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Lactobacillus plantarum strains for multifunctional oat-based foods

Pasquale Russo [a, 1], Maria Lucia Valeria de Chiara [a], Vittorio Capozzi [a, 1], Mattia Pia Arena [a], Maria Luisa Amodio [a], Ana Rascón [b], María Teresa Dueñas [c], Paloma López [d], Giuseppe Spano [a, *]

[a] Department of Agricultural, Food and Environmental Sciences, University of Foggia, via Napoli 25, Foggia 71122, Italy
[b] Food for Science Health Centre, Lund University, Sweden
[c] Department of Applied Chemistry, University of Basque Country, (UPV/EHU), Paseo Manuel de Lardizabal 3, 20018 Donostia, Spain
[d] Department of Molecular Microbiology and Infection Biology, Centro de Investigaciones Biológicas (CIB), Ramiro de Maeztu 9, Madrid 28040, Spain

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ABSTRACT

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

In the present work, whole oat flour was fermented with probiotic *Lactobacillus plantarum* strains to produce new functional foods with improved nutritional and technological features. Viability of the probiotic and the main technological, physico-chemical, nutritional and sensorial parameters were monitored at 7, 14 and 21 days of cold storage. The microbial survival was higher than $5x10^8$ cfu g^{-1} at the end of the shelf life. After the fermentation step, viscosity was higher in products inoculated with the exopolysaccharide-producing *L. plantarum* strain Lp90. However, a subsequent viscosity reduction was detected in all the samples throughout the storage period, consistent with the observed concentration decrease of the oat β-glucan. Vitamin B2 content was about two-fold higher in products fermented by the riboflavin-overproducing LpB2, and in these samples the riboflavin concentration further increased during cold storage.

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 (Arena et al., 2014a; Lam & Cheung, 2013). 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,

Abbreviations: exopolysaccharides, EPS; lactic acid bacteria, LAB.

* Corresponding author.
E-mail address: giuseppe.spano@unifg.it (G. Spano).
[1] Promis Biotech srl, via Napoli 25, 71122 Foggia, Italy.

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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, 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 Lp90Δcps2 were analyzed for their ability to improve the rheological properties of the oat-fermented foods. Viability of the probiotic microorganisms and the main technological, physico-chemical, nutritional and sensorial parameters were monitored during three weeks of storage under refrigerated conditions.

2. Materials and methods

2.1. Microbial strains and growth conditions

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

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

2.2. Fermented oat-based foods

Finely milled whole grain oat flour (Table 1) was provided by Glucanova (Lund, Sweden). A fermented oat product was obtained according to the method previously reported by Coda and co-authors (2012) with some modifications. Briefly, a mixture containing oat flour (18% w/v) and distilled water (82% v/v) was prepared with a final volume of 100 mL in sterile plastic containers with screw cap, and heated at 95 °C in a water bath for 10 min, with manual shaking every 2 min. At this time, mesophilic, yeasts and moulds contamination was checked by plate count on PCA or PDA (Oxoid), respectively. To start the fermentation, cells from starter cultures at late exponential phase were harvested by centrifugation (5000 g × 10 min), washed twice in sterilized saline solution (8.6% NaCl), and inoculated into the food matrix to obtain a concentration of approximately 8×10^8 cfu g^{-1}. The fermentative step was carried out at 37 °C for 16 h. At this time, samples for probiotic assays were stored at 4 °C for 21 days, while control samples were submitted to a thermal treatment of 30 min at 65 °C before storage. Three replicates of each sample were performed and analyzed after 0, 7, 14 and 21 days of cold storage.

Table 1
Nutritional information (g 100 g^{-1}) of the whole grain oat flour.

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

^ kcal 100 g^{-1}.

2.3. Total soluble solids, titratable acidity, and pH

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

2.4. Organic acids and sugars

Organic acids and sugars were extracted by homogenizing 5 g of fresh oat based product with 10 mL of ultrapure water using IKA T18 Ultraturrax (Wilmington, USA) homogenizer at 14,000 g for 10 s. The homogenate was centrifuged at 9000 g for 10 min at 5 °C. The supernatant was filtered with a Grace Pure SPE C189 cartridge (Alltech Italia srl - Grace Division, Milan, Italy) and then with a 0.2 μm filter. Organic acids and sugars were identified using the method of Mena et al. (2011). Samples were injected (10 μL) into a HPLC system (Agilent 1200 series) equipped with an UV detector, set at 210 nm, coupled with a refractive index detector. Separation was achieved on a Rezex ROA-Organic Acid H+(8%) column (300 × 7.80 mm) (Phenomenex, Torrance, USA), using a mobile phase of acidified water (phosphoric acid (0.1%)) with a flow rate of 0.5 mL min^{-1} and an oven temperature of 30 °C. The different organic acids and sugars were characterised and quantified by chromatographic comparison with analytical standards. Sugars and organic acids content was expressed as mg per 100 g of fresh weight.

2.5. Determination of β-glucan concentration

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

2.6. Viscosity

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

2.7. Extraction and quantification of riboflavin

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

2.8. Color analysis

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

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

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

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

2.9. Sensorial quality

A panel of five previously trained panelists carried out the sensory evaluations of fermented oat-based products at all experimental times. Samples were kept at 5 °C until sensorial evaluation, and each panelist was presented with 3 samples from each treatment in a lidded container to avoid loss of aroma. The samples were coded with a random 3-digit number in order to mask the treatment identity and to minimize subjectivity. The sensorial attributes judged during evaluation were: color, odor, off odor, visual quality, overall appearance. A hedonic scale was used that included 5 pictures corresponding to a score from 1 to 5 where: 1 = really poor and 5 = full characteristic.

2.10. Statistical analysis

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

3. Results and discussion

3.1. Formulation of new multifunctional fermented oat-based products

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

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

Starter cultures at middle of exponential phase were inoculated at an initial concentration of 8×10^8 cfu g^{-1} in order to ensure a high cell viability consistent with the probiotic concept. Fermentation was carried out for 16 h, since this time was found to be optimal for the *in situ* riboflavin biofortification of other cereal-based fermented foods (Capozzi et al., 2011; Russo et al., 2014). Foods were stored during three weeks under refrigerated conditions according to previously reported (Angelov et al., 2006; Gupta et al., 2010). Control samples were thermal stabilized after fermentation, and they showed a contamination lower than 10^2 cfu g^{-1} that did not increased during the shelf life (data not shown). After the fermentative step, the microbial population of

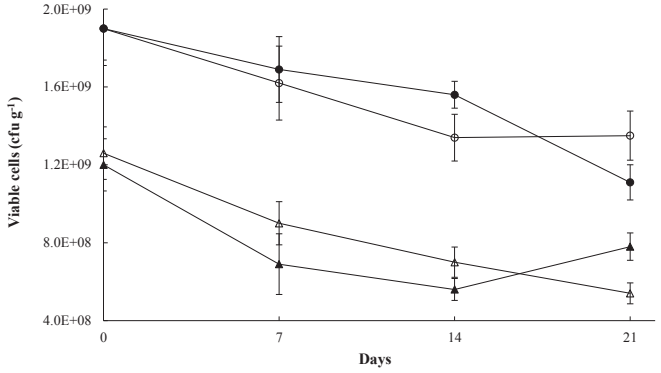

Figure 1. Survival of probiotic *L. plantarum* strains in oat-based foods. Viability of Lp B2 (black circle), Lp UFG9 (white circle), Lp90 (black triangle), and Lp90Δcps2 (white triangle) in probiotic oat-based fermented foods after 0, 7, 14, and 21 days of storage at 4 °C. Experiments were performed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

probiotic products was about 2×10^9 cfu g^{-1} (Fig. 1). This concentration slightly decreased during the storage time to 5.4×10^8 and 1.2×10^9 cfu g^{-1} for Lp UFG9 and Lp90Δcps2, respectively (Fig. 1). This result indicate that, at the time of consumption, the fermented oat foods contained more viable probiotics than the minimum level recommended to obtain the intended health benefits (Champagne, Ross, Saarela, Hansen, & Charalampopoulos, 2011).

3.2. Technological, nutritional, and sensorial attributes

From a technological point of view, all the strains showed optimal acidifying aptitudes. After the fermentation step, the pH of the oat products was about 3.9 (Fig. 2A). In contrast, pH higher than 4.0 were observed in oat-based matrices fermented for 8 h with *L. plantarum* strains (Angelov et al., 2006; Gupta et al., 2010). However, Mårtensson et al. (2001) reported a stronger acidification of an oat-based product after 16 h of fermentation. These findings point out that both starter cultures and time of fermentation are key parameters that should be optimized to obtain acidic foods. Interestingly, the pH values of the oat-based products containing live bacteria further decrease during the storage. The pH declined faster in the first two weeks then stabilized in the last seven days of storage whereas the pH of pasteurized samples ranged between 3.8 and 3.9 at the end of the shelf-life (Fig. 2A). This suggests that 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 et al., 2006; Gupta et al., 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 3). Thus, the overall acceptance of the oat probiotic products was to some extent considered better than the corresponding pasteurized samples (Fig. 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

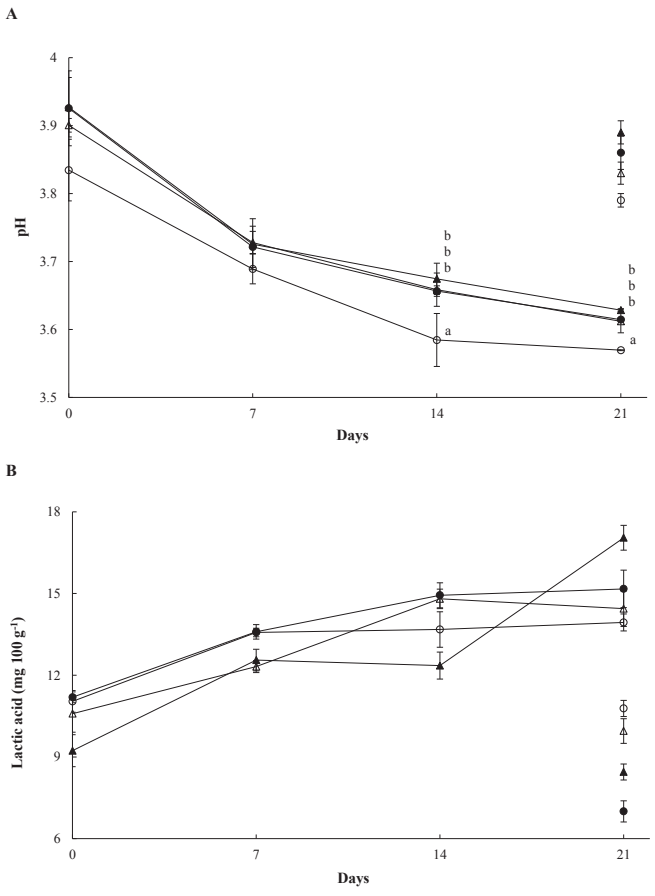

Figure 2. pH and lactic acid concentration during the storage of oat-based foods. pH (A) and lactic acid production (B) of probiotic oat-based products fermented with Lp B2 (black circle), Lp UFG9 (white circle), Lp90 (black triangle), and Lp90Δcps2 (white triangle), after 0, 7, 14, and 21 days of storage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

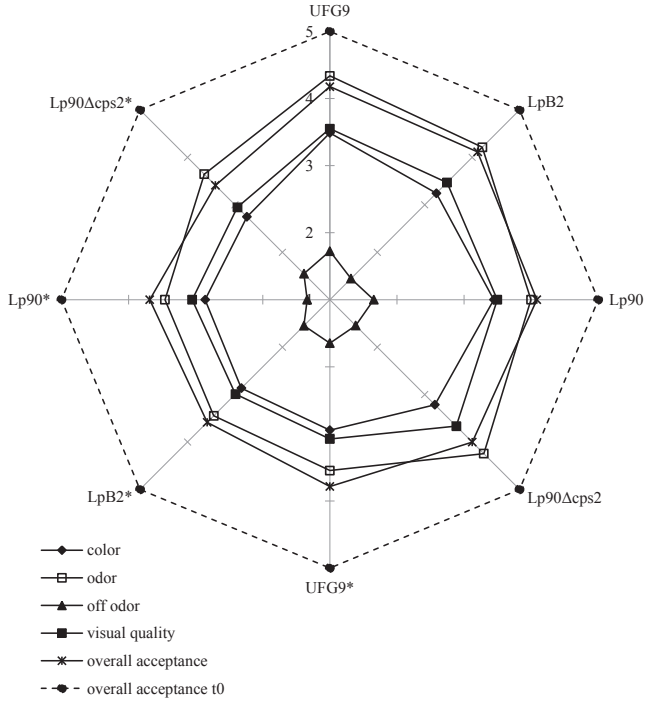

Fig. 3. Sensorial analysis of functional oat-based foods. Sensorial evaluation of oat-based products fermented with Lp B2, Lp UFG9, Lp90, Lp90Δcps2 and the corresponding pasteurized samples (*) after 21 days of storage at 4 °C. The overall acceptance after the fermentation step (dashed lines) is also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

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

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

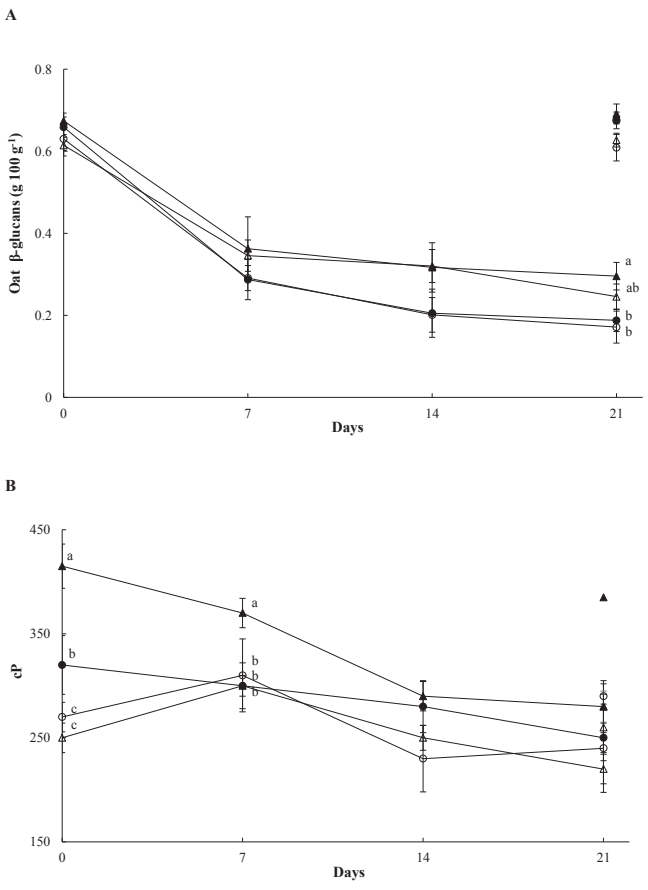

Fig. 4. β-glucan concentration (A) and viscosity (B) in probiotic oat-based foods. β-glucan concentration (A) and viscosity (B) in probiotic oat-based products fermented with Lp B2 (black circle), Lp UFG9 (white circle), Lp90 (black triangle), and Lp90Δcps2 (white triangle), after 0, 7, 14, and 21 days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

With respect to viscosity, samples fermented with Lp90 showed the highest initial level corresponding to approximately 400 cP (Fig. 4B). At the same time, the viscosity of the products fermented with Lp90Δcps2 was the lowest (ca. 260 cP) while values of approximately 300 cP were found in samples fermented with the other *L. plantarum* strains (Fig. 4B). A progressive reduction of the viscosity was noted throughout the storage time (Fig. 4B). Nonetheless, samples fermented with Lp90 showed the highest viscosity at all experimental times, with the final value being approximately 60 cP higher than in oat-based products from Lp90Δcps2 (Fig. 4B). Remarkably, the viscosity of the samples inoculated with Lp90 and pasteurized after the fermentation step was about 400 cP, while for the other thermally-treated samples viscosities ranged between 260 and 290 cP (Fig. 4B). It was reported that EPS-producing LAB improved the texture of different oat-based non-dairy media (Mårtensson, Öste, & Holst, 2002b). Recently, rheological studies showed that EPS from *L. plantarum* enhanced the viscosity of skim milk at lower temperature or at acidic pH (Wang, Zhao, Tian, Yang, & Yang, 2015), probably due to the protective nature of bacterial EPS (de los Reyes-Gavilán et al., 2011). Accordingly, comparison between oat-based foods fermented with Lp90 and the corresponding isogenic no-ropy derivative Lp90Δcps2 suggested that viscosity was positively related to the EPS-production ability of the strain.

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

Recently, it was suggested that the addition of cereal β-glucans into dairy-based food systems could support the survival of probiotic cultures throughout cold storage (Rosburg, Boylston, & White, 2010; Lazaridou, Serafeimidou, Biliaderis, Moschakis, & Tzanetakis, 2014), although the protective effect does not necessarily appear to be associated to the break-down of these polysaccharides (Mårtensson et al., 2002a). However, oat β-glucan and microbial EPS concentration should not excessively decrease during the production or preservation of the food in order to ensure their technological and functional properties.

3.4. Riboflavin biofortification

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

4. Conclusions

In recent years, attempts have been reported to produce probiotic oat-based products with the aim to simultaneously combine the beneficial effects of probiotic microorganisms and oat and its components such as their soluble fibers, β-glucan (Angelov et al., 2006; Gupta et al., 2010). In the present study, potentially probiotic *L. plantarum* strains were used to obtain multifunctional fermented oat foods further improving some nutritional and technological traits. In particular, the products were *in situ* biofortified with riboflavin by inoculating LpB2, while fermentation with the EPS-producer Lp90 seems to have a positive impact on the rheological features of the product although it was apparently lost during the storage. It is crucial to consider that in contrast to heat-stabilized samples, probiotic oat foods showed dynamic modifications of nutritional, physico-chemical and sensorial attributes, although stored under cold conditions. These changes could improve some quality feature, such as the vitamin B2 content that increase during the shelf life of the product. On the other hand, a reduction of the viscosity was observed that was attributable to the degradation of oat β-glucan and/or microbial EPS resulting in a potentially lower functionality. These findings underline that the addition of live microorganisms is a potential concern for the demand of standardized products in a globalized market. Nonetheless, from an industrial perspective some advantages such as the elimination of the pasteurization step should provide clear economic benefits for the producers. Moreover, in this study it was observed that the thermal treatment seems to be negatively linked to the overall acceptance of the products probably due to a higher degree of browning and a lower aromatic profile. Therefore, although the addition of live beneficial microorganisms is an interesting approach to enhance several quality features of oat-based fermented foods, more investigations are required in this field to optimize the conditions to obtain a product with high standard quality level.

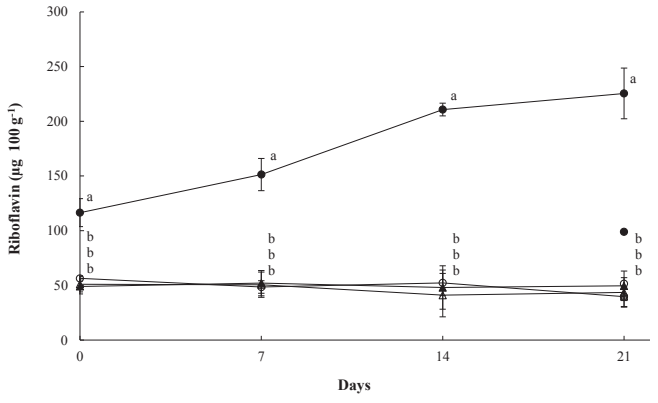

Fig. 5. Riboflavin concentration in probiotic oat-based foods. Riboflavin concentration in probiotic oat-based products fermented with Lp B2 (black circle), Lp UFG9 (white circle), Lp90 (black triangle), and Lp90Δcps2 (white triangle), after 0, 7, 14, and 21 days of storage at 4 °C. The corresponding single spots are the riboflavin concentration in pasteurized samples after 21 days of storage at 4 °C. Experiments were performed in triplicate, and the standard deviations are indicated. Means with different letters at the same time of storage are significantly different according to Tukey's test (P value ≤ 0.05).

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