

Antioxidant Activity and Phenolic Content of Cereal Food Concentrates: Import Control Issues

Almas MUKHAMETOV¹ (✉)

Naila ALIYEVA²

Nazakat MUSAYEVA³

Ulker MAMMADOVA³

Summary

This paper aims to analyze the antioxidant activity and phenolic content of cereal concentrates from corn and oat and to investigate issues related to import regulation. The phenolic composition was examined by liquid chromatography-high-resolution mass spectrometry, and the antioxidant activity was determined using the spectrophotometric method and expressed as μmol of Trolox equivalent antioxidant capacity per kg of extract. The cornmeal flour was found to contain almost twice as much phenolic compounds as the oatmeal flour, despite comparable antioxidant activity (range, 800-900 μmol Trolox kg^{-1} extract). The phenolic content of grain was 550-650 mg CE 100 g^{-1} extract; it was statistically lower compared to flour samples and statistically higher compared to cracker samples. The presence of 9 flavonoids and 4 phenolic acids was found in all tested samples of flour and crackers. The phenolic acids content of grain ranged from 380 to 3010 mg kg^{-1} ; most of them (up to 80-90%) were bound. The incidence of mycotoxins in positive samples (excluding grain) was as follows: 8%, patulin; 4%, nivalenol/deoxynivalenol; 34%, aflatoxins; 6%, zearalenone; 31%, ochratoxin A; 17%, fumonisins. The concentration ranged from 0.5 to 10 $\mu\text{g}/\text{kg}$. The present findings can be useful to optimize food formulations and improve product stability during transportation.

Key words

cereal concentrates, sweet corn, oat, antioxidant activity, phenolics, mycotoxins, import regulation

¹ Department of Technology and Safety of Food Products, Kazakh National Agrarian Research University, Almaty, Kazakhstan

² Department of Economics, Russian School of Economics at Azerbaijan State University of Economics (UNEC), Baku, Azerbaijan

³ Department of Accounting and Auditing, Azerbaijan State University of Economics (UNEC), Baku, Azerbaijan

✉ Corresponding author: alm_mukhametov78@rambler.ru

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Introduction

Cereals are among the major sources of energy and carbohydrates, but they also contain pathogenic contamination and are a source of pathogenic food poisoning. Consumers, industries and governments around the world are increasingly concerned about the ability of national systems to ensure safety, quality and authenticity of domestically produced and marketed foods whose major ingredient is cereals (FAO, n.d.). For that reason, many countries have implemented food safety management reforms to protect consumers better. In particular, Lebanon has integrate market-based food safety management systems based on ISO 22000, HACCP and ISO 9001 standards (Abebe et al., 2020). Indonesia adopted a bottom-up approach that requires a full risk assessment under the FSO/ALOP framework (Wahidin and Purnhagen, 2018). A top-down approach to product quality control has proven to be a successful strategy for developing countries to have more market access to export products not only to the European Union but also to other potential export market destinations (Wahidin and Purnhagen, 2018). In Kazakhstan, food safety management consists of national regulatory controls and public efforts, which guide the decisions and actions of food producers (Aigarinova et al., 2014). The organization responsible for the quality of food products in the market is the General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ).

Control measures for imported agricultural products, grain in particular, are a worldwide strategy to protect countries from harmful pests and diseases. Since developing countries lack an effective national system for monitoring compliance with the norms of production and export of food resources, there is a problem of free trade of food products with countries with established market economies. At the same time, such system has strict selection criteria in countries with advanced economies (Rahmat et al., 2016). The existence of restrictions and norms of global markets can contribute to the improvement and development of each country's own National Food Control System, which would significantly improve the quality and competitiveness of food resources from developing countries. Additionally, high standards of the global market can also drive improving food safety in developing countries, creating the possibility of expanding export platforms (Wieck and Grant, 2021; Quak, 2019).

Each country and region has its own biosecurity requirements and import obligations (Stoneham et al., 2020). In Kazakhstan, the grain industry, which accounts for about 56% of the total cultivated land area, makes a significant contribution to the economy of Central Asia, and yet, it remains dependent on imported grain (Hamidov et al., 2016). To reduce food safety risks associated with grain import, the control measures are applied, including quarantine.

According to the cooperation protocol signed between the Ministry of Agriculture of Kazakhstan and AQSIQ in 2017 (Online.zakon.kz, n.d.), Kazakhstan subjects imported grain to strict quarantine. When it comes to safety, grain treatment is of great importance, but the removal of flour dust is also effective (Martinelli et al., 2020). Particular attention should be paid to de-dusting of the personnel clothes for those, working on grain processing in quarantine zones.

Grain contamination is closely related to environmental conditions not only at the stage of contact with pollutants of living and inanimate nature, but also during storage, transportation and processing of food raw materials. Among such contaminants, a prominent place is occupied by microorganisms, heavy metals (such as cadmium, lead, zinc, copper, arsenic) and factors of production. The high concentrations of heavy metals in cereals, in particular arsenic (from 0.017 mg kg⁻¹), can be due to an application of arsenic-containing pesticides (Adam et al., 2022). The main pathogens of cereal grains are bacteria (*Lactobacillus*, *Acetobacter*, *Bacillus*, etc.), molds (mainly of genera *Aspergillus*, *Fusarium*, and *Penicillium*), and yeasts (*Cryptococcus*, *Candida*) (Erkmen and Bozoglu, 2016; Wachowska et al., 2017). The excessive levels of these contaminants can cause significant damage to organs and result in neurological defects, respiratory disorders, carcinogenicity, gastrointestinal obstruction, and osteoporosis (Mitra et al., 2022). It is known that there have been severe cases of grain damage by mycotoxins, which manifest their toxic effect during germination and metabolic activity of fungal cells (Alshannaq and Yu, 2017). Stringent quality control is required when importing food commodities to maintain their nutritional value and taste qualities.

Cereals are susceptible to contamination of mycotoxins. Many countries regulate the level of mycotoxins in agricultural commodities, especially when they are transported across borders. Because mycotoxins occur naturally and are not completely controllable, certain levels of human food contamination can be expected (Keřińska-Pacelik and Biel, 2021). As such, efforts have been made to minimize their occurrence and avoid adverse effects. These efforts include advanced agronomic and production practices and the use of regulatory restrictions (Quak, 2019). The European Union and the United Kingdom have established guidelines for food safety based on insights from the toxicological and epidemiological research (IHS Markit et al., 2021; Hadjigeorgiou et al., 2013). These guidelines are not always consistent across countries, for they display varying food intakes, availability of commodities, and climatic conditions for mycotoxin exposure. However, the presence of legal restrictions is important in international trade, as they apply to both imported goods and domestic commodities.

Despite research and technological advances, mycotoxins remain a threat to food safety. Globalization and growing trends in agricultural markets also heighten the importance of food supply protection (Black, 2016; Tartian et al., 2017). It required additional efforts in surveillance and monitoring programs, as well as in research on mycotoxin detection. In addition, public policies are likely to be adopted to address the changing risks of exposure to the growing number of commodities that may be contaminated with mycotoxins. Food security calls for unified international standards and mycotoxin regulations.

Research on food quality regarding the biologically active compounds in food is a matter of maintaining people's physical health. The effective recovery of biologically active compounds from food products is an important issue in biotechnological production, and the industry seeks to discover new recovery techniques. In this regard, it would be interesting to investigate

whether the value of grain in the production of its derived concentrates, such as flour and cracker, changes in terms of phenolic compound content. The present study aims to evaluate the value of organic cereal concentrates (based on corn and oats), namely phenolic content and antioxidant activity, and to investigate issues related to import regulation. As the preservation of the nutritional value and quality of raw resources and cereal-based products during the import process is a relevant issue, the control of imported product quality is vitally important. The issue of controlling the import of raw materials and their derivatives directly relates to the quality and purity of these food products. In particular, this is due to frequent exposure of bacterial, viral, and fungal pathogens to grain. The latter produce mycotoxins, which significantly reduce the quality of food. For that reason, this study also concerns quality control using an example of fungal mycotoxin contamination. The objectives of the study are (1) to determine the biochemical profile of organic cereal concentrates based on corn and oats; (2) to examine the qualitative and quantitative characteristics of bioactive compounds in cereal grains and related products (flour and crackers); and (3) to investigate how mycotoxin contamination of imported cereal grain and cereal concentrates is being prevented.

Methods and materials

Cereal material and sample preparation

Samples of sweet corn (*Zea mays* L.) and field grown oats (*Avena sativa* L.) were collected during the 2021 harvest season in Kazakhstan and Azerbaijan.

- Corn grain is small and rounded; it has a pointed tip and horn-shaped endosperm. The grain contains protein (12-15%), starch (up to 75%), and fat (2-4%). It is well suited for making cereals, flour, and cereals. Corn grain samples were taken on agricultural land in the Sheki District of Azerbaijan (latitude: 41°11'31" N; longitude: 47°10'14" W).
- Oat grain is oblong and small, with a sharp tip. It contains up to 13% protein, its content of starch and other carbohydrates is 45%, and its fat content is 3-5%. Oat-based food products have a high content of essential amino acids and vitamins and dietary value. Oat grain samples were taken from the sown areas of the Panfilovsky District of the Almaty region, Kazakhstan (latitude: 44°10'17" N; longitude: 80°00'34" W).
- The grain ground in the Miller-800 laboratory mill (China) was sent for extraction by the maceration process (Xiao et al., 2012). A small sample of dry ice was immediately added to the ground material, which was then stirred periodically for 20 minutes at +5 °C every 5 hours during the next 72 hours.

Extraction of bioactive compounds and biochemical analysis

The series of analyses were conducted to determine the antioxidant activity and the content of phenolic compounds (phenolic acids and flavonoids) in cereal grains (corn and oats) and products (flour and crackers) whose major ingredients were corn and oat. For the chemical analysis, the 50-g samples of cereal-

based products were prepared. All chemicals were purchased from Sigma-Aldrich (Germany). Smaller samples (300 mg) were used for the extraction and chromatographic analysis of phenolic compounds. After the chromatographic analysis, those samples were placed into the 30-mL culture tubes and hydrolyzed using alkaline and acid hydrolysis methods. For alkaline hydrolysis, 1 mL of distilled water and 3 mL of 2M NaOH were added to the ground material in the culture tubes. The samples thus prepared were closed with corks and then subjected to prolonged heating (for 30 min) in a water bath at 95 °C. The tubes were then cooled to room temperature, and the contents were neutralized with 1 mL of 6M HCl (pH 2.4). The phenolic profile was analyzed using a liquid chromatography-high resolution mass spectrometry according to Xiao et al. (2012). The total content of phenolic compounds was expressed as milligrams of catechin equivalents per 100 gram of extract (mg CE 100 g⁻¹ extract). The antioxidant activity (AA) was determined by the spectrophotometric method (Santos and Silva, 2020) and expressed as μmol Trolox equivalent antioxidant capacity per one kilogram of extract (μmol Trolox kg⁻¹ extract).

To prevent mycotoxin contamination, the grain samples underwent gaseous treatment with chlorine dioxide and sulfur dioxide. The preventive measures also include the ammonification process. The long-term exposure (1 day) to relatively high concentrations (400-800 ppm) of gaseous chlorine dioxide and sulfur dioxide was reported to be effective against certain types of fungi. The concentration of mycotoxins in corn and oats was evaluated in accordance with (Mihalcea and Amariei, 2022).

Statistical analysis

The analysis of data was performed by using the one-way ANOVA in Microsoft Excel and Statistica 10 (De Smith, 2021). The experiment was repeated six times. Differences at $P \leq 0.05$ were considered statistically significant according to Student's t-test.

Results

Antioxidant activity and phenolic profile of cereal concentrates based on processed corn and oat

During the analysis of cereal concentrates whose major ingredients were corn and oat, the presence of bioactive compounds was found. A significant proportion of those compounds is constituted by phenolics (Fig. 1).

The antioxidant activity of phenolic compounds varies by product. Oat and corn both have high antioxidant activity, as shown by the analysis of food products based on these two cereals. Fig. 2 shows the total polyphenol content in cereals, cereal concentrates, and cereal-based products. As can be seen, the cornmeal flour contains almost twice as much polyphenols as the oatmeal flour, despite comparable antioxidant activity.

Significant differences were found in the quantity of phenolic compounds between cereals and flour. The phenolic content in cereal grain varies between 550 and 650 mg CE/100-g extract and is statistically lower compared to flour. At the same time, it is significantly higher than in crackers (Fig. 2).

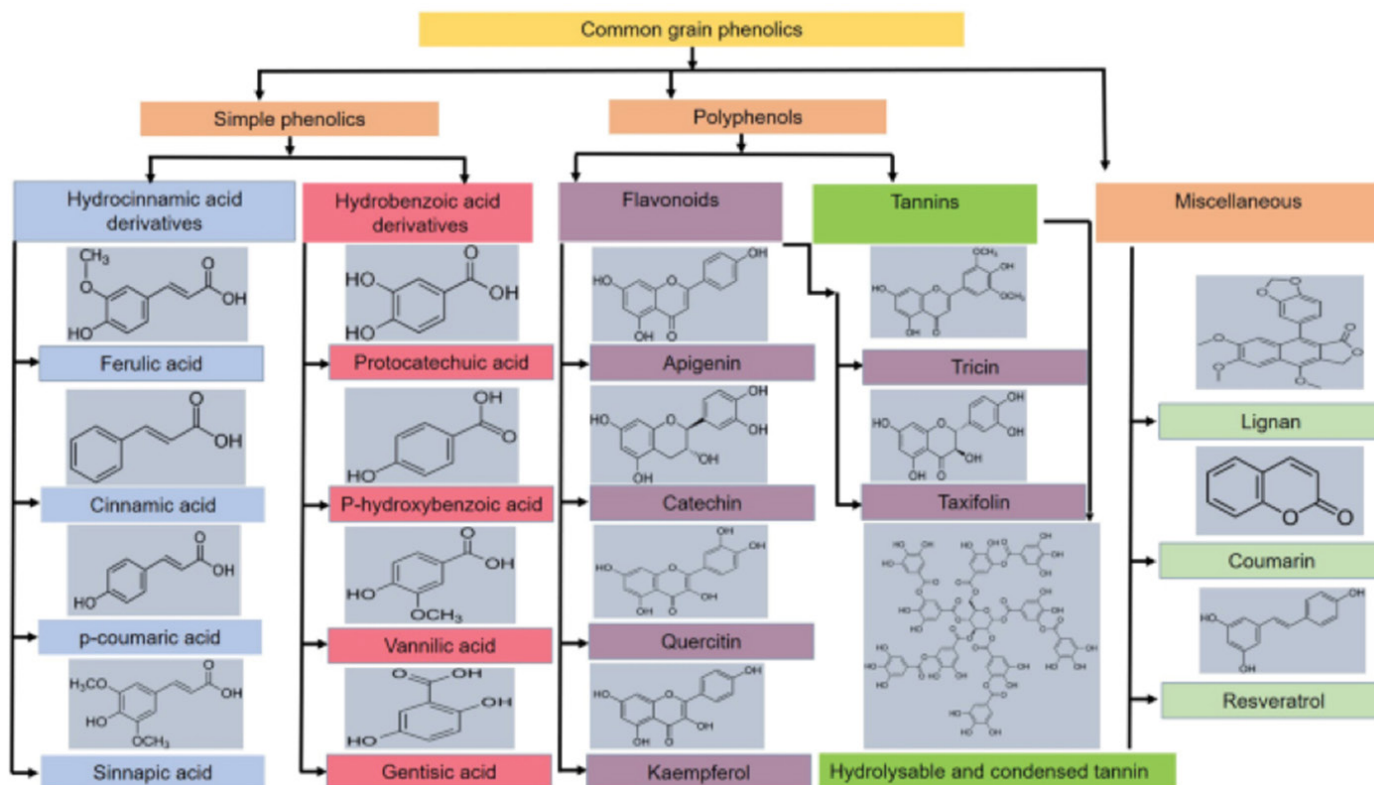


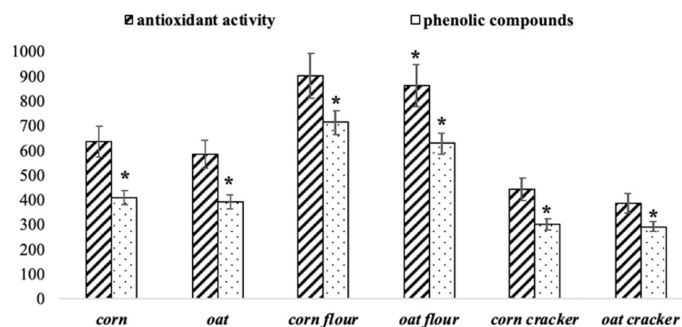
Figure 1. Major bioactive compound present in corn and oat

Following a similar pattern, significant differences were found in antioxidant activity. The likely explanation is that antioxidant compounds, mostly phenolics and carotenoid pigments, are not bound to the cell wall and are usually present in the pericarp and nucleus. Oat and corn crackers had different polyphenol content and antioxidant activity. Furthermore, baked crackers had two to three times lower content of total polyphenols than the flour they were made from, and the antioxidant activity was also lower by 25-28% regardless of the cereal type.

Regarding the qualitative profile of phenolic compounds, there were 9 flavonoids (including apigenin, luteolin and orientin) and 4 phenolic acids (ferulic, 4-hydroxybenzoic, sinapinic and

caffeic acids, in particular) found in the flour and cracker samples based on corn and oats. The benefits of using refined grain in the form of corn or oat flour in food are mainly due to the content of bioactive compounds, which are considered as functional food additives with the ability to exhibit a therapeutic effect. According to the results of this study, the phenolic acids content in corn and oats ranges from 380 to 3010 mg kg⁻¹ depending on the cultivation region. Whole grains of corn and oats are characterized by a higher content of phenolic acids than in shelled grains, due to their localization in the bran layer, pericarp and endosperm. In addition, grain production requires special attention to the bioavailability of phenolic acids, since they can occur both in free and bound form. Thus, bound phenolic acids are characterized by lower bioavailability due to the presence of a significant number of covalent bonds, which are broken only under hyperthermal conditions and during basic or acid hydrolysis. In our experiment, up to 80-90% of phenolic acids in corn and oat grains were bound. In contrast, free phenolic acids have no compounds with the cell wall, being mainly found in starch and pericarp.

In the first stages of the study, we analyzed the quantitative and qualitative content of phenolic compounds, the antioxidant activity of grains, and their food concentrates. However, it was insufficient to determine only the value of grain products, given the industrial importance, the cultivation, and further cross-border transport of the selected crops. These processes account for a significant share of the economy in countries with agro-industrial potential. Therefore, we have traced the issue of regulating grain imports using the example of their contamination with fungal pathogens that produce mycotoxins.



Note: * - represents statistically significant values at $P \leq 0.05$

Figure 1. Antioxidant activity ($\mu\text{mol Trolox/kg extract}$) and phenolic content (mg CE/100-g extract) of the selected cereals (corn and oat) and food products containing thereof (flour and crackers)

Imported food product quality control

At the time of the study, there were no serious issues regarding the quality and safety of imported food products in Kazakhstan and Azerbaijan. In the first half of 2022, the General Administration of Quality Supervision, Inspection and Quarantine strengthened safety supervision over imported food products. Subsequently, 916 batches of unqualified food imported from 49 countries and regions were inspected, with the total weight of 5647 tons (Online.zakon.kz, n.d.). Compared to the same period last year, the number of low-quality batches decreased by 9.7%, indicating a steady improvement in the quality of imported food.

In this study, six major mycotoxins were detected in the grain samples: aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone and nivalenol/deoxynivalenol. According to the World Health Organization (2018), these mycotoxins can cause considerable damage to human health. They belong to a group of aromatic polycyclic compounds with a molecular weight of 200-550 Daltons; the structural formulas are given in Fig. 3.

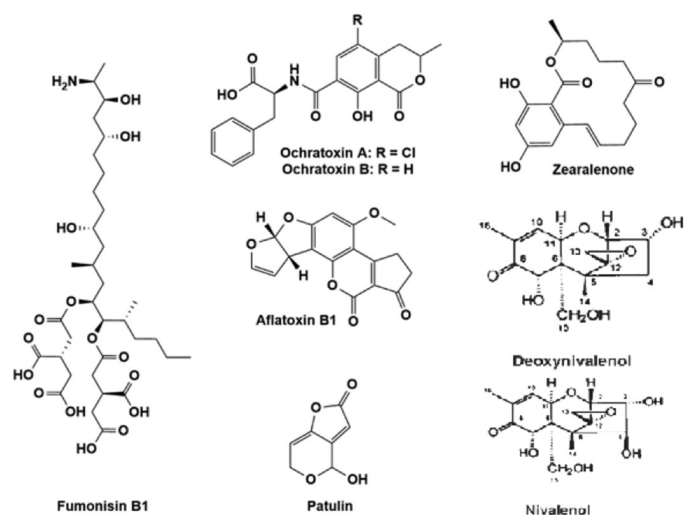


Figure 3. Structural formulas of six mycotoxins found in corn and oat according to WHO

According to the analysis, processed food products have lower mycotoxin concentrations, but the levels can vary from 0.5 to 10 $\mu\text{g kg}^{-1}$ depending on the individual mycotoxin. The likely reason behind this is the varying stability during processing and distribution of mycotoxins. The lower level of mycotoxin contamination in food products can also be related to the fact that producers sort out grains with lower concentrations of mycotoxins. In this study, the incidence of mycotoxins among the corn-positive food products is as follows (Fig. 4): 8%, patulin; 4%, nivalenol/deoxynivalenol; 34%, aflatoxins; 6%, zearalenone; 31%, ochratoxin A; 17%, fumonisins. Since the data for oat concentrates were almost identical, we did not present them in the figure.

Given that fungal growth on cereal grains depends on multiple factors, including temperature, pH, the presence of water and nutrients, and lighting, it is reasonable to assume that climate may be a decisive factor in this process and have a direct effect on the levels of mycotoxins present.

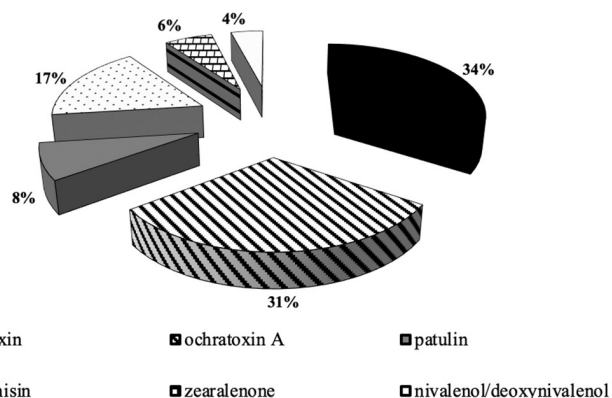


Figure 4. The average quantitative and qualitative composition of mycotoxins in corn concentrates

Discussion

Previous studies point to the relationship between the antioxidant activity of food products and their content of phenolic compounds. The high antioxidant activity of these compounds is determined by the amount and content of polyphenols in corn and oat grains (Călinoiu and Vodnar, 2018). The available literature provides evidence that these cereals, especially whole-grain products and barn containing a substantial amount of polyphenols, have high antioxidant activity (Chen et al., 2021; Martín-Diana et al., 2021). Phenolic compounds have a specific effect on the human body. They are involved in various physiological processes (Fărcaș et al., 2021), although their number varies significantly not only in different types of cereals but also within the same species. As shown earlier, the total phenolic acid content in corn varies from 307.57 mg 100⁻¹ g (Méndez-Lagunas et al., 2020) and 2213 $\mu\text{g g}^{-1}$ (Horvat et al., 2020) to 6637.73 $\mu\text{g/g}$ (Chen et al., 2021). At the same time, the concentration of phenolic compounds in oats is significantly lower and varies between 15.79 mg 40 g^{-1} and 25.05 mg 40 g^{-1} (Soycan et al., 2019).

The results on the concentration of phenolic acids in grain and food concentrates of corn (Fig. 2) completely coincide with the data of our colleagues and vary in the range of 300-700 mg 100 g^{-1} . Barley products and grains contained from 300 to 600 mg 100 g^{-1} , exceeding the literature data several times. Obviously, the conditions of grain cultivation and processing significantly affect this indicator.

The difference can also be noticed in the level of antioxidant activity of cereals. The study by Granato et al. (2018) noted significant difference between antioxidant activity *in vitro* and the actual ability to neutralize free radical molecules, positively affecting health status *in vivo*. This is due to the fact that the technological features of production or its metabolic transformations in a living organism are not taken into account. When analyzing the breeding lines of maize as potential ingredient for the formulation of novel gluten-free products, Bassolino et al. (2022) found that the antioxidant profile and the total antioxidant capacity of many genotypes differed depending on the color of the grain. Another indicator for the selection of the cereal genotypes with the required antioxidant content is the correlation coefficient, which showed that there is no dependence of antioxidant compounds on the pericarp color (Colombo et al., 2021).

In this study, cornmeal crackers with tannins contained 1.5 times more phenols than oatmeal crackers. Corn flour had slightly higher phenol content and antioxidant activity than the confectionery made from it. This finding is in line with the previous research (Parsaei et al., 2018). The cornmeal products taste as good as the oatmeal products and both have beneficial effects on health (Parsaei et al., 2018). Corn crackers were reported to reduce oxidative stress and inflammation and to improve glycemic response (Prasanthi et al., 2017).

To preserve the nutritional value and taste qualities of imported gran material and cereal products, a good quality control system should be in place (Chen et al., 2015). Among the many factors that can reduce the quality of cereal concentrates and finished food products, particular attention should be given to the microbiological factor (Irklienko et al., 2021; Mbow et al., 2019). Grain resources are susceptible to contamination by bacteria (*Lactobacillus*, *Acetobacter*, *Bacillus*, etc.) and fungal pathogens (mainly of genera *Aspergillus*, *Fusarium* and *Penicillium*, *Cryptococcus*, *Candida*) (Erkmen and Bozoglu, 2016; Wachowska et al., 2017). The presence of mycotoxins, which these pathogens produce, can significantly reduce imports. Even though the current incidence rates of mycotoxins are within acceptable limits (World Health Organization, 2018), they remain a problem. Neutralization of fungal mycotoxins and containment of their toxic effects on the body is carried out with the help of antioxidant compounds or other food components. This is the basis of dietary strategies for responding to challenges in the food industry (Galvano et al., 2001). In summary, effective protection of cell membranes from mycotoxin damage depends on the consumption of foods with a high content of compounds with antioxidant activity (vitamins, carotenes, xanthophylls, etc.) that are able to neutralize reactive oxygen species (Mavrommatis et al., 2021). In addition to whole grain products, biologically active compounds and nutrients are also found in fruit and vegetables, the consumption of which contributes to a healthy and balanced diet in general (Liu et al., 2013).

There are several strategies for mycotoxin degradation, one of which involves probiotic microorganisms (Liu et al., 2022). One of the main mechanisms of mycotoxin neutralization is their binding to the cell wall of a microorganism and fermentation processes. In particular, due to the binding of mycotoxins by fermented milk probiotic strains, their neutralization and reduction of absorption in the gastrointestinal tract are achieved. In particular, the probiotic adsorbed up to 90 to 98% of zearalenone, ochratoxin A and aflatoxin B1 when cultured in milk (Alshannaq and Yu, 2017). In addition, given that there were up to 34% aflatoxins, 31% ochratoxin, and 17% fumonisins detected in our grain concentrate samples, the use of probiotic microorganisms is a promising approach to the neutralization of mycotoxins in food. Therefore, it requires further research into the potential of probiotics to adsorb mycotoxins. This approach is supported by other studies showing the effectiveness of aflatoxin B1 neutralization in wheat, barley and corn grains during contact with probiotic microorganisms (Peles et al., 2019; Dovganyuk et al., 2021; Togisbayeva et al., 2022).

Another suggestion would be to increase the antioxidant content of the plant itself. We have shown that the antioxidant activity, and therefore the level of antioxidants, is higher in food concentrates than in whole grains of corn and oats. Hence, future

research will focus on investigating the increase of antioxidant levels in cereal concentrates to neutralize mycotoxins during the import/export process.

Conclusions

Based on the results of the conducted research, the phenolic profile analysis of food products from corn and oat showed a significant reduction of phenolic content in cracker samples as compared to grain, while the flour samples demonstrated significantly higher concentrations. It was found that the flour and cracker samples were found to contain 9 flavonoids and 4 phenolic acids. The analysis of the phenolic acids content of the cereal grain showed that they ranged from 380 to 3010 mg kg⁻¹. Corn and oat both had high antioxidant activity, but the total polyphenol content of cornmeal flour was almost twice the total polyphenol content of the oatmeal flour

Quality control is a necessary measure to preserve the nutritional quality of imported food commodities. Since one factor that can reduce the quality of cereal concentrates and cereal-based foods is fungal contamination, it was reasonable to analyze grain food concentrates for mycotoxin contamination. The incidence of mycotoxins in the corn positive samples (excluding grain) was as follows: 8%, patulin; 4%, nivalenol/deoxynivalenol; 34%, aflatoxins; 6%, zearalenone; 31%, ochratoxin A; 17%, fumonisins. The concentration ranged from 0.5 to 10 µg kg⁻¹. The present findings can be useful to optimize food formulations and improve product stability during transportation. The results of the study confirmed that the value of grain flour in terms of phenolic compound content is higher than that of whole grains. Therefore, it is recommended to transport grain in processed form. In addition, the antioxidant activity, as well as its level, is higher in food concentrates than in whole corn and oat grains. Hence, future research will focus on investigating increasing the level of antioxidants in cereal concentrates to neutralize mycotoxins.

Declaration of Conflicting Interests

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Availability of Data and Materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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ACS88_36