (0.05 M, pH 7.5). After centrifugation, the supernatant was mixed with substrate and
215 kept in 45 °C for 2 h. The colour reagent was added and the released inorganic phosphate was measured
216 by a spectrophotometer. Phytic acid concentration was measured using Megazyme kit K-PHYT 05/07
217 (Megazyme International Ireland Limited, Bray, Ireland) following the manufacturer's instructions.

218

219 **2.9 Total phenols and antioxidant activity**

220 The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined on the
221 methanolic extract (ME) of freeze dried faba bean doughs and the concentration of total phenols was
222 determined as described by Slinkard and Singleton (1997), and expressed as gallic acid equivalent. The
223 free radical scavenging capacity was determined using the stable radical DPPH[•] (Rizzello et al., 2010).
224 and expressed as follows: DPPH scavenging activity (%) = [(blank absorbance – sample absorbance) /
225 blank absorbance] x 100. The value of absorbance was compared with 75 ppm butylated
226 hydroxytoluene (BHT), which was used as the antioxidant reference.

227

228 **2.10 Statistical Analysis**

229 The results were calculated as means of at least three replicates. The data were analyzed by one-way
230 ANOVA; pair-comparison of treatment means was obtained by Tukey's procedure at P < 0.05, using
231 the statistical software Statistica 8.0 (StatSoft Inc., Tulsa, USA). Significantly different data were
232 indicated with a different superscript letter. Biochemical and nutritional properties of the doughs after
233 48 h of fermentation were analyzed through principal component analysis (PCA), using the software
234 Statistica 8.0 (StatSoft Inc., Tulsa, USA).

235

236 **3. Results**

237 **3.1 Faba bean fermentation**

238 Prior to fermentation, cell density of presumptive LAB was 3.6 ± 0.2 and 2.5 ± 0.1 log cfu/g in native
239 and radiated faba bean flours, respectively. After 24 h of fermentation at 25 °C, LAB in inoculated
240 doughs ranged between 8.4 ± 0.4 and 9.5 ± 0.1 log cfu/g (Figure 1S). The lowest value was observed
241 for the dough fermented by *P. pentosaceus* VTT E-153483, while no significant differences were found

for the other doughs. Cell densities of 7.00 ± 0.26 and 3.20 ± 0.15 were observed for NF and IF doughs (Figure 1S).

A further increase of ca. 1 log cycle of LAB cell density was retrieved for both IF and NF doughs after 48 h of incubation. However, also after 48 h of fermentation, the cell number of LAB in the inoculated doughs was significantly higher than the spontaneously fermented controls (Figure 1S). Compared to native flours, the radiation treatment caused a reduction of 1 log cycle of all microbial groups analyzed. Coliforms remained constant below 2 log cfu/g after 24 and 48 h of fermentation in IF dough, while their number increased up to 6.1 ± 0.1 log cfu/g during 24 h spontaneous fermentation of NF (Figure 1S). Compared to the data obtained after 24 h, decreases in coliforms from 1 to 2 log cycles were observed after 48 h of fermentation. Yeasts and moulds were stably lower than 2.6 log cfu/g in all fermented samples throughout all the incubation time.

Based on the kinetics of acidification (Table 1), both *W. cibaria* and *W. confusa* behaved similarly, showing both the shortest latency phase (less than 6 h), V_{max} of approximately 0.1 dpH/h and similar pH variation. *P. pentosaceus* VTTE-153483 and *L. kimchii* VTT E153484 had λ values of ca. 8 h, and showed the highest ΔpH (Table 1).

At the beginning of fermentation, doughs had a pH of ca. 6.20. The pH value was stable in IF dough during the 48 h of incubation, while it decreased in all the other samples from 22 to 30% (Table 2). The lowest pH values at the end of the fermentation were found for doughs fermented with *P. pentosaceus* VTT E-153483 and *Leuc. kimchii* VTT E-153484 (Table 2).

In agreement with pH values, the TTA of E3483, E3484 and E3485 after 24 h were significantly higher than IF and NF, while E3403 showed a TTA similar to NF dough (Table 2). After 48 h, all inoculated doughs had TTA values significantly higher than the controls (Table 2). In both the fermentation times, the doughs fermented with the starters showed similar results with the exception of E3404 which had significantly lower TTA.

266 After the first 24 h, the highest synthesis of lactic acid was observed in E3483 and E3484 with 104.29
 267 ± 4.91 and 97.78 ± 3.63 mmol/kg, respectively (Table 2). A marked further increase (46%) was
 268 observed in E3483 after 48h of fermentation. The lowest lactic acid concentration was obtained when
 269 *W. cibaria* VTT E-153485 was the starter (Table 2).

270 A slight increase in acetic acid concentration occurred in IF during the incubation, whereas it
 271 significantly increased after 48 h in NF dough reaching values higher than E3483 and E3403 (Table 2).
 272 *L. kimchii* VTT E-153484 and *W. cibaria* VTT E-153485 led to the highest production of acetic acid
 273 (Table 2). FQ values after 24 h ranged from 5.64 to 36.80 with E3483 showing the highest value (Table
 274 2). After 48 h of fermentation, FQ remained constant in E3483, E3484, and E3403, while a slight
 275 increase was found in E3485 (Table 2).

276 Peptides and FAA concentrations were analyzed before and after incubation to estimate the degree of
 277 proteolysis (Table 2). During the first 24h of fermentation, the peptide concentration increased for all
 278 the doughs, up to 26 g/kg. Significantly higher concentrations were found in E3485 and E3403
 279 compared to the other doughs. The peptide concentration further increased from 6 to 71% during 48 h
 280 for all the inoculated doughs, while a moderate increase was noticed in NF dough (Table 2).

281 Total FAA (TFFA) concentration after 24 h of fermentation increased from 16 to 97% in all the
 282 samples, and particularly for the inoculated doughs (Table 2). TFFA kept increasing in all the samples
 283 during further incubation until 48 h. The dough started with *W. confusa* VTT E-143403 showed the
 284 highest TFAA concentration of ca. 4g/kg after 48 h.

285 Prior incubation, the most abundant amino acids in faba bean doughs were Glu and Arg, in amounts of
 286 400 ± 19 and 450 ± 23 mg/kg, respectively. After 24 h of fermentation with *P. pentosaceus* VTT E-
 287 153483, *Leuc. kimchii* VTT E-153484 and *W. cibaria* VTT E-153485 several FAA reached
 288 concentrations significantly higher than those found in IF (Thr, Glu, Gly, Ala, Cys, Val, Leu, Phe,
 289 GABA, His and Arg) (Figure 1A). Asp in E3484 was more than 10 times higher than in both the

controls, reaching 142 ± 6 mg/kg. A marked decrease of Tyr (more than 70%) was observed in all the inoculated doughs compared to the controls. In E3403 after 48 h, the amount of all the amino acid was significantly higher than that found in the other fermented doughs with few exceptions (Lys, Tyr and Arg) (Figure 1B). The FAA profiles of the doughs fermented with *Pediococcus* and *Leuconostoc* strains were almost similar, while E3485 was characterized by relevant lower concentration of almost all the essential FAA (Figure 1B).

3.2 Sugars and mannitol

Prior fermentation, verbascose, sucrose and stachyose were the most abundant sugars in not incubated faba bean doughs (Table 3). Raffinose was ca. 0.60%, while glucose, fructose and galactose were not detected. After 24h fermentation, sucrose was not detected in the doughs E3484, E3485, E3403 (Table 3), while it decreased in E3483 (44%) and NF (26%).

Fructose was present after 24 h in E3485 and E3403 (Table 3), but not anymore detectable after 48h, while glucose was detected in IF and NF doughs until the end of fermentation (Table 3). Galactose was retrieved in all the doughs after the first 24 h of fermentation (Table 3). Stachyose, a tetrasaccharide consisting of two α -D-galactose units, one α -D-glucose unit, and one β -D-fructose unit, was found at a concentration of ca. 2.80% at the beginning of incubation. Its concentration progressively decreased below 1% in E3483 and E3485. Although a decrease was observed also in IF and NF doughs, final amounts were markedly higher than the inoculated doughs (Table 3). A similar trend was observed for the pentasaccharide verbascose (Table 3), with the lowest concentration in E3483. Raffinose amount significantly decreased during fermentation, and it was not detectable in all the inoculated doughs at 48h, while in IF and in NF decreases of 43 and 58% were found, respectively (Table 3). At 24 h, the most efficient raffinose consumption was observed in E3484 and E3485 (Table 3).

313 Mannitol was found in all the doughs after 24 h of fermentation. In particular, after 24 h, the highest
314 concentration was found in E3484 and E3485, while no significant differences were found between
315 E3483 and E3403 (Table 3). With the exception of E3483, mannitol further increased in the following
316 24h of fermentation. The highest final concentration was found in E3484. A relevant mannitol
317 concentration was also found in NF fermented dough (Table 3).

318

319 **3.3 Dextran production and viscosity**

320 The preliminary screening of the ability to synthesize EPS showed that all the LAB strains produced
321 slime in different amount on MRS agar supplemented with sucrose, but none of them produced EPS in
322 presence of raffinose, as no sliminess or ropyness was observed. Being raffinose a substrate for
323 levansucrase, but not for glucansucrase, dextran but not fructan synthesis was hypothesized
324 (Semjonovs and Zikmanis, 2008).

325 After 24 h of fermentation, the amount of dextrans in the doughs ranged from 0.24 to 1.47% of dry
326 weight (dw) (Table 4). The highest amount was found in the doughs inoculated with *W. cibaria* and *W.*
327 *confusa*, containing more than 1% (of dw). In the following 24 h the amount further increased in E3483
328 (of ca. 24%), and E3403, while it remained stable in all the other cases.

329 Viscosity of the doughs increased during incubation and, after 24 h, it was similar for the two control
330 doughs IF and NF. Compared to those, viscosity of E3484 and E3485 was significantly lower, while a
331 viscosity ca. 7 fold higher was found in E3403. E3403 had the highest viscosity also at 48 h, followed
332 by E3483, having a value ca. 3 fold lower (Table 4).

333

334 **3.4 *In vitro* protein digestibility**

335 The protein digestibility was determined on faba bean doughs through a protocol mimicking the
336 digestion in the gastrointestinal tract (Akeson and Stahmann, 1964). Prior to fermentation IVPD values

337 ranged from 54.54 to 56.57%, with no difference among the controls and the inoculated samples. A
338 slight but not significant increase was observed in IF dough during incubation, while IVPD
339 significantly increased (from 7 to 18%) during the first 24h of fermentation in all the other samples
340 (Figure 2). Compared to t0, at the end of 48 h of incubation, increases of 10-19% were found in the
341 inoculated doughs. A significant increase was also observed in NF during incubation, although the final
342 value was lower, but not significantly, than those of the inoculated doughs (Figure 2).

343

344 **3.5 Total phenols and antioxidant activity**

345 Prior fermentation, doughs contained ca. 1.57 mmol/kg of total phenols. With the only exception of IF
346 dough, the extracted total phenols increased during incubation (Figure 3A). E3485 was characterized
347 by the highest increase of total phenols in the first 24 h and a final concentration of 3.19 mmol/kg
348 (Figure 3A). Total phenols further increased in E3484 and E3403 after 48 h showing ca. 3.20 mmol/kg,
349 while no significant differences were found for E3485. As shown in Figure 3B, there were no
350 significant differences in the antioxidant activity among the doughs before fermentation, although
351 relevant DPPH scavenging activity was detected (81-83%). Proportionally to the phenols concentration,
352 the antioxidant activity increased during fermentation. Increases up to 14% were observed for the
353 inoculated doughs, especially those fermented with *Leuc. kimchii* VTT E-153484, *W. cibaria* VTT E-
354 153485 (Figure 3B).

355

356 **3.6 Phytase activity and phytic acid concentration**

357 A weak phytase activity (Figure 4A) was found in faba bean doughs before and after 24 h of
358 fermentation. The activity was more than 10 times higher in E3483 and E3484 after 24 h of
359 fermentation. A significant increase was observed in E3485 and E3403, only after 48 h of fermentation.
360 An opposite trend was shown in E3483 and E3484, in which decreases up to 36% were found after 48 h

361 (Figure 4A). The concentration of phytic acid in doughs was ca. 0.40 g/100g before fermentation. With
362 the exception of IF, phytic acid content decreased during fermentation. The most efficient degradation
363 was observed in E3483 and E3484, having final values of ca. 0.18g/100 g of phytic acid. Significantly
364 higher concentrations were found in NF doughs compared to all the inoculated doughs (Figure 4B).

365

366 **3.7 Principal component analysis**

367 Data collected from the nutritional characterization of faba bean doughs were subjected to principal
368 component analysis (PCA) as showed in Figure 5. The first and second factors explained, respectively,
369 the 66.61 and 18.88% of the total variance. Factor 1 clearly separated the inoculated from the control
370 doughs. Inoculated doughs shared several features (lower amount of phytic acid and raffinose while
371 higher content in FAA, and lactic acid with an increase in protein digestibility) and were grouped in the
372 left area of the plot. The difference between NF and IF is explained by the activity of endogenous
373 microbiota which contributed, in NF dough, to the higher synthesis of organic acids and proteolysis
374 compared to IF. A partial reduction of phytic acid and oligosaccharides was observed in the controls,
375 slightly higher for NF than IF. Among the inoculated doughs, E3484 and E3485 were very similar,
376 especially for the amount of lactic acid produced and the high antioxidant activity. Whereas, dough
377 fermented with *W. confusa* VTT E-143403, placed on the bottom left area of the plot, stood out for the
378 highest proteolytic activity and a more balanced ratio of organic acids. E3403 dough also showed the
379 highest viscosity. *P. pentosaceus* VTT E-153483 was located in the middle of the left area because of
380 its discrete ability to influence viscosity and highest lactic acid production. This analysis suggested that
381 the use of *W. confusa* VTT E-143403 represents the best compromise between nutritional and
382 technological properties, with a relevant increase of antioxidant activity.

383

384 **4. Discussion**

385 The growing importance of legumes as food ingredient and the confirmed advantages of fermentation,
386 as an affordable method for improving nutritional and nutraceutical properties require the set-up novel
387 biotechnological protocols for legume bioprocessing applied in the industrial scale.

388 Recent studies revealed that LAB fermentation of different legumes improved the protein digestibility,
389 the mineral and phenols bioavailability, leading to the partial or complete degradation of the
390 antinutritional factors α -galactosides, tannins, phytic acid and trypsin inhibitors and pyrimidine
391 glucosides causing favism (vicine, convicine and its derivatives) (Bartkiene et al., 2014, Chandra-Hioe
392 et al., 2016; Coda et al., 2015; Rizzello et al., 2016a). Another area of growing interest concerning
393 legume flours is the synthesis *in situ* of EPS such as dextrans, which have shown the ability to modify
394 the rheological properties of different fermented legumes, promoting their usability for several food
395 applications (Xu et al., 2017a, 2017b). Additionally, the nutritional advantages of the fortification of
396 cereal-based foods with fermented faba bean as ingredient (e.g. bread and pasta) were also
397 demonstrated (Coda et al., 2017a; 2017b; Rizzello et al., 2017). For all these reasons, the evaluation of
398 the metabolic and pro-technological performances of LAB strains potentially employable as starters for
399 faba bean bioprocessing is crucially important in order to find the best candidates for food and feed
400 applications. A list of the main characteristics of the lactic acid bacteria considered in this study is
401 reported in Table 1S.

402 Fermentation process can rely on the microbiota characterizing raw materials and the environment or
403 can be controlled by inoculation of selected, well-characterized starters. Several research outcomes
404 have shown that the LAB microbiota associated with the food matrix to be processed represents an
405 optimal source for potential starter cultures, thanks to the faster adaptation to the specific matrix, and
406 the marked influence on the nutritional and technological properties (Corbo et al., 2017; Filannino et al.,
407 2018; Sáez et al., 2017).

408 In a former study the LAB previously found to be dominant in spontaneously fermented faba bean
409 doughs mainly belonged to *Pediococcus* and *Leuconostoc* genera and, to a lesser extent, to *Weissella*,
410 *Enterococcus*, and *Lactobacillus* spp. (Coda et al., 2017b). In the present study, four strains, belonging
411 to *P. pentosaceus*, *Leuc. kimchii*, *W. cibaria* and *W. confusa*, originating from faba bean, were applied
412 as starters for fermentation and compared based on characteristics of great interest for the development
413 of specific faba bioprocessing protocols. The four strains are able to produce EPS of the glucan type,
414 most probably dextran. Dextran synthesis was largely reported for LAB strains belonging to *P.*
415 *pentosaceus* (Shukla and Goyal, 2013), *Leuc. kimchii* (Schleifer, 2009), *W. cibaria* and *W. confusa*
416 (Galle et al., 2010; Xu et al., 2017a). Based on previous observation on EPS synthesis *in situ* (Kajala et
417 al., 2016), fermentation was carried out at 25 °C. The sub-optimal temperature for LAB growth was
418 chosen to avoid excessive acidification and, as consequence, potential negative repercussions on the
419 dextransucrase activity.

420 All the starters used in this study showed a growth higher than 2 log cycles during the 48 h of
421 fermentation exceeding, with the exception of *P. pentosaceus* VTT E-153483, a cell density of 9 log
422 cfu/g. A markedly lower LAB cell density was observed in spontaneously fermented dough obtained
423 with irradiated flour, while native, untreated flour harboured a population of ca. 8 log cfu/g at the end
424 of the incubation. In agreement with the previous findings (Coda et al., 2017b), yeasts were at low
425 cellular density before and after fermentation, in all the samples.

426 The *Weissella* spp. strains showed the shortest latency phase and the lowest Vmax, while *P.*
427 *pentosaceus* VTT E-153483 and *Leuc. kimchii* VTT E-153484 showed the highest variation of pH and
428 production of lactic acid. *P. pentosaceus* is a homofermentative species, and its use as starter led to the
429 highest FQ. The marginal concentration of acetic acid found in E3483, lower than that found in the
430 spontaneous fermented control, could be related to the contribution of the indigenous microbiota.

431 Doughs fermented with *Leuc. kimchii* VTT E-153484 had the lowest FQ, due to the relevant amount of

432 acetic acid produced, most likely due to the reduction of fructose to mannitol, as previously reported
433 for *Leuconostoc* spp. (Wisselink et al., 2002). Relevant acetic acid concentration was also observed in
434 E3485, also in this case together with mannitol release. Overall, most *Weissella* spp. are not able to
435 reduce fructose to mannitol (Gänzle, 2015), nevertheless, some *W. cibaria* strains are able to produce
436 mannitol from sucrose (Galle et al., 2010). Indeed, the mannitol production has already been reported
437 as both strain- and substrate- dependent (Galle et al., 2010). The ratio between lactic and acetic acid
438 can positively influence sensorial properties of the final product, therefore a proper FQ is considered an
439 important parameter for starters selection (Gänzle, 2015).

440 Sucrose was the most abundant disaccharide in faba doughs and enabled the formation of EPS.
441 Theoretically, sucrose can be first utilized for microbial growth during the exponential phase, then for
442 EPS production during the stationary phase (Plante and Shriver, 1998). Residual fructose was found at
443 concentration higher than 1% in the two doughs fermented with *Weissella* spp. This is in agreement
444 with previous studies on faba bean showing that, through the activity of glucansucrase on sucrose,
445 glucan is formed, while fructose is released (Xu et al., 2017b). Faba bean contains relevant
446 concentrations of the raffinose family oligosaccharides (RFO), the α -galactosides raffinose, verbascose,
447 and stachiose which can act as antinutritional factor (Teixeira et al., 2012). RFO are not degraded in the
448 gastrointestinal tract due to the lack of α -galactosidase (α -Gal) activity, and, despite favouring the
449 metabolism of beneficial intestinal microorganisms at low concentration, they can be fermented in the
450 large intestine by the intestinal microbiota, causing gastrointestinal symptoms (Teixeira et al., 2012).
451 Fermentation can efficiently lead to raffinose elimination in different legumes including faba bean (Xu
452 et al., 2017a, 2017b). The possibility to degrade RFO during fermentation is an important selection
453 criteria for faba bean starters (Connes et al., 2004). Under the conditions of this study, all the fermented
454 doughs showed a decrease of RFO content. Previously Xu et al. (2017b) showed the role of
455 endogenous α -Gal in RFO degradation in faba bean flour. Therefore, we can hypothesize a combined

activity of the endogenous and microbial enzymatic activity. As the consequence of the RFO hydrolysis, galactose, was detected after the first 24 h of fermentation. Stachyose progressively decreased during fermentation, especially in the inoculated doughs. Verbascose followed a similar trend, nevertheless *P. pentosaceus* VTT E-153483 caused intensive degradation.

The accumulation of raffinose was previously observed and correlated to the partial hydrolysis of verbascose and stachyose during faba bean fermentation (Coda et al., 2015, 2017b; Teixeira et al., 2012). It is hypothesizable that the longer fermentation time applied in this study favoured intensive degradation of raffinose. The capability to use raffinose is not widespread among LAB. However, it has been reported that in *P. pentosaceus* the fermentation of some sugars, including raffinose, is plasmid encoded and the presence of α -Gal is inducible (Gonzalez and Kunka, 1986). Furthermore, *Weissella* spp. strains able to use raffinose were previously characterized (Verni et al., 2017).

Dextran concentration in doughs fermented by *Weissella* strains was notably increased, ranging from 1.02 to 1.70% and relatively close to the theoretical amount expected (1.5%) based on the presence of native sucrose. Theoretically, the total content of residual fructose, liberated from sucrose, and mannitol, should be roughly equal to the content of glucan formed. In our conditions, the content of glucan was lower in E3483 and E3485. Probable reasons for this could be the use of sucrose-liberated glucose for growth and other metabolic activities, and the formation of oligosaccharides by glucansucrase through acceptor reaction, as reported earlier (Xu et al., 2017b). Despite the relative similarity in dextran content, a very different rheological behaviour was found for E3485 and E3403, since the latter showed a markedly higher viscosity. The different thickening ability of EPS might be due to the structure and molecular weight of the dextrans produced by the two strains and, possibly, by the different interactions between the other faba bean components (e.g. starch and proteins) (Xu et al., 2017a, 2018).

479 Together with acidification, proteolysis is considered a LAB key-feature in food biotechnology, since
480 the degradation of native proteins is of great importance for improving the digestibility of polypeptides
481 and bioavailability of free amino acids, but also for the release of potential bioactive peptides (Rizzello
482 et al., 2016b) and for the development of taste and flavor (Leroy and De Vuyst, 2004). A discrete
483 contribution to the proteolysis was given by the presence of endogenous enzymes, responsible for the
484 increase of peptides and amino acids (ca. 16%) occurring during the first 24 h of incubation of the
485 dough obtained with radiated flour, having the lowest microbial cell density among the samples. The
486 use of LAB led to a more intense proteolysis; doughs fermented with *W. cibaria* VTT E-153485 and *W.*
487 *confusa* VTT E-143403 showed the highest peptide release. The use of *W. confusa* VTT E-143403 also
488 induced the highest release of FAA. In previous studies, *Weissella* spp. strains isolated from faba bean
489 displayed high endopeptidase activity (Verni et al., 2017). The final TFAA in faba bean doughs
490 resulted markedly higher than what commonly found in cereals (Lattanzi et al., 2013, Verni et al.,
491 2017). Moreover, the fermentation with *Leuc. kimchii* VTT E-153484 and *W. confusa* VTT E-143403
492 increased the concentration of free Met and Cys, the limiting amino acids of faba bean (Multari et al.,
493 2015).

494 In agreement with the proteolysis driven by the starters, the IVPD values at the end of the fermentation
495 were significantly higher than those of the control doughs. The partial degradation of the proteins
496 makes the sequences more susceptible to further degradation by the digestive enzymes (Rizzello et al.,
497 2016b). *W. cibaria* VTT E-153485, able to cause the highest release of peptides, led to the highest
498 increase of IVPD in the first 24 h of fermentation.

499 The interest for the presence of antioxidant compounds in foods has increased, based on the recognized
500 role in the prevention mechanisms of the oxidative stresses (Adebiyi et al., 2009) and to the ability to
501 prolong the shelf-life, through the inhibition of the oxidative processes (Shahidi and Ambigaipalan,
502 2015). The antioxidant activity found in faba doughs was markedly higher than that found for cereal

doughs, mainly thanks to the high total phenols content (El-mergawi and Taie, 2014). Overall, significant increases of the antioxidant activity were observed in inoculated doughs, with the only exception of that fermented by *P. pentosaceus* VTT E-153483, having the same activity of the spontaneously fermented NF. It was reported that *P. pentosaceus* is particularly efficient to degrade phenol compounds, for example through decarboxylases, leading to a loss of antioxidant activity (Rodríguez et al., 2009). Nevertheless, the contribution to the antioxidant activity provided by the high concentration of peptides and FAA found in inoculated samples, should be considered (Rizzello et al., 2016b).

Phytic acid is widely present in legume flours where it acts as mineral chelator (Campos-Vega et al., 2010) also reducing the digestibility of protein, starch, and lipids (Frias et al., 2003). Phytase activity, catalyzing the hydrolysis of phytic acid, is described as lower in legumes than in cereals (Frias et al., 2003; Steiner et al., 2007). Overall, acidification activates cereal endogenous phytases due to more suitable values of pH, however, it was reported that the acidification conditions did not improve the activity of the legume endogenous phytases (Coda et al., 2015; Gustafsson and Sandberg, 1995). For this reason, the contribution of microbial phytases to the degradation of phytic acid in faba bean is highly relevant. LAB possess phytase activity to a certain extent, largely investigated in previous studies (De Angelis et al., 2003; Zotta et al., 2007). Among the faba bean LAB characterized, *P. pentosaceus* VTT E-153483 and *Leuc. kimchii* VTT E-153484 caused a significant degradation of phytic acid already during the first 24h of fermentation.

All strains showed a proper aptitude (growth, acidification and proteolysis performances) to be applied as starters for faba bean bioprocessing. Investigation of the metabolic differences highlighted crucial information for the choice of the suitable starter strains (to be used as a single or mixed cultures) aiming at obtaining specific biotechnological targets to overcome issues regarding the use of faba bean in human nutrition. Overall, *W. confusa* VTT E-143403 showed the best combination among the

527 characteristics tested (optimal fermentation quotient, high amino acids release and dextran
528 synthesis/texture modifying potential, intense phytase and antioxidant activities).

529

530 **Future prospective**

531 One of the reasons of the rising demand for healthier plant-based food is the increasing awareness of
532 the adverse risks associated with the consumption of animal proteins. Among legumes, faba bean has
533 great potential as functional ingredient. However, despite its positive impact on human health and
534 agroecosystem, faba bean has been underutilized for decades mainly due to the presence of ANF that
535 interfere with nutrient absorption and sometimes cause pathologic conditions. A broad variety of
536 methods were effective in their reduction and some of the strategies proposed resulted in an efficient
537 and affordable way to diminish, and in some cases completely eliminate, ANF. The efficiency of
538 fermentation and its impact on the sensory and technology properties (beside nutritional aspects)
539 strongly depends on the pro-technological and metabolic traits of the starters used, as described by an
540 increasing number of scientific reports on faba bean bioprocessing . The incorporation of faba bean,
541 whether raw or prior treatments, in cereal-based food formulas has been studied over the years. When
542 added as raw flour, it was mostly responsible of increasing the protein content of the composite product,
543 but technological properties were negatively affected and ANF increased. On the contrary, the
544 fortification of bread, pasta, extruded snacks and gluten-free products with fermented faba bean flour
545 allowed an overall improvement of the nutritional, functional and technological qualities. On the basis
546 of the promising results already obtained, a significant increase of the use fermented faba bean in food
547 industry is expected.

548

549 **Conflict of interest**

550 The authors declare no conflict of interest.

551

552 **Funding**

553 This research has been developed under the European Project “BIOPROT – Novel multifunctional
554 plant protein ingredients with bioprocessing” (FP7-ERA-Net - SUSFOOD).

555 **References**

556 AACC (2003). Approved methods of the american association of cereal chemistry, 10th ed. AACC,
557 St. Paul, Minnesota, U.S.A.

558 Adebisi, A.O., Njobeh, P.B., Adebisi, J.A., Gbashi, S., Phoku, J.Z., Kayitesi, E., 2017. Fermented
559 pulse-based food products in developing nations as functional foods and ingredients. In: Chavarri
560 Hueda, M. (Ed), Functional Food - Improve Health through Adequate Food. InTech, Rijeka, Croatia.

561 Adebisi, A.P., Adebisi, A.O., Yamashita, J., Ogawa, T., Muramoto, K., 2009. Purification and
562 characterization of antioxidative peptides derived from rice bran protein hydrolysates. Eur. Food Res.
563 Technol. 228, 553.

564 Akesson, W.R., Stahmann, M.A., 1964. A pepsin pancreatin digest index of protein quality
565 evaluation. J. Nutr. 83, 257-261.

566 Aune, D., Chan, D.S., Lau, R., Vieira, R., Greenwood, D.C., Kampman, E., Norat, T., 2011. Dietary
567 fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of
568 prospective studies. Br. Med. J. 343, d6617.

569 Bartkiene, E., Krungleviciute, V., Juodeikiene, G., Vidmantienė, D., Maknickienė, Z., 2014. Solid
 570 state fermentation with lactic acid bacteria to improve the nutritional quality of lupin and soya bean. J.
 571 Sci. Food Agric. 95, 1336–1342.

572 Campos-Vega, R., Loarca-Piña, G., Oomah, B.D., 2010. Minor components of pulses and their
 573 potential impact on human health. Food Res. Int. 43, 461-482.

574 Chandra-Hioe, M.V., Wong, C.H.M., Arcot, J., 2016. The potential use of fermented chickpea and
 575 faba bean flour as food ingredients. Plant Foods Hum. Nutr., 71, 90–95.

576 Chapagain, T., Riseman, A., 2015. Nitrogen and carbon transformations, water use efficiency and
 577 ecosystem productivity in monocultures and wheat-bean intercropping systems. Nutr. Cycling
 578 Agroecosyst. 101, 107–121.

579 Church, F.C., Swaisgood, H.E., Porter, D.H., Catignani, G.L., 1983. Spectrophotometric assay using
 580 *o*-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins¹. Journal of
 581 Dairy Science, 66, 1219–1227.

582 Coda, R., Melama, L., Rizzello, C.G., Curiel, J.A., Sibakov, J., Holopainen, U., Pulkkinen, M.,
 583 Sozer, N., 2015. Effect of air classification and fermentation by *Lactobacillus plantarum* VTT E-
 584 133328 on faba bean (*Vicia faba* L.) flour nutritional properties. Int. J. Food Microbiol. 193, 34–42.

585 Coda, R., Varis, J., Verni, M., Rizzello, C.G., Katina, K., 2017a. Improvement of the protein quality
 586 of wheat bread through faba bean sourdough addition. Food Sci. Technol. 82, 296-302.

587 Coda, R., Kianjam, M., Pontonio, E., Verni, M., Di Cagno, R., Katina, K., Rizzello, C.G., Gobbetti,
 588 M., 2017b. Sourdough-type propagation of faba bean flour: dynamics of microbial consortia and
 589 biochemical implications. Int. J. Food Microbiol. 248, 10–21.

590 Connes, C., Silvestroni, A., Leblanc, J.G., Juillard, V., de Giori, G.S., Sesma, F., Piard, J.C., 2004.
 591 Towards probiotic lactic acid bacteria strains to remove raffinose-type sugars present in soy-derived
 592 products. *Lait*, 84, 207-214.

593 Corbo, M.R., Racioppo, A., Monacis, N., Speranza, B., 2017. Commercial starters or autochthonous
 594 strains? That is the question. In: Speranza, B., Bevilacqua, A., Corbo, M.R., Sinigaglia, M. (Eds.),
 595 Starter Cultures in Food Production, John Wiley & Sons, UK, pp. 174-198.

596 De Angelis, M., Gallo, G., Corbo, M.R., McSweeney, P.L.H., Faccia, M., Giovine, M., Gobbetti, M.,
 597 2003. Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase
 598 from *Lactobacillus sanfranciscensis* CB1. *Food Microbiol.* 87, 259–270.

599 Di Cagno, R., Coda, R., De Angelis, M., Gobbetti, M., 2013. Exploitation of vegetables and fruits
 600 through lactic acid fermentation. *Food Microbiol.* 33, 1-10.

601 El-Mergawi, R.A.G.A.B., Taie, H.A., 2014. Phenolic composition and antioxidant activity of raw
 602 seeds, green seeds and sprouts of ten faba bean (*Vicia faba*) cultivars consumed in Egypt. *Int. J. Pharm.*
 603 *Biol. Sci.* 5, 609-617.

604 Fernández-Quintela, A., del Barrio, A.S., Macarulla, M.T., Martínez, J.A., 1998. Nutritional
 605 evaluation and metabolic effects in rats of protein isolates obtained from seeds of three legume species.
 606 *J. Sci. Food Agric.* 78, 251–260.

607 Filannino, P., Di Cagno, R., Gobbetti, M., 2018. Metabolic and functional paths of lactic acid
 608 bacteria in plant foods: get out of the labyrinth. *Curr. Opin. Biotechnol.* 49, 64-72.

609 Frias, J., Doblado, R., Antezana, J.R., Vidal-Valverde, C., 2003. Inositol phosphate degradation by
 610 the action of phytase enzyme in legume seeds. *Food Chem.* 81, 233-239.

611 Galle, S., Schwab, C., Arendt, E., Gänzle, M., 2010. Exopolysaccharide-forming *Weissella* strains as
 612 starter cultures for sorghum and wheat sourdoughs. J. Agric. Food Chem. 58, 5834-5841.

613 Gänzle, M.G., 2015. Lactic metabolism revisited: metabolism of lactic acid bacteria in food
 614 fermentations and food spoilage. Curr. Opin. Food Sci. 2, 106-117.

615 Gonzalez, C.F., Kunka, B.S., 1986. Evidence for plasmid linkage of raffinose utilization and
 616 associated α -galactosidase and sucrose hydrolase activity in *Pediococcus pentosaceus*. Appl. Environ.
 617 Microbiol. 51, 105-109.

618 Gustafsson, E.L., Sandberg, A.S., 1995. Phytate reduction in brown beans (*Phaseolus vulgaris* L.). J.
 619 Food Sci. 60, 149-152.

620 Juvonen, R., Honkapää, K., Maina, N.H., Shi, Q., Viljanen, K., Maaheimo, H., Virkki, L., Tenkanen,
 621 M., Lantto, R., 2015. The impact of fermentation with exopolysaccharide producing lactic acid bacteria
 622 on rheological, chemical and sensory properties of pureed carrots (*Daucus carota* L.). Int. J. Food
 623 Microbiol. 207, 109-118.

624 Kajala, I., Mäkelä, J., Coda, R., Shukla, S., Shi, Q., Maina, N. H., Juvonen, R., Ekholm, P., Goyal,
 625 A., Tenkanen, M., Katina, K., 2016. Rye bran as fermentation matrix boosts in situ dextran production
 626 by *Weissella confusa* compared to wheat bran. Appl. Microbiol. Biotechnol. 100, 3499-3510.

627 Katina, K., Maina, N.H., Juvonen, R., Flander, L., Johansson, L., Virkki, L., Tenkanen, M., Laitila,
 628 A., 2009. *In situ* production and analysis of *Weissella confusa* dextran in wheat sourdough. Food
 629 Microbiol. 26, 734-743.

630 Lattanzi, A., Minervini, F., Di Cagno, R., Diviccaro, A., Antonielli, L., Cardinali, G., Cappelle, S.,
 631 De Angelis, M., Gobbetti, M., 2013. The lactic acid bacteria and yeast microbiota of eighteen

632 sourdoughs used for the manufacture of traditional Italian sweet leavened baked goods. *Int. J. Food*
633 *Microbiol.* 163, 71-79.

634 Leroy, F., De Vuys, L., 2004. Lactic acid bacteria as functional starter cultures for the food
635 fermentation industry. *Trends Food Sci. Technol.* 15, 67–78.

636 Multari, S., Stewart, D., Russel, W.R., 2015. Potential of fava bean as future protein supply to
637 partially replace meat intake in the human diet. *Compr. Rev. Food Sci. Food Saf.* 14, 511–522

638 Plante, C.J., Shriver, A.G., 1998. Differential lysis of sedimentary bacteria by *Arenicola marina* L.:
639 examination of cell wall structure and exopolymeric capsules as correlates. *J. Exp. Mar. Biol. Ecol.* 229,
640 35-52.

641 Ramya, K.B., Thaakur, S., 2007. Herbs containing L- DOPA: an update. *Anc. Sci. Life* 27, 50–55.

642 Rizzello, C.G., Nionelli, L., Coda, R., De Angelis, M., Gobbetti, M., 2010. Effect of sourdough
643 fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. *Food Chem.*
644 119, 1079–1089.

645 Rizzello, C.G., Losito, I., Facchini, L., Katina, K., Palmisano, F., Gobbetti, M., Coda, R., 2016a.
646 Degradation of vicine, convicine and their aglycones during fermentation of faba bean flour. *Sci. Rep.*
647 6, 32452.

648 Rizzello, C.G., Tagliazucchi, D., Babini, E., Rutella, G.S., Taneyo Saa, D.L., Gianotti, A., 2016b.
649 Bioactive peptides from vegetable food matrices: Research trends and novel biotechnologies for
650 synthesis and recovery. *J. Funct. Foods.* 27, 549–569.

651 Rizzello, C.G., Verni, M., Koivula, H., Montemurro, M., Seppa, L., Kemell, M., Katina, K., Coda,
652 R., Gobbetti, M., 2017. Influence of fermented faba bean flour on the nutritional, technological and
653 sensory quality of fortified pasta. *Food Funct.* 8, 860-871.

654 Rodríguez, H., Curiel, J.A., Landete, J.M., de las Rivas, B., de Felipe, F.L., Gómez-Cordovés, C.,
655 Mancheño, J.M., Muñoz, R., 2009. Food phenolics and lactic acid bacteria. *Int. J. Food Microbiol.* 132,
656 79-90.

657 Sáez, G.D., Hébert, E.M., Saavedra, L., Zárate, G., 2017. Molecular identification and technological
658 characterization of lactic acid bacteria isolated from fermented kidney beans flours (*Phaseolus vulgaris*
659 L. and *P. coccineus*) in northwestern Argentina. *Food Res. Int.* 102, 605-615.

660 Schleifer, K.H., 2009. *Leuconostocaceae* fam. nov. *Bergey's Manual of Systematics of Archaea and*
661 *Bacteria*.

662 Semjonovs, P., Zikmanis, P., 2008. Evaluation of novel lactose-positive and exopolysaccharide-
663 producing strain of *Pediococcus pentosaceus* for fermented foods. *Eur. Food Res. Technol.*, 227, 851-
664 856.

665 Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices:
666 Antioxidant activity and health effects—A review. *J. Funct. Foods* 18, 820-897.

667 Shukla, R., Goyal, A., 2013. Novel dextran from *Pediococcus pentosaceus* CRAG3 isolated from
668 fermented cucumber with anti-cancer properties. *Int. J. Biol. Macromol.* 62, 352-357.

669 Slinkard, K., Singleton, V.L., 1997. Total phenol analysis: Automation and comparison with manual
670 methods. *Am. J. Enol. Viticul.* 28, 49–55.

Steiner, T., Mosenthin, R., Zimmermann, B., Greiner, R., Roth, S., 2007. Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. *Anim. Feed Sci. Technol.* 133, 320-334.

Teixeira, P.J., Carraca, E.V., Markland, D., Silva, M.N., Ryan, R.M., 2012. Exercise, physical activity, and self-determination theory: a systematic review. *Int. J. Behav. Nutr. Phys. Act.* 9:78.

Tijhuis, M.J., Ezendam, J., Westenbrink, S., van Rossum, C., Temme, L., 2012. Replacement of meat and dairy by more sustainable protein sources in the Netherlands: quality of the diet. *Natl. Inst. for Public Health and the Environment, Netherlands*. Report no. 350123001.

Verni, M., Wang, C., Montemurro, M., De Angelis, M., Katina, K., Rizzello, C. G., Coda, R., 2017. Exploring the microbiota of faba bean: functional characterization of lactic acid bacteria. *Front. Microbiol.* 8.

Weiss, W., Vogelmeier, C., Görg, A., 1993. Electrophoretic characterization of wheat grain allergens from different cultivars involved in bakers' asthma. *Electrophoresis* 14, 805-816.

Windey, K., De Preter, V., Verbeke, K., 2012. Relevance of protein fermentation to gut health. *Mol. Nutr. Food Res.* 56, 184–96.

Wisselink, H.W., Weusthuis, R.A., Eggink, G., Hugenholtz, J., Grobbee, G.J., 2002. Mannitol production by lactic acid bacteria: a review. *Int. Dairy J.* 12, 151-161.

Xu, Y., Wang, Y., Coda, R., Säde, E., Tuomainen, P., Tenkanen, M., Katina, K., 2017a. *In situ* synthesis of exopolysaccharides by *Leuconostoc* spp. and *Weissella* spp. and their rheological impacts in fava bean flour. *Int. J. Food Microbiol.* 248, 63-71.

691 Xu, Y., Coda, R., Shi, Q., Tuomainen, P., Katina, K., Tenkanen, M., 2017b. Exopolysaccharides
692 production during the fermentation of soybean and fava bean flours by *Leuconostoc mesenteroides*
693 DSM 20343. J. Agr. Food Chem. 65, 2805-2815.

694 Xu, Y., Pitkänen, L., Maina, N.H., Coda, R., Katina, K., Tenkanen, M. 2018. Interactions between
695 fava bean protein and dextrans produced by *Leuconostoc pseudomesenteroides* DSM 20193 and
696 *Weissella cibaria* Sj 1b. Carbohydr. Polym. 190, 315-323.

697 Zotta, T., Ricciardi, A., Parente, E., 2007. Enzymatic activities of lactic acid bacteria isolated from
698 Cornetto di Matera sourdoughs. Int. J. Food Microbiol. 115, 165-172.

699 Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K., 1990. Modeling of the bacterial
700 growth curve. Appl. Environ. Microbiol. 56, 1875-1881.

701

702

703 Figure Legends

704 **Figure 1.** Free amino acids concentrations (mg/kg) in faba bean doughs inoculated with *P. pentosaceus*
705 VTT E-153483 (E3483), *Leuc. kimchii* VTT E-153484 (E3484), *W. cibaria* VTT E-153485 (E3485),
706 and *W. confusa* VTT E-143403 (E3403) after 24 (A) and 48 h (B) of fermentation at 25 °C. Two not
707 inoculated doughs, one obtained with native faba bean flour (NF) and one with irradiated flour (IF)
708 were produced and incubated in the same conditions and used as controls. Data are the means of three
709 independent analyses. Error bars indicating the standard deviation are represented. ^{a-c} Values of the
710 same FAA having different superscript letters differ significantly ($P < 0.05$).

711 **Figure 2.** *In vitro* protein digestibility (IVPD) determined on freeze dried faba bean doughs before and
712 after 24 and 48 h of fermentation. IF, irradiated faba bean flour; NF, native faba bean flour; E3483:
713 *Pediococcus pentosaceus* VTT E-153483 (E3483), *Leuc. kimchii* VTT E-153484 (E3484), *W. cibaria*
714 VTT E-153485 (E3485), and *W. confusa* VTT E-143403 (E3403). Data are the means of three
715 independent analyses. Error bars indicating the standard deviation are represented. ^{a-c} Values in the
716 with different superscript letters within the same time of incubation differ significantly ($P < 0.05$).

717 **Figure 3.** Total phenols concentration (A) expressed as mmol of gallic acid equivalents/kg and
718 antioxidant activity (B) expressed as DPPH radical scavenging activity (%) determined on methanolic
719 extracts from freeze-dried faba bean doughs inoculated with *P. pentosaceus* VTT E-153483 (E3483),
720 *Leuc. kimchii* VTT E-153484 (E3484), *W. cibaria* VTT E-153485 (E3485), and *W. confusa* VTT E-
721 143403 (E3403) after 24 (A) and 48 h (B) of fermentation at 25 °C. Two not inoculated doughs, one
722 obtained with native faba bean flour (NF) and one with irradiated flour (IF) were produced and
723 incubated in the same conditions and used as controls. Data are the means of three independent
724 analyses. Error bars indicating the standard deviation are represented. ^{a-c} Values in the with different
725 superscript letters within the same time of incubation differ significantly ($P < 0.05$).

726 **Figure 4.** Phytase activity (A) and phytic acid concentration (B) in freeze-dried faba bean doughs
727 inoculated with *P. pentosaceus* VTT E-153483 (E3483), *Leuc. kimchii* VTT E-153484 (E3484), *W.*
728 *cibaria* VTT E-153485 (E3485), and *W. confusa* VTT E-143403 (E3403) after 24 (A) and 48 h (B) of
729 fermentation at 25 °C. Two not inoculated doughs, one obtained with native faba bean flour (NF) and
730 one with irradiated flour (IF) were produced and incubated in the same conditions and used as controls.
731 One unit (U) of phytase activity was defined as the amount of enzyme required to release 1 µmol of
732 phosphate from Na-phytate per min under the assay conditions. Data are the means of three
733 independent analyses. Error bars indicating the standard deviation are represented. ^{a-c} Values in the
734 with different superscript letters within the same time of incubation differ significantly (P < 0.05).

735 **Figure 5.** Principal component analysis (PCA) on the basis of the biochemical and nutritional
736 characteristics of faba bean doughs inoculated with *P. pentosaceus* VTT E-153483 (E3483), *Leuc.*
737 *kimchii* VTT E-153484 (E3484), *W. cibaria* VTT E-153485 (E3485), and *W. confusa* VTT E-143403
738 (E3403) after 48 h of fermentation at 25 °C. Two not inoculated doughs, one obtained with native faba
739 bean flour (NF) and one with irradiated flour (IF) were produced and incubated in the same conditions
740 and used as controls. aL: lactic acid concentration; aA: acetic acid concentration; Faa: free amino acids;
741 IVPD: *in vitro* protein digestibility; Aox: antioxidant activity; Vis: viscosity; Raf: raffinose
742 consumption; Phy: phytic acid concentration.

743

744

745

746

747

748 **Table 1.** Kinetics of acidification as modelled according to the Gompertz equation as modified by
749 Zwietering et al. (1990) of faba bean dough inoculated with *P. pentosaceus* VTT E-153483, *Leuc.*
750 *kimchii* VTT E-153484, *W. cibaria* VTT E-153485, and *W. confusa* VTT E-143403 and fermented at
751 25 °C for 24 h.

752	Strain	V_{max} (dpH/h)	λ (h)	ΔpH
	<i>P. pentosaceus</i> VTT E-153483	0.12 ± 0.03 ^b	7.94 ± 0.15 ^a	1.38 ± 0.08 ^a
753	<i>L. kimchii</i> VTT E-153484	0.20 ± 0.03 ^a	8.04 ± 0.7 ^a	1.41 ± 0.09 ^a
754	<i>W. cibaria</i> VTT E-153485	0.10 ± 0.0 ^b	4.91 ± 0.5 ^b	0.99 ± 0.05 ^b
755	<i>W. confusa</i> VTT E-143403	0.08 ± 0.01 ^b	5.75 ± 0.6 ^b	0.80 ± 0.04 ^b

756

757 The data are the means of three independent experiments ± standard deviations (n = 3).

758 ^{a-b} Values in the same column with different superscript letters differ significantly (P<0.05).

759

Table 2. Biochemical characterization of faba bean doughs inoculated with *P. pentosaceus* VTT E-153483 (E3483), *Leuc. kimchii* VTT E-153484 (E3484), *W. cibaria* VTT E-153485 (E3485), and *W. confusa* VTT E-143403 (E3403) after 24 and 48 h of fermentation at 25 °C. Two not inoculated doughs, one obtained with native faba bean flour (NF) and one with irradiated flour (IF) were produced and incubated in the same conditions and used as controls.

		pH	TTA	Lactic acid (mmol/kg)	Acetic acid (mmol/kg)	FQ	Peptides (g/kg)	FAA (g/kg)
IF	0	6.14 ± 0.02 ^a	3.1 ± 0.1 ^a	7.26 ± 0.34 ^a	0.45 ± 0.02 ^a	15.97 ± 0.75 ^a	15.80 ± 0.78 ^a	1.58 ± 0.08 ^a
	24	6.21 ± 0.03 ^a	3.5 ± 0.2 ^d	7.73 ± 0.36 ^e	0.71 ± 0.05 ^e	10.87 ± 0.51 ^c	18.31 ± 0.90 ^b	1.83 ± 0.10 ^d
	48	6.08 ± 0.04 ^a	3.7 ± 0.2 ^d	8.49 ± 0.40 ^e	0.78 ± 0.03 ^e	10.82 ± 0.43 ^c	18.88 ± 0.92 ^c	2.41 ± 0.12 ^c
NF	0	6.24 ± 0.04 ^a	2.8 ± 0.1 ^a	7.24 ± 0.30 ^a	0.53 ± 0.02 ^a	13.66 ± 0.74 ^a	15.94 ± 0.76 ^a	1.59 ± 0.07 ^a
	24	5.47 ± 0.03 ^b	7.5 ± 0.4 ^c	43.23 ± 1.73 ^d	7.66 ± 0.38 ^c	5.64 ± 0.24 ^e	18.42 ± 0.91 ^b	2.30 ± 0.12 ^c
	48	4.85 ± 0.03 ^b	11.7 ± 0.6 ^c	66.8 ± 2.84 ^d	9.74 ± 0.45 ^c	6.86 ± 0.37 ^{de}	23.60 ± 1.19 ^b	2.98 ± 0.16 ^b
E3483	0	6.20 ± 0.02 ^a	3.0 ± 0.2 ^a	7.38 ± 0.29 ^a	0.46 ± 0.02 ^a	16.04 ± 0.53 ^a	16.19 ± 0.84 ^a	1.67 ± 0.07 ^a
	24	4.70 ± 0.04 ^c	12.2 ± 0.3 ^a	104.29 ± 4.91 ^a	2.83 ± 0.11 ^d	36.80 ± 1.71 ^a	18.64 ± 0.92 ^b	3.01 ± 0.17 ^a
	48	4.39 ± 0.03 ^c	16.0 ± 0.9 ^a	152.89 ± 6.28 ^a	4.36 ± 0.17 ^d	35.05 ± 1.46 ^a	31.98 ± 1.65 ^a	3.19 ± 0.19 ^b
E3484	0	6.19 ± 0.01 ^a	3.1 ± 0.1 ^a	7.42 ± 0.29 ^a	0.49 ± 0.08 ^a	15.14 ± 0.45 ^a	16.03 ± 0.94 ^a	1.66 ± 0.10 ^a
	24	4.74 ± 0.03 ^c	12.7 ± 0.7 ^a	97.78 ± 3.63 ^a	16.33 ± 0.77 ^a	5.98 ± 0.56 ^e	18.96 ± 0.98 ^b	3.24 ± 0.18 ^a
	48	4.48 ± 0.04 ^c	15.3 ± 0.9 ^a	116.41 ± 4.86 ^b	21.06 ± 0.95 ^a	5.52 ± 0.76 ^e	20.18 ± 1.12 ^b	3.62 ± 0.22 ^a

E3485	0	6.21 ± 0.03 ^a	3.4 ± 0.2 ^a	7.40 ± 0.42 ^a	0.47 ± 0.07 ^a	15.74 ± 0.65 ^a	16.35 ± 0.80 ^a	1.68 ± 0.12 ^a
	24	5.13 ± 0.02 ^b	9.3 ± 0.6 ^b	62.53 ± 3.24 ^c	9.16 ± 0.39 ^b	6.83 ± 0.34 ^d	26.42 ± 1.37 ^a	2.84 ± 0.17 ^b
	48	4.64 ± 0.05 ^b	15.3 ± 0.9 ^a	100.56 ± 4.41 ^c	11.66 ± 0.51 ^b	8.62 ± 0.42 ^d	35.70 ± 1.89 ^a	3.23 ± 0.20 ^b
E3403	0	6.22 ± 0.04 ^a	3.7 ± 0.3 ^a	7.45 ± 0.43 ^a	0.50 ± 0.09 ^a	14.90 ± 0.59 ^a	16.45 ± 0.92 ^a	1.67 ± 0.10 ^a
	24	5.36 ± 0.03 ^b	7.8 ± 0.5 ^c	75.70 ± 3.56 ^b	5.34 ± 0.32 ^{dc}	14.17 ± 0.33 ^b	23.80 ± 1.28 ^a	3.29 ± 0.19 ^a
	48	4.76 ± 0.03 ^b	13.8 ± 1.0 ^b	120.10 ± 5.31 ^b	8.20 ± 0.37 ^c	14.60 ± 0.27 ^b	34.07 ± 1.35 ^a	4.06 ± 0.25 ^a

764 The data are the means of three independent experiments ± standard deviations (n = 3).

765 ^{a-c} Values referring to the same incubation time, in the same column, with different superscript letters, differ significantly (P<0.05).

766

767 **Table 3.** Sugars and mannitol concentration in faba bean doughs inoculated with *P. pentosaceus* VTT E-153483 (E3483), *Leuc.*
768 *kimchii* VTT E-153484 (E3484), *W. cibaria* VTT E-153485 (E3485), and *W. confusa* VTT E-143403 (E3403) after 24 and 48 h of
769 fermentation at 25 °C. Two not inoculated doughs, one obtained with native faba bean flour (NF) and one with irradiated flour (IF)
770 were produced and incubated in the same conditions and used as controls. Data are expressed as % on dry weight.
771

		Glucose	Fructose	Galactose	Sucrose	Stachyose	Verbascose	Raffinose	Mannitol
IF	0	nd	nd	nd	3.08 ± 0.12 ^a	2.84 ± 0.10 ^a	4.57 ± 0.18 ^a	0.61 ± 0.04 ^a	nd
	24	0.56 ± 0.02 ^b	nd	0.66 ± 0.03 ^b	2.94 ± 0.13 ^a	1.81 ± 0.07 ^a	2.61 ± 0.11 ^a	0.36 ± 0.02 ^a	0.12 ± 0.01 ^c
	48	0.82 ± 0.04 ^a	nd	0.91 ± 0.05 ^b	2.91 ± 0.12 ^a	1.55 ± 0.05 ^a	2.43 ± 0.12 ^a	0.34 ± 0.01 ^a	0.12 ± 0.02 ^d
NF	0	nd	nd	nd	3.03 ± 0.11 ^a	2.78 ± 0.06 ^a	4.68 ± 0.15 ^a	0.62 ± 0.04 ^a	nd
	24	0.67 ± 0.03 ^a	nd	0.82 ± 0.04 ^a	2.24 ± 0.09 ^b	1.51 ± 0.06 ^b	2.22 ± 0.09 ^b	0.36 ± 0.02 ^a	0.74 ± 0.03 ^{ab}
	48	0.87 ± 0.03 ^a	nd	0.99 ± 0.06 ^b	1.95 ± 0.06 ^b	1.29 ± 0.04 ^b	1.89 ± 0.07 ^b	0.26 ± 0.01 ^b	0.73 ± 0.03 ^b
E3483	0	nd	nd	nd	3.05 ± 0.12 ^a	2.84 ± 0.09 ^a	4.61 ± 0.16 ^a	0.60 ± 0.03 ^a	nd
	24	nd	nd	0.66 ± 0.03 ^b	1.69 ± 0.07 ^c	1.63 ± 0.05 ^b	1.88 ± 0.08 ^c	0.22 ± 0.01 ^b	0.22 ± 0.02 ^c
	48	nd	nd	1.05 ± 0.06 ^b	1.65 ± 0.05 ^c	0.76 ± 0.03 ^d	0.71 ± 0.06 ^d	nd	0.24 ± 0.03 ^d
E3484	0	nd	nd	nd	3.05 ± 0.12 ^a	2.81 ± 0.08 ^a	4.66 ± 0.16 ^a	0.61 ± 0.03 ^a	nd
	24	nd	nd	0.87 ± 0.05 ^a	nd	1.71 ± 0.06 ^{ab}	2.18 ± 0.08 ^b	0.14 ± 0.01 ^c	0.86 ± 0.04 ^a
	48	nd	nd	1.35 ± 0.07 ^a	nd	1.02 ± 0.05 ^c	1.19 ± 0.08 ^c	nd	1.24 ± 0.10 ^a

E3485	0	nd	nd	nd	3.01 ± 0.11^a	2.83 ± 0.10^a	4.50 ± 0.17^a	0.61 ± 0.04^a	nd
	24	nd	1.50 ± 0.13^a	0.85 ± 0.04^a	nd	1.53 ± 0.05^b	2.16 ± 0.09^b	0.17 ± 0.01^c	0.63 ± 0.03^b
	48	nd	nd	1.01 ± 0.05^b	nd	0.90 ± 0.06^{cd}	1.10 ± 0.09^c	nd	0.99 ± 0.09^{ab}
E3403	0	nd	nd	nd	3.00 ± 0.12^a	2.86 ± 0.10^a	4.60 ± 0.18^a	0.61 ± 0.04^a	nd
	24	nd	1.28 ± 0.10^{ab}	0.62 ± 0.03^b	nd	1.73 ± 0.07^{ab}	2.15 ± 0.08^b	0.29 ± 0.03^b	0.21 ± 0.02^c
	48	nd	nd	0.99 ± 0.05^b	nd	1.02 ± 0.05^c	1.12 ± 0.10^c	nd	0.52 ± 0.03^c

772 The data are the means of three independent experiments $\pm$ standard deviations (n=3).

773 ^{a-d} Values referring to the same incubation time, in the same column, with different superscript letters, differ significantly (P<0.05).

774 nd: not detected

775

776

Table 4. Dextrans concentration (expressed as % of dry weight) and viscosity (Pa/s) as determined on faba bean doughs inoculated with *P. pentosaceus* VTT E-153483 (E3483), *Leuc. kimchii* VTT E-153484 (E3484), *W. cibaria* VTT E-153485 (E3485), and *W. confusa* VTT E-143403 (E3403) after 24 and 48 h of fermentation at 25 °C. Two not inoculated doughs, one obtained with native faba bean flour (NF) and one with irradiated flour (IF) were produced and incubated in the same conditions and used as controls. Data are the means of three independent analyses. Error bars indicating the standard deviation are represented. ^{a-e} Values in the with different superscript letters within the same time of incubation differ significantly ($P < 0.05$).

		Dextrans (%)	Viscosity (Pa/s)
IF	0	nd	0.17 ± 0.02^a
	24	0.24 ± 0.03^d	0.89 ± 0.02^c
	48	0.28 ± 0.04^d	0.77 ± 0.0^c
NF	0	nd	0.16 ± 0.04^a
	24	0.63 ± 0.03^c	0.89 ± 0.04^c
	48	0.66 ± 0.03^c	0.58 ± 0.06^d
E3483	0	nd	0.17 ± 0.02^a
	24	0.60 ± 0.04^c	1.66 ± 0.03^b
	48	0.81 ± 0.03^c	2.17 ± 0.09^b
E3484	0	nd	0.17 ± 0.01^a
	24	0.25 ± 0.03^d	0.39 ± 0.04^d
	48	0.28 ± 0.04^d	0.41 ± 0.09^d
E3485	0	nd	0.16 ± 0.03^a
	24	1.01 ± 0.02^b	0.39 ± 0.06^d

	48	1.02 ± 0.05^b	0.46 ± 0.09^a
E3403	0	nd	0.16 ± 0.04^a
	24	1.37 ± 0.03^a	6.20 ± 0.05^a
	48	1.70 ± 0.03^a	6.73 ± 0.08^a

785

786 The data are the means of three independent experiments $\pm$ standard deviations (n=3).

787 ^{a-d} Values referring to the same incubation time, in the same column, with different superscript letters,
788 differ significantly (P<0.05).

789 nd: not detected

A

B

Table 1S. Main characteristics of the lactic acid bacteria strains selected for faba bean bioprocessing.

Selection criteria	Parameter	Data
<i>Pro-technological characteristics</i>		
Acidification	Kinetics of acidification (λ , Δ pH, Vmax)	Table 1
		Table 2
	Lactic and acetic acid synthesis	Table 2
	Fermentation quotient	Table 2
Proteolytic activity	Peptides and free amino acids release	Table 2, Figure 1
EPS synthesis	Dextran production	Table 4
	Viscosity	Table 4
<i>Nutritional and functional features</i>		
GABA synthesis	GABA concentration	Figure 1
RFO degradation	Verbascose, raffinose, stachyose concentration	Table 3
Protein digestibility	IVPD	Figure 2
Antioxidant activity	Total phenol concentration	Figure 3
	DPPH scavenging activity	Figure 3
Phytic acid degradation	Phytase activity	Figure 4
	Phytic acid concentration	Figure 4

Figure 1S. Cell density of LAB, coliforms, yeasts and moulds determined in faba bean doughs inoculated with *P. pentosaceus* VTT E-153483 (E3483), *Leuc. kimchii* VTT E-153484 (E3484), *W. cibaria* VTT E-153485 (E3485), and *W. confusa* VTT E-143403 (E3403) after 24 (A) and 48 h (B) of fermentation at 25 °C. Two not inoculated doughs, one obtained with native faba bean flour (NF) and one with irradiated flour (IF) were produced and incubated in the same conditions and used as controls. Data are the means of three independent analyses. Error bars indicating the standard deviation are represented. ^{a-d} Values in the with different superscript letters within the same time of incubation differ significantly ($P < 0.05$).