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Deoxynivalenol in the wheat milling process and wheat-based products and daily intake estimates for the Southern Brazilian population

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ABSTRACT

Fusarium head blight of wheat is caused by the *Fusarium* species that produces mycotoxins, such as deoxynivalenol (DON). The distribution of DON in wheat products can lead to high economic and health impacts. The objective of this study was to evaluate the natural distribution of DON in the wheat milling process and wheat-based products, as well as the daily intake estimates for the Southern Brazilian population. The fractions of wheat grains (milled wheat, finished flour and bran) were produced in a mill. Additionally, wheat-derived products, such as pasta, bread and crackers were analyzed. The bran fraction had the highest mean concentration of DON (2278 $\mu g\ kg^{-1}$), followed by milled wheat and finished flour (1895 $\mu g\ kg^{-1}$ and 1305 $\mu g\ kg^{-1}$). The distribution factor in the finished flour (69%) fraction demonstrates that DON was reduced when compared to milled wheat, by contrast of bran fraction that presents higher DON levels (120%). A percentage of 35% bran, 35% finished flour and 30% milled wheat samples would not be in compliance with future Brazilian regulations for DON levels. From the wheat-based products analyzed, 17% of whole bread and 10% of salted cracker products were contaminated with DON, with a median of 437 $\mu g\ kg^{-1}$ and 624 $\mu g\ kg^{-1}$, respectively. The finished flour was the fraction that most contributes to the daily intake of DON in Southern Brazil, representing 89.6% of the provisional maximum tolerable daily intake.

1. Introduction

Fusarium head blight (FHB) caused by different species of *Fusarium*, is a serious worldwide problem in wheat grains. This disease causes agricultural damage by reducing harvest yield due to poor grain quality and affects processed products from infected grains (Parry, Jenkinson, & Mcleod, 1995; Savi et al., 2015; Scussel, Beber, & Tonon, 2011). Moreover, it presents a threat to food safety because of the accumulation of mycotoxins in wheat grains and their products, especially deoxynivalenol (DON), considered to be the most important wheat hazard (McMullen, Jones, & Gallenberg, 1997). The accumulation of DON in human and animal bodies after ingestion of contaminated food can induce development of acute and chronic effects, such as immunosuppression, neurotoxicity, embryotoxicity and teratogenicity (Pestka, 2007; Rotter, Prelusky, & Pestka, 1996; Wijnands & Van Leusden, 2000).

The toxicity of DON has led many countries to set up regulations for its control in wheat grains and their products intended for human or animal consumption. In Brazil, the maximum limits established in 2012 for milled wheat, whole flour and wheat bran were 2000 $\mu g\ kg^{-1}$ and for wheat flour, pasta, crackers and biscuits were 1750 $\mu g\ kg^{-1}$ (ANVISA, 2011). These levels will be progressively reduced and in 2017 will be set at 1000 $\mu g\ kg^{-1}$ and 750 $\mu g\ kg^{-1}$ for the products stated above, respectively (ANVISA, 2013). For unprocessed wheat grain, the maximum limit will be set at 3000 $\mu g\ kg^{-1}$ in 2017 (ANVISA, 2013). It is necessary to mention that no regulation limits have been established for animal feed in Brazil. Nowadays, the European Commission has established the limit of DON equal to 1250 $\mu g\ kg^{-1}$ for unprocessed cereals and

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1750 μg kg^{-1} for unprocessed durum wheat (EC, 2006; 2007). For cereals intended for direct human consumption, cereal flour, bran and germ as end products marketed for direct human consumption as well as pasta (dry), the limit of DON is 750 μg kg^{-1}. In addition, bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals are equal to 500 μg kg^{-1}.

Most of the wheat harvested in the world is subjected to milling, the procedure by which whole wheat grains are ground and their components separated into milled fractions based on particle size. However, the wheat does not have tissue structures within the kernel to act as an effective barrier against fungal invasion and the subsequent synthesis of mycotoxins and the entire milling fraction could be contaminated as a result (Pinson-Gadais et al., 2007).

The natural occurrence of DON in wheat and its products has been reported worldwide, including in Brazil (Bensassi, Zaied, Abid, Hajlaoui, & Bacha, 2010; Mishra, Ansari, Dwivedi, Pandey, & Das, 2013; Santos et al., 2013; Savi, Piacentini, Tibola, & Scussel, 2014). Moreover, DON can be resistant to the wheat milling process and remain in by-products such as spaghetti (Visconti, Haidukowski, Pascale, & Silvestri, 2004), wheat flour and bran (Rodrigues & Naehrer, 2012; Tibola, Fernandes, Guarienti, & Nicolau, 2015), wheat germ and wheat germ oil (Giménez et al., 2013) and bread (Pacin, Ciancio Bovier, Cano, Taglieri, & Hernandez Pezzani, 2010; Zhang & Wang, 2014).

The distribution of DON in wheat fractions and their products can enter in the food chain of animals and humans directly and negatively impact the economy and health. In addition, the distribution of the wheat milling fractions, usually intended for animal feed such as bran, may also present useful support in the future consideration of the legal maximum limit (Tibola et al., 2015). Therefore, the objective of this study was to evaluate the natural distribution of DON in the wheat milling process and in wheat-based products, as well as daily intake estimates for the Southern Brazilian population.

2. Materials and methods

2.1. Sample characterization

Wheat grain samples from different cultivars harvested during the 2014 crop season from Southern Brazil were used. Wheat grains were cleaned and dried in the storage units. The milling process of samples was performed by Embrapa Wheat (Brazilian Agricultural Research Corporation). Each sample (1000 g) was milled in the Laboratory Mill 3100® (Perten, Sweden), to obtain the milled wheat fraction. The same set of samples, composed of 5000 g, were conditioned to a 14% moisture content and milled using a pilot-scale mill Quadrumat Senior® (Brabender, Germany), with a standard setting for hard wheat (AACC, 2000). This milling process produced the following fractions: milled wheat, finished flour (reduction and break flour) and bran (the outer layers of wheat kernel). The milled wheat, finished flour and bran were weighed and mixed separately, before the division of 200 g each sample for mycotoxin analysis (60 samples). Samples were packed in polyethylene bags and stored at 8 °C for DON analysis in the Laboratory of Mycotoxicology and Food Contaminants, Food Science and Technology Department, Center of Agricultural Sciences at the Federal University of Santa Catarina, Brazil. Additionally, wheat-based products, such as pasta of type semolina, eggs and common pasta, sweet common and whole bread and sweet and salted crackers, were purchased from the local retail market in the same period. The products were weighed (200 g each sample) and mixed separately for mycotoxin analysis (30 samples per group, totaling 90 samples).

2.2. Chemicals and reagents

DON standards were supplied by Sigma Aldrich Chemicals (St Louis, MO, USA). The solutions were prepared in acetonitrile at a concentration of 1 mg mL^{-1} and stored at −20 °C until use. Working standard solutions, ranging from 0.15 to 10 μg mL^{-1}, were prepared from suitable dilutions of the stock solution in the mobile phase acetonitrile:water (10:90, v/v) and stored at 4 °C. The solvents acetonitrile and methanol were obtained from Vetec (Duque de Caxias, RJ, Brazil) at LC grade. Water was obtained from a Milli-Q system on 18.2 MΩ/cm (Millipore, Bedford, MA, USA). For the sample clean-up step, an immunoaffinity column of DON-Test (Vicam, Milford, MA, USA) was used according to the manufacturer procedures.

2.3. DON determination

Milled wheat samples were analyzed using the immunoaffinity columns for the cleaning step, according to the Vicam protocol DON Test, N_o G1005 USA (Vicam, 2013), with some modifications. In summary, 25 g of each sample was ground in an industrial blender jar with 100 mL of LC grade water. The mixture was blended for 30 s, followed twice by filtration and cleaning using the immunoaffinity column (DON Test HPLC). This column was first conditioned with 1 mL of LC grade water and the filtrate sample (1 mL) was then loaded in a flow rate of one drop per second. After washing the column with 2.5 mL of LC grade water, the toxin was slowly eluted with 2 mL of 100% LC grade methanol. The eluate was evaporated using a heating block device at 40 °C with a gentle nitrogen stream and the dry residue was redissolved in 200 μL of mobile phase acetonitrile:water (10:90, v/v).

2.4. HPLC-DAD analysis

The determination of DON levels was carried out by high performance liquid chromatography (HPLC), a Shimadzu (Kyoto, Japan), equipped with an isocratic pump (LC-20AT), column oven (CTO-20A), prominence communication bus module (CBM-20A), degasser (DGU-20A), autosampler (SIL-20A) and a detector diode array (DAD) (SPD-M 20A). Chromatographic separations were performed on a C18 reversed-phase column (250 × 4.6 mm, 4μ), Synergi Fusion-RP 80A (Phenomenex, Torrance, USA). The column temperature was maintained at 30 °C. The isocratic mobile phase consisted of acetonitrile:water (10:90, v/v). The retention time of DON was approximately 12.2 ± 0.5 min. The extract (20 μL) was injected into the LC/DAD/UV System set at a wavelength equal to 218 nm and the mobile phase was delivered in a constant flow rate of 1 mL min^{-1}. Quantification of DON levels was performed by measurement of the peak area at DON retention time compared with the standard solutions used for the calibration curve.

2.5. Validation of analytical method

Validation of the analytical method was based on the criteria of linearity, selectivity, sensitivity, reproducibility, limit of detection (LOD) and quantification (LOQ), and recovery. The linearity of the method was confirmed using the calibration curve. The calibration curves were constructed with different DON concentrations from 0.15 to 10 μg mL^{-1}. Linearity was shown with the correlation coefficient (R^2) through linear regression analysis. The selectivity of the method was determined through the comparative analysis of non-spiked blank wheat samples and spiked wheat samples at 250, 1000 and 1500 μg kg^{-1} of DON. The sensitivity of the method was assessed using the LOD (signal-to-noise - S/N ratios of 1/3) and LOQ (S/N 1/10). The recovery process was set by spiking DON-free samples of wheat with DON concentrations of 250, 1000 and