Deoxynivalenol reduction during the frying process of turnover pie covers

M. Samar a, S.L. Resnik a,b, H.H.L. González c,d, A.M. Pacin b,e,f, M.D. Castillo e

a Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
b Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina
c Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina
d Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Buenos Aires, Buenos Aires, Argentina
e Fundación de Investigaciones Científicas Teresa Benedicta de la Cruz. Luján, Provincia de Buenos Aires, Argentina
f Universidad Nacional de Luján, Luján, Provincia de Buenos Aires, Argentina

Received 11 May 2006; received in revised form 7 August 2006; accepted 14 August 2006

Abstract

The effect of the frying process on deoxynivalenol contamination was evaluated. Deoxynivalenol naturally contaminated flour (1200 μg/kg) and fortified flour artificially contaminated (260 μg/kg) were used to prepare turnover pie dough covers. Frying was performed at three temperatures (169 °C, 205 °C and 243 °C) for different times. The final time for cooking at every temperature was established by measuring the colour during the frying process. Deoxynivalenol reduction was greater in the artificially contaminated samples (>66% at 169 °C, 43% at 205 °C and 38% at 243 °C). For the level of 1200 μg/kg, the average percentage of deoxynivalenol reduction, based on medians, was 28% when the dough covers were fried at 169 °C, 21% at 205 °C and 20% at 243 °C.

Keywords: Deoxynivalenol; Mycotoxin; Colour; Turnover pie dough covers

1. Introduction

Certain products such as wheat flour are susceptible to be contaminated with deoxynivalenol (DON) (Samar, Ferro Fontán, Resnik, Pacin, & Castillo, 2003; Scott, Kanhere, Dexter, Brennan, & Trenholm, 1984) as the fungus able to produce it (Fusarium graminearum) is frequently isolated from the whole kernel (González, Pacin, Resnik, & Martinez, 1996; González, Martinez, Pacin, & Resnik, 1999). This secondary metabolite is a mycotoxin frequently found in foods, especially in those elaborated with cereals and it has been demonstrated that different products of massive consumption in Argentina like bakery products and beer are frequently contaminated by DON (Moltó, Samar, Resnik, Martínez, & Pacin, 2000; Pacin, Resnik, Neira, Moltó, & Martínez, 1997; Quiroga et al., 1995; Samar, Neira, Resnik, & Pacin, 2001).

Due to the culinary customs and considering that in Argentina appreciable amounts of wheat based foods are consumed, it is important to establish how processes are able to diminish the contamination by DON in those foods. Previous studies on DON behaviour during the bread elaboration in Argentina have been made and a positive effect was found when increasing the temperature of fermentation in order to reduce the DON contamination (Samar et al., 2001). The DON reduction during bread baking was also quantified (Neira, Pacin, Martínez, Moltó, & Resnik, 1997). In other study, the presence of DON in the different steps of the manufacture of turnover pie dough covers prepared for baking and for frying was studied, with the objective to evaluate the industrial processing effect on DON reduction in a factory (Del Pelo, 2002).
“Empanadas” are prepared with different filling types within the cover (turnover pie cover) and they are baked, or fried in vegetable oil or animal fat (pork or cow). Turnover pie covers are raw dough flattened and disc shaped (main component is wheat flour). They appear in packages of the thermoformed type closed with a flexible film but with no type of modified atmosphere. Each package contains several separated units with polyethylene films. The product must be conserved between 2 and 8 °C, being its shelf life 25 days. The daily average consumption of turnover pie covers varies between 135 and 217 g according to the survey of food consumption by the population at the University of Luján (Pacin, Martinez, Portela, & Neira, 1998).

The Provisional Tolerable Maximum Daily Intake (PTMDI) according to the JECFA last update (Canady et al., 2001) is of 1 μg/kg body weight/day, therefore for a person of 70 kg of weight who consumes daily 217 g of turnover pie covers, the maximum contamination of these covers would not have to surpass a DON concentration of 322.6 μg/kg.

The objective of this work was to evaluate DON contamination during a traditional home frying of turnover pie dough cover of “empanadas”, at three ordinary frying temperatures to establish how much this process diminishes the DON contamination level.

2. Materials and methods

2.1. Treatment and sample preparation

Natural and artificially contaminated flour were used. DON concentration was 1200 μg/kg and 260 μg/kg respectively. Dough was made with the following ingredients: 500 g flour, 75 g bovine fat, and 207 g of warm water. All the ingredients were manually held together, shaped into a ball, covered, rested for 15 min, and then kneaded to obtain a non-sticky and smooth appearance dough. After flattening the ball of dough slightly, it was rolled out until it had 2 mm thickness. Dough circles of 10 cm diameter, 2 mm thickness and 30 g weight, so called “tapas” (covers), were cut.

The covers were cooked with commercial corn oil in a 10 litres capacity frying pan, preheated at the temperature of each assay (169 °C, 205 °C and 243 °C). The most common commercial frying pan has three temperature levels: low, medium and high. Fourteen covers were fried at each assay (169 °C, 205 °C and 243 °C) and in the raw dough (26 samples). The median of the X, Y and Z measured values were calculated for each side of covers, then the median for both sides and finally the median for the replicates of each time and temperature. Using these values the colour functions were calculated for the CIE standard illuminant. The colour functions were calculated from the X, Y, and Z values according to Lozano (1978) and Petriella et al. (1985).

2.3. Chemical analysis for DON

DON extraction of the samples was performed as described by Truckses et al. (1996) with slight modifications according to Samar et al. (2003). The mixture acetone:water (84:16) was adjusted taking into account the water content of each sample.

The water content was determined by weighing 2 g of each sample by triplicate and heating them in a vacuum oven at 60 °C until two successive weight loss measurements, performed every two hours, showed <0.05% weight difference.

Based on contamination levels, two methods were used to clean up the samples, performing the analysis by quintuplicate. At 1200 μg/kg initial DON naturally contaminated samples, extracts of 8 ml were placed in an 8 × 15 mm culture tube and a 2 ml portion was passed through a Mycosep 225 column (Romer Labs. Inc., MO, USA).

Extracts of artificially contaminated samples were cleaned up with a DON test affinity (VICAM, Cebasa S.A., Buenos Aires, Argentina). Briefly, to extract the sample, 25 g of sample were blended with a mixture of 5 g of PEG 8000 and 100 ml of distilled water, homogenised for 1 minute and then filtered by using Whatman No. 1 filter. Two millilitres of the extract were passed over the DON test affinity column with a constant flow of 1 drop/s. The column was washed with distilled water (5 ml) and then dried completely. The column was eluted with methanol (1.5 ml) at very low speed and the eluate collected in an amber vial.

The extracts were evaporated to dryness in a 60 °C water bath under vacuum and stored at −18 °C prior the analysis.

The dried extract residues were derivatized as described by Croteau, Prelusky, and Trenholm (1994). Briefly, the catalyst solution was added to the dried extract and after vortexing, 50 μl of HFBA were added. The tube was placed in a heating block for 20 min. Excess of derivatizing agent was destroyed with 1 ml of sodium bicarbonate solution and 400 μl of toluene were added. After centrifuging, the upper organic layer (480 μl) was transferred to an autosampler vial for GC analysis.

Gas chromatography with electron capture detection (GC-ECD) was performed on a Hewlett-Packard Model 5890 Series II equipped with an HP automatic liquid sam-
pler, an HPG1512 A controller, an HPG1513A injector module and an HP3398A GC-ChemStation. GC separation was achieved with an HP-5 capillary column (30 m × 0.25 mm of i.d. × 0.25 µm thickness of film). The temperature program consisted of holding 1 minute at 80 °C, then increasing the temperature at 30 °C min⁻¹ from 80 to 160 °C, followed by an increase from 160 to 183 °C at 1 °C min⁻¹ and then 183–280 °C at 12 °C min⁻¹ (with 5-min hold). Column head pressure was 12 psi. Nitrogen flow was 1 ml min⁻¹. The temperature of the injector and the detector was 250 and 300 °C respectively. The injection volume was 2 µl. Typical obtained chromatograms are shown in Fig. 1.

The limit of both detection and quantification of DON was 9 and 12 µg/kg, calculated at a signal-to-noise ratio of 3:1 and 5:1 respectively. A standard curve was constructed each day to determine electron capture detector response to DON standard (Samar et al., 2003).

2.4. Statistical analysis

Statistix for Windows (2000) software was used to compare DON contamination in raw dough and in the covers fried at 169 °C, 205 °C and 243 °C.

3. Results and discussion

Using Mycosep 225 as clean up column, the average DON recovery values obtained were 88 (SD: 11%, n = 5) and 74% (SD: 9%, n = 5) in raw and fried dough, respectively (5 min at 169 °C, DON level: 300 µg/kg). DON immunoaffinity columns (VICAM) were used at 260 µg/kg level due to the good recovery obtained at low levels in fried dough (93% (SD: 4%, n = 5) at 85 µg/kg level).

The oil temperature variation in a home pan during the cooking time of dough covers was measured with the calibrated thermocouple for each sample group (14 covers each group). Temperature varied during the frying. For 243 °C the variation range was 240–246 °C; for 205 °C, between 201 °C and 209 °C; and for 169 °C the range was 164–174 °C.

Frying evolution colour was measured in order to establish the final cooking time at each temperature. The colour development at the three temperatures in the CIE x–y chromaticity diagram is shown in Fig. 2. It can be observed that the colour variation along the frying process occurs on the same chromatic curve.

Calculations were made for the CIE standard illuminant. The colour functions were calculated from the X, Y, and Z
values and they were plotted versus cooking time at the three temperatures (results not shown). The \( \Delta E \) colour differences between raw dough and fried covers were selected because of its linearity at the three temperatures with time (Fig. 3). This linearity facilitates the study design where a visual final cooking time was defined, corresponding to a \( \Delta E \) of 35.3 (SD: 0.8) with respect to the raw dough colour. Therefore, the final time at each temperature was determined.

The DON concentration, expressed as \( \mu g \) DON/kg dry flour, and moisture content versus time at the three temperatures of frying (169 °C, 205 °C and 243 °C) are shown on Fig. 4. This behaviour is not similar for both concentrations. This could be a difference between natural or artificial contamination or that a rate of DON reduction is not an easy kinetics.

Fig. 3. \( \Delta E \) functions obtained for turnover pie dough covers fried with respect to the raw dough colour at different temperatures versus cooking time.

Fig. 2. Colour development at the frying process in the CIE \( x-y \) chromaticity diagram taken at the three temperatures.

Fig. 4. Temperature influence on two DON contamination levels during the frying process, expressed as \( \mu g \) DON/kg dry flour, and covers moisture content (dry basis).

The DON concentration, expressed as \( \mu g \) DON/kg dry flour, and moisture content versus time at the three temperatures of frying (169 °C, 205 °C and 243 °C) are shown on Fig. 4. This behaviour is not similar for both concentrations. This could be a difference between natural or artificial contamination or that a rate of DON reduction is not an easy kinetics.

Fig. 5 shows the box plots (based on five analysis) of natural DON contamination in the raw dough and in those fried at different temperatures at the final cooking time. Despite the few data considered which does not allow an accurate statistical treatment, a tendency of DON reduction can be seen. In Table 1 number of analysis, mean,
standard deviation (SD), median and MAD of the DON concentration determined in the raw dough at the different temperature treatments are listed. The greater average percentage of reduction was 28% for the level of 1200 µg/kg and 66% for 260 µg/kg, when the dough was fried at 169 °C.

4. Conclusion

A reduction of DON contamination was observed during the home-made frying process. This DON reduction seems to depend on the frying temperature. A major DON reduction was obtained when the fried covers reached the home-made colour at the minor of the assayed temperatures. The average percentage of DON reduction, based on medians, was 28% for the level of 1200 µg/kg, when the dough covers were fried at 169 °C, 21% at 205 °C and 20% at 243 °C. The observed results lead to conclude that turnover dough pie covers frying process at traditional home pan temperature available diminishes more than 20% initial natural DON contamination.

Acknowledgements

The authors acknowledge the technical assistance provided by Ms Gabriela Cano and Daniela Taglieri as well as the financial support from the Comisión de Investigaciones Científicas of the Province of Buenos Aires, Universidad Nacional de Luján, Universidad de Buenos Aires, CONICET, BID 1201/OC-AR PICTOR 2002-00012, Argentina, and European Union (Contract No. ICA4-CT-2002-10043).

References


