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Assessment of Gluten-Free Food Sourced Heavy Metal Accumulation for Celiac People

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Abstract. This paper shows an evaluation to the study of some heavy metals in certain gluten-free foods for celiac people marketed in Kayseri, Turkey. The concentration values of Cu, Mn, Co, Pb, Cr, Zn, Ni, Cd and Fe in eleven different gluten-free food samples are reported. The determinations were carried out by flame atomic absorption spectrometry after wet digestion method. The average metal concentrations of all the analyzed samples were determined in the range of 0.45-5.12, 1.84-12.2, 1.29-22.5, 0.60-3.01, 0.08-0.18, and 4.61-79.6 mg kg^{-1} for Cu, Mn, Zn, Ni, Cd and Fe, respectively. The accuracy of the method was confirmed by the analysis of a standard reference material (SRM 1570a Spinach Leaves). Metal pollution index was also calculated to examine the overall heavy metal levels in all analyzed samples. The concentrations of the analyzed metals are equivalent to 7.55-35.3% compared to the daily intake offered by international decision authorities. For this reason, the nutritional quality index for gluten-free food products with respect to all the metals seems to be relatively low.

***Keywords*:** Heavy metals, gluten-free foods, celiac disease, flame atomic absorption spectrometry.

Çölyak Hastaları için Glütensiz Gıda Kaynaklı Ağır Metal Birikiminin Değerlendirilmesi

Özet. Bu çalışma, Kayseri'de satışa sunulan çölyak hastaları için bazı glütensiz gıdalarda belirli ağır metallerin çalışılmasına ilişkin bir değerlendirme sunmaktadır. On bir farklı glutensiz gıda numunesinde Cu, Mn, Co, Pb, Cr, Zn, Ni, Cd ve Fe konsantrasyon değerleri rapor edilmiştir. Tespit çalışmaları yaş yakma yöntemini takiben alevli atomik absorpsiyon spektrometresi ile yapılmıştır. Analiz edilen tüm numunelerin ortalama metal konsantrasyonları Cu, Mn, Zn, Ni, Cd ve Fe için sırasıyla 0,45-5,12, 1,84-12,2, 1,29-22,5, 0,60-3,01, 0,08-0,18 ve 4,61-79,6 mg kg^{-1} olarak belirlenmiştir. Yöntemin doğruluğu, standart referans materyalin (SRM 1570a Ispanak Yaprakları) analizi ile teyit edilmiştir. Analiz edilen tüm numunelerde toplam ağır metal seviyelerini incelemek için metal kirliliği endeksi de hesaplanmıştır. Analiz edilen metallerin konsantrasyonları uluslararası karar makamları tarafından önerilen günlük alım miktarı ile karşılaştırıldığında %7,55 ile 35,3'lük kısma denk gelmektedir. Bu nedenle, tüm metallere göre glütensiz gıda ürünlerinin beslenme kalite endeksi nispeten düşük görünmektedir.

***Anahtar Kelimeler*:** Ağır metaller, glütensiz gıdalar, çölyak hastalığı, atomik absorpsiyon spektrometresi.

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1. INTRODUCTION

Celiac disease is a genetically mediated autoimmune disease in which gluten causes gastral inflammation or damage [1]. Gluten refers to the proteins in cereal grains, such as wheat, rye, barley and derivatives. Among other negative effects, celiac disease could cause some problems with absorption of some nutrients including micro and macro elements, and vitamins [2-4]. Recent studies indicate that celiac disease has a high rate and that the prevalence of celiac disease is approximately 2% in the general world population [5,6]. The prevalence of celiac disease in Turkey is 1:100, similar to several European countries and United States [1,7,8]. Historically, celiac disease has been under diagnosed; however, the rate of diagnosis is increasing day by day [1]. Today, the only available and effective treatment for celiac people is a lifelong strict gluten-free diet. Additionally, with the help of novel therapy methods for celiac disease, the gluten detoxification through enzymatic cures come into question [9,10].

Celiac disease was a difficult diagnosis to live with 20 years ago. This was probably due to the limited range of gluten-free products as well as the guided to high quality products. Over the years, the increased prevalence of celiac disease guides to a high demand of gluten-free products and this made a dramatic increase of the gluten-free market. However, it has become easier to find and consume gluten-free products with high amounts and desired quality. There are too many similar gluten-free products in the market all around the world including breads, cereal bars, pastas, crusts, desserts and more.

Currently, there are limited studies available focused on the heavy metal contents in gluten-free foods [2,5,11-14]. In addition, the manufacturers attach the only available information about mineral composition on the label. The World Health Organization (WHO) and some other international authorities have determined and published acceptable levels in food [15,16]. Using these publications and the label data, not only health professionals but also the researchers and celiac people are having chance to compare the right products they need. As they are not synthesized in the body, heavy metals have an important role in human beings life. Trace amounts of some metals are essential micronutrients and have a range of different biochemical functions in human beings. On the other hand, some mineral trace elements are toxic and can cause negative effects in human health even at trace levels. Many analytical techniques have been developed in order to determine heavy metal contents of food and environmental samples such as atomic absorption spectrometry, inductively coupled plasma mass spectrometry and inductively coupled plasma optical emission spectrometry [17-20]. In particular, atomic absorption spectrometry has been a widely used technique due to its simplicity and economy [21].

The purpose of this study was to obtain data on the heavy metal (Cu, Mn, Zn, Ni, Cd and Fe) concentrations in selected gluten-free food samples (Black Wheat, Burger Pasta, Bread, Flour, Noodle, Spaghetti, White cookie, Cocoa cookie, Cake, Plane Pasta, Lasagna) marketed in Kayseri, Turkey. Also, intake levels and percentage of contribution to dietary reference intakes are assessed. There is a lot of study for the intake levels of non-celiac people in literature, however there is limited information for the celiac ones [22-25].

2. EXPERIMENTAL

a. Instrumentation

The metal contents of the digested gluten-free samples were determined by using a Perkin Elmer A 800 flame atomic absorption spectrometer (FAAS) equipped with hollow cathode lamps. Operation conditions of FAAS for each metal are shown in Table 1. A Millipore water purification system was used to acquire purified reverse osmosis water (18.2 MΩ cm resistivity (Millipore, Bedford, MA, USA). A Hettich Universal 320 centrifuge (Tuttlingen, Germany) was utilized for the separation of undissolved parts from aqueous sample phase.

Table 1. FAAS settings.

Element	Wavelength (nm)	Lamp Current (mA)	Slit width (nm)	Calibration range (mg L^{-1})
Cu	324.8	15	0.7	0.5-5
Ni	232.0	30	0.2	0.5-5
Mn	279.5	20	0.2	0.1-2.5
Zn	213.9	10	0.7	0.1-1.0
Cd	228.8	8	0.7	0.1-1.0
Fe	248.3	30	0.2	0.5-5
Co	240.7	30	0.2	0.5-5
Pb	283.3	4	0.7	1-10
Cr	357.9	30	0.7	1-10

b. Reagents and Solutions

Analytical reagent-grade chemicals were preferred for all stages of the developed procedure. Analytical reagent-grade of hydrochloric acid, nitric acid and hydrogen peroxide were obtained from Merck Company (E. Merck, Darmstadt, Germany). All glassware and the plastic laboratory equipments were kept in %10 (v/v) of nitric acid solution bath for 24 hours and rinsed with reverse osmosis water prior to use. The standard solutions of increasing concentration were prepared for each element by diluting their stock solutions of 1000 mg L^{-1} (Sigma Aldrich, St. Louis, MO, USA). Calibration curves for each element were obtained by measuring of absorbance values of the prepared standart solutions. Concentration range of calibration curve is shown in Table 1.Certified reference material (SRM 1570a spinach leaves) was used in the experiments in order to check the accuracy of the digestion procedure.

c. Sampling, digestion and determination

Gluten-free samples were obtained from local markets in Kayseri. The obtained samples were dried at 90 °C for 10 h. homogenized by using an agate homogenizer and stored in sampling bottles. Wet digestion procedure was carried out for the digestion of the samples and extraction of metal ions from food matrix to acidic solution phase. The suggested wet digestion procedure was carried out on the heater plate at ~ 90 ^{o}C. For this purpose, 10 mL of analytical grade concentrated HNO_3 was added on the 1.0 g of SRM 1570a spinach leaves or each of the collected samples and the mixture was evaporated until dryness. After cooling, a solution consist of analytical grade 3 mL of H_2O_2 and 10 mL of HNO_3 was added on the same samples. Then they were again evaporated to near dryness. The resulting mixture was taken with ultra-pure water, centrifuged and filtered through a blue-band filter paper. Last volume of the filtrate was completed to 10 mL with 1 M HNO_3 solution. The suggested digestion method was also applied to blank samples. Concantration of metal ions in last phase were analyzed by using flame atomic absorption spectrometer.

3. RESULTS AND DISCUSSION

Accuracy of the developed digestion and measurement procedure was checked by using the certified reference material SRM 1570a spinach leaves. The obtained results for the studied elements in the certified reference material were within or near the certified values. The results shown in Table 2 proved that our method is free from the interference of matrix components in the food samples. The combined wet digestion and flame atomic absorption spectrometry procedure was preferred due to its accuracy, simple and short sample preparation and analysis time.

Table 2. Analysis of SRM 1570a spinach leaves standard reference material (N=3).

Element	Certified value, (mg kg^{-1})	Found value (mg kg^{-1})
Cu	12.2±0.86	12.1±0.4[a]
Ni	2.14±0.10	UDL[b]
Mn	76.0±1.2	75.4±2.4
Zn	82.3±3.9	79.0±3.0
Cd	2.89±0.07	3.01±0.09

a: mean±standart deviation.
b: Under of the detection limit.

The metal concentrations in the studied gluten-free samples are shown in Table 3. All measurements and calculations were carried out on a dry weight basis. All analysis were conducted in triplicate. The obtained results are calculated and given as mean±standard deviation. Concentrations of cobalt, chromium and lead were found to be under of the detection limits of FAAS.

Table 3. Metal concentrations in analyzed gluten-free food samples (mg kg^{-1}, N=3).

Sample	Cu	Mn	Zn	Ni	Cd	Fe
Black Wheat	1.57±0.11	7.20±0.46	6.83±0.06	1.83±0.21	0.08±0.03	12.0±0.22
Burger Pasta	0.74±0.09	3.72±0.15	1.29±0.13	0.60±0.17	0.12±0.03	44.5±0.66
Bread	1.41±0.11	4.37±0.15	2.58±0.19	1.68±0.42	0.13±0.04	5.79±0.22
Flour	1.25±0.11	5.89±0.15	22.5±1.25	2.42±0.21	0.18±0.03	46.3±0.66
Noodle	0.45±0.05	1.84±0.06	1.61±0.09	0.77±0.10	0.10±0.04	4.61±0.11
Spaghetti	2.63±0.23	4.41±0.13	8.43±0.31	3.01±0.21	0.18±0.03	36.5±0.44
White cookie	0.63±0.09	3.68±0.13	4.93±0.25	1.83±0.21	0.18±0.03	79.6±5.07
Cocoa cookie	5.12±0.34	12.2±0.50	16.3±0.18	3.01±0.21	0.17±0.03	24.6±2.20
Cake	3.18±0.12	6.53±0.23	10.2±0.97	1.36±0.10	0.14±0.01	26.8±0.88
Plane Pasta	0.53±0.05	3.36±0.06	6.71±0.29	0.62±0.10	0.16±0.01	10.1±0.66
Lasagna	0.62±0.06	3.25±0.06	10.0±0.28	1.06±0.10	0.17±0.02	19.7±1.65
Mean	**1.65±0.12**	**5.13±0.19**	**8.30±0.36**	**1.65±0.19**	**0.14±0.03**	**28.2±1.06**

Copper is one of the most important element for living cells and has important roles on the nervous system, lipid and carbohydrate metabolisms and so on [26-28]. Lack of copper to be taken with daily diets, copper deficiency can cause dangerous diseases for living cells [29-31]. Hence accurate analysis of copper concentrations in the consumed diatery samples is an important task for analytical chemists. In our study, copper concentrations in collected gluten-free samples were found between 0.45 and 5.12 mg kg^{-1} (Table 3). In a different experiment conducted on the Gluten-free Foods From Saudi Arabia, the mean concentration of copper was found between 1.4–10.5 mg kg^{-1} [5]. According to The Brasilian Ministry of Health, copper levels should be lower than 10 mg kg^{-1} [32]. Copper concentration in all of the our samples was lower than this recommedation value. World Health Organization (WHO) recommends daily 2-5 mg copper intake for adults [33] and moreover it is difficult to say that food-based copper intake is a health problem because there are no limitation.

Manganese also plays important role in different metabolisms as an essential element in living cells such as protein, fat, nitrogen and inorganic acid metabolism. Moreover it has an important task on the activation of many enzymes used in metabolic processes [29, 31]. The minimum and maximum levels of manganese were 1.84 mg kg^{-1} for dried noodle and 12.2 mg kg^{-1} for dried cocoa cookie samples, respectively (Table 3). According to the National Research Council, the daily adequate intake of manganese from dietary supplements, water, and food should be between 2–5 mg and lower than 11 mg [34].

Zinc is one of the most important essential trace elements for living cells and has a role in the biochemical reactions in the most of the cells and enzymes. For example, Zinc is an important element for growth and development, cell-mediated immunity, neurosensory functions and DNA synthesis and act as the active center of approximately 300 enzymes. As low levels of zinc in humans can cause serious discomfort such as immunological abnormalities, anorexia, skin changes and. growth retardation. High level of zinc can show toxic effects [29, 31, 35]. The mean concentration of zinc in studied samples were found in the range of 1.29 and 16.3 mg kg^{-1}. As the burger pasta sample has the lowest zinc concentration, the cocoa cookies sample has the highest zinc concentration (Table 3). Turkish Food Codex announced that the permitted maximum amount of zinc in foods is 5 mg kg^{-1}. According to WHO, the tolerable daily intake of zinc is 0.3–1.0 mg kg^{-1} [36]. Zinc levels in some of the studied gluten-free samples were higher than these suggested values.

Trace level of nickel is both essential and toxic for living organisms. Nickel plays a important role on the glucose and adrenaline metabolism and production of the red blood cells. But high level of nickel can cause serious health problems such as respiratory system cancer, skin disorder known as nickel-eczema, lung, kidney and cardiovascular diseases [29, 31, 37, 38]. A Ni-poor nutrition of <0.1 mg kg^{-1} dry matter cause Ni deficiency symptoms. Food and water are the main possible sources of nickel for humans. Hence accurate and sensitive analysis of nickel in food samples is a important task. The minimum and maximum levels of nickel were 0.60 mg kg^{-1} for burger pasta and 3.01 mg kg^{-1} for dried spaghetti pasta and cocoa cookie samples, respectively (Table 3). According to The Turkish Official Gazette for Turkish standards, the maximum permitted nickel amounts in some food samples are 0.2 mg kg^{-1} [36]. Nickel levels in all of the studied gluten-free samples were higher than these permitted values.

Cadmium is known as highly toxic element even in low concentrations and has a biological half-life in the range of 10–30 years. It causes adverse effects on the important organs such as kidneys, liver and lungs, even at its very low amounts [39-41]. The World Health Organization has suggested that the provisional allowable intake of cadmium not exceed 0.4–0.5 mg per week or 0.057–0.071 mg per day [42]. Food and water intake are the main possible sources of cadmium for living cells. As can be seen from Table 3, as the highest level of cadmium were in flour, spaghetti pasta and white cookie samples (0.18 mg kg^{-1}), the lowest level was in Black wheat (0.08 mg kg^{-1}) (Table 3).

Iron, which is primarily involved in a wide range of metabolic processes, including oxygen transport, DNA synthesis and electron transport, is vital to the survival of life. However iron levels in different parts of the body should be kept under constant control and regulated tightly because the excess iron in the living structure causes tissue damage as a result of the formation of free radicals. Metabolic diseases born of the high or low amount of iron are among the most common disorders in people. It covers a wide spectrum of diseases with various clinical manifestations ranging from anemia to iron overload and possibly to neurodegenerative diseases [39, 31, 43, 44]. The mean iron concentration in studied gluten-free foods were found in range of 4.61 and 79.6 mg kg^{-1}. As the noodle has the lowest iron concentration, the white cookie has the highest iron concentration (Table 3).

To determine the overall heavy metal concentrations in all analyzed gluten-free samples, metal pollution index (MPI) was calculated (Figure 1). This index was obtained by calculating the geometrical mean of concentrations of all the metals in the samples

$$MPI=(C_1*C_2*C_3*.......*C_n)^{(1/n)}$$

where C_n is the concentration of single metal in the sample. Among different gluten-free food analyzed, cocoa cookie showed the highest value of MPI (4.83) followed by flour (3.85) and the spaghetti (3.52) respectively. As compared to the previous products, noodle sample showed lower value of MPI (0.88).

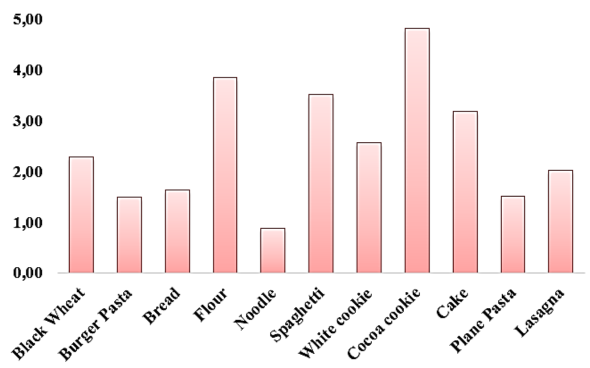

Figure 1. Metal pollution index for analyzed gluten-free food samples.

4. CONCLUSION

In this reported study, the concentration values of Cu, Mn, Co, Pb, Cr, Zn, Ni, Cd and Fe in eleven different gluten-free food samples, which consumed by celiac patients from Turkey, are reported. After wet digestion procedure, concentrations of the analytes in the final phase were measured by FAAS. While the Cd amounts of samples were the lowest for all samples, Fe levels were the highest. Analyzed levels of the trace elements in the gluten-free foods were compared with the recommended values or maximum corresponding levels suggested by the National NAS/NRC, WHO, the Turkish Food Codex, the Brasilian Ministry of Health, the Turkish standards and the EC. The obtained results showed that the amounts of Ni, Zn and Cd in the analyzed samples were higher than the recommended or maximum values.

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