Gliadin Degradation Ability of Artisanal Lactic Acid Bacteria, The Potential Probiotics from Dairy Products

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Abstract: Selected *Lactobacillus* spp. with high protease and acid producing capacity was explored for effective gliadin degradation in wheat sourdough environment. The total titratable acidity (TTA), pH and lactic acid bacteria (LAB) counts were evaluated. At the end of fermentation, the acidity and pH of the sourdough samples reached to 13.49-17.34 and 3.84-3.52 range, respectively. LAB population was enumerated as 10^7-10^9 colony forming unit (CFU)/g dough. Gliadin profiles were examined qualitatively using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional electrophoresis (2-DE) and reverse phase-high performance liquid chromatography (RP-HPLC) techniques. Especially RP-HPLC could be considered as a sensitive technique and is useful to determine the biochemical changes in gliadin fragmentation throughout sourdough fermentation process. LAB inoculated sourdoughs and chemically acidified sourdoughs generally represent similar gliadin degradation patterns. Although the total removal of gliadin toxicity could not be achieved in all dough formulations, it may be beneficial to use LAB to improve the dough and bread quality through the exploration of its bioconversion by-products.

Keywords: Gliadin, sourdough, lactic acid bacteria, probiotic, celiac.

1. INTRODUCTION

The wheat storage protein, gluten, is composed of gliadin and glutenin fractions which have amino acid sequences rich in proline and glutamine residues [1]. However, ingestion of gluten causes celiac, gluten sensitive enteropathy, in genetically susceptible individuals. High proline and glutamine content makes gluten proteins resistant to gastrointestinal digestion enzymes which lack postproline cleaving activity [2,3]. After reaching small intestine, immune response is activated and mucosa structure is damaged. The damage causes malabsorption and several related health problems such as malabsorption, abdominal pain and bloating, chronic diarrhea, growth failure, iron deficiency anemia, nonspecific arthritis, depression and low bone mineral density [4]. Celiac has an incidence of 1 of 100-550 people in European population [5] and gluten-free diet has a key and unique role for the treatment. At this point, the variety, availability, quality and affordability of gluten-free products on the market are of great importance. Therefore, substantial efforts are exerted on the development of new products. Furthermore, gluten contamination of naturally gluten-free foods and residual gluten in gluten-free ingredients exhibit a serious problem.

Sourdough is a fermented semi-product that is the mixture of basically wheat flour and water. It contains LAB, mostly *Lactobacillus* strains, and yeasts. As sourdough fermentation progresses, several microbial and enzymatic bioconversions occur in carbohydrates, lipids and proteins [6]. The protein degradation is mediated by both microbial metabolism and cereal enzymes. As a result of microbial growth, acidity decreases, which makes the proteins more susceptable to degradation. The acidification leads to activation of endogenous proteolytic enzymes of wheat flour, such as aspartic and serine proteinases which have acidic pH optima [7-9]. Together with these wheat enzymes, proteolytic activities of LAB probably result in degradation of gluten proteins, hence amino acid concentration increases. Proteolysis during sourdough fermentation with additional LAB strains has been investigated by several researchers [10-15]. These results have revealed that sourdough fermentation is probably a promising approach to detoxify residual gluten. Additionally, it has been reported that sourdough fermentation enhances the texture, flavor and shelf-life of the final product and also it has a significant effect on nutritional value through a serial of bioconversions [16-19]. Microbial exopolysaccharides are long chain polysaccharides, which consist of branched and repeated units of sugar and sugar derivatives. They have positive effects on dough rheology and bread quality and also they have prebiotic properties [20-24].

LAB isolated from dairy products have been widely investigated for their proteolytic activity [25, 26]. LAB isolates from dairy products have already been used for bread production through sourdough process and

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desirable effects on bread quality were obtained [27-29]. Also, a commercial probiotic preparation was used in sourdough formulation [13]. Since the strains showed proteolytic activity, this preparation was suggested to use for the removal of gluten traces.

The scope of this study is to explore the effectiveness of potential probiotic LAB, isolated from artisanal dairy products in Turkey, for their gliadin degradation capabilities. *Lactobacillus casei* D4 and *Lb. delbrueckii* ssp. *bulgaricus* TY30, were selected for their high proteolytic activity and exopolysaccharide content, respectively [30,31]. Contributions from the enzymatic system of LAB on the hydrolysis of gluten has been primarily important, whereas the exopolysaccharide investigations are underway since these metabolites have importance on improving bread structure.

2. MATERIALS AND METHODS

2.1. Microorganisms and Media

In sourdough formulations, three different LAB strains were used. 1) *Lb. acidophilus* NRRL-B 1910 was selected as a reference strain and obtained from Agricultural Research Service Culture Collection (NRRL, U.S.). 2) *Lb. casei* D4 and 3) *Lb. delbrueckii* ssp. *bulgaricus* TY30 were isolated previously from a traditional cheese (Comlek peyniri) and yoghurt (Toros yogurdu), respectively in previous studies which were conducted at Food Engineering Department of IZTECH [30, 32]. A hundred µL of each LAB were taken from frozen glycerol stocks and inoculated to 5 mL of de Man, Rogosa and Sharpes (MRS) Broth [52.2 g MRS broth (Merck 10661)/L deionised water]. The broths containing *Lb. acidophilus* NRRL-B 1910 and *Lb. casei* D4 were incubated at 37°C for 24 hours (h), and *Lb. delbrueckii* ssp. *bulgaricus* TY30 was incubated at 42°C for 24 h. Subculture was done for each LAB and overnight incubated *Lactobacillus* cells were used for sourdough fermentation.

2.2. Wheat Flour Analyses

Wheat flour was supplied from a local market. Protein content of wheat flour was analyzed according to Kjeldahl method with some modifications in the amounts of chemical solutions [33]. Moisture and ash contents of wheat flour were determined according to ICC Standard No. 110/1 [34] and 104/1 [35], respectively.

2.3. Preparation of Sourdough

The LAB cells of each strain were harvested *via* centrifugation at 5000 $r.min^{-1}$ for 15 min at 4°C. After washing with phosphate buffered saline (PBS) [NaCl (8 g/L), KCl (0.2 g/L), Na_2HPO_4 (1.44 g), KH_2PO_4 (0.24 g/L), pH 7.4], LAB cells were suspended in water and the cell concentrations were adjusted to $2x10^8$ CFU/mL suspension. Wheat flour (200 grams) was mixed manually with tap water (100 mL) that contains $2x10^8$ CFU of related LAB suspension to produce 300 g of dough (Table **1**) [10]. Each *Lactobacillus* strain had the final concentration of $2x10^7$ CFU/g dough. The sample codes with the name of inoculated strain(s) were as *Lb. acidophilus* NRRL-B 1910 (LA), *Lb. casei* D4 (LC), *Lb. delbrueckii* ssp. *bulgaricus* TY30 (LD), *Lb. casei* D4 and *Lb. delbrueckii* ssp. *bulgaricus* TY30 (M1), *Lb. acidophilus* NRRL-B 1910, *Lb. casei* D4 and *Lb. delbrueckii* ssp. *bulgaricus* TY30 (M2), no bacterial inoculation as control (C), chemically acidified dough (CAD). Some dough samples (LA, LC, M2, M1, C and

Table 1: Sourdough Formulations

Code	Wheat flour (g)	Water (ml)	LAB Cell suspension (ml), $2x10^8$ CFU/30 ml		
			Lb. acidophilus NRRL-B 1910	*Lb. casei* D4	*Lb. delbrueckii* ssp. *bulgaricus* TY30
LA	200	70	30	-	-
LC	200	70	-	30	-
LD	200	70	-	-	30
M1	200	40	-	30	30
M2	200	10	30	30	30
C	200	100	-	-	-
CAD	200	100	-	-	-

LA: Dough fermented with *Lb. acidophilus* NRRL-B 1910, LC: Dough fermented with *Lb. casei* D4, LD: Dough fermented with *Lb. delbrueckii* ssp. *bulgaricus* TY30, M1: Dough fermented with *Lb. casei* D4 and *Lb. delbrueckii* ssp. *bulgaricus* TY30, M2: Dough fermented with *Lb. acidophilus* NRRL-B 1910, *Lb. casei* D4 and *Lb. delbrueckii* ssp. *bulgaricus* TY30, C: Dough contains no bacterial inoculation (Control), CAD: Chemically acidified dough.

CAD) were incubated in a glass beaker covered with foil at 37°C for 48 h. On the other hand, the dough inoculated only with *Lb. delbrueckii* ssp. *bulgaricus* TY30 (LD) was fermented at 42°C for 48 h. In order to investigate the differences between acidities developed by chemical acidification and LAB inoculation, dough without LAB inoculation was acidified with lactic and acetic acid (4:1, v/v) to pH 4.22 at 5.5 h after the beginning of the fermentation (CAD).

2.4. Determination of Fermentation Parameters

The progress in fermentation was followed by measuring pH, TTA and LAB concentration of each sourdough sample.

The pH and TTA of sourdough samples were measured at 0, 3, 6, 24 and 48 h of fermentation. Sourdough sample (10 g) was taken and after addition of 90 mL of ultra pure water, homogenization was done by using a bar blender for 40 s. The pH of this homogenate was measured *via* a pH meter with a glass electrode (Hanna Instruments, U.S.). TTA was determined by titration with standardized 0.1 mol/L NaOH [36]. The amount (mL) of 0.1 mol/L NaOH which was consumed during analysis was recorded.

The LAB concentrations of sourdough samples were enumerated at 0, 3, 6, 24 and 48 h of fermentation by suspending 10 g of sourdough sample in 90 mL of sterile deionized water and further homogenized (60 s). After preparing serial dilutions of homogenized samples, double layer pour plate technique with MRS agar (15 g agar/L MRS broth) was used. Plates were incubated for 48 h at 37 and 42°C, the latter was for sample LD. Colonies on plates containing a range of 30-300 colonies were counted.

2.5. Fractional Extraction of Wheat Proteins

Wheat protein fractions were extracted sequentially from sourdough samples taken at 0, 24 and 48 h of fermentation, according to Weiss *et al*. [37] which is the modified procedure of a method described by Osborne [38]. Gliadin extracts were stored at -80°C until further analyses.

2.6. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was applied to gliadin extracts according to Laemmli system [39]. Polyacrylamide gel (12% separating, 4% stacking) was poured between the glass plates (PROTEAN II XL system, Bio-Rad, U.S.). The gliadin extracts were mixed with the sample buffer in a ratio of ca. 1:9 (v/v), according to nanodrop (NanoDrop 8000, Thermo Scientific) measurements and were heated in boiling water for 5 min. After loading 10 µL/well of each sample and 2 µL of marker (#SM0671, Fermentas, Canada) to the wells, the electrophoresis was carried out at a constant current of 32 mA for 30 min and 48 mA for 5 h at 10°C. After the run was over, gel was taken out and kept in a fixer solution until silver staining.

2.7. Two-Dimensional Electrophoresis (2-DE)

Immobilized pH gradient (IPG) strips, nonlinear pH 3-10 and 17 cm (Bio-Rad 163- 2009, U.S.), were used in 2-DE. Gliadin extract, which contained 30 µg of protein, was mixed with rehydration buffer. The strips were incubated with a total amount of 400 µl of that sample mixture at room temperature for 1 h and rehydrated overnight at 25°C. After rehydration, the IPG strips were placed onto the separating gel and isoelectric focusing (IEF) was carried out under stated focusing conditions [10] with some modifications (PROTEAN IEF cell, Bio-Rad, U.S.). Applied voltages and times were 0-300 V, 1 h; 300-500 V, 3h; 500-2000 V, 4h; 2000-8000 V, 32000 V-h and 8000-500 V until the end, at 20°C. After that step, 6 mL of equilibration buffer I [6 mol/L urea, 2% SDS (w/v), 0.375 mol/L Tris-HCl (pH 8.8), 20% glycerol (v/v), 130 mol/m^3 1,4-Dithio-DL-threit(ol) (DTT)] was added for one strip and shaked (40 $r.min^{-1}$, 10 min). Equilibration buffer I was removed and 6 mL of equilibration buffer II [6 mol/L urea, 2% SDS (w/v), 0.375 mol/L Tris-HCl (pH 8.8), 20% glycerol (v/v), 135 mol/m^3 iodoacetamide (freshly added)] was added onto each strip and shaked (40 $r.min^{-1}$, 10 min).

For the second dimension, the separating gel was cast and the low-melt overlay agarose was pipetted to the space above the gel. The equilibrated IPG strip was placed onto the SDS gel. Electrophoresis was carried out at a constant current of 48 mA for 5.5 h at 10°C. Gel was put into a fixer solution until silver staining. Same procedures were also applied to 2-D SDS-PAGE Standard (Bio-Rad 161-0320, U.S.).

2.8. Silver Staining of SDS-PAGE and 2-D Gels and Monitoring

Gel was washed with 50% ethanol three times for 20 min each and then kept in pretreatment solution (0.02% sodium thiosulfate, w/v) for 1 min. It was rinsed with ultra pure water three times for 20 s each and was further treated with silver nitrate solution [0.2% silver

nitrate (w/v), 0.0375% formaldehyde (v/v)] for 20 min. Again, it was washed with ultra pure water twice for 20 s each until color development. Gel was placed in a developing solution [2.25% potassium carbonate (w/v), 0.0004% sodium thiosulfate (w/v), 0.075% formaldehyde (v/v)]. After a few minutes, some ultra pure water was added to slow down the reaction. When gliadins appeared in a satisfactory extend, ultra pure water was added and gel was rinsed twice, 2 min each. Finally, stop solution [50% methanol (v/v), 12% acetic acid (v/v)] was applied and gels were kept in this solution until monitoring. Orbital shaker was utilized during the whole staining procedure. The silver stained gels were monitored by using an imaging system (VersaDoc 4000 MP, Bio-Rad, U.S.). Molecular weights and pIs of gliadins were calculated by using Quantity One 1-D Analysis Software and Bio 2-D Software (Bio-Rad, U.S.), respectively.

2.9. Reverse Phase-High Performance Liquid Chromatography (RP-HPLC)

RP-HPLC analysis of gliadins was done as stated in a previous study [40]. Fifteen µL of filtered (0.45µm) gliadin extract was injected to 5µm, 15 cmx4.6 mm Zorbax SB300-C18 Reverse-Phase column (Agilent Technologies, U.S.) and analyzed for 120 min using a linear acetonitrile (0.1% trifluoroacetic acid, v/v) gradient from 24 to 50% over 108 min (Perkin Elmer Series 200, USA). Column temperature was 60°C and 210 nm was used to monitor column effluent.

2.10. Statistical Analysis

Statistical evaluation of the data was conducted by MINITAB 14 software (Minitab Inc.,U.S.). Analysis of variance (ANOVA) and Tukey's test were used to investigate whether there was a significant difference in means ($p<0.05$). Each analysis was performed in duplicate

3. RESULTS AND DISCUSSIONS

3.1. Flour Characteristics

According to the results of proximate composition analysis, wheat flour had protein content of 10.78 ± 0.14 g/100 g dry matter (DM), moisture level of 11.48 ± 0.09 g/100g and ash content of 0.54 ± 0.008 g/100 g DM (type 550). The wheat flour used in preparation of sourdoughs complied with standards [41] and by these results, it was validated that sourdough fermentation was performed by using a standard wheat flour.

3.2. Changes in pH, TTA, LAB Count

Adaptation of LAB to wheat sourdough environment was followed by the changes in the pH, TTA and LAB inoculum concentrations. During the whole sourdough fermentation process, there was a significant decrease in the pH values of all samples ($p<0.05$) (Figure **1a**). Also, a significant difference was observed between the pH values of sourdough samples at certain fermentation times ($p<0.05$). The decrease in pH from ca. 6.1 to ca. 3.7 was recorded in 48 h of fermentation period. In all LAB inoculated doughs, final pH varies within the range of 3.47-3.80. At 24 h, all inoculated doughs, except sample LD, reached the values similar to those at 48 h. A slight pH drop to 4.55 at 24 h was recorded in the case of LD due to the low growth rate of the strain used. The pH decrease was faster in samples LA, LC, M1 and M2 which contained *Lb. acidophilus* NRRL-B 1910 and/or *Lb. casei* D4. In control dough, the pH values at 24 h and 48 h of fermentation were 4.77 and 3.95, respectively. During the period after chemical acidification, only a small change was observed in the pH of CAD. It is important to mention that although there was a pH drop in both control and LAB inoculated doughs, this reduction was lower than the inoculated doughs, particularly LA, LC, M1 and M2, at 24 h. But at 48 h, while there was not a significant drop ($p<0.05$) in LA, LC, M1 and M2, the pH of control dough decreased to the level similar to other dough samples. These results showed that the pH decrease in control dough was due to its own natural microflora. On the other hand, the pH of CAD remained almost at the same level between 6 and 24 h. It is likely that the reduction of pH by the acid addition inhibited the natural growth of bacteria in wheat flour microflora. In a previous research, final pH of sourdough samples were found as between the values of 3.90 to 3.70 [11]. In another literature, in which probiotic preparations were used in sourdough fermentation, the pH was reached to 3.7 to 4.0 after 24 h [13]. According to a different study, it was observed that fermented doughs had the pH decrease from ca. 6.5 to 4.5-5.0 after 8 h and the pH was in the range of 3.5-4.0 at the end of 24 h [42]. In another study of the same research group samples, which were taken at 6 and 24 h, had pH values of 4.5-5.5 and 3.6-3.8, respectively [43].

Changes in TTA values of all samples during 48 h of fermentation were significant ($p<0.05$) and were in agreement with pH changes. Additionally, a significant difference was observed between the TTA values of sourdough samples at certain fermentation times ($p<0.05$). The TTA values of all LAB inoculated

doughs, which were between 1.75-1.85 range initially, reached to 3.94-14.40 and 11.45-15.70 range after 24 and 48 h of fermentations, respectively (Figure **1b**). In a previous study, it was found that a rye sourdough fermentation reached a TTA value of 17 ± 2.5 at the end of 24 h fermentation [44].

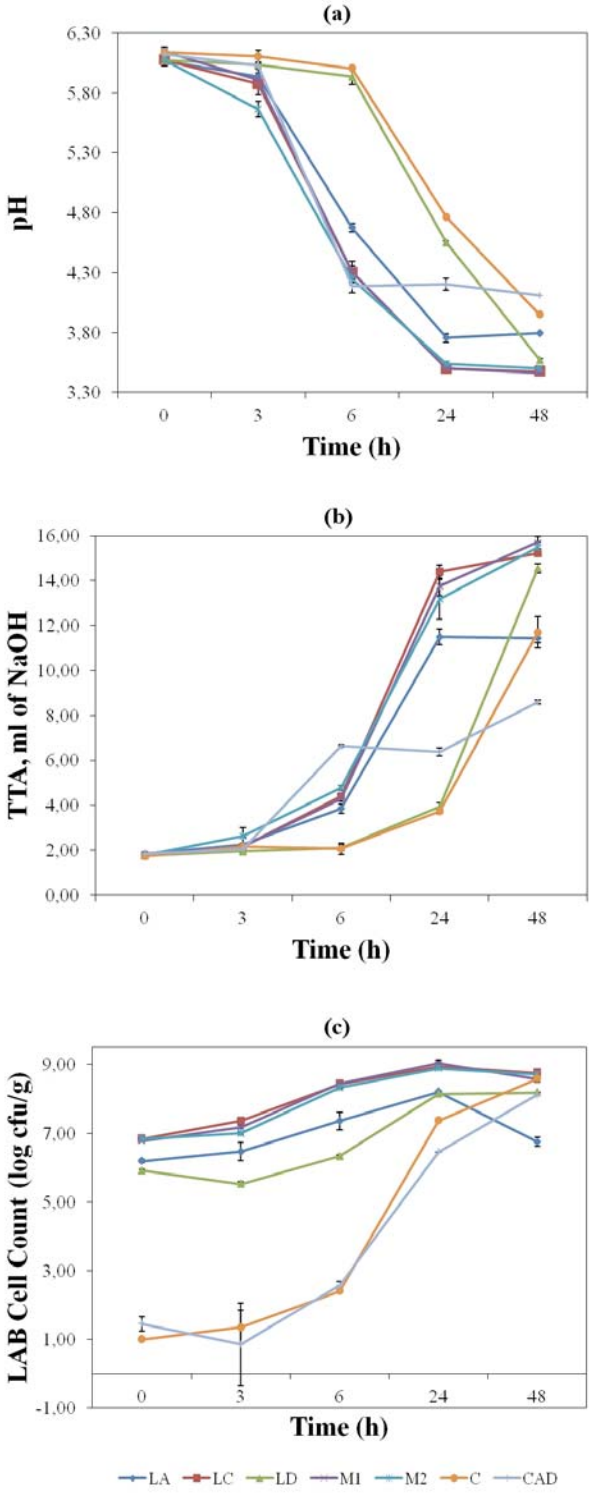

Figure 1: Changes in (**a**) pH, (**b**) TTA and (**c**) LAB counts (log CFU/g) of sourdough samples during fermentation.

In all samples, during 48 h of fermentation, changes in LAB concentrations were found significant ($p<0.05$) and also a significant difference was observed between the LAB counts of sourdough samples at certain fermentation times ($p<0.05$). The LAB population of inoculated doughs was counted as ca. $1.62x10^8$-$1.09x10^9$ CFU/g dough at the end of 24 h of fermentation (Figure **1c**). After 24 h, LAB population of samples started to decrease except the one in the sample LD. The enumeration results of control and CAD showed that a LAB growth, which constituted the microflora of wheat flour, occured and the concentrations reached to $2.7x10^3$, $3.2x10^7$ and $3.8x10^8$ CFU/g for control sample and to $1.0x10^2$, $1.4x10^6$ and $3.2x10^7$ for CAD at 6, 24 and 48 h, respectively. In previous studies in the related literature, the LAB content of sourdoughs at the end of 24 and 48 h were found as ca. 10^9 CFU/g sourdough [10, 11].

3.3. SDS-PAGE Profiles of Gliadin Extracts

The SDS-PAGE results of gliadins extracted from samples which were taken at the beginning (0 h) of sourdough fermentation shows that gliadin bands were located in the 42-29 kDa range (Figure **2**). Previous studies and literature findings have indicated that gliadins are divided into three groups according to their mobilities in SDS-PAGE gel as ω-gliadins (50,000-75,000), γ-gliadins and α/β-gliadins (M_rs 30,000-45,000) [45, 38]. According to this information, it can be said that our gliadin extracts contain γ-gliadins and α/β-gliadins. The absence of ω-gliadins could be due to the extraction procedure used in this study. The SDS-PAGE gel also contains bands which have lower M_rs such as 21,000- 10,000. This is because of the occurrence polymers related to the glutenins in the alcohol-extractable gliadin fraction. They differ from the alcohol-unextractable glutenins in having lower molecular weights and higher contents of low molecular weight (LMW) subunits. They are called as “aggregated gliadins” or “LMW glutenin” [46].

In all gliadins, which were extracted from inoculated sourdough samples taken at 0 h of fermentation, the bands at 38 and 37 kDa existed (Figure **2**). With the progress of fermentation, changes occurred in the protein structure and these two bands disappeared (A). When different sample bands were compared at 24 h, except for the sample LD, bands with molecular weights of 38, 37 and 28 kDa (A and B) disappeared. On the other hand, four new bands with molecular weights in the range of 27-25 kDa formed (C). The same changes occured in sample LD, more slowly (48

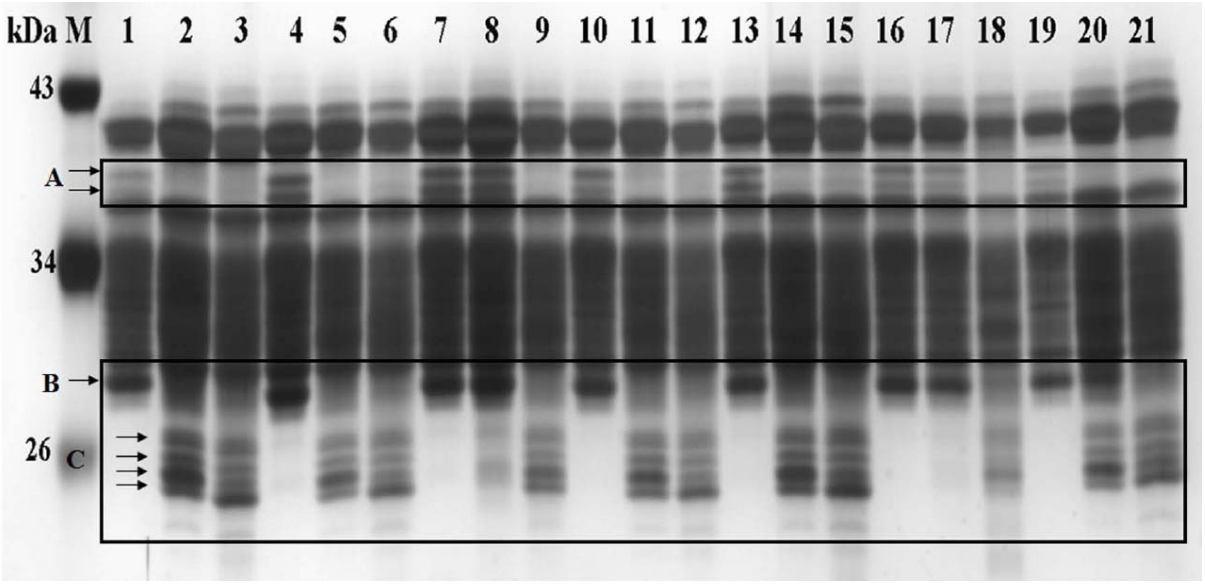

Figure 2: SDS-PAGE gel (12%) of gliadin fractions from sourdough samples. M represents the molecular weight marker; Lanes 1, 2, 3 correspond to sample LA at 0, 24 and 48 h; Lanes 4, 5, 6 correspond to sample LC at 0, 24 and 48 h; Lanes 7, 8, 9 correspond to sample LD at 0, 24 and 48 h; Lanes 10, 11, 12 correspond to sample M2 at 0, 24 and 48 h; Lanes 13, 14, 15 correspond to sample M1 at 0, 24 and 48 h; Lanes 16, 17, 18 correspond to sample C at 0, 24 and 48 h; Lanes 19, 20, 21 correspond to sample CAD at 0, 24 and 48 h.

h). In control dough, the same changes occurred as in the LD, but only two new bands formed (27 and 25 kDa) instead of four bands. CAD had also the same band appearance as the inoculated doughs. Although time for fermentation differs, these changes occurred during sourdough fermentation in all samples and also in chemically acidified and control doughs. Because of this reason, the gliadin band modification could be attributed to protein degradation which is not specific to any bacterial species used. Wheat flour enzymes such as aspartic proteinases and serine carboxypeptidases which are active at acidic pH ranges, 3.0-4.5 and 4.0-6.0, respectively, are likely to be responsible for the degradation [7, 9, 47].

3.4. 2-DE of Gliadin Extracts

The 2-D gel images of gliadins extracted from sample LC at 0 h and 24 h, M1 at 24 h and LD at 48 h are shown in Figure **3**. On 2-D gels of gliadin extracts of samples taken at the beginning of fermentation (0 h) 51 spots were detected. In agreement with previous studies [38] in which the location of gliadins stated as 35-50 kDa and pI from 6.5 to 9, the gliadin spots located mostly in 30-42 kDa range and at pI 5.7-9.8. After 24 h of fermentation two spots (spots 1 and 2) with molecular weight of ca. 39 kDa and pI of 6.4 and 6.7 and one spot (spot 3) with molecular weight of ca. 38 kDa and pI of 7.1 were disappeared. Also newly formed very small spots were observed in a wide region (region 4) which were particularly located in the ranges of ca. 30-29 kDa and pI of 6.5-8.9 and ca. 28-27 kDa and pI of 8.9-9.5. Obtained results from 2-DE showed similarity with SDS-PAGE findings but it gives more detailed representation of the locations of the gliadins. The spot disappearance and formation occurred after 24 h in LA, LC, M1, M2 and CAD, and after 48 h in control and LD. Changes occurred in all samples and also in chemically acidified and control doughs. The results seemed to indicate that there was a positive correlation between gliadin degradation and acidity development. The disappearance and formation of the spots were due to the effect of acidification (CAD) and acidity development by LAB inoculation. LAB gradually shifted the pH to ca. 3.5-4.0 and during this period wheat flour proteolytic enzymes reached their optimum pH values. Activated enzymes catalyzed the breakdown of proteins to peptides. Since no different spot alteration occurred in CAD, protein degradation might not be specific to any bacterial species used.

3.5. RP-HPLC

Since similar changes were observed in all chromatograms, only the representative chromatograms of gliadin extracts of sample LC (0, 24 and 48 h) were given in Figure **4**. In all dough samples, the peak appearance of gliadin fractions underwent some significant changes, which occurred due to the structural changes of proteins, as fermentation progressed. The main alterations in chromatograms of

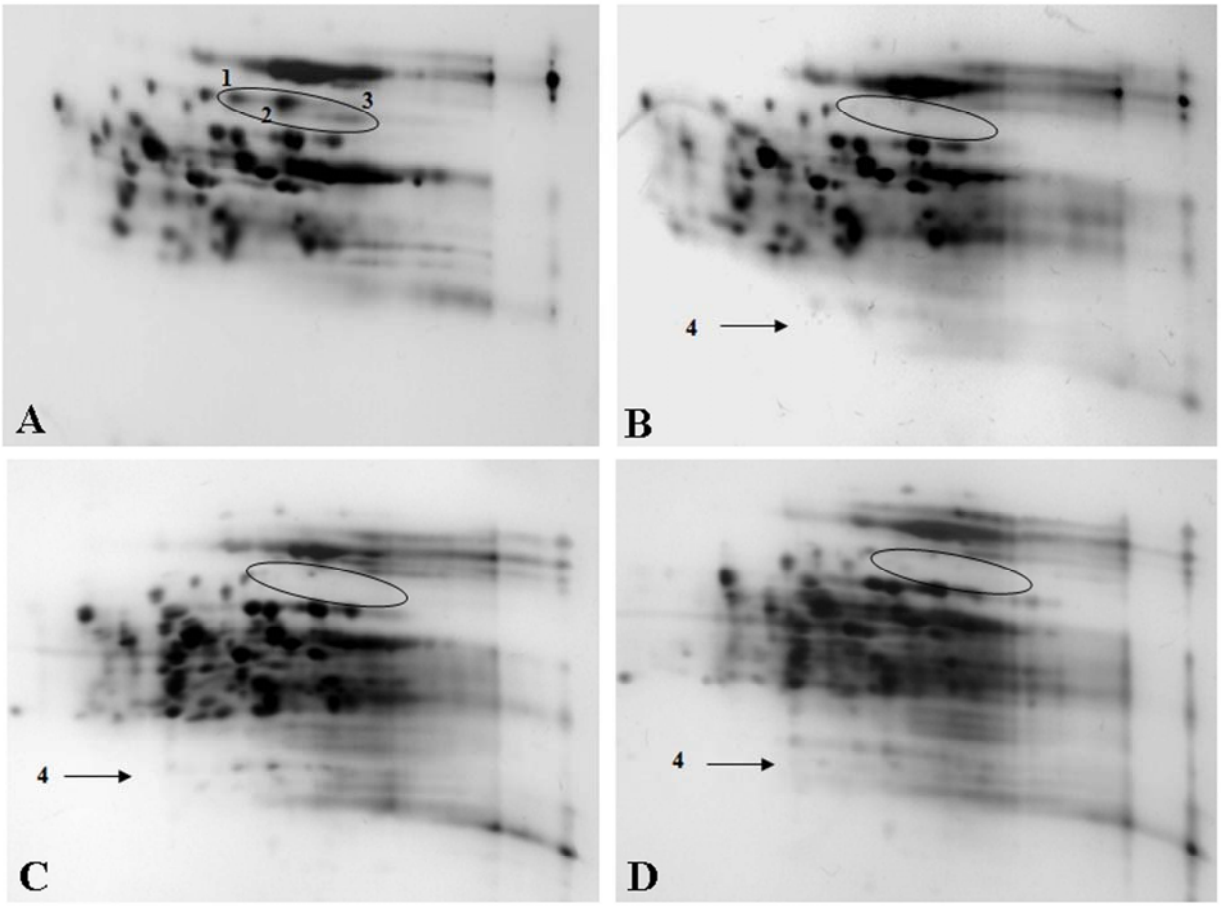

Figure 3: Enlarged views of 2-D gels of gliadin extracts from (**A**) LC at 0 h (**B**) LC at 24 h (**C**) M1 at 24 h and (**D**) LD at 48 h.

samples LA, LC, M1 and M2 were observed after 24 h and 48 h of fermentations. The peaks 3 (Retention time, R_t=83.58) and 4 (R_t=85.45) disappeared, also peaks 5 (R_t=91.45) and 6 (R_t=94.22) lost their intensities after 24 h. Samples LA, LC, M1 and M2 further had additional changes such as the disappearance of peaks 1 (R_t=66.26), 2 (R_t=77.16 and 77.92), 5 and 6 at 48 h. In control dough, CAD and LD no distinctive changes were investigated after 24 h. At the end of 48 h of fermentation, the peaks 3 and 4 disappeared, also peaks 1, 2, 5 and 6 lost their intensities. These results were in accordance with SDS-PAGE and 2-DE results. While no differences in bands and/or spots were observed in gel for LC at 24 and 48 h in electrophoresis gels, the disappearance of peaks 2, 5 and 6 could be observed in RP-HPLC chromatograms. So it is possible to conclude that RP-HPLC was more versatile than electrophoretic methods.

4. CONCLUSION

A group of LAB isolated from dairy products were examined for their gliadin degradation ability into a non-dairy medium. The decrease in pH and the increase in total acidity were recorded. Characterization of protein profiles obtained from SDS-PAGE, 2-DE and RP-HPLC techniques provided information on molecular weight and pI of gliadin fractions degraded. The degradation capability of sourdoughs with “LAB inoculation” and “chemical acidification” were found to be superior to “non inoculated & not acidified” (control) dough samples in variation with pH. The results seemed to indicate that LAB contribute to this degradation with the developing acidity and proteolytic activity which enhance the breakdown of proteins. Activation of endogenous proteolytic enzymes of wheat flour also contributes to this process in high acidic environments. In addition to *Lb. casei* and *Lb. acidophilus*, their mixed culture combinations have also been identified for higher adaptation and gliadin degradation capability in wheat-based medium. This study has been based on a M.Sc. thesis [48] and it has basically dealt with the structural changes of gliadin in wheat dough. Studies on bread making with culture combinations including high exopolysaccharide producer *Lb. delbrueckii* ssp. *bulgaricus* together with *Lb. casei* are underway and will be the context of the next publication

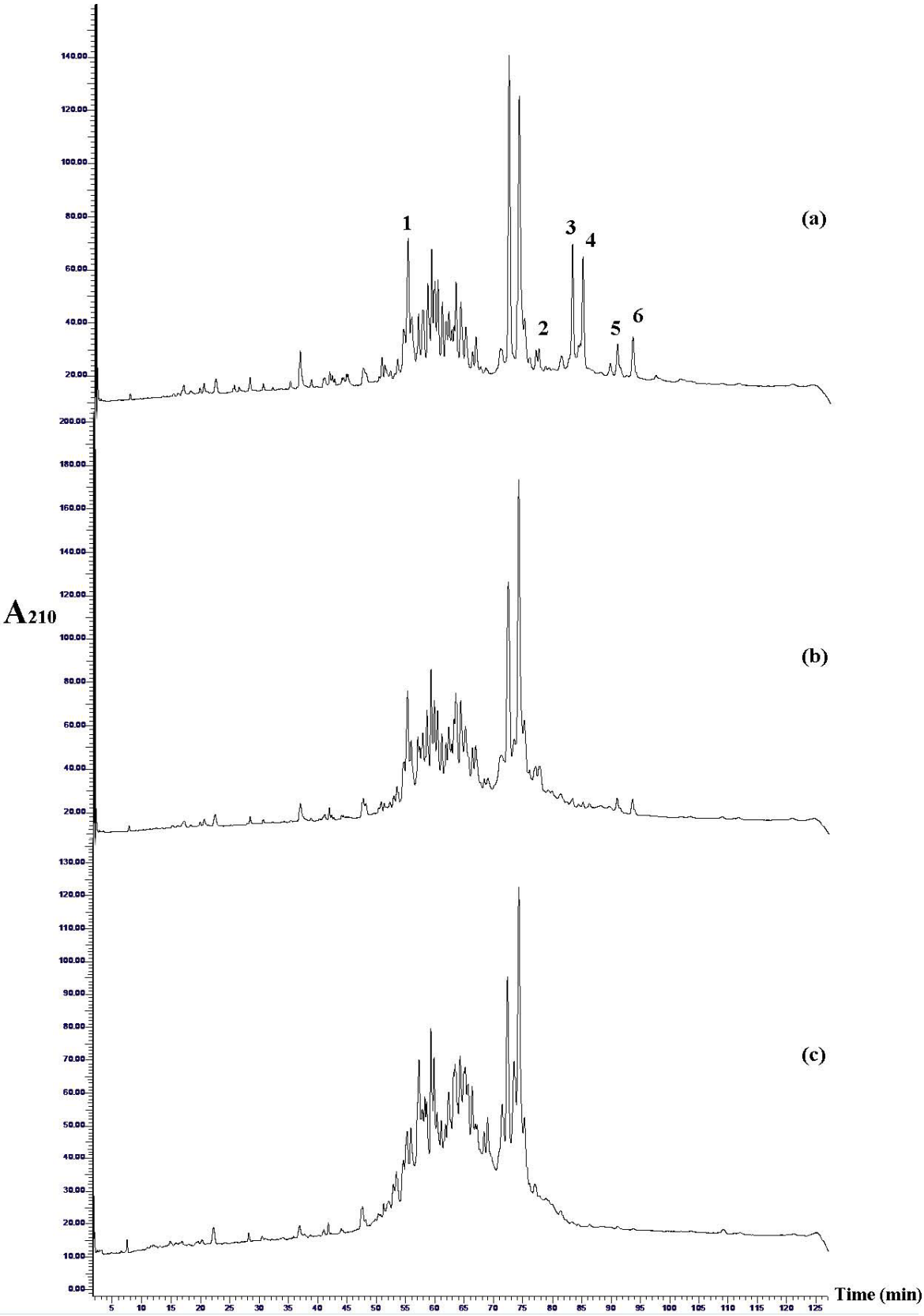

Figure 4: RP-HPLC chromatograms of sample LC at fermentation times of (**a**) 0 h (**b**) 24 h (**c**) 48 h.

elsewhere. In the case of naturally gluten-free raw materials and incorporation into bread formulations, and utilization of above strains in related sourdough fermentations should be evaluated both for

contaminating gluten removal and improving their quality further.

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ABBREVIATIONS

2-DE	=	Two-dimensional electrophoresis
CAD	=	Chemically acidified dough
CFU	=	Colony forming unit
IEF	=	Isoelectric Focusing
IPG	=	Immobilized pH gradient
LAB	=	Lactic acid bacteria
M_r	=	Relative molecular mass
MRS	=	de Man, Rogosa and Sharpes
pI	=	Isoelectric point
RP-HPLC	=	Reverse phase-high performance liquid chromatography
Rt	=	Retention time
SDS-PAGE	=	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TTA	=	Total titratable acidity

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