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Deoxynivalenol reduction through the processing of whole grain cookies

Redução de desoxinivalenol através do processamento de biscoitos integrais[1]

Reducción de desoxinivalenol mediante el procesamiento de galletas integrales

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Abstract

Whole-grain products are increasingly integrated into consumers diets due to the presence of the bran fraction that concentrates phenolic and antioxidant compounds. However, the bran

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may also contain mycotoxins, toxic compounds, harmful to health and undesirable in food. For wheat, the mycotoxin deoxynivalenol (DON) is the most prevalent. The objective of this work was to evaluate the reduction of DON after the processing of whole wheat cookies. Five commercial wheat, harvest 2017/18, naturally contaminated by *Fusarium* spp. with DON content higher or as established to Brazilian legislation (1000 ppb), were provided by Embrapa Trigo from Passo Fundo/RS. The cookies were baking mostly using flour, sugar and fat. The detection of DON was performed using the ELISA immunoenzymatic assay. Cookie production reduced the contamination of DON in the samples, and two products were in compliance with Brazilian legislation. The reduction of contamination by DON may have occurred due to the dilution effect by the ingredients in samples 1 and 3. The reduction of contamination by DON of the other samples (2, 4 and 5) was attributed to thermal degradation due to the baking temperature. Cookie processing is a complementary strategy to reduce the DON content in wheat products.

Keywords: Mycotoxins; Wheat bran; Whole wheat flour; Maximum tolerated limits and ELISA.

Resumo

Os produtos integrais estão cada vez mais integrados na dieta dos consumidores devido a presença da fração de farelo que concentra compostos fenólicos e antioxidantes. Entretanto essa fração também pode conter micotoxinas, compostos tóxicos, prejudiciais à saúde e indesejáveis em alimentos. Dentre os contaminantes do trigo, a micotoxina desoxinivalenol (DON) é considerada a mais prevalente. O objetivo do trabalho foi avaliar a redução de DON após o processamento de biscoitos integrais. Cinco amostras de trigo comercial, safra 2017/18, naturalmente contaminadas por *Fusarium* spp. com conteúdo de DON acima ou dentro do estabelecido pela legislação brasileira (1000 ppb), foram fornecidas pela Embrapa Trigo de Passo Fundo/RS. Os biscoitos foram elaborados utilizando farinha, açúcar e gordura. A detecção de DON foi feita através do ensaio imunoenzimático ELISA. A produção dos biscoitos reduziu a contaminação de DON nas amostras, sendo que dois produtos apresentaram valores em conformidade com a legislação brasileira. A redução da contaminação por DON pode ter ocorrido pelo efeito de diluição pelos ingredientes nas amostras 1 e 3. A redução no conteúdo de DON das demais amostras (2, 4 e 5) foi atribuída a degradação térmica, devido a temperatura de forneamento. O processamento de biscoitos mostra-se uma estratégia complementar para reduzir o teor de DON em produtos derivados de trigo.

Palavras-chave: Micotoxinas; Farelo de trigo; Farinha de trigo integral; Limites máximos tolerados e ELISA.

Resumen

Los productos integrales se integran cada vez más en las dietas de los consumidores debido a la presencia de la fracción de salvado que concentra compuestos fenólicos y antioxidantes. Sin embargo, esta fracción también puede contener micotoxinas, compuestos tóxicos, nocivos para la salud e indeseables en los alimentos. Entre los contaminantes del trigo, la micotoxina deoxinivalenol (DON) se considera el más prevalente. El objetivo del trabajo fue evaluar la reducción de DON luego del procesamiento de galletas integrales. Cinco muestras de trigo comercial, cosecha 2017/18, contaminadas naturalmente por *Fusarium* spp. contenido de DON por encima o según la legislación brasileña (1000 ppb), fueron proporcionados por Embrapa Trigo de Passo Fundo/RS. Las galletas se elaboraron con harina, azúcar y grasa. La detección de DON se realizó mediante el ensayo inmunoenzimático ELISA. La producción de las galletas redujo la contaminación de DON en las muestras y dos productos presentaron valores de acuerdo con la legislación brasileña. La reducción de la contaminación por DON puede haber ocurrido debido al efecto de dilución de los ingredientes en muestras 1 y 3. La reducción en el contenido de DON de las otras muestras (2, 4 y 5) se atribuyó a la degradación térmica, debido a la temperatura de suministro. El procesamiento de galletas es una estrategia complementaria para reducir el contenido de DON en los productos de trigo.

Palabras clave: Micotoxinas; Salvado de trigo; Harina integral; Límites máximos tolerados y ELISA.

1. Introduction

Use Wheat (*Triticum aestivum* L.) is an important cereal due to its forms of consumption (human and animal nutrition) and is considered an energy source for humans, as it is present in the food as a raw material for bread, pasta, cookies and cakes (Tibola; Fernandes; Guarienti, 2016). Whole grain products are increasingly integrated into consumers diets due to the knowledge of their properties and beneficial health effects related to the presence of the bran fraction that concentrates phenolic and antioxidant compounds (Liu et al., 2015). However, it is also known that bran can also contain mycotoxins, toxic compounds products of the secondary metabolism of toxigenic fungi, harmful to health, undesirable in food. Mycotoxins are mainly distributed in the surface layers of the wheat grain, thus

concentrated in the bran fraction. For wheat, deoxynivalenol (DON) is the most prevalent mycotoxin (Tibola et al. 2015). Known as vomitoxin, in chronic intake of foods with high concentrations, DON can inhibit protein synthesis and induces immunosuppressive effects. As a result, health effects are vomiting, temporary acute nausea, diarrhea, abdominal pain, headache, dizziness, fever, rejection of food and weight loss in animals, among others (Liu et al. 2016).

Reducing contamination from food processing can be a viable alternative, as DON content can be reduced by ingredients dilution effect, as well as by baking temperature. The ingredients dilution contributes to the degradation of the DON, as it changes the moisture and the chemical composition of the product (Schaarschmidt; Fauhl-Hassek, 2018). The heat treatment can be influenced by the product shape, ingredients and quality, combined with processing conditions such as temperature and time (Generotti et al. 2017). Cookies are good food matrix to be studied since the reduced weight and diameter provides greater heat exchange surface when compared to breads, which facilitates the homogeneous incidence of temperature in the product (Suman et al., 2012). The current Brazilian legislation establishes maximum tolerated limits of DON in whole wheat flour of 1000 ppb and bakery products of 750 ppb (ANVISA, 2017).

Then, the objective of the study was to reduce deoxynivalenol through the processing of whole wheat cookies, in order to obtain products in compliance with the current Brazilian legislation for DON content. This work was presented at the Brazilian Online Cereals and Bakery Congress (CBCP) 2020 and selected by the organizing committee for publication in the RSD.

2. Methodology

2.1 Material

Five samples of commercial wheat, harvest 2017/2018, naturally contaminated by *Fusarium* spp., were provided by Brazilian Agricultural Research Corporation - Embrapa Wheat from Passo Fundo/RS.

Whole wheat flour was obtained by milling the whole grain in a refrigerated mill (Marconi, MA090-CFT, Brazil). To avoid cross-contamination between samples, wheat was ground from the lowest to the highest DON content.

2.2 Cookies making procedure

The cookies were produced according to Protonotariou et al. (2016) with modifications. The ingredients used in the formulation of the cookies were: 100.0 g of whole wheat flour, 35.3 g of vegetable fat, 28.3 g of granulated refined sugar, 1.1 g of salt, 1.3 g of sodium bicarbonate and 12.5 ml distilled water.

Initially, vegetable fat (35.3 g), sugar (28.3), salt (1.1 g), distilled water (12.5 mL) and sodium bicarbonate (1.3 g) were mixed (Kitchen Aid, USA) for 3 min at speed 2 until a cream was formed. Then, the whole wheat flour (100 g) was added and mixing for an additional 3 min at speed 4. The dough was shaped at 7 mm of thick and 6 cm of diameter. The cookies were baked in an electric oven at 190 °C for 12 min. and analyzed 30 min after baking.

2.3 Spread factor and moisture content of cookies

The diameter (width) and thickness of the cookies were recorded with a digital Pachymeter, and the spread factor was determined according to Mancebo, Picón and Gómez (2015), as a ratio to the thickness of the cookies.

Cookies were manual grounded, and the moisture content was determined in an oven with air circulation at 105 ºC for 4 h, according to AACCI method 44-15.02 (2010).

2.4 Mycotoxin detection

The detection of DON in whole wheat flour and cookies was carried out using the immunoenzymatic assay ELISA (AgraQuant®) as recommended by the manufacturer (Romer Labs Methods, 2014). A fraction (10.0 g) of whole wheat flour or grounded cookies was diluted in reverse osmosis water (200 mL), homogenized with constant agitation for 3 min, followed by filtration on filter paper. The filtrate was used for DON analysis in ELISA. The absorbance was determined in a microplate reader (Mindray, MR-96A, China) with a 450 nm filter.

2.5 Deoxynivalenol stability

The dilution effect of the ingredients was determined by calculating DON (µg/kg) in dry weight (d.b.) considering the whole wheat flour and cookies. Equation 1 is the theoretical

DON value in the cookies based on flour DON content. Equation 2 is the real DON values in the cookies. Real value higher of theoretical value the reduction is by dilution effect. Real value inferior then theoretical the reduction effect is by thermal degradation.

$$THEORETICAL\,VALUE = \frac{flour\ DON * flour\ weight}{(flour\ mass) + (mass\ of\ ingredients)} \quad (1)$$

$$REAL\,VALUE = \frac{cookie\ DON}{1\ g\ cookie * (1 - cookie\ moisture)} \quad (2)$$

Of which: flour mass (g) = flour weight (g) - flour moisture

mass of ingredients (g) = weight of dry ingredients (g)

2.6 Statistical analysis

The analyzes were conducted in duplicate and the results submitted to the analysis of variance (ANOVA) at the 5% level of significance, followed by the Tukey test with 95% confidence interval, for comparison of means.

3. Results and Discussion

DON contamination can reach high levels in wheat crop due to environmental conditions in Brazil. DON content (µg/kg) of whole wheat flour and whole wheat cookies analyzed are shown in Table 1, as well as the percentage of mycotoxin reduction. The DON content of 3 flours sample was higher than allowed by Brazilian legislation. The high DON content can be related to the climate of the harvested period (2017/2018) – excessive rain during flowering – causing a high incidence of *Fusarium* spp. As a result, high mycotoxin content can be produced.

Table 1 - DON content in whole wheat flour and whole wheat cookies.

Sample[1]	DON (µg/kg)		Reduction (%)	DON dry weight (µg/kg)	
	Flour	Cookie		Theoretical	Real
1	566.3^{e}	481.1^{b}	15.0	354.6	491.7
2	799.5^{d}	46.4^{c}	94.2	523.1	47.4
3	1423.7^{c}	999.1^{a}	29.8	931.7	1019.8
4	2362.6^{b}	1050.3^{a}	55.5	1552.2	1072.3
5	3890.9^{a}	827.6^{ab}	78.7	2553.1	841.8

[1]Different letter in the column differs by the Tukey test at 5% probability.
Source: Authors (2020).

In Table 1 is possible to observe the reduction in the content of DON in the cookies produced in relation to whole wheat flour from the same sample. Reduction of DON content of 2, 4 and 5 samples are related to thermal degradation since real value decreased compared to its theoretical counterpart. Most contaminants resist food processing temperatures in the range of 80 to 121 ºC (Kabak, 2009) therefore, the application of high temperatures (above 150 °C) can assist in the degradation of those contaminants. Although DON is a thermostable toxin, drastic heating can partially degrade it, as evidenced by Vidal et al. (2015), Scudamore et al. (2009) and Suman et al. (2012). Vidal et al. (2014) observed significant differences in DON content after bread baking at temperatures above 170 ºC. Generotti et al. (2017) reduction 68% of DON content in whole wheat cookies baked at 200 ºC for 8 min. Thus, it can suggest the temperature of about 190 ºC for 12 min is effective to reduce DON content in cookies.

In addition to the processing conditions, product characteristics such as size and shape influence the contaminant contents, as it affects the incidence of heat in the food (Generotti et al. 2017). According to Vidal et al., (2014) the maximum temperature inside the product (98 ºC), independent of oven temperature, while outside of products can reach up to 30 ºC higher. The authors noticed that the size of the product has a relevant effect on the DON reduction after cooking, due to the heat easily transfer in small products (Vidal et al. 2015). Thus, the heat exchange surface of the cookies is significantly greater in relation to the breads (Suman et al. 2012) which leads to the temperature being reached homogeneously, causing a greater effect to the heat treatment. Samples 2, 4 and 5 had the lowest values of diameter (6.81, 6.85 and 6.86 cm respectively) and spread factor (2.89, 3.00 and 3.14 respectively), demonstrating a relationship between product size and reduction in DON content (94.2, 55.5 and 78.7% respectively).

DON reduction of samples 1 and 3 can be attributed to dilution effects of the ingredients, as the real value is higher than its theoretical counterpart. It can be related to the initial DON content, since the degradation of contaminants is dependent on the initial content in the samples (Vidal et al. 2015), and also the moisture content and the pH of the mass (Kabak, 2009). Sample 1 had the lowest initial DON content (566.3 µg/kg) and, therefore, obtained the smallest reduction (15%). On the other hand, it is known that the pH of the dough (expressed as sodium bicarbonate content) helps to reduce the toxin, especially in samples with high initial concentrations (Generotti et al., 2017), which occurred in sample 3, which had a high initial DON content (1423 µg/kg) and may have been influenced by the added bicarbonate content (0.7% in relation to the mass).

Whole wheat cookies from samples 1 and 2 complied with the Brazilian legislation. The baking process can be considered an effective alternative to DON reduction; however, it is important to observe the initial DON content in the flour.

4. Conclusion

The production of whole wheat cookies caused a significant reduction in deoxynivalenol content and can be used as a complementary strategy to reduce the content of mycotoxin in bakery products. Two samples of cookies were presented in accordance with the Brazilian mycotoxin legislation in bakery products. Therefore, further studies need to be performed in order to evaluate the influence of individual ingredients in the reduction of deoxynivalenol content.

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