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Research Paper / Araştırma Makalesi

Gluten Status in Gluten-Free Pastry and Bakery Products Produced in Istanbul, Turkey

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ABSTRACT

Gluten is a complex protein that forms the basis of bakery products, including pastry products, with its elasticity. Gluten proteins are constituted by gliadin and glutenin. Gliadin, which is in the water-insoluble protein group, is very difficult to digest. Many immune disorders influence a growing number of people in relation to the consumption of wheat flour-based foods. The aim of this study is to determine the status of gluten-free products with the legal limits of the gluten-free products sold in pastry shops and bakeries in Istanbul, Turkey. Ninety samples in total including gluten-free bread, cakes, cookies, snacks, and cereals were collected from various regions of Istanbul in November 2020. The samples obtained were examined by ELISA (Enzyme-Linked Immunosorbent Assay) for the presence of gluten. As a result, the presence of gluten was found to be lower than 5 ppm in 61 samples (67.7%), between 5-20 ppm in 8 samples (8.8%), and 20 ppm and above in 21 samples (23%). Within the scope of Turkish Food Codex Regulation on Food Labeling and Consumer Information Number 29960, some foods were inappropriate products which offered to consumption as gluten-free in patisseries and bakeries. It could be thought that risks might be reduced to the maximum extent with hygiene and sanitation training in food businesses, analysis in terms of gluten in raw materials and final products, control of contamination from raw materials, personnel and environment during the production phase, and gluten analysis at critical points.

Keywords: Gluten, Contamination, Gluten intolerance, ELISA

İstanbul İlinde Pastane ve Fırınlarda Üretilen Glutensiz Ürünlerde Gluten Varlığı

ÖZ

Gluten, esneklik özelliği ile pastacılık ürünleri dahil olmak üzere unlu mamullerin temelini oluşturan kompleks bir proteindir. Gluten proteinleri gliadin ve glutenin tarafından oluşturulur. Suda çözünmeyen protein grubunda yer alan gliadinin sindirimi oldukça zordur. Birçok bağışıklık bozukluğu, buğday unu bazlı gıdaları tüketen insanları artan oranda etkilemektedir. Bu çalışmanın amacı, İstanbul ilinde pastane ve fırınlarda glutensiz olarak tüketime sunulan ürünlerin yasal limite (20 ppm) uygunluğunun araştırılmasıdır. 2020 yılının Kasım ayında, İstanbul'un farklı bölgelerinde glutensiz ekmek, kek, kurabiye, atıştırmalık ve tahıl gevrekleri olmak üzere 90 adet örnek toplanmıştır. Gluten varlığı analizi için örnekler Enzim Bağlı İmmünosorbent Testi (ELISA, Enzyme-Linked Immunosorbent Assay) ile incelenmiştir. Sonuç olarak, incelenen örneklerin gluten varlığı 61 (%67.7) adedinde 5 ppm'den düşük, 8 (%8.8) adedinde 5-20 ppm arasında ve 21 (%23) adedinde ise 20 ppm ve üzerinde tespit edilmiştir. Pastane ve fırınlarda glutensiz olarak tüketime sunulan ürünlerin 29960 sayılı Türk Gıda Kodeksi Gıda Etiketleme ve Tüketicileri Bilgilendirme Yönetmeliği kapsamına uygun olmadığı belirlenmiştir. Gıda işletmelerinde hijyen ve sanitasyon eğitiminin son derece önemli olduğu düşünülmektedir. Ek olarak, hammadde ve son üründe gluten yönünden

analizlerin yapılması, üretim aşamasında hammadde, personel ve ortam kaynaklı kontaminasyonunun kontrolü ve kritik noktalarda gluten analizi ile risklerin azami ölçüde azalacağı düşünülmektedir.

Anahtar Kelimeler: Gluten, Kontaminasyon, Gluten intolerans, ELISA

INTRODUCTION

The word gluten means glue derived from the Latin word “glue” [27]. Gluten substance is a storage protein in wheat consisting of “gluten and gliadin” fractions. Based on dry matter, gluten contains 75-86% protein while gliadins protein makes up about 30% of all proteins [35, 36]. Gluten is generally separated into two classes: soluble fraction in alcohol called gliadins (monomer unit) which contribute to the cohesiveness and extensibility of the gluten, and insoluble-glutenin (polymer unit, soluble in dilute acids and bases) which play a role in the maintenance of the elasticity and strength of the gluten. Both proteins constitute 80–85% of gluten proteins and define viscoelastic properties of dough [35]. In reference to Codex Alimentarius, ‘Gluten is a protein fraction of wheat, rye, barley, or their crossed varieties and derivatives thereof, being insoluble in water and NaCl 0.5 mol/L’ [4].

The structure and organization of glutenin and gliadin has difference, despite their similar utilitarian relationship [36]. It is stated that the effects of gluten proteins on dough and bread are mostly combined [38]. Gluten contributes to the appearance and crumb structure of many bakery products. When gluten is removed from the bread formulation, low volume, friability, color, and other quality defects occur in the bread [12].

The characteristics of the products covered by the Turkish Food Codex Regulation on Gluten-Free Foods are defined in two sections. The first part, in foodstuffs defined as gluten reduced, the gluten substance in the dry part is 200 mg/kg; in the second part, in food products defined as gluten-free, the gluten substance should not exceed 20 mg/kg in the dry part. Instead of gluten-free foods used as an alternative to basic foods such as flour and bread, they should contain the same number of vitamins and minerals as the food products they consume [33].

Gliadins include intra-molecular disulfide bonds, the breaking of which leads to opening of the protein molecule and are responsible for binding property of gluten [3, 18]. Glutenin is multi-chained and appeared to be mainly polymerized by disulfide bonds [7]. They are mainly responsible for the viscoelasticity properties of dough and gliadins provide to dough extensibleness [19, 35]. The relevant ratio between proteins is responsible for important rheological properties as viscosity, extensibility, and elasticity [14, 17]. The functional properties of gluten proteins depend on their physical and chemical properties that affect their behavior in food systems [6].

Grain and cereal products containing gluten protein, as the main source of energy and nutrients in nutrition can cause discomfort in many people. The source of toxic effects of gluten protein is prolamins which are known as “gliadin” in wheat, “secalin” in rye, “hordein” in barley and “avenin” in oats [21]. Gluten-related disorders are known to affect about 10% of the general population. The only known treatment today is avoiding consumption of gluten during the lifelong. Therefore, accurate and reliable information about the presence of gluten in foods is of great importance [10]. However, the foods made from wheat flour can negatively impact human health, inducing adverse reactions in genetically vulnerable individuals [37].

Gluten-related diseases (GRD) are also called reactions that occur by consuming gluten protein. Dermatitis herpetiformis (DH), gluten ataxia, wheat allergy, non-celiac gluten sensitivity (NCGS), celiac disease (CD) are gluten-related diseases [30, 31]. Celiac disease (CD) is commonly known as GRD in which genetic and environmental factors in addition to gluten intolerance are the primary causes of natal and adaptative immune responses [1, 24]. CD is typical by damaged and shortened small intestine mucosa, partial, or total intestinal villi atrophy and nutrient malabsorption [16]. The prevalence of CD globally is estimated about 2% in the general population and 0.3–2.9% in children [23, 29].

Additionally, the gluten-free diet is practiced by many people and is gaining vital importance and popularity. Regarding this issue, it is known that following a search for "gluten-free diet" on the internet, 4.2 million results were displayed, and result in an informational search for “gluten-free diet and weight loss”, over 5 million results [13, 15].

On the other hand, cross contamination can occur in packaged food products, as well as in institutions that produce large capacity food. It is due to the untrained and carelessness of kitchen personnel at catering, hotel, restaurant, and cafe where food is produced. Notably, by the reason of not to paying attention to the use of counter and equipment can spread gluten protein through direct or cross contamination. Thus, it can occur in such gluten-free food products [8]. Within the scope of Turkish Food Codex Regulation on Food Labeling and Consumer Information No 29960, some foods are inappropriate products which offered to consumption as gluten-free in patisseries and bakeries.

In this study, it was aimed to investigate the suitability of gluten presence according to legal limits with the R5 ELISA RIDASCREEN Gliadin method in pastry and bakery products sold as gluten-free in Istanbul, Turkey.

MATERIALS and METHODS

In this study, 90 gluten-free products that were produced and sold on site were directly collected to able to analyze all samples for three various days as materials in different 8 cities of Istanbul in November 2020. The sampling was randomly selected from the products produced by the boutique patisseries located in the regions where gluten-free products notably are manufactured, which is frequently preferred in terms of gastronomy in Istanbul. Since the products are not packaged products, they were collected under the hygiene conditions in line with the options from their own products.

Table 1. Type and distribution of the samples

Region	Number of Samples	Collection Date	Type of Samples
Plant 1	2 (%2,2)	08.11.2020	Cake, Cookie
Plant 2	13 (%14,4)	08.11.2020 16.11.2020	Bread, Cookie, Snack
Plant 3	2 (%2,2)	15.11.2020	Cracker
Plant 4	1 (%1,1)	15.11.2020	Cracker
Plant 5	19 (%21,1)	08.11.2020	Bread, Cake, Cookie, Cracker, Snack
Plant 6	28 (%31,1)	15.11.2020	Cake, Cookie, Cracker, Snack
Plant 7	3 (%3,3)	08.11.2020	Bread, Cookie, Snack
Plant 8	22 (%24,4)	08.11.2020	Cake, Cookie, Snack
Total		90 (%100)	

*Plant number indicates each different collection point.

Table 2. Type and distribution of the samples

	Bread	Cake	Cookie	Snack	Crispy
	Bread (n:5)	Cake (n:20) Brownie (n:6) Muffin (n:5) Cakepop (n:4)	Cookie (n:32)	Bar (n:4) Truf (n:6) Wafer (n:1)	Cracker (n:5) Grissini (n:2)
Total	5	35	32	11	7

Following the sampling, samples were analyzed in the Laboratory of the Department of Food Hygiene and Technology at the Istanbul University - Cerrahpasa Faculty of the Veterinary Medicine under the cold chain considering the contamination risks in the thermo-box.

The Sandwich ELISA immunological method, which uses the R5 antibody, which is accepted to be the most sensitive, was chosen for examination of gluten analysis methods performed in recent years internationally. Gluten-free foods were analyzed with the R5 Sandwich ELISA, R5 ELISA Ridascreen Gliadin method, which is given as the ELISA R5 Mendez Method gluten determination method in the gluten detection method section of the Codex Alimentarius (39). The samples were analyzed by R7001 Ridascreen Gliadin (R-Biopharm AG, Darmstadt, Germany) test kit and R7006 Cocktail solution (R-Biopharm AG, Darmstadt, Germany) and 96% EtOH (Merck) according to supplier's protocol, respectively.

To prepare samples, 40% EtOH was applied to remove possible gliadin contamination of the equipment during analysis of the laboratory's existing work areas and instrument/equipment in the work area. It is the pre and past version of the analysis. Subsequently, if there are packaged products, it needs to be cleaned with 40% EtOH, just in case. Additionally, analysis grade gloves were used. Even when giving numbers to the tubes, contamination was considered.

Finally, result of test was calculated by RIDA SOFT Win Z9999 (Version 1.79, R-Biopharm, Darmstadt, Germany) software.

RESULTS and DISCUSSION

In this study, the presence of gluten was investigated in a total of 90 food samples, including bread, cake, cookie, snacks and crisp, produced and sold in patisseries and bakeries manufacturing in different regions of Istanbul. Information on the region taken, the number of samples, dates of purchase and sample types of the samples taken for analysis is given (Table 1).

It has been determined that the products in the bread category contain 60% gluten between 20-80 ppm and 40% gluten above 80 ppm. Although there is no contamination between 20-80 ppm of the products in the cake category, the presence of gluten contamination at a rate of 5.7% above 80 ppm was detected. It was determined that the products in the cookie category had 18.75% contamination between 20-80 ppm and 15.6% contamination above 80 ppm. Gluten contamination was detected at a rate of 28.5% between 20-80 ppm in the friable category and 14.2% above 80 ppm (Table 3).

In summary, in line with the results of the study, it was determined that 21 (23.31%) of bakery and pastry food products offered for gluten-free consumption contain 20 ppm gluten, which is the legal limit value specified in the Codex Alimentarius [4]. It was determined that 8 (8.8%)

products were between 5-20 ppm, while the other 61 (67.7%) products were below 5 ppm (Tablo 3).

As a result of the examination in terms of legal limit value in terms of main ingredients, 100% of the product containing corn flour, 60% of the product containing buckwheat, 40% of the product containing almond flour, 5% of the product containing hazelnut flour, The legal limit was determined above 20 ppm for 75% of the product, 75% of the product containing chickpea flour, and 100% of the other flour-mixed products. When the main raw materials of gluten-free products containing gluten above the limit value are examined, buckwheat flour at 60%, flour mixture at 100%, chickpea flour at 75%, corn flour at 100%, almond flour at 40%, hazelnut flour at 5%. It was determined that 75% of einkorn flour was used (Table 4).

Table 3. Gluten amounts of samples according to the legal limit

Product Type	Total Product Number	Product Number Over Legal Limit	<5 ppm	5-20 ppm	20-80 ppm	>80 ppm
Bread	5	5	-	-	3	2
Cake	35	2	29	4	-	2
Cookie	32	11	18	3	6	5
Snack	11	-	10	1	-	-
Crispy	7	3	4	-	2	1
Total	90	21 (23.3%)	61 (67.7%)	8 (8.8%)	11 (12.2%)	10 (11.11%)

Table 4. Type and rate of the main component of gluten-free products over the legal limit

Main component	Product number	≥20 ppm	Positive rate (%)
Corn flour	1	1	%100
Buckwheat	5	3	%60
Almond flour	10	4	%40
Hazelnut floır	20	1	%5
Walnut flour	11	-	0
Coconut flour	7	-	0
Einkorn flour	4	3	%75
Chickpea flour	4	3	%75
Mix flour	6	6	%100
Other	22	-	0
Total	90	21	(23.3%)

When the studies conducted were examined, it was found that they were generally correlated with our study. Within the scope of studies conducted in Turkey, gluten contamination was found that 17.5% of 200 gluten-free labeled foods had gluten above the legal limit of 20 ppm. When the mentioned contamination sources are examined, it was determined that it is sourced from food raw materials with 68% buckwheat content, 23% grain mixtures, 6% corn and 3% rice origin [2].

In Finland, 59 natural gluten-free and 24 wheat starch-based products were collected in the market in different years. As a result of the study, in 13 (22%) of 59 natural gluten-free products, it was determined that 11 (45.8%) of 24 wheat-starch-based gluten-free products contain gluten above 20 ppm [5]. According to the study conducted in Brazil, 180 gluten-free food products obtained from 60 different food services were analyzed by ELISA; stated that 2.8% of the samples and at least 6.7% of the food services were contaminated with gluten above the legal limit of 20 ppm [9].

Bustamente et al. (2017) evaluated the gluten content of grain-based gluten-free foods from 1998 to 2016 in their study [43]. The products are divided into 8 categories as flour, breakfast cereal, bakery products, pasta, bread, dough, snacks, and yeasts. 3141 grain-based gluten-free products sold in Spain between 1998-2016 were selected, and the products were divided into 2 subgroups as those with gluten-free logo and without gluten content on the label. Analysis was carried out using 2 different ELISA kits between 1998 and 2016. The Transia Plate Gluten kit, which was extracted with 40% EtOH between 1998 and 2001, and using the ω-gliadin antibody, was used for analysis. Between 2001-2016, Ridascreen R 7001 gliadin test kit and INGEZIM gluten quick kit using R5 antibody were used for analysis. Gluten was detected in 371 of 3141 products. Yeast category was determined as 22.2%, breakfast cereal category with 21.5%, as the categories with the highest contamination. According to another study conducted in Brazil; As a result of the analysis of 130 samples from gluten-free bakery products by ELISA, the gluten contamination rate was determined to be 21.5% [8]. According to a study conducted in India; Gluten contamination was found above the legal limit in 36.7% of the products made using gluten-free flour in 160 food samples offered for sale in the supermarkets. In addition, it was stated that 35.9% of naturally gluten-free flour samples and 85% of oat flour were contaminated with gluten [28]. As a result of the ELISA analysis of 275 food samples with gluten-free labels and 186 food samples without gluten-free labels supplied from supermarkets in the United States of America; It was found that 1.1% and 19.4%, respectively, were contaminated with gluten [32]. In another study, samples were taken from 22 gluten-free grains, seeds and flour containing no gluten-free label was analyzed with the Ridascreen Gliadin sandwich R5 enzyme-linked immunosorbent test. As a result, it was determined that

13 of the 22 samples (59%) were below 5 ppm, in 9 (41%) it ranged between 2.9 - 8.5 ppm, and 7 (32%) of them contained gluten above 20 ppm [34]. Approximately 88% (n:133) of oat samples were found to be contaminated above 20 ppm, after confirming that oat-containing samples obtained from commercial outlets in Canada were heavily contaminated with gluten [20]. As a like our study, Verma et al. (40) analyzed gluten contamination by Sandwich ELISA sensitive to gliadin and prolamins in wheat coated with R5 antibody in 32 gluten-free products in Italy. It was found that 6 (19.4%) products that do not contain gluten due to the nature of the raw materials and 1 (3%) product with the gluten-free logo contain more than 20 ppm of gluten. The contaminations were found in buckwheat, oat, and lentil-based products. Gluten amounts range from 30 ppm to 53 ppm. It has been emphasized that these amounts will cause damage to the intestinal mucosa of celiacs. Moreover, gluten contamination was investigated in simultaneously purchased gluten-free food products to examine the awareness of gluten-related ailments by food preparation and service personnel in Ireland. It has been determined that 2.7% of 260 gluten-free food samples offered for consumption in restaurants contain between 21-100 ppm, 7.7% above 100 ppm and 10% in total contains gluten [26].

Gluten contamination was detected above 20 ppm limit value of 0.5% from 205 gluten-free bread, pasta, pastry, biscuits, pizza, breakfast cereals and foods covered with breadcrumbs collected in four countries, including Italy, Spain, Norway, and Germany [11]. Furthermore, 78 gluten-free labeled samples from the United States were analyzed using the gliadin competitive enzyme-linked immunosorbent assay. While 48 (61.5%) samples were lower than 10 ppm for gluten; 16 samples (20.5%) were found to contain 20 ppm and above gluten ranging from 20.3 to 60.3 ppm. It was stated that gluten content in 5 of 8 breakfast cereal samples was above 20 ppm [22]. To verify whether the gluten content of several commercial food products sold in Brazil comply with the labeling, Méndez ELISA R5 sandwich method analyzed 437 samples; it has been stated that it is 70% gluten-free, contains 26% gluten and is not labeled for gluten at 4% [25].

Another study was conducted on 84 food samples including 52 labeled gluten-free foods (L-GFF) and 32 naturally gluten-free foods (N-GFF), regarding six various categories (cake, cookies and cakes, baker`s yeast, dried vegetables, dried fruits, and cereals). To determine their gluten content, samples were analyzed using a sandwich enzyme immunosorbent assay (R5 ELISA Ridascreen® gliadin Mendez), considering 20 mg/kg (ppm) as the contamination limit. As a result of the test, the contamination rate was 23.8% (L-GFF: 21.9%, N-GFF: 25%). The cake, cookies, and baker's yeast products did not result in any contamination. The contamination rate was 5.3% in dried vegetables, 25% in dried fruits, and 42.1% in cereals. However, all oat samples were the most contaminated food. L-GFF locally manufactured were more often contaminated than those imported (28.6 vs. 16.7%) [41].

Verma et al. (42) investigated the gluten variety with the Ridascreen R-7001 Gliadin Sandwich ELISA kit in products with gluten production and gluten-free logo in the markets in Italy, as a continuation of the previous study of them (41). Between April and October 2016, the most preferred 200 product purchases from the markets in Ancona, Italy, are the two-component sections with the gluten-free logo and gluten-free products by nature. 93 products with the gluten-free logo and 107 gluten-free products were collected. According to analysis, more than 20 ppm of gluten was found in 1% of the guidelines with the gluten-free logo, in 8% of the gluten-free guidelines, and in 9% of a total of 200 manufacturers. It has been stated that gluten contamination is found in gluten-free products in Italy.

Table 5. Comparison of gluten contamination results for some countries with Turkey

	Collin et al. [5]	Lee et al. [22]	Mattioni et al. [25]	Sharma et al. [32]	Verma et al. [40]	Verma et al. [42]	Bustamente et al. [43]	Guennouni et al. [41]	Our study
Country	Finland	USA	Brazil	USA	Italy	Italy	Spain	Morocco	Turkey
Number of labeled gluten-free	83	78	437	275	32	93	3141	84	90
Number of gluten-free over the limit	28.9%	20.2%	30%	1.1%	3%	1%	12%	42.1%	23.3%

CONCLUSION

Individuals with gluten sensitivity prefer foods such as bread, crackers, biscuits, and cereals as the basic carbohydrate source that should be taken daily in a healthy diet. To produce healthy and reliable gluten free products, cross contamination prevention and control plans should be implemented from production to consumption. The gluten-free product production line should be separated. Food personnel should be educated. Additionally, packaging should be in appropriate features and during storage, packaging, and transportation. Direct/indirect contact with gluten-containing products should be prevented during harvest, preparation and serving.

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