Appl Microbiol Biotechnol
DOI 10.1007/s00253-012-4520-3

APPLIED MICROBIAL AND CELL PHYSIOLOGY

Fermentation of quinoa and wheat slurries by *Lactobacillus plantarum* CRL 778: proteolytic activity

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Received: 10 July 2012 / Revised: 6 October 2012 / Accepted: 15 October 2012
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Abstract Quinoa fermentation by lactic acid bacteria (LAB) is an interesting alternative to produce new bakery products with high nutritional value; furthermore, they are suitable for celiac patients because this pseudo-cereal contains no gluten. Growth and lactic acid production during slurry fermentations by *Lactobacillus plantarum* CRL 778 were greater in quinoa (9.8 logcfu/mL, 23.1 g/L) than in wheat (8.9 logcfu/mL, 13.9 g/L). Lactic fermentation indirectly stimulated flour protein hydrolysis by endogenous proteases of both slurries. However, quinoa protein hydrolysis was faster, reaching 40–100 % at 8 h of incubation, while wheat protein hydrolysis was only 0–20 %. In addition, higher amounts of peptides (24) and free amino acids (5 g/L) were determined in quinoa compared to wheat. Consequently, greater concentrations (approx. 2.6-fold) of the antifungal compounds (phenyllactic and hydroxyphenyllactic acids) were synthesized from Phe and Tyr in quinoa by *L. plantarum* CRL 778, an antifungal strain. These promising results suggest that this LAB strain could be used in the formulation of quinoa sourdough to obtain baked goods with improved nutritional quality and shelf life, suitable for celiac patients.

Keywords Quinoa · Proteolysis · *Lactobacillus* · Fermentation

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Introduction

The development of new food products with improved quality and health benefits has been gaining attention in recent years. In this regard, there has been an increasing interest on the use of the pseudo-cereal quinoa (*Chenopodium quinoa*) for the formulation of new foods (Bhargava et al. 2006). Quinoa has been grown in the South American plateau for centuries and introduced in Europe, North America, Asia, and Africa, recently. This Andean grain-like crop is considered an excellent source of high-quality proteins due to its balanced essential amino acid composition (Mujica and Jacobsen 2006; Ranhotra et al. 1993). Moreover, quinoa has greater protein content than other cereals; for instance, quinoa flour contains nearly 16 % of proteins (Ruales and Nair 1994), while white wheat flour tends to be about 10 % (Enriquez et al. 2003), corn flour 8.13 % (Edema et al. 2005), and rice flour 8.75 % (Ju et al. 2001). In this sense, quinoa flour represents a valuable ingredient which can be used as partial substitute of wheat flour to producing more nutritious bakery goods. In addition, as quinoa is a naturally gluten-free seed, it can be used for formulating new bakery products suitable for celiac patients. Concerning this aspect, Hager et al. (2012) formulated gluten-free bread using different pseudo-cereals; however, quinoa bread showed inferior quality than the wheat counterpart. Moreover, Coda et al. (2010) developed new bread using a mixture of pseudo-cereals (including quinoa) which showed good sensorial characteristics. Other products such quinoa fruit beverage suitable for celiac patients was formulated by Bicudo et al. (2012), while a quinoa beer was developed by de Meo et al. (2011).

Published online: 06 November 2012

Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB) and yeasts (De Vuyst and Vancanneyt 2005). The use of sourdough offers many advantages over baker's yeast, such as enhanced flavor, prolonged preservation, and improved dough structure (De Vuyst and Neysens 2005). Sourdough properties depend on the production of organic acids (lactic and acetic acids) and the proteolytic activity of LAB during the fermentation process. LAB are microorganisms fastidious from the nutritional point of view as they require some vitamins, amino acids, and bases to grow. Moreover, these microorganisms have complex proteolytic systems which allow peptide transport and further degradation by the intracellular peptidases (Hebert et al. 2000). Therefore, the flour's composition (free amino acids, vitamins, and proteins) will determine the growth parameters of each LAB strain grown in these substrates (Lee et al. 2001).

During dough fermentation, wheat proteins (glutenins and gliadins) are scarcely degraded by cereal and microbial proteases, probably due to their low solubility with respect to globulins and albumins (Lagrain et al. 2010). In aqueous solution, these proteins form a tridimensional gel (gluten) which is difficult to access by proteolytic enzyme (Gänzle et al. 2008). The use of gluten-free flours as quinoa in sourdough could be an advantage since its main proteins (globulins and albumins) are more hydrophilic than gluten and therefore more accessible for protease enzymes. The hydrolysis of quinoa proteins by LAB could enhance their growth and metabolic activity toward the release of organic acids, small peptides, and amino acids. In this sense, the aim of this study was to assess and compare the fermentative and, mostly, the proteolytic activity of *Lactobacillus plantarum* CRL 778 in slurries prepared from wheat and quinoa flours. The *Lactobacillus* strain used in this work was selected due to its antifungal properties, technological characteristics, and potential use as starter culture in the bread industry (Gerez et al. 2009, 2010; Dallagnol et al. 2011).

Materials and methods

Microorganism and growth conditions

L. plantarum CRL 778 was isolated from homemade wheat dough and belongs to the Culture Collection (CRL) of Centro de Referencia para Lactobacilos, Tucumán, Argentina. The LAB strain was grown in MRS broth at 37 °C for 16 h and transferred twice prior to inoculating the slurries.

Slurry fermentation and sampling

L. plantarum CRL 778 strain was inoculated (2 %) in MRS broth and incubated for 16 h at 37 °C. Cells were harvested by centrifugation at 8,000×*g* for 10 min, washed twice, and resuspended in sterile distilled water. This cell suspension (3.0×10^9 cfu/mL) was inoculated (2 %, *v*/*v*) in two types of slurries (S): quinoa (QS) and wheat (WS). The slurries were prepared by mixing 200 mL of tap water, 2 g of anhydrous dextrose (cerelose; Adama, S.A., Argentina), and 100 g of the different flours: commercial wheat flour (000 type) and quinoa flour. The latter was prepared in the laboratory using commercial quinoa seeds (*C. quinoa* Wild) from Bolivia. The seeds were washed several times with cold water to remove saponins until there was no more foam in the wash water; then, they were dried at 48 °C for 24–48 h until reaching an A_W=0.30–0.35, which was measured with a water activity meter (AquaLab LITE, Decagon, USA). Finally, the quinoa seeds were ground and sifted to obtain the flour. Slurries inoculated with *L. plantarum* CRL 778 (WS_{778} and QS_{778}) were adjusted to pH6.0 with NaOH 2 N and fermented at 30 °C with soft stirring (Shaker Vicking model Dubnoff, Argentina) in 24 h. Samples were withdrawn at different times (0, 2, 4, 6, 8, 12, and 24 h) for analysis. Slurries without inoculation of *L. plantarum* CRL 778 (WS_C and QS_C) were performed and incubated under similar condition and used as the negative control.

To support the fact that the obtained cells corresponded to *L. plantarum* CRL 778, a naturally antibiotic [streptomycin (Strp), spectinomicin (Spct)] resistant strain of *L. plantarum* CRL 778^R was obtained and then grown in the slurries (${WS_{778}}^R$ and ${QS_{778}}^R$) in a similar way to the wild-type strain. The resistant strain was used only for comparison and corroborate that the obtained cell count after the fermentation was of that obtained with the wild type. All other results shown in this manuscript were done using the wild-type *L. plantarum* CRL 778.

To obtain the resistant strain, *L. plantarum* CRL 778 was grown in MRS broth with an increasing concentration of Strp (0.01–1.0 g/L). After 24 h of incubation, the culture with maximum antibiotic concentration in which the strain grew was used to inoculate a new culture with an increasing concentration of Strp. The process was repeated until a variant resistant to 1.0 g/L of Strp was obtained. A colony of the resistant strain was picked from the MRS agar plate containing 0.5 g/L of Strp, and this process was repeated using Spct. Finally, a $Strp^+/Spct^+$-resistant strain (*L. plantarum* CRL 778^R), which was able to grow at 0.5 g/L of these antibiotics in MRS agar plates, was obtained.

Microbial counts

One milliliter of each slurry sample (WS_{778}, QS_{778}, ${WS_{778}}^R$, and ${QS_{778}}^R$) was suspended in 9 mL of sterile physiological solution and homogenized in the vortex.

To count the number of *L. plantarum* CRL 778 cells, aliquots (0.1 mL) in serial tenfold dilutions from each

homogenate were spread on MRS agar with 0.01 g/L cycloheximide to inhibit yeast strains. Plates were prepared in duplicate and incubated at 30 °C for 48 h. For each slurry (WS_{778} and QS_{778}), up to 15 bacterial colonies were randomly picked up from the countable plates and streaked to purity. The cell morphology of LAB isolates was examined using a light microscope (CX, Olympus, Tokyo, Japan) under oil immersion (×100) and the Gram and catalase reactions of the LAB isolates were checked.

For *L. plantarum* CRL 778^R counting, aliquots (0.1 mL) in serial tenfold dilutions from each homogenate were spread on MRS agar with 0.01 g/L cycloheximide and 0.5 g/L of Strp and Spct. Plates were prepared in duplicate and incubated at 30 °C for 48 h. The antibiotics allowed distinguishing the inoculated *L. plantarum* CRL 778^R from possible natural-appearing LAB present in the flour's autochthonous microbiota.

pH assessment

The pH values of each slurry (WS_{778}, QS_{778}, WS_C, and QS_C) were determined with a pH meter (Hanna Instruments, FC200B, USA).

Organic acid determination

Lactic and acetic acids were determined by HPLC (Knauer Smartline, Berlin, Germany) in the supernatants of slurries (WS_{778}, QS_{778}, WS_C, and QS_C) obtained by precipitation with 0.6 mmol/L trichloroacetic acid (Sigma) and centrifugation (17,000×*g* for 20 min at 4 °C). Samples were filtered (0.45-μm filters; Ministart high flow, Sartorius) and 20-μL aliquots were injected into an ion exclusion Aminex HPX-87H column (300×7.8 mm, Bio-Rad, USA). Chromatographic separation was performed under the following conditions: isocratic mobile phase, H_2SO_4 (5 mmol/L); flow rate, 0.6 mL/min; and column temperature, 45 °C. A refractive index detector (Knauer Smartline) connected to the software (Peak Simple II) was used for data analysis. The concentrations of organic acids were expressed as millimoles per liter of slurry.

Soluble proteins

Soluble protein concentrations in supernatants obtained by centrifugation (17,000×*g*, 10 min, 4 °C) from each slurry (WS_{778}, QS_{778}, WS_C, and QS_C) were determined with the Bradford method (Bradford 1976) using bovine serum albumin (Sigma) as the standard. The concentration was determined spectrophotometrically (595 nm) using a microplate reader (VersaMax ELISA Microplate Reader, Molecular Devices, USA) and commercial Bradford solution (Bio-Rad Laboratories, Hercules, CA).

Hydrolysis of proteins by SDS-PAGE

The hydrolysis of wheat and quinoa protein extracts was monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according to the methods of Schagger and Von Jagow (1987). Before loading the gels, 500 μL of each sample (WS_{778}, QS_{778}, WS_C, and QS_C) from different time intervals (0, 8, 12, and 24 h) was adjusted to pH 11 with 5 N NaOH, stirred for 1 h to allow the solubilization of proteins, and then mixed with 5× Laemmli buffer (Laemmli 1970). The denatured samples were incubated at room temperature for 12 h, heated to 100 °C (5 min), and centrifuged at 17,000×*g* for 10 min. Fifteen micrograms protein aliquots and 5 μg of a molecular weight marker (M) were loaded into the lanes of a vertical gel (10 %) electrophoresis unit (Mini-Protean II; Bio-Rad Laboratories, Richmond, CA). Gel electrophoresis was carried out at 70 mV. The gels were stained with 0.1 % (*w*/*v*) Coomassie brilliant blue R-250 (Sigma) at room temperature. The molecular weight values of the protein fractions were estimated using broad-range Protein Marker (14.4–116 kDa; SM0431, MBI Fermentas). The protein-stained bands were quantified using a Quanti-Scan software, version 2.1 (Biosoft, USA). A linear relationship between the stain intensity and the protein concentration was observed with each band. The relative ratios among the protein bands of a sample were calculated from these stain intensity values.

Peptide analysis

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to separate quinoa and wheat peptides as previously described by Vermeulen et al. (2006). Samples of slurries (WS_{778}, QS_{778}, WS_C, and QS_C) were centrifuged (17,000×*g*, 10 min, 5 °C) and the peptides present in the supernatants were separated by molecular exclusion with a 3-kDa filter (Amicon Ultra Centrifugal Filter Devices-0.5, Millipore, USA). A Shimadzu liquid chromatograph (Shimadzu Corporation, SSI, Kyoto, Japan) equipped with a UV–Visible detector and a C18 column (250×4.6 mm, 300 Å, 5 μm; Grace, Research AG S.A., USA) was used to elute sample aliquots of 20 μL. The binary solvent system consisted of solvent A containing 0.1 % (*v*/*v*) trifluoroacetic acid (TFA) in water and solvent B containing 0.1 % (*v*/*v*) TFA in acetonitrile at a flow rate of 1.0 mL/min. Before injection, the column was equilibrated with 2 % B for 5 min. The samples were eluted with the following solvent gradient: 0–25 min, gradient from 2 to 20 % B; 25–40 min, gradient from 20 to 80 % B. Peptides were detected at 257 nm.

Total free amino acids

The concentrations of total free amino acids in slurries were determined using the ninhydrin–cadmium method

(Cd–ninhydrin) described by Baer et al. (1996), with modifications in the sample preparation. The slurry supernatants were clarified by mixing equivalent volumes of the sample and perchloric acid (7 %, *w*/*v*) and incubated at 4 °C for 1 h. Then, the samples were centrifuged (17,000×*g*, 10 min, 4 °C) and neutralized with 1.5 volume of 0.43 M KOH to precipitate perchloric acid. Clarified supernatants (100 μL) were mixed with 200 μL H_2O and 600 μL of the Cd–ninhydrin reagent in a capped microtube; this mixture was heated at 84 °C for 5 min, cooled, and centrifuged (17,000×*g* for 10 min). The treated samples were added (200 μL) into each well of a microplate (Plates Costar® UV-Transparent Microplates). A blank (200 μL) of a solution containing 300 μL Cd–ninhydrin reagent and 300 μL H_2O was added to the plate, as well as a standard solution containing 0.1–10 mmol/L glycine. Analyses were performed in duplicate. The plate was read with a microplate reader (VersaMax ELISA Microplate Reader) at 490 nm. Total free amino acids were expressed in grams per liter of slurry.

Individual free amino acids

Free amino acids were determined in the supernatants of slurries previously clarified by molecular exclusion as described before. These supernatants were transformed into *o*-phthaldialdehyde (OPA) derivatives and the concentration of amino acids was determined by RP-HPLC. The OPA reagent contained 200 mg of OPA, 9 mL methanol, 1 mL sodium borate buffer (0.4 mmol/L, pH 10), and 160 μL β-mercaptoethanol. This reagent was prepared 24 h before use and kept at 8 °C. The amino acids used as standards (Sigma) were treated with OPA reagent in the same way as the samples. The chromatographic separation was carried out using a Shimadzu liquid chromatograph equipped with a C18 column (Gemini 5u, C18 110A, 150×4.6 mm) and a Shimadzu fluorescence detector (excitation, 340 nm; emission, 460 nm). The binary solvent system consisted of solvent A, sodium phosphate buffer (40 mmol/L) at pH 6.0, and solvent B, acetonitrile/methanol/water (45:45:10, *v*/*v*/*v*). The elution gradient was carried out at 30 °C with a flow rate of 1 mL/min. The injection volume of the derivatized amino acids was 10 μL. The concentration of amino acids was expressed in grams per liter of slurry.

Phenyllactic acid and hydroxyphenyllactic acid concentrations by HPLC

Amino acid catabolism by *L. plantarum* CRL 778 was also checked. Thus, antifungal compound production derived from Phe and Tyr degradation, namely, phenyllactic acid (PLA) and hydroxyphenyllactic acid (OH-PLA), respectively, was analyzed in slurry supernatants. The same method previously described for organic acid determination was used; however, in this case, an UV detector (Knauer Smartline) programmed to 210 nm was employed. The concentrations of PLA and OH-PLA were expressed in milligrams per liter of slurry.

Statistical analyses

Assays were determined in two independent experiments; mean values±standard deviations are given. Data were compared using analysis of variance and Dunnett's *t* test. Statistical significance ($p<0.05$) was determined using Minitab-12 software.

Results

Growth and fermentative activity

The agar plates of QS_{778}, WS_{778}, QS_{778}^{R}, and WS_{778}^{R} samples showed similar colonies with homogeneous shapes to those expected for *L. plantarum* CRL 778. On the contrary, plates from non-inoculated samples (QS_C and WS_C) showed different types of colonies consistent with the presence of a heterogenic microbiota. Colonies from all plates were randomly picked and observed under an optical microscope. For QS_{778}, WS_{778}, QS_{778}^{R}, and WS_{778}^{R}, only one kind of cell morphology was observed corresponding to short, non-mobile, Gram(+) bacilli like to those observed for pure CRL 778 cultures. In addition, plate counts from 24-h fermented slurries showed similar results between *L. plantarum* CRL 778 and *L. plantarum* CRL 778^{R} grown in quinoa (approx. 9.80 log cfu/mL) and wheat (approx. 8.90 log cfu/mL). These results confirmed that *L. plantarum* CRL 778 was able to displace the autochthonous microbiota of quinoa and wheat since only one kind of colony grew showing parallel counting in both plates, supplemented or not with Strp and Spct antibiotics.

On the other hand, QS_C and WS_C samples showed mixed microbiota formed mainly of long Gram(+) or Gram(−) bacilli, some of them sporulated as well as some Gram(+) diplococci. The indigenous microbiota was initially higher in quinoa slurry (approx. 5.0 log cfu/mL) than in wheat slurry (approx. 2.0 log cfu/mL).

The growth of *L. plantarum* CRL 778 in the samples was followed for 24 h at 37 °C. Both QS_{778} and WS_{778} showed similar cell counts (8.9–9.2 log cfu/mL) at 6 h of incubation (Fig. 1). After this period, the wheat slurry culture reached stationary phase while that in quinoa continued to grow, showing a maximum cell count of 9.80 log cfu/mL at 12–24 h of incubation.

The acidification rate was similar in both, QS_{778} and WS_{778}, reaching pH values of 3.9 and 4.2, respectively, at

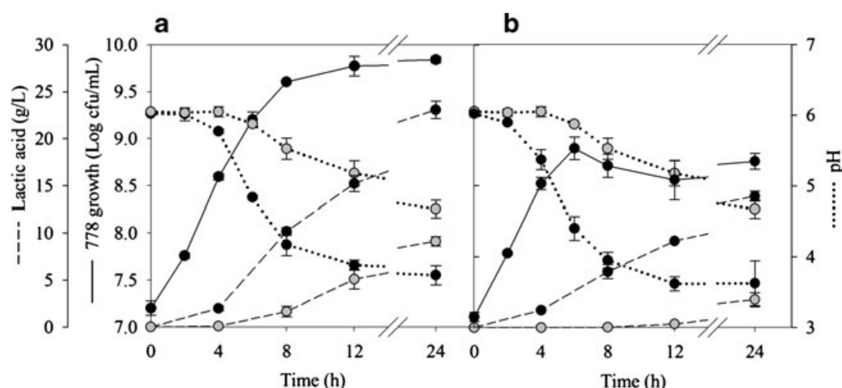


Fig. 1 Viability, pH, and lactic acid values in quinoa (**a**) and wheat (**b**) slurries incubated at 30 °C for 24 h. Inoculated slurries—QS_{778} and WS_{778} (*filled black circle*); non-inoculated (controls)—QS_C and WS_C (*filled gray circle*)

8 h of incubation (Fig. 1). On the contrary, no significant pH decrease ($p>0.05$) was observed for the controls before 12 h of incubation (pH≥5.2). At 24 h, the pH values were approx. 3.6 in ferments (QS_{778} and WS_{778}) and approx. 4.7 in the controls (QS_C and WS_C).

Lactic acid production was nearly 1.65-fold higher in QS_{778} than in WS_{778} throughout the incubation period; after 24 h, 23 and 14 mmol/L lactic acid were determined in the quinoa and wheat slurries, respectively. Lower lactic acid concentrations were detected in the control samples (9.11 and 2.97 mmol/L in QS_C and WS_C at 24 h, respectively).

The acetic acid concentration was low (0.56–0.83 g/L) in all samples, without significant differences among them (data not shown).

Soluble protein analysis

At the beginning of fermentation, soluble protein concentrations were higher (1.5-fold) in quinoa (6.32 g/L) than in wheat (4.36 g/L) samples (Fig. 2). The soluble protein concentration in QS_{778} decreased after 4 h of fermentation, detecting only 0.98–0.55 g/L at 12–24 h, respectively. In contrast, the protein concentration in WS_{778} increased two-fold (approx. 8 g/L) after 8 h incubation. To corroborate whether the pH drop produced during slurry fermentation by *L. plantarum* CRL 778 could be influencing protein solubility, the pH of the fermented samples were adjusted to 6.0. Soluble proteins determined in slurries with identical pH decreased 0.2 g/L after 24 h of incubation in wheat samples (Fig. 2), indicating that acidic pH (3.9–4.2) was responsible for the increase of soluble proteins observed in WS_{778}. On the contrary, the concentration of soluble proteins in QS_{778} was independent of the pH value of the slurry during the incubation period.

Changes in soluble protein profiles of QS_C and WS_C were less pronounced with respect to QS_{778} and WS_{778}. In QS_C, a slow decrease from 6.32 g/L (0 h) to 1.59 g/L (24 h) throughout the incubation period was observed. On the other hand, in WS_C, a slight increase (approx. 1.38-fold) of soluble proteins with respect to the initial value was detected at the end of the incubation period (24 h).

Electrophoretic analysis

SDS-PAGE analysis of the quinoa samples showed 16 protein bands with different molecular weights, the 20- and 28-kDa bands being the most intense ones. These bands are the acidic (A_{11s}) and basic (B_{11S}) fractions of the main quinoa protein globulin 11S or chenopodin (Fig. 3a). On the whole, quinoa proteins were more degraded than those coming from wheat, showing hydrolysis percentages between 40 and 100 % at 8 h of incubation with *L. plantarum* CRL 778, except for bands of 57 and 66 kDa which were not hydrolyzed even after 24 h of fermentation. In general, protein degradation in QS_C was lower than in inoculated

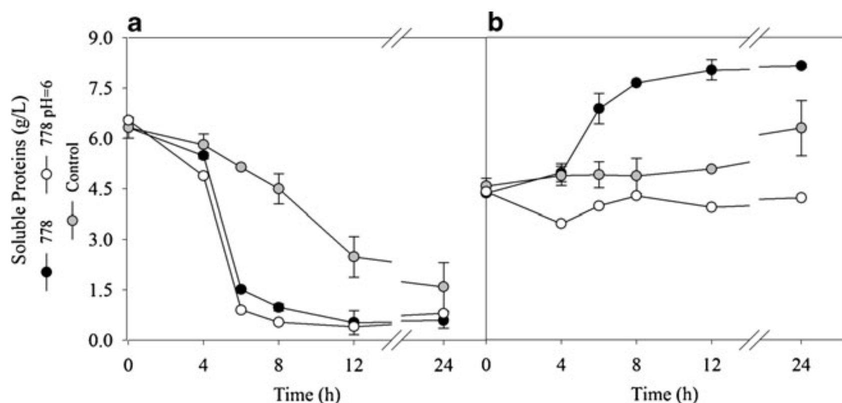


Fig. 2 Soluble proteins and total free amino acid values in quinoa and wheat slurries incubated at 30 °C for 24 h. Proteins were determined with (6.0) and without adjustment of the pH value

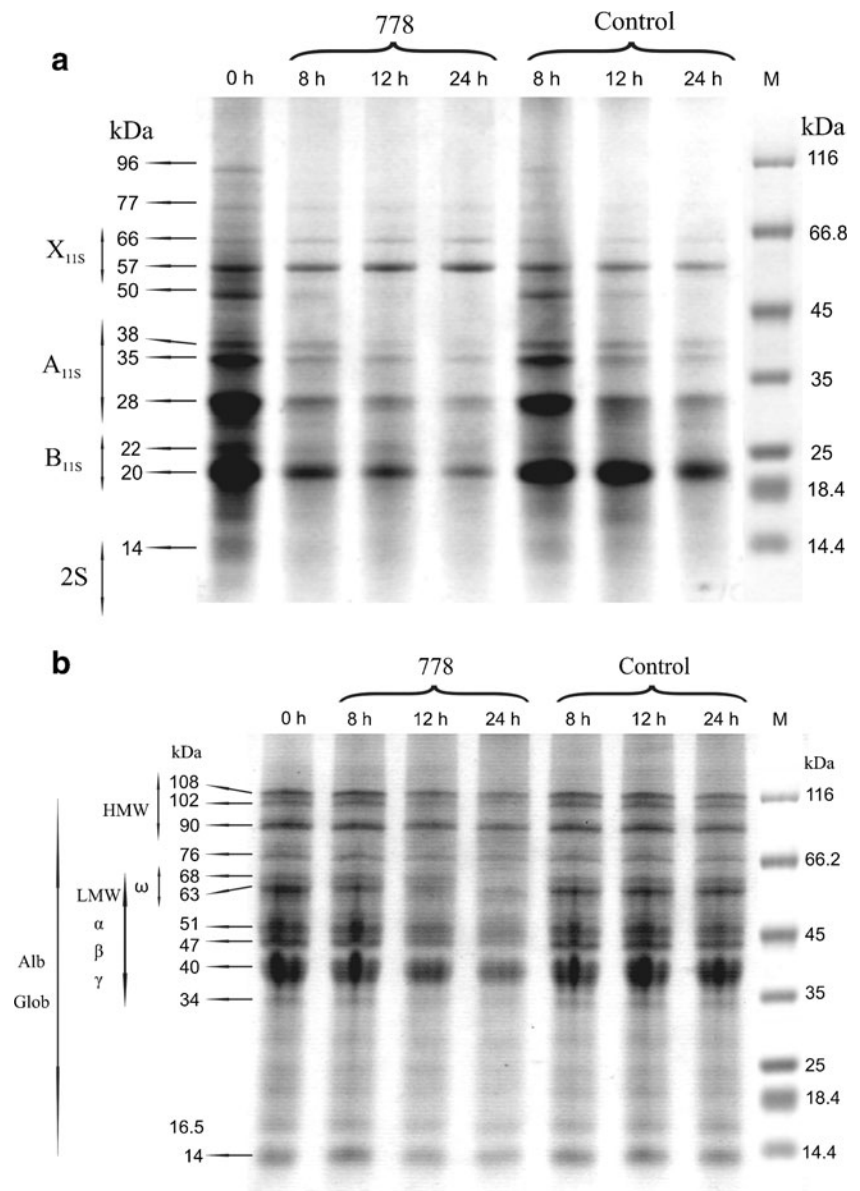


Fig. 3 SDS-PAGE patterns of protein degradation by *L. plantarum* CRL 778 from quinoa (**a**) and wheat (**b**) slurries incubated at 30 °C for different time intervals (0, 8, 12, and 24 h). *X11S, A11S* (acidic) and *B11S* (basic) quenopodin fractions, *M* molecular weight marker, *HMW* high molecular weight glutenins, *LMW* low molecular weight glutenins, *Alb* albumins, *Glob* globulins. *α-, β-, γ-, and ω-gliadins the different gliadin fractions*

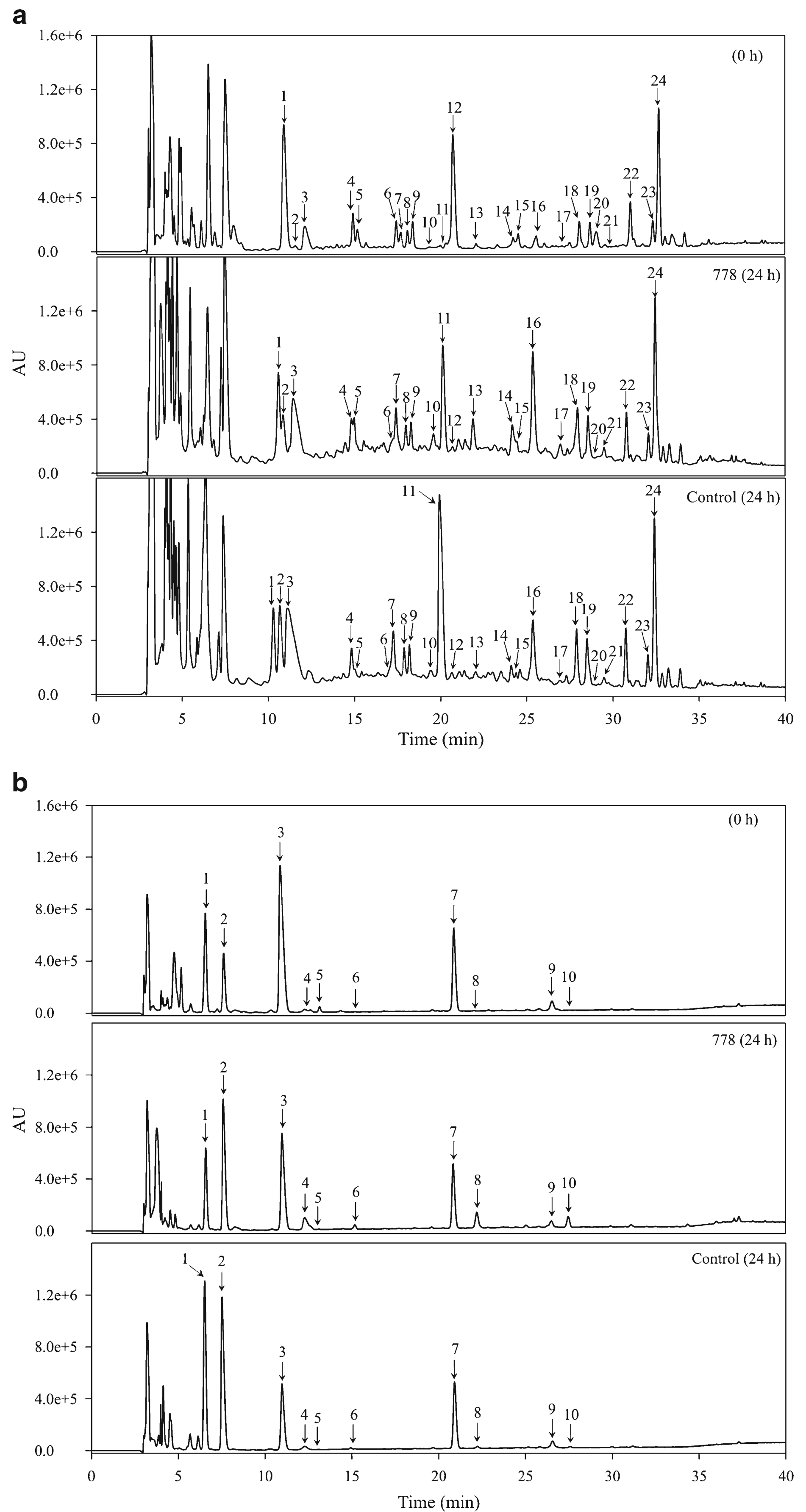
samples; however, the 57- and 66-kDa fractions were hydrolyzed (30–70 %, respectively) in the non-inoculated quinoa slurry.

In wheat, 12 electrophoretic bands with molecular weights between 14 and 108 kDa were detected at the beginning of fermentation. These bands corresponded to the low- and high molecular weight fractions of glutenins and gliadins (α, β, γ, and ω); the fractions of albumins and globulins would be distributed throughout the gel (Di Cagno et al. 2002; Fig. 3b). Scarce protein degradation was observed during the first hours of incubation with *L. plantarum* CRL 778, the 63-kDa fraction being the first to be cleaved, showing 20 % hydrolysis at 8 h. However, after 24 h, all wheat protein fractions were degraded, although at different extents (58–100 %), with the 68-kDa fraction being completely hydrolyzed. With respect to WS_C, protein degradation was low, showing only 10–15 % hydrolysis for several bands (34, 47, 76, 90, 102, and 108 kDa) after 24 h.

Peptide analysis

Protein hydrolysis was further analyzed by RP-HPLC. Samples of inoculated and control slurries were filtered with 3-kDa exclusion membranes to determine those peptides resulting from proteolysis that could not be observed by SDS-PAGE. On the whole, more peptides with MW> 3 kDa were detected in quinoa than in wheat slurries (Fig. 4a, b) through the fermentation. Twenty-four peptides were arbitrarily chosen for their analysis in quinoa. The most intense peak which showed similar area in all chromatograms [0 h, 778 (24 h), and control (24 h)] was used for

Fig. 4 Reverse-phase HPLC patterns of soluble peptides present in quinoa (**a**) and wheat (**b**) slurries incubated at 30 °C for 24 h



comparing and measuring the area of the remaining ones, attributing to this peak a relative area of 100 % (Fig. 4). The most intense peaks were 1 (77 %), 12 (70 %), 22 (28 %), and 4 (21 %) at the beginning of the incubation period. After 24 h of fermentation, QS_{778} showed five new peaks, named 2, 10, 11, 17, and 21, with relative intensities of 24, 11.5, 67, 10, and 7 %, respectively. Moreover, an increase between 19.5 and 35 % was observer for peaks 3, 5, 7, 13, 14, 18, and 19, while the areas of peptide 16 increased 64 % with respect to the beginning of fermentation. On the contrary, peaks 6, 12, and 20 were completely hydrolyzed at 24 h.

Although changes on the peptide profiles were also observed in QS_C after 24 h, the intensity of the peptides was different from that observed for QS_{778}. Peptides corresponding to peaks 16 (37 %), 14 (9.2 %), 17 (1.8 %), and 5 (1.3 %) were significantly ($p<0.05$) smaller, while peaks 2 (47.6 %) and 11 (113.8 %) were greater than in QS_{778}.

In wheat, less amounts of peptides were observed with respect to quinoa, ten of them being analyzed in detail (Fig. 4b). Peptide 3 was the most intense at the beginning of fermentation and was considered as 100 % of intensity; the other most intense peptides were 1 (67 %), 7 (56 %), and 2 (40 %). After 24 h of fermentation with *L. plantarum* CRL 778, four new peptides were observed (4, 6, 8, and 10), while peak 2 duplicated its area and peptide 3 diminished 37 %.

Changes in the peptides patterns of WS_C were also observed, although in lesser amount than for WS_{778}; the area of peptide 4 increased only 2.5 times, while no changes were observed for peaks 6, 8, and 10. Moreover, the area of peak 3 was 54 % lower than in the control sample. On the contrary, peptide 1 was more intense (115 %) in WS_C with respect to T0.

Amino acid analysis

The concentration of total free amino acids at the beginning of fermentation was 3.5-fold higher in quinoa (2.30 g/L) than in wheat (0.55 g/L). However, these concentrations increased during the incubation of quinoa slurries since in QS778 these reach approx. 5 g/L (12–24 h) and in QSC 6.73 g/L (24 h; Fig. 5a). On the contrary, no significant ($p>$ 0.05) changes in the total amino acid concentrations were detected in wheat samples (Fig. 5b).

Sixteen amino acid concentrations were determined by RP-HPLC, six of them corresponding to essential amino acids (Lys, Leu, Ile, Phe, Thr, and His). At the beginning of fermentation, the most abundant amino acids (in grams per liter) present in quinoa were Arg (0.220), Glu (0.200),

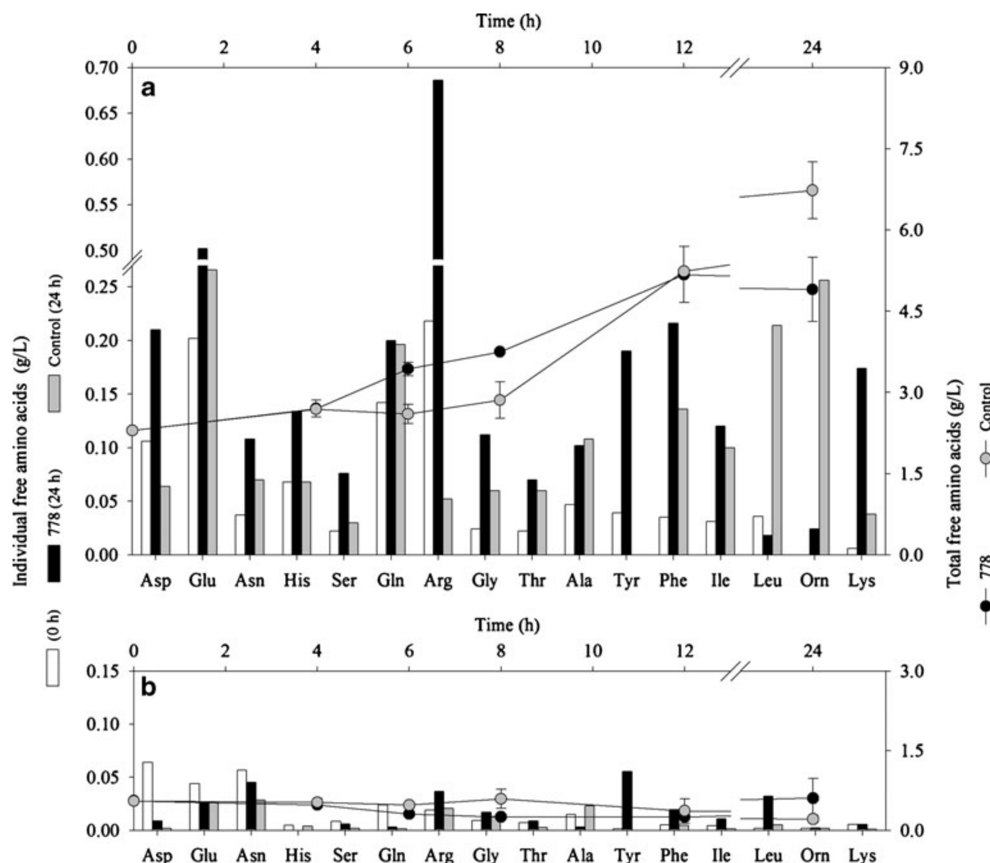


Fig. 5 Profiles of free amino acids present in quinoa (**a**) and wheat (**b**) slurries after fermentation with *L. plantarum* CRL 778 incubated at 30 °C for 24 h

Gln (0.140), Asp (0.110), and His (0.068), while in wheat were Asp (0.064), Asn (0.056), and Glu (0.044).

In QS_{778}, all amino acids initially present in the sample increased their concentrations at least twice, except for Leu that was reduced to half after 24 h. The amino acids (in grams per liter) Arg (0.69), Glu (0.50), Asp (0.21), Phe (0.21), Gln (0.20), Tyr (0.19), and Lys (0.17) increased their concentrations in the highest extent.

In wheat slurry fermentation with the CRL 778 strain, the amino acids Arg, Gly, Tyr, Phe, Ile, and Leu were released, while Asp, Glu, Asn, His, Gln, and Ala were consumed, although in a different extent.

Interestingly, Phe and Tyr, the PLA and OH-PLA precursors, respectively, were released in both QS_{778} and $WS_{778.}$ However, the concentrations of these amino acids were 11.10 and 3.45 times higher in QS_{778} than in WS_{778} at 24 h of incubation.

Changes on the amino acid profiles were also observed in the quinoa and wheat control samples after 24 h of incubation. In QS_C, the concentrations of almost all analyzed amino acids increased, however in a lower extent than in QS_{778}, except for Leu and Orn which were released in higher quantities.

PLA and OH-PLA production

The concentrations of both PLA and OH-PLA metabolites were approx. 2.65-fold higher in QS_{778} than in WS_{778} after 24 h of incubation (Table 1). PLA synthesis was not observed in the control samples, while OH-PLA occurred in both QS_C and WS_C, their concentrations being 1.83- to 1.55-fold lower than in the respective fermented slurries with *L. plantarum* CRL 778.

Discussion

Quinoa, a free-gluten pseudo-cereal, has high nutritional value as it contains all the essential amino acids displaying a biological value superior to that of cereals (Gorinstein et al. 2002; Vega-Gálvez et al. 2010). Because of this, the production of quinoa in the South American plateau may contribute to the economic development of this region.

Table 1 PLA and OH-PLA concentrations (in milligrams per liter) in quinoa and wheat slurries fermented by *L. plantarum* CRL 778 at 30 °C for 24 h

Samples	PLA	OH-PLA
QS_{778}	45.5±07.5	63.9±10.9
WS_{778}	17.5±01.2	23.5±05.5
QS_C	nd	35.0±07.5
WS_C	nd	15.1±03.1

nd not detected

The use of quinoa in bakery could be interesting for producing more balanced foods with respect to amino acid content than conventional wheat breads and also for developing gluten-free products suitable for celiac patients (Moroni et al. 2009; Alvarez-Jubete et al. 2010). The fermentation of quinoa by LAB could contribute to enhancing bread storage periods by releasing organic acids (Moore et al. 2008) as well as increasing its nutritional value by releasing small peptides and amino acids, which are better absorbed in the intestine.

In this work, *L. plantarum* CRL 778, previously selected due to its ability for producing antifungal metabolites (Gerez et al. 2009), allowed accelerating the fermentation process and inhibiting the growth of deteriorating indigenous microbiota in quinoa. This finding was supported by the use of an antibiotic-resistant strain, *L. plantarum* CRL778^R. These results confirm the robustness of the *L. plantarum* species previously reported by Minervini et al. (2010).

The differences on the microbial counts found between flours could be explained by several factors including the place where the grains were cultured (climate, altitude), the interactions with other organisms in their ecological niches, and human manipulation during flour processing. Moreover, the composition of the flour itself (sugars, proteins, and moisture) could also influence microbial growth (Gobbetti 1998; Wick et al. 2003; Van der Meulen et al. 2007). In this work, the quinoa sugar concentration determined for glucose/fructose/maltose+sucrose was 1.4:0.143:4.23% (*w*/*w*), respectively. Similar results on quinoa sugar concentrations were demonstrated by Repo-Carrasco et al. (2003). These fermentable sugar concentrations are four times higher than those reported for wheat and probably affect microbial survival in quinoa.

L. plantarum CRL 778 grew and produced lactic acid in a higher extent in quinoa than in wheat slurries despite the fact that this strain was isolated from the latter one. The poorer growth in wheat can be explained by the lowest amount of nutrients due to the process of flour manufacture in which the germ and brand, containing the highest amount of oils, minerals, and vitamins (B and E), are lost. The remaining part, the grain, contains mainly starch, gluten proteins, and cellulose. Quinoa flour, instead, is produced with whole grains, saponin cleaning being the only previous treatment. Moreover, quinoa has higher amounts of fermentative sugars than wheat, which contributes to bacterial growth.

L. plantarum CRL 778 released a higher concentration of lactic acid in quinoa than in wheat; however, this result was not correlated with a greater drop in the pH value with respect to the wheat-fermented sample. Gänzle et al. (2008) showed that growth and lactate production were positively related with the buffering capacity of flour since

pH values below 4 inhibited bacterial growth. Protein and amino acids are important buffering compounds in vegetables because they have anionic and cationic groups that can be ionized. Hence, the greatest buffering capacity of quinoa flour could be, in part, related to its highest concentrations of free amino acids (tenfold more than wheat), hydrophilic peptides, and proteins. The food buffer capacity is also influenced by the concentrations of potassium, calcium, and magnesium salts of organic acids such as malate, citrate, and glycerate (Playne and McDonald 1966). Although these compounds were not determined in this work, the initial titratable acidity was threefold higher in quinoa slurry than wheat slurry, suggesting the presence of more buffering organic acid concentrations in the pseudo-cereal.

Wheat-soluble proteins increased their concentration as a consequence of the pH drop during fermentation; this process was reversible as their solubility decreased when the pH was adjusted. This fact can be explained as it has already been established that lactic acid can depolymerize and solubilize glutenin macropolymers (Gänzle et al. 2008). At low pH values, there is a net positive charge that may affect hydrophobic interactions and break H^+ breaches, increasing the proportion of gluten proteins detected in the soluble fraction (Clarke et al. 2004). However, when the pH was adjusted to 6.0, a slight decrease in the concentration of wheat-soluble proteins was observed during fermentation.

Lactic acid bacteria have a complex proteolytic system which is, in general, composed of a cell wall-associated proteinase, an efficient transport system, and several intracellular peptidases which degrade peptides into amino acids. However, in *L. plantarum* species, no cell wall-associated proteinases were reported (Kleerebezem et al. 2003). Moreover, Gerez et al. (2006) showed that *L. plantarum* CRL 778 degraded wheat proteins poorly when grown in a sterile medium (free of endogenous proteases) which had these proteins as the sole nitrogen source. Therefore, we suggest that the first step on wheat and quinoa protein hydrolysis could be mainly attributed to the cereal endogenous proteases that become functional at a low pH (Thiele et al. 2002; Poutanen et al. 2009).

In general, proteolysis was higher in quinoa than in wheat, as can be observed on SDS-PAGE patterns. This fact could be related to the higher solubility of quinoa proteins with respect to wheat gluten, as was referred above in "Introduction." However, it also could be related with a higher activity of the endogenous proteases of quinoa with respect to those of wheat. Quinoa proteases have an optimal activity at a higher pH range (4.0–6.0; Lorenz and Nyanzi 1989) than the aspartic proteases (AP; pH3.0–40) of wheat (Loponen et al. 2004), being probably active at the earlier stages of the fermentation process. The AP are enzymes related to gluten that show maximum activities at pH3.0 in which especially the high molecular weight glutenins and, to a lesser extent, the low molecular weight glutenins and gliadins are hydrolyzed (Bleukx et al. 1998; Bleukx and Delcour 2000).

The acid and basic fractions of quinoa chenopodins and other high- and low molecular weight protein fractions were highly degraded after fermentation, while the bands corresponding to 57 and 66 kDa were not hydrolyzed. Brown et al. (1982) reported that chenopodins are synthesized as long precursors that are afterwards cleaved by a posttranslational process; therefore, it is possible that the 57- and 66-kDa fractions correspond to chenopodins that escaped this process (Brinegar and Goundan 1993), giving rise to a chimerical protein (named X_{11S}) that cannot be degraded by the endogenous pseudo-cereal proteases.

Regarding the proteolytic activity of LAB, several authors showed that these microorganisms had an important role on the proteolysis of wheat during fermentation. Zotta et al. (2006) have shown that LAB could degrade mainly high molecular weight glutenins during sourdough fermentation. On the other hand, Gerez et al. (2012) reported that a combination of *L. plantarum* and *Pediococcus* strains could partially degrade gliadins during sourdough fermentation. In addition, Di Cagno et al. (2002) showed that lactobacilli could hydrolyze wheat proteins and release amino acids in higher concentrations than when the dough was chemically acidified. In this respect, a secondary hydrolysis of quinoa and wheat protein fractions by *L. plantarum* CRL778 peptidases was evidenced in this work as changes in the peptide and amino acid profiles in the fermented flours. The capacity of *L. plantarum* to degrade peptides and release amino acids has been well documented for different proteins, which include those of wheat (Zotta et al. 2006), soy (Aguirre et al. 2008), and milk (Milesi et al. 2008); however, this is the first evidence showing the role of a LAB strain on the proteolysis of quinoa during fermentation.

L. plantarum CRL 778 was able to release Lys during quinoa fermentation in a higher amount with respect to wheat, which could be due to the higher Lys concentration in pseudo-cereals than in wheat, rice, and corn proteins (Borges et al. 2010). In addition, the highest amounts of free Arg, Glu, and Asp were detected in SQ_{778}. Despite the fact that the named amino acids are non-essential, Wu (2010) suggested that they should be taken into account for the establishment of the hypothetical "ideal protein" due to their relevance on human health. Asparagine, together Glu, is used for ATP synthesis in enterocytes, contributing to preserve the intestinal mucosa integrity (Watford 2008). On the other hand, Arg is essential for embryonic survival and in adults can contribute to improve endothelial function in atherothrombosis (Loscalzo 2004).

Amino acid catabolism was observed in all samples as assessed by the presence of PLA and OH-PLA, compounds which are known to inhibit the growth of fungi in bakery

products (Valerio et al. 2004; Gerez et al. 2009). Interestingly, the highest amounts of these acids were found in the inoculated quinoa and wheat slurries, the greatest amount being detected in SQ_{778}. This fact could be related with the greater release of the precursor amino acids Phe and Tyr in quinoa by the evaluated *L. plantarum* CRL 778 strain. A novel functional quinoa-supplemented bakery product with increased concentration of free amino acids could be formulated by fermentation with *L. plantarum* CRL 778. Moreover, this product could have a longer storage period than other gluten-free products due to their major concentrations of PLA and OH-PLA.

In this work, the ability of an *L. plantarum* strain to degrade quinoa peptides and release amino acids and antifungal compounds (PLA and OH-PLA) using quinoa as the sole fermentative substrate was shown. The comparative analysis with wheat fermentation showed the advantage of using quinoa for enhancing the release of antifungal compounds and free amino acids. Moreover, this work showed that quinoa is an excellent substrate to formulate new gluten-free food with elevated nutritional value.

Acknowledgments We acknowledge the financial support of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT), and Ministerio de Ciencia, Tecnología e Innovación Productiva (MINCyT) from Argentina. We thank Dr. Fernanda Mozzi and Dr. Raúl Raya (Centro de Referencia para Lactobacilos, Argentina) for helpful suggestions and Dr. Jorge Palacios for the technical support.

References

Aguirre L, Garro MS, Savoy De Giori G (2008) Enzymatic hydrolysis of soybean protein using lactic acid bacteria. Food Chem 111:976–982

Alvarez-Jubete A, Auty M, Arendt EK, Gallagher E (2010) Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. Eur Food Res Technol 230:437–445

Baer A, Ryba I, Meyer J, Butikofer U (1996) Microplate assay of free amino acids in Swiss cheeses. Food Sci Technol 29:58–62

Bhargava A, Shukla S, Ohri D (2006) *Chenopodium quinoa*—an Indian perspective. Ind Crop Prod 23:73–87

Bicudo MOP, Vasques EC, Zuim DR, Candido LMB (2012) Elaboration and characterization of fermented drink from quinoa water soluble extract and pulp fruit. B CEPPA 30:19–26

Bleukx W, Delcour JA (2000) A second aspartic proteinase associated with wheat gluten. J Cereal Sci 32:31–42

Bleukx W, Brijs K, Torrekens S, Van Leuven F, Delcour JA (1998) Specificity of a wheat gluten aspartic proteinase. Biochim Biophys Acta 1387:317–324

Borges JT, Bonomo RC, Paula CD, Oliveira L, Cesário MC (2010) Características físico-químicas, nutricionais e formas de consumo da quinoa (*Chenopodium quinoa* Willd.). Temas Agrarios 15:9–23

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brinegar C, Goundan S (1993) Isolation and characterization of chenopodin, the 11S seed storage protein of quinoa (*Chenopodium quinoa*). J Agric Food Chem 41:182–185

Brown JWS, Ersland DR, Hall TC (1982) Molecular aspects of storage protein synthesis during seed development. In: Khan AA (ed) The physiology and biochemistry of seed development, dormancy, and germination. Elsevier, New York, pp 3–42

Clarke CI, Schober TJ, Dockery P, O'Sullivan K, Arendt EK (2004) Wheat sourdough fermentation: effects of time and acidification on fundamental rheological properties. Cereal Chem 81:409–417

Coda R, Rizzello CG, Gobbetti M (2010) Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of a functional bread enriched of γ-aminobutyric acid (GABA). J Food Microbiol 137:236–245

Dallagnol AM, Font de Valdez G, Catalán C, Mercado MI, Rollán G (2011) Effect of biosynthetic intermediates and citrate on the phenyllactic and hydroxyphenyllactic acids production by *Lactobacillus plantarum* CRL 778. J Appl Microbiol 111:1447–1455

De Meo B, Freeman G, Marconi O, Booer C, Perretti G, Fantozzi P (2011) Behaviour of malted cereals and pseudo-cereals for gluten-free beer production. J Inst Brew 117:541–546

De Vuyst L, Neysens P (2005) The sourdough microflora: biodiversity and metabolic interactions. Trends Food Sci Technol 16:43–56

De Vuyst L, Vancanneyt M (2005) Biodiversity and identification of sourdough lactic acid bacteria. Food Microbiol 24:120–127

Di Cagno R, De Angelis M, Lavermicocca P, De Vincenzi M, Giovannini C, Faccia M, Gobbetti M (2002) Proteolysis by sourdough lactic acid bacteria: effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. Appl Environ Microbiol 68:623–633

Edema MO, Sanni LO, Sanni AI (2005) Evaluation of maize–soybean flour blends for sour maize bread production in Nigeria. Afr J Biotechnol 4:911–918

Enriquez N, Peltzer M, Raimundi A, Tosi V, Pollio ML (2003) Characterization of wheat and quinoa flour in relation to their breadmaking quality. J Argent Chem Soc 91:47–54

Gänzle MG, Loponen J, Gobbetti M (2008) Proteolysis in sourdough fermentations: mechanisms and potential for improved bread quality. Trends Food Sci Technol 19:513–521

Gerez CL, Rollán GC, De Valdez GF (2006) Gluten breakdown by lactobacilli and pediococci strains isolated from sourdough. Lett Appl Microbiol 42(5):459–464

Gerez CL, Torino MI, Rollán GC, Font de Valdez G (2009) Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. Food Control 20:144–148

Gerez CL, Torino MI, Obregozo MD, Font de Valdez G (2010) A ready-to-use antifungal starter culture improves the shelf life of packaged bread. J Food Prot 73:758–762

Gerez CL, Dallagnol AM, Rollán G, Font de Valdez G (2012) A combination of two lactic acid bacteria improves the hydrolysis of gliadin during wheat dough fermentation. Food Microbiol 32:427–430

Gobbetti M (1998) The sourdough microflora: interactions of lactic acid bacteria and yeasts. Trends Food Sci Technol 9:267–274

Gorinstein S, Pawelzik E, Delgado-Licon E, Haruenkit R, Weisz M, Trakhtenberg S (2002) Characterization of pseudocereal and cereal proteins by protein and amino acid analyses. J Sci Food Agric 82:886–891

Hager AS, Wolter A, Czerny M, Bez J, Zannini E, Arendt EK, Czerny M (2012) Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts. Eur Food Res Technol 235:333–344

Hebert EM, Raya RR, De Giori GS (2000) Nutritional requirements and nitrogen-dependent regulation of proteinase activity of *Lactobacillus helveticus* CRL 1062. Appl Environ Microbiol 66:5316–5321

Ju ZY, Hettiarachchy NS, Rath N (2001) Extraction, denaturation and hydrophobic properties of rice flour proteins. Food Chem Toxicol 66:229–232

Kleerebezem M, Boekhorst J, Van Kranenburg R, Molenaar D, Kuipers OP, Leer R, Tarchini R, Peters SA, Sandbrink HM, Fiers MW, Stiekema W, Lankhorst RM, Bron PA, Hoffer SM, Groot MN, Kerkhoven R, De Vries M, Ursing B, De Vos WM, Siezen RJ (2003) Complete genome sequence of *Lactobacillus plantarum* WCFS1. Proc Natl Acad Sci U S A 100:1990–1995

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lagrain B, Goderis B, Brijs K, Delcour JA (2010) Molecular basis of processing wheat gluten toward biobased materials. Biomacromolecules 11:533–541

Lee K, Lee J, Kim YH, Moon SH, Park YH (2001) Unique properties of four lactobacilli in amino acid production and symbiotic mixed culture for lactic acid biosynthesis. Curr Microbiol 43:383–390

Loponen J, Mikola M, Katina K, Sontag-Strohm T, Salovaara H (2004) Degradation of HMW glutenins during wheat sourdough fermentations. Cereal Chem 81:87–93

Lorenz K, Nyanzi F (1989) Enzyme activities in quinoa (*Chenopodium quinoa*). Int J Food Sci Technol 24:543–551

Loscalzo J (2004) L-Arginine and atherothrombosis. J Nutr 134:2798–2800

Milesi MM, McSweeney PL, Hynes ER (2008) Viability and contribution to proteolysis of an adjunct culture of *Lactobacillus plantarum* in two model cheese systems: cheddar cheese-type and soft-cheese type. J Appl Microbiol 105:884–892

Minervini F, De Angelis M, Di Cagno R, Pinto D, Siragusa S, Rizzello CG, Gobbetti M (2010) Robustness of *Lactobacillus plantarum* starters during daily propagation of wheat flour sourdough type I. Food Microbiol 27:897–908

Moore MM, Dal Bello F, Arendt EK (2008) Sourdough fermented by *Lactobacillus plantarum* FST 1.7 improves the quality and shelf life of gluten-free bread. Eur Food Res Technol 226:1309–1316

Moroni AV, Dal Bello F, Arendt EK (2009) Sourdough in gluten-free bread-making: an ancient technology to solve a novel issue? Food Microbiol 26:676–684

Mujica A, Jacobsen SE (2006) La quinua (*Chenopodium quinoa* Willd.) y sus parientes silvestres. In: Moraes RM, Øllgaard B, Kvist LP, Borchsenius F, Balslev H (eds) Botánica Económica de los Andes Centrales. Instituto de Ecología UMSA, Bolivia, pp 449–457

Playne MJ, McDonald P (1966) The buffering constituents of herbage and of silage. J Sci Food Agric 17:264–268

Poutanen K, Flander L, Katina K (2009) Sourdough and cereal fermentation in a nutritional perspective. Food Microbiol 26:693–699

Ranhotra GS, Gelroth JA, Glaser BK, Lorenz KJ, Johnson DL (1993) Composition and protein nutritional quality of quinoa. Cereal Chem 70:103–105

Repo-Carrasco R, Espinoza C, Jacobsen SE (2003) Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kaniwa (*Chenopodium pallidicaule*). Food Rev Int 19:179–189

Ruales J, Nair BM (1994) Effect of processing on in vitro digestibility of protein and starch in quinoa seeds. Int J Food Sci Technol 42:1–11

Schagger H, Von Jagow G (1987) Tricine–sodium dodecyl sulfate-ployacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 166:368–379

Thiele C, Gänzle MG, Vogel RF (2002) Contribution of sourdough lactobacilli, yeast and cereal enzymes to the generation of amino acids in dough relevant for bread flavour. Cereal Chem 79:45–51

Valerio F, Lavermicocca P, Pascale M, Visconti A (2004) Production of phenyllactic acid by lactic acid bacteria: an approach to the selection of strains contributing to food quality and preservation. FEMS Microbiol Lett 233:289–295

Van der Meulen R, Grosu-Tudor S, Mozzi F, Vaningelgem F, Zamfir M, Font de Valdez G, De Vuyst L (2007) Screening of lactic acid bacteria isolates from dairy and cereal products for exopolysaccharide production and genes involved. Int J Food Microbiol 118:250–258

Vega-Gálvez A, Miranda M, Vergara J, Uribe E, Puente L, Martínez EA (2010) Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* Willd.), an ancient Andean grain: a review. J Sci Food Agric 90:2541–2547

Vermeulen N, Gänzle MG, Vogel RF (2006) Influence of peptide supply and co-substrates on phenylalanine metabolism of *Lactobacillus sanfranciscensis* DSM20451 and *Lactobacillus plantarum* TMW1.468. J Agric Food Chem 54:3832–3839

Watford M (2008) Glutamine metabolism and function in relation to proline synthesis and the safety of glutamine and proline supplementation. J Nutr 138:2003–2007

Wick M, Böcker G, Stolz P, Lebault JM (2003) Influence of several process parameters on sourdough fermentation. Acta Biotechnol 23:51–61

Wu G (2010) Functional amino acids in growth, reproduction, and health. Adv Nutr 1:31–37

Zotta T, Piraino P, Ricciardi A, McSweeney PL, Parente E (2006) Proteolysis in model sourdough fermentations. J Agric Food Chem 54:2567–2574