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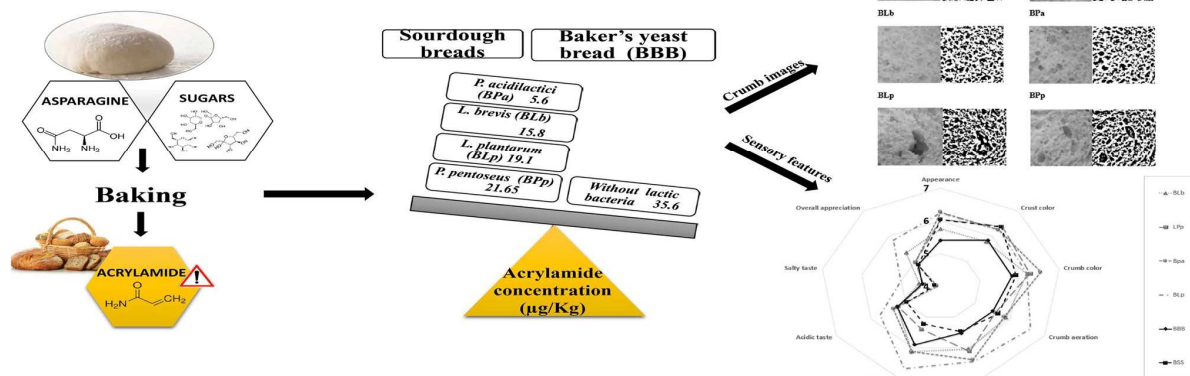
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Title: Assessment of lactic acid bacteria application for the reduction of acrylamide formation in bread



1 **Assessment of lactic acid bacteria application for the reduction of acrylamide formation**
2 **in bread**

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18 **Abbreviations:**

19 SS, spontaneous sourdough; SLb, sourdough inoculated with *L.brevis* S12; SLp, sourdough inoculated with
20 *L.plantarum* S28; SPp, sourdough inoculated with *P.pentoseus* S14; Spa, sourdough inoculated with
21 *P.acidilactici* S16; DSS, dough made with spontaneous sourdough; DLb, dough inoculated with *L.brevis* S12
22 sourdough; DLp, dough inoculated with *L.plantarum* S28 sourdough; DPp, dough inoculated with *P.pentoseus*
23 S14 sourdough; DPa, dough with *P.acidilactici* S16 sourdough; DBB, Baker's yeast dough made without the

24 addition of sourdough; BSS, bread made by using spontaneous sourdough without LAB strains; BLb, bread
25 made with *L.brevis* S12 sourdough; BLp, bread made with *L.plantarum* S28 sourdough; BPP, bread made with
26 *P.pentoseus* S14 sourdough; BPa, bread made with *P.acidilactici* S16 sourdough; BBB, Baker's yeast bread;
27 TTA, Total titratable acidity; LAB, Lactic acid bacteria; DY, dough yield.

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54 **Abstract**

55 Four isolated lactic acid bacteria LAB strains *Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus*
56 *pentoseus*, and *Pediococcus acidilactici* were selected and used to inoculate sourdough in order to reduce
57 acrylamide content. After fermentation for 16 h, all of the inoculated sourdoughs showed lower pH values
58 compared to the spontaneous sourdough. This acidification was accompanied by a significant ($p<0.05$) increase
59 in the concentration of reducing sugars. The baked bread samples made with the tested LAB strains showed
60 significantly reduced acrylamide content, in particular for the sample inoculated with *P.acidilactici* (5.64 $\mu\text{g}/\text{kg}$),
61 compared to the bread sample prepared with baker's yeast (35.6 $\mu\text{g}/\text{kg}$). The resulting breads were also evaluated
62 for several other quality parameters. The highest softness was registered for the breads obtained from the
63 fermentation by *P.acidilactici* (2704 g). The different tested strains also influenced the color, the void fraction,
64 and the cell density of the breads. The sensory evaluation indicated that the crust color, crumb aeration, as well
65 as the salty and acidic tastes were not significantly affected by sourdough incorporation. However, breads made
66 from LAB sourdoughs were more appreciated by the tasters. This study proved the suitability use of the selected
67 *P.acidilactici* strain for industrial-scale bread production.

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69 **Keywords:** Sourdough bread; Lactic acid bacteria; Acrylamide; Sensory evaluation

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84 1. Introduction

85 Bread is one of the staple foods for human nutrition. It supplies a considerable portion of the nutrients required
86 for growth, maintenance of health and well-being. The baker's yeast bread is the most consumed type in Tunisian
87 diet (EI, 2014). During bread baking, some hazardous byproducts can be formed, such as acrylamide. This
88 compound has been recognized as a neurotoxic and potentially carcinogen substance for humans (IARC, 1994).
89 Acrylamide has been detected in a wide range of cooked and fried carbohydrate rich foods, particularly crisp
90 bread, potato chips, breakfast cereals, and coffee (Gökmen et al., 2007). The most probable route of acrylamide
91 formation is through Maillard reactions involving the amino acid asparagine and reducing sugars (Mottram et al.,
92 2002). It also can be formed through several other pathways, such as the reaction between aspartic acid and
93 reducing sugars (Yaylayan & Stadler, 2005), thermal degradation of amino acids and proteins (Keramat et al.,
94 2011), as well as decarboxylation and deamination of asparagine (Granvogl & Schieberle, 2006).

95 A wide range of researchers representing national food safety authorities, academia, and food manufacturers
96 have sought to better understand the mechanisms of acrylamide formation and to find ways to minimize its
97 formation in foods (EFSA, 2011).

98 Previous studies have indicated that the occurrence of acrylamide in heat-processed foods may depend on
99 specific factors, such as the initial concentrations of asparagine, reducing sugars, pH, water activity, time and
100 temperature of the heating process (Gökmen et al., 2007).

101 Over the last decades, there have been many attempts to reduce acrylamide formation by the optimization of
102 processing conditions. Indeed, the HEATOX report by Hellenäs et al. (2005) showed that acrylamide synthesis
103 increased at higher baking temperature (200–260°C) and with time (10–25 min). Another strategy for reducing
104 the acrylamide content depended on the choice of appropriate raw materials. Wang et al. (2017) suggested that
105 the generation of acrylamide can be mitigated by reducing the presence of degraded starch in flour.

106 Several studies indicated that fermentation processes performed with lactic acid bacteria (LAB) and yeast could
107 reduce the acrylamide content in bread. This effect is mainly related to a decrease of pH rather than to the
108 consumption of precursor nutrients (asparagine and reducing sugars) by microorganisms growing in sourdough
109 (Fredriksson et al., 2004; Bartkiene et al., 2013b; Wang et al., 2017).

110 Moreover, the use of lactic acid bacteria in sourdough improved the properties of dough and enhanced the flavor
111 and texture of bread (Saeed et al., 2014). It was also reported that the use of sourdough extended the
112 microbiological shelf life of bread by controlling and inhibiting spoilage organisms during fermentation, due to
113 the lower pH value and antimicrobial metabolites of LAB (Cizeikiene et al., 2013). Likewise, lactic acid

114 sourdough had a higher content of biogenic compounds, lower level of anti-nutritional factors and a better value
115 of glycemc response, as well as improved the uptake of minerals (Gobbetti et al., 2012).

116 So, the challenge of this study was to find a way to reduce the acrylamide content in Tunisian bread without
117 compromising its organoleptic properties. Thus, a lactic fermentation was conducted using four LAB strains,
118 previously isolated from Tunisian flours and identified. They were tested to decrease the acrylamide
119 concentration in bread. Up to our knowledge, no research has been conducted on acrylamide reduction in
120 Tunisian food. For this purpose, the known acrylamide precursors (asparagine and reducing sugars) were
121 measured in fermented dough. At the same time, the influence of lactic acid fermentation on sensory features of
122 the final products was studied.

123

124 **2. Materials and methods**

125 **2.1. Materials**

126 The flour used (type PS-7 and moisture content 14.43%) was purchased from the mill SOTUMIS (Ennakhla)
127 situated in Tunisia. Chemicals and analytical standards were mainly purchased from Sigma-Aldrich (Dublin,
128 Ireland). All analytical standards had a purity of at least 95%.

129

130 **2.2. Microorganisms**

131 Fresh yeast was supplied by Rayen Food Industries. The strains of lactic acid bacteria (LAB) used in this study
132 (*Lactobacillus brevis* strain S12 (MF458471), *Lactobacillus plantarum* strain S28 (MF458477), *Pediococcus*
133 *pentoseus* strain S14 (MF4584872), and *Pediococcus acidilactici* strain S16 (MF458474) were previously
134 isolated, identified by 16S ribosomal RNA (rRNA) gene from various Tunisian bakery flours and selected basing
135 on their acidification activity according to the method described by Alfonzo and al. (2013) where LAB cells
136 were assessed to reduce pH of a flour extract after 24 h, 48 h and 72 h of inoculation (Data not shown).

137

138 **2.3. Preparation of sourdough**

139 Experimental sourdough bread was manufactured using the previously described selected strains of LAB
140 (*L.brevis* S12, *L.plantarum* S28, *P.pentoseus* S14 and *P.acidilactici* S16) based on their acidification capacity.
141 The LAB strains were cultivated in MRS broth (1% maltose w/v, 1% lactose w/v, pH 5.6) at 30°C for 18 h.
142 Bacterial cells were harvested by centrifugation (10,000 × g, 10 min, 4°C), washed twice with 50 mM sterile
143 potassium phosphate buffer (pH 7.0) before being resuspended in tap water. Each strain was individually

144 characterized and inoculated into the dough. The sourdough was made according to Nionelli et al., (2014).
145 Briefly, for preparing 100 g of sourdough, 62.5 g of flour were mixed with 37.5 mL of tap water containing 10^9
146 CFU/g of LAB cells. The obtained sourdough was incubated at 30°C for 16 h. A spontaneous sourdough (SS)
147 prepared without inoculation with the selected LAB strains was used as a control.

148

149 **2.4. Making of bread**

150 The dough was made according to the recipe of Nionelli et al., (2014). In all of the experiments, 20% (w/w) of
151 sourdough was added to dough. Baker's yeast was added at the amount of 2% (w/w). Dough yield (DY = (dough
152 weight / flour weight) \times 100) was fixed at 160. After that, dough samples were left to ferment for 1 h 30 min.

153 The control bread was made according to the same formulation, but without the addition of sourdough. All bread
154 samples were prepared in triplicates. All breads were baked at 220°C for 20 min.

155

156 **2.5. Analytical methods**

157 **2.5.1. Determination of pH and total titratable acidity**

158 The values of pH and total titratable acidity (TTA) were determined after mixing 10 g of sourdough or dough
159 with 90 mL of distilled water using a Stomacher mixer. The pH was measured using a pH meter and the
160 acidification capacity (Δ pH) was calculated as the difference between pH after fermentation and pH before
161 fermentation. The mixture was used to measure the TTA expressed as the amount (mL) of 0.1 M NaOH solution
162 needed to reach the pH value of 8.3.

163

164 **2.5.2. Determination of reducing sugars**

165 The sugar content (glucose, fructose, and sucrose) of freeze-dried doughs was determined by high-performance
166 liquid chromatography (HPLC) system (Waters 2695 Separations Module) according to the method of Bartkiene
167 et al. (2013). Five grams of each sample were mixed with 20 mL of deionized water. The filtrate was placed in a
168 water bath for 1 h at 75°C, in order to inactivate the enzymes, before being centrifuged at $13500 \times g$ for 10 min.

169 The obtained supernatants were analyzed by a HPLC system equipped with an AKTA purification system, an
170 analytical column (Phenomenex Luna 5 μ m NH₂, 150 \times 4.6 mm (Torrance, CA, USA)) and a refractive index
171 detector (Waters 2414). The compounds were quantified using a calibration curve of the corresponding standards
172 ranging between 50 and 500 mg/mL. Sucrose, glucose, and D-fructose standards were obtained from Sigma-
173 Aldrich.

174

175 2.5.3. Analysis of asparagine

176 Asparagine was extracted by the method reported by Rizzello et al. (2010). Each sample (2 μ L) was injected into
177 HPLC coupled with a Shimadzu LC-20AD evaporative light scattering detector (ELSD). The mobile phase was
178 composed of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in 98% acetonitrile) with a
179 flow rate of 0.7 mL/ min at 30°C, and the injection volume was 10 μ L. The gradient starting from 0 % B for 5
180 min, 15 % B for 7 min, 30 % B for 5 min, and finally followed by a 18 min maintained at 0% B. Asparagine was
181 quantified using a calibration curve made with L-asparagine (Sigma-Aldrich).

182

183 2.5.4. Determination of acrylamide by LC-MS/MS

184 The extraction of acrylamide was carried out on bread samples according to the method of Bartkiene et al.
185 (2013). It was conducted with acetonitrile and QuEChERS salt mixture (4 g anhydrous $MgSO_4$ (Sigma Aldrich)
186 and 0.5 g of NaCl (Analytika Ltd (Check Republic)). The extracts were then added to 50 mg of PCA-sorbent
187 (SSELECTRA Bulk Sorbents) and 150 mg anhydrous $MgSO_4$ to obtain pure extracts. These latter were than
188 analyzed with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The quantitative
189 analysis was performed by QTRAP 5500 LC-MS/MS instrument (AB SCIEX, Framingham, MA, USA) coupled
190 to an Acquity UPLC system (Waters, Milford, MA, USA). The separation of acrylamide was obtained with Luna
191 3 μ m HILIC column (100 \times 3.00 mm) (Phenomenex, Torrance, CA, USA). The solvent system was composed of
192 0.1% formic acid in water and 0.1% formic acid in methanol with a flow rate of 0.3 mL/min at 30°C, and the
193 injection volume was 10 μ L. The conditions selected for the MS/MS detection were as follows: curtain gas
194 (Nitrogen): 30.0 psi; ion spray: 5500 V; temperature: 400°C, nebulizer gas (Nitrogen): 40 psi; nebulizer gas
195 (Nitrogen): 50 psi. Acrylamide was quantified using a calibration curve made with internal standard (acrylamide-
196 d_3) dissolved in acetonitrile and chromatograms were acquired and processed by using the Analyst software,
197 version 1.5 (AB SCIEX).

198

199 2.5.5. The evaluation of bread quality

200 The hardness of bread samples was determined with TVT 6700 texture analyzer (Perten Instruments, North Ryde
201 BC, NSW, Australia). All samples were prepared and baked on the day of test. Three slices of bread (20 mm
202 thick) were compressed by using a cylindrical compression probe with 75 mm diameter. The following settings
203 were selected: test speed 5 mm/s, 50% deformation of the sample and two compression cycles with a break of 12

204 s. Data was analyzed using the TexCal software. Color parameters (L^* , a^* , b^*) of the crust and crumb parts of
205 bread samples were determined in triplicate by using a CR-300 colorimeter (Minolta, Japan).

206 The crumb features of breads were evaluated after 24 h of storage. Images of three slices were acquired using a
207 Nikon D3100 digital camera (55 mm lens; level of sensitivity: ISO1600; shutter speed: 1/640 s). Two 20 mm \times
208 20 mm square fields of view were evaluated in each image. Segmentation was performed manually, by
209 binarization of 8-bit greyscale images into black-and-white images using the Otsu ImageJ thresholding algorithm
210 implemented with Fiji 1.51 software package. After that, the void fraction (the fraction of the total area
211 corresponding to the bread pores) and cell density (number of cells/mm²) were extracted and calculated.

212 Sensory analysis of baked breads was accomplished after 1 hour of manufacturing by a semi-trained panel (38
213 panelists consisting of students (male and female) from the High School of Food Industries of Tunisia). The
214 panelists evaluated the sensory properties of the bread samples based on their degree of acceptance (scale of 1-8,
215 with 8 being the highest score). The sensory attributes were discussed with the panelists during the introductory
216 sensory training sessions. Before the sensory evaluation, the loaves were cut into 1.5 cm thick slices and were
217 then served in random order. The elasticity, crust and crumb colors, acidic and salty taste, appearance, volume
218 and crumb aeration were considered as sensory attributes. All sensory evaluation tests were performed in
219 accordance to the ethical and professional guidelines described in the document issued by the Institute of Food
220 Science and Technology (IFST, 2015).

221

222 **2.5.6. Statistical analysis**

223 All experiments were repeated three times and illustrated as the mean values \pm standard deviations. Statistical
224 analyses were performed using the IBM SPSS Statistics software version 23.0. The data were analyzed using
225 one-way analysis of variance (ANOVA), followed by the Duncan's test with the significance level set at $p < 0.05$
226 to establish the significance of differences between the samples. Pearson correlation analysis was also
227 performed.

228

229 **3. Results and discussion**

230 **3.1. Physico-chemical analysis of sourdough**

231 The values of pH, TTA, the content of sugars (glucose, fructose, and sucrose) and free asparagine in spontaneous
232 and inoculated fermented sourdoughs were determined, as presented in Table 1.

233 After 16 h of fermentation, the pH of all sourdough samples had decreased from 6.36 ± 0.06 to 4.03 ± 0.06 in SS
234 - 3.55 ± 0.04 in SLb. Moreover, the highest ΔpH (2.81) ($p < 0.05$) was found in the sourdough prepared with
235 *L.brevis* S12 (SLb), whereas the lowest ΔpH value (2.33) was measured in the spontaneous sourdough (SS). In
236 fact, LAB are recognized to considerably decrease the pH values in sourdoughs, but the variation of pH depends
237 on the LAB strain used (Elsanhoty et al., 2016). According to Cizeikiene et al., (2013), adding sourdough to
238 bread could control and notably inhibit spoilage organisms through lowering the pH value during fermentation.
239 Besides, TTA was significantly ($p < 0.05$) increased after fermentation, from 2.5 mL to 5.2–12.2 mL. The
240 comparison of results highlighted that all sourdoughs inoculated with the selected LAB strains had significantly
241 ($p < 0.05$) higher TTA values than those observed for spontaneous sourdough (SS). Furthermore, a high negative
242 correlation ($r = -0.953$) between the pH and TTA values was found after fermentation. These findings are in
243 agreement with the results of Mamhoud et al. (2016). Concerning the analysis of reducing sugars, a significant
244 ($p < 0.05$) increase of their concentration (from 612 ± 38 to 966 ± 21 mg/100g dry weight (d.w.) and from $266 \pm$
245 48 to 563 ± 12 mg/100g d.w. for glucose and fructose, respectively) was shown after 16 h of fermentation.
246 Interestingly, *P.pentoseus* S14 was the key strain for the formation of reducing sugars at concentrations reaching
247 1529 mg/100g d.w. after fermentation (74.15%). The use of LAB caused a significant ($p < 0.05$) increase of the
248 glucose concentration (718–966 g/100g d.w.) compared to spontaneous sourdough (529 g/100g d.w.) after
249 fermentation. This observed increase of glucose concentration in sourdough could be related to the degradation
250 of sucrose and starch by cereal amylases and LAB (Bartkiene et al., 2013).
251 Before fermentation, the concentration of sucrose was estimated to be 460 ± 71 mg/100g d.w. After 16 h of
252 fermentation, a significant ($p < 0.05$) decrease of sucrose amount was noticed in sourdoughs, ranging from $413 \pm$
253 22 mg/100g d.w. in DSS to 173 ± 5 mg/100g d.w. in SLp). The sharpest decrease of sucrose concentration was
254 recorded in the sourdough inoculated with *L.plantarum* S28 (62.39%), compared to the results for spontaneous
255 sourdough, which showed only a very weak decrease (9.78%).
256 The fermentation of dough plays a major role in the control of the acrylamide formation rate related to the
257 presence of asparagine (Dastmalchi et al., 2016). In this context, the content of free asparagine was determined
258 in all sourdough samples before and after fermentation (Table 1) in order to investigate whether the quantity of
259 this amino acid was reduced by fermentation.
260 Before fermentation, the content of free asparagine was found to be 16.1 ± 0.6 mg/100g d.w. Nevertheless, all
261 LAB strains were significantly ($p < 0.05$) able to increase the asparagine content after 16h of fermentation. That

262 could be explained by the proteolytic activity of the selected starters (Bartkiene et al., 2013, Mamhoud et al.,
263 2016).

264

265 3.2. Physico-chemical analysis of dough

266 The variation of pH, the concentrations of reducing sugars and asparagine in the dough samples were measured
267 after fermentation and are presented in Table 2. The Baker's yeast dough (DBB), which was made without the
268 addition of sourdough, was considered as a control.

269 The pH values obtained using the selected LAB strains and spontaneous sourdough are reported in Table 2. As
270 expected, the sourdoughs made with the selected LAB strains caused a significantly ($p<0.05$) more pronounced
271 decrease of pH (4.13–4.68) in comparison to DSS (4.97) and DBB (5.77). Diana et al., (2014) reported that the
272 addition of sourdough to the dough formulation resulted in a linear increase in the acidity of the product. These
273 results are consistent with those obtained by Suhr and Nielsen. (2004) who attested that the pH value reached 5
274 using yeast and 4.4 – 4.8 using sourdough in the dough formulation.

275 After 1h 30 min of fermentation, it was noticed that the amount of reducing sugars in the control dough (DBB)
276 was significantly ($p<0.05$) higher than that of other doughs except for the DLp.

277 Indeed, the amount of fructose was significantly ($p<0.05$) higher in DBB (532 ± 11 mg/100g d.w.) compared to
278 the other dough samples (from 230 ± 7 mg/100g d.w. to 326 ± 10 mg/100g d.w.). These results can be explained
279 by the fact that fructose is used as an electron acceptor by LAB and thus is reduced to mannitol (Wisselink et al.,
280 2002).

281 Sucrose was not detected in any of the analyzed samples. This observation can be explained by several studies
282 that already revealed the presence of a very active invertase enzyme in yeast, which promotes rapid breakdown
283 of sucrose to glucose and fructose (Koschwanez et al., 2011).

284 The concentration of asparagine in dough samples is shown in Table 2. The effects of LAB strains on the amount
285 of asparagine depended on the particular strains. The amount of asparagine is ranged from 9.9 ± 0.4 mg/100g
286 d.w. in DPp to 16 ± 0.9 mg/100g d.w. in DBB, and these values were similar to those reported previously (Wang
287 et al., 2017). A significant decrease ($p<0.05$) of asparagine content was shown in DSS, DPp, and DPa (14.2 ± 0.2
288 mg/100g d.w., 9.9 ± 0.4 mg/100g d.w., and 10.5 ± 0.1 mg/100g d.w., respectively), compared to DBB (16 ± 0.9
289 mg/100g d.w.), which is fermented with yeast only. The results showed that fermentation of wheat dough with
290 LAB reduced the concentration of asparagine. These results are in agreement with those of Wang et al., (2017).

291 In addition, Fredriksson et al., (2004) reported that during bread dough fermentation amino acids are assimilated
292 by yeast or LAB and metabolized as a source of nitrogen.

293

294 3.3. Analysis of acrylamide

295 All bread samples were evaluated for their acrylamide levels and the results are presented in Figure 1. To better
296 understand the mechanism of acrylamide formation and its relationship to dough fermentation, the physico-
297 chemical characteristics and composition of sourdough were assessed.

298 The concentration of acrylamide in bread samples varied from 5.64 µg/kg to 35.6 µg/kg. The highest acrylamide
299 content was detected in the control bread sample (BBB). According to European Commission, for this type of
300 bread (Wheat based soft bread), the indicative value of acrylamide was estimated to 80 µg/kg (EC, 2013).
301 However, bread is classified as the main food contributing to the daily ingestion of acrylamide which can cause a
302 public health problem (EFSA, 2011) and Tunisians are big consumers of bread; on average 70 Kg/person/year
303 (Benaours, 2017).

304 All LAB strains were able to significantly ($p < 0.05$) reduce the acrylamide content of bread. Especially, the
305 *P.acidilactici* strain S16 allowed to decrease the acrylamide levels by 84.2% (to 5.64 µg/kg) compared to the
306 control bread (35.6 µg/kg). The other LAB strains (*L.brevis* S12, *L.plantarum* S28, and *P.pentoseus* S14)
307 provided significantly ($p < 0.05$) decreased acrylamide levels (reduction by 55.6%, 49.2%, and 39.2%,
308 respectively) compared to the control sample. Thus, the effect of LAB fermentation on acrylamide reduction
309 depended on the particular LAB strain used in sourdough and its adaptability or competition with yeast in the
310 absorption of nutrients (Dastmalchi et al., 2016).

311 Bartkiene et al., (2013) showed that the fermentation with a commercial *L.casei* strain could reduce the
312 acrylamide content in bread samples by 29.4% on average. In the same way, Baardseth et al. (2006) found that
313 LAB could reduce the acrylamide level in French fries by 71% after 120 min of fermentation.

314 Besides, a significant decrease ($p < 0.05$) of acrylamide content was noticed in bread leavened with spontaneous
315 sourdough. This result was consistent with the findings by Bartkiene et al., (2013) and Forstova et al., (2013).

316 The concentration of glucose and sucrose in sourdoughs was significantly ($p < 0.05$) slightly correlated to the
317 formation of acrylamide ($r = 0.565$ and $r = -0.617$, respectively). These results were in agreement with those of
318 Surdyk et al., (2004). Sugars seems to be the most important ingredient that influences the acrylamide formation
319 during baking (Baardseth et al., 2006; Dastmalchi et al., 2016). Unlike sugars, the asparagine content of
320 sourdoughs did not show a clear correlation with acrylamide concentration.

321 Numerous studies suggested that lowering pH values by microorganisms is one of the solutions to preventing the
322 Maillard reaction, the main path of acrylamide formation (Bartkiene et al., 2013; Keramat et al., 2011;
323 Dastmalchi et al., 2016). The first step in Maillard reaction is the generation of a Schiff base that can form 3-
324 aminopropionamide, a precursor of acrylamide (Granvogl et al., 2004).

325

326 3.4. Characteristics of the experimental breads

327 The color parameters (L^* , a^* , b^*) of crust and crumb obtained from the bread samples are listed in Table 3. As it
328 can be seen, the crust lightness (L^*) of inoculated sourdough breads (BLb, BLp, and BPa) was significantly
329 lower ($p < 0.05$) than that of control bread (BBB). Moreover, all sourdoughs inoculated with LAB caused
330 significant changes in the “ a^* ” values of crusts, with BPp showing the lowest value. Statistical analysis indicated
331 that the “ L^* ” and “ b^* ” parameters of crusts correlated positively ($r = 0.486$ and $r = 0.510$, respectively, $p < 0.05$)
332 with the acrylamide levels in breads. These results were in agreement with those of Surdyk et al., (2004). The
333 crumbs of BLp, BLb, and BPp bread samples had significantly ($p < 0.05$) high values of luminosity (L^*) (74.21,
334 73.85, and 74.49) compared to the BSS sample (70.11). A few differences were registered for the b^* and a^*
335 parameters of the crumb.

336 The highest hardness (5149 ± 119 g) was observed for the bread made with yeast only (BBB) (Table 3). The
337 softest breads were those obtained from sourdoughs fermented with *P.pentoseus* S14 (1815 ± 216 g) and
338 *P.acidilactici* S16 (2704 ± 42 g). As reported by Dastmalchi et al., (2016), the addition of sourdoughs reduced
339 the firmness of bread, which depended mainly on the acidification level of sourdough.

340 The crumb structure of breads was evaluated by digital image analysis (Figure 2). According to Table 3, the
341 observed void fraction and cell density were significantly ($p < 0.05$) different from BBB. This finding confirmed
342 that the final characteristics of the breads were influenced by the starter strains and their interactions.

343

344 3.5. Sensory attributes of breads

345 The results of the sensory analysis of the experimental breads are shown in Figure 3. The crust color, crumb
346 aeration, and detection of salty or acidic taste were not significantly ($p < 0.05$) affected by the addition of
347 sourdough. Moreover, the appearance of bread samples showed an increasing trend towards average scores when
348 LAB sourdoughs were added. The BPa bread sample had the higher appearance score of 6.27, compared to BSS
349 and BBB (6.03 and 5.41, respectively). Likewise, the crumb color and the volume attributes increased when
350 sourdoughs were used. Organic acids, alcohols, esters, carbonyl compounds, carbon dioxide, and

351 exopolysaccharides produced by LAB during sourdough fermentation are the probable factors resulting in the
352 improved volume, texture, flavor, and aroma of baked bread samples (Saeed et al., 2014).

353

354 4. Conclusion

355 In essence, our proposed strategy provides an effective way to reduce acrylamide formation in bread by using
356 selected lactic acid bacteria strains for fermentation of dough. The most pronounced reduction of acrylamide
357 formation (by 84.7%) was obtained in bread made with *P.acidilactici* strain S16. At the same time, the influence
358 of lactic acid fermentation on the sensory properties of the final bread samples was studied. The obtained results
359 indicate that the incorporation of selected lactic acid bacteria in the bread preparation can improve the texture
360 and flavor of bread. Selected *P.acidilactici* strains could be potentially developed for further applications in
361 fermented products. Finally, a continued effort is recommended to investigate the effects of other lactic bacteria
362 strains on acrylamide synthesis during the baking of bread.

363

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367

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- 447

Table 1. The physico-chemical parameters and composition of sourdoughs after fermentation for 16 h.

| Samples | pH | Total titratable acidity (mL NaOH) | Glucose (mg/100 g d.w.) | Fructose (mg/100 g d.w.) | Total reducing sugars (mg/100 g d.w.) | Sucrose (mg/100 g d.w.) | Asparagine (mg/100g d.w.) |
|---------|--------------------------|------------------------------------|-------------------------|--------------------------|---------------------------------------|-------------------------|---------------------------|
| SS | 4.03 ± 0.06 ^c | 5.2 ± 0.3 ^a | 529 ± 18 ^a | 250 ± 3 ^b | 779 ± 15 ^a | 413 ± 22 ^d | 18.0 ± 1 ^a |
| SLb | 3.55 ± 0.04 ^a | 12.2 ± 0.5 ^d | 751 ± 26 ^{bc} | 274 ± 4 ^b | 1025 ± 22 ^b | 203 ± 8 ^b | 20.0 ± 0.3 ^b |
| SLp | 3.65 ± 0.1 ^{ab} | 11.0 ± 0.4 ^{bc} | 718 ± 62 ^b | 196 ± 5 ^a | 914 ± 57 ^c | 173 ± 5 ^a | 17.7 ± 0.5 ^a |
| SPp | 3.69 ± 0.04 ^b | 10.3 ± 0.4 ^b | 966 ± 21 ^d | 563 ± 12 ^d | 1529 ± 19 ^d | 223 ± 19 ^{bc} | 19.8 ± 0.4 ^c |
| SPa | 3.57 ± 0.02 ^a | 11.6 ± 0.4 ^{cd} | 793 ± 11 ^c | 356 ± 4 ^c | 1149 ± 7 ^e | 241 ± 10 ^c | 18.7 ± 0.4 ^a |

The data are presented as the mean values ± standard errors ($n = 3$). The mean values followed by the same letter within column are not significantly different ($p < 0.05$); SS: spontaneous sourdough; SLb: sourdough inoculated with *L. brevis* S12; SLp: sourdough inoculated with *L. plantarum* S28; SPp: sourdough inoculated with *P. pentoseus* S14; SPa: sourdough inoculated with *P. acidilactici* S16.

Table 2. The pH values and concentrations of glucose, fructose, total reducing sugars and asparagine in different dough samples after 90 min of fermentation.

| Samples | pH | Glucose (mg/100g d.w.) | Fructose (mg/100g d.w.) | Total reducing sugars (mg/100 g d.w.) | Asparagine (mg/100g d.w.) |
|---------|--------------------------|------------------------|-------------------------|---------------------------------------|---------------------------|
| DSS | 4.97 ± 0.01 ^d | 826 ± 23 ^{bc} | 230 ± 7 ^b | 1057 ± 30 ^c | 14.2 ± 0.2 ^b |
| DLb | 4.49 ± 0.02 ^b | 833 ± 20 ^{bc} | 262 ± 6 ^c | 1096 ± 21 ^c | 15.7 ± 0.2 ^c |
| DLp | 4.13 ± 0.04 ^a | 1494 ± 26 ^d | 258 ± 8 ^c | 1752 ± 29 ^e | 14.8 ± 0.4 ^{bc} |
| DPp | 4.52 ± 0.03 ^c | 500 ± 14 ^a | 232 ± 6 ^b | 732 ± 19 ^a | 9.9 ± 0.4 ^a |
| DPa | 4.68 ± 0.04 ^c | 471 ± 19 ^a | 326 ± 10 ^d | 797 ± 14 ^b | 10.5 ± 0.1 ^b |
| DBB | 5.77 ± 0.09 ^e | 748 ± 31 ^b | 532 ± 11 ^e | 1281 ± 39 ^d | 16 ± 0.9 ^c |

The data are presented as the mean values ± standard errors ($n = 3$). The mean values followed by the same letter within the column are not significantly different ($p < 0.05$). DSS: dough made with spontaneous sourdough; DLb: dough inoculated with *L. brevis* S12 sourdough; DLp: dough inoculated with *L. plantarum* S28 sourdough; DPp: dough inoculated with *P. pentoseus* S14 sourdough; DPa: dough with *P. acidilactici* S16 sourdough; DBB: Baker's yeast dough made without the addition of sourdough.

Table 3. The characteristics of bread loaves.

| Samples | Crust color | | | Crumb color | | | Hardness (g) | Void fraction (%) | Cell density (cells/mm ²) |
|------------|---------------------------|----------------------------|---------------------------|---------------------------|----------------------------|---------------------------|-------------------------|---------------------------|---------------------------------------|
| | L* | a* | b* | L* | a* | b* | | | |
| BLb | 60.33 ± 0.47 ^b | 11.25 ± 0.64 ^c | 34.64 ± 0.33 ^b | 73.85 ± 0.41 ^b | -1.27 ± 0.05 ^{bc} | 14.13 ± 0.24 ^a | 4338 ± 212 ^b | 35.11 ± 1.5 ^b | 2888.82 ± 254.99 ^a |
| BLp | 60.91 ± 0.39 ^b | 10.62 ± 0.33 ^c | 35.23 ± 0.33 ^b | 74.21 ± 0.19 ^b | -1.01 ± 0.02 ^{cd} | 15.95 ± 0.49 ^b | 4188 ± 444 ^b | 47.04 ± 0.6 ^d | 4764.22 ± 112.03 ^{cd} |
| BPp | 68.71 ± 0.21 ^e | 5.94 ± 0.05 ^a | 34.42 ± 0.19 ^b | 74.49 ± 0.23 ^b | -1.64 ± 0.09 ^a | 14.97 ± 0.64 ^b | 1815 ± 216 ^a | 43.99 ± 2.32 ^d | 3949.54 ± 104.56 ^{bc} |
| Bpa | 58.49 ± 0.15 ^a | 10.33 ± 0.37 ^{bc} | 32.73 ± 0.32 ^a | 71.09 ± 0.43 ^a | -0.84 ± 0.06 ^a | 14.38 ± 0.2 ^a | 2704 ± 42 ^a | 34.89 ± 1.23 ^b | 3217.14 ± 386.02 ^{ab} |
| BSS | 63.77 ± 0.44 ^d | 9.56 ± 0.05 ^b | 34.91 ± 0.66 ^b | 70.11 ± 0.67 ^a | -1.14 ± 0.2 ^{cd} | 13.85 ± 0.07 ^a | 4060 ± 57 ^b | 31.15 ± 0.90 ^a | 5256.74 ± 27.67 ^d |
| BBB | 63.09 ± 0.07 ^d | 9.58 ± 0.38 ^b | 35.36 ± 0.11 ^b | 74.89 ± 0.30 ^b | -1.53 ± 0.1 ^{ab} | 14.63 ± 0.18 ^a | 5149 ± 119 ^b | 39.57 ± 0.87 ^c | 3831.8 ± 659.82 ^b |

Data are presented as the mean value ± standard error ($n = 3$); the mean values followed by the same letter within column are not significantly different ($p < 0.05$); BSS: bread made with spontaneous sourdough but without LAB strains; BLb: bread made with *L. brevis* S12 sourdough; BLp: bread made with *L. plantarum* S28 sourdough; BPp: bread made with *P. pentoseus* S14 sourdough; BPa: bread made with *P. acidilactici* S16 sourdough; BBB: Baker's yeast bread.

Figure captions

Fig. 1. Acrylamide content in different bread samples. BSS: bread made by using spontaneous sourdough without LAB strains; BLb: bread made with *L. brevis* S12 sourdough; BLp: bread made with *L. plantarum* S28 sourdough; BPp: bread made with *P. pentoseus* S14 sourdough; BPa: bread made with *P. acidilactici* S16 sourdough; BBB: Baker's yeast bread.

Fig. 2. Representative images of the crumb samples from experimental breads (unprocessed digital images (left) and binary images thresholded using the Otsu ImageJ algorithms (right)). BSS: bread made by using spontaneous sourdough without LAB strains; BLb: bread made with *L. brevis* S12 sourdough; BLp: bread made with *L. plantarum* S28 sourdough; BPp: bread made with *P. pentoseus* S14 sourdough; BPa: bread made with *P. acidilactici* S16 sourdough; BBB: Baker's yeast bread.

Fig. 3. A spider web chart of the sensory analysis data for experimental breads. BSS: bread made by using spontaneous sourdough without LAB strains; BLb: bread made with *L. brevis* S12 sourdough; BLp: bread made with *L. plantarum* S28 sourdough; BPp: bread made with *P. pentoseus* S14 sourdough; BPa: bread made with *P. acidilactici* S16 sourdough; BBB: Baker's yeast bread.

Figure 1.

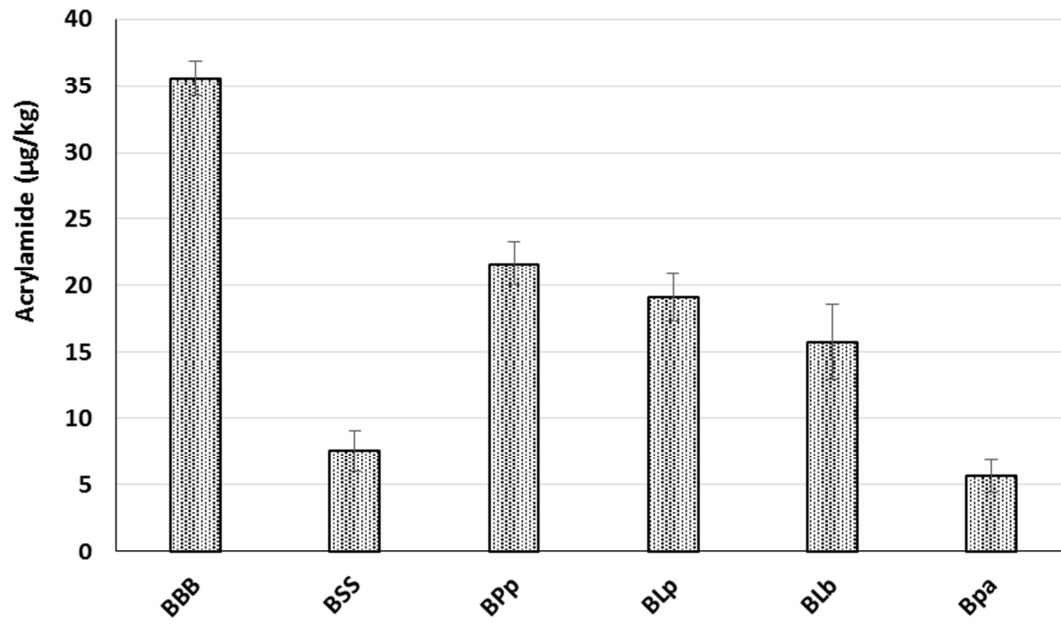


Figure 2.

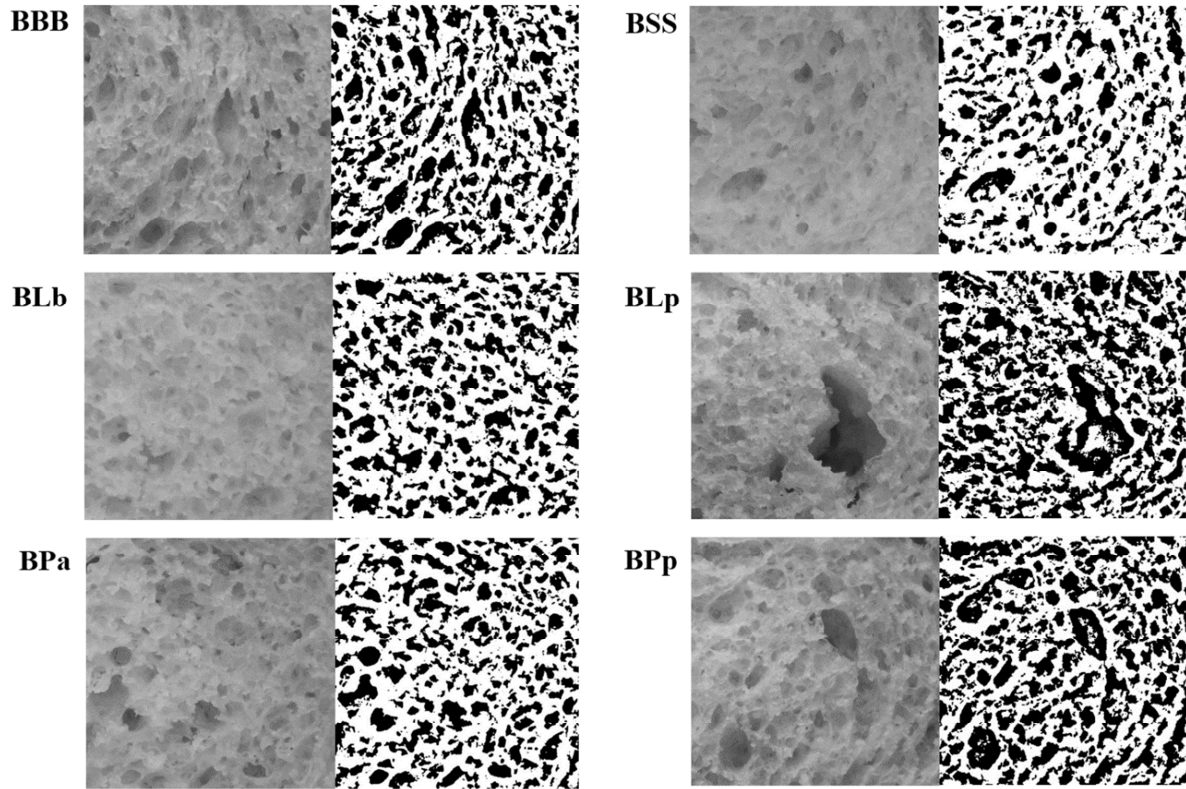
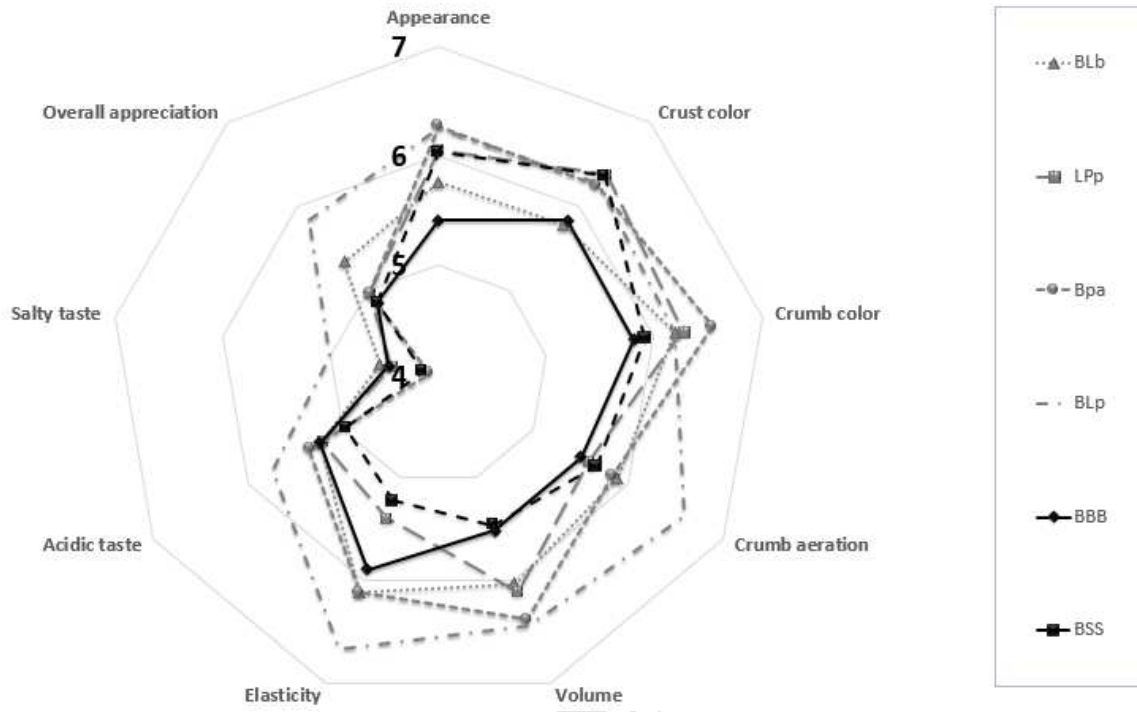


Figure 3.



Highlights

- Four lactic acid bacteria were inoculated separately in bread sourdough.
- Incorporation of lactic acid bacteria in bread reduced acrylamide content.
- Acrylamide amount is correlated to sugar concentration in sourdough.
- Incorporation of lactic acid bacteria in bread can improve its texture and flavor.