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Enzyme bread improvers affect the stability of deoxynivalenol and deoxynivalenol-3-glucoside during breadmaking

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ABSTRACT

The stability of deoxynivalenol (DON) and deoxynivalenol-3-glucoside (DON-3-glucoside) during the breadmaking process was studied. Some enzymes used in the bakery industry were examined to evaluate their effects on DON and DON-3-glucoside. The level of DON in breads without added enzymes was reduced (17–21%). Similarly, the addition of cellulase, protease, lipase and glucose-oxidase did not modify this decreasing trend. The effect of xylanase and α-amylase on DON content depended on the fermentation temperature. These enzymes reduced the DON content by 10–14% at 45 °C. In contrast, at 30 °C, these enzymes increased the DON content by 13–23%. DON-3-glucoside levels decreased at the end of fermentation, with a final reduction of 19–48% when no enzymes were used. However, the presence of xylanase, α-amylase, cellulase and lipase resulted in bread with greater quantities of DON-3-glucoside when fermentation occurred at 30 °C. The results showed that wheat bran and flour may contain hidden DON that may be enzymatically released during the breadmaking process when the fermentation temperature is close to 30 °C.

1. Introduction

Deoxynivalenol (DON), also known as vomitoxin, is one of the most common contaminants in cereals (Cano-Sancho, Valle-Algarra, et al., 2011). DON can be found at relatively high concentrations in wheat and wheat-containing products (such as bread and pasta) (Cano-Sancho, Valle-Algarra, et al., 2011). In addition, wheat and wheat-containing products are considered to be the major source of human intake of DON (Cano-Sancho, Gauchi, Sanchis, Marín, & Ramos, 2011). Although DON is not classifiable by the International Agency for Research on Cancer (IARC, 1993) due to its carcinogenicity to humans, DON has been linked to human gastroenteritis (Pestka, 2010).

Wheat grains contaminated with DON may also contain deoxynivalenol-3-glucoside (DON-3-glucoside), a plant metabolite of DON (Berthiller et al., 2009). Reported levels of DON-3-glucoside are variable; however, the ratio of DON-3-glucoside/DON concentrations is similar among assays, ranging from 10 to 30% (Berthiller et al., 2009; Dall'Asta, Dall'Erta, Mantovani, Massi, & Galaverna, 2013). Moreover, Berthiller et al. (2011) have shown that DON-3-glucoside can be hydrolysed to DON by several lactic acid bacteria that may be present in the intestines. Thus, the FAO/WHO Expert Committee (JEFCA) considered DON-3-glucoside to be an additional contributing factor to total dietary exposure to DON (JEFCA, 2010).

Due to the high presence of DON and DON-3-glucoside in raw wheat, studying the stability of DON and DON-3-glucoside during the breadmaking process is critical. Contradictory reports exist regarding the fate of DON during this process. First, results on the effect of fermentation on DON are contradictory: while some studies have suggested that DON concentrations are reduced during fermentation (Neira, Pacin, Martínez, Moltó, & Resnik, 1997; Zachariasova, Vaclavikova, Lacina, Vaclavik, & Hajslova, 2012), other studies have shown that DON concentrations significantly increase after fermentation (Bergamini et al., 2010; Lancova et al., 2008; Vidal, Morales, Sanchis, Ramos, & Marín, 2014). Studies that have examined DON concentrations after baking are also contradictory: some studies have observed reductions in DON levels (Bergamini et al., 2010; Neira et al., 1997), while other studies have reported no changes or even increases in DON levels (Simsek,

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Burgess, Whitney, Gu, & Qian, 2012; Zachariasova et al., 2012). However, inconsistencies may exist because these studies have been conducted on different scales: some studies have been conducted in laboratories, while other studies have been conducted at the industrial level (Bergamini et al., 2010). Moreover, Vidal, Sanchis, Ramos, and Marín (2015), using small items, demonstrated that DON levels may be reduced only in the external part of the loaves due to the reduced heat transmission. Thus, the size of the baked items may also provide an explanation for the inconsistent results reported for baking studies (Vidal, Morales, et al., 2014). Four publications show also contradictory results for DON-3-glucoside after the fermentation and baking steps (Generotti et al., 2015; Suman, Manzitti, & Catellani, 2012; Vidal, Marín, Morales, Ramos, & Sanchis, 2014; Vidal, Morales, et al., 2014; Zachariasova et al., 2012). Recently, Vidal et al. (2015) showed that DON-3-glucoside may be released under mild baking conditions of temperature and time (for instance, 140 °C for 35 min or 200 °C for less than 10 min) but reduced under harsher baking conditions (i.e., longer periods of time and higher temperatures).

The increases in DON and DON-3-glucoside concentrations reported during breadmaking may be due to enzymatic activity (Simsek et al., 2012; Vidal, Morales, et al., 2014). Enzymes may hydrolyse mycotoxins bound to carbohydrates or to other components related to the ingredients of the recipe formulations causing an increase in mycotoxin concentrations at the end of the breadmaking process. For example, Zhou, Schwarz, He, Gillespie, and Horsley (2008) detected higher DON levels in barley samples after treatment with protease, xylanase and cellulase. Simsek et al. (2012) obtained the same result for wheat samples after they were treated with xylanase. Finally, Zachariasova et al. (2012) found that α-amylase caused no changes in DON-3-glucoside levels when malt samples were treated for more than 6 h.

Given that hydrolytic enzymes may affect the release of DON during breadmaking, the objective of this study was to assess the effects of different enzymes (xylanase, α-amylase, cellulase, protease, lipase and glucose oxidase that are commonly used in breadmaking) on DON and DON-3-glucoside levels during bran bread production.

2. Materials and methods

2.1. Initial levels of DON and DON-3-glucoside in flour and bran

Flour and bran wheat were purchased from a flour mill in Lleida (Spain) and were analysed for natural DON and DON-3-glucoside contaminations. The initial DON concentration in the flour was 251 ± 30 μg/kg (n = 3); DON-3-glucoside was not detected. In the bran, DON and DON-3-glucoside concentrations were 2004 ± 72 and 579 ± 61 μg/kg (n = 3), respectively.

2.2. Dough preparation and baking

A flour + bran mix was prepared (200 g of bran/1000 g of flour) and used for the bread experiments. Therefore, the concentrations of DON and DON-3-glucoside were 651 ± 13 and 137 ± 23 μg/kg (n = 6), respectively, in the mix of flour + bran.

To each flour + bran mix (156 g), 2.3 g of salt, 4.7 g of sucrose, 4.7 g of lard and 6.2 g of commercial compressed yeast (*Saccharomyces cerevisiae*, Levanova, Lesaffre Ibérica, S.A., Spain) were added. The dough was obtained by adding 83 mL of water to the mixture. Different doughs were prepared, containing six different enzymes (xylanase, α-amylase, cellulase, protease, lipase and glucose oxidase) plus a control. The enzyme concentrations were adjusted according to breadmaking standards: 1 U of xylanase/g flour (Oliveira, Telis-Romero, Da-Silva, & Franco, 2014), 10 U of α-amylase/g flour (Kim, Maeda, & Morita, 2006), 35 mU of cellulase/g flour (Haros, Rosell, & Benedito, 2002), 10 U of protease/g flour (Harada, Lysenko, & Preston, 2000), 1 U of lipase/g flour (Moayedallaie, Mirzaei, & Paterson, 2010) and 10 U of glucose oxidase/g flour (Hanft & Koehler, 2006). The enzymes were added in powder form. Moreover, the second fermentation was conducted separately at 30 or 45 °C. Thus, 14 treatments were tested in the study, and the experiment was repeated three times.

Dough was manually kneaded until it was held together with a non-sticky, smooth and satiny appearance and had optimum handling properties. Rounded pieces weighing 250 g each were prepared. From this point, thermoprobes (Thermo Bouton, Proges Plus, France) were used in the dough to record fermentation and baking temperatures; specifically, probes were placed in the centre of the loaf and close to the surface. Doughs were covered with a damp cloth, and the first fermentation was conducted at 30 °C for 15 min. Next, the pieces were placed in moulds, where the dough further fermented for 1 h at 30 or 45 °C. After fermentation, a sample of 25 g was taken from each proofed dough. Samples were lyophilized and stored at −20 °C until mycotoxin analysis. The proofed doughs were then baked in an oven (Eurofred PE46SVR, Eurofred, Spain). Baking conditions were 180 °C and 75 min. Such conditions were used to obtain suitable bread based on previous experiments. After baking, a representative sample was taken, lyophilized and stored at −20 °C until the analyses were performed.

2.3. Chemicals, reagents and enzymes

Mycotoxin (DON and DON-3-glucoside) standards were supplied by Sigma (Sigma-Aldrich, Alcobendas, Spain). Acetonitrile, methanol and ethanol were purchased from J.T. Baker (Deventer, The Netherlands). All solvents were LC grade. 3,5-Dinitrosalicylic acid (DNS) (⩾98%), sodium azide (⩾99.5%), starch (from potato), *o*-dianisidine (peroxidase substrate), Triton X-100 (laboratory grade), copper (II) acetate (⩾99.5%), caseinate (from bovine milk) and trichloroacetic acid (⩾99.0%) were supplied by Sigma. Malic acid (⩾99%), 2,2,4-trimethylpentane (⩾99.5%) and acetic acid (100%) were supplied from VWR Prolabo (Llinars del Vallès, Spain). Sodium hydroxide (⩾99.5%), sulphuric acid (⩾96%) and sodium chloride (⩾99.5%) were supplied by Fisher Bioreagents (New Jersey, USA). Sodium and potassium tartrate (⩾99%) and Tris buffer (reagent grade) were supplied by Scharlau (Barcelona, Spain). Sodium carbonate (⩾99.5%) and Folin's reagent were supplied by Panreac (Castellar del Valles, Spain). Filter paper (Whatman No. 1) was purchased from Whatman (Maidstone, UK). Immunoaffinity chromatography (IAC) columns for DON (DONPREP®) extract clean-up were purchased from R-Biopharm (Rhone LTD Glasgow, UK). Pure water was obtained from a Milli-Q apparatus (Millipore, Billerica, MA, USA). The six enzymes used in the study, namely, xylanase (from *Trichoderma longibrachiatum*), α-amylase (*Aspergillus oryzae*), cellulase (*Aspergillus niger*), protease (*Aspergillus oryzae*), lipase (*Aspergillus niger*) and glucose oxidase (*Aspergillus niger*), were purchased from Sigma.

2.4. Preparation of mycotoxin standard solutions

DON standard solution was dissolved in ethanol at a concentration of 10.0 μg/mL and stored at 4 °C. The concentration in the stock solution was confirmed by UV spectrometry according to the AOAC Official methods of analysis. Working standards (2.5, 1.0, 0.5, 0.1 and 0.05 μg/mL) were prepared by appropriate dilution of known volumes of the stock solution with the mobile phase and were used to obtain calibration curves in the appropriated chromatographic system. The DON-3-glucoside standard was dissolved in acetonitrile at a concentration of 10.0 μg/mL and stored at 4 °C