Structural changes of gliadins during sourdough fermentation

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ABSTRACT

Gluten intolerance, celiac disease (CD), is a chronic inflammatory disorder induced in genetically susceptible people by the ingestion of gliadins of wheat and prolamins of rye and barley. During sourdough fermentation, gliadin fractions are likely to be hydrolyzed by complex mechanism of protease and peptidase activity of probiotic lactic acid bacteria (LAB), particularly *Lactobacillus* strains, and endogenous enzymes of wheat flour. The aim of this study was to investigate the changes in gliadin structure during sourdough fermentation performed with selected LAB strains. *Lactobacillus* strains inoculated, control and chemically acidified wheat flour sourdough formulations were prepared and fermentation was started. During fermentation, total titratable acidity and pH profiles were followed and LAB were enumerated. Gliadin fractions were extracted from dough samples that were taken during definite fermentation times and were analysed by SDS-PAGE and two-dimensional (2-D) electrophoresis. It was observed that LAB inoculated sourdough samples showed an increasing acidity profile which was an indication of adaptability of inoculated strains to the dough environment. Results of LAB counts that were parallel with acidity trends confirmed this statement. Modifications were observed in the gliadin bands on SDS-PAGE gels as fermentation progressed. 2-D electrophoresis results also provided similar information. These disappearance and formation of the bands and spots are probably due to the effect of acidification. LAB gradually shifts the pH to ≈ 3.5-4.0 and wheat flour proteolytic enzymes reach their optimum pH values during this period. Activated enzymes catalyze the breakdown of proteins to peptides. Since no different band alteration occurred in chemically acidified dough, it is likely that protein degradation is not specific to any bacterial species used.

Keywords: Gluten Intolerance; Gluten; SDS-PAGE; 2-D Electrophoresis

INTRODUCTION

Celiac disease is an autoimmune disease which occurs in genetically predisposed people and caused after the consumption of wheat, rye and barley. CD has an incidence of 1 of 100–550 people in the European population [1]. Gluten proteins, mostly gliadin, have high proline and glutamine content and they are partially cleaved by the enzymes of the human digestive tract [2, 3]. When gluten reaches the small intestine, it activates an immune response and mucosal damage occurs. In order to avoid this damage-related malabsobtion and other symptoms, individuals who have gluten intolerance should obey a lifelong gluten-free diet. Although gluten-free market consists of several products, they mostly have poor quality characteristics than their gluten containing counterparts.

Sourdough fermentation considered as an alternative to gluten-free product variety by including proteolytic lactic acid bacteria. Besides, sourdough fermentation affects nutritional properties, texture and flavor, also changes in the protein structure occur with the help of LAB and wheat flour endogenous enzymes. During sourdough fermentation, as a result of acidity increase, significant increase in positive net charge and electrostatic repulsion and reduction in disulfide bonds lead to an increase in the gluten solubility [4, 5]. On the other hand, under acidic conditions, wheat flour enzymes such as aspartic proteinases and carboxypeptidases, are activated [6, 7]. Under these conditions, gluten proteins become more susceptible to degradation and primary proteolysis of proteins to peptides takes place [6, 8]. After the peptide formation, they are also hydrolyzed to amino acids by LAB (secondary hydrolysis). LAB proteolytic system consists of an extracellular proteinase, peptide transport systems, and intracellular peptidases [9]. Proteins are degraded by cell wall-associated extracellular proteinases and peptides are taken into the cell via transport systems. After all, peptides are degraded with several intracellular peptidases [10, 11, 12].

The objective of the study was to perform sourdough fermentation with previously isolated lactic acid bacteria strains and to evaluate the changes in the structure of gliadin proteins during sourdough fermentation.

MATERIALS & METHODS

Lactic acid bacteria strains

Lactobacillus acidophilus NRRL-B 1910 (NRRL, U.S.), *Lactobacillus casei* D4, *Lactobacillus delbrueckii* ssp. *bulgaricus* TY30 (Food Engineering Department, IZTECH, Turkey) [13, 14] were used in sourdough fermentation.

Preparation of sourdough samples

Sourdough samples were obtained by mixing wheat flour and water, contains cell suspensions, in 2:1 (w/v) ratio. In Table 1, all the sourdough formulations were given with their codes. Five different sourdough fermentations were performed with the inoculation of previously selected LAB strains as individual strains and as mixed culture and the final concentration of each strain was $2x10^7$ cfu /g dough. Control sample (C) with no LAB inoculation and chemically acidified dough (CAD) were also prepared. All sourdough samples were incubated at 37°C for 48 h, differently sample inoculated only with *Lb. delbrueckii* ssp. *bulgaricus* was incubated at 42°C.

Table 1. Sourdough formulations

Sourdough Sample Code	LAB Cell suspension			Lactic acid:acetic acid (4:1, v/v)
	Lb. acidophilus NRRL-B 1910	*Lb. casei* D4	*Lb. delbrueckii* ssp. *bulgaricus* TY30	
LA	+	-	-	-
LC	-	+	-	-
LD	-	-	+	-
M1	-	+	+	-
M2	+	+	+	-
C	-	-	-	-
CAD	-	-	-	+

Determination of fermentation parameters

In order to follow fermentation progress, changes in total titratable acidity (TTA) and pH were measured at 0, 3, 6, 24 and 48 h of fermentation. Concentrations of LAB were determined after incubation on MRS agar medium at 37°C or 42°C for 48 h.

Extraction of gliadin

In order to use in electrophoretic evaluation, gliadins were fractionally extracted from sourdough samples that were taken at 0, 24 and 48 h of fermentation [15, 16]. Ethanol soluble gliadins were stored at -80°C until further analysis.

SDS-PAGE of gliadin extracts

SDS-PAGE was used in order to evaluate the alterations in gliadin patterns of sourdough samples during sourdough fermentation [17]. 10 µl of gliadin extract-sample solution mixture for each sample was loaded to the wells of 15% separating gel and electrophoretic run was carried out under constant current of 32 mA for 30 min and 48 mA for 5 h at 10°C.

Two-dimensional electrophoresis of gliadins

Selected gliadin fractions were separated according to both isoelectric points (pI) and molecular weights by 2-D electrophoresis [16, 18]. Gliadin extracts were loaded to nonlinear pH 3-10 immobilized pH gradient (IPG) strips (Bio-Rad, U.S.) during rehydration step and isoelectric focusing was carried out [19]. After equilibration of IPG strips, second separation was carried out according to molecular weights by placing the IPG strip on an acrylamide gel and applying constant current of 48 mA for 5.5 h at 10°C.

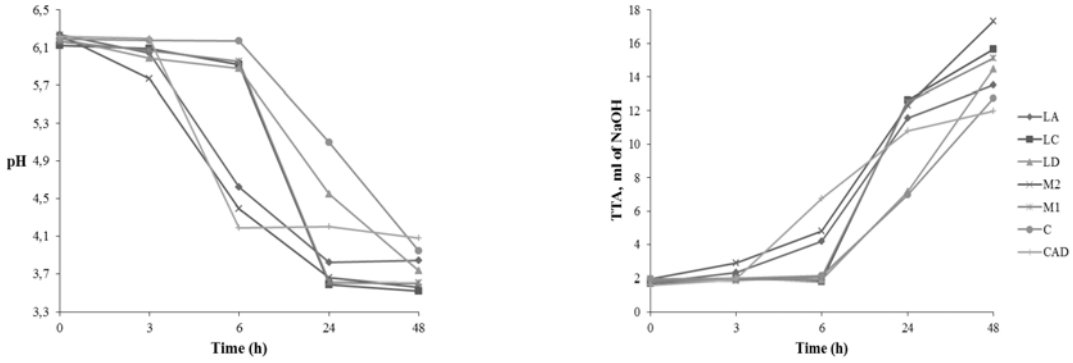
Staining and monitoring the SDS-PAGE and 2-D Gels

SDS-PAGE and 2-D gels were silver stained and monitored by an imaging system (VarsaDoc, Bio-Rad, U.S.). Molecular weights and pI values of gliadin bands and spots were evaluated by Quantity One 1-D Analysis Software (Bio-Rad, U.S.) and Bio 2-D Software (Bio-Rad, U.S.).

RESULTS & DISCUSSION

Fermentation parameters

In Figure 1, the pH and acidity profiles of sourdough samples were given. At the end of fermentation, pH was decreased from 6.1-6.2 range to 3.8-3.5, total titratable acidity was reached the 13.49-17.34 range, and the LAB population was counted as 10^8-10^9 cfu/g dough. The slowest pH decrease was observed in sample LD due to the low growth rate of the inoculated strain. The pH decrease was faster in samples LA and M2 which contain *Lb. acidophilus* NRRL-B 1910. It should be noted that there are also acidity increase in control dough and it can be attributed to the activity of wheat flour microflora.



(a) (b)

Figure 1. pH (a) and TTA (b) profiles of sourdough samples

SDS-PAGE gel of gliadins

According to SDS-PAGE gel given in Figure 2, after 24 h of fermentation, some bands around 38, 37 and 28 kDa disappeared, on the other hand, new bands with molecular weights in the range of 27-25 kDa formed in the samples fermented with individual *Lb. acidophilus* NRRL-B 1910, *Lb. casei* D4 strains and mixed cultures, and also in CAD; the same changes happened after 48 h for sample fermented with *Lb. delbrueckii* ssp. *bulgaricus* TY30 and control dough.

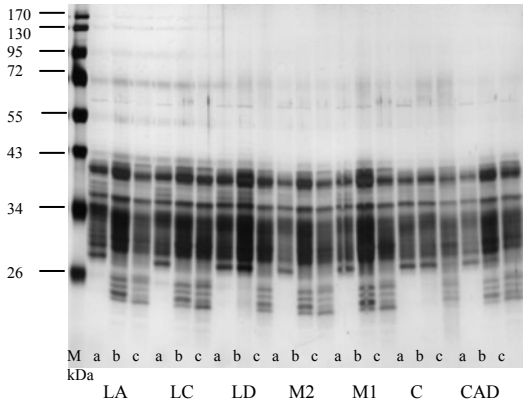
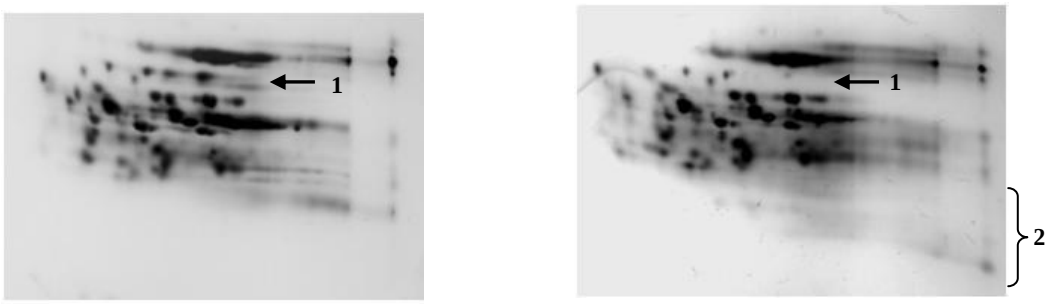


Figure 2. SDS-PAGE gel of gliadins extracts from sourdough samples. M represents marker and sample codes are given under the lanes. Fermentation times are represented as a, 0 h; b, 24 h; c, 48 h.

Two-dimensional electrophoresis gels

After 24 h of fermentation spots with molecular weight of ≈ 39 kDa and pI of 6.4 and 6.7; molecular weight of ≈ 38 kDa and pI of 7.1 were disappeared (region 1 in Figure 3). Also newly formed very small spots which

were distributed in two wide regions one with the range of ≈ 30-29 kDa and pI of 6.5-8.9 and the other in the range of ≈ 28-27 kDa and pI of 8.9-9.5 were observed (region 2 in Figure 3).
As seen from the results, 2-DE findings agreed with SDS-PAGE results. The degradations occurred in all gliadins and were parallel with acidity increase. The spot disappearance and formation occurred in LA, LC, M1, M2 and CAD after 24 h, in control and LD after 48 h. Changes occurred in all samples and also in chemically acidified and control doughs.



(a) (b)

Figure 3. 2-D electrophoresis gels of gliadins from sourdough fermented with *Lb. casei* at 0 h (a) and 24 h.

CONCLUSION

Gluten-free diet is the only treatment of celiac disease and gluten-free products are of great importance in terms of strict adherence to this diet and complete withdrawal of gluten. In this perspective, sourdough fermentation can be considered as a promising alternative in terms of removal of total or residual gluten.
In this study, sourdough fermentations with the inoculation of several LAB were performed. It was observed that the selected LAB were adapted to wheat dough environment and with their growth, pH decreased and acidity increased. According to SDS-PAGE results, band modifications related with gliadin hydrolysis were observed. As a result of hydrolysis, new bands formed and previously existed bands disappeared. Moreover, changes of the spot pattern in 2-D gels agreed with these results. Since no strain related specific changes were discovered, the degradations occurred in gliadins during sourdough fermentation were probably resulted from the activation of the wheat flour endogenous proteolytic enzymes. The lactic acid bacteria contribute to this degradation by producing acidity and enhancing the breakdown. Complete characterization of degraded gliadins could be done with immunochemical and spectrometric techniques to investigate if the hydrolyzed fragments contain toxic parts.

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