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PROTEOMIC VIEW ON GLUTEN STRUCTURE IN DIFFERENT TYPES OF FLOUR AND BREAD SAMPLES BY USING BOTTOM UP PROTEOMICS AND FT-IR SPECTROSCOPY

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ABSTRACT

In the current study, some proteomic methods containing 2D-PAGE and FT-IR techniques were performed to screen gliadin, the subunit of gluten protein, and transformation between α and β sheet forms of this protein was evaluated. The protein concentration of the samples varied between flour types and also cooked form of these samples. We focused on Amide A, Amide I and phosphorylated protein regions on the spectrums achieved by FT-IR. Gliadin structure was dramatically differed when the raw material was formed in baked form. While Amide A vibration which is related to N-H streching increased for the bread form of the white flour, Amide I which is related to C=O streching decreased when the raw material changed in the cooked form. It can be concluded that the type of flour used in bread production and the type of baking were effective on gluten structure and amount of the final product.

Keywords: Gluten, gliadin, proteomics, 2D-PAGE, FT-IR

FARKLI TİPTE UN VE EKMEK ÖRNEKLERİNDE GLUTEN YAPISININ PROTEOMİK VE FT-IR SPEKTROSKOPİSİ ARAÇLARI İLE ARAŞTIRILMASI

ÖZ

Bu çalışma kapsamında; gluten proteininin alt birimi olan gliadin 2D-PAGE ve FT-IR tekniklerini içeren bazı proteomik yöntemler aracılığı ile araştırılmış ve bu proteinin α ve β yaprak formları arasında dönüşüm olduğu tespit edilmiştir. Denemelerde yer alan örneklerin protein konsantrasyonları un çeşidine ve örneklerin pişmiş formuna göre değişiklik göstermiştir. FT-IR ile elde edilen spektrumlarda Amid A, Amid I ve fosforile protein bölgeleri olmak üzere üç alan çalışılmıştır. Bu spektrum bilgisinden yola çıkarak, gliadin yapısının, ham maddenin ürüne dönüşme sürecinde önemli ölçüde farklılaştığı gözlenmiştir. Beyaz unun ekmek formunda N-H bağı ile ilgili

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Amid A titreşiminde artış gözlenirken, un örnekleri pişmiş forma dönüştüğünde C=O bağı titreşimi ile bağlantılı olan Amid I bölgesinde azalma tespit edilmiştir. Çalışma sonuçlarından yola çıkarak, ekmek üretiminde kullanılan un çeşidinin ve pişirme şeklinin nihai ürünün gluten yapısı ve miktarı üzerinde etkili olduğu söylenebilir.
Anahtar kelimeler: Gluten, gliadin, proteomiks, 2D-PAGE, FT-IR

INTRODUCTION

Gluten is a protein in the structure of wheat, barley, rye and oat. This protein is responsible for the unique viscoelastic properties of wheat and the quantity/quality of proteins being strongly related to the bread-making quality of flour (Primo-Martin et al., 2003; Ronda et al., 2017). Gluten is digested by proteases in the human digestion system, the products of this metabolism are Pro/Gln-rich peptides of up to 30-40 amino acid length. Some part of gluten protein and peptides arrive to the large intestine and the microbiota of large intestine participate in the metabolism of gluten proteins (Caminero et al., 2014; Bascuñán et al., 2020). In large intestine the microbial activity on gluten proteins result with some metabolites which show enflamation effect on the intestinal wall. Gluten term refers to a protein complex contains gliadins (prolamins) and glutenins as major subunits. Both of these proteins have high proline and glutamine aminoacids, which are responsible from Coeliac Disease (Celiac Disease, CD) (Caminero et al., 2012; Dunaevsky et al., 2021). Gliadins which play a key role in CD contain around 40% glutamine and around 14% proline (Dziuba et al., 2014).

Gluten is the sticky part of dough, which obtained after washing the dough with water and separated from water soluble proteins; albumins and globulins. This protein complex has 15000-141000 Da molecular weight, forms from prolamin (gliadin) and glutenin and insoluble in water. It is known that the ratios of gliadin and glutenin in the gluten structure are almost equal (Artik, 1988).

Gliadin protein is controlled by six loci on chromosomes, which belong to the 1st and 6th homology groups of hexaploid *Triticum aestivum* L. genome. Gliadins are shown in α-, β-, γ-, ω- forms in acidic polyacrylamide gel electrophoresis (Dziuba et al., 2014; Solé-Jamault et al., 2022). While gliadin was detected on 2D gel image with 46 bands, only 8 bands were detected on 1D SDS gel (Artik, 1988).

Proteomic analysis emerged in the last two decades and among these methods, two dimensional (2-D) electrophoresis allow us to separate up to thousand proteins in an analyze (Dziuba et al., 2014). 1B-encoded ω-gliadins are located approximately at 50000 Da on 1D gel, and 1A-encoded ω-gliadins, α-gliadins, β-gliadins and the low molecular weight gluten subunits rank among 36000 to 48000 Da (DuPont et al., 2005).

Food allergy is an important health concern occurs with proteins resistant to heat, digestion, proteolysis and acid. Cereals are the good sources due to containing protein, fiber, vitamins and minerals. In the world, the most consumed crops are wheat, corn and rice. Among these crops solely wheat has a future to form dough due to having gluten in its structure (Shewry et al., 2002).

Most food allergies can be defined as IgE-mediated diseases, but Celiac Disease is known as cell-mediated disease of food intolerance (Diaz-Amigo and Yeung, 2010). The gliadin proteins have rich proline and glutamine content and humans inherently lack endopeptidases to cleave bonds between proline and glutamines. Gliadin can not be fully digested by digestive tract enzymes leads to the generation of many polypeptides, which are immunogenic to patients genetically susceptible to CD (Rajpoot and Makharia, 2013).

When making a gluten-free bread nutritional quality is significant and depends on the ingredients and additive combination, but also processing can provide a way to improve bread quality (Matos and Rosell, 2014). Additionally, the information about the threshold of gluten contamination in gluten-free products is essential to ascertain these products to guarantee a safe diet. A study performed to research this

confusion has offered the limit of 20 ppm for products naturally gluten-free and of 100 ppm for products rendered gluten-free in order to provide a safe diet (Gibert et al., 2006). So, the gluten-free products are not supposed to be fully gluten-free and the processes, making these products less gluten containing are desired to date.

Wheat starch and wheat gluten has technological properties as dough strengtheners, stabilizers, thickeners, surface finishing agents, texturizing agents (Diaz-Amigo and Yeung, 2010). But 30% of human population, carry HLA-DQ2 and DQ8 genes which are responsible from CD. Although the people having these genes are 30% of the total population, only 3% of them develop this disease (Caminero et al., 2015). This means that, environmental factors have a significant role to display Coeliac Disease. The CD is known as a chronic small intestinal immune-mediated entheropathy caused by gluten proteins. The current remedy for dealing with the CD is a strict lifelong gluten-free diet (Caminero et al., 2014). The flours produced for CD patients generally combined of corn flour, rice flour or buckwheat flour supplemented with xanthan gum. According to the literature, there is a tendency to remove gluten from daily diet due to causing some diseases and obesity. Currently gluten-free product industry should find new technologies to suffice this demand and the first step of this rank is certainly understanding the alterations in the gluten structure during the process (Gomez and Sciarini, 2015; Stantiall and Serventi, 2017).

The CD was believed to be a rare disease, now it is accepted as a global disease and affects almost 0.6-1.% of the World population. This disease is now emerging in the East and Asian countries after Europe, USA and Middle East (Rajpoor and Makharia, 2013). Today besides CD, people suffer from non-coeliac gluten sensitivity as well (Volta et al., 2013). CD disease is emerging in Turkey and the other countries and has also become a public health problem. While there is a great amount of patients with CD disease, to date only a fraction of them have been diagnosed. Some of the key issues in management of CD disease, it is vital to provide reliable industrial production and affordable gluten-free food, and food labeling of gluten contents (Rajpoot and Makharia, 2013).

We performed some proteomic methods containing 2D-PAGE and FT-IR techniques to screen gliadin, the subunit of gluten protein, and we evaluated transformation between α and β sheet forms of this protein. In the current study, the overall aim was to exhibit the effects of raw material (flour) and the baking method on the gluten structure and amount of gluten in bread.

MATERIALS AND METHODS

Material

The materials of this study were flour and bread samples. While the flour and commercial bread samples were obtained from local markets in Ankara-Turkey; white bread (made from white flour) and whole wheat bread (made from whole wheat flour) was produced by lab-scale production. The samples were selected in order to screen the effect of the raw material and cooking method on the α- and β-gliadin forms of the gluten protein. The commercial whole wheat bread and commercial stone oven whole wheat bread ingredients were same and contained; whole wheat flour (100%), water, cracked wheat, yeast, wheat sour, salt, preservative (calcium propionate, sorbic acid), antioxidant (ascorbic acid) (the proportions of the ingredients in the products are not known due to the commercial nature of the products).
The samples used in the study are seen in Table 1.

As it is seen on the Table 1, we had a chance to compare the raw materials (sample 3 and 5) and their breads (sample 2 and 4). Furthermore, we could also exhibit the differences between the baking style of whole wheat breads (samples 1 and 6).

All of the reagents used in the study were proteomics or molecular biology grade. Glycerol and agarose were from Sigma-Aldrich (St Louis, Missouri), ampholytes and dithiothreitol (DTT) were from Fluka (St Gallen, Switzerland), bovine serum albumin (BSA) was from Thermo Scientific (Rockford, Illinois), 3-[(3-cholamidopropyl)dimethylammonio]-1-

propanesulfonate (CHAPS) was from Amresco (Solon, Ohio), sequencing grade modified porcine trypsin (Promega, Madison, WI, USA), trifluoro acetic acid (TFA) (Supelco, Bellefonte, USA), substance P, angiotensin, renin, ACTH (adrenocorti cotropic hormone), glu-fib (glu-1-fibrinopeptide B) (Sigma, St. Louis, USA) and protein ladder was from New England BioLabs (UK). Pro-Q Diamond and Pro-Q Emerald were from Life Technologies (Thermo- Fisher Scientific, USA). Other reagents and chemicals were purchased from Bio-Rad (USA).

Table 1. The samples used in this project study

	Sample	Source
1	Commercial whole wheat bread	Obtained from local market
2	The white bread made from sample 3	Made in lab scale
3	Wheat flour	Obtained from a local market
4	Whole wheat bread made from sample 5	Made in lab scale
5	Whole wheat flour	Obtained from local market
6	Commercial whole wheat stone oven bread	Obtained from local market

Methods

Lab-scale bread making

The breads produced by white flour and whole wheat flour contained 500 g flour, 350 mL water, 30 g salt, 20 g sugar, 20 g lyophilized yeast. After the dough mixture was fermented for 1 hour, it was baked at 250°C for 30 minutes. For the bread making procedure, the method offered by Salvador et al. (2006) was used with some modifications.

Gliadin isolation from flour and bread samples

In order to isolate gliadin from the samples the method offered by Di Cagno et al., (2002) was performed. Tris-HCl buffer (pH:8.8 and 50 mM) was added to 1 g of the sample (during sampling, the bread samples were first crushed and homogenized) and then incubated at 4 °C for 1 hour. The sample was vortexed every 15 minutes and this step was repeated twice. At the end of the time, the mixture was centrifuged at 20000 x g for 20 minutes and the supernatant containing albumins and globulins were removed. Pelet was resuspended with 75% ethanol and incubated at room temperature. The mixture was vortexed every 15 minutes and then centrifuged at the same conditions. The supernatant which contains the gliadin proteins was seperated in another tube and preserved at 4 °C until used for the further experiments.

Quantitation of protein with Bradford Method

After gliadin isolation, the solution was concentrated by using Millipore 0.5K ultrafiltration tubes and Bradford Assay (Bradford, 1976) was used for protein concentration measurement.

Two-dimensional gel electrophoresis (2D-PAGE)

2D-PAGE experiments were performed with 7 cm linear immobilized pH gradient (IPG) strips (pH 3-10, Bio-Rad). We have six groups which are commercial whole wheat bread, white flour bread, white flour, lab-scale made whole wheat bread, whole wheat bread flour and commercial traditional stone oven made whole wheat bread. Each group was studied by three technical replications. The Bradford assay results provided the protein amounts of the samples and the proportions of the samples which includes 40 ug protein amount were prepared and uploaded onto the IPG strips and rehyrated at 50V by using a Protean IEF Cell (Bio-Rad) at room temperature. After that IEF part was performed (250 V for 15 minutes, 4 kV for 3 hours and 4 kV until reaching 20 kV/h). Just before second dimensional separation IPG strips were incubated with equilibration buffer I (6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% SDS, 20% glycerol, and 2% DTT) for 15 minutes on a shaker at room temperature and then an additional incubation was done for 15 minutes with equilibration buffer II (6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% SDS, 20% glycerol, and (2.5% iodoacetamide, bromophenol blue). The strips were loaded onto polyacrylamide gels (4% stacking and 12.5% running gels) for the second dimensional

seperation which was done with Mini Protean 3 Cell (Bio-Rad, USA) by 100 V for 15 minutes and 150 V until the bromophenol blue dye reached to the bottom of the gels. Gels were stained with Oriole Fluorescent Gel Stain (Bio-Rad) for 90 minutes.

Fourier Transform Infrared (FT-IR) Spectroscopy Application

The liquids that were contained gliadin (solubilized in ethanol) were frozen at -80^{o}C for twelve hours. After freezing, gliadin samples were lyophilized with Millrock Technology freeze-drier. Lyophilized powder was loaded ATR-FT/IR and the spectra were recorded on a Bruker Tensor 27 FT-IR spectrophotometer using an ATR-FT/IR cell, the internal reflection element of which was a ZnSe plate. Spectra were recorded as the average of 64 scans at 4 cm^{-1} resolution, using the empty cell as blank. In all cases, sample analyses were performed as triplicate.

Statistical Analysis

Significant differences between samples were calculated using t-test analysis (SPSS Statistics 24.0) The p value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Quantitation of Proteins and 2D-PAGE Results

As it declared in the literature, white wheat flour contains about 80% starch and 10% protein. The indigestible oligosaccharides (fructo-oligosaccharides and fructans) constitute 13.4 % of the dietary fiber in wheat. Among wheat proteins, gluten constitutes 80% of the total protein, and comprises two major groups: the glutenins and the gliadins (prolines) (El-Salhy et al., 2015). It is known that gliadins responsible for allergenic reactions, therefore this protein were focused on the current study (Czaja-Bulsa and Bulsa, 2017). Bradford Assay was performed after gliadin isolation in order to screen the protein concentration of the samples and the results were evaluated within the framework of raw material and products. During the bread making, it was required to add additional materials such as yeast, salt, sugar and water and use different baking methods, so we observed different protein values from different samples. It is expected that the protein quantity is halved after baking process which means bread will contain only 50% of the total protein of the raw material, flour (Sivam et al., 2010). In bakery different temperatures and time applications are used to produce breads. In our study, it was observed that the baking process caused a decrease in the amount of protein. As it is seen in Table 2; when compared the white bread sample (0.5 ug/mL protein content) and white wheat flour (1.9 ug/mL); there is a 73.7% protein loss during bread making process. Similarly, 31.5% loss was detected for the cooked form (whole wheat bread) of the whole wheat flour. The protein contents of the samples were evaluated with t-test (Table 3). As it is seen on the Table 3, it was observed that both raw materials had the same amount of protein content. However the lab-scale made bread samples (sample 2 and sample 4) had significantly different amount of protein from their raw materials (sample 3 and sample 5, respectively) ($p<0.05$). When the sample 1 (commercial whole wheat bread) and sample 6 (commercial stone oven whole wheat bread) were compared, it can be concluded that these samples were statistically different from each other on protein content, amide A, amide I and phosphorilated protein regions ($p<0.05$) (Table 4, 5 and 6). As it is declared in "Material" section, these two commercial bread samples ingredients were same but the cooking style was different; sample 1 was baked by a standard oven while the sample 6 was baked by a stone oven.

In the bakery, loaf quality is related the quality and quantity of gluten protein of the raw material. Therefore gluten-free diet needs to a substitute material to create the fixed, open foam crumb structure. Xanthan and hydroxypropyl methylcellulose (HPMC) are the hydrocolloids used in bakery to increase gas retention and loaf volume. While HPMC has ability to improve gluten-free loaf quality by increasing loaf volume and reducing crumb hardness of the rice cassava bread without the addition of alternative proteins, xanthan can increase dough elasticity and reduce extensibility (Crockett et al., 2011; Gomez and

Sciarini, 2015). Some additives like enzymes and other proteins are used to improve the quality of gluten-free breads, but the combinations and the proper amounts need to be optimized to reduce the cost and to enhance the overall consumer acceptability (Smerdel et al., 2012).

Table 2. The protein concentration results after gliadin isolation (1; commercial whole wheat bread, 2; White flour bread, 3; White flour, 4; house-made whole wheat bread, 5; whole wheat bread flour, 6; commercial traditional stone oven made whole wheat bread)

Sample Name	Protein Concentration (ug/ml)	Sample Name	Protein Concentration (ug/ml)
1	1.3±0.13	4	1.3±0.06
2	0.5±0.06	5	1.9±0.03
3	1.9±0.13	6	0.8±0.06

Table 3. The comparison of the samples with t-test analysis to screen the amount of protein differences of the samples in the raw material, baking method and the return of the raw material to the cooked form frame.

Comparison criteria	Sample pairs included to test	t-test value	Interpretation of p value	Result
Raw material difference	Sample 3 (Wheat flour) and Sample 5 (Whole wheat flour)	1	p=1	No difference
Baking method	Sample 1 (Commercial whole wheat bread) and Sample 6 (Commercial whole wheat stone oven bread)	0.013	p<0.05	Significant
Comparison of raw material with baked form	Sample 3 (Wheat flour) and Sample 2 (The white bread made from sample 3)	0.015	p<0.05	Significant
	Sample 5 (Whole wheat flour) and Sample 4 (Whole wheat bread made from sample 5)	0.002	p<0.05	Significant

Table 4. The comparison of the samples with t-test analysis to screen the Amide I region differences of the samples in the raw material, baking method and the return of the raw material to the baked form frame.

Comparison criteria	Sample pairs included to test	t-test value	Interpretation of p value	Result
Raw material difference	Sample 3 (Wheat flour) and Sample 5 (Whole wheat flour)	0.312	p > 0.05	Not significant
Baking method	Sample 1 (Commercial whole wheat bread) and Sample 6 (Commercial whole wheat stone oven bread)	0.030	p<0.05	Significant
Comparison of raw material with baked form	Sample 3 (Wheat flour) and Sample 2 (The white bread made from sample 3)	0.016	p<0.05	Significant
	Sample 5 (Whole wheat flour) and Sample 4 (Whole wheat bread made from sample 5)	0.065	p>0.05	Not Significant

Table 5. The comparison of the samples with t-test analysis to screen the Amide A region differences of the samples in the raw material, baking method and the return of the raw material to the baked form frame.

Comparison criteria	Sample pairs included to test	t-test value	Interpretation of p value	Result
Raw material difference	Sample 3 (Wheat flour) and Sample 5 (Whole wheat flour)	0.074	$p > 0.05$	Not significant
Cooking method	Sample 1 (Commercial whole wheat bread) and Sample 6 (Commercial whole wheat stone oven bread)	0.023	$p<0.05$	Significant
Comparison of raw material with cooked form	Sample 3 (Wheat flour) and Sample 2 (The white bread made from sample 3)	1.96072E-18	$p<0.05$	Significant
	Sample 5 (Whole wheat flour) and Sample 4 (Whole wheat bread made from sample 5)	1	$p=1$	No difference

Table 6. The comparison of the samples with t-test analysis to screen the phosphorylated protein region differences of the samples in the raw material, baking method and the return of the raw material to the baked form frame.

Comparison criteria	Sample pairs included to test	t-test value	Interpretation of p value	Result
Raw material difference	Sample 3 (Wheat flour) and Sample 5 (Whole wheat flour)	1	$p=1$	No difference
Baking method	Sample 1 (Commercial whole wheat bread) and Sample 6 (Commercial whole wheat stone oven bread)	0.035	$p<0.05$	Significant
Comparison of raw material with baked form	Sample 3 (Wheat flour) and Sample 2 (The white bread made from sample 3)	1.04488E-32	$p<0.05$	Significant
	Sample 5 (Whole wheat flour) and Sample 4 (Whole wheat bread made from sample 5)	1	$p=1$	No difference

As we saw in the current study, while the gluten protein amount was detected same for both white flour and whole wheat flour; the protein concentrations of the bread samples produced by these raw materials were different from each other. This result may indicate, the process and the raw material of the bread can effect the protein structure of the final product. We showed in this study although the raw material contains the same amount of protein, their products can be differ from each other on the protein amount and the structure. Besides, raw material protein

content and the structure can be changed amoung the baking process (Table 2). These results are important and exhibits a chance to select an alternative application ways to produce low-gliadin/gluten-content products for the consumers which suffer from CD. A similar declaration was reported by Stantiall and Serventi, (2017). Although they searched about the effect of the parameters on the glycemic index, they reported the bread developed with several emulsifiers, vegetable margarine and egg contained a much higher proportion of readily digestible starch and combination of ingredients, processing and technology.

The wheat flour proteins are composed by mostly gliadins, albumins and globulins. These proteins can be separated by using solubility in solutions such as water, salt or alcohol. After isolation of the target protein one-dimensional methods, 2D-PAGE or HPLC should be applied to exhibit protein purity or quantity (DuPont, Chan, Lopez, & Vensel, 2005). Basically gluten proteins or prolamins are the storage proteins of wheat (*Triticum aestivum* L.) and are insoluble in water, but can be divided into alcohol soluble gliadins and alcohol insoluble glutenins (Lagrain et al., 2013).

In literature, the quality of the wheat is thought to be in correlation with glutenin subunits. Liu et al., (2012) searched about glutenin accumulation patterns and their relationships with wheat quality for three wheat cultivars (Jimai 20, Jin 411 and Zhoumai 16). They reported there is a possible relationship between low molecular weight glutenin subunits and gluten quality. They also declared that 2D-PAGE technique was more efficient than SDS-PAGE. In our study we also chosed to perform 2D-PAGE analysis to exhibit protein spots (Figure 1).

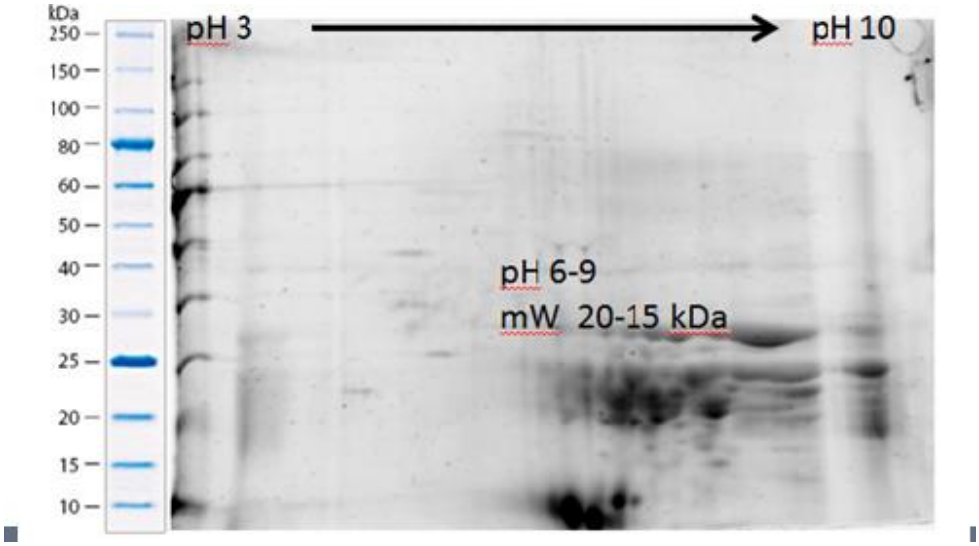

Figure 1. Gliadin protein profile that is separated by 2D-PAGE; General profile of gliadin which isolated from lab scale made bread by wheat flour.

According to the PDQuest analysis, 143 spots were matched from six groups and both upregulation/ downregulation of expression levels and presence/ absence of protein spots between groups were determined. Protein spots are mostly distributed around pH 6 - 9. In the current study, 2D-PAGE experiment was performed to see gliadin isolation success and also to observe differences between sub-groups of the samples. According to Secundo and Guerrier, (2005), protein profile of our isolated proteins was similar with pure gliadin proteome. Therefore it is possible to say our protein solution contains mostly gliadin. But it is required to perform mass spectrometry application to identify protein spots content.

In addition to that the proteom profile of the groups (1; commercial whole wheat bread, 2; white flour bread, 3; white flour, 4; lab-scale made whole wheat bread, 5; whole wheat bread flour, 6; commercial stone oven made whole wheat bread) showed main differences. According to this assay it is possible to say; the process during bread making may affect the gliadin structure. It is fact that 3D structure of proteins is important for allergic reaction since protein docking.

FT-IR Results

Fourier Transform Infrared Spectroscopy is a kind of device the principle of which is a continuous source of light used to produce light over a broad range of infrared wavelengths. It can be shown that if the intensity of light is measured and plotted as a function of the position of the movable mirror therefore by using FT-IR, it is possible to get information about bound structure. In FTIR spectroscopy, the light is directed onto the sample of interest, and the intensity is measured using an infrared detector (Igci et al., 2017).

We focused on three parts on FT-IR spectrum that are Amide A region (3000-3600 cm^{-1}), Amide I region (1600-1700 cm^{-1}) and phosphorylated protein region (1230-1245 cm^{-1}) due to analyse the protein profiles of the samples and get an idea on the effect of different applications such as flour type and baking style on the gliadin structure. Figure 2, 3, 4, 5 and 6 show the details of this assay.

The Amide A region is generally associated between the N-H streching and does generally not related to the backbone of the protein structure but is in the relationship between the hydrogen bonds (Ji et al., 2020). As seen in the Figure 2, at 3643 wavelength this bond vibration was observed similar for whole wheat flour and the bread made from this raw material with 0.006 value. However Amide A vibration increased for the white bread when compared to its raw material (the value was 0.008 for the white flour and 0.014 for the white bread).

Another important region for the evaluation of the secondary structure of the proteins is Amide I band. This band is usually screened between at 1600-1700 cm^{-1}. In Figure 4, at 1654 cm^{-1}, this band decreased when the raw material was changed in the cooked product; the value was; 0.402 for white flour and 0.366 for white bread. Further, the Amide I band was detected as 0.362 for whole wheat flour and 0.339 for the bread made from this raw material. Amide I band is an indicator for C=O streching vibration which is directly related to the backbone conformation of the proteins (Ji et al., 2020). Figure 3 and Figure 5 shows the cluster analysis of the Amide A and Amide I regions respectively. This analysis indicated the gliadin structure differs after the applied procedures. Finally the phosphorylated protein region was searched at 1241 cm^{-1}; which was recorded as 0.005, 0.006 and 0.001 for the samples respectively whole wheat bread, white bread and whole wheat stone bread. This result is significant due to this second structure was detected for only the cooked form of the samples (Figure 6).

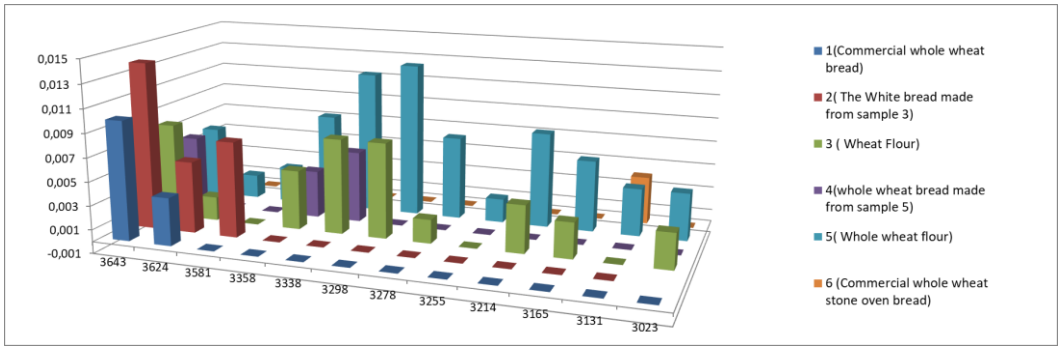

Figure 2. FT-IR spectrum results. Amide A region spectrum results of all samples and wavenumber of the region.

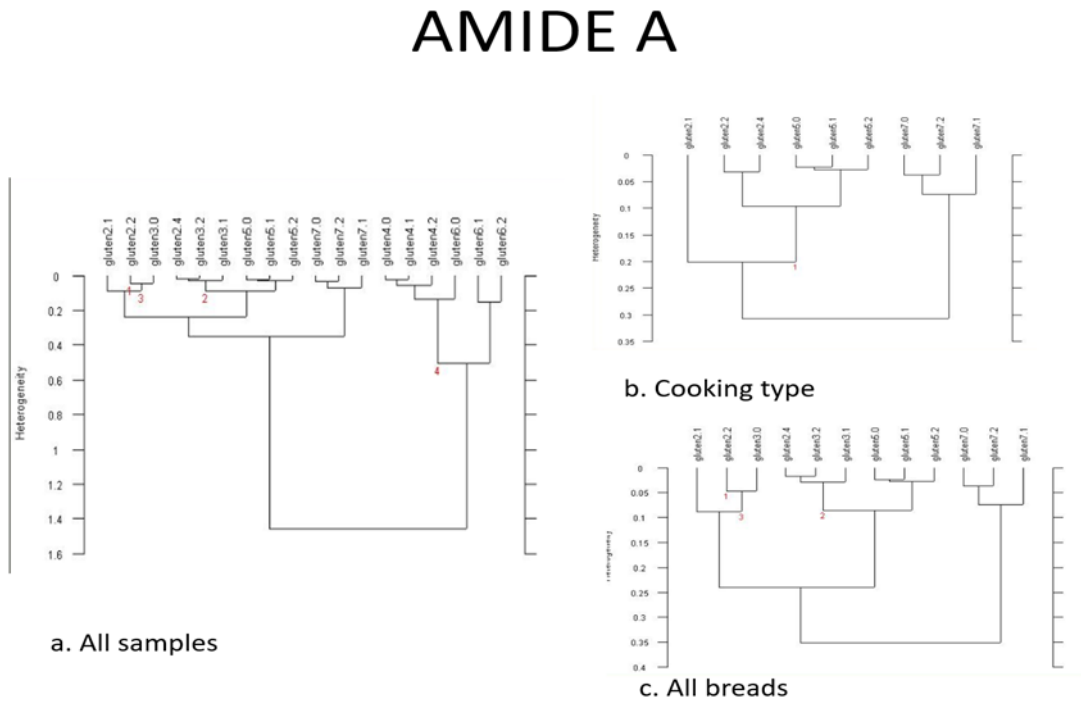

Figure 3. Clustering results of different groups of Amide A region spectrum (OPUS 5.5 software) a. All samples b. Cooking Type c. All breads

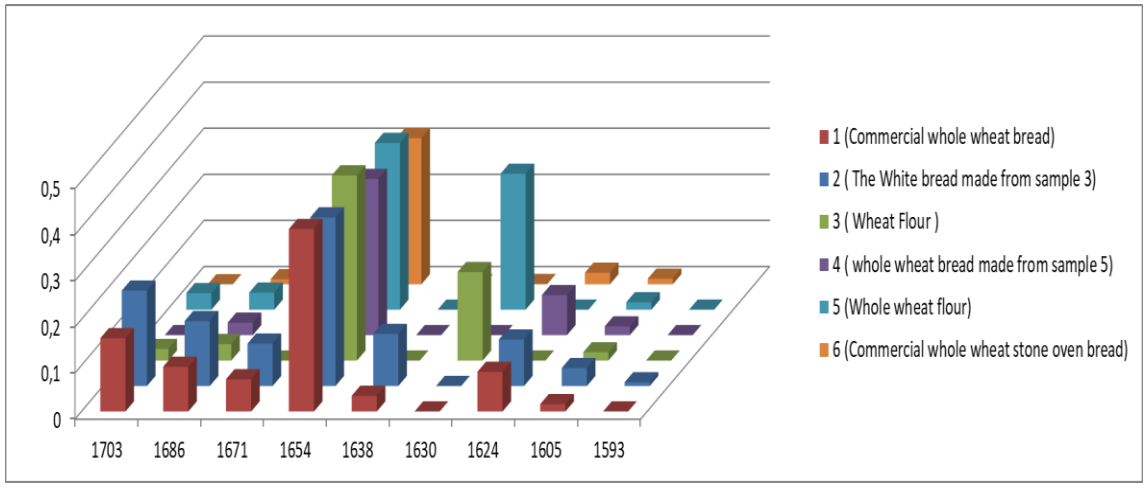

Figure 4. FT-IR spectrum results. Amide I region second derivative spectrum results and plotting results of the spectrum.

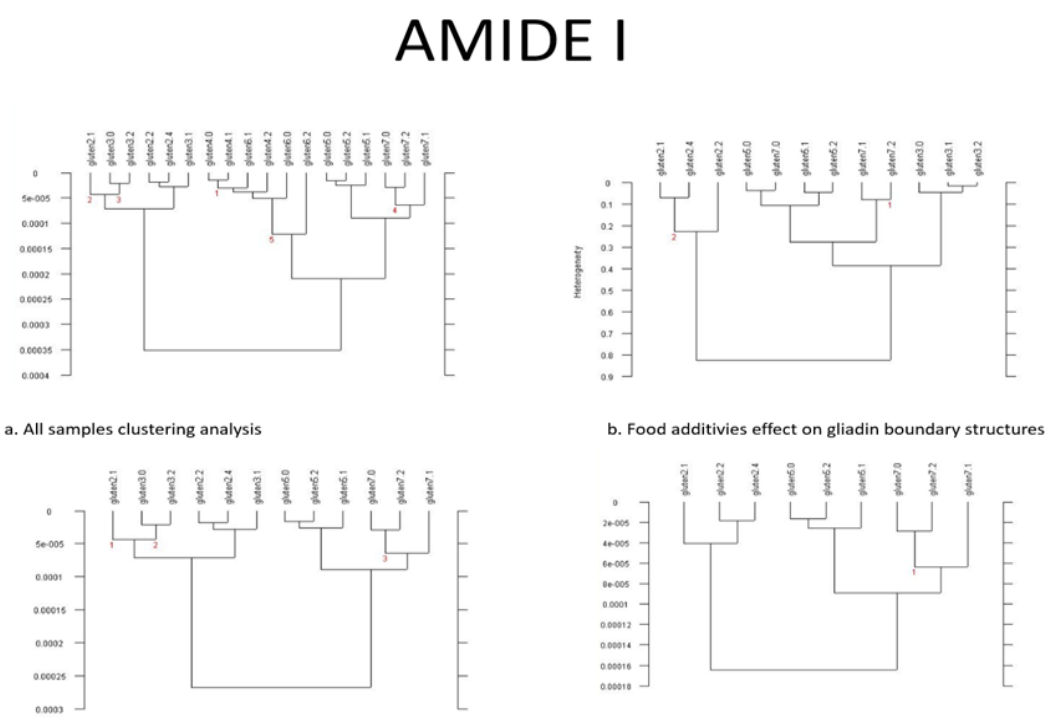

Figure 5. Clustering results of different groups of spectrum (OPUS 5.5 software) a. All samples clustering analysis b. Food additive effect on gliadin boundary structures

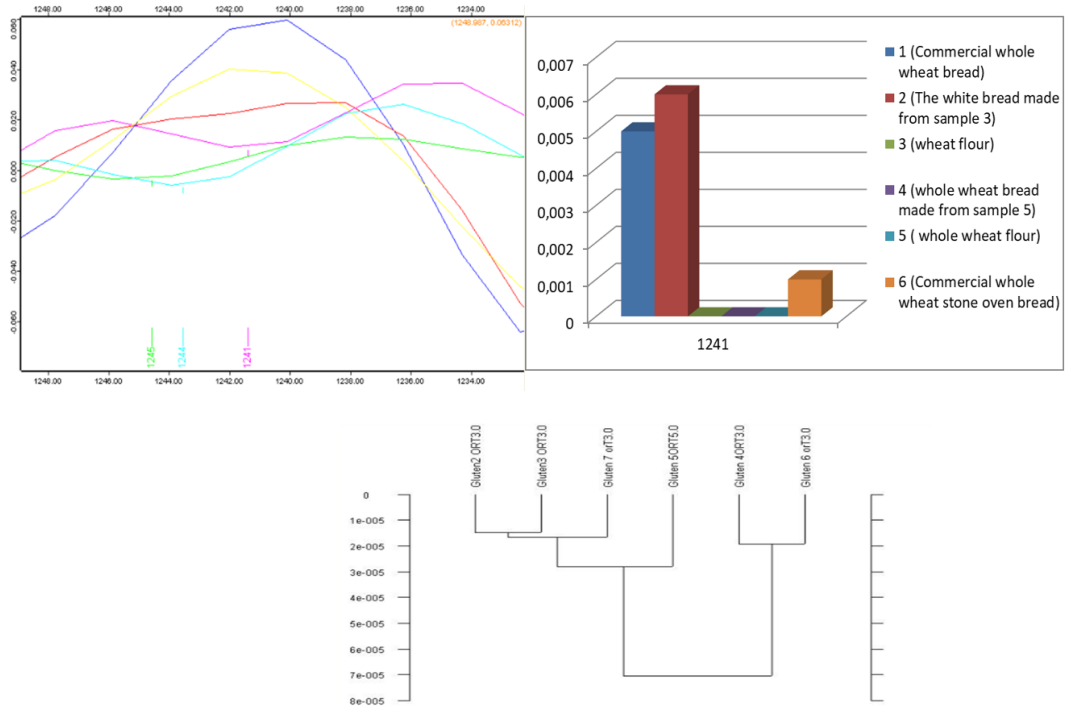

Figure 6. Clustering results of different groups of phosphorylated protein region spectrum (OPUS 5.5 software. The average value of the spectrums used for each group.

The starting point of this study was to understand and discuss the effect of raw material type and the process conditions on the gluten protein structure of bread. The studies conducted to date show that wheat-based food processing generally develops and sets the gluten protein network. Delcour et al. (2012) declared heat-induced gluten aggregation proceeds through cross-linking within and between its protein fractions. Besides SH-disulfide cross-links, other covalent bonds can be also formed. Therefore gluten functionality may be impacted biochemically (Delcour et al., 2012). Our results showed some data which are required to improve with further experiments; -By the differences in food additives and baking type, it is possible to change gliadin structure, - Cluster analysis indicates that food additives dramatically change gliadin structure, -Gliadin structure is also changed by thermal application. Our cluster analysis, which based on Amide A, Amide I and phosphorylated protein region results in distinction of flour from other groups.

It is the common way to use additives to improve the quality of gluten-free products. But selecting the proper component or protein is a time consuming and costly process (Smerdel et al., 2012). Currently gluten-free products are made by using buckwheat, corn or rice flour. However, making dough has been always an inconvenient process due to missing gluten protein. However xanthan is a rescuer to bind components in the dough, we still need an alternative strategy to make gluten-free products. It is a significant point to understand gluten metabolism and the effects of the production procedure to find a solution. We believe that we exhibited some useful knowledge on comparing the gluten content of raw and cooked materials and this is a very good clue to determine the best convenient process for CD patients.

Until now, there are lots of study about allergic response to gluten which is one of the main protein of wheat. Owing to the extensive usage of gluten in human diets, its allergic or intolerance effect on human metabolism is one of the big problem of the food industry especially for bread segment. Allergic response to the gluten are thought to be caused by gliadin fraction which are solubilized or not. And also the other fact about the immune response is that the characterization of protein by immune cell is based on the protein structure. Protein structure is important in order to know the active epitope region of the intolarated protein. Even if the role of gliadin or its derivative (subtypes) in eliciting the adverse reactions in CD or gluten intolareted ones are still far from being completely explained. Recent developments in proteomics area provide several explanations for understanding the aspects of wheat protein-related diseases since it is possible to analyze protein/peptide by getting their subtypes or their boundary structure. By the recent development it is possible to validate gluten amount in the flour but there is little knowledge on gliadin in the bread. In this study gliadin isolation from different kind of bread and flour was performed and its purity was calculated by 2D-PAGE. In addition to that 2D-PAGE results show us there are differences in gliadin sub-groups between samples. Therefore, it is possible to say that flour type, the ingredients used in bread making process and baking temperature are important for gliadin structure.

After we realized the differences between groups in 2D-PAGE, FT-IR spectroscopy was applied to the samples to see if there were any secondary differences between different baking applications. According to van Herper (2006) α-type of gliadins can cause T-cell response than other type. Thus we aimed to see which application would result in less gliadin/gluten concentration. According to our results whole wheat stone oven bread shows least α-helix structure according to Amide I second derivative analysis. Additionaly we can declare that the ingredients used in bread making process may increase α-helix structure of gliadin.

CONCLUSIONS

Gliadin structure can be characterized by mass spectrometry and other proteomic technologies like FT-IR spectroscopy, by the aspect of its identification and quantification that can be important for its biochemical, immunological and toxicological effect on wheat intolerated people.

The main point of understanding CD and also solving this problem is to exhibit the metabolism and textural changes in gluten protein both in production process and human body. This study focused on screening the technological effect on gluten structure which is in relation with immune-response of gluten intolerated people. This pre-trial showed us that it is highly possible to suggest most convenient production methods for gluten-free products in the continuing part of the study.

In recent years, the studies have gained speed due to increasing in CD and need for gluten-free diets. The molecular weight of gluten, composed of gliadins and glutenins, is between 15000 and 141000 Da and this protein complex is considerably affected by heating or treatments. The gluten metabolism in human body is realized by digestion of gluten to subunits then absorption in small intestine. But, this metabolism is different in CD patients. The gluten allergic people comprise 30% of total population and only 3% of them arise as CD patient. This means that environmental factors have a significant effect on appearance of CD besides of genetic factors. The main external factor is microbial flora of large intestine of CD patients. It is known that people who show an allergic reaction to some foods are strong candidates for the CD as well. As we understand from the literature and the results of the studies there is a great need for new experiments on environmental effect on CD. We started from this point and aimed to exhibit protein profiles of raw and processed breads and analyze the results. Following this study, it can be concluded that further investigation of the technological applications and contents of the products and the raw materials will contribute to this issue.

CONFLICT OF INTEREST

The author(s) declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS CONTRIBUTIONS

Evrim GÜNEŞ ALTUNTAŞ, Hatice YILDIZHAN and Mohammed Reza DASTOURI were performed the study experiments. Duygu ÖZEL DEMİRALP was responsible for the execution of the work. All the authors were involved to wrote the manuscript and all authors read and approved the final manuscript.

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