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Formulation and processing factors affecting trichothecene mycotoxins within industrial biscuit-making

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ABSTRACT

Food processing, especially thermal treatment, may have implications on mycotoxins in products available for consumers. This research work aimed to study how mycotoxin levels may be influenced by modifying the technological parameters of both whole grain and cocoa biscuit-making processes. The study was mainly focused on the following mycotoxins: deoxynivalenol, deoxynivalenol-3-glucoside, and the minor metabolite culmorin. Special emphasis was given to the recipe formulation, and to the baking conditions, using an industrial-scale operation, starting from naturally contaminated raw materials. Exploiting the power of Design of Experiments (DoE) and a dedicated LC-MS/MS method, the complexity of the different processes was investigated. The models obtained within this study showed a high goodness-of-fit suggesting that the pH and the baking time play important roles for minimizing mycotoxins in the final products, while the recipe formulation has an impact on the mycotoxins extractability by affecting the biscuit microstructure.

1. Introduction

Grain quality and safety are key issues in the bakery production chain. Wheat is one of the most susceptible cereals for contamination with mycotoxins (Pleadin et al., 2013). Due to the global importance of wheat and wheat-based foods in the diet, it is a major concern that mycotoxins in crops can persist during processing and contaminate final products. Serious health effects can be observed in humans and animals due to consumption of mycotoxin contaminated food commodities (De Ruick, De Boevre, Huybrechts, & De Saeger, 2015).

Over the last decade, attention to both occurrence and modification of mycotoxins during bakery production has greatly increased. Trichothecene mycotoxins are secondary metabolites produced mainly by *Fusarium* head blight pathogens (FHB), such as *F. graminearum* and *F. culmorum*. Among them, deoxynivalenol (DON) is the most frequently found toxin in wheat in the more temperate regions of the World (Galvano, Ritieni, Piva, & Pietri, 2005; Larsen, Hunt, Perrin, & Ruckenbauer, 2004). On account of its chemical and thermal stability, a wide range of cereal-based foods have been reported to be contaminated by this toxin (Visconti & Pascale, 2010; Zinedine, Soriano, Moltó, & Mañes, 2007). Based on its toxic effects, the European Commission has set a maximum level of 500 μg/kg for DON in cereals products for human consumption (EC, 2006).

Among the metabolites often co-occurring with DON in contaminated crops, recent studies reported the presence of culmorin – a sesquiterpene, biosynthesized from *trans*-farnesyl pyrophosphate (Hanson & Nyfeler, 1976, Ghebremeskel & Langseth, 2000; Uhlig et al., 2013). Its effects on human and animal health are still being evaluated. More than ten hydroxy-culmorins were reported in the literature, with a significant difference in production between the two mentioned *Fusarium* species. For example, it is reported that the 15-hydroxy-culmorinis are mainly produced by *F. culmorum* strains (Langseth, Ohebremeskel, Kosiak, Kolsaker, & Miller, 2001).

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Food processing, especially thermal treatment, can have an impact on mycotoxin levels, but details of the exact effects and modification often remain unclear: while the stability of mycotoxins during various baking process practices has been studied and documented worldwide, the possible inactivation of mycotoxins during baking and the influence of raw material composition on this effect are still to be clarified (Kabak, 2009; Suman & Generotti, 2015; Karlovsky et al., 2016). Results obtained on DON reduction after thermal treatment differ depending on the product type, size and on the selected conditions; some researchers reported a relevant DON reduction in the finished product (Samar, Resnik, Gonzalez, Pacin, & Castillo, 2007; Voss & Snook, 2010), on the other hand other works suggested a high stability in products treated in a temperature range of 170–300 °C (Gärtner, Munich, Kleijer, & Mascher, 2008; Lancova et al., 2008; Numanoglu, Uygun, Koksel, & Solfrizzo, 2010).

In recent years, the final contribution to the exposure load by masked mycotoxins, such as deoxynivalenol-3-glucoside (DON3Glc), has been considered (Berthiller et al., 2013; Suman et al., 2013). Technological processes may indeed affect masked mycotoxins through two different mechanisms: a) conjugation with other molecules of the matrix such as sugars, proteins or lipids; b) release of parent compounds from masked forms. Consistently, the co-occurrence of DON3Glc in flour could be considered a reasonable explanation of the increase in DON content observed by several authors upon dough fermentation (Bergamini et al., 2010; Lancova et al., 2008; Suman, Manzitti, & Catellani, 2012). According to recent studies, there is a need for a specific legislation covering both parent and masked forms, as also recognized by European regulatory bodies (EFSA, 2014).

The present study was aimed at verifying the effect of technological parameters on DON, DON3Glc, and culmorin, during the production of two representative bakery products (whole grain biscuits and cocoa biscuits). The model was developed using statistical Design of Experiment (DoE) enabled an exploration of the relationship between the analytical responses and the multiple parameters (independent variables) that may affect the desired output (Telford, 2007). This model can therefore establish an optimal process design for mycotoxin control, which at the same time produces a final product that is acceptable to consumers. Aside from monitoring some specific mycotoxin levels, two LC-MS/MS multi-mycotoxin methods were used and compared, to allow the simultaneous detection and quantification of all the major mycotoxins and other secondary fungal metabolites in cereals. Finally, to better understand the influence of the microstructure on mycotoxin extraction capability, morphological studies were conducted by Environmental Scanning Electron Microscopy (ESEM).

2. Materials and methods

2.1. Chemicals

Methanol and acetonitrile (both LC gradient grade) were purchased from J.T. Baker (Deventer, The Netherlands) and ammonium acetate (MS grade) and glacial acetic acid (p.a.) were obtained from Sigma-Aldrich (Vienna, Austria). Water was purified successively by reverse osmosis and a Milli-Q plus system from Millipore (Molsheim, France). Mycotoxin standards were obtained either as gifts from various research groups or from the following commercial sources: RomerLabs®Inc. (Tulln, Austria), Sigma-Aldrich (Vienna, Austria), Iris Biotech GmbH (Marktred-witz, Germany), Axxora Europe (Lausanne, Switzerland) and LGCPromochem GmbH (Wesel, Germany). DON3Glc was isolated from wheat treated with DON (Berthiller et al., 2005). All solutions were stored at −20 °C and were brought to room temperature before use. OASIS® HLB 3 cc (60 mg) extraction cartridges were purchased from Waters (Manchester, UK). Glass vials with septum screw caps were purchased from Phenomenex (Torrance, CA, USA). Centrifugal filter units (Ultrafree MC 0.22 μm, diameter 10 mm) were obtained from Millipore (Billerica, MA, USA).

2.2. Samples

For the production of both whole grain and cocoa biscuits, three samples of naturally contaminated wheat bran, obtained from wheat lots during 2015 campaign, were selected and analyzed for DON by LC-MS. Analysis using LC-MS yielded mean ± standard deviations of 600 ± 16 μg/kg (bran A), 1050 ± 48 μg/kg (bran B) and 1500 ± 92 μg/kg (bran C). Industrial whole grain flours ready for the experiments were obtained by mixing the contaminated bran (7–10%) with common wheat flours contaminated at about 150–250 μg/kg (depending on the lots). The final concentrations of DON in the mixes are reported in Table 1.

2.3. Moisture content determination

All mycotoxin results from these commodities are presented on a dry matter (d.m.) basis. The moisture content was measured by heating 5 g ground sample in a thermostatic oven at 105 °C for 6 h.

2.4. Experimental design

Design of Experiments (DoE) was exploited to study synergistic effects of some technological parameters on the mitigation of mycotoxins in the final product, according to previous work done in our group (Generotti et al., 2015; Suman et al., 2012). In this research work, the following parameters were selected: a) DON contamination level on wheat bran; b) dextrose, margarine, egg and milk content (as percentage in recipe); c) pH value (as sodium bicarbonate content); d) baking time and e) baking temperature. Each variable was modified within a range set according to the technological requirements, taking into consideration the organoleptic acceptability of the final product as a boundary (Table 2). In particular, organoleptic evaluations were executed with an internal trained group of six panelists, applying a comparative test with respect to the industrial product obtained with the standardized process already in place. Experimental data were statistically elaborated by using a multi-variate approach (MODDE software, version 9.1; Umetrics, Umea, Sweden)

2.5. Dough preparation and biscuit-making

Doughs were prepared starting from standard wheat flour/bran, and addition of cocoa powder for the cocoa recipe in order to obtain a final dough of about 1000 ± 30 g. For the whole grain biscuits, the ingredients were wheat flour (60%), bran (7%), potassium bitartrate, glucose syrup and salt. Eggs, margarine and dextrose were added depending on the value reported in recipe obtained from the experimental design (Table 4 – supplementary material). Sodium bicarbonate was added in order to reach the appropriate pH value. Water amount ranged from 1.6% to 5%, depending on the technological requirements. For cocoa biscuits, wheat flour, bran, cocoa powder, margarine, glucose syrup and salt were used. Milk and dextrose amount were also added, based on the values in the model generated by Design of Experiment (Table 5 – supplementary material). Again, sodium bicarbonate was added to reach an appropriate pH value, while the optimal amount of water added to each dough sample was based on internal technological knowledge.

The process for whole grain and cocoa biscuit production consisted essentially of the following steps: creaming, dough prepara-

Table 1
Design of the experiments and corresponding analytical results for the effects of the screening variables on the deoxynivalenol (DON) levels throughout the wholegrain biscuit-making process.

Experiment number	$NaHCO_3$ (g)	DON in initial wholegrain flour (μg/kg d.m.)*	Baking stage		
			Time (min)	Temperature (°C)	DON in wholegrain biscuits (μg/kg d.m.)*
1	0	219 ± 8	5	180	119 ± 13
2	0	304 ± 9	5	200	263 ± 0
3	0	219 ± 8	8	180	186 ± 3
4	0	304 ± 9	8	200	274 ± 1
5	0	219 ± 8	8	200	172 ± 13
6	0	304 ± 9	8	180	213 ± 20
7	0	219 ± 8	5	200	165 ± 0
8	0	304 ± 9	5	180	228 ± 1
9	9	219 ± 8	8	200	129 ± 7
10	9	304 ± 9	8	180	208 ± 2
11	9	219 ± 8	5	200	105 ± 3
12	9	304 ± 9	5	180	155 ± 7
13	9	219 ± 8	5	180	154 ± 1
14	9	304 ± 9	5	200	192 ± 4
15	9	219 ± 8	8	180	117 ± 3
16	9	304 ± 9	8	200	138 ± 2
17	4	237 ± 19	6.5	190	188 ± 7
18	4	237 ± 19	6.5	190	189 ± 1
19	4	237 ± 19	6.5	190	203 ± 15

* Data expressed as mean value ± standard deviation on dry matter basis (d.m.) of a final number of four replicates.

Table 2
Processing conditions assumed during wholegrain and cocoa biscuit experiments in the pilot-scale plant.

Treatment	Wholegrain biscuit making			Cocoa biscuit making		
	Minimum	Optimum	Maximum	Minimum	Optimum	Maximum
DON bran level (μg/kg)	600	1050	1500	600	1050	1500
Dextrose (%)	15	19	23	15	19	23
Milk (%)	–	–	–	5	6.5	8
Eggs (%)	4	6	7	–	–	–
Margarine (%)	10	15	20	–	–	–
pH value	5	6.5	8	5	6.5	8
Baking time (min)	5	6.5	8	5	6.5	8
Baking temperature (°C)	180	190	200	180	190	200

tion and baking. First, wheat flour was mixed with all solid powder ingredients using a test planetary kneader XBE10 (Dito Electrolux, Stockholm, Sweden) for 2 min. Dextrose and margarine were mixed separately by using another test planetary kneader BE5 (DitoSama Electrolux, Stockholm, Sweden) for 3 min (creaming step). At a later stage, cream and powders were mixed together for 3 min. The resulting dough was shaped, and rounded pieces of about 4 cm diameter (approximately 10 g) were obtained and rested for 10 min at room temperature. Baking was performed in a pilot-scale dynamic oven (Tagliavini, Parma, Italy). Fig. 4 (supplementary material) summarizes the biscuit-making process. Nineteen different tests for each process were performed in duplicate (Table 4 and 5, respectively). Mycotoxin content was analyzed in the initial whole grain flours, dough (before baking) and finished product (after baking). Before the mycotoxin content analysis, samples were stored at −20 °C. Each sample was extracted in duplicate and each extract was measured in duplicate.

2.6. Sample extraction and instrumental conditions – method A

Samples were extracted according to a previously published procedure (Lattanzio, Della Gatta, Suman, & Visconti, 2011) with slight modifications. Briefly, a total of 10.00 g of flour or dough or biscuit sample were extracted with 100 ml of an acetonitrile/water (84:16, v/v) mixture by homogenization at a medium-to-high speed for 2 min using a mixer (Oster, New York, USA). The extract was allowed to settle for 15 min. Afterwards, 5 ml were poured into a 10 ml vial, and evaporated to dryness under a nitrogen stream. The extract was reconstituted with 100 μl of ^{13}C-DON internal standard solution (100 ng/ml in methanol) and 900 μl of water. Each extraction cartridge column was activated using 2 ml of methanol, and 2 ml of methanol:water (10:90, v/v). The sample extract was then slowly passed through the OASIS® HLB 3 cc (60 mg) (Waters, Manchester, UK) column using a vacuum chamber system. A solution of methanol:water (20:80, v/v) was used for washing, followed by elution with 1 ml of methanol. The eluate was evaporated under a gentle stream of nitrogen, and the residue was dissolved in 200 μl of eluent A (methanol:water, 20:80 v/v, 0.5% acetic acid, and 1 mM ammonium acetate) prior to UHPLC-MS/MS analysis.

Ultrahigh-performance liquid chromatography (UHPLC) was performed using a Dionex Ultimate® 3000 LC systems (Thermo Fisher Scientific Inc., Waltham, MA, USA) and a Kinetex Biphenyl column (2.6 μm; 100 × 2.10 mm; Phenomenex). The flow rate of the mobile phase was 400 μl/min, and the injection volume was 20 μl. The column oven was set to 30 °C. A linear binary gradient composed of (A) water (0.5% acetic acid, 1 mM ammonium acetate) and (B) methanol (0.5% acetic acid, 1 mM ammonium acetate) was employed. The gradient was as follows: 0–4 min to 40% B; 4–20 min to 80% B; 20–22 min, isocratic step 80% B; finally, a re-equilibration step at 10% B (the initial value) was performed for another 3 min, bringing the total analysis time to 25 min. Before UHPLC-MS/MS analysis, all samples were filtered through centrifugal filter units for clarification.

ESI-MS/MS was carried out by a Q-Exactive (Thermo Fisher Scientific Inc., Waltham, MA, USA) mass spectrometer. Experiments were performed in full MS data scan for quantification and data-dependent scan for confirmation both in positive and negative polarities in two separate chromatographic runs per sample with the following settings: the capillary temperature was set to 300 °C; the sheath gas and auxiliary gas flow rates were set to 40 and 10 units, respectively; the spray voltage was set to 3500 kV; and the S-lens RF level was set to 55 V. All equipment control and data processing were performed by Excalibur software (Thermo Fisher Scientific Inc., Waltham, MA, USA). Mycotoxin measurements in all the samples were performed using isotopically labeled standards and calibration vs. matrix-matched standards. Spiking experiments were performed on flour, dough and biscuits: the mean recovery for DON was 104 ± 2% in flour, 100 ± 2% in dough and 111 ± 3% in biscuits. Concerning DON3Glc recovery, the mean values were 98 ± 4% in flour, 79 ± 3% in dough and 81 ± 3% in biscuits. Data were corrected for recovery percentage.

2.7. Sample extraction and instrumental conditions – method B

Sample preparation was carried out according to Malachová, Sulyok, Beltrán, Berthiller, and Krska (2014). Briefly, 20 ml of extraction solvent (acetonitrile/water/acetic acid, 79/20/1, v/v/v) were added in a 50 ml tube with 5.00 g of sample and mixed for 90 min. Then, the extract was centrifuged and injected for analysis without further pre-treatment.

Detection and quantification was performed with a QTrap 5500 MS/MS system (Sciex, Foster City, CA, USA) and a 1290 Series UHPLC system (Agilent Technologies, Waldbronn, Germany) according to a previously reported work conducted by our group (Generotti et al., 2015).

2.8. Environmental scanning electron microscope (ESEM) analysis

Morphological analysis was carried out by using an environmental scanning electron microscope (ESEM) Quanta™250 FEG (FEI Company, Oregon, USA), in order to verify and control potential matrix interferences on mycotoxin extraction capability due to the differences in the microstructure. ESEM microscopy was used to record the images of whole grain biscuits and cocoa biscuits differentiated by the absence/presence of milk in recipe. Samples were placed on double-sided adhesive carbon tape fixed to metal sample holder, mounted in the microscope chamber. All the micrographs were acquired using an accelerating voltage of 7 kV under different magnifications (114x, 800x, and 1600x).

3. Results and discussion

This study investigated the influence of the industrial biscuit-making process on DON, DON3Glc and culmorin levels in whole grain and cocoa biscuits. The trials were designed to explore the impact of several technological factors on mycotoxin content (in order to minimize their content in the final product), and to set their optimal ranges. Three wheat bran and cocoa batches containing different initial DON amount, respectively, were chosen and mixed with a blank wheat flour, in order to simulate a medium-to-low and a high contamination. The statistical model required 19 single experiments per technological process; in each experiment, DON, DON3Glc, and culmorin levels were measured by LC-MS/MS in the starting raw materials, before and after baking step, exploiting two distinguished methods (method A, B), as previously explained.

3.1. Statistical evaluation of the two analytical methods

Results were acquired by applying two independent analytical methods. Both methods were LC-MS based, although they differed for the extraction procedure and the range of detectable metabolites. Method A measured most of the major mycotoxins, such as DON, and DON3Glc, but not culmorin, which was detected by method B.

The percentages of mycotoxin reduction were used for the statistical evaluation and data were expressed on a dry matter basis (d.m.), corrected according to recipe formulation.

Referring to the MODDE output settings, method A seemed to be more efficient than method B according to two parameters linked to fitting and prediction capability (R2 and Q2), as reported in Table 6 (supplementary material). Furthermore, model A seemed to have a greater robustness than model B. This aspect was also confirmed by ANOVA plot, especially for the cocoa system, standard deviation of the regression being much larger than the standard deviation of the residuals (data not shown). For this reason, data from method A (Table 1) were chosen to perform further statistical evaluation (except for the culmorin results). All values were collected in a variable importance plot (VIP) that enables an understanding of the effect of each factor on the mycotoxin final content. On the other hand, the range of factor acceptability was assessed using a response contour plot (RCP).

3.2. DON and DON3Glc trends during wholegrain biscuit-making

Taking into consideration the MODDE outputs, DON levels seemed to be more affected by the recipe formulation than the thermal treatment, as shown in Fig. 1. Basically, the VIP would suggest that the pH value (related to sodium bicarbonate content – Table 1) had the greatest effect on the final DON concentration. A potential reduction within the total processing up to 10% may be feasible still remaining in an acceptable range of technological and organoleptic properties: as shown by the RCP (Fig. 2); an increase of the pH conditions in the dough can result in a reduction of free DON concentrations (due to its instability under alkaline conditions) in the final biscuit, as already observed by Suman et al. (2012).

A second aspect inferred from the VIP plot (Fig. 1), is that the initial DON contamination of wheat bran affected the evolution of DON content in the finished product, as similarly reported by Vidal, Morales, Sanchis, Ramos, and Marín (2014). By contrast, statistical analysis suggested that baking parameters (time/temperature) had a negligible effect on DON stability, in disagreement with another previous study on bakery products (rusks) conducted by our group (Generotti et al., 2015). This can be explained by the extent of the different thermal treatments applied for biscuit-making and rusk-making.

Baking time and temperature only become relevant in the model when all the other variables are set as constants, as reported in Table 7 (supplementary material): considering experiments carried out within the most severe time/temperature baking conditions, reduction ranged from 9 to 68%. In particular, the greatest effect induced by baking time/temperature was observed for samples containing higher initial DON amounts, with the baking step being performed at 200 °C for 8 min. This temperature/time combination still remains in an acceptable range of technological and organoleptic conditions. A similar reduction trend was observed for DON3Glc (Table 7), when the baking step is assumed as the main parameter. Reduction ranged from 31 to 75% and decrease seemed to be affected by initial DON contamination level, according again to previous scientific studies (Kostelanska et al., 2011; Simsek, Burgess, Whitney, Gu, & Qian, 2012).

DON3Glc average values measured along the different replicated experiments ranged from 10 to 18 μg/kg in the flour to 4–

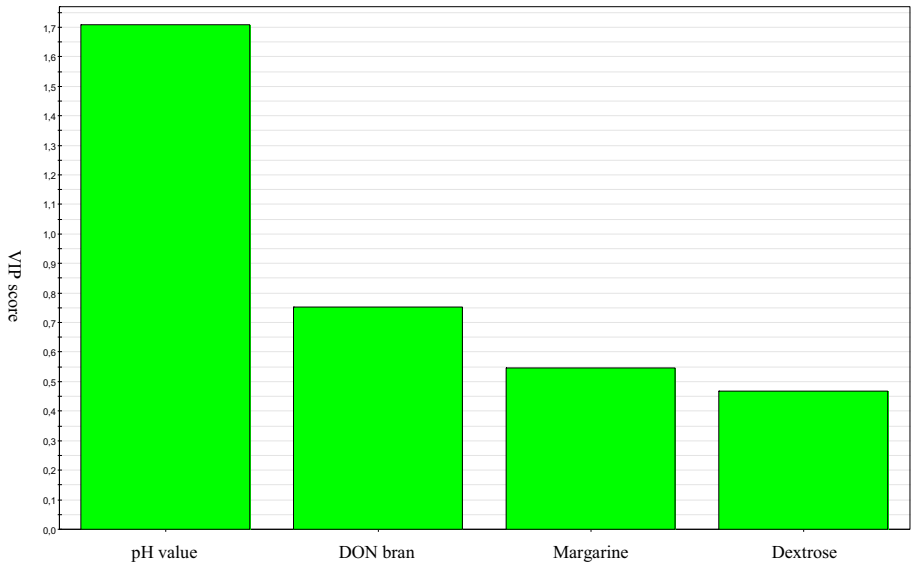

Fig. 1. Variable Importance Plot (VIP) obtained for the data referred to DON concentration during wholegrain biscuit-making process.

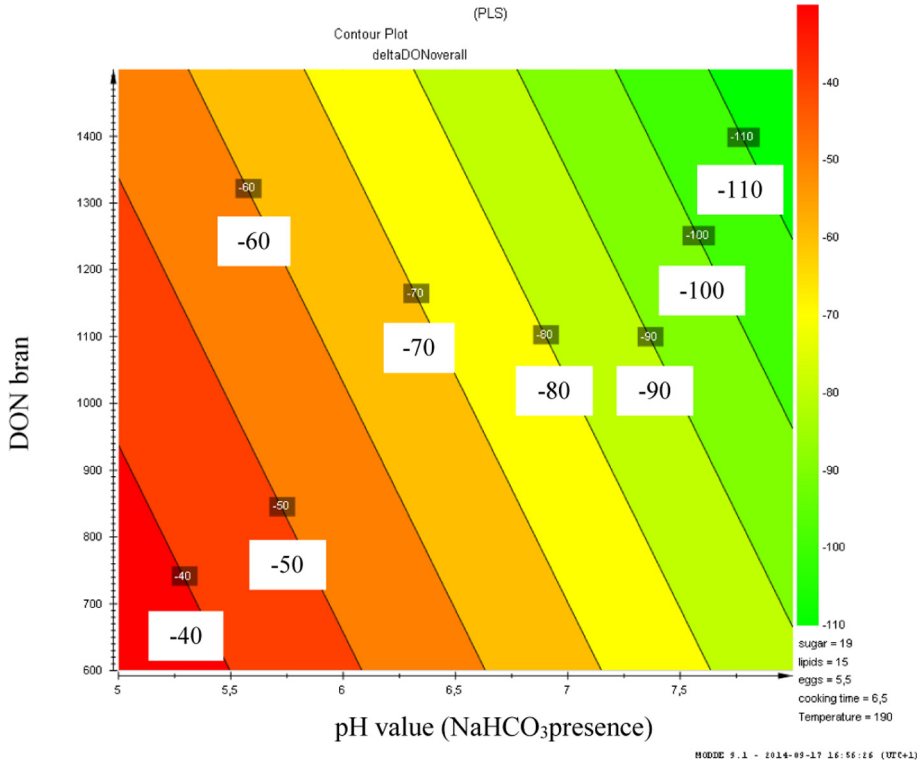

Fig. 2. Response Contour Plot obtained using the results of the wholegrain biscuit-making experiments: DON bran vs. pH value ($NaHCO_3$ presence).

6 μg/kg in the final biscuits: however, since these overall levels were very low in the samples, when the experimental uncertainty is taken into account, the model may not be considered as completely reliable for DON3Glc mitigation, due to the lack of relevant cause-effect phenomena to be related to the changes in the production parameters.

3.3. DON and DON3Glc trends during cocoa biscuit-making

Samples obtained from the cocoa biscuit-making experiments were analyzed for DON and DON3Glc. Concerning DON, the VIP plot showed that this mycotoxin is significantly influenced by milk content, while baking parameters seemed to have smaller influences. As the composition of milk should not chemically affect the stability of DON so directly, a possible modification of sample extractability due to different biscuit microstructure was taken into consideration by application of Environmental Scanning Electron Microscopy (ESEM) under different magnifications. Different samples obtained with or without milk in the recipe were compared (Fig. 3). In general, milk seemed to affect the texture to some extent, as the more milk in recipe, the more complex the matrix. As shown in Fig. 3(B), starch seemed to be embedded into the matrix with a reduced area: milk may negatively affect the extractability of mycotoxins. In this case, considering milk as a constant in the

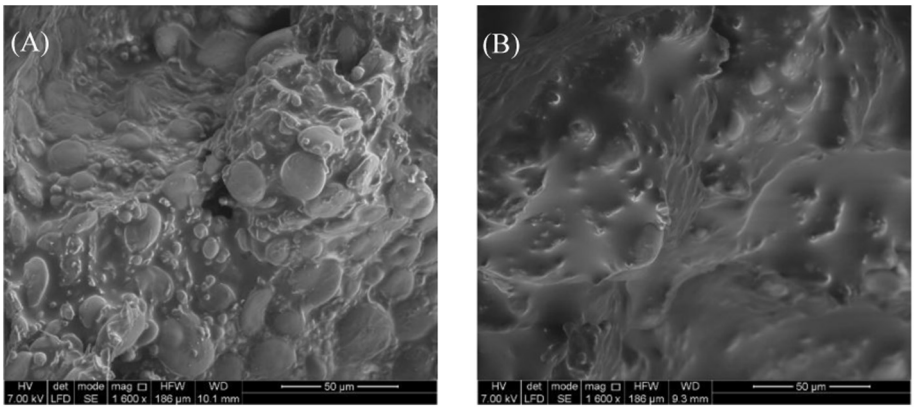

Fig. 3. Scanning electron micrographs of cocoa biscuits: (A) matrix without milk, (B) matrix with milk under magnification (1600x).

Table 3
Thermal overall effect in cocoa biscuit processing on DON levels.

Experiment number	DON bran (μg/kg)	Baking stage		Reduction DON	Time/temperature combination power
		Time (min)	Temperature (°C)		
4	1500	8	180	25%	Medium
6	1500	5	200	17%	Low
7	1500	8	200	27%	High
10	600	5	180	68%	Very low

model, the baking parameters become the most relevant factors with a reduction up to 27% (Table 3). Although the greatest reduction was reported for a baking step at 200 °C for 8 min, as obtained for whole grain biscuits, the reduction percentage was smaller here, on account of the higher structural complexity of the matrix: the strong influence of the recipe can be shown by considering experiments n.12 and n.15, characterized by higher milk content and where, in both cases, a DON increase was observed. As for whole grain biscuit production, the model cannot be successfully applied to DON3Glc, on account of the initial comparable concentration in bran.

3.4. Other metabolites: Occurrence of culmorin

In spite of a rich scientific literature on mycotoxins in commodities intended for human consumption, little or no information is available with respect to culmorin compounds and their thermal stability. It has previously been mentioned that culmorins often co-occur with type-B trichothecenes (Ghebremeskel & Langseth, 2000). In this study, culmorin and 15-hydroxy-culmorin were detected in all biscuit samples, at a concentration level positively related to the concentration of DON (R^2 coefficients: 0.81–0.99 and 0.86–0.96 for whole grain and cocoa biscuits, respectively), in agreement with the literature (Uhlig et al., 2013). This correlation can be inferred from the VIP plot (Fig. 5 – supplementary material), as DON concentration in the starting raw material exerts the main influence on culmorin contamination both in whole grain and cocoa model. The concentration of culmorin in the samples were up to 245 μg/kg and 92 μg/kg for whole grain biscuits in flours and baked products, respectively, while it reached 109 μg/kg for cocoa model in baked biscuits. 15-hydroxy-culmorin occurred in all samples at concentrations ranging from 18 μg/kg to 111 μg/kg with higher levels in whole grain biscuits than cocoa products. Processing, in particular thermal treatment, seemed to decrease culmorin and 15-hydroxy-culmorin content in the final products. In our model for whole grain and cocoa biscuit production, the percentage of degradation ranged between 25% and 80%, with the largest reduction at 180 °C for 8 min, in products formulated with flour with high concentration of DON.

4. Conclusions

The present study investigated the effect on the final mycotoxin content due to the possible modifications of technological parameters and recipe ingredients in the bakery process. Starting from naturally contaminated raw material, the power of dedicated DoE approach coupled with mass spectrometry based methods was exploited. The models fitted the data well and provided important information for the optimization of the industrial production process. Among the information provided: (1) increasing pH value (expressed as sodium bicarbonate content) induced a DON decrease during whole grain biscuit production, particularly when the initial contamination was high; (2) an increase in time during the baking phase, in an acceptable technological range, can effectively reduce DON and DON3Glc content in the final product; (3) the recipe formulation can contribute to the mycotoxin extractability, by affecting the biscuit microstructure.

Furthermore, for the first time, the evolution of culmorin-related compounds during baking was considered: culmorin concentration was positively correlated to DON, while a reduction up to 80% was reported. Nonetheless, the combined effects among fungal metabolites need to be further investigated.

This study represents a tangible example of how careful control of industrial lines may mitigate mycotoxin impact, especially when their level in the raw material is close to the legal limit (for example in the case of a specific adverse year-campaign), through appropriate management of cereal processing techniques.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.02.115.

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