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Determination of some heavy metal deposits in gluten-free foods in Turkish market with ICP-OES

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Abstract: In recent years, celiac disease has become known to the public with the development of medical diagnosis possibilities and increased awareness of the celiac disease. The only cure for celiac disease is a gluten-free diet that must be strictly followed for perpetuity. Food products with gluten content lower than 20 ppm are considered gluten-free products. With the increase in the number of people with celiac disease and people on a gluten-free diet, the amount and variety of gluten-free products have increased significantly. Rice and corn are the raw materials of gluten-free products. However, this situation causes people who follow a gluten-free diet to be exposed to a uniform diet and deficient in the intake of essential elements such as copper, iron, and manganese. In this study, 41 gluten-free products in Turkey were examined with ICP-OES as copper, iron, manganese, zinc, and strontium content, and the results were reported. The samples with the highest Cu concentration were corn and puffed rice at 51.45 mg/kg and vermicelli at 32.40 mg/kg. Chocolate sauce and cornflakes were the samples at 137.43 mg/kg and 117.20 mg/kg, respectively, with the highest Fe concentration. The highest Mn concentration was precooked chickpea flour at 20.80 mg/kg. The highest strontium concentration value was 12.85 mg/kg in carob flour. The samples with the highest Zn concentration were determined in yeast at 91.98 mg/kg and pistachio croquant at 85.50 mg/kg.

Keywords*:* Celiac disease; essential elements; gluten; gluten-free diet.

1. Introduction

The importance of consuming macro elements in human nutrition and their effects on health have been revealed in many studies [1]. Although there is a variety of information about the chemical composition of foods for people without celiac disease, very little data on the chemical composition of gluten-free foods have been reported in the literature. As stated in recent studies, celiac disease is becoming more common, increasing incidence in the elderly. The only solution to this situation is an unconditional gluten-free diet that will be applied for life [2].

Celiac disease (CD) is a lifelong autoimmune disease characterized by an inappropriate immune response to gluten-fed, primarily wheat (gliadin), rye (hordein), and barley (secalin). Celiac disease (also known as gluten-sensitive enteropathy) derives from koeliakos, meaning 'suffering in the gut.' This condition occurs in genetically predisposed individuals who carry the human lymphocyte antigen (HLA)

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- DQ2 or - DQ8. Genetic studies have found the prevalence of CD for first-degree relatives to be around 10% [3,4]. Erosion of intestinal villi (villous atrophy (VA)) with dietary gluten intake leads to reduced capacity to absorb most nutrients. CD manifests many symptoms and causes untreated patients, infertility, and osteoporosis [5]. When gluten is removed from the diet, symptoms improve, and a strict gluten-free diet normalizes mortality rates.

CD diagnosis rates are increasing critically in many countries, including developed countries. The increase in diagnosis has been attributed to improvements in the accuracy of diagnostic testing and better awareness of far-reaching symptoms. Despite the increase in diagnosis, CD remains undiagnosed or misdiagnosed in most cases because of the variable nature of CD symptoms. It is estimated that approximately 50% of people with CD are asymptomatic [6,7]. Signs and symptoms of CD occurring outside the intestinal system include fatigue, neurological conditions (including depression); reproductive diseases; oral problems; and skeletal issues. On the other hand, in untreated celiac patients, significant disorders such as malignancies, other autoimmune conditions (for example, insulin-dependent diabetes), iron deficiency anemia (DEA), and osteoporosis can occur.

Wheat is one of the world's most important food crops, cultivated, consumed, and traded worldwide. One common wheat species (*Triticum aestivum L.*) is frequently used to describe many other cultivated wheat species and genotypes. Wheat grain contains 8-15% protein, of which 10-15% is albumin/globulin and 85-90% is gluten. Gluten is a mixture of hundreds of proteins, primarily gliadin and glutenin. Different wheat varieties' protein content and the composition and distribution of gluten proteins differ. The gliadin and glutenin proteins are collectively referred to as prolamins, which represent seed proteins that are insoluble in water but extractable in aqueous ethanol and are characterized by high levels of glutamine (38%) and proline residues (20%) [8].

Gluten is necessary to determine the dough quality of other baked goods such as bread, pasta, cakes, pastries, and biscuits. Gluten can act as a heat-stable, binding and expanding agent and is commonly used as an additive in processed foods for improved texture, flavor, and moisture retention. Therefore, processed meats, seafood, vegetarian products, candies, ice creams, fillings, and coatings used in confectionery are relatively less apparent sources of gluten [9]. The unusual rheological and functional properties of gluten depending on the ratio of glutenins to gliadins and the interactions of these structures. Each ingredient has different functions that are very important in determining the viscoelastic properties (capture of carbon dioxide released during the leavening of bread) and the quality of the end product. For example, purified, hydrated gliadins contribute more to dough viscosity and extensibility, while hydrated glutenins are cohesive and contribute to dough strength and elasticity [10].

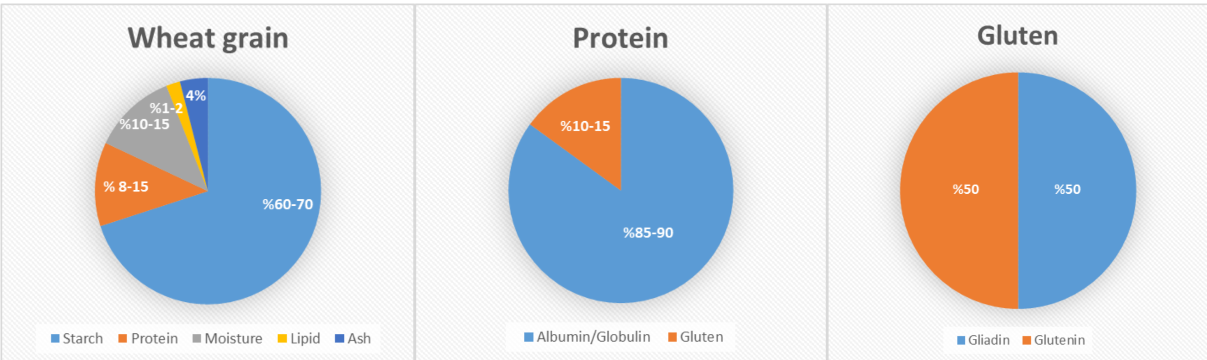

Figure 1. Wheat grain protein content

Wheat is an important staple food due to its high nutritional properties, long shelf life, and a source of various nutrients and a fermentable substrate for the human colon microflora, which benefits the host. It forms the basis of all daily meals and is consumed worldwide. Information on gluten intake in the general population is scarce because there is a lack of detailed information about the gluten content of food products. Calculations of dietary gluten intakes are approximate as the amount of grain protein in gluten-containing grains is usually calculated and estimated from recipe information. In developed countries, the average daily gluten intake varies between 5 and 20 g/day [8].

A gluten-free diet (GFD) is free of wheat, rye, and barley and their derivatives. GFD usually consists of naturally gluten-free products (e.g., fruit and vegetables, meat and dairy products) and gluten-free options (e.g., gluten-free bread, biscuits, and pasta). It has been argued that an absolute gluten-free diet is unrealistic and that occasional gluten consumption may not pose a significant risk for many people with CD. Most CD patients can tolerate small amounts of gluten, but whether the amount of gluten handled has any harmful effects differs between individuals [11]. Products labeled "gluten-free" are allowed to contain up to 20 mg/kg of gluten [12]. Wheat starch, processed to contain less than 20 mg/kg of gluten, is often used in gluten-free food production [13]. Products containing <20 mg/kg of gluten are considered safe for people with CD, and most can tolerate these products. However, some patients require a rigorous gluten-free diet to avoid symptoms. People with CD may also consume products labeled "low gluten." In the United Kingdom, these products are allowed to contain up to 200 mg/kg of gluten and are considered safe for most people with CD [12].

Essential heavy metals perform biochemical and physiological functions in plants and animals. They are critical components in several key enzymes and play crucial roles in various oxidation-reduction reactions. Copper (Cu), cobalt (Co), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn) elements are essential nutrients required for various biochemical and physiological functions. Insufficient intake of these micronutrients can result in multiple diseases [1]. Some enzymes play a crucial role in detoxification and damage repair in biological systems' essential parts of cellular organelles and metabolism. The literature has reported that components such as these enzymes are affected by heavy metals. [14]. Metal ions interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that can lead to cell cycle modulation, carcinogenesis, or apoptosis. [15]. Heavy metal-induced toxicity and carcinogenicity involve many mechanistic aspects, some of which are not clearly explained or understood. However, it is known that each metal has unique physicochemical properties that contribute to its specific toxicological mechanisms of action [16].

This study aims to obtain data on trace elements in gluten-free foods (pasta, bread, flour, etc.) found in celiac patients' diets that must comply. Considering the high number of celiac patients in Turkey, this information is urgently needed to estimate the daily nutrient intakes in celiac patients from the available food consumption data. Trace element intake levels for non-celiac patients have been reported in many studies, whereas there is relatively limited information for celiac patients [17,18]. In addition, in this study, any toxic elements in gluten-free foods and daily metal intake from diets created according to reference intake levels were investigated. The study included 41 different products from gluten-free products made for celiac patients.

2. Materials and Methods

2.1. Collection and Preparation of Samples

In this study, 41 gluten-free samples were obtained from different supermarkets and virtual markets in Sakarya, Turkey. The samples were dried in an oven at 70 °C, then ground in a rice mortar and homogenized to prepare gluten-free products for analysis. The gluten-free ground products were stored in polyethylene containers until the research day. The chemicals used in the experiments were of suprapur quality (Merck, Darmstadt, Germany) of HNO_3 (65%) and H_2O_2 (30%) (E. Merck, Darmstadt, Germany).

2.2. Standard Reference Material Analysis

Recovery values were determined using standard reference material. Each sample was prepared for inductively coupled plasma-optical emission spectrometry (ICP-OES) measurements, including duplications, blanks, and certified standards. Analysis of the standard reference material (Tomato leaves NIST 1573a) for gluten-free samples allows the determination of accuracy and precision at a wide range of element concentrations.

2.3. Microwave Thawing

The dissolution process was performed in the Start D Microwave Digestion System (Milestone, Japan) model microwave. 0.2 g of the prepared homogeneous gluten-free food samples were weighed and transferred into Teflon cups. 6 mL of HNO_3 and 2 mL of H_2O_2 were added to the gluten-free food samples, transferred to Teflon containers, and the dissolution process was started. Conditions for thawing in the microwave: 250 W 2 min, 0 W 2 min, 250 W 6 min, 400 W 5 min, 550W 8 min, cool 8 min. After dissolution, the clear solution was transferred to balloon flasks and made up to 10 mL of distilled deionized water (Millipore Milli-Q 18.2 MΩ.cm). Samples that would wait more than 24 hours for analysis were kept in the refrigerator at 4°C [19].

2.4. ICP-OES Analysis

Arcos FHE-16 Model ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer, Spectro Analytical Instruments, Kleve, Germany) was used to determine the concentrations of all studied elements (Table 5). A standard solution (multi-element standard) was prepared for analysis, with 0.10-0.25-0.5-1-2-5 and 10 mg/kg ranges. 0.5 mg/kg of stock solution and washing water were added between every ten samples. The operating parameters of the ICP-OES device are listed in Table 1.

Table 1. Element determination operating parameters with ICP-OES

Device	**Spectro Arcos 165 ICP-OES**
Viewing Height (mm)	12
Wavelength	Cu: 324.754 nm, Fe: 238.204 nm, Mn: 257.611 nm, Zn: 213.856 nm, Sr: 407.771 nm
Iteration	3
RF power (w)	1450
Spray chamber	Cyclonic
Nebulizer	Modified lichte model
Nebulizer flow (L/min)	0.8
Plasma torch	Quartz, fixed 3.0 mm injector tube
Reread time	50 sec per repeat
Plasma gas flow (L/min)	13
Auxiliary gas flow (L/min)	0.7
Sample aspiration rate (mL/min)	2.0
Sample pump speed (rpm)	25

3. Results and Discussion

3.1. Standard Reference Substance Analysis and Results

In order to determine the applied method's accuracy, the same analyses were made with the standard reference material (Tomato leaves 1573a). A confidence level of 104.48-97.64% was determined between the values obtained by the method used for these metals. The reference material was tested with three different acid ratios, and the best results were obtained by adding 6 mL of HNO_3 and 2 mL of H_2O_2 and dissolving it in the microwave. Obtained ICP-OES results are given in Table 2.

Table 2. Measurement results of tomato leaves NIST1573a certified reference material with ICP-OES (mg/kg)

Element	Value Found	Reference Value	% Recovery
Cu	4.77±0.13	4.70 ± 0.14	104.48
Fe	366±8	368 ± 7	99.45
Mn	242±6	246 ± 8	98.37
Sr	83 ±1.2	85 ± 1.9	97.64
Zn	30.4±0.8	30.9 ± 0.7	98.38

Table 3 shows the Cu, Fe, Mn, Sr, and Zn concentration values in gluten-free food samples produced for the consumption of celiac patients. Samples were obtained from 41 gluten-free foodstuffs, and the results were given in mg/kg units.

Cu concentration range was determined as 0.15-51.45 mg/kg in all analyzed foods. The highest Cu concentration was determined as 51.45 mg/kg popcorn and crunch and 32.40 mg/kg vermicelli, as indicated in the table. The lowest Cu concentration was 0.15 mg/kg in rice flour and 0.40 mg/kg in bread flour. Cu is involved in critical processes in the human body, such as mitochondrial activities, metabolism activities at the cellular level, and absorption and storage of iron. The average daily tolerable copper amount in healthy adults is 0.9 mg. If copper is consumed more than 10 mg daily, its toxicity can be seen. It is difficult to assess the health risk of the element Cu for celiac patients and gluten-free food consumers because when studies on food consumption are examined, it has been found that there is no standard maximum value for this element [20].

It was observed that the Fe concentration value analyzed in all food samples varied between 0.60 mg/kg and 137.43 mg/kg. Chocolate sauce and corn flakes were the samples with the highest Fe concentrations, with 137.43 mg/kg and 117.20 mg/kg, respectively. It was determined that cornmeal and banana quinoa pudding samples had 0.60 mg/kg Fe concentration and showed the lowest value. While the daily amount of iron required for healthy adults is 8 mg for men and 18 mg for women, toxic effects can be observed in more than 45 mg of consumption. When examined in terms of dietary intake, iron deficiency is critically essential, and excessive iron storage in the body can cause hereditary disorders.

The Mn element was investigated in 41 gluten-free food samples, and the concentration range was determined as 0.02 mg/kg to 20.80 mg/kg. Among the analyzed food samples, maize flour semolina had the lowest Mn concentration, with 0.02 mg/kg. The highest Mn concentration was observed in precooked chickpea flour at 20.80 mg/kg. Adequate daily intake of Mn should be 2.3 mg for adult men and 1.8 mg for adult women. Intakes of more than 11 mg may cause toxic effects.

The Sr element was analyzed in the gluten-free food samples analyzed in the study, and it was determined that the determined value was between 0.12 mg/kg and 12.85 mg/kg. Among the samples examined, the foods with the lowest Sr concentration were pastry flour, rice flour, and yeast (0.12 mg/kg, 0.19 mg/kg, 0.19 mg/kg). The highest strontium concentration value was 12.85 mg/kg in carob flour. The average Sr concentration value of 41 gluten-free food samples collected within the scope of the study was determined as 4.69 mg/kg. Since Sr is not included in essential elements, studies on daily intake have not been conducted.

The Zn concentration was between 1.95-91.98 mg/kg in the gluten-free food samples examined. The samples with the lowest Zn concentration were determined as 1.95 mg/kg of banana quinoa pudding and 2.01 mg/kg of pudding. The samples with the highest Zn concentration were 91.98 mg/kg of yeast and 85.50 mg/kg of pistachio croquant. According to the Turkish food codex, the maximum Zn allowed in foods is 5 mg/kg. The daily zinc intake for healthy adults is 11 mg for men and 18 mg for women. Toxic effects can be seen in zinc intakes over 40 mg.

Table 3. Metal concentrations in analyzed gluten-free food samples (mg/kg)

Sample	Cu	Fe	Mn	Sr	Zn
1. Raw buckwheat flour	4.15 ±0.12	18.42 ±2.18	12.73 ±1.12	3.95±0.02	30.70 ±3.43
2. Bread flour	0.40 ±0.03	7.60 ±0.28	1.40 ±0.10	5.74±0.03	7.43 ±1.02
3. Pastry flour	0.46 ±0.01	63.23 ±4.59	6.20 ±0.34	0.12±0.01	12.50 ±1.14
4. Cocoa cake flour	0.43 ±0.56	22.70 ±2.32	0.44 ±0.04	4.81±0.15	5.23 ±0.46
5. Carob flour	0.45 ±0.43	76.82 ±5.21	1.60 ±0.16	12.85±2.28	8.24 ±1.21
6. Cornmeal	0.41 ±0.13	0.60 ±0.07	0.05 ±0.01	3.39±0.06	2.50 ±0.19
7. Precooked pea flour	3.51 ±0.34	39.76 ±3.36	9.40 ±1.22	10.04±1.12	61.60 ±4.51
8. Precooked bean flour	1.72 ±0.08	49.35 ±4.29	15.07 ±2.18	7.79±0.08	25.80 ±2.83
9. Precooked red lentil flour	4.80 ±0.31	42.74 ±2.78	4.64 ±0.04	4.34±0.04	26.10 ±2.10
10. Precooked chickpea flour	2.42 ±0.54	38.01 ±3.65	20.80 ±3.42	11.25±1.12	25.42 ±1.62
11. Precooked yellow lentil flour	1.60 ±0.21	29.43 ±2.34	3.21 ±0.17	5.54±0.06	26.80 ±2.56
12. Rice flour	0.15 ±0.06	11.03 ±2.21	6.51 ±0.68	0.19±0.0.4	24.80 ±3.22
13. Flour	0.45 ±0.07	34.69 ±3.83	0.03 ±0.01	0.21±0.03	31.29 ±2.41
14. Pretzel	0.42 ±0.05	6.63 ±0.06	0.05 ±0.01	4.44±0.02	8.23 ±0.82
15. Bread	0.44 ±0.06	13.50 ±2.34	0.06 ±0.02	5.31±0.04	7.61 ±0.24
16. Brown bread	0.46 ±0.02	22.65 ±4.61	3.92 ±0.04	7.69±0.05	15.30 ±1.45
17. Couscous	0.41 ±0.03	1.30 ±0.06	0.04 ±0.01	3.51±0.08	2.14 ±0.03
18. Semolina mix	1.60 ±0.08	1.35 ±0.06	0.10 ±0.01	3.71±0.03	13.22 ±3.12
19. Pasta	10.45±1.33	2.15 ±0.08	0.05 ±0.02	3.39±0.04	12.60 ±2.41
20. Yeast	0.30 ±0.02	25.80 ±3.27	0.24 ±0.04	0.19±0.02	91.98 ±6.32
21. Corn bulgur	7.51 ±0.94	5.80 ±0.06	0.04 ±0.03	3.45±0.03	16.30 ±2.35
22. Cornstarch	0.43 ±0.05	16.90 ±2.71	0.05 ±0.02	0.19±0.01	2.77 ±0.02
23. Cornmeal semolina	0.44 ±0.03	5.24 ±0.05	0.02 ±0.01	0.21±0.02	2.81 ±0.05
24. Popcorn and crunch	51.45±5.37	0.94 ±0.07	0.06 ±0.02	4.51±0.03	23.55 ±3.41
25. Tarhana soup	0.45 ±0.05	95.55 ±8.37	8.30 ±0.62	4.04±0.04	14.10 ±1.39
26. Sesame pretzel	0.44 ±0.04	15.30 ±2.46	0.03 ±0.01	4.49±0.07	47.90 ±3.53
27. Noodle	32.40±4.31	5.05 ±0.07	0.05 ±0.03	3.79±0.86	18.52 ±2.45
28. Olive Thyme Cookies	0.44 ±0.23	7.40 ±0.05	0.02 ±0.01	5.02±0.59	2.01 ±0.02
29. Bitter almond paste	0.43 ±0.02	31.32 ±3.21	0.06 ±0.02	4.89±0.02	13.10 ±2.11
30. Baby biscuits	0.44 ±0.03	87.70 ±5.32	0.44 ±0.03	4.61±0.04	7.62 ±0.97
31. Chocolate	0.41 ±0.02	31.50 ±4.10	0.06 ±0.01	5.11±0.62	7.70 ±0.08
32. Chocolate sauce	7.53 ±0.07	137.43 ±6.72	7.01 ±0.64	7.81±1.10	13.50 ±1.13
33. Pistachio croquant	4.90 ±0.05	8.05 ±1.14	5.14 ±0.05	5.43±0.53	85.50 ±5.32
34. Cocoa cookies	0.45 ±0.02	7.23 ±1.14	1.15 ±0.03	4.64±0.03	12.12 ±1.46
35. Cocoa mini cake	0.44 ±0.03	30.93 ±3.45	0.04 ±0.02	5.11±0.04	11.20 ±0.98
36. Whipped Cream	0.41 ±0.02	3.56 ±0.05	0.05 ±0.03	3.91±0.05	2.02 ±0.05
37. Chickpea powder	3.23 ±0.08	16.22 ±2.27	6.85 ±0.05	4.59±0.02	19.02 ±3.45
38. Protein bar	2.10 ±0.05	27.01 ±3.61	4.50 ±0.02	8.81±1.12	8.04 ±2.34
39. Pudding	0.42 ±0.03	41.11 ±4.47	0.03 ±0.01	3.61±0.05	2.01 ±0.05
40. Cornflakes	0.45 ±0.04	117.20 ±7.42	0.06 ±0.03	4.13±0.02	5.30 ±0.03
41. Banana Quinoa Pudding	0.44 ±0.03	0.60 ±0.06	0.02 ±0.01	3.49±0.04	1.95 ±0.07
Average value	3.67 ±	29.26 ±	2.94 ±	4.64±	18.45±

Compared to the literature, Mehder et al. [21] found 10.5 mg/kg in chocolate petals for Cu, 2009 mg/kg in rice flicks for Fe, 100.3 in spaghetti for Mn, and 41.3 in snacks for Zn as maximum concentrations. Sr was not examined in this study. Also, in another study, Orecchio et al. [20] found 1.8

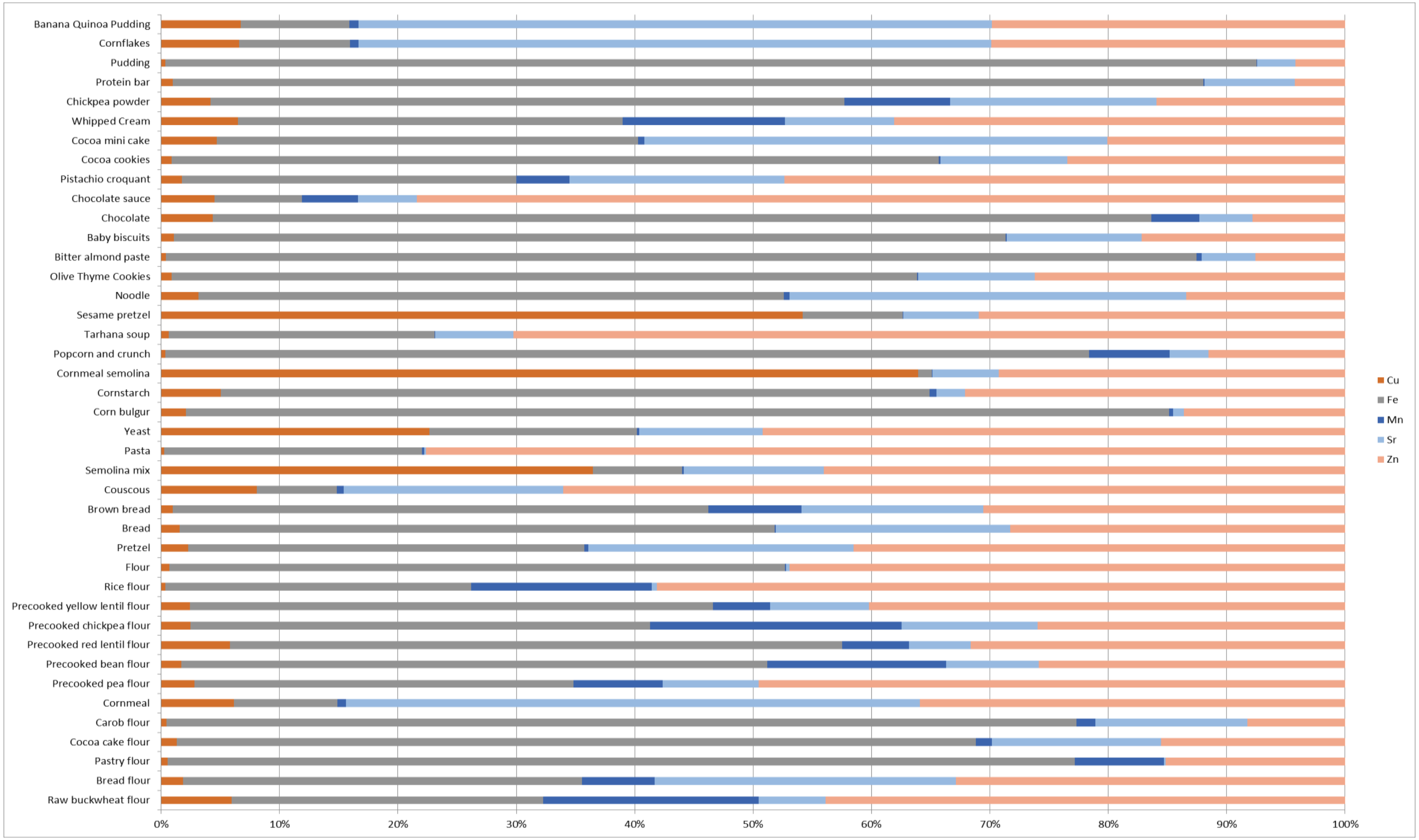

Figure 2. Measurement result for metal percentages of gluten-free foods

mg/kg in croutons for Cu, 7.5 mg/kg in croutons for Mn, 4.7 mg/kg in croutons for Sr, and 137 mg/kg in shortbread for Zn as maximum concentrations. In this study, however, Fe was not examined. Compared with our study, 51.45 mg/kg in popcorn and crunch for Cu and 12.85 mg/kg in carob flour for Sr are the maximum concentrations found, showing that celiac patients with Cu or Sr deficiency can easily use these foods.

3.2. Metal Pollution Index

The metal pollution index (MPI) of 41 gluten-free food samples used in this study was calculated and given in figure 3. The metal pollution index (MPI) was calculated by taking the geometric mean of the concentrations of all the samples used in the study.

$$MPI= (C1 . C2 . C3. \ldots\ldots .Cn)1/n \qquad (1)$$

Among the metal pollution indices obtained within the scope of the study, gluten-free foods with the lowest index value were determined as corn flour semolina, banana quinoa pudding, and cornmeal (0.58 mg/kg, 0.61 mg/kg, 0.64 mg/kg). Among the analyzed samples, precooked chickpea flour at 14.04 mg/kg, chocolate sauce at 15.02 mg/kg, and precooked pea flour at 15.21 mg/kg stand out as the samples with the highest metal pollution index.

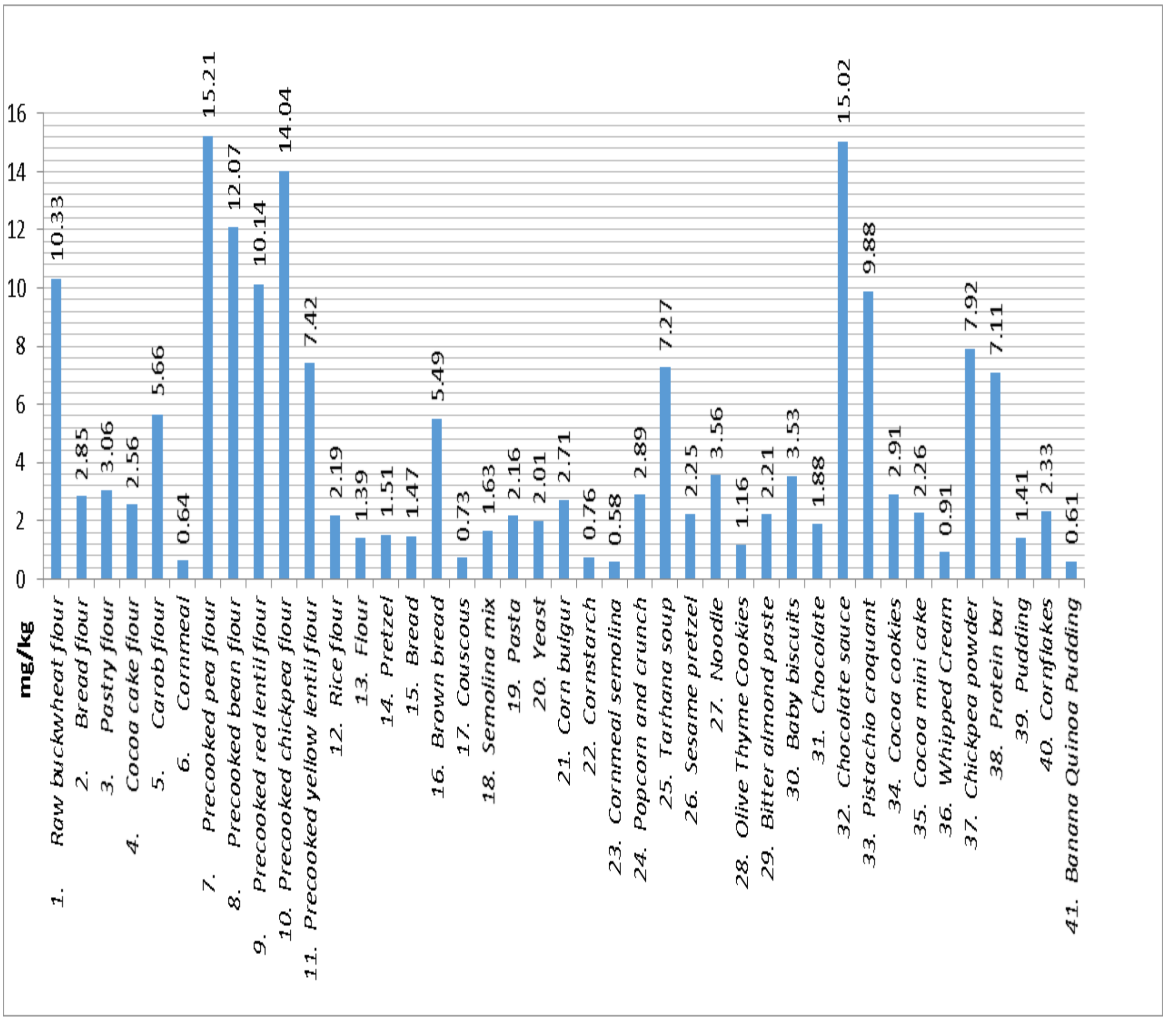

Figure 3. Metal contamination index in gluten-free food samples

3.3. Analytical Performance Parameters

The analytical parameters of the study are presented in Table 4. According to the results, the calibration lines formed in the 0.1-10 mg/kg measurement range have high regression coefficients, and the measurability limits of each element were calculated using the calibration lines. Zn has the lowest measurability limit, while Fe has the highest value. In addition, the equations of each calibration line are given in the table.

Table 4. Analytical performance parameters

	Limit of detection (LOD) (µg/kg)	**Linear range (mg/kg)**	**Equation**	**R^2**
Cu	8.73	0.1-10	$y=2.9\times10^5x+6.8\times10^5$	0.9956
Fe	12.36	0.1-10	$y=3.2\times10^5x+6.4\times10^5$	0.9944
Mn	0.73	0.1-10	$y=10\times10^5x+1.7\times10^5$	0.9940
Sr	39.77	0.1-10	$y=90\times10^5x+20\times10^5$	0.9787
Zn	0.67	0.1-10	$y=6\times10^5x+2.2\times10^5$	0.9868

3.4. Uncertainty Assessment

Estimating measurement uncertainty in a laboratory is a vintage pointer that is used to show the distribution level of a test result. Errors arising from the different experimental conditions like instrumental calibration and operation, purity of chemicals and solvents, sampling procedures, standard and sample solution preparations, and environmental conditions, including temperature, humidity, light, etc., can be justified for the analytical methods by performing the uncertainty assessment along with the suggested validation parameters. Although it is possible to make calculations in the light of the EURACHEM CITAC Guide and GUM guidelines for uncertainty budget estimation [22-27], the NORDTEST approach [28,29] was deemed appropriate because NIST CRM was used in this study. This study estimated Cu, Fe, Mn, Sr, and Zn measurement uncertainty. While performing the NORDTEST method, the calculations are as follows:

$$u(Rw) = Rw/2 \quad (2)$$

$$RMS_{bias} = (\Sigma(bias)^2/N))^{1/2} \quad (3)$$

$$u(C_{ref}) = \Sigma(C_{ref})^2/N)^{1/2} \quad (4)$$

$$u(bias) = (RMS_{bias}{}^2 + u(C_{ref})^2)^{1/2} \quad (5)$$

$$u_c = (u(Rw)^2 + u(bias)^2)^{1/2} \quad (6)$$

$$U = 2xu_c \quad (7)$$

Here, N is the number of rounds, u(x) is the standard uncertainty, u(Rw) is the standard uncertainty component for the within laboratory reproducibility, $u(C_{ref})$ is the mean of the standard uncertainty of the assigned values, RMS_{bias} is the root mean square of the individual bias values, u(bias) is method and laboratory bias, u_c is combined standard uncertainty, and U is the expanded uncertainty. According to the Nordtest approach, all parameters are shown in Table 5. The table shows Cu had the highest MU (6.9%), and Fe had the lowest MU (4.9%).

Table 5. Calculated Nordtest guide parameters

	u(Rw)	**u(Cref)**	**RMS_{bias}**	**u(bias)**	**u_c**	**U(%)**
Cu	2.7	1.5	1.5	2.1	3.4	6.9
Fe	2.2	0.9	0.5	1.1	2.5	4.9
Mn	2.5	1.6	1.6	2.3	3.4	6.8
Sr	1.5	1.1	2.4	2.6	3.0	5.9
Zn	2.6	1.1	1.6	2.0	3.3	6.6

3.5. Daily Metal Intake

To understand the effects of a gluten-free diet on human health, daily intake of macroelements is critical for celiac patients and people on a gluten-free diet. It is imperative to obtain this information. Daily intake depends on the amount of food consumed by the individual following the diet and the number of elements found in gluten-free foods.

$$DIM = (C_{metal} \times D_{foodintake}) \quad (2)$$

C_{metal} in the formula refers to the heavy metal concentration in gluten-free foods. $D_{foodintake}$ refers to the heavy metal concentration in the daily food intake of a gluten-free individual. Following a gluten-free diet, the daily food consumption amount was accepted as 100 g, and daily dietary intakes are given in Table 6.

Table 6. Percentage of contribution to the daily reference intake for nutrients in gluten-free food samples

Sample	Cu	Fe	Mn	Sr	Zn
Raw buckwheat flour	45.23	23.39	54.73	1.89	27.31
Bread flour	4.46	9.66	6.25	2.58	6.76
Pastry flour	4.98	77.77	27.32	0.06	11.25
Cocoa cake flour	4.73	28.38	2.02	2.21	4.81
Carob flour	4.92	94.48	7.15	5.14	7.49
Cornmeal	4.68	0.73	0.23	1.69	2.31
Precooked pea flour	39.76	49.31	41.41	4.01	54.81
Precooked bean flour	20.31	61.19	66.31	3.42	23.22
Precooked red lentil flour	54.71	52.99	20.41	2.21	23.49
Precooked chickpea flour	27.03	47.51	90.39	4.51	22.87
Precooked yellow lentil flour	18.24	36.78	14.12	2.77	24.11
Rice flour	2.39	13.89	28.64	0.09	22.32
Flour	5.17	43.01	0.23	0.11	27.84
Pretzel	5.06	8.35	0.32	1.95	7.41
Bread	4.75	17.02	0.13	2.65	6.84
Brown bread	5.11	28.31	17.24	3.46	13.91
Couscous	4.99	1.65	0.23	1.75	1.96
Semolina mix	19.84	1.69	0.51	1.85	11.89
Pasta	104.49	2.71	0.23	1.69	11.34
Yeast	3.66	31.97	1.09	0.13	81.86
Corn bulgur	72.54	7.32	0.27	1.72	14.84
Cornstarch	4.57	21.13	0.32	0.10	2.54
Cornmeal semolina	4.49	6.61	0.23	0.12	2.58
Popcorn and crunch	497.01	1.19	0.18	2.29	21.19
Tarhana soup	4.99	117.52	35.85	2.02	12.84
Sesame pretzel	4.81	18.98	0.18	2.24	43.12
Noodle	313.08	6.36	0.23	1.93	16.85
Olive Thyme Cookies	5.55	9.33	0.32	2.31	1.84
Bitter almond paste	5.16	39.15	0.27	2.34	11.74
Baby biscuits	3.96	108.76	2.02	2.21	6.85
Chocolate	5.16	39.36	0.18	2.24	6.93
Chocolate sauce	81.02	166.29	30.69	3.43	12.15
Pistachio croquant	54.21	10.14	22.92	2.38	76.11
Cocoa cookies	5.51	9.11	5.17	2.04	10.91
Cocoa mini cake	4.72	38.35	0.36	2.24	10.08
Whipped Cream	5.64	4.52	0.23	1.95	1.84
Chickpea powder	35.21	20.27	23.15	2.29	17.31
Protein bar	22.81	33.76	20.11	3.87	7.31
Pudding	5.14	50.56	0.18	1.73	19.93
Cornflakes	4.87	140.62	0.27	1.89	4.83
Banana Quinoa Pudding	4.57	0.77	0.23	1.67	1.79

An individual consuming an accepted daily amount of 100 g of gluten-free food is 2.39 to 497.01% copper, 0.73% to 166.29% iron, 0.13 to 90.39% manganese, 0.06 to 4.51% strontium, and It is estimated to take 1.84% to 81.86% zinc.

4. Conclusion

In recent years, with the increase in the diagnosis of celiac disease and learning of its symptoms, celiac disease is more known, and its precautions have been more applicable to individuals. Celiac disease, the only cure for a lifelong strict gluten-free diet, has enabled the gluten-free food market to grow significantly—the variety of gluten-free food is expanded and accessible in almost every region.

The need for gluten-free raw materials, which arises due to the increased variety and consumption of gluten-free foods, is mainly procured from rice and corn. This situation causes gluten-free individuals and celiac patients to have a uniform diet. Along with this type of nutrition, individuals who follow this diet are deficient in essential elements such as copper and iron. They generally have to use supplementary foods in addition to their diet.

For people who have to follow a strict gluten-free diet for life, it is necessary to eliminate the disadvantages of uniform nutrition by increasing gluten-free products' nutritional values and mineral contents. In this context, the following items list the measures that can be taken to eliminate these disadvantages.

In order to prevent diseases such as iron deficiency anemia caused by the deficiency of essential elements, which is common in celiac patients, gluten-free foods should be obtained from foods with high important element content.

The nutritional value of basic consumption materials such as bread, pasta, and pastry can be increased using materials rich in minerals and high nutritional value, such as buckwheat and oats. Hazelnuts, almonds, etc. flour obtained from nuts are included in the scope of gluten-free flour and can increase the nutritional value of gluten-free foods.

Flours produced from legumes with high protein content, such as chickpeas and beans, are included in the scope of gluten-free flour. With the bread and pastries made with these flours, it can be ensured that people who have to follow a gluten-free diet for life receive the essential nutritional elements ultimately.

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