Influence of technological operation on decrease of concentration of ochratoxin A during processing of Slavonian kulen

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Summary

The aim of this study was to investigate the influence of specific technological operations (smoking, fermentation, drying and ripening) on decrease of the concentration of ochratoxin A (OTA) in samples of Slavonian Kulen which was previously contaminated with ochratoxin A (75 μg kg^{-1}). The samples of raw kulen were prepared according to the traditional processing procedures and divided in two groups (referent and contaminated with OTA). Basic chemical composition, salt content, weight loss and OTA concentrations were determined in all processing stages (before stuffing, after smoking, fermentation, drying and ripening) as well as in raw materials for kulen production (meat, salt and spices). OTA concentrations were determined by HPLC-FD method. OTA concentrations in raw stuffing (1.02 μg kg^{-1}) originates from meat, red powder paprika spice and garlic, increased linearly during the processing to 2.41 μg kg^{-1} in referent samples and in samples contaminated with OTA to 175.82 μg kg^{-1}. This increase in OTA concentrations are related to weight loss during processing and consequently with the decrease of moisture content in samples. OTA concentrations expressed on dry matter showed no increase during processing for both samples groups, from which it can be concluded that none technological operation neither its duration or concentration added substances had any impact on OTA concentration in both samples groups.

Keywords: *decrease of the concentration of OTA, Slavonski kulen, technological operations*

Introduction

Ochratoxin A (OTA) is mycotoxin that represents the important secondary metabolite produced by different moulds belonging to species of *Aspergillus* and *Penicillium* (Moss, 2000; Iacumin et al., 2009). As many studies have shown OTA teratogenic, neurotoxic, genotoxic, immunotoxic and nephrotoxic properties International Agency for Research on Cancer clasified this contaminant into Group 2B as possible human carcinogen (I.A.R.C., 1993). Contamination of feed and subsequently of food from animal origin by mycotoxins could present a serious hazard to humans and animals. Among farmed animals, pigs are known to be particularly sensitive to OTA accumulation, with the highest distribution in kidney, than liver, muscle and the lowest in fat (Lusky et al., 1993; Gareis and Scheuer, 2000; Curtui et al., 2001; Malagutti et al., 2005).

In traditional Croatian fermented sausage "Slavonski Kulen", OTA may be present due to natural contamination of animal feed and consequently pork meat used as a raw material (Pleadin et al., 2013; Perši et al., 2014.) or be produced by certain moulds of *Aspergillus* and *Penicillium* genera, which may spontaneously grow on the surface of "Slavonski Kulen" during its long ripening (Frece et al., 2010).

It is mycotoxin of concern as there is a high potential for its production under appropriate temperature and moisture conditions of storage (Amézqueta et al., 2009). Critical limits for products contaminated with toxigenic molds and production and accumulation of toxic secondary metabolites: aw < 0.9, crack formation on product surfaces and temperature lower than 20 °C (Assefa et al., 2011). Data revealed that among mycotoxins OTA is a moderately stable molecule able to survive most food processing to some extent that may occur in final products and once it has been formed in a food it would be difficult to remove it by most forms of processing (Moss, 1996). Food processing may involve boiling, baking, frying, roasting, fermentation and some other, and the degree to which OTA is destroyed depends on parameters such as pH, temperature and the other ingredients present, with those that utilize the highest temperatures having greatest effects. Studies also shown that some processes reduce OTA concentrations more or less, some significantly, but do not eliminate them completely (Bullerman and Bianchini, 2007).

The aim of this study was to investigate the influence specific technological operations (smoking, fermentation, drying and ripening) to decrease the concentration of OTA

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in samples of Slavonian Kulen previously contaminated with ochratoxin A.

Materials and methods

Sample preparation

The technological process of preparation and production of Slavonian Kulen was conducted in a controlled environment, laboratory and pilot plant for kulen production and application of ripening chamber with programmable and automated control of technological parameters . Kulen stuffing was prepared according to traditional recipes (Kovačević et al., 2010): pork meat (first and second category) (91.8 %) , pig back fat (5 %), garlic (0.2 %), red hot paprika powder (0.4 %) , sweet red paprika powder (0.6 %) and NaCl (2 %). Half of raw stuffing were stuffed in a pig's appendix, (lat. *intestinum caecum*). The other half of stuffing were weighed and contaminated with the ochratoxin A at a concentration of 75 µg kg -1. A total of 14 samples of raw kulen were divided into two groups (with and without the addition of ochratoxin A) and subjected to the traditional production process (Kovačević et al., 2010; Babić et al., 2011). During the production process in eight stages (raw stuffing , 14 (after the smoking process), 30, 60, 90, 120, 150 and 180 day)) physic-chemical analysis, measured of the weight loss and the concentration of the OTA were carried out. Also, the OTA was determined in raw materials for the preparation of the stuffing (meat, salt, garlic, red hot paprika powder and red sweet paprika powder).

Determination of physico-chemical properties

Produced Slavonian kulen sampels were homogenized using Grindomix GM 200 (Retsch, Germany) and prepared for determination of physico-chemical properties. They were analysed using standard analytical methods: ISO 1442:1997 (water), HRN ISO 1443:1999 (fat), HRN ISO 937:1999 (protein), and HRN ISO 3496:1999 (collagen). Sodium chloride content was determined using the titration method (Trajković el al., 1983). All chemicals used for the analyses were of an analytical grade.

Determination of OTA concentration

All Slavonian kulen samples were first homogenised using Grindomix GM 200 (Retsch, Germany) and then with UltraTurrax DI 25 basic homogenisator (IKA®Werke, GmbH&Co.KG). To 5 g of the homogenized sample 7.5 ml of 1% aqueous sodium hydrogen carbonate solution was added and mixed for 2 min. Then 17.5 ml of methanol was added, homogenized for 30 min with rotation, centrifuged, and salted out and degreased. In 10 ml of sample, 10 ml of hexane was added, stirred and left to separation of layers. Extraction procedure was repeated with 10 ml of hexane, and in 5 ml of defatted sample 250 g of 0.4 N $AgNO^3$ solution was added. After precipitation of a white powder (AgCl) samples were centrifuged for 5 min at 3000 rpm at room temperature and then purified by solid phase extraction with the use of immunoaffinity columns Ochraprep® (R-Biopharm, Rhône Ltd), according to instruction manual.

High performance liquid chromatography (HPLC)-based OTA analysis with fluorescent detection (FD) was performed using a Shimadzu instrument (Kyoto, Japan). The instrument consists of SCL 10 APV control module, CTO 10-ASVP thermostat, SPD M10AVP diode array detector, SIL-10 ADVP auto-sampler, LC-10 ATVP quaternary pump and RF-10.

AXLVP fluorescent detector, and is supported by the LP Class Software Vers. 5.032. The analysis made use of SUPELCOSIL™ column LC-18, size 25 cm × 4.5 mm, filled with 5 µm-sized particles. As a mobile phase having a flow rate of 1 mL/min, acetonitrile/water/isopropanol/ acetic acid solution (46:46:6:2) was used. The column temperature was set at 40 °C, while the detector excitation and emission wavelengths were set at 333 nm and 460 nm, respectively. The sample injection volume was 100 µL.

HPLC-FD method was previously validated by determining the limit of detection (LOD) and quantification (LOQ), linearity, selectivity, yield and repeatability of measurements. The implementation of validation procedures were described in Perši et al., 2014.

Results and discussion

Basic chemical compositions of Slavonian kulen during processing are given in Table 1. During the processing (after 6 months of ripening), the mass fraction of moisture deceased significantly from 68-69% in both groups of samples to 24 - 25% . Decrease of mass fraction of moisture resulted with average weight loss of production of 56.5%. Reducing the moisture content resulted with a proportional increase in dry matter in samples of sausage, or increase of mass fraction of basic component (protein, fat, collagen) and salt (Table 1).

Results of the determination of OTA concentration in Slavonian kulen samples are shown in Tables 2 and 3 and Figure 1 and 2. Raw stuffing of the referent sample had OTA concentration of 1.02 µg kg^{-1} which may originate from pig meat (0.97 µg kg^{-1}), red powdered paprika spice (5.62 µg kg^{-1}) and garlic (2.10 µg kg^{-1}). OTA concentration increased linearly in all processing stages for both sample groups (Figure 1 and 2) which is related with the increase of weight loss with the increase of processing time and increase of dry matter content.

OTA concentrations expressed on dry matter (Table 2, Figure 1 and 2) showed no increase during processing for all samples, from which it can be concluded that none technological operation neither its duration or concentration of added substances (spices, salt) had any impact on OTA concentration in all samples and for all processing stages. According to this results, the preservation methods used in Slavonian kulen production (smoking, fermentation, drying and ripening) can't reduce the OTA concentration in Slavonian kulen samples during processing. The decrease of health risk

Table 1 Basic chemical compostion and salt content of Slavnian kulen with (OTA) or without (REF) contamination with OTA during smoking. fermentation and ripening

		Days of processing							
		0	14	30	60	90	120	150	180
Moisture (%)	REF	68.91 ± 0.21	55.67 ± 0.11	48.92 ± 0.09	43.22 ± 0.15	38.60 ± 0.11	32.27 ± 0.13	29.77 ± 0.08	24.41 ± 0.11
	OTA	68.23 ± 0.15	56.12 ± 0.31	49.14 ± 0.12	43.72 ± 0.11	37.57 ± 0.17	33.33 ± 0.09	30.67 ± 0.11	25.02 ± 0.14
Total fat (%)	REF	8.67 ± 0.08	15.92 ± 0.23	17.92 ± 0.11	18.82 ± 0.07	17.39 ± 0.20	18.21 ± 0.12	19.32 ± 0.12	21.54 ± 0.09
	OTA	9.21 ± 0.11	15.83 ± 0.18	17.71 ± 0.06	18.55 ± 0.11	17.73 ± 0.18	17.59 ± 0.10	18.94 ± 0.10	20.89 ± 0.15
Total protein(%)	REF	19.19 ± 0.07	22.32 ± 0.11	23.87 ± 0.12	24.12 ± 0.08	34.84 ± 0.20	37.62 ± 0.14	39.12 ± 0.20	41.78 ± 0.22
	OTA	19.36 ± 0.05	22.48 ± 0.08	23.73 ± 0.10	24.36 ± 0.12	35.27± 0.15	38.38 ± 0.17	40.67 ± 0.16	42.12 ± 0.24
Collagen (%)	REF	1.08 ± 0.31	1.19 ± 0.27	1.56 ± 0.33	2.05 ± 0.41	2.06 ± 0.29	2.43 ± 0.25	2.55 ± 0.33	3.01 ± 0.27
	OTA	1.12 ± 0.25	1.08 ± 0.26	1.49 ± 0.28	1.99 ± 0.31	1.89 ± 0.20	2.41 ± 0.29	2.57 ± 0.40	2.97 ± 0.21
Salt (NaCl) (%)	REF	1.92 ± 0.03	2.71 ± 0.03	3.29 ± 0.06	3.63 ± 0.01	3.75 ± 0.04	4.12 ± 0.02	4.53 ± 0.10	4.75 ± 0.04
	OTA	1.89 ± 0.04	2.58 ± 0.05	3.33 ± 0.04	3.72 ± 0.03	3.81 ± 0.05	4.23 ± 0.05	4.57 ± 0.05	4.80 ± 0.06

Values are means ±SD of triplicate

Table 2 Changes in OTA concentration during the processing of Slavnian kulen with or (OTA) or without (REF) contamination with OTA

		Days of processing							
		0	14	30	60	90	120	150	180
Okratoksin A (μgkg^{-1})	REF	1.21	1.41	1.63	1.81	2.01	2.14	2.23	2.41
	OTA	74.39	102.91	119.08	131.85	145.54	155.89	162.25	175.82

Table 3 Changes in OTA concentration during the processing of Slavnian kulen with (OTA) or without (REF) contamination with OTA expressed on dry matter

		Days of processing							
		0	14	30	60	90	120	150	180
Okratoksin A (μgkg^{-1})	REF	3.23	3.72	3.21	3.01	2.82	3.22	3.42	3.22
	OTA	234.17	240.53	234.13	233.28	229.12	237.82	230.02	234.49

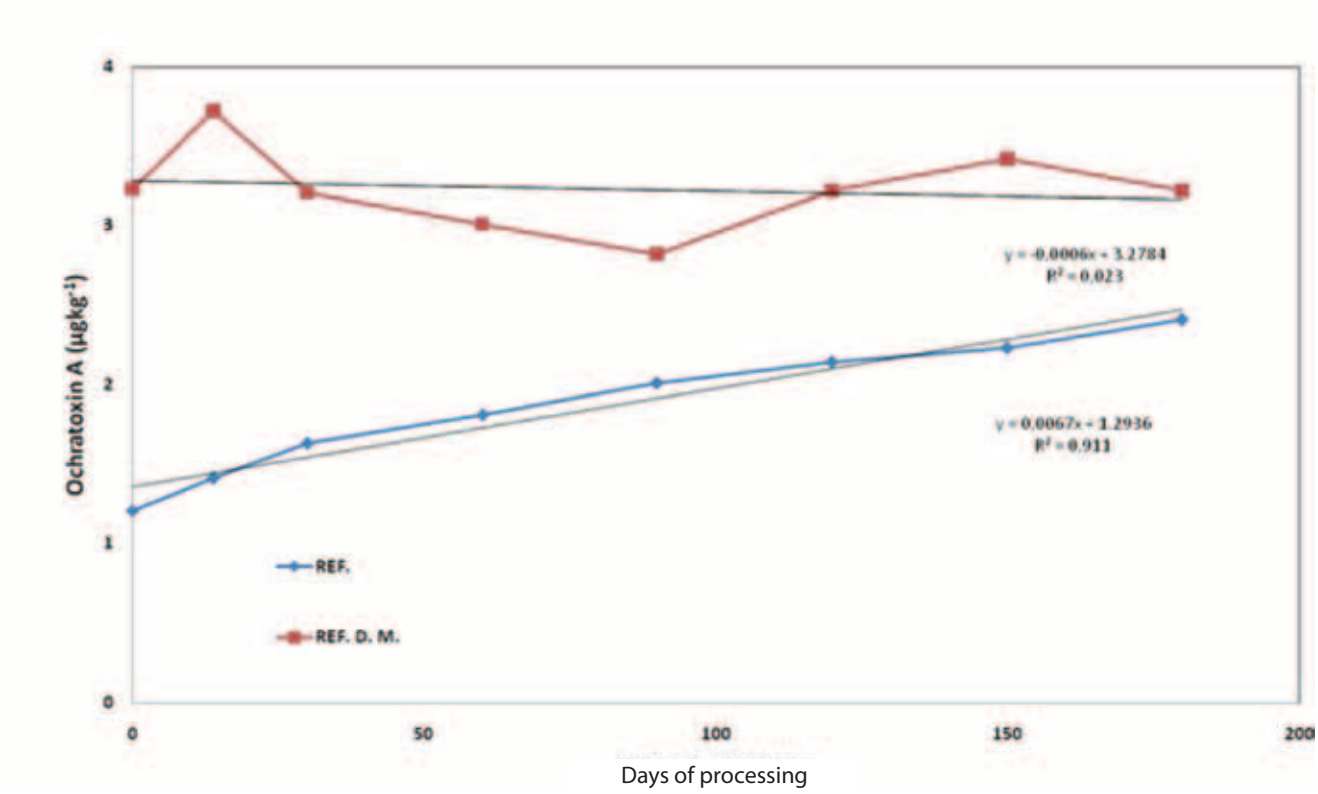

Figure 1 Changes in OTA concentration during the processing of Slavnian kulen with or (OTA) or without (REF) contamination with OTA

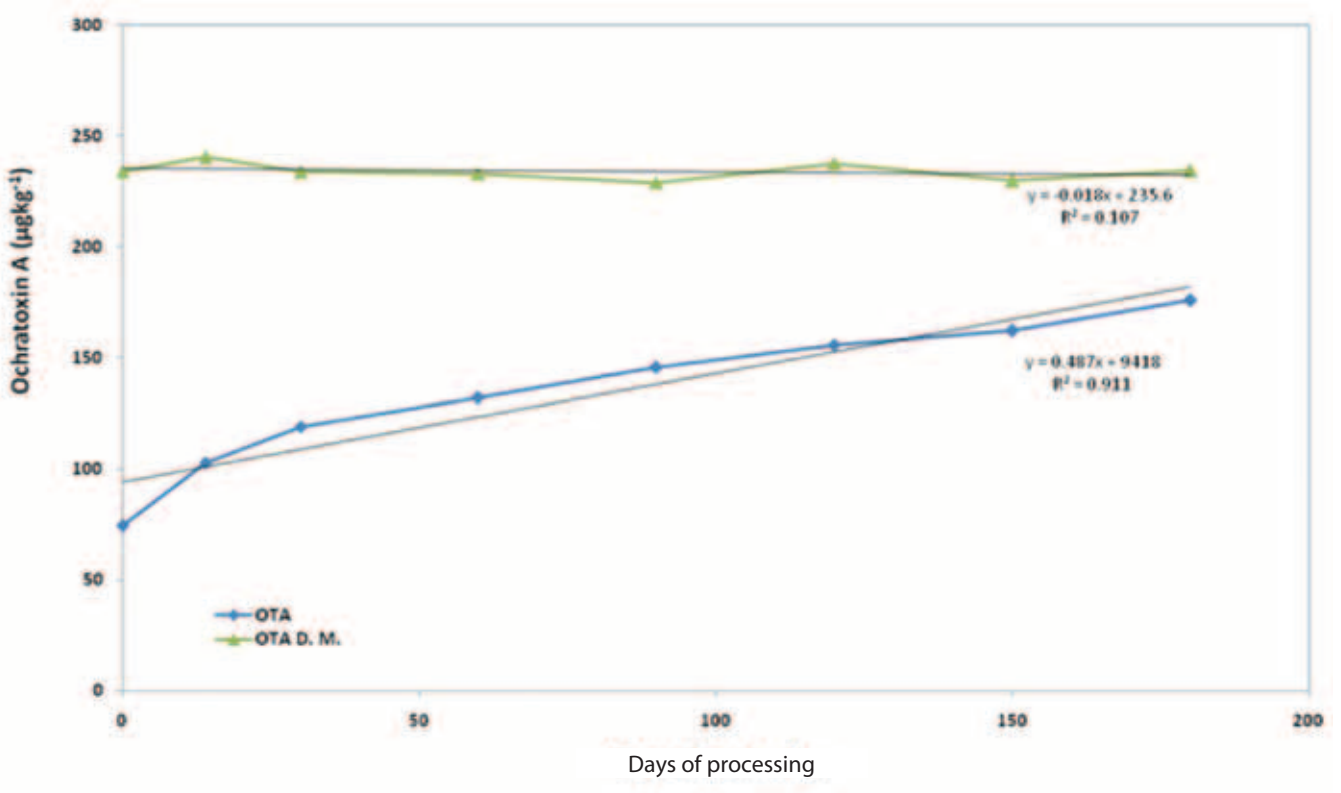

Figure 2 Changes in OTA concentration during the processing of Slavnian kulen with (OTA) or without (REF) contamination with OTA expressed on dry matter

from OTA concentration in Slavonian kulen samples can be achived only by using the raw materials (meat, spices and salt) which are not contaminated with OTA.

Conclusion

Small concentration of OTA in Slavonian kulen raw stuffing may originate from raw materials for kulen production (meat, salt, garlic, red hot paprika powder and red sweet paprika powder). Continuous linear increase of OTA concentration during the processing of Slavonian kulen is related to weight loss (decrease in moisture content) during the drying and ripening. OTA concentrations expressed on dry matter of the samples showed that the technological operation (preservation method) used in Slavonian kulen production (smoking, fermentation, drying and ripening), addition of spices and NaCl had no impact on increase/decrease of OTA concentration during six months kulen processing.

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