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# Effect of cereal products supplementation with american blueberries, cranberries and cinnamon on the formation of type A and B trichothecenes group

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## Abstract

**Introduction.** Mycotoxins – secondary mould metabolites with undesirable effects for humans – are common in the environment. These toxins are mainly produced by fungi of the genera Penicilium, Aspergillus and Fusarium.
**Objective.** The aim of this study was to evaluate the applicability of various sources of antioxidants (blueberries lyophilisate, cranberries lyophilisate and cinnamon powder), at 5 different concentrations (3%, 5%, 10%, 20%, 30%), to inhibit the formation of mycotoxins during the storage of cereal products. Analysed cereal samples included selected cereal grains, bran and cereal products intended for consumption by children.
**Results.** The results showed that supplementation of oat brans with the highest concentrations of blueberry lyophilisate resulted in a significant decrease in the mycotoxins levels; specifically: 20% concentration reduced the level of HT-2 toxin by 10.7% in one sample, while 30% concentration reduced it by 9.4% and 17.4% in 2 other samples. A similar result was measured for oat bran samples supplemented with the cranberry lyophilisate: specifically, 20% concentration significantly reduced the level of HT-2 toxin by 10.6% in one sample, while 30% concentration reduced it by an average of 18.0%±6,0% in 5 other samples. Finally, cinnamon powder supplementation caused a significant reduction in HT-2 levels in all stored samples, even at its lowest concentration. 30% supplementation resulted in HT-2 reduction in cereal samples by 67.1% – 76.1%, in wheat bran samples by 57.5% – 69.2%, in oat bran samples by 83.4% – 87.0% and by 55.0% – 100% in samples of cereal products intended for consumption by children.
**Conclusions.** Natural products used in the experiment (blueberry, cranberry, cinnamon) inhibited the formation of mycotoxins from the group of trichothecenes.

## Key words
trichothecenes, cereals, antioxidant potential, cereals products, mycotoxin reduction, natural phenolic compounds

## INTRODUCTION

Mycotoxins – secondary mould metabolites with undesirable effects for humans – are common in the environment. Their presence is not related to the development of industry or transport. which in the common understanding are considered to be the main factors responsible for the occurrence of food contamination. These toxins are mainly produced by fungi of the genera *Penicilium*, *Aspergillus* and *Fusarium*. Their occurrence is associated with the inevitable presence of mould in the environment [1]. Mycotoxins may enter the food chain indirectly and directly: indirectly by consuming the meat or milk of animals fed with the contaminated products, or directly by consuming plant products contaminated in the field or during storage or processing [2]. Vegetable products such as cereals, oilseeds, coffee, fruits, spices and nuts, as well as animal products (milk, meat, offal), are all susceptible to mycotoxin contamination [3]. The most frequently occurring mycotoxins include aflatoxins, ochratoxin A, patulin and a group of toxins produced by certain fungi species of the genus *Fusarium* (trichothecenes, fumonisins, moniliformin (MON), zearalenone (ZEA) and its derivatives). Four types are distinguished among the trichothecenes: A, B, C and D. Type A trichothecenes, produced by F. *sporotrichiodies* and F. *Poae*, include T-2 toxin, HT-2 toxin and diacetoxyscirpenol (DAS). Type B trichothecenes include deoxynivalenol (DON), nivalenol (NIV) and fusarenone-X (FUS). Type A trichothecenes are generally considered more toxic than Type B trichothecenes [4]. The maximum acceptable levels of aflatoxins, ochratoxin A, patulin, deoxynivalenol and zearalenone, fumonisins (sum $B_1$ and $B_2$), citrin and ergot alkaloids are established by the European Commission Regulation (EC) No. 1881/2006 from 19 December 2006 [5]. In 2013, the European Commission issued recommendations on the presence of T-2 and HT-2 toxin in cereals and cereal products [6].

The growth of *Fusarium* and the production of mycotoxins depend on multiple environmental factors, which include substrate composition, its consistency, presence of

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micronutrients, presence of accompanying microflora, and above all, humidity and temperature.

Numerous studies are available on the effect of temperature on the growth of selected *Fusarium* species and the production of T-2 and HT-2 toxins. Mylona and Magan [7] reported 25 °C as optimal for *Fusarium* to produce the T-2 and HT-2 toxin in oats. Similarly, a range between 20 – 30 °C was reported by Medina and Magan [8].

Several detoxification methods are known to prevent the mycotoxin contamination of food products; however, most of them are not being commonly applied due to their high costs or impracticality [9]. In order to limit losses during post-harvest storage of food products, synthetic organic compounds are currently applied. Unfortunately, many of those compounds have the potential for adverse impact on the environment, consumers safety and development of resistance in pest species. As a result, there is an increasing need for safer, ecological and biodegradable alternatives [10]. There are several compounds of natural and synthetic origin that have antioxidant properties and could be implemented to counteract the formation of mycotoxins.

Highbush blueberry and cranberry are a rich source of bioactive compounds with high antioxidant potential. Blueberries are characterized by a high content of polyphenolic compounds, anthocyanins. Levels of anthocyanins vary, ranging 25–495 mg/100g, depending on variety, fruit maturity and size, climate and growing conditions, as well as handling post-harvest storage time and conditions [11]. Cranberries are an exceptionally rich source of phenolic compounds. Their high antioxidant activity is due to the presence of 3 main classes of flavonoids: anthocyanins, flavonols and flavan-3-ols [12]. Spices are a separate group with natural high antioxidant potential, containing essential oils, glycosides, alkaloids, bitterness, mucus and tannins. Cinnamon spice is obtained from the tree bark of cinnamomum genus and is particularly rich in antioxidants [13].

Numerous studies have shown that essential oils, obtained from raw plant material, demonstrate a wide range of biological and pharmacological activities. The bioactive constituents of cinnamon are cinnamaldehyde, eugenol, linalool and 1,8-cneolol. Cinnamon oil is recommended for indigestion, severe cramps or bloating, due to its ability to suppress gastrointestinal motility [14]. In addition, essential oils are currently extensively studied due to their antimicrobial and antioxidant properties [15, 16]. Studying the preventive effect of essential oils against mildew showed that *Ocimum basilicum* oil at a concentration of 4.8 µL/g significantly reduces the growth of *Fusarium verticillioides* and the formation of fumonisin in artificially infected corn grain [17].

Chemical preservatives are currently used to protect the food commodities against toxic fungal spoilage. Available data suggest that some known natural compounds can become successful alternatives. In addition, increasing awareness among consumers put pressure on the food industry and food scientists to improve food quality and safety. For those reasons, essential oils, oleoresins and their components extracted from aromatic plants, natural plant extracts and spices attracted increased attention [2, 17].

Numerous studies are available regarding cereals and methods applied to inhibit the fungal growth and mycotoxin production, providing information on species, tested cereals and general growth conditions. Unfortunately, the literature is limited regarding processed foods, including cereal products intended for infants and young children

## OBJECTIVE

The aim of this study was to evaluate the effect of blueberries lyophilizate, cranberries lyophilizate and cinnamon powder as natural sources of bioactive substances, including antioxidants, on the formation of mycotoxins. The results of the above tests may have practical application in the food industry to ensure the health safety of products exposed to mycotoxin contamination.

## MATERIALS AND METHOD

Samples consisted of cereals (wheat, oats), brans (wheat, oats) and cereal products intended for consumption by infants and young children. Wheat and oat grains were obtained directly from the producers (south-eastern Poland). Wheat and oat bran, as well as cereal products intended for consumption by children, were purchased in retail (Krakow). The wheat bran and oat bran did not contain any artificial flavours or additives, and consisted of 100% of the respective type of grain. Cereal products for infants and children included instant porridge products intended for infants aged from 4 months and for small children, containing a mixture of various cereals (wheat, oats, rye, barley and millet). Highbush (American) blueberry lyophilizate (*Vaccinium corymbosum L*), cranberries lyophilizate (*Vaccinium macrocarpon)* and Ceylon cinnamon powder (*Cinnamomum verum*) were purchased in a health food store. Freeze-dried blueberry and cranberry have a relatively high antioxidant capacity, are widely popular among consumers, and combine well with cereal products. Cinnamon has a significant antioxidant potential as well as an accepted taste and aroma.

**Chemicals.** Trifluoroacetic acid anhydride (TFAA) and 15-Acetoxyscirpenol (15-MAS) metanol, aceton, Trolox, dipotassium peroxodisulfate were purchased from Sigma, Krakow, Poland. N-(trimethylsilyl)-imidazol (TMSI) and trimethylchlorosilane (TMCS) were obtained from Fluka, Krakow, Poland. T2-Toxin (T-2), HT2-Toxin (HT-2), scirpenol (SCI), 15-monoacetoxyscirpenol (15-MAS), diacetoxyscirpenol (DAS), triacetoxyscirpenol (TAS), fusarenon X (FUS-X), B-trichothecene-mixture solution: nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), were purchased from Riedel-de-Haen, Germany.

**Sample preparation.** The experiment was carried out in 2 stages. The first stage included the baseline assessment of the antioxidant potential, the degree of Type A and B trichothecenes contamination, and the level of microbiological purity (total number of moulds) in all raw materials: cereal and bran samples, cereal products for children, blueberry and cranberry lyophilizate and cinnamon powder. The moisture content of each individual sample was determined according to the method described in ISO 712:2009 report [18]. Samples were analyzed for total number of colonies according to the method described in the standard [19, 20]. The second stage involved only samples deemed contaminated.

Each cereal sample was milled and supplemented with blueberry lyophilizate, cranberry lyophilizate and cinnamon powder at 5 different concentrations (3%, 5%, 10%, 20%, 30%). In order to provide favourable conditions for fungal growth, humidity was set at 19% by adding an appropriate amount of distilled water. Samples were then stored at 28 °C for 14 days. After this storage period, the antioxidant potential and the levels of Type A and B trichotheses were determined. A total of 189 samples were tested and a total of 2,272 analyses made (Fig. 1).

**Determination of the antioxidant potential.** The antioxidative potential of the tested samples was determined by measuring their quenching capacity of ABTS · + free radical and expressed in micromoles of trolocyte equivalents (TEAC – Trolox Equivalent Antioxidant Capacity) per 1 g sample (µmol TE/g) [21]. 3 g of each tested sample was extracted with 40 ml of 0.08 M HCl in 80% methanol at 18–22 °C for 2 h. The extract was then centrifuged at 1,500 × g for 15 min. The supernatant was preserved, and the sample re-extracted with 40 ml of 70% acetone for a further 2 h. After centrifugation (1,500 × g, 15 min) the supernatant was combined with the

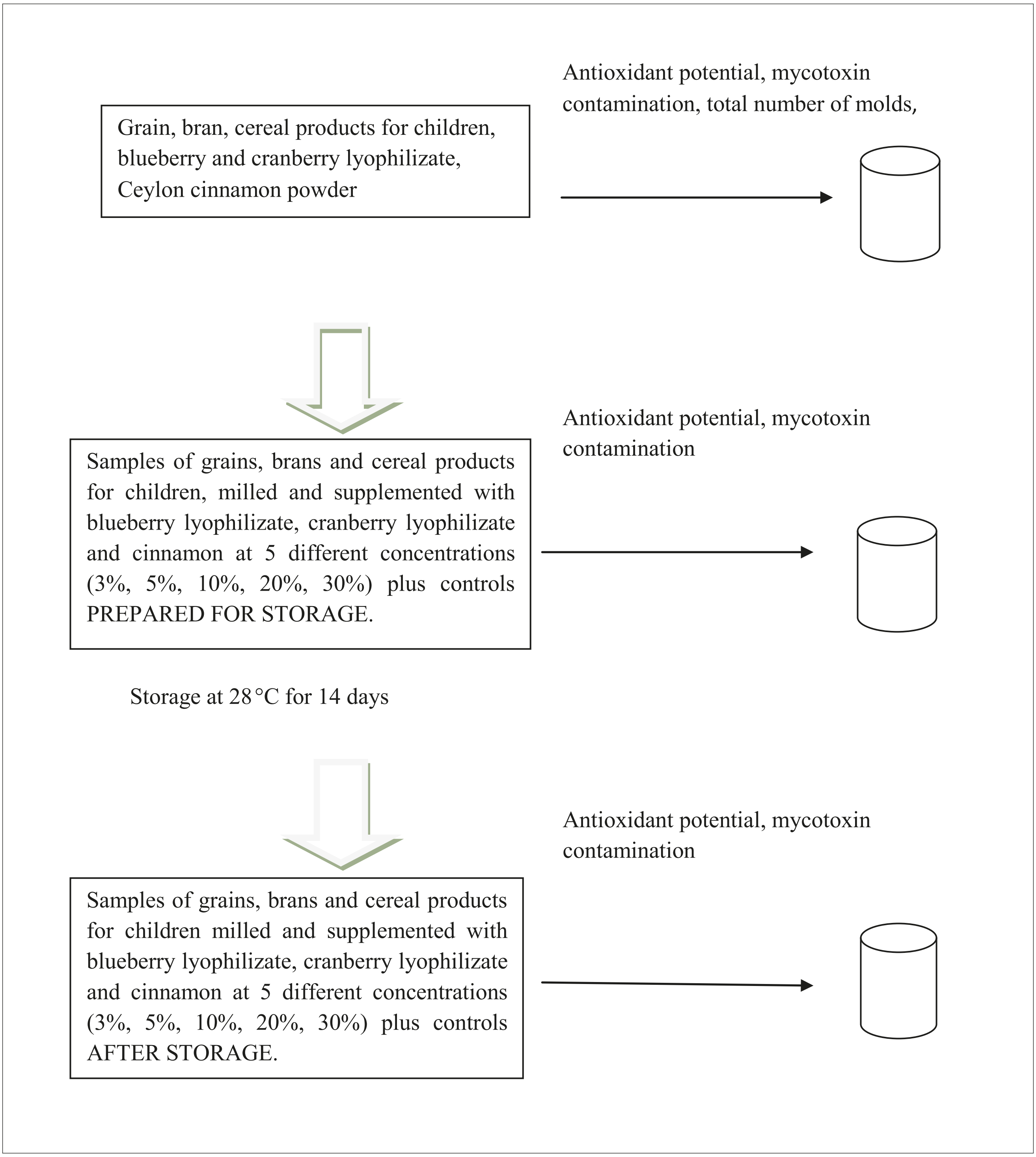


**Figure 1.** Schematic diagram of experimental procedure

previous one. Extract was then stored at -20 °C. ABTS·+ was dissolved in a solution of dipotassium peroxysulfate and then diluted until its absorbance at 734 nm was 0.740 – 0.750. Samples containing 0.35 ml of methanol-acetone extracts were made up to 1 ml with acetone-methanol solution (1:1), mixed with 2 ml of ABTS solution and incubated at 30 °C for 6 min. The absorbance was then measured at 734 nm relative to the methanol-acetone (1:1) solution. Standard 1 M Trolox solution was prepared to create a methanol-diluted standard curve. Each sample was prepared in triplicate.

**Mycotoxins analysis.** Samples were analyzed for the presence of trichothecenes according to Jeleń and Wąsowicz [22]. 25 g of each sample was extracted in 100 ml acetonitrile for 2 hrs, and then filtered through a paper filter.

To analyse the type A trichothecenes (T-2, HT-2, DAS, SCI, 15 MAS, TAS), 1 ml of the filtrate was evaporated to dryness and then re-disolved in 200 ml of trifluoroacetic acid (TFA). Obtained solution was then heated at 60 °C for 20 minutes. After cooling, the solution was again evaporated to dryness and finally redissolved in 1 ml of isooctane.

To analyse the type B trichothecenes (DON, 3-Ac-DON, FUS-x, NIV, 15-Ac-DON), 100 µl of trimethylchlorosilane (TMCS) was added to 1 ml of the filtrate. Obtained solution was incubated for 20 min at room temperature. 400 µl of isooctane and 0.5 ml of water were then added, the sample vortexed and incubated for 10–20 min until phase separation. The isooctane layer was used for the analysis. Isooctane sample extracts were analysed using a 2-dimensional gas chromatograph coupled with a Time-of-Flight Mass Spectrometer (GCxGC-TOFMS) Pegasus 4D (Leco). Each sample was prepared in triplicate.

**Enumeration of total moulds by colony count.** The fungal colony count was calculated using the plate count method described by the appropriate ISO [19, 20]. For each sample, a stock sample solution was prepared followed by a series of 10-fold dilutions. 0.1 mL of each dilution (including the stock solution) was aseptically innoculated on previously prepared DRBC agar plates or DG18 agar plates. Inculum was then evenly spread until all excess liquid was absorbed. The seeded plates were incubated at 25 °C±1 °C for 5 days, without inverting the plates. Depending on the growth rate, colonies were counted after 2–5 days of incubation.

**Equipment.** Quantitative analysis applied 2-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC × GC–TOF-MS, Pegasus 4D LECO, St. Joseph, MI, USA). The GC was equipped with a DB-5 column (25 m × 0.2 mm × 0.33 µm, Agilent Technologies, Santa Clara, CA, USA) and Rxi®-17 (1.2 m × 0.1 mm × 0.1 µm, Restek Bellefonte, PA, USA) as a second column. For the first 1 minute, the main oven temperature was kept at 80 °C, the oven was then heated at a rate of 20 °C/min – 170 °C (3 min), from then on at 4 °C/min to 240 °C (4 min), followed by the rate of 10 °C/min – 270 °C (5 min). A secondary oven was run at temperatures 10 °C higher than the main oven. Transfer line was 290 °C. Injection port temperature was 280 °C, splitless mode (60 s), helium flow 0.8 ml/min, ion source temperature 240 °C. Spectra were collected at a rate 50 scans/s in a range of 100 -700 Da. For 2-dimensional analysis, modulation time was optimized and set at 5 s. Carry 50 UV spectrophotometer (Varian, USA) was used to measure absorbance.

**Statistical analysis.** Results were analysed using one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$. The correlation as assessed between the antioxidant activity and mycotoxins content.

## RESULTS AND DISCUSSION

The analyzed raw materials demonstrated various antioxidant potential (Tab. 1). The highest antioxidant activity was measured for the cinnamon 1,654.80±105.21 µmol TE/g. For comparison, the literature reports 1,119.90±199.2 µmol TE/g as the average antioxidant activity for cinnamon [13]. Significantly lower antioxidant activity was measured for the cranberry lyophilizate 18.50±0.87 µmol TE/g. Those levels are supported by results published for assorted cranberry varieties (ranging from 9.3±1.2 – 16.4±1.2 µmol TE/g) by Borowska et al. [23]. Finally, the average antioxidant potential of American blueberry lyophilizate was 19.37 ± 2.11 µmol TE/g, similar to that measured for the cranberries. Comparable average levels of 18.50±4.04 µmol TE/g were demonstrated by Rodrigues et al. [24] for assorted blueberry varieties.

The average antioxidant potential of oat grain was 16.84±0.53 µmol TE/g and of wheat grain 12.39±0.41 µmol TE/g. The average antioxidant activity of wheat bran was 9.84±0.24 µmol TE/g and of oat bran was 6.27±0.23 µmol TE/g, comparable to 9.65±0.32 µmol TE/g and 6.45±0.14 µmol TE/g, determined respectively by Filipiak-Florkiewicz et al. [25]. Lastly, to the best knowledge of the authors of the current study, the data presented is the first to report the average antioxidant potential of cereal products intended for consumption by children. The measured level was 26.78±1.31 µmol TE/g, higher than that of cereals and bran.

In all cereal and cereal products analyzed samples, detectable amounts of mycotoxins were found for the Type A trichothecenes, namely, HT-2 toxin and diacetoxyscirpenol (DAS). The levels of contamination with HT-2 mycotoxin ranged from 77.93 – 137.18 µg $kg^{-1}$ (Tab. 1). HT-2 toxin was found in the oat grain sample in the amount of 85.3 µg $kg^{-1}$, and in the wheat grain sample at 77.93 µg $kg^{-1}$. In oat bran samples, the presence of this toxin was found at 94.21 and 100.70 µg $kg^{-1}$. In contrast, the content of HT-2 toxin in the wheat bran samples was higher and amounted to 137.18 µg $kg^{-1}$ and 131.67 µg $kg^{-1}$. DAS toxin was found only in the wheat grain sample at 148.1 µg $kg^{-1}$. No detectable levels of mycotoxins were found in cereal products intended for children.

On 27 March 2013, the European Commission established the indicative values for T-2 and HT-2 toxins [6]. These values for unprocessed cereals are 1,000 µg $kg^{-1}$ for oats and 100 µg $kg^{-1}$ for wheat. For oat bran the value is 200 µg $kg^{-1}$, and for wheat bran 100 µg $kg^{-1}$. The results for the oat grain sample in the current study showed lower than the index value (8.5% of the index value) levels of HT-2 toxin, while the content of this toxin in the wheat grain sample was much higher (77.9% of the index value).

In contrast, the content of HT-2 mycotoxin measured in oat and wheat grains from Croatia [26] were significantly lower, averaging at 7 µg $kg^{-1}$ and 9 µg $kg^{-1}$, respectively. Similarly, lower levels of trichothecenes were found in wheat grain in studies conducted in Poland [27] and Lithuania [28]. The level of HT-2 toxin determined in oat brans was significantly lower than the acceptable values (47.1% and 50.4% of the

**Table 1.** Antioxidant potential, mycotoxin conten, and total number of moulds in the research material.

| Sample | Antioxidant potential [μmol TE/g] | Mycotoxin levels [μg kg$^{-1}$] | | Total number of moulds [CFU] |
|---|---|---|---|---|
| | | **HT-2** | **DAS** | |
| Oat grain | 16.84±0.53 | 85.30±5.79 | <LOQ | 4.1 x $10^5$ |
| Wheat grain | 12.39±0.41 | 77.93±0.33 | 148.1±0.89 | 2.6 x $10^5$ |
| Oat bran A* | 6.11±0.25 | 94,21±1.87 | <LOQ | 2.7 x $10^3$ |
| Oat bran B* | 6.43±0.38 | 100.70±2.69 | <LOQ | 3.1 x $10^3$ |
| Wheat bran A* | 9.67±0.29 | 137.18±3.22 | <LOQ | 2.1 x $10^3$ |
| Wheat bran B* | 10.01±0.33 | 131.67±2.39 | <LOQ | 2.3 x $10^3$ |
| Cereal products intended for children A* | 27.89±1.12 | <LOQ | <LOQ | 61 |
| Cereal products intended for children B* | 25.44±0.99 | <LOQ | <LOQ | 70 |
| Cereal products intended for children C* | 23.87±1.02 | <LOQ | <LOQ | 82 |
| Cereal products intended for children D* | 22.52±1.25 | <LOQ | <LOQ | 93 |
| American blueberry lyophilisate | 19.37±2.11 | <LOQ | <LOQ | 1.1 x $10^2$ |
| Cranberry lyophilisate | 18.50±0.87 | <LOQ | <LOQ | 1.2 x $10^2$ |
| Ceylon cinnamon powder | 1654.80±105.21 | <LOQ | <LOQ | 1 x$10^2$ |

*A, B, C and D represent different brands

index value). In contrast, the level of HT-2 toxin in wheat bran exceeded the indicative value (131% and 137% of the index value). Obtained results regarding the content of HT-2 toxin in the wheat and oat bran samples were also higher than those reported by Rodrigeuez-Carrasco et al. [29].

The total number of moulds in all tested products ranged from 61 colonies to 4.1 x $10^5$ [CFU] (Tab. 1), with the highest overall number of moulds found in cereal grain samples. The content of mould in oat grain was 4.1 × $10^5$ [CFU], higher than the value obtained for wheat (2.6 × $10^5$ [CFU]). In the bran group, colonies grew in both oat and wheat bran samples, 3.1 × $10^3$ [CFU] – 2.1 × $10^3$ [CFU]. Higher colonies counts were found for oat bran, an average of 2.9 × $10^3$ [CFU] than wheat, where the average value was 2.2 × $10^3$ [CFU]. Cereal products for children were characterized by much lower counts, between 61 – 93 [CFU]. The grain contamination level was higher than values reported by Stuper-Szablewska & Perkowski [27] at 1.13 – 2.67 Log CFU per g. Twaróżek et al. [30] also reported a lower average total number of moulds in wheat grain at 1.2 × $10^4$ [CFU]. On the other hand, measured wheat bran mycotoxin contamination was less than the 1.5 × $10^4$ [CFU] reported by Czerwińska and Kubiak [31] for bran produced from a raw material originating from organic farming. Interestingly, wheat bran obtained from raw material originating from traditional cultivation was characterized by a lower degree of mould contamination than that found in the current study.

The analysis showed a positive correlation between the CFU count in analyzed grain samples and levels of mycotoxin contamination. However, this correlation was either absent for bran samples or inverse.

Supplementation of cereal samples with plant material rich in antioxidants increased the antioxidant potential of oat and wheat both grain and bran, due to the higher antioxidant potential of freeze-dried blueberries and cranberry (Tab. 2). Interestingly, the antioxidant potential of cereal products intended for children decreased when supplemented with those lyophilizates at 20% and 30%. This was due to the relatively lower antioxidant potential of used supplement compared to the enriched product. Cinnamon significantly increased the antioxidative potential of all samples.

The presented study focused on the applicability of various plant sources of antioxidants (blueberry, cranberry and cinnamon) to inhibit the formation of mycotoxins and showed some inhomogeneous results, depending on the sample as well as the type and amount of supplement added. The 20% addition of blueberry lyophilizate influenced the level of HT-2 toxin only in one sample of oat bran, inhibiting its formation by 10.7% in relation to the control (Tab. 3). 30% concentration of blueberry lyophilizate reduced it by 9.4% and 17.4% in both oat bran samples. Interestingly, supplementation of cereal products intended for children with blueberries (at different levels of enrichment) caused an increase in the level of HT-2 toxin in 3 out of 4 samples. It should be noted that the original product had a higher antioxidant potential than the additive, leading to a significant decrease in the antioxidative potential of the supplemented product. No significant changes in the level of mycotoxins were measured for 2 cereal samples (oats, wheat) and 2 samples of wheat bran and one cereal product intended for consumption by children. Correlation analysis showed a strong negative correlation in 90% of all tested samples. Only one sample of cereal products intended for children showed no linear relationship (r = 0.220).

The addition of cranberry lyophilizate had a statistically significant effect on the content of HT-2 toxin in 6 out of 10 samples tested (Tab. 4). A 20% concentration significantly reduced the level of HT-2 toxin by 10.6% in one sample, while 30% concentration reduced it by an average of 18.0%±6.0% in 5 other samples. Cranberry lyophilizate – like the effect of supplementation with blueberry lyophilizate – did not inhibit the formation of mycotoxins in any of the samples of cereal products intended for children. Moreover, in 3 out of 4 samples (30% supplementation) the level of contamination increased. Correlation analysis showed a very strong negative correlation for 80% of the tested samples. Two sample of cereal products intended for children showed no linear relationship (r = 0.263 and r = -0.079).

Cinnamon powder supplementation caused a significant dose-dependent reduction in HT-2 levels in all stored samples

**Table 2.** Effect of supplementation with antioxidant-rich compounds [μmol TE/g] on antioxidative potential of samples.

| Level of Supplementation<br>Sample type | Without supplementation | 3% | 5% | 10% | 20% | 30% |
|---|---|---|---|---|---|---|
| *American blueberry lyophilizate* | | | | | | |
| Oat grain | 16.84$^{a}$±0.53 | 16.89$^{a}$±0.42 | 16.92$^{a}$ ± 0.42 | 17.02$^{a}$±0.62 | 17.54$^{a}$±0.58 | 17.77$^{a}$±0.53 |
| Wheat grain | 12.39$^{a}$±0.41 | 12.24$^{a}$±0.45 | 12.36$^{a}$±0.39 | 12.71$^{a}$±0.47 | 13.51$^{a}$±0.49 | 14.32$^{a}$±0.55 |
| Oat bran A* | 6.11$^{a}$±0.25 | 6.41$^{a}$±0.12 | 6.72$^{a}$±0.18 | 7.44$^{b}$±0.22 | 8.69$^{b}$±0.27 | 10.05$^{c}$±0.29 |
| Oat bran B* | 6.43$^{a}$±0.38 | 6.74$^{a}$±0.15 | 7.15$^{a}$±0.22 | 7.81$^{a}$±0.21 | 9.08$^{b}$±0.38 | 10.43$^{b}$±0.26 |
| Wheat bran A* | 9.67$^{a}$±0.29 | 9.89$^{a}$±0.27 | 10.01$^{a}$±0.32 | 10.64$^{a}$±0.33 | 11.69$^{a}$±0.41 | 12.80$^{a}$±0.43 |
| Wheat bran B* | 10.01$^{a}$±0.33 | 9.85$^{a}$±0.25 | 10.10$^{a}$±0.29 | 10.65$^{a}$±0.32 | 11.73$^{a}$±0.43 | 12.87$^{a}$±0.33 |
| Cereal products intended for children A* | 27.89$^{a}$±1.12 | 27.26$^{a}$±0.65 | 27.20$^{a}$±0.39 | 26.84$^{a}$±0.87 | 25.97$^{b}$±0.69 | 25.22$^{b}$±0.58 |
| Cereal products intended for children B* | 25.44$^{a}$±0.99 | 24.89$^{a}$±0.51 | 24.64$^{a}$±0.47 | 24.01$^{b}$±0.39 | 23.70$^{b}$±0.34 | 23.35$^{b}$±0.99 |
| Cereal products intended for children C* | 23.87$^{a}$±0.62 | 23.09$^{a}$±0.47 | 23.21$^{a}$±0.57 | 23.15$^{a}$±0.44 | 22.82$^{b}$±0.41 | 22.50$^{b}$±0.36 |
| Cereal products intended for children D* | 21.52$^{a}$±0.84 | 21.79$^{a}$±0.42 | 21.62$^{a}$±0.56 | 21.29$^{a}$±0.69 | 20.99$^{a}$±0.45 | 20.16$^{a}$±0.62 |
| *Cranberry lyophilizate* | | | | | | |
| Oat grain | 16.84$^{a}$±0.53 | 17.18$^{a}$±0.38 | 17.67$^{a}$±0.52 | 17.93$^{a}$±0.64 | 18.02$^{ab}$±0.66 | 18.45$^{b}$±0.69 |
| Wheat grain | 12.39$^{a}$±0.41 | 12.11$^{a}$±0.37 | 12.18$^{a}$±0.47 | 12.64$^{a}$±0.42 | 13.37$^{a}$±0.51 | 13.99$^{a}$±0.52 |
| Oat bran A* | 6.11$^{a}$±0.25 | 6.42$^{a}$±0.11 | 6.82$^{a}$±0.22 | 7.36$^{a}$±0.23 | 8.66$^{b}$±0.25 | 9.93$^{c}$±0.27 |
| Oat bran B* | 6.43$^{a}$±0.38 | 6.76$^{a}$±0.13 | 7.18$^{a}$±0.19 | 7.89$^{b}$±0.22 | 9,11$^{c}$±0.27 | 10.36$^{d}$±0.28 |
| Wheat bran A* | 9.67$^{a}$±0.29 | 9.85$^{a}$±0.23 | 9.99$^{a}$±0.28 | 10.61$^{a}$±0.29 | 11,49$^{a}$±0.39 | 12.60$^{b}$±0.41 |
| Wheat bran B* | 10.01$^{a}$±0.33 | 9.90$^{a}$±0.26 | 10.14$^{a}$±0.33 | 10.70$^{a}$±0.31 | 11.73$^{a}$±0.33 | 13.96$^{b}$±0.39 |
| Cereal products intended for children A* | 27.89$^{a}$±1.12 | 27.18$^{a}$±0.65 | 26.93$^{a}$±0.89 | 26.53$^{a}$±0.76 | 25.75$^{b}$±0.55 | 24.83$^{c}$±0.64 |
| Cereal products intended for children B* | 25.44$^{a}$±0.99 | 24.72$^{a}$±0.74 | 23.86$^{a}$±0.59 | 23.43$^{a}$±0.47 | 23.25$^{a}$±0.42 | 22.99$^{b}$±0.31 |
| Cereal products intended for children C* | 23.87$^{a}$±0.62 | 23.71$^{a}$±0.42 | 23.63$^{a}$±0.51 | 23.33$^{a}$±0.35 | 22,25$^{a}$±0.38 | 22.26$^{a}$±0.38 |
| Cereal products intended for children D* | 21.52$^{a}$±0.84 | 22.40$^{a}$±0.48 | 22.32$^{a}$±0.57 | 22.18$^{a}$±0.59 | 21.72$^{a}$±0.52 | 21.31$^{a}$±0.56 |
| *Ceylon cinnamon powder* | | | | | | |
| Oat grain | 16.84$^{a}$±0.53 | 65.84$^{b}$±1.68 | 98.74$^{c}$±2.78 | 180.51$^{d}$±4,89 | 344.28$^{e}$±7.93 | 508.03$^{f}$±11.89 |
| Wheat grain | 12.39$^{a}$±0.41 | 61.18$^{b}$±1.84 | 94.09$^{c}$±2.51 | 175.37$^{d}$±4.38 | 336.72$^{e}$±9.43 | 500.83$^{f}$±14.02 |
| Oat bran A* | 6.11$^{a}$±0.25 | 55.64$^{b}$±1.52 | 88.34$^{c}$±2.22 | 176.02$^{d}$±3.99 | 334.51$^{e}$±7.89 | 494.28$^{f}$±12.87 |
| Oat bran B* | 6.43$^{a}$±0.38 | 55.93$^{b}$±1.29 | 89.15$^{c}$±2.19 | 170.46$^{d}$±4.22 | 333.36$^{e}$±8.09 | 501.62$^{f}$±11.57 |
| Wheat bran A* | 9.67$^{a}$±0.29 | 58.96$^{b}$±1.57 | 91.90$^{c}$±2.17 | 173.21$^{d}$±4.17 | 337.41$^{e}$±9.57 | 496.58$^{f}$±10.57 |
| Wheat bran B* | 10.01$^{a}$±0.33 | 58.57$^{b}$±1.26 | 91.79$^{c}$±2.21 | 172.85$^{d}$±3.97 | 333.97$^{e}$±10.17 | 495.61$^{f}$±11.25 |
| Cereal products intended for children A* | 27.89$^{a}$±1.12 | 76.67$^{b}$±2.45 | 109.51$^{c}$±2.96 | 190.51$^{d}$±3.78 | 353.11$^{e}$±6.27 | 515.72$^{f}$±10.35 |
| Cereal products intended for children B* | 25.44$^{a}$±0.99 | 72.68$^{b}$±3.02 | 104.17$^{c}$±3.22 | 182.89$^{d}$±5.11 | 340.35$^{e}$±7.46 | 497.81$^{f}$±11.43 |
| Cereal products intended for children C* | 23.87$^{a}$±0.62 | 74.15$^{b}$±2.88 | 107.68$^{c}$±3.74 | 191.48$^{d}$±4.57 | 359.09$^{e}$±8.14 | 526.71$^{f}$±9.83 |
| Cereal products intended for children D* | 21.52$^{a}$±0.84 | 72.36$^{b}$±2.09 | 105.59$^{c}$±2.66 | 188.67$^{d}$±4.71 | 354.82$^{e}$±6.95 | 520.96$^{f}$±9.45 |

*A, B, C and D represent different brands
$^{a,b}$ – statistically significant difference between mean values designated by the identical letters in rows, $p < 0.05$

**Table 3.** The HT-2 toxin content [μg kg$^{-1}$] in samples supplemented with the blueberry lyophilizate after storage at 28 ° C and 19% humidity for 14 days

| Level of supplementation<br>Sample type | Without supplementation | With 3% american blueberry lyophilizate | With 5% american blueberry lyophilizate | With 10% american blueberry lyophilizate | With 20% american blueberry lyophilizate | With 30% american blueberry lyophilizate |
|---|---|---|---|---|---|---|
| Oat grain | 90.09$^{a}$±3.76 | 89.60$^{a}$±4.01 | 90.34$^{a}$±4.31 | 90.45$^{a}$±3.71 | 87.17$^{a}$±4.13 | 85.35$^{a}$±4.45 |
| Wheat grain | 88.95$^{a}$±3.81 | 88.94$^{a}$±3.94 | 90.85$^{a}$±3.82 | 91.85$^{a}$±3.42 | 89.43$^{a}$±4.10 | 83.63$^{a}$±3.97 |
| Oat bran A* | 101.14$^{a}$±4.66 | 105.67$^{a}$±5.27 | 104.48$^{a}$±5.09 | 101.84$^{ab}$±5.06 | 98.81$^{ab}$±3.22 | 91.61$^{b}$±4.03 |
| Oat bran B* | 112.27$^{a}$±5.26 | 118.04$^{a}$±5.31 | 115.61$^{a}$±5.36 | 113.33$^{a}$±4.53 | 100.28$^{b}$±4.14 | 92.76$^{b}$±4.21 |
| Wheat bran A* | 154.41$^{a}$±5.76 | 150.96$^{a}$±7.60 | 156.72$^{a}$±8.04 | 154.07$^{a}$±7.37 | 142.69$^{a}$±7.50 | 141.17$^{a}$±6.20 |
| Wheat bran B* | 152.71$^{a}$±6.70 | 154.53$^{a}$±7.60 | 152.15$^{a}$±7.50 | 153.99$^{a}$±8.18 | 146.88$^{a}$±6.96 | 139.87$^{a}$±7.06 |
| Cereal products intended for children A* | 7.12$^{a}$±0.29 | 6.94$^{a}$±0.35 | 7.22$^{a}$±0.33 | 7.69$^{ab}$±0.42 | 8.54$^{b}$±0.38 | 9.86$^{c}$±0.47 |
| Cereal products intended for children B* | 10.32$^{a}$±0.61 | 10.61$^{a}$±0.57 | 9.94$^{a}$±0.49 | 12.74$^{b}$±0.63 | 13.54$^{bc}$±0.72 | 14.74$^{c}$±0.69 |
| Cereal products intended for children C* | 12.22$^{a}$±0.62 | 12.55$^{a}$±0.61 | 13.14$^{ab}$±0.59 | 12.83$^{ab}$±0.68 | 13.03$^{ab}$±0.64 | 14.34$^{b}$±0.68 |
| Cereal products intended for children D* | 22.33$^{a}$±0.68 | 22.79$^{a}$±0.74 | 21.74$^{a}$±0.96 | 22.54$^{a}$±0.91 | 21.73$^{a}$±1.02 | 22.24$^{a}$±0.78 |

*A, B, C and D represent different brands
a,b – statistically significant difference between mean values designated by the identical letters in rows, $p < 0.05$

**Table 4.** HT-2 toxin content [μg kg$^{-1}$] in samples supplemented with cranberry lyophilizate,after storage at 28° C and 19% humidity for 14 days.

| Level of supplementation<br>Sample type | Without supplementation | With 3% cranberry lyophilizate | With 5% cranberry lyophilizate | With 10% cranberry lyophilizate | With 20% cranberry lyophilizate | With 30% cranberry lyophilizate |
|---|---|---|---|---|---|---|
| Oat grain | 89.79$^{a}$±3.61 | 89.65$^{a}$±4.35 | 90.13$^{a}$±4.24 | 90.32$^{a}$±3.50 | 84.87 $^{ab}$±3.42 | 76.82$^{b}$±3.86 |
| Wheat grain | 88.55$^{a}$±3.22 | 88.00$^{a}$±3.17 | 90.65$^{a}$±3.69 | 92.50$^{a}$±3.71 | 87.20$^{a}$±4.09 | 84.63$^{a}$±3.33 |
| Oat bran A* | 115.08$^{ac}$±5.44 | 117.74$^{ab}$±4.53 | 114.13$^{ac}$±5.39 | 112.84$^{ac}$±4.21 | 104.06 $^{c}$±4.98 | 89.11 $^{d}$±3.67 |
| Oat bran B* | 118.81$^{a}$±4.66 | 121.09$^{a}$±2.82 | 119.44$^{a}$±5.09 | 117.61$^{a}$±3.81 | 106.21 $^{b}$±5.08 | 87.66 $^{c}$±4.02 |
| Wheat bran A* | 143.62$^{a}$±3.95 | 141.67$^{a}$±7.08 | 142.28$^{a}$±5.82 | 141.19$^{a}$±5.32 | 135.04$^{a}$±5.27 | 133.06$^{a}$±5.55 |
| Wheat bran B* | 140.67$^{a}$±3.90 | 138.16$^{a}$±5.99 | 139.87$^{a}$±5.45 | 140.22$^{a}$±5.23 | 134.87$^{a}$±4.61 | 132.25$^{a}$±5.28 |
| Cereal products intended for children A* | 7.87$^{a}$±0.35 | 9.08$^{b}$±0.43 | 10.06$^{c}$±0.31 | 11.39$^{d}$±0.39 | 9.94$^{bc}$±0.35 | 12.88$^{e}$±0.44 |
| Cereal products intended for children B* | 11.71$^{a}$±0.57 | 12.26$^{a}$± 0.62 | 13.09$^{a}$±0.51 | 12.81$^{a}$± 0.39 | 12.94$^{a}$±0.45 | 10.09$^{b}$± 0.74 |
| Cereal products intended for children C* | 12.56$^{a}$±0.53 | 12.14$^{a}$± 0.52 | 11.84$^{a}$±0.54 | 12.19$^{a}$±0.45 | 12.83$^{a}$±0.59 | 10.92$^{b}$± 0.71 |
| Cereal products intended for children D* | 24.63$^{a}$±0.74 | 23.93$^{a}$± 0.76 | 24.34$^{a}$±1.14 | 24.91$^{a}$±1.07 | 25.44$^{a}$±1.12 | 25.77$^{a}$± 0.91 |

*A, B, C and D represent different brands
a,b – statistically significant difference between mean values designated by the identical letters in rows, $p < 0.05$

compared to the original value (Tab. 5). 3% supplementation resulted in a reduction ranging from 20.3% – 23.3% for cereal samples and 10.1% – 14.0% for other samples; 5% caused a reduction ranging from 22.1% – 40.5% in all samples. Favourable changes in HT-2 content in samples supplemented with a 10% addition of cinnamon were even higher, ranging from 33.3% – 56%. A 20% addition of cinnamon caused a reduction ranging from 37% – 70.4%, while 30% caused a reduction ranging from 55% – 87%. Moreover, the supplementation of products intended for children completely inhibited the formation of mycotoxins. When analyzing the changes in individual groups for this additive, the content of HT-2 toxin decreased from 67.1% – 76.1% in cereal samples, from 57.5% – 69.2% in wheat bran, from 83.4% – 87.0% in oat bran, and from 55% – 100% in products cereal for children. Correlation analysis showed a strong negative correlation in all tested samples.

Taken together, the results obtained in the current thus show that the addition of compounds with a high antioxidant potential, such as blueberries, cranberry and cinnamon, in an amount that results in an increase of the overall antioxidant potential of the food sample, may inhibit the formation of mycotoxins.

Horvath et al. [32] found that the essential oils of mint and cinnamon effectively inhibit the growth of *Fusarium mycelium*. Dambolen et al. [33] found the inhibitory effect of essential oils from basil on the production of fumonisin $B_1$ by *F. verticillioides* on maize seeds. Dambolen et al. [34] found that thymol, carvacrol and isoeugenol are most effective on the inhibition of fumonisin $B_1$ formation. Hussain et al. [35] studied the antifungal activity of some chemical compounds (benzoic acid, propionic acid and copper sulfate), herbs/spices (clove, clove oil) and plants (garlic onion) at various concentrations which were shown to inhibit growth of toxin-producing *Aspergillus flavus* and *Aspergillus parasiticus*.

In a study by Císarová and Tančinová [36] on the antifungal activity of some essential oils, oregano was found to display highest antifungal activity against *Aspergillus flavus* strains, and to inhibit the formation of aflatoxin $B_1$. Research by Heidtmann-Bemvenuti et al. [37] showed that natural antifungal agents inhibit the production of fungal biomass and their toxins, suggesting that natural compounds can be considered as alternative synthetic antifungal agents for effective inhibition of *F. graminearum* and production of trichothecenes. Ferulic acid applied at high concentrations

**Table 5.** HT-2 toxin content [μg kg$^{-1}$] in samples supplemented with cinnamon powder after storage at 28° C and 19% humidity for 14 days.

| Level of supplementation<br>Sample type | Without supplementation | With 3% ceylon cinnamon powder | With 5% ceylon cinnamon powder | With 10% ceylon cinnamon powder | With 20% ceylon cinnamon powder | With 30% ceylon cinnamon powder |
|---|---|---|---|---|---|---|
| Oat grain | 88.69$^{a}$±3.61 | 68.04$^{b}$±2.75 | 59.81$^{c}$±2.52 | 49.93$^{d}$±17.04 | 33.18$^{e}$±1.36 | 21.17$^{f}$±0.78 |
| Wheat grain | 88.05$^{a}$±4.05 | 70.18$^{b}$±3.21 | 62.74$^{c}$±2.47 | 53.63$^{d}$±1.51 | 39.71$^{e}$±1.05 | 28.94$^{f}$±1.17 |
| Oat bran A* | 98.02$^{a}$±4.26 | 85.51$^{b}$±3.04 | 58.30$^{c}$±2.07 | 42.78$^{d}$±1.34 | 31.62$^{e}$±1.14 | 16.23$^{f}$±0.64 |
| Oat bran B* | 102.83$^{a}$±4.64 | 90.17$^{b}$±4.41 | 75.04$^{c}$±3.51 | 51.36$^{d}$±1.67 | 30.47$^{e}$±0.65 | 13.38$^{f}$±0.62 |
| Wheat bran A* | 162.26$^{a}$±4.68 | 140.29$^{b}$±4.53 | 121.21$^{c}$±3.18 | 98.78$^{d}$±2.96 | 92.48$^{e}$±2.76 | 69.04$^{f}$±2.56 |
| Wheat bran B* | 158.22$^{a}$±5.47 | 136.09$^{b}$±4.43 | 102.28$^{c}$±3.89 | 91.49$^{d}$±3.72 | 72.71$^{e}$±3.62 | 48.72$^{f}$±2.04 |
| Cereal products intended for children A* | 8.28$^{a}$±0.38 | 7.22$^{b}$±0.32 | 6.45$^{c}$±0.31 | 5.71$^{cd}$±0.42 | 5.22$^{d}$±0.33 | nd |
| Cereal products intended for children B* | 10.21$^{a}$±0.42 | 9.11$^{b}$±0.25 | 7.74$^{c}$±0.31 | 6.45$^{d}$±0.31 | 5.76$^{d}$±0.41 | nd |
| Cereal products intended for children C* | 12.03$^{a}$±0.41 | 10.81$^{b}$±0.38 | 9.21$^{c}$±0.39 | 8.02$^{d}$±0.38 | 6.82$^{e}$±0.28 | 5.41$^{f}$±0.25 |
| Cereal products intended for children D* | 24.11$^{a}$±0.93 | 20.81$^{b}$±0.78 | 16.43$^{b}$±0.69 | 12.51$^{c}$±0.59 | 9.72$^{c}$±0.41 | 5.56$^{d}$±0.33 |

*A, B, C and D represent different brands
a,b – statistically significant difference between mean values designated by the identical letters in rows, $p < 0.05$
nd – non detected (<LOQ)

**Table 6.** Correlation coefficient between the antioxidant activity and measured HT-2 toxin content

| Type of supplementation<br>Sample type | Blueberry lyophilizate | Cranberry lyophilizate, | Cinnamon powder |
|---|---|---|---|
| Oat grain | -0.962 | -0.731 | -0.952 |
| Wheat grain | -0.751 | -0.683 | -0.952 |
| Oat bran A* | -0.898 | -0.963 | -0.924 |
| Oat bran B* | -0.940 | -0.942 | -0.968 |
| Wheat bran A* | -0.793 | -0,970 | -0.928 |
| Wheat bran B* | -0.956 | -0.910 | -0.930 |
| Cereal products intended for children A* | -0.955 | -0.870 | -0.943 |
| Cereal products intended for children B* | -0.924 | -0.079 | -0.961 |
| Cereal products intended for children C* | -0.842 | 0.263 | -0.950 |
| Cereal products intended for children D* | 0.220 | -0.785 | -0.948 |

(20–25 mM) inhibited the production of fumonisin by *Fusarium verticillioides* and *Fusarium proliferatum* [38].

Available literature provides data confirming not only antifungal properties of these compounds but also antibacterial. Shan et al. [39] showed the antibacterial activity of cinnamon, oregano, clove, pomegranate peel and grape seed extracts against *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica* in raw pork. A recent study by Tamkute [40] also showed a high effectiveness of the addition of cranberry water and alcohol extracts against pathogenic *Listeria monocytogenes,* and some other bacteria (*Brochothrix thermospacta, Pseudomonas putida, lactic acid bacteria*, etc.) in pork meat and meat products during storage in cold stores for 16 and 40 days.

## CONCLUSIONS

The natural products used in study (blueberry, cranberry, cinnamon) inhibited the formation of mycotoxins from the group of trichothecenes. A direct correlation was also found between mycotoxin formation and antioxidant potential. It should also be noted that the addition of these compounds may affect the flavour and aroma of the final product. Therefore, assessing the consumer acceptance will be required before releasing such a food product.

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