Food and Chemical Toxicology 125 (2019) 289–304

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Numerical optimization of temperature-time degradation of multiple mycotoxins

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ARTICLE INFO

Keywords:
Mycotoxins
Numerical modeling
Optimization
Thermal degradation
Thermal stability

ABSTRACT

Mycotoxins are potent food contaminants that exert significant deleterious effects on human and animal health, yet, there is limited and often conflicting data on their thermal stability. The present study systematically investigated the thermal degradation patterns of multiple mycotoxins as a function of temperature and time, in pure form and spiked into a food matrix (maize flour), using a numerical modelling approach. Mycotoxins under investigation included aflatoxins (AFs), fumonisins (FBs), zearalenone and its analogue α and β epimers (ZEAs), ochratoxins (OTs), T-2 toxin (T-2), alternariol monomethyl ether (AME) and sterigmatocystin (STEG). A set of statistically-designed experiments were conducted, and a second-order optimization function fitted to the experimental data. The resultant models were well fit with R^2 values ranging from 0.87 to 0.99 and 0.89 to 0.99, for pure mycotoxin standards and spiked maize flour, respectively. It was also possible to statistically determine the optimum degradation conditions which were 216.57 °C/63.28 min and 210.85 °C/54.71 min for pure mycotoxins and spiked into maize flour, respectively. Our observations herein could be critical for food safety applications targeted at reducing or at best eliminating completely multi-mycotoxins in food using heat processing while limiting the destruction of food quality factors.

1. Introduction

Mycotoxins are poisonous biochemical compounds of fungal origin that contaminate various food and feed commodities on a global scale. They have been implicated as major environmental hazards due to their perpetual proliferation in food and feed products, and subsequent possible lethal effects on humans and animals (Enyiukwu et al., 2014; Gbashi et al., 2017b; Makun et al., 2012; B. P. Njobeh et al., 2010; Zain, 2011). One of the mycotoxins, aflatoxin B_1 (AFB_1), has been classified as the most noxious naturally-occurring carcinogen known to man (FAO, 2004). Although mycotoxins have been estimated in a wide range of food and feed commodities (Chilaka et al., 2016; Mircea et al., 2008; Yang et al., 2014), maize in particular is a favoured food substrate for incessant contamination (often at levels above regulatory limits) due to its susceptibility to attack by mycotoxigenic fungal species (Makun et al., 2012; Njobeh et al., 2010a,b, 2012). This is of great concern because maize and maize-based products are staple foods for billions of people, and as well often constitute a major component of animal feed (du Plessis, 2003; Ranum et al., 2014). In South Africa for instance, daily consumption of maize and maize-based meals can reach up to 328 g/person (du Plessis, 2003; Ranum et al., 2014). Because of that, maize is an ideal reference matrix for investigating various mitigation approaches in mycotoxicology (Brown, 1999; Lauren and Smith, 2001; Raters and Matissek, 2008).

Different approaches have been investigated for the degradation of mycotoxins, some of which include gamma irradiation (Hooshmand and Klopfenstein, 1995), UV irradiation (Murata et al., 2008), thermal processing (Bretz et al., 2006; Kabak, 2009; Raters and Matissek, 2008), microbial and enzyme degradation (Adebo et al., 2017; Ji et al., 2016), plasma-based degradation (ten Bosch et al., 2017), microwave-induced argon plasma degradation (Park et al., 2007), oxidative degradation using ozone (McKenzie et al., 1997), and many others (Doyle et al., 1982; Juodeikiene et al.,

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https://doi.org/10.1016/j.fct.2019.01.009
Received 20 September 2018; Received in revised form 10 January 2019; Accepted 11 January 2019
Available online 14 January 2019
0278-6915/

Abbreviations

AAD	Average absolute deviation
A_f	Accuracy factor
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFs	Aflatoxins
AME	Alternariol monomethyl ether
B_f	Bias factor
CCD	Central composite design
CE	Collision energy
DF	Desirability factor
ESI^+	Electron spray ionization
FA	Formic acid
FB_1	Fumonisin B_1
FB_2	Fumonisin B_2
FB_3	Fumonisin B_3
FBs	Fumonisins
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
LC-MS/MS	Liquid chromatography couple to tandem mass spectrometry
MRM	Multiple reaction monitoring
MS	Mass spectrometry
OTs	Ochratoxin A and ochratoxin B
Q1	Quadruple 1
Q3	Quadruple 3
Ret. time	Retention time
RPM	Revolutions per minute
RSM	Response surface methodology
STEG	Sterigmatocystin
T1L	Linear effect of temperature
T1L by T2L	Interaction effect temperature and time
T1Q	Quadratic effect of temperature
T2	T-2 toxin
T2L	Linear effect of time
T2Q	Quadratic effect of time
ZEAs	Zearalenone, α-zearalenone, and β-zearalenone

2012). Amongst these, heat treatment remains a cheap, simple and sustainable approach for mitigating the prevalence of mycotoxins, and has had a good track record of effectively reducing other contaminants present in food (Kabak, 2009; Méndez-Albores et al., 2013; Wu et al., 2017; Yazdanpanah et al., 2005). However, because of its unsophisticatedness and everyday use, a critical understanding of the goal-specific applications of thermal processing is often unintentionally neglected (Sindelar and King, 2013). This perhaps could be the reason for the limited and conflicting studies on thermal stability of many mycotoxins (Kabak, 2009; Raters and Matissek, 2008; Turner et al., 2009).

The efficacy of a thermal processing system is known to be in proportion to the amount of heat energy supplied and time of exposure. Higher temperatures and longer heating times are known to result in greater degradation of mycotoxins (Gbashi et al., 2017a; Kabak, 2009). However, due to the detrimental effects higher temperatures have on important food quality factors, it is important to optimize these parameters in order to achieve maximum degradation of these toxins at the lowest possible temperature and time conditions. Moreover, the notoriously incessant prevalence of mycotoxins even in heat-processed foods (Stoloff and Trucksess, 1981), has necessitated the systematic re-investigation of their response to heat treatment.

In this regard, numerical modelling is a useful optimization approach that has found wide applicability in thermal processes in biological systems (Abakarov and Nuñez, 2013; Sendín et al., 2010). Amidst various approaches, central composite design (CCD) is one of the most commonly used numerical optimization techniques in food processing. This is because it offers the advantage of a reduced number of experimental runs, and provides a function and empirical relationship between the objective function (response variable) and the various control variables, as well as provides details on the effects of different control variables on a response variable (Gbashi et al., 2016; Goncalves et al., 2006; Khuri and Mukhopadhyay, 2010; Uma et al., 2010). The present study adopts a CCD numerical modelling approach to systematically investigate, and optimize the temperature-time degradation patterns of multiple mycotoxins in pure and spiked form (maize flour).

2. Materials and methods

2.1. Materials

Mycotoxin reference standards, AFB_1, aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), ochratoxin A (OTA), ochratoxin B (OTB), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), fumonisin B_3 (FB_3), zearalenone (ZEA), α-zearalenone (α-ZEA), β-zearalenone (β-ZEA), T2-toxin, AME, and STEG were purchased from the Council for Scientific and Industrial Research (CSIR). Solvents used included high grade acetonitrile, methanol and formic acid which were purchased from Sigma, Aldrich (South Africa). Ultrapure water was obtained from a Milli-Q Gradient A10 dispensing system (Millipore, Billerica, MA, USA). Maize flour was purchased from a Shoprite grocery store in Johannesburg (South Africa).

2.2. Methods

2.2.1. Experimental design

A set of experiments was statistically designed based on the response surface methodology (RSM) using Statistica version 7 statistical software (StatSoft, USA). Specifically, the central composite design (CCD) (Hossain et al., 2015) approach was adopted because it permits building second-order optimization models without the need for a complete three-level factorial experimental design (Goncalves et al., 2006; Tamhane, 2014). Accordingly, a 2-factor, 1 block experimental design was achieved, which consisted of 10 experimental levels (Table 1); 2 levels of fractional factorial design for each of the factors studied, a replicated center-point to improve the precision of the model, and a set of axial points (i.e. α and $-\alpha$) that permits rotatability of the model and ensures estimation of response curvature. Rotatability of the model is desirable because it allows for equal variance of prediction for all points equal-distance from the center

Table 1
Two-factor, 1 block standard order CCD experimental design for temperature-time degradation of mycotoxins.

S/No	Temperature (°C)	Time (min)	RSM Codes	Comment
1	120.00	15.00	−1, −1	Factorial level
2	120.00	55.00	−1, +1	Factorial level
3	200.00	15.00	+1, −1	Factorial level
4	200.00	55.00	+1, +1	Factorial level
5	103.43	35.00	-α, 0	Axial point
6	216.57	35.00	+α, 0	Axial point
7	160.00	6.72	0, -α	Axial point
8	160.00	63.28	0, +α	Axial point
9	160.00	35.00	+1, +1	Center point
10	160.00	35.00	+1, +1	Center point

point irrespective of the direction. The experimental region was selected based on preliminary laboratory trials (Gbashi et al., 2017a).

After conducting the experiments, a second-order optimization model described by Equation (1) (Adebo et al., 2018) was fitted to the experimental data using the method of least squares (MLS) which generates the lowest possible residuals (Bas and Boyaci, 2007).

$$Z = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j \quad (1)$$

Where z is the response variable i.e. degradation (%), x_i and x_j are the factors, temperature (°C) and time (min), respectively, β_0 is the model constant, β_i, β_{ii} and β_{ij} are the coefficients for the linear, quadratic and interaction terms (Adebo et al., 2018).

The model fitness and adequacy were determined by evaluating the coefficient of determination (R^2), adjusted R^2, Pearson's correlation coefficient (r), average absolute deviation (AAD), accuracy factor (A_f) and the bias factor (B_f). Model parameters and significance were determined at a probability level of 95% (i.e. $p < 0.05$). The various mathematical functions used to compute these parameters are presented in the Equations (2)–(7) (Adebo et al., 2018; Morshedi and Akbarian, 2014).

$$R^2 = \frac{\sum_{i=1}^{n} (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2} \quad (2)$$

Where R^2 is the coefficient of determination, n is the sample size, $\bar{y}$ is estimated mean value, y_i and $\hat{y}$ are the experimental and predicted values, respectively.

$$R^2 adj = 1 - \frac{k-1}{k-p}(1 - R^2) \quad (3)$$

Where $R^2 adj$ is the adjusted coefficient of determination, R^2 is the coefficient of determination, p is number of regression coefficients and k is total number of observations.

$$r = \frac{\sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^{n} (y_i - \bar{y})^2}} \quad (4)$$

Where r is the Pearson's correlation coefficient, n is the sample size, x_i and y_i are single measurements indexed with i for predicted and experimental values, respectively, and $\bar{x}$ and $\bar{y}$ are the means for the predicted and experimental variables, respectively.

$$AAD = \frac{\left[\sum_{i=1}^{n} \left(\frac{y_{i\,exp} - y_{i\,cal}}{y_{i\,exp}}\right)\right]}{n} \quad (5)$$

Where AAD is the average absolute deviation, n is the sample size, and $y_{i\ exp}$ and $y_{i\ cal}$ are the individual experimental and predicted values indexed with i, respectively.

$$B_f = 10^{\frac{1}{n}} \sum_{i=1}^{n} log\left(\frac{y_{i\,cal}}{y_{i\,exp}}\right) \quad (6)$$

Where B_f is the bias factor, n is the sample size, and $y_{i\ exp}$ and $y_{i\ cal}$ are the individual experimental and predicted values indexed with i, respectively.

$$A_f = 10^{\frac{1}{n}} \sum_{i=1}^{n} \left[log\left(\frac{y_{i\,cal}}{y_{i\,exp}}\right)\right] \quad (7)$$

Where A_f is the accuracy factor, n the sample size, and $y_{i\ exp}$ and $y_{i\ cal}$ are the individual experimental and predicted values indexed with i, respectively.

After the model-fit and validation of the model adequacy, the resultant quadratic optimization models were used for optimization and computation of the globally optimal conditions for the degradation of real samples i.e. pure mycotoxin standards and spiked maize flour,

Table 2
MRM transitions, optimized MS conditions and retention times of multi-mycotoxins; Ret. time – retention time; Q1 – quadrupole 1; Q3 – quadrupole 3; CE – collision energy.

S/No	Mycotoxin	Recovery (%)[a]	Ret. time (min)	Precursor (*mz*)	Products (*mz*)	Q1 Pre Bias (V)	CE (eV)	Q3 Pre Bias (V)
1.	AFB_1	110.35	8.5510	313.00	241.00	−22.000	−41.000	−23.000
					285.10	−22.000	−24.000	−29.000
2.	AFB_2	101.50	8.3040	315.00	259.10	−22.000	−31.000	−25.000
					287.00	−23.000	−26.000	−30.000
3.	AFG_1	101.15	8.0770	329.00	243.00	−12.000	−28.000	−23.000
					311.10	−16.000	−24.000	−14.000
4.	AFG_2	95.950	7.8280	331.00	245.10	−12.000	−32.000	−24.000
					313.00	−12.000	−24.000	−20.000
5.	AME	99.500	10.318	273.00	128.10	−10.000	−49.000	−21.000
					115.05	−18.000	−54.000	−19.000
6.	FB_1	105.70	8.1060	722.20	352.20	−34.000	−42.000	−11.000
					334.30	−20.000	−42.000	−11.000
7.	FB_2	109.00	9.1740	706.10	336.30	−20.000	−38.000	−22.000
					318.30	−26.000	−41.000	−22.000
8.	FB_3	93.600	8.8490	706.30	336.30	−40.000	−39.000	−11.000
					354.40	−20.000	−35.000	−24.000
9.	OTA	119.40	10.312	403.80	239.00	−15.000	−27.000	−24.000
					221.00	−12.000	−38.000	−21.000
10.	OTB	108.65	9.5580	370.10	205.00	−13.000	−22.000	−21.000
					324.10	−13.000	−14.000	−22.000
11.	STEG	99.450	10.520	324.90	310.00	−22.000	−24.000	−30.000
					281.10	−22.000	−40.000	−27.000
12.	T-2 Toxin	119.50	10.027	467.20	245.10	−13.000	−11.000	−16.000
					305.20	−22.000	−11.000	−20.000
13.	ZEA	118.70	10.260	319.10	185.00	−12.000	−27.000	−30.000
					187.10	−15.000	−21.000	−19.000
14.	a-ZEA	127.60	8.8020	323.10	277.20	−17.000	−17.000	−18.000
					305.20	−24.000	−9.000	−20.000
15.	B-ZEA	134.55	8.3920	323.10	277.20	−16.000	−16.000	−18.000
					305.20	−16.000	−11.000	−20.000

[a] Extraction recovery for mycotoxins spiked into the maize matrix.

using the Minitab 17 global optimization function. The 3-D surface plots and Pareto charts were used to examine the degradation patterns of the analytes, with and without matrix interference.

2.2.2. Sample preparation

Spiking of maize matrix was performed as described by Sameni et al. (2014) with little modifications. To obtain a spiked maize concentration of 2 μg/g, exactly 2 mL of multi-mycotoxin standards stock solution (1 μg/mL) was added to 1 g of blank maize flour (previously tested on LC-MS/MS) contained in a 16 mL glass vial (25 × 50 mm). The spiked sample was thoroughly mixed and left overnight in the dark in a fume hood to allow for slow evaporation of the solvent at ambient conditions and for the mycotoxins to be absorbed into the matrix. For the pure standards, 2 mL of the multi-mycotoxin stock solution (1 μg/mL) was transferred into a 16 mL glass vial and dried under similar conditions.

2.2.3. Thermal treatment

Thermal treatment of samples was achieved using a GC 600 Vega Series 2 oven (Carlo Erba Instruments, Italy) with an automatic temperature control unit (± 1 °C). The oven was allowed to equilibrate at a desired temperature for 5 min before samples were introduced. Samples were placed inside the oven which was maintained at the preset temperature. After heating for the desired time, samples were retrieved immediately and allowed to cool under ambient conditions. The vials containing pure mycotoxin standards were then reconstituted in 1 mL of methanol, thoroughly vortexed for 3 min, allowed to stand and vortexed again for 2 min, filtered through a 0.22 μm syringe filter into a 1.5 mL clear HPLC vial for subsequent analysis on LC-MS/MS.

The thermally-treated spiked maize samples were extracted using the method of Bertuzzi et al. (2011) with slight modifications. To a 1 g of maize flour, 2 mL of methanol/acetonitrile (50:50, v/v) was added, and placed on a bench shaker (LABCON GmbH, Heppenheim, Germany) for 1 h. After which samples were centrifuged at 4000 RPM, and the supernatant was filtered through a 0.22 μm syringe filter into a 1.5 mL HPLC vial for subsequent analysis on LC-MS/MS.

2.2.4. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)

Chromatographic separation followed by quantification of multi-mycotoxins was accomplished using a Shimadzu LC-MS/MS 8030 equipment (Shimadzu Corporation, Tokyo, Japan), which consists of an LC-30AD Nexera chromatograph connected to a SIL-30 AC Nexera autosampler and a CTO-20 AC Prominence Column Oven. Analytes were separated on a Raptor™ ARC-18 column (2.7 μm, 2.1 mm × 100 mm) (Restek Corporation, Pennsylvania USA), thermostated at 40 °C. The mobile phases used consisted of (A) 0.1% formic acid (FA) in deionized water, and (B) 0.1% FA in methanol/acetonitrile (50:50 v/v), and was delivered at a constant flow rate of 0.2 mL/min. The gradient elution program began with 10% B for 0.1 min, ramped to 95% B in 8.4 min, kept constant for 3 min, and the initial condition (10% B) was re-instated for 1 min, after which, the column was allowed to re-equilibrate for 4.5 min for the next run, bringing these to a total run time of 17 min.

Following chromatographic separation, analytes were detected and quantified on a Shimadzu triple-quadrupole MS model 8030 (Shimadzu Corporation, Kyoto, Japan) operated in positive ionization mode with an electron spray ionization (ESI^+) source. The interface nebulizing gas flow rate was 3 L/min, DL temperature was 250 °C, heat block temperature was 400 °C, and drying gas flow rate was 15 L/min. Data acquisition was by means of a multiple reaction monitoring (MRM) method operated using optimized MS conditions for the analytes (Table 2). The Shimadzu LabSolutions software was used for subsequent processing and visualization of the data.

Table 3
Temperature-time degradation of pure multi-mycotoxin standards.

Mycotoxin	120 °C/15 min (%)	120 °C/55 min (%)	200 °C/15 min (%)	200 °C/55 min (%)	103.43 °C/35 min (%)	216.57 °C/35 min (%)	160 °C/6.72 min (%)	160 °C/63.28 min (%)	160 °C/35 min (%)	Average
AFB_1	$17.15^{a} \pm 12.29$	$11.56^{a} \pm 3.32$	$92.86^{d} \pm 0.53$	$98.73^{d} \pm 0.20$	$3.05^{a} \pm 2.86$	$100.00^{d} \pm 0.00$	$48.73^{bc} \pm 1.26$	$66.07^{c} \pm 4.65$	$41.49^{b} \pm 2.44$	53.29 ± 3.06
AFB_2	$30.94^{a} \pm 17.07$	$18.78^{a} \pm 7.06$	$62.44^{ab} \pm 0.17$	$89.49^{b} \pm 2.99$	$22.95^{a} \pm 16.16$	$85.88^{b} \pm 5.59$	$30.94^{a} \pm 28.68$	$32.90^{a} \pm 13.78$	$22.27^{a} \pm 1.80$	44.07 ± 10.37
AFG_1	$12.26^{a} \pm 6.42$	$13.85^{a} \pm 4.03$	$97.35^{e} \pm 0.20$	$99.23^{e} \pm 0.00$	$5.89^{a} \pm 2.87$	$100.00^{e} \pm 0.00$	$38.51^{b} \pm 2.73$	$71.77^{d} \pm 1.30$	$52.22^{c} \pm 2.23$	54.56 ± 2.2
AFG_2	$16.54^{ab} \pm 2.08$	$6.54^{a} \pm 0.13$	$67.94^{d} \pm 0.06$	$89.46^{b} \pm 0.31$	$6.27^{a} \pm 0.25$	$100.00^{d} \pm 0.00$	$12.98^{a} \pm 8.49$	$29.79^{c} \pm 4.09$	$27.12^{bc} \pm 2.11$	39.63 ± 1.95
AME	$7.32^{ab} \pm 8.75$	$2.24^{a} \pm 1.55$	$55.29^{d} \pm 1.78$	$89.22^{e} \pm 0.98$	$6.79^{ab} \pm 4.08$	$93.86^{e} \pm 0.40$	$17.58^{bc} \pm 4.03$	$29.70^{c} \pm 1.15$	$24.78^{c} \pm 1.46$	36.31 ± 2.69
FB_1	$10.01^{a} \pm 13.55$	$6.37^{a} \pm 8.40$	$100.00^{c} \pm 0.00$	$100.00^{c} \pm 0.00$	$18.12^{a} \pm 12.66$	$100.00^{c} \pm 0.00$	$88.47^{c} \pm 0.41$	$93.29^{c} \pm 0.00$	$52.12^{b} \pm 5.8$	63.15 ± 4.53
FB_2	$19.94^{b} \pm 2.82$	$24.10^{b} \pm 2.33$	$100.00^{e} \pm 0.00$	$100.00^{e} \pm 0.00$	$1.59^{a} \pm 0.00$	$100.00^{e} \pm 0.00$	$88.50^{d} \pm 1.06$	$95.23^{e} \pm 0.37$	$54.41^{c} \pm 1.00$	64.86 ± 0.84
FB_3	$19.62^{a} \pm 5.61$	$7.93^{a} \pm 4.78$	$99.42^{c} \pm 0.24$	$99.71^{c} \pm 0.06$	$15.45^{a} \pm 15.41$	$100.00^{c} \pm 0.00$	$83.97^{c} \pm 2.24$	$96.33^{c} \pm 0.35$	$59.58^{b} \pm 1.74$	64.67 ± 3.38
OTA	$10.42^{ab} \pm 10.72$	$5.50^{ab} \pm 3.76$	$38.79^{cd} \pm 1.32$	$48.32^{de} \pm 0.75$	$4.74^{a} \pm 3.57$	$58.78^{e} \pm 0.63$	$12.23^{ab} \pm 8.65$	$23.67^{bc} \pm 0.25$	$15.87^{a} \pm 0.61$	24.26 ± 3.36
OTB	$22.57^{abc} \pm 6.58$	$2.23^{a} \pm 0.54$	$29.12^{bc} \pm 0.27$	$38.81^{cd} \pm 3.90$	$10.07^{ab} \pm 1.81$	$50.50^{d} \pm 2.96$	$10.64^{ab} \pm 5.31$	$10.93^{ab} \pm 5.84$	$8.93^{ab} \pm 7.19$	20.42 ± 3.82
STERIG	$17.04^{a} \pm 5.77$	$30.41^{b} \pm 2.92$	$94.42^{f} \pm 0.44$	$99.23^{f} \pm 0.07$	$17.97^{a} \pm 0.51$	$100.00^{f} \pm 0.00$	$62.31^{d} \pm 2.04$	$79.25^{e} \pm 0.44$	$47.60^{c} \pm 3.16$	60.91 ± 1.71
T2-Toxin	$12.92^{ab} \pm 13.27$	$4.74^{a} \pm 4.04$	$84.37^{de} \pm 1.85$	$95.94^{e} \pm 0.96$	$6.34^{a} \pm 6.29$	$97.53^{e} \pm 0.89$	$34.54^{bc} \pm 4.17$	$65.89^{d} \pm 1.85$	$40.11^{c} \pm 7.26$	49.15 ± 4.51
ZEA	$13.63^{a} \pm 0.62$	$20.11^{a} \pm 3.49$	$84.44^{d} \pm 1.50$	$94.97^{e} \pm 0.27$	$19.24^{a} \pm 2.67$	$100.00^{e} \pm 0.00$	$33.88^{b} \pm 3.01$	$53.26^{c} \pm 1.03$	$39.15^{b} \pm 1.28$	50.96 ± 1.54
α-ZEA	$12.35^{a} \pm 2.93$	$20.41^{ab} \pm 3.56$	$88.98^{e} \pm 0.77$	$94.76^{e} \pm 0.70$	$12.50^{a} \pm 4.26$	$100.00^{e} \pm 0.00$	$32.71^{c} \pm 4.33$	$53.26^{d} \pm 0.28$	$31.37^{bc} \pm 3.48$	49.59 ± 2.26
β-ZEA	$15.78^{a} \pm 1.34$	$18.52^{a} \pm 0.85$	$88.07^{d} \pm 1.06$	$96.31^{e} \pm 0.28$	$22.72^{a} \pm 2.82$	$100.00^{e} \pm 0.00$	$32.25^{b} \pm 0.07$	$57.41^{c} \pm 4.17$	$34.00^{b} \pm 1.73$	51.67 ± 1.37

Key: Values represent means of duplicate determinations of the percentage degradations ± standard deviation of the means. Significant differences among the sample treatments as a function of time and temperature are indicated as superscripted alphabets on the means, and were compared using Tukey's pairwise multiple comparison test following a one-way ANOVA. Values in the same row followed by the same alphabet are not significantly different ($p > 0.05$).

Table 4
Temperature-time degradation of multi-mycotoxins spiked into maize matrix.

Mycotoxin	120 °C/15 min (%)	120 °C/55 min (%)	200 °C/15 min (%)	200 °C/55 min (%)	103.43 °C/35 min (%)	216.57 °C/35 min (%)	160 °C/6.72 min (%)	160 °C/63.28 min (%)	160 °C/35 min (%)	Average
AFB_1	$2.80^{a} \pm 3.07$	$6.23^{a} \pm 2.30$	$85.91^{e} \pm 0.00$	$99.37^{f} \pm 0.13$	$5.74^{a} \pm 4.54$	$100.00^{f} \pm 0.00$	$17.80^{b} \pm 0.38$	$59.49^{d} \pm 1.47$	$46.48^{c} \pm 0.47$	47.09 ± 1.37
AFB_2	$18.57^{a} \pm 14.84$	$30.79^{abc} \pm 11.36$	$72.51^{cd} \pm 12.82$	$90.84^{d} \pm 0.00$	$14.38^{a} \pm 17.00$	$100.00^{d} \pm 0.00$	$23.65^{ab} \pm 4.74$	$62.22^{bcd} \pm 18.18$	$45.81^{abc} \pm 3.30$	50.97 ± 9.14
AFG_1	$4.00^{a} \pm 4.19$	$5.93^{a} \pm 3.98$	$91.30^{e} \pm 0.00$	$100.00^{e} \pm 0.00$	$1.29^{a} \pm 3.29$	$100.00^{e} \pm 0.00$	$11.91^{a} \pm 12.44$	$59.91^{b} \pm 1.89$	$45.03^{b} \pm 2.17$	46.6 ± 3.11
AFG_2	$2.66^{a} \pm 3.68$	$6.72^{a} \pm 2.36$	$67.64^{c} \pm 2.43$	$89.37^{d} \pm 0.00$	$3.54^{a} \pm 2.43$	$97.55^{d} \pm 0.22$	$14.28^{ab} \pm 6.26$	$27.20^{b} \pm 8.47$	$22.41^{b} \pm 0.42$	36.82 ± 2.92
AME	$28.39^{a} \pm 2.63$	$23.12^{a} \pm 6.11$	$29.50^{a} \pm 1.78$	$51.36^{b} \pm 0.99$	$31.71^{a} \pm 7.60$	$62.66^{b} \pm 2.91$	$28.79^{a} \pm 2.63$	$25.58^{a} \pm 0.64$	$18.84^{a} \pm 3.19$	33.33 ± 3.17
FB_1	$52.93^{ab} \pm 1.27$	$51.75^{ab} \pm 11.24$	$100.00^{e} \pm 0.00$	$100.00^{e} \pm 0.00$	$41.86^{a} \pm 5.02$	$100.00^{e} \pm 0.00$	$64.76^{b} \pm 4.62$	$96.78^{c} \pm 0.00$	$96.59^{c} \pm 0.00$	78.3 ± 2.46
FB_2	$43.58^{ab} \pm 4.80$	$52.80^{b} \pm 5.00$	$100.00^{d} \pm 0.00$	$100.00^{d} \pm 0.00$	$41.70^{a} \pm 5.25$	$100.00^{d} \pm 0.00$	$65.00^{c} \pm 0.84$	$97.43^{d} \pm 0.26$	$94.82^{d} \pm 0.16$	77.26 ± 1.81
FB_3	$55.24^{a} \pm 6.95$	$59.35^{ab} \pm 3.10$	$100.00^{c} \pm 0.00$	$100.00^{c} \pm 0.00$	$49.73^{a} \pm 5.67$	$100.00^{c} \pm 0.00$	$69.50^{b} \pm 2.04$	$98.34^{c} \pm 0.38$	$95.94^{c} \pm 0.49$	80.9 ± 2.07
OTA	$25.25^{a} \pm 2.43$	$33.67^{a} \pm 6.04$	$56.41^{b} \pm 1.60$	$84.63^{c} \pm 0.65$	$25.92^{a} \pm 2.90$	$93.84^{c} \pm 0.18$	$33.25^{a} \pm 6.16$	$31.78^{a} \pm 4.20$	$25.80^{a} \pm 1.16$	45.62 ± 2.81
OTB	$17.26^{ab} \pm 15.10$	$21.49^{ab} \pm 3.12$	$46.43^{b} \pm 2.21$	$80.03^{c} \pm 0.78$	$16.24^{a} \pm 12.76$	$92.36^{c} \pm 0.13$	$21.81^{ab} \pm 7.48$	$21.35^{ab} \pm 9.44$	$29.50^{ab} \pm 0.85$	38.5 ± 5.76
STERIG	$5.13^{a} \pm 0.21$	$7.14^{a} \pm 0.36$	$56.46^{c} \pm 4.12$	$94.32^{d} \pm 0.21$	$9.35^{a} \pm 0.21$	$98.09^{d} \pm 0.14$	$11.71^{a} \pm 2.56$	$39.32^{b} \pm 2.35$	$14.83^{a} \pm 5.22$	37.37 ± 1.71
T2-Toxin	$8.95^{a} \pm 6.27$	$6.90^{a} \pm 3.37.00$	$52.93^{b} \pm 14.97$	$84.98^{c} \pm 0.30$	$12.93^{a} \pm 9.29$	$95.61^{c} \pm 2.19$	$19.12^{a} \pm 13.20$	$29.29^{ab} \pm 6.15$	$25.65^{ab} \pm 2.95$	37.37 ± 6.52
ZEA	$14.15^{a} \pm 0.12$	$17.19^{a} \pm 11.2$	$56.40^{b} \pm 1.61$	$84.54^{c} \pm 1.13$	$20.60^{a} \pm 4.23$	$93.13^{c} \pm 0.30$	$21.78^{a} \pm 2.44$	$43.98^{b} \pm 2.03$	$27.13^{a} \pm 2.24$	42.1 ± 2.81
α-ZEA	$5.88^{ab} \pm 0.00$	$17.40^{bc} \pm 0.66$	$57.60^{e} \pm 0.66$	$88.13^{f} \pm 0.72$	$2.98^{a} \pm 2.88$	$96.90^{f} \pm 0.61$	$14.26^{abc} \pm 4.10$	$34.95^{d} \pm 7.65$	$18.97^{c} \pm 0.18$	37.45 ± 1.94
β-ZEA	$3.16^{b} \pm 0.00$	$19.62^{b} \pm 2.89$	$57.53^{d} \pm 0.58$	$90.30^{e} \pm 0.05$	$8.88^{a} \pm 5.36$	$97.51^{e} \pm 0.37$	$19.62^{b} \pm 1.00$	$32.26^{c} \pm 1.73$	$18.58^{b} \pm 1.24$	38.61 ± 1.47

Key: Values represent means of duplicate determinations ± standard deviation of the means. Significant differences among the sample treatments as a function of time and temperature are indicated as superscripted alphabets on the means, and were compared using Tukey's pairwise multiple comparison test following a one-way ANOVA. Values in the same row followed by the same alphabet are not significantly different ($P > 0.05$).

3. Results and discussion

3.1. Thermal degradation of mycotoxins

Results for the thermal stability of pure multi-mycotoxin standards as well as mycotoxins spiked into the maize matrix as a function of temperature and time are presented in Tables 3 and 4. Clearly, thermal degradation of all studied mycotoxins increased proportionately with increasing temperature and exposure time (Appendices A & B). For pure mycotoxin standards, OTB was the most thermally stable mycotoxin with an average degradation of 20%, followed by OTA (24%), AME (36%), AFG_2 (40%), AFB_2 (44%), T2 (49%), α-ZEA (50%), ZEA (51%), β-ZEA (52%), AFB_1 (53%), AFG1 (55%), STEG (61%), FB_1 (63%), FB_3 and FB_2 (65% each). When mycotoxins were spiked into the maize matrix, AME showed the highest resilience to heat treatment with an average percentage degradation of 33%, followed by AFG_2 and STEG (37% each), T2 and α-ZEA (37% each), OTB and β-ZEA (39% each), ZEA (42%), OTA (46%), AFG_1 and AFB_1 (47% each), AFB_2 (51%), FB_2 (77%), FB_1 (78%), and FB_3 (81%). It can be observed that mycotoxins that were relatively stable to heat treatment have a compact molecular structural configuration, *i.e.*, OTA, AME, and AFG_2, whereas the more structurally spread-out mycotoxins were more thermolabile, *i.e.*, FB_3, FB_2 and FB_1 (De Souza et al., 2013; Lerda, 2011). A more compact structural configuration could possibly imply a more difficult thermal break down (Cremer et al., 2000; Gibbs et al., 1998). Generally, evaluation of the results presented in Table 2 revealed that the temperature-time conditions that resulted in the most degradation of all classes of the mycotoxins was a temperature of 216.57 °C at a time of 35 min.

Although all mycotoxins showed a differential degradation pattern for pure mycotoxin standards compared with mycotoxins spiked into maize matrix, an independent-samples Student's t-test showed that only FB_1, FB_2, FB_3, and AME had statistically significant ($p \leq 0.05$) differences between pure standards and spiked maize matrix (Appendix C).

A temperature of 216.57 °C for 35 min was sufficient to completely eradicate all the aflatoxins (AFs) in the absence of matrix interference, except for AFB_2 which had over 86% degradation (Table 3). Whereas when spiked into maize matrix, all AFs where completely degraded at similar condition (216.57 °C for 35 min), except for AFG_2, which had over 97% degradation (Table 4). Our results agree with Yazdanpanah et al. (2005), who reported that roasting nuts which contained AFs at 90–150 °C for 30–120 min resulted in reduced AF levels that ranged from 17 to 63%, depending on the temperature and exposure time (Yazdanpanah et al., 2005). The *Fusarium* group of toxins, FBs, were the most thermolabile of all tested mycotoxins for both spiked and unspiked standards. Average degradation for FBs ranged from 79 to 82% (Table 5), with FB_3 being the most susceptible analogue in this group. Scott and Lawrence (1987) made similar observations, where heating maize meal at 190 °C for 60 min degraded FBs by 60–80%, whereas, when the conditions were increased to 220 °C and 25 min, FBs were almost completely eliminated. Our observations also agree with reports by Castelo et al. (1998), who noted that roasting of artificially-contaminated maize meal at 218 °C for 15 min eliminated FB_1 from the food samples (Scott and Lawrence, 1987).

In the food industry, particularly the coffee industry, the thermal stability of ochratoxins (OTs) is of particular interest, because OTs have been reported to be prevalent even in processed ready-to-eat coffee (FAO, 2001; Kabak, 2009; Studer-Rohr et al., 1995; Vanesa and Ana, 2013). It has not been possible to conclusively establish the thermal stability of OTs based on available literature. Tsubouchi et al. (1987) considers OTA as relatively heat stable, because only 0–12% of OTA was degraded in beans when roasted at 200 °C for 10–20 min (Tsubouchi et al., 1987). Whereas, other reports consider OTA as relatively thermolabile (Perez de Obanos et al., 2005; Studer-

Table 5
Regression model fit coefficients and validation indices for thermal degradation for pure mycotoxins standards.

Mycotoxin	$z(x, y) = C_{00} + C_{10}x + C_{20}x^2 + C_{01}y + C_{02}y^2 + C_{11}xy$												
	c_{00}	c_{10}	c_{20}	c_{01}	c_{02}	c_{11}	R^2	R^2 Adj	Residual	ρ	AAD	B_f	A_f
AFB_1	13.68	−0.22	0.00	−1.84	0.02	0.00	0.98	0.97	37.59	0.99	0.16	1.06	1.15
AFB_2	297.85	−3.41	0.01	−3.01	0.02	0.01	0.87	0.82	139.67	0.93	0.33	1.06	1.31
AFG_1	−92.07	0.79	0.00	−0.03	0.01	0.00	0.96	0.95	65.97	0.98	0.27	0.71	1.67
AFG_2	177.89	−2.51	0.01	−1.19	0.00	0.01	0.98	0.98	27.99	0.99	0.25	1.02	1.24
AME	163.93	−2.25	0.01	−1.63	0.00	0.01	0.99	0.99	14.46	0.99	0.51	1.05	1.30
FB_1	−76.53	1.25	0.00	−2.64	0.04	0.00	0.95	0.84	245.39	0.94	0.47	1.17	1.26
FB_2	−124.16	1.85	0.00	−2.53	0.04	0.00	0.98	0.97	37.25	0.99	0.60	1.05	1.08
FB_3	−116.69	1.83	0.00	−2.48	0.03	0.00	0.91	0.88	170.19	0.95	0.54	1.18	1.24
OTA	97.4	−1.33	0.01	−0.81	0.00	0.00	0.96	0.95	18.86	0.98	0.38	1.10	1.32
OTB	199.85	−2.28	0.01	−1.84	0.00	0.01	0.92	0.89	28.28	0.96	0.51	0.97	1.58
STEG	−3.12	0.01	0.00	−1.14	0.03	0.00	0.98	0.97	28.41	0.99	0.09	0.96	1.10
T2-Toxin	20.96	−0.41	0.00	−1.5	0.01	0.01	0.95	0.94	80.65	0.98	0.58	1.16	1.23
ZEA	85.18	−1.36	0.01	−0.39	0.01	0.00	0.98	0.98	23.06	0.99	0.09	1.01	1.08
α-ZEA	118.92	−1.83	0.01	−0.83	0.02	0.00	0.98	0.97	30.04	0.99	0.11	1.00	1.12
β-ZEA	146.69	−2.06	0.01	−1	0.01	0.00	0.97	0.96	43.93	0.98	0.13	1.01	1.13

Key: c_{00} is a constant, c_{10} and c_{01} are the linear coefficients of x (temperature) and y (time), respectively, c_{20} and c_{02} are the quadratic coefficients of x and y, respectively, and c_{11} is the interaction coefficient.

Rohr et al., 1995). Ultimately, our findings reveal that the thermal stability of OTs is strongly influenced by matrix interactions (Table 5). As pure standards, OTs were highly resilience to heat treatment, such that a temperature-time condition of 216.57 °C and 35 min (a condition at which almost all the other mycotoxins completely degrade) resulted in only about 50% degradation of the OTs. However, when OTs were spiked into maize matrix and subjected to similar conditions (i.e. 216.57 °C and 35 min), there was at least 94 and 92% degradation for OTA and OTB respectively. The mechanism of thermal degradation of OTA at higher temperatures (> 250 °C) has been described as partial isomerization on the molecules, which results in the formation of a less toxic diastereomer (Studer-Rohr et al., 1995).

Based on our results, T2 and ZEA and its analogues (ZEAs) can be regarded as mid-thermally stable mycotoxins. The average thermal degradation of ZEAs ranged from 48 to 50% for pure standards, and 36–41% for spiked maize. Likewise, T2 had an average degradation of 48 and 32% for pure standards and spiked maize respectively, which agrees with the findings by Schmidt et al. (2017). The thermal stability of the relatively understudied, AME and STEG, were also investigated in the present study. AME demonstrated a significantly strong thermal stability. In fact, it had the highest thermal stability for spiked maize (32%), second only to the OTs for pure standards with an average degradation of 35%. Just like the OTs, the thermal stability of STEG is strongly matrix-influenced. When in pure form, STEG had an average degradation of 60%, however, when spiked into maize matrix it had an average degradation of 35%. To the best of our knowledge, this is the first report on the thermal degradation of AME and STEG.

3.2. Matrix effect on thermal stability of mycotoxins

The effect of matrix interference on the thermal stability of mycotoxins is indisputably substantial. Depending on the mycotoxin and thermal conditions, maize matrix either accelerated or suppressed the degradation of mycotoxins (Tables 3 and 4). The mycotoxins most susceptible to matrix-enhanced degradation included FBs, OTs and AFB2. STEG, T2 and the other mycotoxins demonstrated less susceptibility, and perhaps some level of matrix-suppressed degradation (Appendix C). This phenomenon can be more clearly visualized on the compound 3-D surface plots (Fig. 1), which is discussed in greater detail in the succeeding sections of this work. Raters and Matissek (2008) also observed that the presence of matrix components (starch) led to increased degradation of AFB_1 in spiked maize matrix compared to pure-form AFB_1. In fact, they noted that when AFB_1 was spiked into a proteinous matrix (*i.e.*, soybean), thermal degradation was much more increased compared to starch (Raters and Matissek, 2008). Other possible matrix constituents that may interfere with the thermal stability of mycotoxins are polyphenols and moisture (Boudra et al., 1995; Howard et al., 1998; Raters and Matissek, 2008).

Previous studies have shown that mycotoxin-matrix interactions during thermal degradation entails a number of physicochemical possibilities such as breakdown or modification the chemical structures of the analytes, or heat-assisted binding of the toxins to matrix components (Dhanasekaran et al., 2011; Nicolás-Vázquez et al., 2010; Seefelder et al., 2001), which could render the toxin undetectable during routine analysis and perhaps less toxic to humans and animals. For example, during thermal processing of maize or maize-based foods, it is known that fumonisins can bind to various components within the food matrix or react with other ingredients within the food such as reducing sugars (Kabak, 2009; Seefelder et al., 2001). Lu et al. (2002) showed that the incubation of FB1 with D-glucose resulted in the formation of N-carboxymethyl-FB1, a reaction product of FB1 and reducing sugars. The four primary products of the FB1-reducing sugars reaction have been characterized as Nmethyl-FB1,N-carboxymethyl-FB1,N-(3-hydroxyacetonyl)-FB1 and N-(2-hydroxy, 2-carboxyethyl)-FB1 using nuclear magnetic resonance and electrospray mass spectroscopy (Lu et al., 2002).

In another study, Seefelder and co-authors (2001) reported the formation of hydrolyzed fumonisin B1 when samples containing FB1 and sucrose were thermally processed. The authors also observed that N-(carboxymethyl)fumonisin B1, which was formed via thermal processing of samples containing FB1 and d-glucose is less toxic compared to FB1, the parent toxin. This heat-accentuated binding of FBs to matrix components could be the reason for the herein observed thermolabile nature of FBs.

The presence of residual moisture in sample matrices is known to enhance the thermal degradation of mycotoxins via formation of a carboxylic acid terminal on the molecule (Kabak et al., 2006). This terminal is formed by addition of a water molecule to the lactone ring of the molecules, which subsequently undergoes heat-induced decarboxylation (Kabak et al., 2006). Boudra et al. (1995) showed that when OTA was present in wheat matrix and dry-heated at 100 °C for 40–160 min there was no observable degradation, however, wet-heating at the same temperature (100 °C) for only 120 min resulted in over 50% degradation of OTA. It has been advanced that availability of moisture during heating of AFs hydrolyses its lactone ring, which

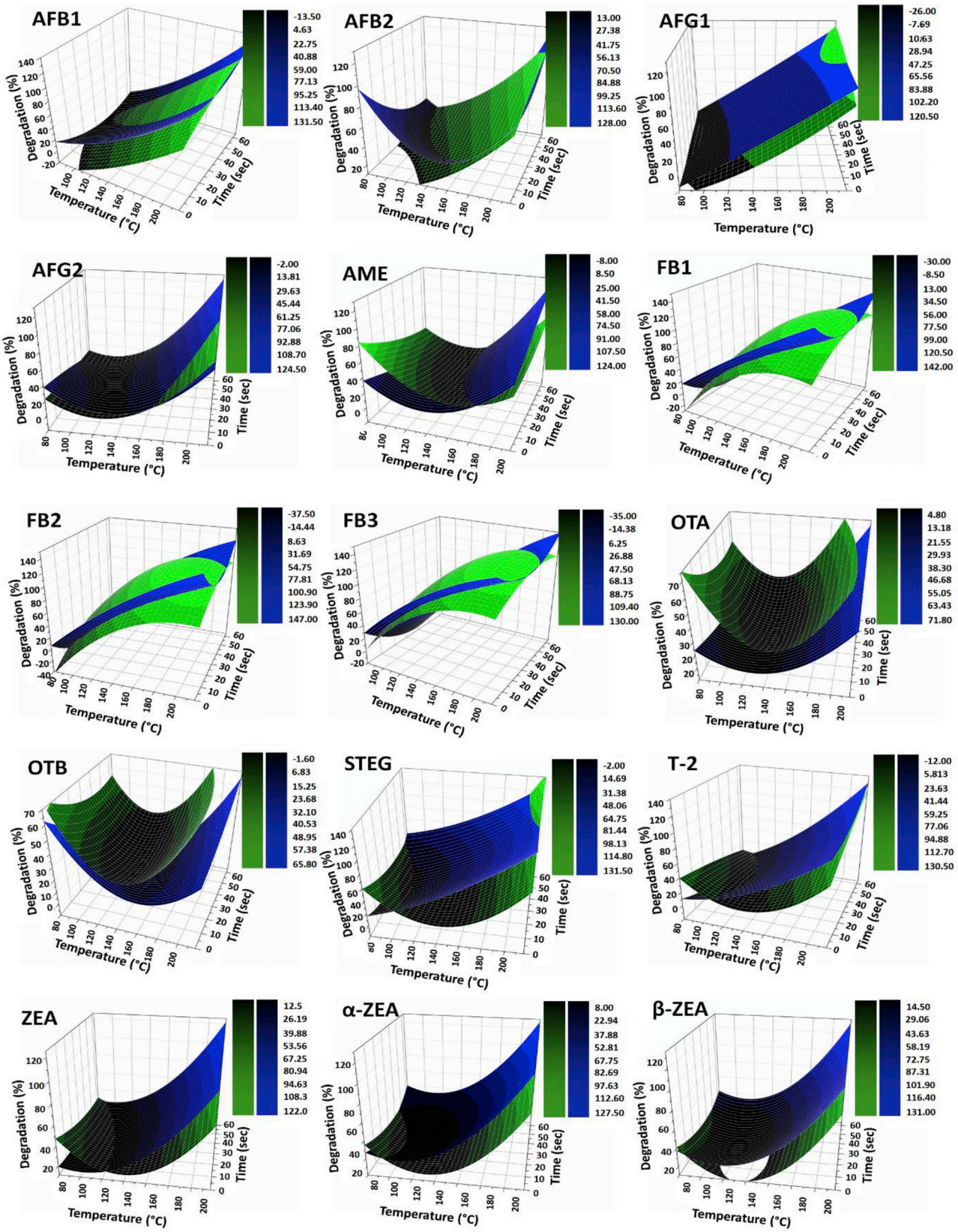


Fig. 1. Compound response surface plots for thermal degradation for pure mycotoxins and mycotoxins spiked into maize matrix.

makes them highly-chemically unstable, as such, subsequent mild heating (> 100 °C) allows for clipping of the lactone ring of the molecule (Raters and Matissek, 2008; Samarajeewa et al., 1990).

3.3. Numerical modelling of the degradation of mycotoxins

3.3.1. Model fitting to experimental data

In order to describe the empirical relationship between the studied

Table 6
Regression model fit coefficients and validation indices for thermal degradation for mycotoxin standards spiked into maize flour.

Mycotoxin	$z(x, y) = C_{00} + C_{10}x + C_{20}x^2 + C_{01}y + C_{02}y^2 + C_{11}xy$												
	c_{00}	c_{10}	c_{20}	c_{01}	c_{02}	c_{11}	R^2	R^2 Adj	Residual	ρ	*AAD*	B_f	A_f
AFB_1	−54.76	0.08	0.00	0.53	−0.01	0.00	0.96	0.95	69.52	0.98	0.36	1.10	1.32
AFB_2	21.82	−0.63	0.00	0.34	0.00	0.00	0.92	0.89	98.83	0.96	0.49	1.09	1.33
AFG_1	−56.35	0.02	0.00	0.64	−0.01	0.00	0.94	0.92	119.28	0.97	0.83	1.28	1.37
AFG_2	154.33	−2.43	0.01	−0.72	0.00	0.01	0.99	0.98	25.17	0.99	2.52	1.19	1.48
AME	247.13	−2.7	0.01	−1.83	0.01	0.01	0.89	0.86	27.38	0.95	0.13	0.99	1.12
FB_1	−227.76	3.09	−0.01	1.59	−0.02	0.00	0.93	0.9	54.12	0.96	0.07	1.00	1.07
FB_2	−248.21	3.18	−0.01	2.08	−0.02	0.00	0.95	0.94	38.52	0.98	0.06	1.00	1.06
FB_3	−189.89	2.68	−0.01	1.57	−0.02	0.00	0.94	0.92	34.64	0.97	0.06	1.00	1.06
OTA	264.86	−3.26	0.01	−1.53	0.01	0.01	0.95	0.93	42.44	0.98	0.11	1.00	1.12
OTB	177.39	−2.36	0.01	−0.69	−0.01	0.01	0.92	0.89	76.27	0.96	0.35	1.04	1.32
STEG	261.22	−3.51	0.01	−2.28	0.01	0.01	0.99	0.99	11.36	1.00	0.13	1.01	1.13
T2-Toxin	179.08	−2.45	0.01	−1.27	0.00	0.01	0.96	0.95	48.15	0.98	0.19	1.00	1.20
ZEA	189.42	−2.49	0.01	−1.29	0.01	0.01	0.99	0.98	14.12	0.99	0.11	1.01	1.10
α-ZEA	189.15	−2.76	0.01	−1.22	0.01	0.01	0.99	0.98	18.02	0.99	0.47	1.05	1.28
β-ZEA	210.15	−3.01	0.01	−1.18	0.01	0.01	0.98	0.98	23.59	0.99	0.23	1.05	1.22

Key: c_{00} is a constant, c_{10} and c_{01} are the linear coefficients of x (temperature) and y (time), respectively, c_{20} and c_{02} are the quadratic coefficients of x and y, respectively, and c_{11} is the interaction coefficient.

objective variables, *i.e.*, mycotoxin degradation (%) and control variables of temperature (°C) and exposure time (min), the quadratic regression function (Equation (1)) was fitted to the experimental data. The resultant second-order model coefficients for the objective variables are presented in Tables 5 and 6 (Columns 2–7). These models provide a strong approximation to the true relationship between our objective variables and the control variables.

By means of these models, the compound 3-D surface plots (Fig. 1) were generated using OriginPro 8.5 software (OriginLab, Massachusetts, US). These plots present a visual interpretation of the degradation patterns of each mycotoxin, as well allows the comparison of the degradation profile of pure mycotoxins and mycotoxins spiked into maize. Many of the degradation patterns in our data described by these plots are consistent with a first order reaction kinetics. On the plots, the blue surfaces represent the degradation profile of pure mycotoxin standards, while the green surfaces represent degradation profile of corresponding mycotoxin spiked into maize matrix. The colour bands correspond to the value-ranges of the objective variable. Accordingly, lighter regions represent higher degradation, whereas, more intense (darker) regions corresponds to lower degradation.

3.3.2. Factor effects

In contrast to conventional optimization methods, RSM is able to make available details on the magnitude and significance of individual and pairwise factor effects on the objective variable(s) (Gbashi et al., 2016; Uma et al., 2010). Accordingly, our model fit gave the various factor effects for pure mycotoxin standards and mycotoxins spiked into maize matrix as shown in Fig. 2a and b and Appendix D. The Pareto charts of standardized factor effects (Fig. 2a and b) graphically indicates the magnitude and importance of each effect. The red reference line indicated on the chart distinguishes between insignificant and significant effects at $\alpha = 0.05$. Any effect that is below this line is insignificant (Gbashi et al., 2016). It can be seen that the linear effect of temperature (T1L) was significantly higher ($p < 0.05$) across all response variables for pure mycotoxin standards and mycotoxins spiked into maize matrix, and ranged from 75.89 to 25.08, and 80.24 to 18.28 for pure mycotoxin standards and mycotoxins spiked into maize matrix (Appendix D). This suggests that higher temperatures and shorter exposure times are more effective for mycotoxin degradation, compared to lower temperatures and longer exposure times. The other factor effects for pure mycotoxin standards and mycotoxins spiked into maize matrix, respectively, included quadratic effect of temperature (T1Q) that ranged from 35.76 to −10.49, and 39.46 to −25.5; Linear effect of time (T2L): ranged from 12.63 to −2.56, and 21.27 to 3.01; Quadratic effect of time (T2Q): ranged from 32.29 to −1.81, and 11.26 to −15.66; and interactive effect of temperature and time (T1L by T2L): ranged from 19.61 to −4.29, and 17.92 to −4.61.

3.3.3. Optimization of the thermal degradation of multi-mycotoxins

Using the multi-response numerical optimization function of the Minitab 17 statistical software (Pennsylvania, US), it was possible to derive the optimum conditions for the thermal degradation of individual mycotoxins, *i.e.*, the most efficient combination of temperature-time for the maximum degradation of individual mycotoxins (Appendix E). An approximation of these solutions can also be visually extrapolated from the surface plots (Fig. 1). For all mycotoxins, the optimum conditions varied from 178.21 °C/63.28 min to 216.57 °C/63.28 min for pure standard and 207.43 °C/24.43 min to 216.57 °C/63.28 for spiked maize matrix. Within the experimental range and model resources, it was not possible to achieve 100% degradation of pure-standard OTs due to their high thermal stability. A similar situation was observed for AME spiked into the maize matrix. As such, in order to at least have an idea of the region in which conditions for 100% degradation of these mycotoxins would fall, we interrogated the corresponding models beyond the available model resources, which gave values of 239 °C/63.28 for pure-standard OTs, and 230 °C/40.5 min for AME spiked into maize matrix. Although these values are not very far from the experimental range, it should be noted that because these values fall outside of the experimental range, their predictability may not be reliable, except confirmed by laboratory analysis. Essentially, the further away the solution is from the experimental range, the greater the variance and the less the precision (STAT503, 2018).

Beside optimization for individual objective variables, it is important to derive the global (synchronous) optimal solution for all the objective variables. This is critical because mycotoxins co-occur in nature in food commodities and exert synergistic effects (Adekoya et al., 2018; Serrano et al., 2012; Smith et al., 2016). As such, a single compromise optimum that accounts for the maximum eradication of all mycotoxins present is a more meaningful solution for food safety and health applications. In this regard, using the global optimization function of the Minitab 17 statistical software, the compromise multi-objective optimum solution for our objective variables was 216.57 °C/63.28 min and 210.85 °C/54.71 min for pure mycotoxin standards and spiked maize matrix, respectively. The desirability factor (DF) in all cases was 1.00.

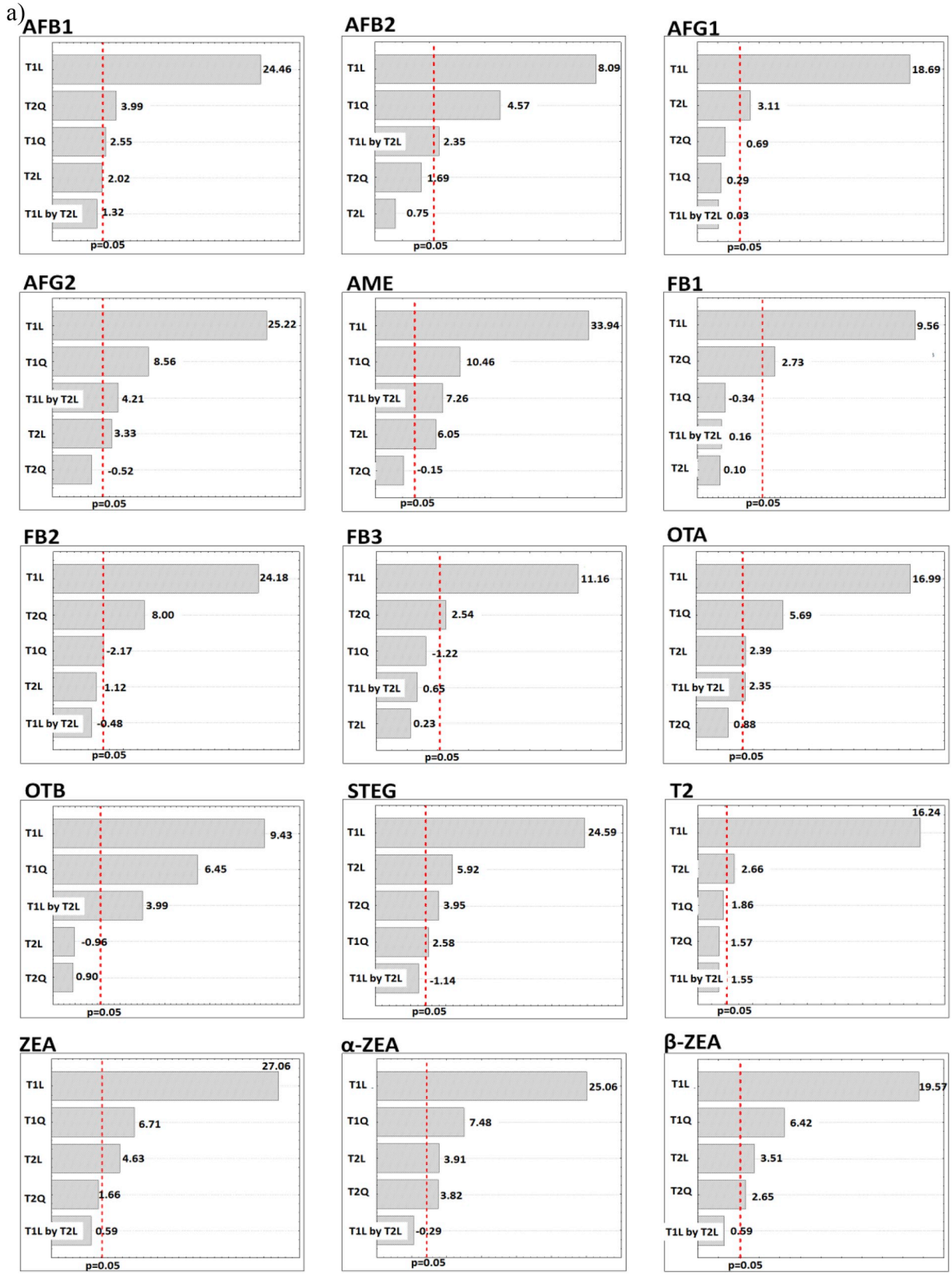


Fig. 2a. Pareto charts of standardized factor effects for model fit of pure mycotoxin standards. T1L – Linear effect of temperature; T1Q – Quadratic effect of temperature; T2L – Linear effect of time; T2Q – Quadratic effect of time; and T1L by T2L - Interaction effect temperature and time.

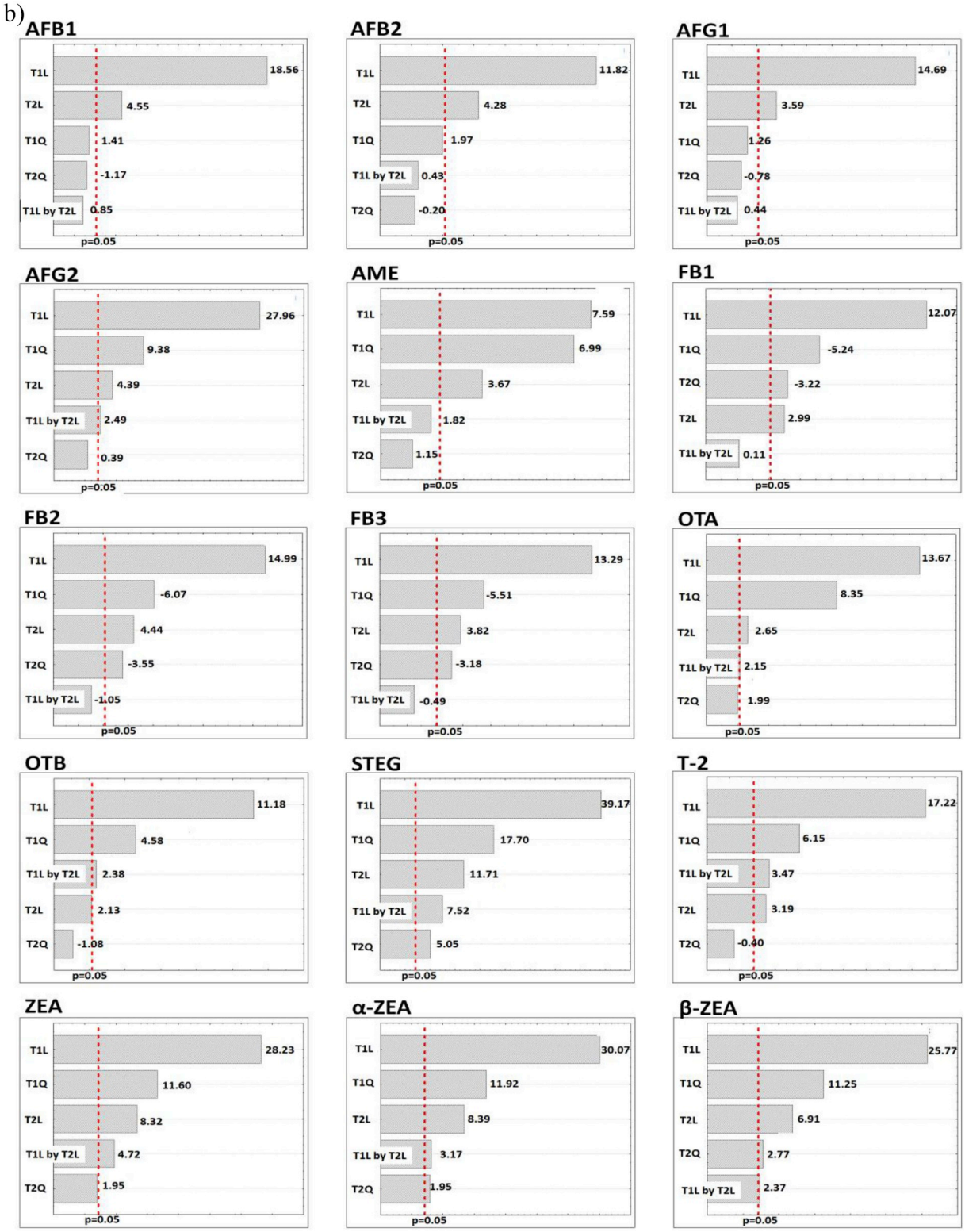


Fig. 2b. Pareto charts of standardized factor effects for model fit of mycotoxins spiked into maize matrix. T1L – Linear effect of temperature; T1Q – Quadratic effect of temperature; T2L – Linear effect of time; T2Q – Quadratic effect of time; and T1L by T2L - Interaction effect temperature and time.

3.3.4. Validation of model

To determine the adequacy of the models and ensure that they offer a good approximation of the true systems, we examined some important validation indices from the model fit which are presented in Tables 5 and 6 (columns 8–14). Significance was determined at a 95% probability level i.e. $\alpha = 0.05$. Computed values of the coefficients of multiple determination (R^2) ranged from 0.87 to 0.99, and 0.89 to 0.99 for pure mycotoxin standards and mycotoxins spiked into maize matrix, respectively. This implies that our models describe between 87 and 99% of the variability in our data, reflective of its acceptability and significance (Adebo et al., 2018). Also, the adjusted R^2 fell within less than ± 0.05 of the R^2 values for all the models. The closeness of the R^2 and adjusted R^2 values, and their nearness to unity indicated that our empirical models were well

fitted to the actual data (Adebo et al., 2018; Babu and Srivastava, 2007; Morshedi and Akbarian, 2014). The Pearson's correlation coefficient (r) ranged from 0.91 to 0.99, and 0.95 to 1.00 for pure mycotoxin standards and mycotoxins spiked into maize matrix, respectively, indicating a strong relationship between the predicted values and the experimental values.

The degree of variability in the dataset was estimated using the average absolute deviation (*AAD*), which ranged from 0.09 to 0.6, and 0.06 to 0.83 for pure mycotoxin standards and mycotoxins spiked into maize matrix, respectively. It should be noted that *AAD* values for AFG_2 in spiked maize matrix (2.25) was not included in the above ranges because it was considered as an outlier. Outliers were determined using the Q-Q plots in SPSS by a step of 1.5 × IQR (interquartile range) (Marr, 2018). *AAD* values closer to zero are desirable as it indicates agreements between the predicted and experimental values (Adebo et al., 2018). The bias factor (B_f) and accuracy factor (A_f) were also examined for our model fits. A_f estimates the relative deviation of predicted values from the experimental values (Dominguez and Schaffner, 2007). Our A_f values ranged from 1.08 to 1.68, and 1.06 to 1.48 for pure mycotoxin standards and mycotoxins spiked into maize matrix respectively. An A_f value of 1.12 indicates an averagely 12% variation between observed and predicted values (Oscar, 2009). Similarly, the B_f measures the relative deviation of predicted and experimental values (Dominguez and Schaffner, 2007). Our B_f values ranged from 0.71 to 1.57, and 0.99 to 1.28 for 48 for pure mycotoxin standards and mycotoxins spiked into maize matrix, respectively. The closer the values of A_f and B_f to unity, the stronger the predictability of the associated models (Adebo et al., 2018; Desobgo et al., 2015).

4. Conclusion

The present study profiled temperature-time degradation patterns of multiple mycotoxins with and without the effect of a matrix. Mycotoxins such as OTs, AFB_2, ZEA and its analogues, STEG and the FBs showed a strongly enhanced degradation when spiked into maize matrix. AME demonstrated a very high thermal stability generally irrespective of matrix interference, while the FBs were the most thermolabile amongst the studied mycotoxins irrespective of the presence of a food matrix. Moreover, we have demonstrated for the first time the thermal stability of the mycotoxins, AME and STEG. In order to optimize the degradation of mycotoxins, the RSM optimization function was fitted to our experimental data, which yielded well-fit models that clearly define the empirical relationship between our control variables (temperature and time) and our objective variable (i.e. mycotoxin degradation). The resultant global optimum solution for multi-mycotoxin degradation was 216.57 °C/63.28 min and 210.85 °C/54.71 min for pure mycotoxin standards and spiked maize matrix, respectively. Besides optimization, our models can be useful in estimating any desired degradation outcome within the experimental domain. This could find applicability in food processing for goal-specific thermal processing of purportedly mycotoxin contaminated foods for food safety and optimum quality.

Further research however, is required to determine the effect of thermal treatment at the obtained optimal conditions on vital food quality factors. Also, other but more efficient thermal processing techniques such as micronisation (high intensity infrared heating) could be investigated as alternatives to conventional oven heating methods. It would also be interesting to investigate the effects of some specific matrix parameters such as pH and moisture, on the thermal degradation patterns of the mycotoxins. Furthermore, there is need to establish the identities of degradation products via high resolution mass spectrometry (HRMS). We do hope that our observations reported herein would positively contribute to the debate on thermal stability of some mycotoxins such as OTs and AFs.

Acknowledgement

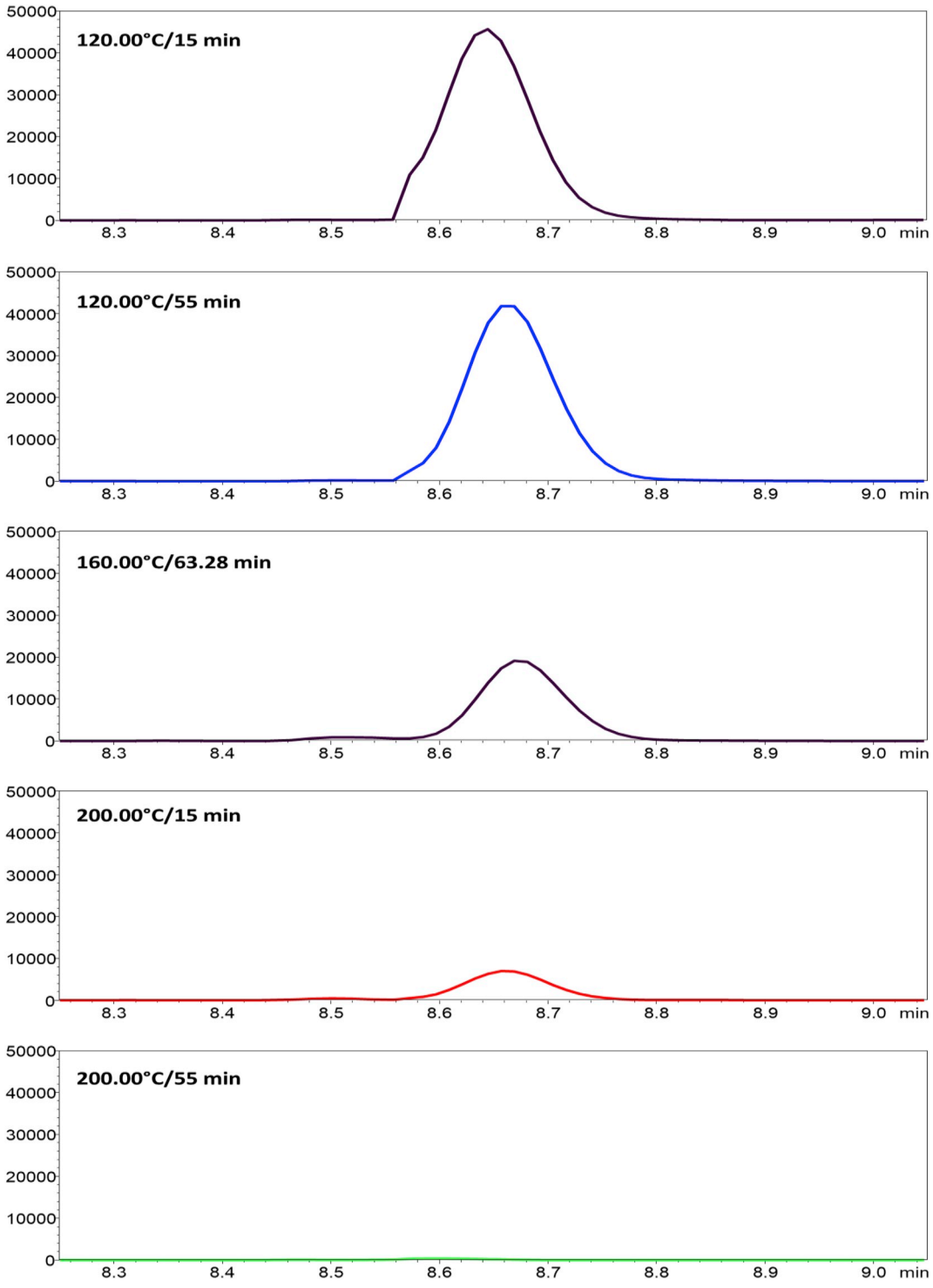
This work was financially supported via the Global Excellence and Stature (GES) Fellowship of the University of Johannesburg granted to the main author (S. Gbashi) as well as support from the South African National Research Foundation (NRF) via the Research and Technology Funding (RTF). Dr Riaan Meyer and Mr Darryl Harris from Shimadzu South Africa are acknowledged for their technical assistance. MYTOX-SOUTH (the Ghent University International Thematic Network) is duly acknowledged for its technical support and provision of a traineeship scholarship to the main author.

Transparency document

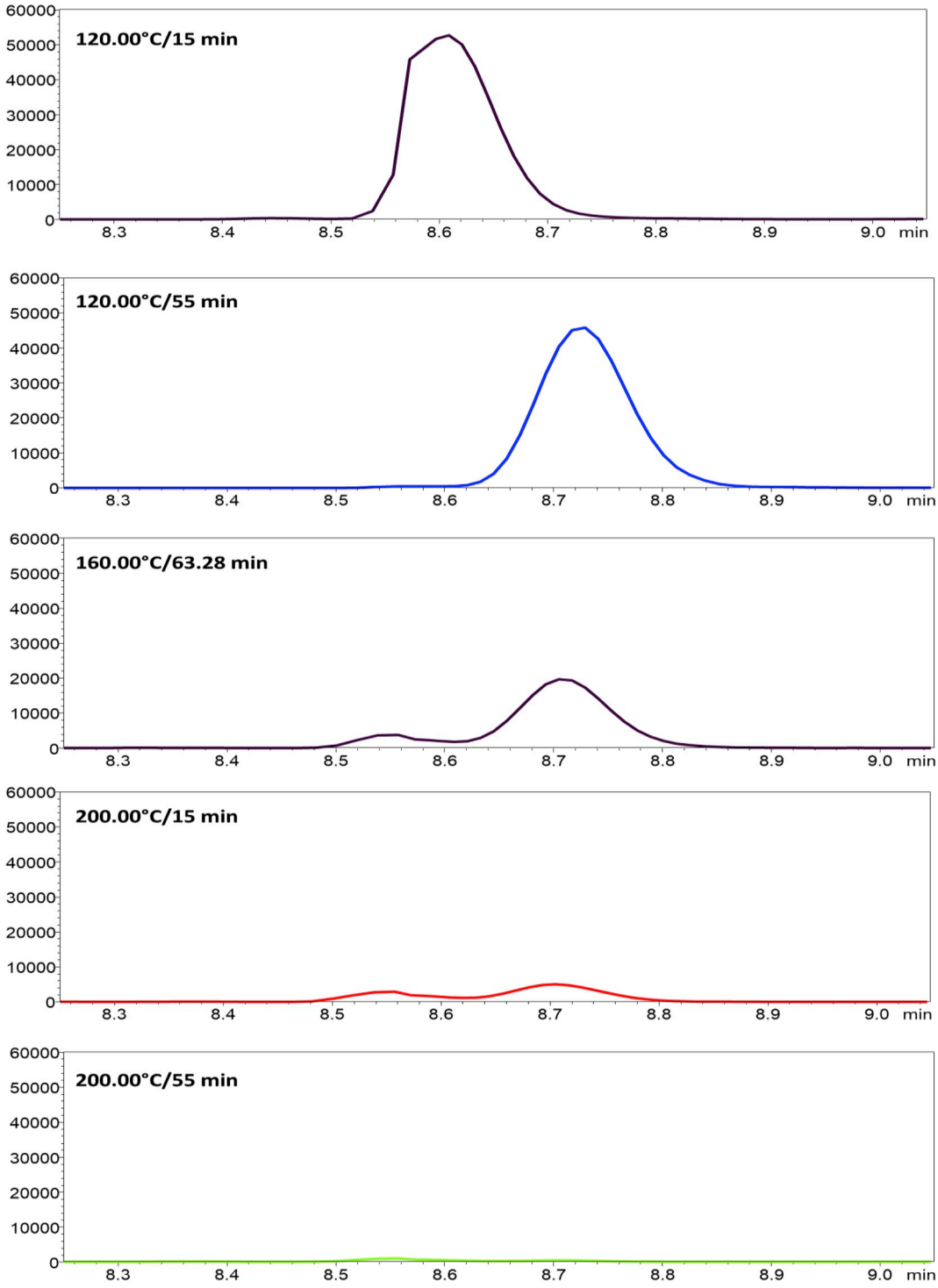
Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.01.009.

Appendix

Appendix A. Single ion chromatogram of pure AFB1 standard at different temperature-time conditions



Appendix B. Single ion chromatogram of AFB1 spiked into maize matrix at different temperature-time conditions



Appendix C. Independent-samples t-test for average thermal degradation of pure mycotoxin and mycotoxins spiked into maize matrix

Mycotoxin	Mycotoxin Standards	Mycotoxin spiked into maize matrix	F-value	p-value	Significance
AFB_1	53.29 ± 3.06	47.09 ± 1.37	0.10	0.75	not sig.
AFB_2	44.07 ± 10.37	50.97 ± 9.14	0.33	0.57	not sig.
AFG_1	54.56 ± 2.2	46.6 ± 3.11	0.16	0.69	not sig.
AFG_2	39.63 ± 1.95	36.82 ± 2.92	0.03	0.87	not sig.
AME	36.31 ± 2.69	33.33 ± 3.17	7.85	0.01	Sig.

FB_1	63.15 ± 4.53	78.3 ± 2.46	7.65	0.01	Sig.
FB_2	64.86 ± 0.84	77.26 ± 1.81	4.82	0.04	Sig.
FB_3	64.67 ± 3.38	80.9 ± 2.07	7.12	0.02	Sig.
OTA	24.26 ± 3.36	45.62 ± 2.81	1.04	0.32	not sig.
OTB	20.42 ± 3.82	38.5 ± 5.76	2.93	0.11	not sig.
STERIG	60.91 ± 1.71	37.37 ± 1.71	0.05	0.82	not sig.
T2-Toxin	49.15 ± 4.51	37.37 ± 6.52	0.58	0.46	not sig.
ZEA	50.96 ± 1.54	42.1 ± 2.81	0.36	0.56	not sig.
α-ZEA	49.59 ± 2.26	37.45 ± 1.94	0.06	0.81	not sig.
β-ZEA	51.67 ± 1.37	38.61 ± 1.47	0.03	0.87	not sig.

Appendix D. CCD regression model fit factor effects for thermal degradation of mycotoxins

Mycotoxins	Factor effects for pure mycotoxin standards					Factor effects for mycotoxins spiked into maize matrix				
	Temp. (L)	Temp. (Q)	Time (L)	Time (Q)	1L by 2L	Temp. (L)	Temp. (Q)	Time (L)	Time (Q)	1L by 2L
AFB1	74.99*	10.34*	6.2	16.21*	5.73	77.39*	7.8	18.96*	−6.43	5.01
AFB2	47.80*	35.76*	4.42	13.27	19.61*	58.77*	12.94	21.27*	−1.32	3.05
AFG1	75.89*	1.54	12.63*	3.73	0.14	80.24*	9.13	19.63*	−5.6	3.39
AFG2	66.72*	29.94*	8.82*	−1.81	15.76*	70.14*	31.12*	11.02*	1.31	8.83*
AME	64.52*	26.32*	11.49*	−0.37	19.51*	18.28*	26.29*	3.01	6.29	13.57*
FB1	74.85*	−3.49	0.79	28.32*	1.82	44.38*	−25.50*	11.03*	−15.66*	0.59
FB2	73.79*	−8.77*	3.42	32.29*	−2.08	46.52*	−24.94*	13.77*	−14.57*	−4.61
FB3	72.78*	−10.49	1.52	21.93*	5.99	39.13*	−21.45*	11.23*	−12.39*	−2.06
OTA	36.90*	16.34*	5.19*	2.53	7.23*	44.54*	35.98*	8.64*	8.62	9.90*
OTB	25.08*	22.68*	−2.56	3.18	15.01*	48.84*	26.48*	9.29	−6.24	14.68*
STEG	65.56*	9.08*	10.53*	20.88*	−4.29	66.00*	39.46*	19.73*	11.26*	17.92*
T2-Toxin	72.91*	11.04	11.93*	9.32	9.87	59.74*	28.22*	11.09*	−1.84	17.05*
ZEA	64.97*	21.32*	11.11*	5.27	2.03	53.05*	28.84*	15.64*	4.85	12.55*
α-ZEA	68.68*	27.13*	10.73*	13.87*	−1.14	63.82*	33.46*	17.83*	8.13*	9.50*
β-ZEA	64.84*	28.15*	11.64*	11.62*	2.75	62.60*	36.16*	16.78*	8.90*	8.16*

Key: *Statistically significant ($p \leq 0.05$) factors. Temp. (L) – linear effect of temperature, Temp. (Q) – quadratic effect of temperature, Time (L) – linear effect of time, Time (Q) – quadratic effect of time, 1L by 2L – interaction effect temperature and time.

Appendix E. Individually optimized experimental conditions for pure mycotoxins and mycotoxins spiked into maize matrix

Mycotoxin	Mycotoxin standards			Spiked maize		
	Temp (°C)	Time (sec)	Degrad (%)	Temp (°C)	Time (sec)	Degrad (%)
AFB_1	193.09	63.28	100.00	200.32	63.28	100.00
AFB_2	203.04	63.28	100.00	202.19	63.28	100.00
AFG_1	196.26	63.28	100.00	199.70	63.28	100.00
AFG_2	204.71	63.28	100.00	206.21	63.28	100.00
AME	204.47	63.28	100.00	216.57	63.28	80.05
	–	–	–	230.00*	63.28*	100.00
FB_1	206.46	20.10	100.00	208.57	59.78	100.00
FB_2	207.95	48.50	100.00	181.14	58.94	99.97
FB_3	178.21	63.28	100.00	207.43	24.43	100.00
OTA	216.57	63.28	71.73	207.13	63.28	100.00
	239.00*	63.28*	100.47	–	–	–
OTB	216.57	63.28	65.72	213.46	63.28	100.00
	239.00*	63.28*	100.26	–	–	–
STEG	205.66	51.14	100.00	197.41	63.28	100.00
T2-Toxin	194.93	63.28	100.00	206.79	63.28	100.00
ZEA	202.26	63.28	100.00	204.34	63.28	100.00
α-ZEA	200.05	63.28	100.00	202.69	63.28	100.00
β-ZEA	198.52	63.28	100.00	202.96	63.28	100.00

Key: * Out-of-experimental range optimal conditions.

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