1 ***Lactobacillus plantarum* strains for multifunctional oat-based foods**

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27 **Abstract**

28 Fermented oat-based foods offer attractive prospects within the market of non-dairy functional
29 products, since they are suitable substrates for the delivery of probiotic microorganisms, and are
30 significant sources of dietary fiber, both insoluble and soluble such as β -glucan, good quality fat
31 and other phytochemicals important for human health

32 In the present work, whole oat flour was fermented with probiotic *Lactobacillus plantarum* strains
33 to produce new functional foods with improved nutritional and technological features. Viability of
34 the probiotic and the main technological, physico-chemical, nutritional and sensorial parameters
35 were monitored at 7, 14 and 21 days of cold storage. The microbial survival was higher than 5×10^8
36 cfu g⁻¹ at the end of the shelf life. After the fermentation step, viscosity was higher in products
37 inoculated with the exopolysaccharide-producing *L. plantarum* strain Lp90. However, a subsequent
38 viscosity reduction was detected in all the samples throughout the storage period, consistent with
39 the observed concentration decrease of the oat β -glucan. Vitamin B2 content was about two-fold
40 higher in products fermented by the riboflavin-overproducing LpB2, and in these samples the
41 riboflavin concentration further increased during cold storage.

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43 **Highlights**

- 44 • Fermented oat-based foods are suitable substrates for delivery probiotic LAB.
- 45 • Riboflavin-overproducing LpB2 increased the vitamin B2 content of oat-based products.
- 46 • EPS-producing Lp90 seems to improve some rheological features.

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48 **Key words:** oat-based foods; probiotic; oat β -glucan; exopolysaccharides; riboflavin

49 **Chemical compounds studied in this article:** Riboflavin (PubChem CID: 493570)

50 **Abbreviations:** Exopolysaccharides (EPS); Lactic Acid Bacteria (LAB)

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1. Introduction

Fermented beverages are a significant component within the functional food market (Marsh, Hill, Ross, & Cotter, 2014). Dairy-based functional beverages are now consolidated products (Özer & Kirmaci, 2010), and the development of non-dairy fermented drinks is a growing segment of this sector (reviewed by Prado, Parada, Pandey, & Soccol, 2008; Rivera-Espinoza & Gallardo-Navarro, 2010; Gupta & Abu-Ghannam, 2012). Cereals are optimal substrates for the growth of lactic acid bacteria (LAB) with probiotic potential, and they are suggested as matrices for a number of new food formulations with claims of health benefits (Charalampopoulos, Wang, Pandiella, & Webb, 2002). In particular, oat is a major source of non-digestible dietary fibers, including β -glucan. These molecules have been gaining interest for the preparation of functional foods due to their multiple functional and bioactive properties (Brennan & Cleary, 2005). Oat β -glucan has been reported to have beneficial effects on insulin resistance, dyslipidemia, hypertension, obesity, and for their applications in cancer treatment and prevention (Anderson et al., 2009; El Khoury, Cuda, Luhovyy, & Anderson, 2012; Aleem, 2013; Tosh, 2013). Moreover, cereal non-digestible long chain β -glucans have been investigated for their potential as novel prebiotics (Lam & Cheung, 2013; Arena et al., 2014a). In order to exploit these beneficial properties, fermentable oat-based yogurt-like products with high final β -glucan content have been developed (Mårtensson, Andersson, Andersson, Öste, & Holst, 2001). Over the years, the functional potential of oat beverages has been further increased by formulating fermented oat drinks as carriers for probiotic LAB (Angelov, Gotcheva, Kuncheva, & Hristozova, 2006; Kedia, Vázquez, & Pandiella, 2008; Gupta, Cox, & Abu-Ghannam, 2010; Luana et al., 2014). Similarly, oat has been added as a functional ingredient to enhance the quality of non-dairy probiotic mixtures (Coda, Lanera, Trani, Gobbetti, & Di Cagno, 2012; Mridula & Sharma, 2015).

The health benefits of probiotic-enriched foods are expressed either directly through the interactions of ingested live microorganisms with the host (Bron, Van Baarlen, & Kleerebezem, 2012) or

indirectly as the result of the intake of microbial metabolites synthesized during fermentation (Gobbetti, di Cagno, & De Angelis, 2010).

LAB producing B-group vitamins have been described for their potential for the production of functional cereals (Capozzi, Russo, Dueñas, López, & Spano, 2012). Recently, riboflavin over-producing *Lactobacillus plantarum* and *Lactobacillus fermentum* strains were used to considerably increase the vitamin B2 content of cereal-based fermented foods, thus representing a convenient and efficient food-grade biotechnological approach for the production of riboflavin-enriched foods (Capozzi et al., 2011; Russo et al., 2014).

Another interesting feature related to the functional feasibility of microbial food cultures is the ability of some LAB to produce exopolysaccharides (EPS) (Ruas-Madiedo, Salazar, & de los Reyes-Gavilán, 2009). From a technological perspective, EPS show thickening properties and EPS-producing *Pediococcus parvulus* 2.6 was employed to improve viscosity, texture, and mouthfeel of fermented oat-based products (Mårtensson et al., 2002a). Human trials of the oat-based products fermented by *P. parvulus* 2.6 resulted in a decrease in serum cholesterol levels and increased counts of faecal *Bifidobacterium* spp. (Mårtensson et al., 2005). Moreover, EPS obtained from *P. parvulus* 2.6 seems to enhance some probiotic properties of LAB strains *in vitro* models (Russo et al., 2012). Thus, EPS produced by LAB are considered promising molecules in the functional food area as well as prebiotic fermentable substrates able to modulate the intestinal microbiota (Salazar, Gueimonde, de los Reyes-Gavilán, & Ruas-Madiedo, 2015).

In this work, four strains of *L. plantarum* previously characterized for their probiotic aptitudes were used to ferment a whole-grain oat substrate in order to obtain a new oat-based product with improved functional, nutritional and technological features. In particular, the riboflavin over-producing *L. plantarum* B2 and its parental strain *L. plantarum* UFG9 were investigated to increase the vitamin B2 content of the probiotic foods. Similarly, the exopolysaccharides producing *L. plantarum* Lp90 and the corresponding isogenic mutant Lp90 Δ cps2 were analyzed for their ability to improve the rheological properties of the oat-fermented foods. Viability of the probiotic

104 microorganisms and the main technological, physico-chemical, nutritional and sensorial parameters
105 were monitored during three weeks of storage under refrigerated conditions.

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107 2. Materials and methods

108 2.1 Microbial strains and growth conditions

109 Four strains of *Lactobacillus plantarum* were used as starter for oat whole flour fermentation,
110 namely, strain *L. plantarum* UFG9 and its roseoflavin-resistant derivative Lp B2, deposited at the
111 Spanish Type Culture Collection (CECT, Paterna, Spain) with the code number CECT8328 (Arena
112 et al., 2014b); strain Lp90 and its isogenic Lp90Δcps2 mutant (Caggianiello et al., 2015,
113 unpublished results).

114 All the *L. plantarum* strains were routinely grown on MRS broth (Oxoid, Basingstoke, UK) at 37
115 °C.

116 2.2 Fermented oat-based foods

117 Finely milled whole grain oat flour (Table 1) was provided by Glucanova (Lund, Sweden). A
118 fermented oat product was obtained according to the method previously reported by Coda and
119 coauthors (2012) with some modifications. Briefly, a mixture containing oat flour (18% w/v) and
120 distilled water (82% v/v) was prepared with a final volume of 100 mL in sterile plastic containers
121 with screw cap, and heated at 95 °C in a water bath for 10 min, with manual shaking every 2 min.
122 At this time, mesophilic, yeasts and moulds contamination was checked by plate count on PCA or
123 PDA (Oxoid), respectively. To start the fermentation, cells from starter cultures at late exponential
124 phase were harvested by centrifugation (5,000 g x 10 min), washed twice in sterilized saline
125 solution (8.6% NaCl), and inoculated into the food matrix to obtain a concentration of
126 approximately 8×10^8 cfu g⁻¹. The fermentative step was carried out at 37 °C for 16 h. At this time,
127 samples for probiotic assays were stored at 4 °C for 21 days, while control samples were submitted
128 to a thermal treatment of 30 min at 65 °C before storage. Three replicates of each sample were
129 performed and analyzed after 0, 7, 14 and 21 days of cold storage.

130 **2.3 Total soluble solids, titratable acidity, and pH**

131 One gram of sample was used to measure the pH and the titratable acidity (TA), with an automatic
132 titrator (T50 M Terminal, Mettler Toledo, Switzerland). TA was obtained by measuring the volume
133 of 0.1N NaOH used to reach a final pH of 8.2.

134 **2.4 Organic acids and sugars**

135 Organic acids and sugars were extracted by homogenizing 5 g of fresh oat based product with 10
136 mL of ultrapure water using IKA T18 Ultraturrax (Wilmington, USA) homogenizer at 14,000 g for
137 10 seconds. The homogenate was centrifuged at 9,000 g for 10 minutes at 5 °C. The supernatant
138 was filtered with a Grace Pure SPE C189 cartridge (Alltech Italia srl - Grace Division, Milan, Italy)
139 and then with a 0.2 µm filter. Organic acids and sugars were identified using the method of Mena et
140 al. (2011). Samples were injected (10 µL) into a HPLC system (Agilent 1200 series) equipped with
141 an UV detector, set at 210 nm, coupled with a refractive index detector. Separation was achieved on
142 a Rezex ROA-Organic Acid H+(8%) column (300 × 7.80 mm) (Phenomenex, Torrance, USA),
143 using a mobile phase of acidified water (phosphoric acid (0.1%)) with a flow rate of 0.5 mL min⁻¹
144 and an oven temperature of 30 °C. The different organic acids and sugars were characterised and
145 quantified by chromatographic comparison with analytical standards. Sugars and organic acids
146 content was expressed as mg per 100 g of fresh weight.

147 **2.5 Determination of β-glucan concentration**

148 The β-glucans content was determined by using an enzymatic kit according to the manufacturer's
149 instructions (Megazyme International Ireland, Wicklow, Ireland).

150 **2.6 Viscosity**

151 Rheological measurements were performed with a rotational Brookfield LV, DV-II-Pro viscometer
152 (Harlow, England). Approximately 50 g of each sample were placed in the concentric cylindrical
153 cup. For the analysis the spindle n° 3 was used, applying a speed equal to 40 g for 30 sec at a
154 temperature of 5 °C. The viscosity was expressed in centipoise (cP).

155 **2.7 Extraction and quantification of riboflavin**

156 The riboflavin content of oat-based products was analytically determined as previously reported
 157 (Russo et al., 2014). Briefly, 5 g of samples were mixed with 25 mL 0.1 M HCl and hydrolysed by
 158 autoclaving at 121 °C for 30 min. Then, the pH was adjusted to 4.5 with 4 M sodium acetate and the
 159 samples were submitted to enzymatic treatment by adding a 5-mL solution containing α-amylase
 160 (420 U), papain (12 U), acid phosphatase (22 U), and 0.1% of glutathione (all purchased from
 161 Sigma Aldrich). After 1 h in an ultrasonic bath, the samples were diluted to a final volume of 50 mL
 162 with 0.01 M HCl, and quantified by HPLC according to Jakobsen (2008).

163 2.8 Color analysis

164 The most common way to express colour results is the *Commission Internationale de l'Eclairages*
 165 (CIE) L*a*b*, which uses the following colour parameters: L*, indicating lightness (0 = black, 100
 166 = white), a* (-a* = greenness, +a* = redness), and b* (-b* = blueness, +b* = yellowness). Samples
 167 colour was measured using a Spectrophotometer (CM-2600d Konica Minolta, Japan) to obtain L*,
 168 a* and b* values. In addition the colour appearance (Chroma, C*) and hue angle (h°) and total
 169 colour differences (TCD; (ΔE*)) were calculated:

$$170 \quad \text{Chroma} = \left(\sqrt{a^{*2} + b^{*2}} \right) \quad (1)$$

$$171 \quad \text{hue angle} = \arctan (b^*/a^*) \quad (2)$$

$$172 \quad \Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (3)$$

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174 2.9 Sensorial quality

175 A panel of five previously trained panelists carried out the sensory evaluations of fermented oat-
 176 based products at all experimental times. Samples were kept at 5 °C until sensorial evaluation, and
 177 each panelist was presented with 3 samples from each treatment in a lidded container to avoid loss
 178 of aroma. The samples were coded with a random 3-digit number in order to mask the treatment
 179 identity and to minimize subjectivity. The sensorial attributes judged during evaluation were: color,

180 odor, off odor, visual quality, overall appearance. A hedonic scale was used that included 5 pictures
181 corresponding to a score from 1 to 5 where: 1 = really poor and 5 = full characteristic.

182 **2.10 Statistical analysis**

183 A two-way ANOVA by using StatGraphics Centurion XVI.I (StatPoint Technologies, Inc., USA),
184 was performed to evaluate significant differences among treatment triplicates. Mean values were
185 separated applying the Tukey test with significant difference when $P \leq 0.05$.

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187 **3. Results and discussion**

188 **3.1 Formulation of new multifunctional fermented oat-based products**

189 In this work, new multifunctional fermented oat-based products were obtained from a mixture of
190 whole oat flour (18%) and water (82%) submitted to a gelatinization step of 95 °C for 15 min,
191 which was previously reported to produce yogurt-like beverages (Coda, Lanera, Trani, Gobbetti, &
192 Di Cagno, 2012). After this step, the environmental microbial contamination was lower than 100
193 cfu g⁻¹, as showed by plates count (data not shown). Fermentation of the oat matrix was performed
194 by inoculating the riboflavin-overproducer LpB2 or the exopolysaccharides (EPS) producer Lp90 in
195 order to evaluate their contribution to enhance some nutritional or technological features of the
196 product, respectively.

197 In particular, the riboflavin-overproducer LpB2 was selected after exposure of the parental strain Lp
198 UFG9 to the selective pressure of the riboflavin-analogue roseoflavin (Arena et al., 2014b). On the
199 other hand, Lp90, the first *L. plantarum* of wine origin, whose genome is available (Lamontanara et
200 al., 2015), was characterized by a ropy phenotype due to the production of EPS that was lost after
201 deletion of its *Cps2* cluster in its isogenic mutant Lp90Δcps2 (Caggianiello et al., unpublished
202 results). Oat foods fermented with *L. plantarum* UFG9 and Lp90Δcps2 were obtained as
203 corresponding control samples. It is crucial to emphasize that LpB2 and Lp90 were previously
204 characterized for their probiotic properties by using *in vitro* and *in vivo* models (Arena et al., 2014b;
205 Russo et al., 2015; Caggianiello et al., unpublished results).

206 Starter cultures at middle of exponential phase were inoculated at an initial concentration of 8×10^8
207 cfu g⁻¹ in order to ensure a high cell viability consistent with the probiotic concept. Fermentation
208 was carried out for 16 hours, since this time was found to be optimal for the *in situ* riboflavin
209 biofortification of other cereal-based fermented foods (Capozzi et al., 2011; Russo et al., 2014).
210 Foods were stored during three weeks under refrigerated conditions according to previously
211 reported (Angelov, Gotcheva, Kuncheva, & Hristozova, 2006; Gupta, Cox, & Abu-Ghannam,
212 2010). Control samples were thermal stabilized after fermentation, and they showed a
213 contamination lower than 10^2 cfu g⁻¹ that did not increased during the shelf life (data not shown).
214 After the fermentative step, the microbial population of probiotic products was about 2×10^9 cfu g⁻¹
215 (Figure 1). This concentration slightly decreased during the storage time to 5.4×10^8 and 1.2×10^9
216 cfu g⁻¹ for Lp UFG9 and Lp90Δcps2, respectively (Figure 1). This result indicate that, at the time of
217 consumption, the fermented oat foods contained more viable probiotics than the minimum level
218 recommended to obtain the intended health benefits (Champagne, Ross, Saarela, Hansen, &
219 Charalampopoulos, 2011).

220 3.2 Technological, nutritional, and sensorial attributes

221 From a technological point of view, all the strains showed optimal acidifying aptitudes. After the
222 fermentation step, the pH of the oat products was about 3.9 (Figure 2A). In contrast, pH higher than
223 4.0 were observed in oat-based matrices fermented for 8 hours with *L. plantarum* strains (Angelov,
224 Gotcheva, Kuncheva, & Hristozova, 2006; Gupta, Cox, & Abu-Ghannam, 2010). However,
225 Mårtensson et al., (2001) reported a stronger acidification of an oat-based product after 16 hours of
226 fermentation. These findings point out that both starter cultures and time of fermentation are key
227 parameters that should be optimized to obtain acidic foods. Interestingly, the pH values of the oat-
228 based products containing live bacteria further decrease during the storage. The pH declined faster
229 in the first two weeks then stabilized in the last seven days of storage whereas the pH of pasteurized
230 samples ranged between 3.8 and 3.9 at the end of the shelf-life (Figure 2A). This suggests that
231 probiotic bacteria were metabolically active at refrigerated storage conditions. Agreeing with these

findings, previously reported dynamics of pH showed a progressive reduction within the shelf life of oat-based fermented beverages (Angelov, Gotcheva, Kuncheva, & Hristozova, 2006; Gupta, Cox, & Abu-Ghannam, 2010). Consistently, it was found a significant production of lactic acid that increased from about 11 mg 100 g⁻¹ after the fermentation to about 15 mg 100 g⁻¹ after 21 days of storage (Figure 2B). According to the pH pattern, the higher increment of lactic acid was observed within the first two weeks of storage, while concentrations of about 10 mg 100 g⁻¹ were found in pasteurized samples (Figure 2B). In addition, unpasteurized oat foods showed a slight increase of acetic acid (about 1.3 mg 100 g⁻¹) and malic acid (about 300 µg 100 g⁻¹) during the shelf-life of the product (data not shown). As expected, the content of glucose and sucrose slightly decrease at each experimental time in foods containing live bacteria, while higher concentrations were observed in thermal-treated samples (data not shown).

Color parameters were mainly influenced by storage time rather than treatment. However, pasteurized samples maintained lower lightness and higher a* values at the end of shelf life, showing a slightly appearance of browning compared to the probiotic treated samples (data not shown). This browning, probably due to the thermal treatment, was also perceived by the panelists who confirmed a slightly lower visual quality of pasteurized samples (Figure 3). Moreover, the sensorial analysis indicated a higher perception of desirable odors in oat probiotic products that was attributable to the production of organic acids (Figure 3). Thus, the overall acceptance of the oat probiotic products was to some extent considered better than the corresponding pasteurized samples (Figure 3). However, different flavours were developed depending on the probiotic starter cultures suggesting that the right combination of microorganisms and substrates could lead to palatable probiotic products (Salmerón, Thomas, & Pandiella, 2014).

3.3 Rheological and functional traits: oat β-glucan and microbial EPS

Viscosity is a crucial property for yielding desired sensory attributes such as mouth-feel and flavor release. It is well known that cereal β-glucans contribute to increase the viscosity and texture of some foods and, therefore, these fibers are often deliberately added into the food chain as functional

258 ingredients (Ahmad, Anjum, Zahoor, Nawaz, & Dilshad, 2012). Similarly, EPS-producing LAB
259 strains have been proposed as microbial food cultures due to their ability to produce *in situ* these
260 biopolymers (Ryan, Ross, Fitzgerald, Caplice, & Stanton, 2015). In addition, increasing scientific
261 evidences support the positive role of oat β -glucan and microbial EPS on human health as well
262 prebiotic molecules (El Khoury, Cuda, Luhovyy, & Anderson, 2012; Aleem, 2013; Salazar,
263 Gueimonde, de los Reyes-Gavilán, & Ruas-Madiedo, 2015). Therefore, in the present study the β -
264 glucan concentration and viscosity of the oat-based product were monitored during 21 days of cold
265 storage.

266 No differences were observed between the content of β -glucan in oat flour and in products after
267 fermentation (data not shown). As shown in figure 4A the oat β -glucan content of unpasteurized
268 samples was markedly reduced in the first week of storage, and then slightly decreased until the end
269 of the shelf life. However, no significant differences in the final level of oat β -glucan were detected
270 between samples inoculated with Lp90 and its mutant strain (Figure 4A). As expected, the β -glucan
271 concentration of pasteurized samples after 21 days of storage was similar to that detected after the
272 fermentative step (Figure 4A). In contrast to our results, β -glucan content remained almost constant
273 throughout the shelf life of oat-based probiotic products (Angelov, Gotcheva, Kuncheva, &
274 Hristozova, 2006; Gupta, Cox, & Abu-Ghannam, 2010), while Mårtensson et al., (2002a) found that
275 some reductions in the oat β -glucan levels were associated to the strains used in the fermentation
276 process. Accordingly, a consumption of β -glucans in both barley and oat fiber concentrates was
277 observed during the fermentation with different LAB strains (Lambo, Öste, & Nyman, 2005).

278 With respect to viscosity, samples fermented with Lp90 showed the highest initial level
279 corresponding to approximately 400 cP (Figure 4B). At the same time, the viscosity of the products
280 fermented with Lp90 Δ cps2 was the lowest (ca. 260 cP) while values of approximately 300 cP were
281 found in samples fermented with the other *L. plantarum* strains (Figure 4B). A progressive
282 reduction of the viscosity was noted throughout the storage time (Figure 4B). Nonetheless, samples
283 fermented with Lp90 showed the highest viscosity at all experimental times, with the final value

284 being approximately 60 cP higher than in oat-based products from Lp90 Δ cps2 (Figure 4B).
285 Remarkably, the viscosity of the samples inoculated with Lp90 and pasteurized after the
286 fermentation step was about 400 cP, while for the other thermally-treated samples viscosities ranged
287 between 260 and 290 cP (Figure 4B). It was reported that EPS-producing LAB improved the texture
288 of different oat-based non-dairy media (Mårtensson, Öste, & Holst, 2002b). Recently, rheological
289 studies showed that EPS from *L. plantarum* enhanced the viscosity of skim milk at lower
290 temperature or at acidic pH (Wang, Zhao, Tian, Yang, & Yang, 2015), probably due to the
291 protective nature of bacterial EPS (de los Reyes-Gavilán et al., 2011). Accordingly, comparison
292 between oat-based foods fermented with Lp90 and the corresponding isogenic no-ropy derivative
293 Lp90 Δ cps2 suggested that viscosity was positively related to the EPS-production ability of the
294 strain.

295 The reduction of viscosity during the shelf life seems to be attributable to some amylolytic activity
296 of live microorganisms, since no changes were observed in the content of β -glucan and viscosity in
297 pasteurized samples. However, it was reported that EPS production by some LAB strains decreased
298 after prolonged incubation probably due to the presence of glycohydrolases that catalyzed the
299 degradation of polysaccharides (Pham, Dupont, Roy, Lapointe, & Cerning, 2000). The observed
300 reduction of oat β -glucan concentration could play a detrimental effect on the viscosity of oat foods
301 under refrigerated storage. Accordingly, bacteria from yogurt starter cultures were able to hydrolyze
302 and depolymerize β -glucans when lactose became a limiting nutrient, determining a decrease in the
303 viscosity that negatively affected the rheological properties of the food (Gee, Vasanthan, & Temelli,
304 2007). A similar relationship could be deduced by Gupta and coauthors (2010) who did not observe
305 changes in β -glucan concentration nor viscosity of probiotic oat beverages during storage.

306 Recently, it was suggested that the addition of cereal β -glucans into dairy-based food systems could
307 support the survival of probiotic cultures throughout cold storage (Rosburg, Boylston, & White,
308 2010; Lazaridou, Serafeimidou, Biliaderis, Moschakis, & Tzanetakis, 2014), although the protective
309 effect does not necessarily appear to be associated to the break-down of these polysaccharides

310 (Mårtensson et al., 2002a). However, oat β -glucan and microbial EPS concentration should not
311 excessively decrease during the production or preservation of the food in order to ensure their
312 technological and functional properties.

313 **3.4 Riboflavin biofortification**

314 The riboflavin content of samples inoculated with non-overproducing strains was about 50 $\mu\text{g } 100$
315 g^{-1} regardless of the pasteurization or not of the product (Figure 5). In contrast, after fermentation
316 with LpB2, the riboflavin level increased up to about 120 $\mu\text{g } 100 \text{ g}^{-1}$ (Figure 5). According to this
317 finding, strains of *L. plantarum* and *L. fermentum* were able to produce an increase of the vitamin
318 B2 content of about two- to three-fold in pasta and bread, after a fermentative step of approximately
319 16 hours (Capozzi et al., 2011; Russo et al., 2014). In the present study it was observed that the
320 amount of vitamin B2 further enhanced throughout the shelf-life of the product reaching a final
321 concentration of 225 $\mu\text{g } 100 \text{ g}^{-1}$ after 21 days of cold storage (Figure 5). This additional increase
322 was not observed in the pasteurized sample fermented with LpB2 that showed a content of
323 riboflavin of approximately 100 $\mu\text{g L}^{-1}$ (Figure 5). This result indicated that live microorganisms
324 were metabolically able to further produce vitamin B2 even under cold conditions, thus improving
325 the nutritional value of the product during storage. Therefore, taking into account an approximate
326 intake of 150 g serving of food, this product could supply between 180 and 330 μg of vitamin B2,
327 corresponding to about 15-30 % of the daily-recommended intake. In addition, the ability of LpB2
328 to synthesize vitamin B2 in co-culture systems with Caco-2 cells (Arena et al., 2014b) and to
329 colonize the gut of gnotobiotic zebrafish larvae, suggest that this strain could contribute to further
330 increase the riboflavin supply in the gut environment (Russo et al., 2015).

331

332 **4. Conclusions**

333 In recent years, attempts have been reported to produce probiotic oat-based products with the aim to
334 simultaneously combine the beneficial effects of probiotic microorganisms and oat and its

335 components such as their soluble fibers, β -glucan (Angelov, Gotcheva, Kuncheva, & Hristozova,
336 2006; Gupta, Cox, & Abu-Ghannam, 2010). In the present study, potentially probiotic *L. plantarum*
337 strains were used to obtain multifunctional fermented oat foods further improving some nutritional
338 and technological traits. In particular, the products were *in situ* bio-fortified with riboflavin by
339 inoculating LpB2, while fermentation with the EPS-producer Lp90 seems to have a positive impact
340 on the rheological features of the product although it was apparently lost during the storage. It is
341 crucial to consider that in contrast to heat-stabilized samples, probiotic oat foods showed dynamic
342 modifications of nutritional, physico-chemical and sensorial attributes, although stored under cold
343 conditions. These changes could improve some quality feature, such as the vitamin B2 content that
344 increase during the shelf life of the product. On the other hand, a reduction of the viscosity was
345 observed that was attributable to the degradation of oat β -glucan and/or microbial EPS resulting- in
346 a potentially lower functionality [†]. These findings underline that the addition of live microorganisms
347 is a potential concern for the demand of standardized products in a globalized market. Nonetheless,
348 from an industrial perspective some advantages such as the elimination of the pasteurization step
349 should provide clear economic benefits for the producers. Moreover, in this study it was observed
350 that the thermal treatment **seems to be negatively** linked to the overall acceptance of the products
351 probably due to a higher degree of browning and a lower aromatic profile. Therefore, although the
352 addition of live **beneficial microorganisms is an interesting** approach to enhance several quality
353 features of oat-based fermented foods, more investigations are required in this field to optimize the
354 conditions to obtain a product with high standard quality level.

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513 **Table 1. Nutritional information (g 100 g⁻¹) of the whole grain oat flour**

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Nutritional information	g 100 g ⁻¹
Energy	360 ^
Protein	13.4
Carbohydrate total	61.5
Fat	7
- Saturated fat	1
Moisture	7
Salt	0
Dietary fiber total	7.1
β-glucan	3.5

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516 ^ kcal 100 g⁻¹

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529 **Legend to figures**

530 **Figure 1. Survival of probiotic *L. plantarum* strains in oat-based foods.** Viability of Lp B2
531 (black circle), Lp UFG9 (white circle), Lp90 (black triangle), and Lp90Δcps2 (white triangle) in
532 probiotic oat-based fermented foods after 0, 7, 14, and 21 days of storage at 4 °C. Experiments were
533 performed in triplicate, and the standard deviations are indicated.

534 **Figure 2. pH and lactic acid concentration during the storage of oat-based foods.** pH (A) and
535 lactic acid production (B) of probiotic oat-based products fermented with Lp B2 (black circle), Lp
536 UFG9 (white circle), Lp90 (black triangle), and Lp90Δcps2 (white triangle), after 0, 7, 14, and 21
537 days of storage at 4°C. The corresponding single spots are the pH (A) and lactic acid production (B)
538 in pasteurized samples after 21 days of storage at 4 °C. Experiments were performed in triplicate,
539 and the standard deviations are indicated. Means with different letters at the same time of storage
540 are significantly different according to Tukey's test (P value ≤ 0.05).

541 **Figure 3. Sensorial analysis of functional oat-based foods.** Sensorial evaluation of oat-based
542 products fermented with Lp B2, Lp UFG9, Lp90, Lp90Δcps2 and the corresponding pasteurized
543 samples (*) after 21 days of storage at 4 °C. The overall acceptance after the fermentation step
544 (dashed lines) is also showed.

545 **Figure 4. β-glucan concentration (A) and viscosity (B) in probiotic oat-based foods.** β-glucan
546 concentration (A) and viscosity (B) in probiotic oat-based products fermented with Lp B2 (black
547 circle), Lp UFG9 (white circle), Lp90 (black triangle), and Lp90Δcps2 (white triangle), after 0, 7,
548 14, and 21 days of storage at 4 °C. The corresponding single spots are the β-glucans concentration
549 (A) and viscosity (B) in pasteurized samples after 21 days of storage at 4°C. Experiments were
550 performed in triplicate, and the standard deviations are indicated. Means with different letters at the
551 same time of storage are significantly different according to Tukey's test (P value ≤ 0.05).

552 **Figure 5. Riboflavin concentration in probiotic oat-based foods.** Riboflavin concentration in
553 probiotic oat-based products fermented with Lp B2 (black circle), Lp UFG9 (white circle), Lp90
554 (black triangle), and Lp90Δcps2 (white triangle), after 0, 7, 14, and 21 days of storage at 4 °C. The

555 corresponding single spots are the riboflavin concentration in pasteurized samples after 21 days of
556 storage at 4 °C. Experiments were performed in triplicate, and the standard deviations are indicated.
557 Means with different letters at the same time of storage are significantly different according to
558 Tukey's test (P value ≤ 0.05).

Figure 1

Figure 1

Figure 2

Figure 2

A

B

Figure 3

Figure 4

Figure 4

A

B

Figure 5