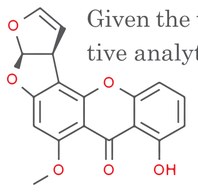
DETERMINATION OF STERIGMATOCYSTIN IN TRADITIONAL DRY-FERMENTED SAUSAGES USING LC-MS/MS METHOD

Tina Lešić[1]*, Ana Vulić[1], Nina Kudumija[1], Nada Vahčić[2], Manuela Zadravec[3], Jelka Pleadin[1]

[1] *Croatian Veterinary Institute, Laboratory for Analytical Chemistry, Savska cesta 143, Zagreb 10000,Croatia*
[2] *Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia*
[3] *Croatian Veterinary Institute, Laboratory for Feed Microbiology,Savska Cesta 143, 10 000 Zagreb, Croatia*
**lesic@veinst.hr*

13th International Scientific and Professional Conference
WITH FOOD TO HEALTH
16th and 17th September 2021
13 hranom do zdravlja with food to health
OSIJEK, CROATIA 2021

INTRODUCTION



Given the toxicity of sterigmatocystin (STC) as a AFB_1 precursor, and insufficient data on its occurrence in food, there is a need for STC detection in different food matrices and the development of sensitive analytical methods (*1*). Dry-fermented meat products, that have a long production tradition in many European countries, are during ripening overgrown by moulds so these products can be contaminated with STC directly through production by the moulds of the *Aspergillus* genus or indirectly through contaminated animal feed and spices used in their production (*2,3*). In this study, the method for STC determination in dry-fermented meat products based on the extraction and clean-up using immunoaffinity columns followed by liquid chromatography with tandem mass spectrometry (LC–MS/MS) was developed and the method was applied in the analyses of household-produced dry-fermented sausages.

MATERIALS AND METHODS

Sausage samples

Samples of 5 different types of traditionally household produced dry- fermented sausages (n=47) from two Croatian regions (West - Istria and East - Slavonia) are obtained from the Croatian markets.

Sample preparation and LC-MS/MS analysis

Preparation of samples was done by high- specific immunoaffinity columns EASI- EXTRACT STERIGMATOCYSTIN (R- Biopharm Rhone Ltd, Glasgow, Scotland) according to manufacturer instructions.
Prepared samples were analysed using high performance liquid chromatograph 1260 Infinity (Agilent Technologies, Santa Clara, USA) coupled with triple qudropole mass spectrometer 6410 QQQ (Agilent Technologies, Santa Clara, SAD). The chromatographic separation was performed on Gemini analytical column, 150 x 4.6 mm with 5 µ- size particles coupled with pre-column SecurityGuard™ Catridges Gemini C18 4 x 3.0 mm ID (Phenomenex, Torrance, USA).

Sausage samples

Immunoaffinity columns

LC-MS/MS analysis

Table 1. STC ion transitions and optimised parameters for MS

Precursor ion	325.1
Fragmentor voltage (V)	130
Product ions	310.0
	281.0
Collision energy (eV)	25
	40
Polarity	+
Source temperature (°C)	350
Gas flow rate (L/min)	12
Nebulizer (psi)	20
Capillary voltage (V)	4000 (+)
	2000 (-)

Method validation

LC-MS/MS method was validated based on the estimation of limit of detection (LOD) and limit of quantification (LOQ) for measurements in the field of contaminants in feed and food via blank samples (*4*). Blank sausage samples were spiked on the level 0.1 µg/kg of STC. For each batch a 5-point calibration curve was prepared in concentration range of 0.1 ng/mL to 10 ng/mL, where linearity was tested. The recovery was determined by virtue of analysis 10 blank samples spiked with STC at 0.1 µg/kg. The matrix effect was evaluated by comparing the peak areas of 0.5 ng/mL standard solution and the blank matrix post extraction spiked at the same level.

RESULTS

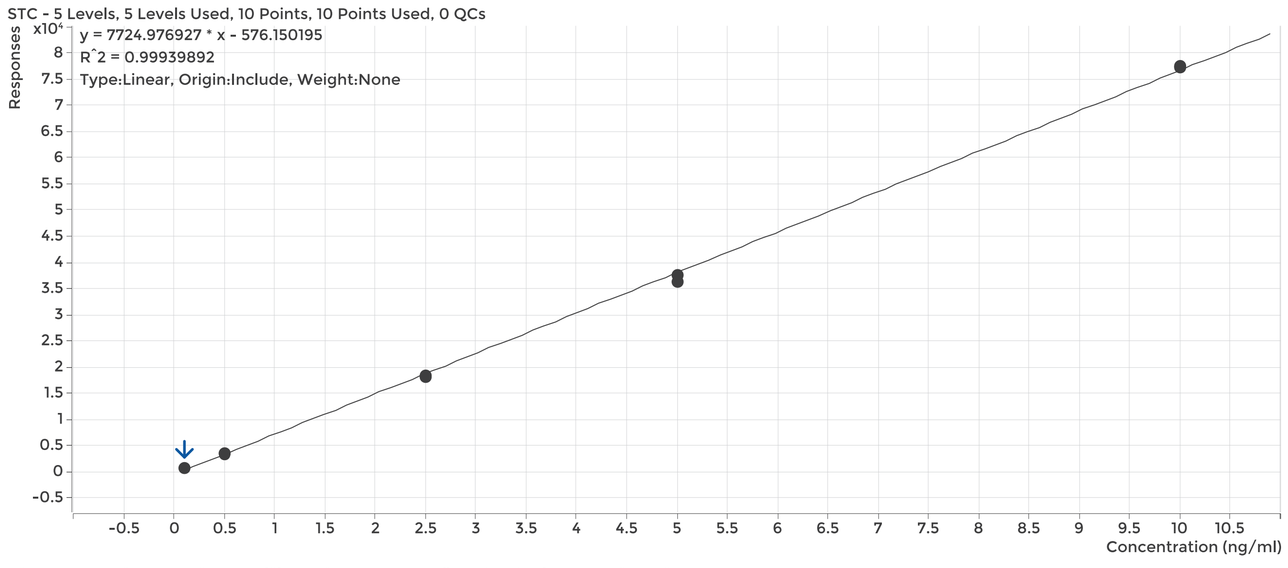


Figure 1. Five-point calibration curve for STC determination

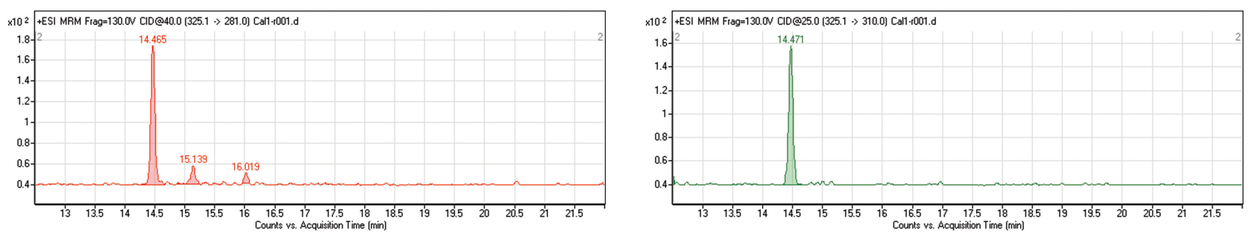


Figure 2. MRM chromatogram of STC standard solution (0.1 ng/ml)

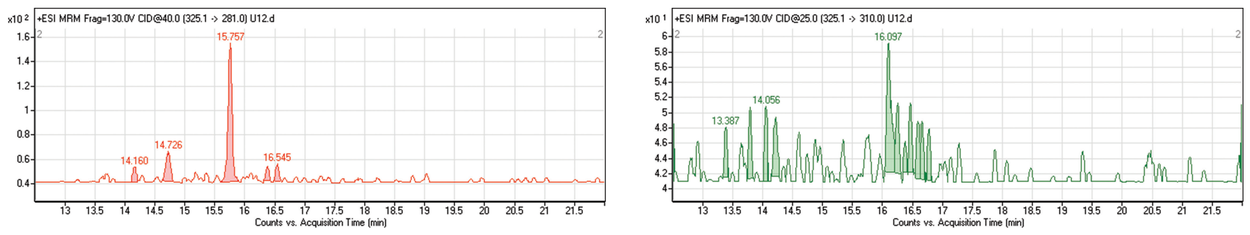


Figure 3. MRM chromatogram for STC in blank sausage sample

Table 2. Validation results

Validation parameter	STC
LOD µg/kg	0.02
LOQ µg/kg	0.06
Correlation coefficient (R^2)	0.999
Recovery (%)	114.4
Matrix effect (%)	7.7

CONCLUSIONS

STC was not detected in any of the analysed sausage samples and one of the reasons behind it can be the fact that moulds of the *Aspergillus* genus are more frequently isolated in meat products that ripen longer and in warmer regions, so that these types of products should be further investigated.

ACKNOWLEDGMENT

This work has been supported by the Croatian Science Foundation under the project "Mycotoxins in traditional Croatian meat products: molecular identification of mycotoxin-producing moulds and consumer exposure assessment" (No. IP-2018-01-9017).

REFERENCES

1. EFSA (2013). *EFSA Journal* 11, 3254.
2. Lešić et al. (2019). *CJFTB* 14, 105-112.
3. Versilovskis, A. & De Saeger, S. (2010). *Mol. Nutr. Food Res.* 54, 136-147.
4. Wenzl et al. (2016). Publications Office of the European Union. EUR 28099.