



Presence of mycotoxins, heavy metals and nitrate residues in organic commercial cereal-based foods sold in the Greek market

Adriana Skendi¹ · Maria Papageorgiou¹ · Maria Irakli² · Dimitris Katsantonis²

Received: 9 December 2018 / Revised: 3 May 2019 / Accepted: 28 May 2019 / Published online: 10 June 2019
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Abstract

The aim of the present work was to report on the occurrence of contaminants and trace elements in *organically* grown commercial cereal products, available in the Greek market. Moreover, the level of contamination of domestic organic cereals was compared with that of imported ones. To this end, the level of contamination by heavy metals (Pb, Cd), nitrates, trace elements (Cu, Fe, Mn, Zn, Cr, Ni, Co) and mycotoxins (aflatoxins, deoxynivalenol, zearalenone, ochratoxin, and T-2) were reported. Seventy-six organic cereals and derived products of domestic and imported origin were tested including rice, barley, oat, wheat, maize and two samples made of a mixture of grains. The levels of contamination were calculated and compared to the recommended or regulated maximum levels. The maximum levels were exceeded for lead (2), cadmium (1), aflatoxins (4), and ochratoxin (1). Contamination of samples by nitrate was below the allowed level. The level of contamination in domestic organic samples was similar to that of imported ones, except for cadmium with imported samples being more contaminated. The findings of this study are important in terms of risk to human health, but they do not support nor reject the hypothesis that organic cereals are less contaminated than the conventional ones.

Keywords Heavy metals · HPLC · Mycotoxins · ICP-OES · Organic cereal products

1 Introduction

Many consumers are turning to organic foods in the hope of finding a healthy and environmentally friendly alternative and avoid food safety issues. Generally, organic products are perceived as containing less contaminants and more nutrients than conventional products (Hoefkens et al. 2009). The increasing productivity of organic farming

currently raises the question of the safety of these products. Cereals and their products represent the main source of food in many countries, so food safety issues and potential health risks related to them represent a serious concern. Health risks from a contaminant depend on average daily exposure. A potential health risk for the individual or the population exists if the exposure is greater than the daily dose that causes adverse effects.

Heavy metals are among the major contaminants of food supply. Their toxic effects, even at very low concentrations, represent a potential danger for human health. Cadmium (Cd) has been classified in category 1 (carcinogenic to humans) by the International Cancer Research Agency (IARC 1993). Heavy metals can contaminate crops in industrialized areas as a result of air and soil pollution and the European Commission has set limits for Cd, lead (Pb) and mercury (Hg) (EC 2006, 2008, 2015). The current maximum limit of Pb in cereals is 0.2 mg/kg whereas in processed cereal-based foods and baby foods for infants and young children it decreases to 0.050 mg/kg wet weight (EC 2015). On the other hand, the limit for Cd in cereals excluding bran, germ, wheat and rice is 0.1 mg/kg while in bran, germ, wheat and rice it is 0.2 mg/kg wet weight (EC

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00003-019-01231-7>) contains supplementary material, which is available to authorized users.

✉ Adriana Skendi
andrianaskendi@hotmail.com; antrskn@teiemt.gr

✉ Maria Papageorgiou
mariapapage@food.teithe.gr

¹ Department of Food Technology, Alexander Technological Educational Institute of Thessaloniki (ATEITH), P.O. Box 141, GR-57400 Thessaloniki, Greece

² Hellenic Agricultural Organization, Demeter, Institute of Plant Breeding and Genetic Resources, P.O Box 60458, Themi, 57001 Thessaloniki, Greece

2008). According to Harcz et al. (2007), the Cd level was similar while Pb occurred at higher concentrations in organic than in conventional food samples. In addition, Hernández-Martínez and Navarro-Blasco (2012) reported the presence of a higher content of lead and cadmium in organic cereals in comparison with conventional ones.

Iron (Fe), copper (Cu), zinc (Zn), nickel (Ni), chromium (Cr), and manganese (Mn), although considered as essential metals, exert toxic effects on human health if present in higher concentrations than the permissible limits. A high intake of Fe, especially in combination with high Mn intake, was found to be related with increased risk for Parkinson disease (Powers et al. 2003). It was reported that organic crops contain 21% more iron and 29% more magnesium than the conventional ones (Rembiałkowska 2007). Moreover, the levels of Zn and Cu were found to be higher in conventionally produced wheat samples than organically produced ones (Arslanbaş and Baydan 2013). In the European Union, there are currently no maximum levels for Ni and Cr in food. It was shown that semolina samples obtained from organic farming systems showed higher concentrations of Ni and Zn with respect to those obtained from conventional ones whereas the opposite was observed for the presence of Cr (Zaccone et al. 2010). Nitrate represents another contaminant in food that results from the nitrogen supplied to crops from soil. The amount of nitrate in crops depends on a large number of factors that are irrelevant to the farming system such as cultivar, plant disease, soil type, geographical location, climate, planting/harvest dates, nitrate present in irrigation water and groundwater, storage and post-harvest processing (Magkos et al. 2003). Rembiałkowska (2007) reports that organic products contain less nitrates than their conventional counterparts. The nitrate level allowed in processed cereal-based foods and baby foods for infants and young children is 200 mg NO₃/kg (EC 2006).

On the other hand, mycotoxins that comprise secondary metabolites of many species of fungi are found responsible for the contamination of a variety of crops. Cereals can be contaminated with different fungal species that might produce various mycotoxins in the same cereal. Mycotoxins and particularly deoxynivalenol (DON) and aflatoxins (AFs) (such as aflatoxin B1 [AFB1], B2 [AFB2], G1 [AFG1], and G2 [AFG2]), zearalenone (ZEA) and ochratoxin A (OTA) are the most important contaminants of the cereal crops (Irakli et al. 2017; Martins et al. 2018; Skendi et al. 2016). Limits were established by current EU regulation for these mycotoxins in all cereals and their products intended for direct human consumption (EC 2006, 2007, 2010). Moreover, T-2 toxin is found to contaminate cereals (Pleadin et al. 2017) but European Commission (EC) legal limits have not been yet agreed upon. Controversy exists in the literature related to the

contamination of organic cereals compared to the conventionally grown ones. Higher contamination with deoxynivalenol was observed in organic wheat compared to conventional (Malmauret et al. 2002). In the study of Harcz et al. (2007) there were found no significant differences in DON content in cereals from the two farming systems. Contradictory findings do not permit definitive conclusions about the safety of organic cereals (Biffi et al. 2004). No significant differences were obtained between ZEA concentrations in conventionally and organically produced cereals, even if the average levels were systematically higher for the conventional cereals (Harcz et al. 2007).

In Greece there is no systematic monitoring of pollutants in organic cereals and their derived products and reports in the literature are scarce (Karavoltos et al. 2002, 2008; Tsumbaris and Tsoukali-Papadopoulou 1994). Moreover, they are focused mainly on the study of a single contaminant at a time giving only partial information about the extent of the contamination. To our knowledge, the present study presents the first study about multi-contaminants in organic cereals and their derived products in the Greek market.

The objective of the present work is to present the results of occurrence of nitrate, heavy metals and mycotoxin content in organic cereal products in the retail market in Greece and to compare them with the established allowed limits of European Union. Furthermore, to evaluate the levels of contaminants in terms of the type of cereals as well as of their origin (domestic or imported). Thus, monitoring for the presence of the heavy metals, nitrates and mycotoxins will provide useful data for the literature as well as will rise the consumers' awareness about the safety of organic products.

2 Materials and methods

2.1 Reagents

AFs and DON standards were purchased from Supelco (Bellefonte, PA, USA) and Sigma Aldrich (Steinheim, Germany), respectively. Methanol, dichloromethane, acetonitrile and water of HPLC (High-Performance Liquid Chromatography) gradient grade were purchased from Chem-Lab (Zedelgem, Belgium) and chloroform from Mallinckrodt Baker B.V. (Deventer, The Netherlands). Solid phase extraction (SPE) cartridges were Oasis HLB (200 mg/6 mL) from Waters (MA, USA). Cuvette tests to be applied for the quantitative determination of NO₃-N were LCK 339 (measurement range 0.23–13.50 mg/L; detection wavelength: 350 nm) whereas the enzyme-linked immunosorbent assay (ELISA) kits AgraQuant Ochratoxin

Assay 2/40, AgraQuant Zearalenone Assay 25/1000, and AgraQuant T-2 Toxin Assay 25/500, were from Romer (Romer Labs, Singapore). Single element standard solutions, 1000 mg/L, were used for Cu, Fe, Mn, Zn, Cd, Pb, Co, Cr, Ni (Sigma Chem, St. Louis, USA). All other chemicals and reagents were of analytical grade (for trace metal analysis) and all glassware and plastic containers were washed properly [first with nitric acid and then with ultra-pure water from an ELGA PurelabFlex apparatus (ELGA Process Water, Marlow, UK)] in order to ensure that any contamination does not occur.

2.2 Sample collection

A total of 76 samples (seeds and their products) from six different cereals (wheat, rye, barley, oat, rice and maize) were collected from local market in Thessaloniki, Greece. The organic products were either EU-imported (54) or of domestic origin (22). Sampling was done at random from different supermarkets and specialized stores within the city area. The chosen samples were seeds, flakes, flour, mixed cereal flakes and pasta. They derived from rice (26), wheat (18), maize (6), barley (6), rye (4), oat (14) and mixtures of rye, oat, barley and wheat (2). The obtained samples were first milled using a laboratory mill (Retsch, model ZM 1000, Haan, Germany) equipped with a 0.5 mm sieve, then properly homogenized and sealed in plastic bags and finally stored in freezer till analysis.

2.3 Determination of heavy metals and trace elements

Two subsamples (6 g) from each milled sample were placed in crucibles and the ashing process was carried out in a muffle furnace (Betriebs W.C. Heraeus, Hanau, Germany) (at 650 °C for 5 h). After cooling, the ash was rinsed with 1 M nitric acid. The ash suspension was filtered and the filtrate made up to the volume of 6 mL with 1 M nitric acid. Appropriate dilution of the solution with 1 M nitric acid was performed before analysis. Blank solution (method blank) was prepared by applying the same procedure and reagent solution without sample. The sample solutions were analysed for heavy metal and trace element contents, as wet weight basis, by Perkin-Elmer Optima 2100 DV (ICP-OES) Inductively Coupled Plasma Optical Emission Spectrometer (Perkin-Elmer, Waltham, Mass., USA). Operating conditions comprise nebulizer flow 0.8 L/min, auxiliary gas flow 15 L/min, sample uptake rate 1.50 mL/min, plasma power 1300 W and integration time 15 s. Measurements were performed in axial view for Cd (228.802), Co (228.616), Cr (267.716), Ni (231.604), Pb (220.353), Cu (327.393), Fe (238.204), Mn (257.610) and Zn (206.200) at the appropriate wavelength (nm). The

method for the determination of the abovementioned metals was developed according to the IUPAC guidelines (IUPAC 1995). First, the instrument was calibrated for each one of the abovementioned elements with a six-point calibration curve. The standard solutions used were prepared by diluting the respective stock solutions. Water acidified with nitric acid was used as the calibration blank. Then, the linearity of the calibration curve for each element calculating the coefficient of determination ($R^2 > 0.9999$ for each element studied) was evaluated. The values of the limit of detection (LOD) for each element studied was calculated as recommended from IUPAC (1995): $LOD = 3 SD/S$, where SD is the standard deviation of analytical responses and S is the slope of calibration curve. The detection limits were 0.0027, 0.0042, 0.0097, 0.0046, 0.0014, 0.0059, 0.0100, 0.0150 and 0.0070 mg/kg for Cd, Pb, Cu, Fe, Mn, Zn, Cr, Ni, and Co, respectively. A recovery study was performed by spiking several samples (including spiked calibration blanks) with different amounts of the standard solutions and the recovery of the method obtained for each element was satisfactory with values in the range of 86–105%. Moreover, the analysis of heavy metals and trace elements resulted in a satisfactory repeatability (RSD < 10%). In general, two subsamples were prepared from each sample and three separate readings were made for each solution.

2.4 Determination of mycotoxins

Samples were examined for the presence of OTA, ZEA and T-2 toxin using commercial ELISA kits following the procedure described in the AgraQuant Assay kit manual. Absorption was measured with a Neogen[®] model 321Plus (Awareness technology, USA) microwell reader.

For the detection of the levels of DON and all aflatoxins (AFB1, AFB2, AFG1, and AFG2) an HPLC system was employed. Mycotoxins were extracted from the samples, cleaned-up and analysed as reported in Skendi et al. (2016). The reported method was tested for different matrix effects in order to check if is applicable to test the type of samples under investigation. Recovery tests for each cereal species, and its products showed acceptable results.

The equipment used was an Agilent 1200 series system (Agilent Technology, Urdorf, Switzerland) equipped with a quaternary pump, a membrane degasser, a column oven, a rheodyne injection valve with 20 μ L loop linked with a diode-array detector (DAD) and a fluorescence detector (FLD) and a photochemical UVE reactor (LCTech GmbH, Dorfen, Germany). Mycotoxins were separated in an Eclipse XDB-C₁₈ column (150 \times 4.6 mm, 5 μ m), kept at 40 °C and chromatograms were recorded in an Agilent Chemstation software (version B.04.01, Agilent

Technologies). All the samples were extracted and analysed in duplicate.

2.5 Determination of nitrate

Ten grams of each milled sample were hand mixed with 20 mL of distilled water and then shaken for 15 min in a shaker at high speed. The mixture was centrifuged (4000 rpm for 10 min at 4 °C) and Hg₂Cl₂ (mercury(I) chloride) was added to the supernatant, then let rest for 30 min and then centrifuged again. Celite was added to supernatant in order to remove proteins and the mixture was agitated for 5 min and then centrifuged (4000 rpm for 10 min at 4 °C). Thereafter, the supernatant was filtrated (using filter paper Whatman No. 1) and the filtrate was stored overnight in freezer (− 20 °C). On the measuring day, the filtrate was let to defrost at room temperature and then centrifuged at 12,500 rpm (10 min at 4 °C). Extracts were tested for nitrate according to standardized cuvette test procedures (Hach Lange GMB, Düsseldorf, Germany). The UV absorption was measured by using Hach Lange DR2800 (Germany) spectrophotometer.

2.6 Statistical analysis

The results were statistically evaluated using the SPSS 25 package. Non-parametric test was performed for independent samples in SPSS. Kruskal–Wallis test was used to detect evidence if samples of the cereal groups (cereal species i.e. rice, wheat, oat, maize, rye, barley, mixture; cereal origin i.e. imported domestic) with similar distribution have the same median ($p < 0.05$), followed by Dunn's pairwise tests adjusted using the Bonferroni correction. Differences were considered significant when $p < 0.05$. Samples below the LOD were assigned a value of 0 concentration.

3 Results and discussion

3.1 Heavy metals and nitrate in organic cereal food samples

Cadmium concentration in cereal samples varied widely (Fig. 1). No rice or wheat samples surpassed the allowed limit for the Cd content (0.2 mg/kg) (EC 2006, 2015). The highest concentration of 0.199 mg/kg was observed in a wheat sample (domestic durum wheat pasta) followed by 0.147 mg/kg in a rice sample (imported glutinous white rice). Two oat samples exceeded the allowed limit for the presence of Cd (oat bran with 0.110 mg/kg, and “crunchy” granola with 0.107 mg/kg). The independent samples Kruskal–Wallis test revealed that the medians of Cd are the

same across the cereal species ($p > 0.05$). Table 1 presents the mean value and the variation (minimum and maximum values) of the analytes in the present study and relevant data reported in the literature. Studies on conventionally grown samples are also included in Table 1 in a separate column in an attempt to depict available data on cereal samples rather than make comparisons between conventional and organic cereals due to limitations arising from the diversity of the applied analytical techniques, the number of the samples analyzed, the type of processing etc.

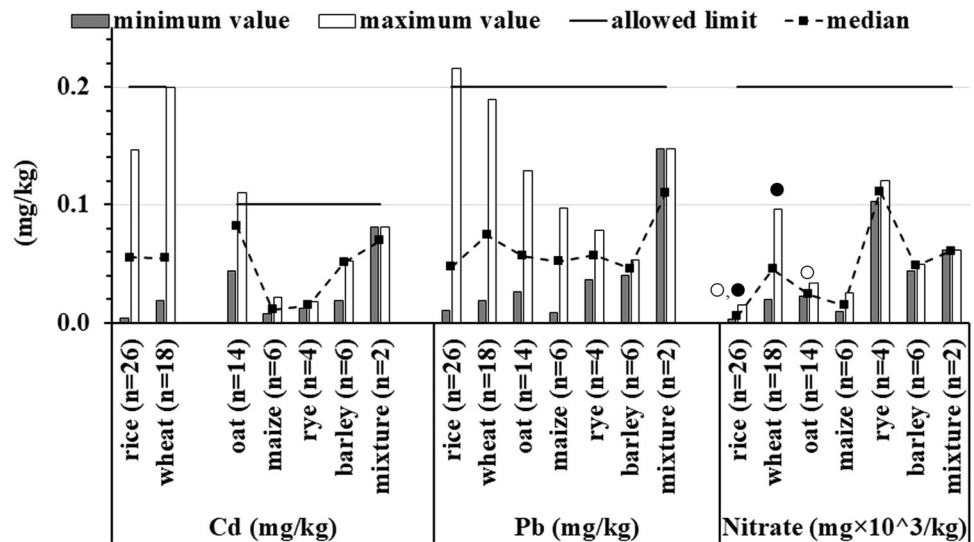
The highest value of Cd observed in this study for wheat samples (0.199 mg/kg) was similar to the maximum value (0.188 mg/kg) (Table 1) observed for conventional winter wheat in the study of Harcz et al. (2007). It was reported that durum wheat accumulates more Cd than other cereals (Perrier et al. 2016). Several factors affect the accumulation of Cd in grains such as weather conditions and soil properties, geographical pattern and genotype (Kubo et al. 2008) resulting in an accumulation of levels higher than the legal limit. Moreover, higher values could be related with possible contamination with Cd during processing (Hernández-Martínez and Navarro-Blasco 2012).

The presence of Pb should not exceed 0.2 mg/kg in cereals (EC 2006). The amounts of Pb measured are shown in Fig. 1. The distribution as well as the medians of Pb are the same across the cereal species ($p > 0.05$) according to Kruskal–Wallis test. Maize, rye and barley samples showed concentrations less than 0.1 mg/kg. One basmati rice sample (imported) exceeded the allowed limit (0.215 mg/kg), whereas the highest value among the wheat samples was 0.190 mg/kg. There are several studies reporting higher levels of Pb in cereal products than in the present study (Table 1).

The lowest concentration of nitrate observed in the cereal samples analysed were in the range of 2.9–102.4 mg/kg, whereas the highest concentration varied from 15.5 to 120.6 mg/kg with the lowest values observed in rice and the highest in rye samples (Fig. 1). The values obtained are higher than those reported by McNaraara et al. (1971). No cereal sample exceeded the limit set by legislation (200 mg/kg). The Kruskal–Wallis test showed that the distribution and the medians of nitrates across the cereal species are not the same ($p = 0.000$) with pairwise comparisons revealing significant differences in the medians of rice and oat ($p = 0.022$) as well as rice and wheat ($p = 0.002$). Oat and wheat samples showed higher mean values than rice.

In an attempt to identify possible differences between the domestic and imported cereals the independent samples Kruskal–Wallis test showed that the distribution of the Cd, Pb and nitrates as well as the medians of nitrates and Pb are the same ($p > 0.05$). Contrary, the median of Cd between the imported and domestic cereals differed significantly

Fig. 1 Content of cadmium, lead and nitrate in samples categorized per cereal origin and their maximum levels allowed by the legislation. Only median values with the same symbol differ significantly from each other ($p \leq 0.05$), as determined by the Kruskal–Wallis test followed by a Dunn’s pairwise tests adjusted using the Bonferroni correction



($p = 0.042$) with imported showing the highest levels of Cd.

3.2 Trace elements in organic cereal food samples

Variation of trace elements in the samples under investigation are shown in Table 2. It was found in the literature that organic fertilizers had limited or no impact on grain concentrations of Cd, Cu, Mn whereas resulted in higher grain concentrations of Zn (Hamner and Kirchmann 2015). The highest value of Cu concentration among all samples was higher than those observed in the literature (Table 1). The highest value of Cu (19.6 mg/kg) was observed for a long-grain white rice (imported), whereas the lowest value (0.9 mg/kg) was observed for a sample of “breakfast corn flakes” (imported). Although the overall distribution of Cu is not the same across the groups of cereal species, only maize–oat groups showed different distribution. The medians of Cu are the same across groups of cereals ($p < 0.05$) because the overall test showed no differences across the groups.

The variation of the Zn content among the samples ranged between 3.48 and 31 mg/kg with the lowest value observed in “breakfast rye flakes” (imported origin) and the highest in a rice sample (domestic origin). The medians of Zn differed only between rice and oat samples ($p = 0.022$), with oat showing the higher level between them. Concentrations of Mn varied from 1.28 to 34.03 mg/kg showing significant difference only between rice and oat ($p = 0.022$) and wheat and oat ($p = 0.009$). The highest value of Fe (66.5 mg/kg) was observed in an oat bran sample (imported origin), whereas the lowest value (4.21 mg/kg) was observed in a glutinous white rice (imported origin). Although distribution ($p = 0.004$) and

medians ($p = 0.006$) of Fe differed across the groups of cereal species, significant differences ($p = 0.022$) among the medians were observed only between rice and oat. It is recognized that trace elements are mainly located in the outer part of the grains, so it was expected that the highest value should be observed in the samples with bran (“oat bran” sample). The values observed in this study are higher than those observed from Dong Phuong et al. (1999) (Table 1).

There are currently no maximum levels for Cr, Ni and Co in food. The highest mean value of Cr was found in maize samples (1.45 mg/kg), whereas the lowest in oat samples (0.87 mg/kg). The lowest mean concentration of Co was observed for maize samples (0.05 mg/kg), whereas the highest was exhibited from oat samples. Pairwise comparisons among the medians of each cereal species, for each element (Cr, Ni and Co), revealed that only medians of Co between rice and oat differ significantly ($p = 0.022$). An obvious observation from data in Table 1 is that the amount of Cr and Ni, in the organic cereals of the present study was higher than that reported for conventional cereals in the literature.

In general, the group of domestic cereal samples showed similar median values of trace elements to those of imported origin ($p > 0.05$ as determined from Kruskal–Wallis test).

3.3 Mycotoxin content in organic cereal food samples

The variation of concentration of the AFs and DON is shown in Table 3. Statistical analysis based on the pairwise comparisons revealed that significant differences exists only between the medians of AFs in rice and oat, AFG2 in rice–maize and wheat–maize and AFB1 in rice–maize. In

Table 1 Means and minimum and maximum values of measurements of cereal samples reported in the literature

Contaminant	Conventional (mean ^a ; min–max ^c)	Organic (mean ^a ; min–max ^a)	References
Cd (µg/kg)	61.7; nr–188	60; nd–199	Present study
	nr; 19–81	66.8; nr–117	Harcz et al. (2007)
	nr; 2.4–252	9.7–53.4; nr	Hamnér and Kirchmann (2015)
	33.0–49.4; nr		Karavoltzos et al. (2008)
	7.1; 4.4–11.8	18.5; 16.6–28.5	Škrbić et al. (2005)
	0.5–73; nd–77		Tsoumbaris and Tsoukali-Papadopoulou (1994)
	20.3; nd–178.0		Hernández-Martínez and Navarro-Blasco (2012)
	34.5; 3.6–69.7		Rahman et al. (2014)
	3–11; 3–48		Al-Saleh and Shinwari (2001)
	nr; 10–130		Fu et al. (2008)
			Dong Phuong et al. (1999)
			Brizio et al. (2016)
Pb (µg/kg)	42.1; nr–287	80; 10–220	Present study
	10.8; 6.4–19.3	100.0; nr–328	Harcz et al. (2007)
	64.6–63.1; nr	26.1; 21.4–51.6	Hernández-Martínez and Navarro-Blasco (2012)
	16–419; 2–1248	8.8–29.2; nr	Karavoltzos et al. (2008)
	134.8; 23.1–1529		Tsoumbaris and Tsoukali-Papadopoulou (1994)
	355; 167–745		Rahman et al. (2014)
	nr; 10–570		Al-Saleh and Shinwari (2001)
			(Fu et al. (2008)
			Brizio et al. (2016)
			Present study
Fe (mg/kg)	4.0–12.0; 2.1–25.8	21.8; 4.2–66.5	Dong Phuong et al. (1999)
			Present study
Co (µg/kg)	3.6–21.7; 1.9–42	50; nd–160	Present study
	169.4; 55–420		Rahman et al. (2014)
			Fu et al. (2008)
Cr (mg/kg)	0.06–0.41; 0.02–0.74	1.08; 0.19–3.27	Present study
	0.20; 0.06–0.42		Rahman et al. (2014)
			Fu et al. (2008)
Ni (mg/kg)	0.1–0.3; 0.1–0.8	3.3; 0.6–23.8	Present study
	0.5; 0.2–0.8		Rahman et al. (2014)
	0.1–0.9; 0.1–2.0		Fu et al. (2008)
			Dong Phuong et al. (1999)
Cu (mg/kg)	nr; 1.7–4.8	3.3; 0.9–19.6	Present study
	1.6–9.3; 1.0–13.0		Hamnér and Kirchmann (2015)
	3.3; 2.8–4.5		Rahman et al. (2014)
	2.2–5.2; 1.1–7.9		Fu et al. (2008)
			Dong Phuong et al. (1999)
Zn (mg/kg)	13.4–17.8; 6.9–24.5	14.1; 3.5–31.0	Present study
	nr, 19.9–42.3		Rahman et al. (2014)
	18.5–26.5; 13.8–32.5		Hamnér and Kirchmann (2015)
Mn (mg/kg)	nr; 5.7–49.0	11.5; 1.3–34.0	Present study
	5.9–24.4; 2.7–51.7		Hamnér and Kirchmann (2015)
	9.4, 5.8–12.7		Rahman et al. (2014)
	9.9–23.7; 5.9–32.6		Fu et al. (2008)
			Dong Phuong et al. (1999)

Table 1 (continued)

Contaminant	Conventional (mean ^a ; min–max ^c)	Organic (mean ^a ; min–max ^a)	References
AFs (µg/kg)	nr; n.d–0.4	1.3; nd–13.0	Present study
	0.1; 0.1–0.5	nr; nd–4.0	Hernández-Martínez and Navarro-Blasco (2010)
	nr; 0.4–24.5		Kabak (2012)
	1.3; 1.1–2.1	1.4; 1.0–3.0	Şengül et al. (2016)
DON (µg/kg)		243; nd–1729	Kuzdraliński et al. (2013)
	nr; 58–565		Present study
	354, nr –284	200; nr–1184	Skendi et al. (2016)
ZEA (µg/kg)	697; 220–2150	636; 250–1700	Harcz et al. (2007)
			Kuzdraliński et al. (2013)
OTA (µg/kg)	0.8, nr–232	2.3; nd–14.7	Present study
		11.2; nr–86	Harcz et al. (2007)
T–2 (µg/kg)	1.8; 0.3–4.5	0.5; nd – 4.9	Present study
	0.2–0.8; 0.1–1.8		Baydar et al. (2007)
	0.3; 0.1–1.1		Kabak (2009)
	3.2; 1–5.7	3.6; 1.4–5.8	Kabak (2012)
T–2 (µg/kg)		nd; nd–nd	Kuzdraliński et al. (2013)
	123; 34–311	95; 32–154	Present study
			Kuzdraliński et al. (2013)

nr not reported, nd not detected, refers to levels below the LOD (limit of detection)

^aMeans: in the case of multiple mean values (i.e. for cereals of different origin, cultivar, or product) being reported in the reference the lowest and the highest mean values are included herewith

^bRange of data: minimum and maximum values of data reported in the reference

Table 2 Variation (minimum–maximum value observed) of trace elements in cereal samples categorized per cereal origin (mg/kg wet weight)

Trace element	Rice	Wheat	Oat	Maize	Rye	Barley	Mixed
No. samples	26	18	14	6	4	6	2
Cu	1.48–19.6 (3.81/2.28)	1.2–6.95 (2.56/2.02)	1.63–5.56 (3.99/4.34)	0.9–1.2 (1.07/1.09)	1.29–3.68 (2.49/2.49)	2.07–5.03 (3.59/3.67)	4.85–4.97 (4.91/4.91)
Fe	4.21–62.11 (13.8/9.72) [○]	9.48–59.17 (24.60/17.55)	20.5–66.5 (36.48/34.85) [○]	5.46–17.52 (10.41/8.25)	11.73–12.86 (12.29/12.29)	11.69–30.42 (23.85/29.44)	33.15–33.27 (33.21/33.21)
Mn	3.23–27.56 (8.34/5.31) [○]	3.15–30.77 (11.64/10.91) [●]	14.96–34.03 (23.79/23.57) ^{○●}	1.28–4.08 (2.29/1.50)	5.2–7.94 (6.57/6.57)	4.53–8.6 (6.66/6.87)	14.70–14.85 (14.78/14.78)
Zn	5.17–31.0 (10.7/9.19)	4.83–29.44 (13.88/13.41)	16.84–30.81 (22.69/21.06) [○]	4.56–12.74 (8.86/9.29)	3.48–8.56 (6.02/6.02)	12.04–28.09 (18.72/16.02)	16.00 - 16.25 (16.13/16.13)
Cr	0.31–1.78 (0.98/0.89)	0.33–2.37 (1.30/1.17)	0.19–2.22 (0.89/0.75)	0.49–3.27 (1.45/0.61)	0.78–1.33 (1.06/1.06)	0.31–1.56 (0.97/1.03)	0.92–1.05 (0.99/0.99)
Ni	0.65–3.29 (1.88/1.79)	0.88–5.37 (2.31/1.59)	2.77–23.82 (9.45/5.34)	0.77–3.09 (1.56/0.82)	0.65–1.15 (0.9/0.9)	1.22–3.14 (2.07/1.86)	2.00–2.18 (2.09/2.09)
Co	0.00–0.1 (0.04/0.02) [○]	0.03–0.1 (0.05/0.06)	0.05–0.16 (0.11/0.10) [○]	0.00–0.03 (0.11/0.00)	0.02–0.07 (0.05/0.05)	0.07–0.09 (0.08/0.07)	0.08–0.10 (0.09/0.09)

The mean/median values of the measurements are shown in parentheses

*Within one line, medians of groups of cereals with the same symbol differ significantly from each other (p ≤ 0.05), as determined by the Independent Samples Kruskal–Wallis test followed by Dunn’s pairwise tests adjusted using the Bonferroni correction

general, 65.8% of the samples were found contaminated with at least one AF. Four samples, representing 5.3% of the total samples examined, exceeded the limits set by the

European Union legislation for the presence of AFs. From the four samples, two were of imported origin; whole grain flour corn flakes and corn grain for popcorn found with

Table 3 Concentrations of aflatoxins (AFs) and DON in cereal products categorized per cereal origin determined with HPLC

Cereal	No. samples		AFs ($\mu\text{g}/\text{kg}$)	AFB1 ($\mu\text{g}/\text{kg}$)	AFB2 ($\mu\text{g}/\text{kg}$)	AFG1 ($\mu\text{g}/\text{kg}$)	AFG2 ($\mu\text{g}/\text{kg}$)	DON ($\mu\text{g}/\text{kg}$)
Rice	26	% Positive	30.8	7.7	15.4	23.1	7.7	84.6
		Min–max ($\mu\text{g}/\text{kg}$)	nd–0.6	nd–0.4	nd	nd–0.6	nd–0.2	nd–278.2
		Median	nd [○]	nd [○]	nd	nd	nd [○]	57.9 [○]
		Above the limit	0	0				0
wheat	18	% Positive	22.2	22.2	44.4	33.3	0.0	100.0
		Min–max ($\mu\text{g}/\text{kg}$)	nd–1.3	nd–0.9	nd–0.1	nd–1.1	nd	71–475
		Median	0.001	nd	nd	nd	nd [●]	186.4
		Above the limit	0	0				0
Oat	14	% Positive	71.4	71.4	28.6	85.7	28.6	100.0
		Min–max ($\mu\text{g}/\text{kg}$)	0.6–2.2	nd–2	nd–0.2	nd–0.6	nd–0.2	299.2–568.1
		Median	1.00 [○]	0.40	nd	0.58	nd	396.3 [○]
		Above the limit	0	0				1
Maize	6	% Positive	100.0	100.0	100.0	66.7	100.0	66.7
		Min–max ($\mu\text{g}/\text{kg}$)	1.3 - 13	0.5 - 8.5	0.1 - 0.9	nd–2	0.7 - 1.9	nd–493.3
		Median	4.51	1.02 [○]	0.55	0.92	1.64 ^{○,●}	123.5
		Above the limit	2	1				0
Rye	4	% Positive	50.0	50.0	100.0	100.0	50.0	100.0
		Min–max ($\mu\text{g}/\text{kg}$)	1.5–7.4	nd–6	0.1–0.7	0.8–1.2	nd–0.2	50–134
		Median	4.45	2.98	0.37	1.00	0.10	95.95
		Above the limit	1	1				0
Barley	6	% Positive	66.7	66.7	0.0	100.0	0.0	100.0
		Min–max ($\mu\text{g}/\text{kg}$)	0.7–4	nd–2.8	nd	0.7–1.7	nd	152.3–1728.7
		Median	3.48	1.73	nd	1.29	nd	204.8
		Above the limit	1	1				1
Mixture	2	% Positive	0.0	0.0	0.0	100.0	0.0	100.0
		Min–max ($\mu\text{g}/\text{kg}$)	0.5–0.5	nd	nd	0.5–0.5	nd	355.6–355.6
		Median	0.53	nd	nd	0.53	nd	342.5
		Above the limit	0	0				0
Total	76	% Positive	65.8	36.8	34.2	52.6	18.4	92.1
		Min–max ($\mu\text{g}/\text{kg}$)	nd–13.0	nd–8.5	nd–0.9	nd–2	nd–1.9	nd–1728.7
		Median	0.64	nd	nd	0.53	nd	166.8
		Above the limit	4	3				2

A positive sample refers to samples containing the analyte \geq LOD

nd not detected, refers to levels below the LOD (limit of detection)

Values are means of at least duplicate measurements. Within one column, medians of groups of cereals with the same symbol differ significantly from each other ($p \leq 0.05$), as determined by the Independent Samples Kruskal–Wallis test followed by Dunn's pairwise tests adjusted using the Bonferroni correction

13.00 and 4.51 $\mu\text{g}/\text{kg}$ AFs, respectively. The two other samples over the limits were of domestic origin; whole grain rye flour with 7.43 $\mu\text{g}/\text{kg}$ and whole grain barley flour with 4.04 $\mu\text{g}/\text{kg}$. AFB1 contaminated 36.8% of the total samples. Three samples, one of imported origin (whole grain cornflakes 8.54 $\mu\text{g}/\text{kg}$) and two samples of domestic origin (whole grain rye flour 5.95 $\mu\text{g}/\text{kg}$ and whole grain barley flour 2.75 $\mu\text{g}/\text{kg}$), exceeded the limit set in the European legislation for AFB1. The other aflatoxins, AFB2, AFG1 and AFG2, contaminated 34.2, 52.6 and 18.4% of total samples (Table 3). Contamination with

more than one aflatoxin was observed. Only 26 samples representing 34.2% of the total samples were totally free of AFs. Sixteen samples (10.5%) were contaminated with only one AFs, eighteen (23.7%) were contaminated with two AFs, eight (10.5%) were contaminated with three AFs, and eight samples (10.5%) were contaminated with four AFs. Kuzdraliński et al. (2013) reported that the number of mycotoxin-positive samples and the concentrations of aflatoxins was higher in organic farming in comparison to conventional production (Table 1). Conventional samples of Şengül et al. (2016) showed higher presence of AFs

Table 4 Concentrations of mycotoxins OTA, ZEA and T-2 in cereal products categorized per cereal origin determined with ELISA

Cereal	No. samples		OTA ($\mu\text{g}/\text{kg}$)	ZEA ($\mu\text{g}/\text{kg}$)	T-2 ($\mu\text{g}/\text{kg}$)
Rice	26	% Positive	53.8	92.3	0.0
		Min-max ($\mu\text{g}/\text{kg}$)	nd-2.2	0.3-14.7	nd
		Median	0.02	1.09	nd
		Above the limit	0	0	0
Wheat	18	% Positive	33.3	88.9	0.0
		Min-max ($\mu\text{g}/\text{kg}$)	nd-1.2	0.2-4.4	nd
		Median	nd	0.87	nd
		Above the limit	0	0	0
Oat	14	% Positive	85.7	100.0	0.0
		Min-max ($\mu\text{g}/\text{kg}$)	nd-4.9	0.2-7.7	nd
		Median	0.87	1.45	nd
		Above the limit	1	0	0
Maize	6	% Positive	33.3	100.0	0.0
		Min-max ($\mu\text{g}/\text{kg}$)	nd-0.7	0.8-6.4	nd
		Median	nd	4.05	nd
		Above the limit	0	0	0
Rye	4	% Positive	0.0	100.0	0.0
		Min-max ($\mu\text{g}/\text{kg}$)	n.d.	1-2.5	nd
		Median	nd	1.77	nd
		Above the limit	0	0	0
Barley	6	% Positive	33.3	100.0	0.0
		Min-max ($\mu\text{g}/\text{kg}$)	nd-0.1	1.2-2.6	nd
		Median	nd	2.14	nd
		Above the limit	0	0	0
Mixture	2	% Positive	100.0	100.0	0.0
		Min-max ($\mu\text{g}/\text{kg}$)	0.5-0.5	2.9-2.9	n.d.
		Median	0.88	2.90	n.d.
		Above the limit	0	0	0
Total	76	% Positive	50.0	94.7	0.0
		Min-max ($\mu\text{g}/\text{kg}$)	nd-4.9	0.2-14.7	n.d.
		Median	0.02	1.25	n.d.
		Above the limit	1	0	0

A positive sample refers to samples containing the analyte \geq LOD

nd non-detected, refers to levels below the LOD (limit of detection)

Values are means of at least duplicate measurements

Within one column, medians of groups of cereals with the same symbol differ significantly from each other ($p \leq 0.05$), as determined by the Independent Samples Kruskal-Wallis test followed by Dunn's pairwise tests adjusted using the Bonferroni correction

whereas those of Kabak (2012) reported lower values than those reported in the present study.

About, 92.1% of the samples were contaminated with DON at concentration range below the LOD to 1728.7 mg/kg. Two samples (2.6% of the total samples) exceeded the allowed limits. Both samples were of imported origin, one was "oat flakes" with DON concentration of 565.46 $\mu\text{g}/\text{kg}$ and the other "barley flakes" with 1728.73 $\mu\text{g}/\text{kg}$. Pairwise comparisons revealed that a significant difference exists only between the rice and oat ($p < 0.05$). Moreover, all samples were examined for the presence of OTA, ZEA and

T-2 toxin (Table 4). The Kruskal-Wallis test showed that the distribution as well as the median of OTA and ZEA are the same across cereal species ($p > 0.05$). None of the samples was found contaminated with T-2 toxin. OTA was found in 50% of the samples; but only one sample ("whole grain oat flour") of domestic origin exceeded the allowed level (4.86 $\mu\text{g}/\text{kg}$). No rye samples were found contaminated with OTA. The 94.7% of total samples was contaminated with ZEA, but no sample exceeded the allowed level (Table 4).

In general, all the samples were contaminated with at least one of the examined toxins (OTA, ZEA, AFs, DON). Sixteen samples (21.1% of the total samples) were contaminated with at least two mycotoxins, thirty-six samples (50% of the total samples) were contaminated with three toxins, twenty-one samples (30% of the total samples) were contaminated with all four toxins, namely AFs, DON, OTA and ZEA. The domestic samples showed similar levels of contamination with the studied mycotoxins ($p < 0.05$ as determined by the Kruskal–Wallis test) when compared to imported samples. Organic crops may be more vulnerable to contamination from fungi because in organic production the use of synthetic fungicides is not allowed. Controversy exists related to the amount of the mycotoxins in cereals from organic farming compared to conventional ones. When comparing values with those from literature, one should keep in mind that they may be affected by factors such as variety, growing year and geographical location. Moreover, the contamination with mycotoxins is related to inappropriate post-harvest storage conditions that facilitate mold growth.

4 Conclusions

The reported data do not support nor reject the hypothesis that organic cereals are less contaminated with the contaminants examined in the present study than the conventional ones. Clearly, the organic samples of the present study are not to be recommended to be used as infant foods. Moreover, not a clear statement can be given about the contamination of organic cereals in the Greek market when compared with the data reported in the literature, since results are diverse and are not reported on a single contaminant and/or single type of cereal or cereal product.

Contrary to the general consumer perception of organic products to be healthier and safer than conventional products, the health risk for consumers might be real for the contamination by mycotoxins and heavy metals. Continuous efforts are needed in routine monitoring of raw materials and processed products, regardless of them being organically grown or not, in order to minimize the levels of contaminants in cereal foods. Furthermore, the contribution to daily intake of contaminants from organic cereals and their products consumed in Greece could be considered to be rather small. A large-scale survey of organic products will be needed in order to estimate precisely the contribution to the daily intake of contaminants and the possible risks to the Greek consumers.

The imported samples of this study have similar amounts of contaminants to those of Greek origin with exception of the presence of Cd, in which case the imported samples were found to be more contaminated.

The results of the present study showed that consumers that consume organic cereals are exposed to lower levels of heavy metals, nitrates as well as mycotoxins compared to conventional ones reported in the literature.

Acknowledgements This work was supported by the European Social Fund and national resources through the Public Investment Program, Research Project ARCHIMEDES III, subproject OΠΣ 383572 “Monitoring mycotoxins and radioactivity level in cereal products of Greece”.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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