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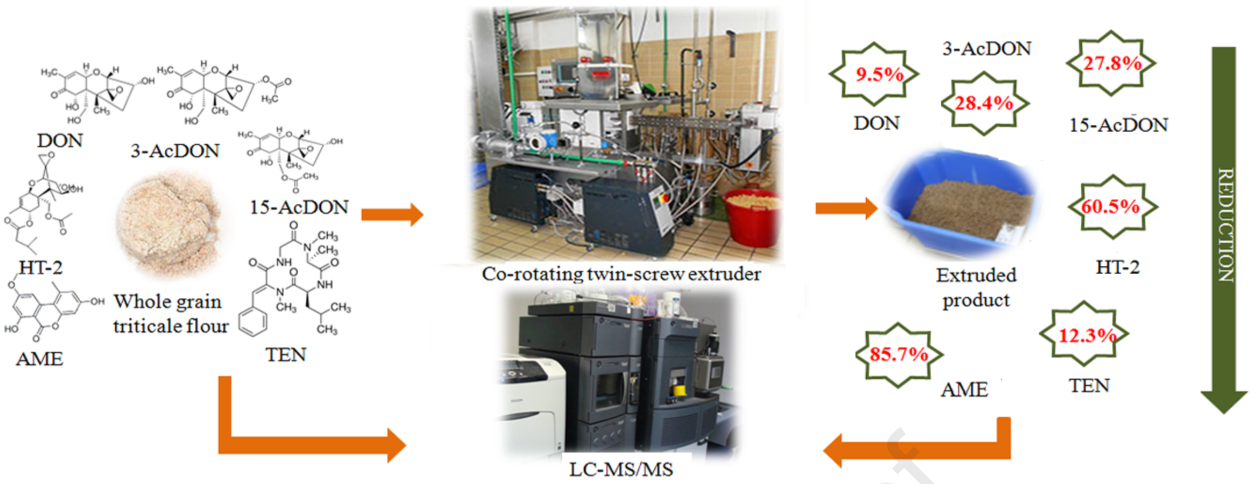
DON
3-AcDON
15-AcDON
HT-2
Whole grain triticale flour
AME
TEN
Co-rotating twin-screw extruder
LC-MS/MS
9.5%
DON
3-AcDON
28.4%
27.8%
15-AcDON
Extruded product
60.5%
HT-2
85.7%
AME
12.3%
TEN
REDUCTION

Effects of extrusion process on *Fusarium* and *Alternaria* mycotoxins in whole grain triticale flour

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Abstract

Effects of extrusion processing parameters of co-rotating twin-screw extruder – screw speed (*SS*= 500, 650, 800 rpm), feed rate (*FR*= 22, 26, 30 kg/h), and moisture content of the material (*MC*= 20, 25, 30 g/100 g), on the reduction rate of deoxynivalenol (DON), 3- and 15-acetyldeoxynivalenol (3- and 15-AcDON), HT-2 toxin (HT-2), tentoxin (TEN) and alternariol monomethyl ether (AME), in whole grain triticale flour were investigated, together with the physico-chemical characterization of obtained products. The die temperature of the extruder ranged between 113 and 151 °C, the pressure at the die was from 2.7 to 7.9 MPa, the mean retention time of material in the barrel was between 4 and 11 s, torque ranged between 39.6 to 59.4 Nm, while the specific mechanical energy ranged from 66.9 to 125 kWh/t. Optimal parameters for lowering the concentration of each investigated mycotoxins were: *SS*= 650 rpm, *FR*= 30 kg/h, *MC*= 20 g/100 g, with a reduction of 9.5, 27.8, 28.4, 60.5, 12.3 and 85.7% for DON, 3-AcDON, 15-AcDON, HT-2, TEN and AME, respectively. Present study is the first report for the fate of mycotoxins (3-AcDON, 15-AcDON, HT-2, TEN and AME) studied less during extrusion process of naturally contaminated whole grain triticale flour.

Keywords: whole grain triticale flour, co-rotating twin-screw extruder, mycotoxins reduction, LC-MS/MS.

Abbreviations

DON – deoxynivalenol

3-AcDON – 3-deoxynivalenol

15-AcDON – 15-deoxynivalenol

HT-2 – HT-2 toxin

TEN – tentoxin

AME – alternariol monomethyl ether

BD –bulk density (g/mL)

FR – feed rate (kg/h)

MC – moisture content (g/ 100 g)

P - pressure at the die (MPa)

PH – pellet hardness (kg)

RT – retention time in the barrel (s)

SME – torque (Wh/kg)

SS – screw speed (rpm)

Tend – die temperature (°C)

WAI- water absorption index (g/g)

WSI – water solubility index (g/100 g)

rAME – reduction of alternariol monomethyl ether (%)

rDON – reduction of deoxynivalenol (%)

r15-AcDON – reduction of 15-acetyldeoxynivalenol (%)

r3-AcDON – reduction of 3-acetyldeoxynivalenol (%)

rHT-2 – reduction of HT-2 toxin (%)

rTEN – reduction of tentoxin (%)

1. Introduction

Cereals worldwide are at risk to be contaminated by mycotoxins both in the field as the result of infection by different fungi or after harvest, as a consequence of ineffective drying or poor storage conditions. Mycotoxins are toxic secondary metabolites of filamentous fungi, mainly *Aspergillus* species (spp.), *Penicillium* spp. and *Fusarium* spp. (Agriopoulou, Stamatelopoulou, & Varzakas, 2020; Oliveira, Zannini, & Arendt, 2014). Among all of the fungal secondary metabolites currently known, only a few groups of mycotoxins are important from the safety and economic points of view; namely aflatoxins (AFs), mainly produced by *Aspergillus* spp., ochratoxin A (OTA), produced by *Aspergillus* and *Penicillium* spp., and zearalenone (ZEN), fumonisins (FBs) and trichothecenes (deoxynivalenol (DON), T-2 and HT-2 toxin (T-2, HT-2), diacetoxyscirpenol (DAS)), primarily produced by many *Fusarium* spp. (Agriopoulou et.al., 2020; Streit et al., 2012). In recent years, less studied *Alternaria* toxins gained more and more interest due to their possibility to cause toxic effects on animal and human health (EFSA, 2016). *Alternaria* spp. produces around 70 different mycotoxins, but the most relevant are tenuazonic acid (TeA), tentoxin (TEN), alternariol (AOH), alternariol monomethyl ether (AME), and altenuene (ALT). Consumers are mainly exposed to *Alternaria* toxins through processed foods or fruits (EFSA, 2016). The presence of mycotoxins in cereals is recognized as a worldwide concern since cereals represent one of the main parts of the human diet and animal nutrition (Babič et al., 2021; Janić Hajnal et al.,, 2019;). Since, mycotoxins are heat stable, during common processing methods of cereals (primary and secondary processes), reduction of their content may have occurred, but they may not be destroyed (Bullerman, & Bianchini, 2007; Agriopoulou et.al., 2020; Wan, Bingcan, & Rao, 2020). Postharvest approaches for the reduction of mycotoxins are important topics in food safety research. Various methods (physical, chemical, and biological) have been applied to prevent mycotoxin production, or reduce mycotoxin content (Liu et al., 2020). According to previously published studies recently reviewed by Schaarschmidt & Fauhl-Hassek (2018, 2021), extrusion could be effective as a physical detoxification approach in reducing some mycotoxins in wheat and maize (Liu et al., 2020; Schaarschmidt & Fauhl-

Hassek, 2018; 2021; Wan et al., 2020). The potential reduction of aflatoxin B1, B2, G1, and G2 levels by extrusion in corn-based products was investigated by Massarolo et al. (2021). The extrusion process combines a high temperature, high pressure, and short time process and can be used for the production of a range of cereal products and animal feeds. Generally, the extrusion process results in chemical changes and modifications (protein denaturation, starch gelatinization, polymer cross-linking, Maillard reactions, etc.), both for food components and present contaminants (Singha, Singh, Muthukumarappan, & Krishnan 2018; Torbica, Belović, Popović, & Čakarević, 2021). However, the extent of mycotoxin contamination reduction in a finished product depends on several factors, including the type of extruder, the extrusion conditions (extruder temperature, screw speed, feed rate, pressure, and residence time in the extruder), moisture content of the raw materials or extrusion mixture, chemical structure of mycotoxins, its initial content in the raw material, as well as depend on the potential matrix effects (Schaarschmidt & Fauhl-Hassek, 2018, 2021; Wan et al., 2020). The stability of mycotoxins during extrusion and the ability of extrusion processes to reduce the content of mycotoxins in extruded products have been studied to promote the degradation of the mycotoxins, mostly *Fusarium* toxins (Schaarschmidt & Fauhl-Hassek, 2018; 2021). The first report presenting the possibility of reduction of *Alternaria* toxins in wheat by extrusion was published by Janić Hajnal et al.(2016). The studies published so far regarding the possibilities of mycotoxins reduction during the extrusion process mostly referred to maize and wheat (Schaarschmidt & Fauhl-Hassek, 2018; 2021), rarely to barley, oats, and rice, while there is almost no data available related to the possibility of reducing of mycotoxins in triticale grain (×*Triticosecale*) during processing. One of the possible reasons is that triticale (produced by cross-breeding wheat and rye) is a less represented cereal in the world, and another reason is that it has been mostly used as animal feed, and less for human nutrition and biofuel production (Gagiu, 2018). However, the world production of triticale has kept increasing during the last few years. Between 2000 and 2019, the largest cultivating countries of triticale grain were: Poland, Germany, France, Belarus, China (≤ 201,870,707 t in total), Hungary, Australia, Lithuania, Russian Federation, Spain (≤ 32,169,927 t in total); Austria, Czechia, Sweden, Romania, Denmark (≤ 18,168,817 t in total) and Turkey, Chile, Brazil, Serbia, Switzerland (≤ 7,755,711 t in total). The Republic of Serbia is among the 20 largest producers of triticale in the world, with an annual production of 102,231 tons in 2019 (FAO, 2019). Considering that triticale is often contaminated with mycotoxins (Gagiu, 2018), in this study the influence of extrusion parameters on reduction of examined mycotoxins content in whole grain triticale flour was investigated.

Modern mathematical approaches such as Response Surface Methodology (RSM) to optimize the extrusion process (Singha & Muthukumarappan, 2017; Kojić et. al., 2019) can be used to regulate the quality of the extrudate and evaluate the effect of extrusion variables on the reduction of mycotoxins. The present work aims to optimize the extrusion process and evaluate the effect of different extrusion process variables (screw speed, feed rate, and moisture content of the material) on the quality of extrudates and on the reduction of mycotoxins using whole grain triticale flour that was naturally contaminated with mycotoxins. *Fusarium* and also less studied *Alternaria* toxins were investigated in the study. This work provides for the first time important data on the reduction effect of *Fusarium* and *Alternaria* toxins during the extrusion process, which is commonly used for the production of animal feed and food.

2. Material and methods

2.1 Material

Approximately 300 kg of triticale grain (×*Triticosecale*) naturally contaminated by mycotoxins was provided by the Institute of Field and Vegetable Crops, Novi Sad (Serbia) and finely ground using a hammer mill (model 9FQ-50, XT Machinery, China) driven by 22 kW electric motor and equipped with 16 hammers arranged in four rows and with the sieve of 1 mm diameter. To achieve an adequate homogeneity level of the ground material before sampling for the analysis and the extrusion processing, the whole grain triticale flour was mixed in a Muyang SLHSJ0.2A double-shaft paddle mixer (Muyang, Yangzhou, China) for 90 s. Mixing homogeneity of triticale flour was assured by Microtracer® method, using external tracers for mixing homogeneity testing (Clark, Behnke, & Poole, 2007), and also, twelve subsamples were taken for investigations of different mycotoxins levels in whole grain triticale flour.

2.2 Extrusion conditions

Co-rotating twin-screw extruder (Bühler BTSK-30, Bühler, Uzwil, Switzerland) with a total barrel length of 880 mm consisted of 7 sections and length/diameter ratio of 28:1 was used for the extrusion of the ground triticale grain. The extruder was equipped with two tempering tools for controlling water temperature for jacketed heating/cooling of barrel's sections. The first tempering tool controlled the temperature of sections 2, 3, and 4 (60 °C) while another tempering tool was used for controlling the temperature of sections 6 and 7 (set at 100 °C). The die plate with one 6 mm diameter opening and cone inlet (total die open area of 28.26 mm^2) was used. In this experiment, the same screw configuration was used as presented by Kojić et al. (2019). The scheme of the co-rotating twin-screw extruder is presented in Fig. 1. Screw speed, feed rate, and moisture content of the material in the extruder barrel were varied during extrusion according to the applied experimental design (Table 1). A total of fifteen extruded samples were obtained (TS-1 to TS-15). Targeted moisture content in the barrel was achieved by adding water at the end of section 1 of the barrel using a cavity pump. Sensors for measuring the pressure and temperature of the die were positioned at the die head. All extrusion data, including die temperature, pressure at the die, motor load, and specific mechanical energy were read directly from the PLC screen of the extruder. The final length of the product was obtained by the rotational knife that faced the die outlet and was fitted with six knives, with a rotational speed set at 1100 rpm. Drying and subsequent cooling of the extrudates were done in a fluidized bed vibro dryer/cooler (model FB 500 x 2000, Amandus Kahl GmbH & Co. KG, Germany).

2.3 Chemicals and reagents

A mixed trichothecene standard solution in acetonitrile (DON, 3-AcDON, 15-AcDON, T-2, HT-2, DAS), produced by Trilogy (Washington, MO, USA) and individual standards of TEN, AOH, AME, ZEN, OTA, FB1, and FB2 (Romer Labs, Tulln, Austria) were used. The stock standard solutions and the working standard solutions were prepared in acetonitrile and stored in amber glass vials at –20 °C. The certified purity of individual standard substances was between 98.5 ± 1.5% and 99.5 ± 0.5%. Working standard solutions of known concentrations were prepared by the appropriate dilution of the stock standard solution. Acetonitrile, methanol (Honeywell, Seelze, Germany), acetic acid (Sigma-Aldrich, Steinheim, Germany), and ammonium acetate (Merck, Darmstadt, Germany) were of pro analysis or LC-MS purity. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

2.4 Moisture content

Moisture content in whole grain triticale flour sample and extruded product samples was determined according to ISO method (ISO, 2009), and expressed on a dry weight basis.

2.5 Bulk density of extrudates

The bulk density (BD) of each extruded product sample was measured with a bulk density tester (Tonindustrie, West und Goslar, Germany) in triplicate.

2.6 Hardness determination

The hardness of the extrudates (PH) was determined using a Texture Analyser (model TA.HDPlus, Stable Micro Systems Ltd, Godalming, Surrey, UK) equipped with the 50 kg load cell. The single extrudate was diametrically positioned between a plate and movable cylindrical probe (diameter 45 mm). Test settings were as follows: pretest speed: 2.0 mm/s; test speed: 0.16 mm/s; post-test speed: 10 mm/s; distance: 2.5 mm; trigger force: 100 g. The maximum peak force from the force-time graph was considered as an indication of hardness. Hardness was expressed in kg as the mean of the results of 20 extrudates from each trial.

2.7 Water Absorption Index and Water Solubility Index

Water absorption index (WAI) and water solubility index (WSI) were determined by the method of Anderson, Conway, & Peplinski (1970) with slight modification. In brief, 0.2 g of ground extrudates was suspended in 5 mL of distilled water in weighed 15 mL glass centrifuge tube. The tube was stirred on a Vortex mixer (VELP Scientifica Srl, Italy) for 2 min and then centrifuged (Eppendorf Centrifuge 5804 R, Hamburg, Germany) at 5000 × g for 20 min at room temperature (25 °C). The supernatant was decanted into an evaporating dish of known weight. The gel obtained after decantation of the supernatant was measured and WAI was calculated using equation (1):

$$WAI\ \text{(g/g)} = weight\ of\ gel\ /\ weight\ of\ sample \qquad (1)$$

The WSI was determined using equation (2) from the weight of dry solids after evaporation of supernatant from the WAI test at 105 °C in drying oven (UNB 400, Memmert, Germany):

$$WSI\ \text{(g/100g)} = weight\ of\ dissolved\ solids\ in\ the\ supernatant\ /\ weight\ of\ sample \times 100 \qquad (2)$$

WAI and *WSI* were expressed as the mean of the results of four repetitions from each trial.

2.8 Sample preparation for LC-MS/MS analysis

The sample preparation procedure consisted of a simple one-step sample extraction which was previously described in detail by Babič et al. (2021), Topi, Tavčar-Kalcher, Pavšič-Vrtač, Babič, & Jakovac-Strajn (2019) and Topi, Babič, Pavšič-Vrtač, Tavčar-Kalcher, & Jakovac-Strajn (2021).

2.9 LC-MS/MS analysis

For the determination of 13 mycotoxins (DON, 3-AcDON, 15-AcDON, DAS, HT-2, T-2, ZEN, FB1 and FB2, OTA, AOH, AME, and TEN) ultra-performance liquid chromatography coupled with a triple-quadrupole mass spectrometer (UPLC-MS/MS) was used with electrospray ionization (ESI) interface and MassLynx software for data collection and processing (Waters, Milford, MA, USA). The LC-MS/MS method for quantification of above mentioned mycotoxins was described, in detail by Babič et al. (2021), Hojnik et al.(2019), Topi et al. (2019; 2021).

2.10 Method validation

For detected mycotoxins in whole grain triticale flour (DON, 3-AcDON, 15-AcDON, HT-2, TEN, and AME), the method was validated in terms of matrix effect, linearity, trueness, precision, limit of detection (LOD), and limit of quantification (LOQ) by an in-house quality control procedure, in the manner described in detail in our previous studies (Janić Hajnal et al., 2015; 2016; 2019), for both matrices (whole grain triticale flour and extruded product samples). The used spiking levels (four) of each mycotoxin (DON, 3-AcDON, 15-AcDON, HT-2, TEN, and AME) into both matrices (whole grain triticale flour and extruded product samples) for method validation are presented in Table 2 and Table 3. The LOD of the single analytes was determined at a signal-to-noise ratio of 3:1. A value 3.3 times the LOD was selected as the LOQ.

2.11 Statistical analysis

2.11.1 Principal component analysis (PCA)

Principal component analysis (PCA) was conducted to elucidate and identify the acquired data. The analysis of variance (ANOVA) was accomplished, with a particular purpose to inquire the effects of the factor variables over the responses. The calculation of the ANOVA, based on the gathered experimental results was done using StatSoftStatistica 13.3® software (Statistica, 2013).

2.11.2 Response surface methodology (RSM)

The impact of the three extrusion factor variables: *SS* (500, 650, and 800 rpm), *FR* (22, 26, and 30 kg/h), and *MC* (20, 25, and 30 g/100 g) on the extrusion of the whole grain triticale flour were investigated according to the experimental plan presented in Table 1. The scopes of these factors were ascertained by the preliminary trial. The experimental data employed for the analysis were produced by the Box and Behnken (BB) experimental design , which was utilized to restrict the sample size to 15 which was adequate to assessing second order polynomial (SOP) coefficients (Singha & Muthukumarappan, 2017).

2.11.3 Standard score

Standard scores were evaluated for different mycotoxins reduction trials, according to the applied extruding process. The ranking method was based according to the ratio of the raw data and the extreme values for each response (Kojić et al., 2019), following the equation (3):

$$\overline{x}_i = \frac{x_i - \min_i x_i}{\max_i x_i - \min_i x_i}, \forall i \text{, where } x_i \text{ represents the raw data.} \qquad (3)$$

3. Results and discussion

3.1 Evaluation of the LC-MS/MS method

The validation data of the analytical method for the determination of quantified mycotoxins are given in Table 2. All investigated mycotoxins showed slight signal suppression, except for AME, which showed strong signal suppression in both matrices (whole grain triticale flour and extruded product samples). The other exception relates to the HT-2 toxin, which showed slight signal enhancement in extrusion product samples. Method exhibited good linearity, with linear regression coefficient (r^2) above 0.9945.

Through recovery studies, the trueness of the analytical method was evaluated. The apparent recoveries (R_A) and the sample preparation recoveries (R_E) for target analytes were calculated as described in our previous studies (Janić Hajnal et al. 2015; 2016; 2019). It can be seen (Table 2) that the R_A and the R_E for all target analytes were above 70% in both matrices. The only exception showed AME for R_A, for the reason that AME strongly suppresses the analytical signal.

Repeatability and within-laboratory reproducibility were used for expression of the precision of method used, for both whole grain triticale flour and extruded product samples. Precision gave relative standard deviation (RSD) values within the range of 1.9 – 14.6% and 2.6 – 18.0%, respectively, fulfilling the criteria of RSD ≤ 20% and indicating a good precision of the method used (Table 3).

LODs and LOQs for both matrices (whole grain triticale flour and extruded product samples) were as follows: 15 µg/kg and 50 µg/kg for DON, 0.9 µg/kg and 3 µg/kg for 3-AcDON, 15-AcDON and HT-2 toxin, and 3.8 µg/kg and 12.5 µg/kg for TEN and AME, respectively.

3.2 Determination of mycotoxins content

The examined mycotoxins were quantified by an external matrix-matched calibration procedure (separate calibrations were prepared for both whole grain triticale flour and extruded product samples), to compensate for the matrix effects. The following mycotoxins were quantified: DON, 3-AcDON, 15-AcDON, HT-2, TEN, and AME. The results obtained were corrected for sample preparation recovery (R_E) and were expressed on a dry matter basis. Initial water content on a dry weight basis was 10.9 g/100 g in naturally contaminated whole grain triticale flour, while initial concentrations (average values of twelve measurements) of quantified mycotoxins expressed on a dry matter basis were 274.4 ± 36.4 µg/kg, 2.86 ± 0.24 µg/kg, 4.86 ± 0.43 µg/kg, 4.59 ± 0.42 µg/kg, 29.8 ± 1.78 µg/kg, and 16.7 ± 5.37 µg/kg, for DON, 3-AcDON, 15-AcDON, HT-2, TEN and AME, respectively. All extruded product samples were analyzed in duplicate.

The water content of extruded product samples was ranged from 8.05 to 14.1 g/100 g on a dry weight basis, while the final concentration expressed on a dry matter basis of quantified mycotoxins in extruded product samples were ranged from 229.0 to 274.1 µg/kg for DON, from 1.12 to 2.81 µg/kg for 3-AcDON, from 2.60 to 4.47 µg/kg for 15-AcDON, from 1.81 to 3.47 µg/kg for HT-2, from 23.5 to 29.3 µg/kg for TEN and from 1.37 to 7.83 µg/kg for AME.

3.3 Reduction of mycotoxins by extrusion processing

The effects of extrusion process variables – screw speed (SS), feed rate (FR), and moisture content (MC) – on observed responses (DON, 3-AcDON, 15-AcDON, HT-2, AME, and TEN reduction rate, *P, Tend, TR, SME, Torque, TR, BD, PH, WAI,* and *WSI*) were determined (Table 4). Reduction of quantified mycotoxins during the extrusion process is expressed as a percentage reduction concerning its initial concentration in the whole grain triticale flour, and it is used in all the performed statistical analyses. Process variables (*SS*, *FR*, and *MC*) were varied according to BB experimental design (Table 4) and the range of observed responses was: *P* from 2.7 to 7.9 MPa, *Tend* from 113 to 151 °C, *SME* from 66.9 to 125 kWh/t, *Torque* from 39.6 to 59.4 Nm, mean retention time in the barrel (*TR*) from 4 to 11 s, *BD* from 0.538 to 0.596 g/mL, *PH* from 6.7 to 19.4 kg, *WAI* from 3.5 to 5.4 g/g and *WSI* from 9.1 to 12.5 g/100 g. Reduction of all investigated mycotoxins was achieved in all trials (extruded product samples) (Table 4). Reduction of DON ranged from 0.12 to 16.6%, while for 3-AcDON, 15-AcDON and HT-2, ranged from 1.7 to 32.8%, from 1.7 to 45.7%, and from 24.3 to 60.5%, respectively. Further, the reduction of TEN ranged from 1.7 to 21.2%, while for AME ranged from 53.2 to 91.8%. The maximum reduction rates (TS-15) for DON and AME of 16.6 and 91.8%, respectively, were obtained at the following process parameters: SS=500 rpm, *FR*= 26 kg/h, and *MC*=20 g/100 g. At the highest screw speed (800 rpm), the lowest feed rate (22 kg/h), and a medium moisture content of the raw material (25 g/100 g), the highest reduction rate (32.8%) of 3-AcDON was achieved (TS-6), while the maximum reduction rate of 45.7% for 15-AcDON (TS-1) was obtained at the highest screw speed (800 rpm), the medium feed rate (26 kg/h), and at the highest moisture content of the raw material (30 g/100 g). Further, at the medium screw speed (650 rpm), the highest feed rate (30 kg/h) and the lowest moisture content of whole grain triticale flour (20 g/100 g), the highest reduction rate (60.5%) of HT-2 toxin was obtained (TS-14). Regarding TEN, its maximum reduction rate (TS-12) during the extrusion process of 21.2% was achieved at the highest screw speed (800 rpm), the medium feed rate (26 kg/h), and at the lowest moisture content (20 g/100 g) of the raw material. If several mycotoxins are found in the raw material, the substantial aim is to minimize their concentrations to the lowest possible level, with a modest effect on the quality of the final product. Having this in mind, the implementation of a suitable mathematical methodology is vital for optimizing the quality of the final result.

3.4 Principal component analysis

Firstly, the PCA analysis pursued to the acquired experimental data set has illustrated a partitioning among samples, as suggested by the factor variables and it was applied as a tool in exploratory data analysis to describe and distinguish response variables (Fig. 2). The conclusion of the PCA analysis interpreted the first three principal components, counting for 67.3% of the total variance, which can be perceived as sufficient for data explanation. *Tend*, *P*, *SME*, Torque, *WAI*, reduction of TEN (*rTEN*) and *rAME* had been more potent for the primary principal component evaluation (contributing: 15.8; 11.0; 14.0; 9.3; 17.0; 8.3 and 8.9%, accordingly, based on correlations), while *P*, *BD*, *WSI*, *r3-AcDON, r15-AcDON, rHT-2* and *rTEN* had been more crucial for the second principal component computation (9.9; 15.5; 14.8; 23.3; 12.3; 10.9 and 7.7%, respectively). The most powerful factors for PC3 calculation were Torque, *PH*, *rDON* and *r3-AcDON*, and *rHT-2* (with a share of 10.3; 24.3; 26.4; 10.5 and 8.1%, individually). The PCA plot (Fig. 1) pointed out well segregation between samples. Samples acquired by higher screw speed are positioned at the right side of the chart; these samples are classified by higher *P*, *RT*, *Tend*, *Torque*, *SME*, and *WAI*, and also by the augmented reduction of DON, AME, and TEN content.

3.5 Response surface method

ANOVA evaluation was performed on the developed SOP models, and each of them was investigated on the effects of input variables (Table 5). The analysis demonstrated that the linear terms of *SS* and *MC* were the most significant variables in the SOP model for *Tend* computation (statistically significant at p<0.01 and p<0.05, accordingly) while the impact of interchange term *SS* × *MC* was significant at p<0.05 level. *P* evaluation was mostly affected by the linear terms of *SS* and *FR* in the SOP model (statistically significant at p<0.01 and

$p<0.05$ levels, respectively). The linear terms of *SS* and *FR*, as well as the quadratic term of *FR* were the most influential for *SME* calculation (statistically significant at $p<0.01$ level), while the linear term of *MC* and the interactive terms *SS* × *MC* and *MC* × *FR* were influential at the statistically significant level of $p<0.05$. *Torque* was mostly impacted by the linear terms of *SS* and *MC* in the SOP model (statistically significant at $p<0.01$ level), while the linear term of *FR* and the quadratic terms of *SS* and *FR* significantly contributed to *Torque* evaluation ($p<0.05$). *BD* and *PH* were altered by the non-linear term of *SS* × *MC* ($p<0.05$ level), while *PH* was also influenced by the linear term of *MC* (statistically significant at $p<0.01$ level).

The prior studies were focused on the increase of *SS*, which conducted the lowered *BD* of extrudates (Ding et al., 2006; Filli et al., 2012; Gulati et al., 2016). This was also displayed in Table 5. The higher *SS* augmented the elasticity of the dough in the extruder tube, which decrease *BD* (Fletcher et al., 1985). The raise of MC guided to an increase in *BD* (Ding et al. 2005; Gulati et al., 2016; Liu et al., 2011), and this was also corroborated in this study (Table 5).

WAI and *WSI* were impacted by the quadratic term of *SS*, while *WAI* was also influenced by the linear term of *SS* (statistically significant at $p<0.01$ level). These results are in consent with earlier studies for the following samples: an extruded mixture of maize bite and spell (Jozinović et al., 2016), quinoa (Dogan and Karwe, 2003), amaranthus (Menegassi et al., 2011), sorghum (Mahasukhonthachat et al., 2010), corn grits with buckwheat and chestnut (Jozinović, 2012), and corn-wheat extrudate (Sobota et al. 2010).

The analysis explained that the quadratic terms of *MC* were the most effective for *r3-AcDON* and *rHT-2* calculation in SOP models (statistically significant at $p<0.05$ level). *rTEN* was mostly influenced by the linear terms of *SS* and *MC*, and also by the quadratic term of *SS* and the non-linear term of *SS* × *FR* in the SOP model ($p<0.01$ level), while the linear term of

FR and the non-linear term of quadratic term of *SS* × *MC* significantly contributed to *rTEN* calculation, statistically significant at $p<0.05$ level. *rAME* was mostly influenced by the linear term of *SS* in SOP model computation ($p<0.05$).

All SOP models had an insignificant lack of fit tests, which means that all the models represented the data satisfactorily. The r^2 values were very suitable and showed a good fit of the model to experimental results.

3.6 Optimization study of the extruder parameters, performed by standard score

The optimal score was determined by averaging the scores for all mycotoxins reduction variables:

$$\text{Score (MC,FR,SS)} = \frac{\overline{rDON} + \overline{r3-AcDON} + \overline{r15-AcDON} + \overline{rHT-2} + \overline{rTEN} + \overline{rAME}}{6} \quad (4)$$

The maximum score function displayed the optimal factor variables, and also the optimum for mycotoxins reduction variables. Standard score evaluation results were presented in Fig. 3. The best scores were reached in sample TS-14, while the optimized parameters were as follows: *SS* = 650 rpm, *FR* = 30 kg/h, and *MC* = 20 g/100 g. The obtained parameters for extrusion process were: *Tend* = 151 °C, *P* = 5.9 MPa, *SME* = 107.8 Wh/kg, *Torque* = 59.4 Nm and *RT* = 7.5 s, while the physico-chemical properties of the optimal sample were: *BD* = 0.589 g/mL, *PH* = 19.4 kg, *WAI* = 5.0 g/g and *WSI* = 9.1 g/100 g (Table 4). The reduction rates of examined mycotoxins at optimal extrusion conditions were as follows: 9.5, 27.8, 28.4, 60.5, 12.3, and 85.7%, for DON, 3-AcDON, 15-AcDON, HT-2, TEN, and AME, respectively. The other two samples which were close to optimal score were samples TS-13 and TS-2, which gained scores of 0.689 and 0.657, respectively (Fig. 3). Sample TS-13 was produced using extruder parameters: *SS* = 650 rpm, *FR* = 22 kg/h and *MC* = 20 g/100 g, while sample TS-2 was obtained using parameters: *SS* = 650 rpm, *FR* = 22 kg/h and *MC* = 30 g/100 g. The physical-chemical properties of sample TS-13 are characterized by the lowest pellet hardness of 6.7 kg compared to other produced pellets, while the values for *WAI* and *WSI* were approximately at the same level as in the sample TS-14. Sample TS-2 had the largest bulk density (g/mL) relative to other extruded produced and medium hardness, while *WAI* was one unit lower than *WAI* of sample TS-14 (Table 4). Regarding mycotoxins reduction by above mentioned extrusion conditions, in extrudates TS-13, reduction by 14.9, 30.8, 35.5, 45.7,12.9, and 81.4 %, for DON, 3-AcDON, 15-AcDON, HT-2, TEN, and AME, respectively were achieved, while in samples TS-2 reduction of 13.0, 32.4, 5.5, 57.0, 12.4 and 83.4% for DON, 3-AcDON, 15-AcDON, HT-2, TEN and AME, respectively were obtained.

As some mycotoxins are highly toxic, maximum limit levels have been established to protect consumers' health. Among examined mycotoxins, Commission regulation (EC) No. 1881/2006 set maximum levels in foodstuffs (EC, 2017) just for DON. Further, Commission recommendation No. 165/2013 (EU, 2013) on the presence of T-2 and HT-2 toxin in cereals and cereal products has issued recommended levels for the sum of HT-2 and T-2, which is 50 µg/kg. Accordingly to the presence of the mycotoxins in original triticale flour before the extrusion process, only DON and HT-2 content can be used for evaluation of possible risks for human health. The maximum permitted level for DON stated in EC (2017) for cereals intended for direct human consumption, cereal flour is 750 µg/kg. The content of DON in triticale flour before extrusion in our study was 274.4 µg/kg, and after the process, the DON content was reduced under optimized conditions for a maximum of 16.6%. Also HT-2 content was lower as 50 µg/kg before and after the extrusion. DON and HT-2 content in triticale flour can be evaluated as non-risk flour for human consumption. Evaluation of the other mycotoxins content cannot be done, since maximum permitted level or indicative recommend level data for them are not known.

The obtained results in this study may have a great contribution to the further selection of appropriate extrusion parameters depending on the mycotoxins present in whole grain triticale flour. It is well known that all of the investigated mycotoxins (DON, 3-AcDON, 15-AcDON, HT-2, AME, and TEN) may potentially affect human and animal health. Most of the data on their toxicity concern their effects when present alone. The investigated trichothecenes (DON, 3-AcDON, 15-AcDON, and HT-2) are potent inhibitors of protein, DNA, and RNA synthesis, causing teratogenic, neurotoxic, embryotoxic, and immunosuppressive effects. The effects of short-term consumption of contaminated food could lead to nausea and vomiting followed by abdominal pain, diarrhea, headache, dizziness, and fever (He et al., 2007; Chen et al., 2020). From the perspective of human health, only DON is classified by International Agency for Research on Cancer in Group 3 not classifiable as to its carcinogenicity (Ostry, Mlir, Toman, & Grosse, 2017). *Alternaria* toxins may have acute or chronic toxic effects and pronounced fetotoxic, teratogenic, and mutagenic effects (Bhunia, 2018). Hence, in terms of human and animal health protection, any achieved reduction of mycotoxins contamination, both individual and combined mycotoxins, is of great importance. If whole grain triticale flour is contaminated with a high level of the following individual mycotoxins DON, 3-AcDON, 15-AcDON, and HT-2, for their maximum reduction, extrusion processing parameters used for the production of samples TS-15, TS-6, TS-1, and TS-14 (Table 4), should be applied, respectively. Further, if a sample is contaminated with *Alternaria* toxins, extrusion processing parameters for the production of TS-12, should be used for the maximum reduction of AME and TEN. Contamination of whole triticale flour with more than one mycotoxin would require a compromise solution such as the choice of extrusion parameters that most reduce the concentrations of mycotoxins present and at the same time achieve a satisfactory quality of the final products. In the present study, extrusion processing parameters applied for the sample TS-14, followed by TS-13 and TS-6, resulted in the best reduction of the sum of mycotoxins present in whole grain triticale flour.

3.7. Comparison of the obtained results with the literature data

The obtained results in this study could not be completely compared to the published data, since to the best of the authors' knowledge there is no previously published data regarding the fate of a larger number of co-occurred mycotoxins in triticale during the extrusion process. Regarding the reduction of *Fusarium* toxins content by the extrusion process, the so far published data relate mainly to the reduction of DON in wheat and FBs in maize (Schaarschmidt & Fauhl-Hassek, 2018; 2021). Changes in DON content during the extrusion process (twin-screw extruder) of whole grain wheat flour ranged from +1 to – 23%, while the reduction rate of DON during the extrusion process (twin-screw extruder) of soaked wheat grains ranged from 6 to 10% (Schaarschmidt & Fauhl-Hassek, 2018). Reduction of DON content during the extrusion process of whole grain triticale flour (0.12 – 16.6%) in this study is in agreement with the published findings so far. On the other hand, by extrusion of spiked wheat grits with laboratory-scale single-screw extruder, reduction of DON content was ranged from 3 to 60%, depending on applied extrusion process parameters (Wu, Lohrey, Cramer, Yuan, & Humpf, 2011). Further, Pleadin et al. (2019) reported, that depending on applied temperature profiles in dosing/compression/ejection zone of the laboratory scale single-screw extruder (135/150/150 °C; 135/170/170 °C and 135/190/190 °C), the following reduction rates of DON were obtained: 51, 61 and 71 % for wheat; 73, 80 and 87% for oat; and 55, 60 and 66% for maize. Similar results were obtained regarding DON reduction during the extrusion process of maize (Schaarschmidt & Fauhl-Hassek, 2021). Namely, by extrusion of maize grits using laboratory-scale twin-screw extruder, the reduction rate of DON was ranged from 22 to 53%, while by using laboratory-scale single-screw extruder for extrusion of whole grain maize grits, its reduction was from 55 to 66%. Contrary to the above mentioned findings, by extrusion of maize grits with a pilot-scale twin-screw extruder, the reduction rates of DON were ranged from 3 to 13%. The so far published study regarding the fate of DON during the extrusion process indicated, that the reduction rate of DON increased at higher temperatures, and lower moisture content of the raw material. Our findings are in agreement with the available data on the fate of DON during extrusion, since the maximum reduction rate of 16.6% (T-15) was achieved at a similar condition (Table 4).

Concerning the fate of *Alternaria* toxins during the extrusion process, only the fate of AME can be compared to our previous study (Janić Hajnal et al., 2016). Namely, the reduction rates of AME depending on applied process parameters in this study ranged between 53.2 to 91.8%, while in our previous study its reduction rate was very similar (62.8 to 94.5%). Moreover, the maximum reduction rate of AME of 91.8% in the present study (pilot-scale twin-screw extruder) is very similar to the obtained reduction rate of AME (94.5%) by extrusion of whole grain wheat flour using a pilot-scale single screw extruder (Janić Hajnal et al., 2016). In the present study, AME reduction was mostly affected by the linear term of screw speed of twin-screw extruder ($p<0.05$), while in our previous study the level of AME reduction was mostly influenced by the linear term of moisture content of the whole grain wheat flour and the quadratic term of screw speed of single-screw extruder (Janić Hajnal et al., 2016). Findings of present and previous studies indicate that the level of reduction of AME content during the extrusion of small grain cereals (whole grain triticale flour, whole grain wheat flour) is very similar by using both types of extruders, although the process parameters, to achieve the maximum reduction of the AME content differ among the types of extruders used.

4. Conclusions

To the best of our knowledge, the results of this study represent the first report regarding the fate of a larger number of mycotoxins, as well as the first data about the fate of some less studied mycotoxins (3-AcDON, 15-AcDON, HT-2, TEN, and AME) during the extraction process of whole grain triticale flour. The best standard scores were obtainedby using extrusion process parameters with the medium screw speed , the highest feed rate , and the lowest moisture content of raw material , which provide the optimal reduction rates of present mycotoxins in the final product.. In brief, due to the combined action of heat, pressure, and shear force, the extrusion process has conditionally a high potential for mycotoxins reduction. However, based on the so far published data, as well as on the obtained results, it can be concluded that due to the complex interaction of the various parameters, the effect of the extrusion process on different mycotoxins still needs to be determined in detail for each combination of ingredient composition, as well as for applied parameters setting. In the case of contamination of the raw material with a large number of mycotoxins, it is not possible to achieve the maximum reduction of the content of each present mycotoxins by extrusion process. For this reason, there is a need to find a compromise solution, i.e. optimal extrusion conditions that will provide a satisfactory reduction of mycotoxins present, as well as a satisfactory quality of the final products.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures captions

Fig. 1. Scheme of the co-rotating twin-screw extruder

Fig. 2. PCA ordination of variables based on component correlations, presented in the first and the second factor plane.

Abbreviations: *MC* ¬ moisture content (g/ 100 g); *FR* ¬ feed rate (kg/h); *SS* ¬ screw speed (rpm); *Tend* – die temperature (°C); *P* - pressure at the die (MPa); *SME* – torque (Wh/kg); *RT* ¬ retention time in the barrel (s), *BD* ¬ bulk density (g/mL); *PH* ¬ pellet hardness (kg); *WAI*- water absorption index (g/g); *WSI* ¬ water solubility index (g/100 g); rDON – reduction of deoxynivalenol (%); *r3-AcDON* – reduction of 3-acetyldeoxynivalenol (%); *r15-AcDON* – reduction of 15-acetyldeoxynivalenol (%); *rHT-2* ¬ reduction of HT-2 toxin (%); *rTEN* – reduction of tentoxin (%); *rAME* – reduction of alternariol monomethyl ether (%).

Fig. 3. Standard score analysis for mycotoxins reduction by extrusion processing of whole grain triticale flour.

Table 1
Independent extrusion parameters and their levels.

Experimental factor	Factor's level		
	low	center	high
Screw speed (rpm)	500	650	800
Feed rate (kg/h)	22	26	30
Moisture content (%)	20	25	30

Table 2

Recovery data of the employed analytical method based on the solvent (R_A) and matrix-matched (R_E) calibration curves and matrix effect (SSE).

Anaytes		Whole grain triticale flour			Extruded product		
	Spiking level (µg/kg) [a]	R_A [b]	R_E [c]	SSE [d]	R_A [b]	R_E [c]	SSE [d]
DON	50 – 400	80.3	102.3	73.3	90.2	100.1	90.2
3-AcDON	3.0 – 24	88.7	109.5	81.1	89.4	97.0	92.2
15-AcDON	3.0 – 24	92.6	95.4	97.1	99.0	101.7	97.4
HT-2	3.0 – 24	78.9	89.4	88.2	98.8	94.0	105.2
TEN	12.5 – 100	88.3	105.1	84.1	92.0	101.0	91.1
AME	12.5 – 100	32.3	103.3	31.3	31.6	98.6	32.1

[a] Concentration range of analytes for standard, matrix-matched calibration curves and calibration curves of spiked samples (µg/kg).

[b] R_A - Apparent recovery (%) calculated by the slope of spiked sample-prepared curve/slope of the solvent calibration curve.

[c] R_E - Sample preparation recovery (%) calculated by the slope of spiked sample-prepared curve/slope of matrix-matched calibration curve.

[d] SSE-matrix effect (%) calculated by the slope of matrix-matched calibration curve/slope of the solvent calibration curve.

Table 3

Precision data of the examined mycotoxins.

Analytes		Whole grain triticale flour		Extruded product	
	Spiking level (μg/kg)	Repeatability[a] RSD (%)	Within-laboratory reproducibility[b] RSDs (%)	Repeatability[a] RSD (%)	Within-laboratory reproducibility[b] RSDs (%)
DON	50	14.6	17.8	11.9	14.1
	100	10.9	12.0	3.68	6.74
	200	10.3	11.8	2.90	4.64
	400	4.32	7.81	1.97	2.57
3-AcDON	3	11.1	15.3	8.66	10.0
	6	10.2	11.2	7.79	9.55
	12	9.38	10.3	7.85	8.74
	24	5.89	8.25	5.98	6.79
15-AcDON	3	7.71	12.7	4.04	10.9
	6	4.35	6.95	3.79	5.37
	12	3.79	6.36	3.14	4.47
	24	3.31	6.21	2.75	4.38
HT-2	3	13.8	18.0	13.55	12.8
	6	13.2	14.5	12.4	9.13
	12	7.07	7.46	5.56	8.78
	24	6.92	6.89	3.94	6.88
TEN	12.5	6.01	7.38	4.86	6.24
	25	3.27	10.1	6.26	7.35
	50	6.69	10.1	2.58	2.94
	100	3.93	4.29	3.93	5.18
AME	12.5	4.79	9.81	2.99	9.82
	25	4.86	5.53	1.92	5.68
	50	2.52	2.94	2.37	4.93
	100	4.22	9.74	4.77	9.38

[a] Results expressed as mean (RSD) (n = 6).

[b] Results expressed as mean (RSD_s) (n = 3×6).

Table 4
Technological parameters of extrusion and reduction of mycotoxins.

Sample	Factors			Process responses					Product responses									
	SS	*FR*	*MC*	*Tend*	*P*	*SME*	Torque	*RT*	*BD*	*PH*	*WAI*	*WSI*	*rDON*	*r3-AcDON*	*r15-AcDON*	*rHT-2*	*rTEN*	*rAME*
TS-1	800	26	30	116	3.0	87.8	46.2	7.0	0.580	14.8	3.7	10.6	0.12	8.6	45.7	24.3	2.9	53.2
TS-2	650	22	30	118	3.1	66.9	39.6	6.0	0.596	12.7	4.1	9.2	13.0	32.4	5.5	57.0	12.4	83.4
TS-3	650	30	30	118	4.1	71.1	44.0	6.0	0.551	14.6	3.5	10.9	13.3	24.3	4.6	56.8	2.4	65.4
TS-4	500	26	30	113	2.7	68.9	44.0	6.0	0.565	13.3	3.6	11.7	13.8	31.2	37.0	44.5	9.0	61.4
TS-5	650	26	25	132	5.4	90.8	44.0	5.8	0.572	14.3	4.4	11.0	9.1	6.0	13.3	35.8	3.4	83.8
TS-6	800	22	25	132	4.4	113.5	46.2	5.0	0.571	14.7	4.5	11.0	15.1	32.8	33.6	47.0	12.6	76.3
TS-7	650	26	25	129	5.3	91.2	44.0	6.8	0.569	13.8	3.8	12.5	9.7	5.5	13.0	39.1	3.0	84.0
TS-8	800	30	25	134	4.8	94.7	55.0	5.5	0.589	14.7	4.0	11.1	8.8	30.8	35.4	47.7	4.7	76.1
TS-9	650	26	25	131	5.5	90.7	44.0	6.8	0.565	14.5	3.9	11.0	11.1	5.7	13.0	37.6	3.3	83.0
TS-10	500	22	25	129	6.1	88.6	44.0	6.0	0.547	10.5	4.1	11.1	15.4	23.6	1.7	34.9	5.2	55.5
TS-11	500	30	25	132	5.8	88.0	44.0	4.0	0.564	15.4	4.1	11.8	13.4	1.7	12.2	45.1	1.7	83.8
TS-12	800	26	20	140	5.6	125.0	57.2	6.5	0.542	13.3	5.4	11.1	15.5	18.5	16.6	26.2	21.2	88.7
TS-13	650	22	20	139	5.6	119.3	46.2	5.5	0.556	6.7	5.2	9.3	14.9	30.8	35.5	45.7	12.9	81.4
TS-14	650	30	20	151	5.9	107.8	59.4	7.5	0.589	19.4	5.0	9.1	9.5	27.8	28.4	60.5	12.3	85.7
TS-15	500	26	20	147	7.9	111.9	52.8	11	0.538	13.7	5.1	10.1	16.6	16.5	8.1	43.9	9.4	91.8

SS: screw speed (rpm); *FR*: feed rate (kg/h); *MC*: moisture content (g/100 g); *Tend*: die temperature (°C); *P*: pressure at the die (MPa); *SME*: specific mechanical energy(Wh/kg); Torque (Nm); *RT*:mean retention time in the barrel (s), *BD*: bulk density (g/mL); *PH*: pellet hardness (kg); *WAI*: water absorption index (g/g); *WSI*: water solubility index (g/100 g); *rDON*: reduction of deoxynivalenol (%); *r3-AcDON*: reduction of 3-acetyldeoxynivalenol (%); *r15-AcDON*: reduction of 15-acetyldeoxynivalenol (%); *rHT-2*:reduction of HT-2 toxin (%); *rTEN*: reduction of tentoxin (%); *rAME*: reduction of alternariol monomethyl ether (%).

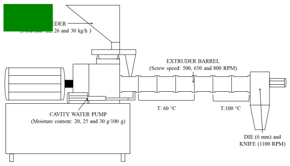
Table 5

ANOVA evaluation of technological parameters and reduction of mycotoxins (sum of squares).

	df	*Tend*	*P*	*SME*	Torque	*RT*	*BD*	*PH*	*WAI*	*WSI*	*rDON*	*r3-AcDON*	*r15-AcDON*	*rHT-2*	*rTEN*	*rAME*
SS	1	1568.0^{+}	1869.7^{+}	3582.8^{+}	218.4^{+}	3.8	0.0006	0.7	4.1^{+}	0.9	33.4	1.1	2.2	4.9	106.4^{+}	886.2^{*}
SS^2	1	3.4	135.1	6.1	33.8^{*}	4.8^{**}	0.0001	0.7	0.4^{*}	4.8^{*}	1.0	353.4	122.6	68.0	118.9^{+}	13.2
MC	1	36.1^{*}	26.3	89.1^{*}	87.1^{+}	0.0	0.0001	48.1^{+}	0.2^{**}	0.7	22.2	153.1	2.3	81.0	60.5^{+}	25.9
MC^2	1	11.9	6.7	3.1	0.3	6.8^{**}	0.0002	0.7	0.0	2.1^{**}	17.8	654.0^{*}	0.5	644.0^{*}	4.3	27.7
FR	1	0.1	300.1^{*}	505.6^{+}	49.0^{*}	1.1	0.0006	2.6	0.1	0.1	48.0	39.2	653.4	68.0	32.5^{*}	0.4
FR^2	1	1.9	0.1	142.5^{+}	33.8^{*}	0.0	0.0003	0.0	0.0	0.9	3.8	37.4	235.6	183.8	10.9^{**}	232.6
SS × *MC*	1	36.0^{*}	13.0	61.6^{*}	19.4^{**}	1.0	0.0015^{*}	29.4^{*}	0.0	0.9	8.0	6.5	9.6	55.7	21.6^{*}	124.3
SS × *FR*	1	25.0^{*}	175.6^{**}	8.4	1.2	7.6^{**}	0.0000	0.9	0.0	1.0	39.7	151.3	0.0	1.6	80.3^{+}	6.5
MC × *FR*	1	0.3	13.3	82.8^{*}	19.4^{**}	1.6	0.0000	5.8	0.1	0.1	4.4	99.0	18.9	22.4	4.8	203.1
Error	5	22.9	171.7	28.4	15.7	5.7	0.0009	12.8	0.3	1.7	66.0	459.9	1793.5	364.0	11.6	576.6
r^2		0.987	0.937	0.994	0.967	0.828	0.786	0.874	0.948	0.872	0.728	0.752	0.364	0.763	0.974	0.722

SS: screw speed (rpm); *FR*: feed rate (kg/h); *Tend*; *MC*: moisture content (g/100 g); *Tend*: die temperature (°C); *P*: pressure at the die (MPa); *SME*: specific mechanical energy (Wh/kg); Torque (Nm); *RT*: mean retention time in the barrel (s), *BD*: bulk density (g/mL); *PH*: pellet hardness (kg); *WAI*: water absorption index (g/g); *WSI*: water solubility index (g/100 g); *rDON*: reduction of deoxynivalenol (%); *r3-AcDON*: reduction of 3-acetyldeoxynivalenol (%); *r15-AcDON*: reduction of 15-acetyldeoxynivalenol (%); *rHT-2*: reduction of HT-2 toxin (%); *rTEN*: reduction of tentoxin (%); *rAME*: reduction of alternariol monomethyl ether (%).

$^{+}$Statistically significant at $p<0.01$ level; *Statistically significant at $p<0.05$ level; **Statistically significant at $p<0.10$ level.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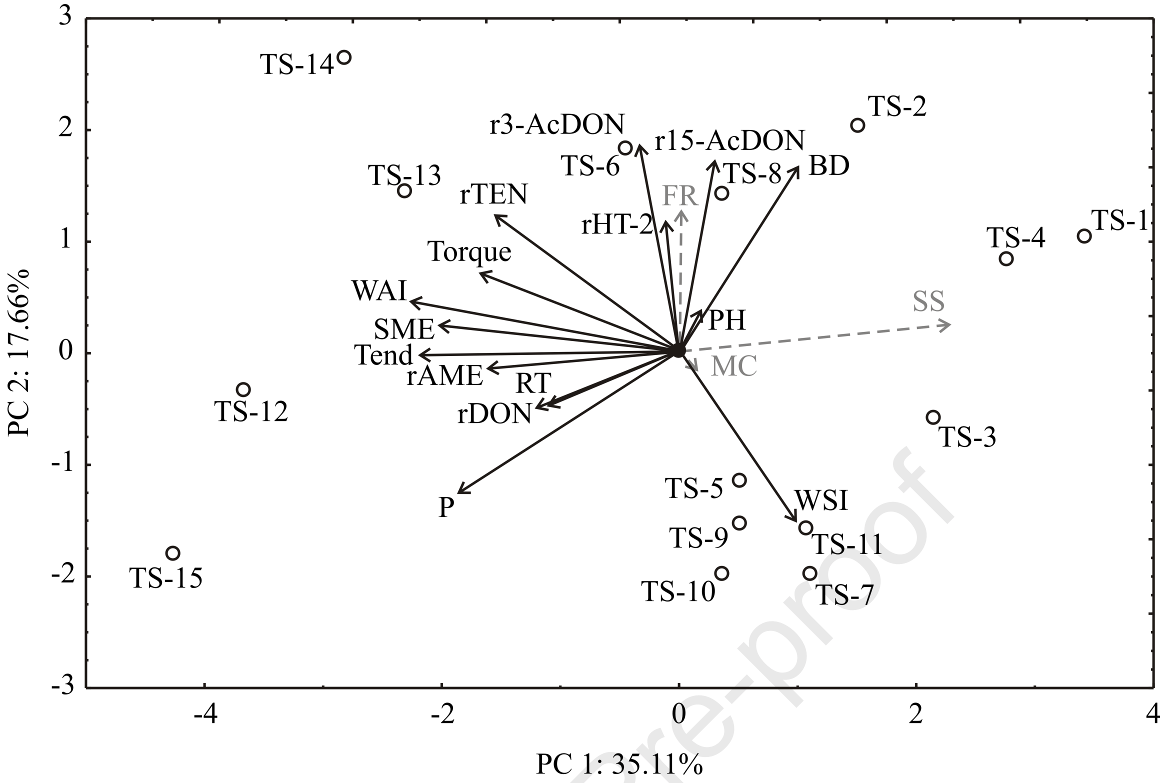
(Moisture content: 20, 25 and 30 g/100 g)
DIE (6 mm) and
KNIFE (1100 RPM)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TS-4
Torque
WAI
PH
SS
SME
Tend
MC
rAME
RT
rDON
TS-12
TS-3
TS-5
WSI
P
TS-9
TS-11
TS-15
TS-10
TS-7
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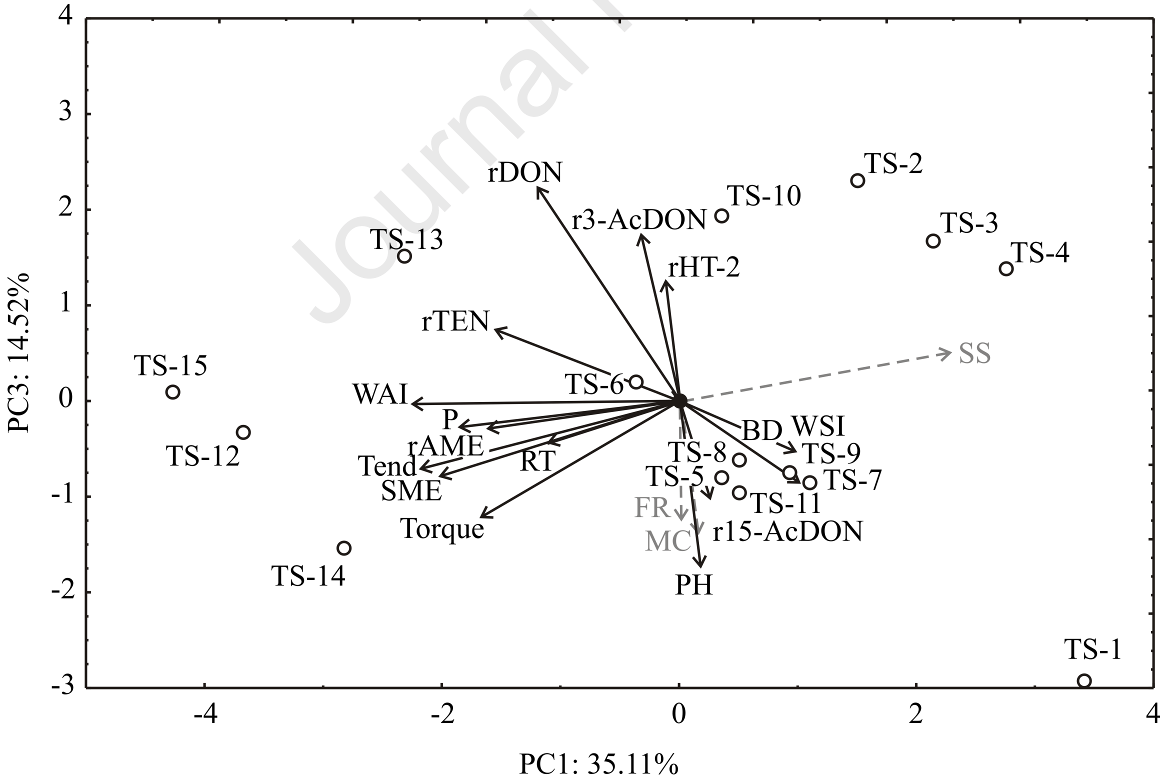
PC 1: 35.11%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rHT-2
rTEN
SS
TS-15
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WAI
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BD
WSI
TS-12
rAME
TS-8
TS-9
Tend
RT
TS-5
TS-7
SME
TS-11
FR
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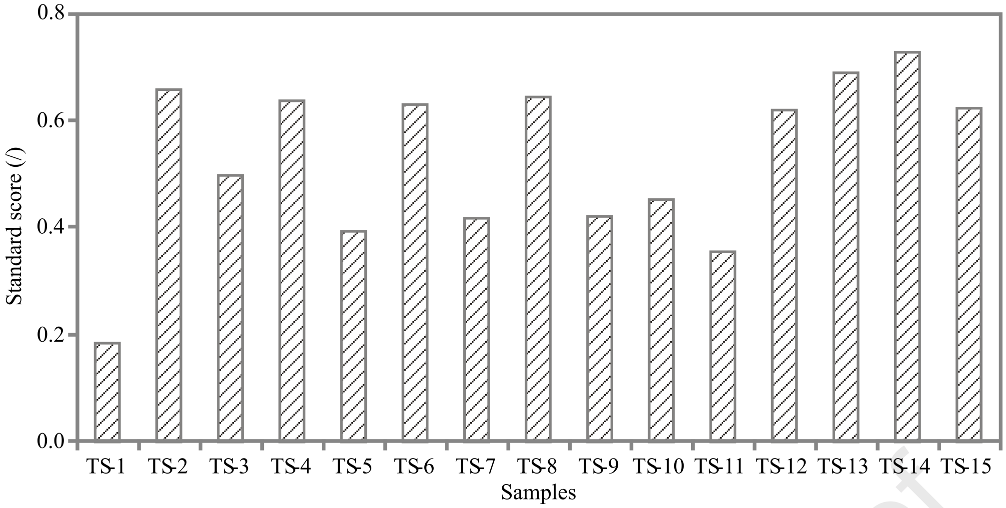
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TS-11
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TS-13
TS-14
TS-15
Samples

Highlights

- Reduction of mycotoxins by extrusion processing of triticale flour was investigated
- *Fusarium* and *Alternaria* toxins were analyzed by the validated LC-ESI-MS/MS method
- PCA, RSM and standard score were used to evaluate the effect of process parameters
- Optimal reduction rate of DON, 3-AcDON, 15-AcDON, HT-2, TEN and AME was determined

CRediT author statement

Elizabet Janić Hajnal: Conceptualization, Methodology, Resources, Formal analysis, Writing - Original Draft, Writing - Review & Editing**. Janja Babič:** Formal analysis**,** Writing - Original Draft Writing - Review & Editing. **Lato Pezo:** Conceptualization**,** Writing - Original Draft, Writing - Review & Editing. **Vojislav Banjac:** Investigation, Formal analysis. **Radmilo Čolović:** Investigation, Formal analysis**. Jovana Kos:** Conceptualization**,** Formal analysis, Writing - Review & Editing. **Jelena Krulj:** Formal analysis**. Katarina Pavšič-Vrtač:** Formal analysis, Writing - Review & Editing**. Breda Jakovac-Strajn:** Conceptualization, Resources, Writing - Review & Editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.