71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

OCHRATOXIN A IN DRY-FERMENTED SAUSAGES: ANALYSIS AND EXPOSURE ASSESSMENT

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I. INTRODUCTION

Dry-fermented sausages are traditional meat products highly appreciated for their sensory properties worldwide. These products have a composition and are subject to environmental conditions during their drying and ripening processes that favour the growth of moulds on the surface, which are crucial for developing the sensory properties of the final product. However, the contamination and growth of undesired moulds can potentially produce mycotoxins, particularly those of the genera *Aspergillus, Penicillium, Fusarium* and *Alternaria* [1].

In 2020, EFSA published a scientific opinion [2] regarding the public health risks associated with the presence of OTA in food and the levels of exposure from consuming contaminated food. The opinion identified 'preserved meat products' as the most important contributors to the chronic dietary exposure to OTA among 11 food categories considered. However, this output was based on a small dataset (54 samples) providing information on preserved meat, out of a total of 73,891 data points on OTA in various types of food, resulting in high uncertainty.

Therefore, it is essential to better understand the levels of mycotoxins present in ingested food of interest and the exposure related to consumption habits, frequency, and quantity of the food consumed [3]. Such studies would be of great value in assessing the advisability of establishing maximum OTA limits on certain meat products. In this context, the aim of the present work was to assess the exposure to Ochratoxin A associated with the consumption of dry-fermented sausages.

II. MATERIALS AND METHODS

Samples. Sixty-five dry-fermented meat products from different Spanish producers, including 26 *fuet*, 19 *llonganissa* and 20 *chorizo* samples, were studied. Homogenized samples were analyzed by UPLC MS/MS using liquid extraction combined with solid-phase extraction (SPE) cartridges. The limit of detection (LOD) and limit of quantification (LOQ) of the analytical method were 1 µg/kg and 3 µg/kg, respectively.

Consumption of dry-fermented sausages was estimated from an online survey involving 400 Spanish consumers, including 50% men and 50% women. The age distribution was 35% aged 18-35, 40% aged 36-50, and 25% aged 51-70. Participants were asked about the frequency and quantity of consumption of these products and certain consumption practices (e.g., with or without casing, removal/non-removal of mouldy parts). The annual consumption level was estimated based on the frequency of consumption and the portions consumed as self-reported by consumers. The level of exposure was calculated based on consumer habits regarding dry-fermented sausages (fuet, *llonganissa*, and *chorizo*) using the 'left-censored data' methodology, a conservative approach applied in mycotoxin risk assessments [2]. Deterministic OTA concentration values associated with two cut-off values were used: half of the detection limit of the analytical technique (i.e., 0.5 µg/kg as the lower limit) and the equivalent of the quantification limit (3 µg/kg as the upper limit). Based on body weight statistics for the adult population in 2023, the level of exposure to OTA, expressed in nanograms per kg of body weight per day (ng/kg_bw/day), was calculated by gender and three age groups (18-44, 45-64, and 65+). The risk for consumer health was assessed through the calculation of Margin of Exposure (MoE) by dividing the $BMDL_{10}$ (minimum confidence limit (10%) for the reference dose) by the estimated exposure value to OTA. The $BMDL_{10}$ recommended by EFSA [2] was used: 4.73 µg OTA/kg_bw for non-neoplastic effects and 14.5 µg OTA/kg_bw for neoplastic effects.

III. RESULTS AND DISCUSSION

OTA was below the limit of detection in all 65 samples of dry-fermented sausages analysed. Considering the overall consumption of dry-fermented sausages, the level of chronic exposure to Ochratoxin A (OTA) varies depending on the consumer group and the cut-off OTA concentration used. For women, applying the lower limit of OTA, chronic exposure to the mycotoxin ranged from 0.12 to 0.38 ng/kg_bw per day, based on average daily consumption. This exposure could reach up to 3.5 ng/kg body weight per day for the 95th percentile (P_{95}) consumption of dry-fermented

sausages. In men, the level of exposure was slightly lower compared to women, likely due to their higher body weight. In this case, the exposure values ranged from 0.11 to 0.15 ng/kg_bw per day and reached up to 3.5 ng/kg_bw per day for the 95th percentile (P_{95}) of dry-fermented sausages consumption. Margin of Exposure (MoE) estimates lower than the values recommended by EFSA were obtained by combining extreme consumption scenarios (P_{95}, 97.5 g/day, every day) for the combination of dry-fermented sausages studied across all age ranges of men and women, considering the higher cut-off OTA contamination (Table 1). However, it is worth mentioning that this level of contamination, which corresponds to the limit of quantification of the analytical methodology applied, was not detected in any of the samples analysed. This result aligns with existing studies in the scientific literature, where OTA is not detected in most of the meat products analysed, and when detected, it generally has values below 3 µg/kg [4], which is the value used in this study for the estimates.

Table 1. Values of the Exposure Margins (MoE) for the different age groups of men and women in relation to the non-neoplastic [(a)] and neoplastic [(b)] effects of OTA associated with the total consumption of dry-fermented sausages. The ranges correspond to estimates calculated from the upper limit (3 µg/kg) [(c)] and lower limit (0.5 µg/kg) [(d)] of OTA concentration in dry-fermented sausages in which mycotoxin was not detected analytically. Values equal to or greater than 200 for non-neoplastic effects and greater than 10,000 for neoplastic effects are considered sufficient margins to conclude non-health effects of concern.

	Women			Men		
	14-44 years	**45-64 years**	**65+ years**	**14-44 years**	**45-64 years**	**65+ years**
Non-neoplastic effects						
Average	4,533-27,198	6,116-36,694	2,065-12,390	5,257-31,539	5,501-33,005	6,846-41,073
P_{50}	7,703-46,216	10,789-64,734	2,065-12,390	10,294-61,762	10,132-60,795	10,953-65,717
P_{95}	1,219-7,317	1,670-10,021	1,405-8,432	1,372-8,235	1,333-7,999	2,475-14,849
Neoplastic effects						
Average	13,896-83,378	18,748-112,486	**6,330**-37,981	16,114-96,684	16,863-101,179	20,985-125,911
P_{50}	23,613-141,676	33,074-198,445	**6,330**-37,981	31,555-189,333	31,061-186,368	33,576-201,457
P_{95}	**3,738**-22,429	**5,120**-30,721	**4,308**-25,849	**4,207**-25,244	**4,087**-24,522	**7,587**-45,520

[(a)]: with respect to $BDML_{10}$ of 4.73 µg OTA/kg body weight per day due to the increased incidence of microscopic renal lesions due to OTA exposure in pigs (EFSA, 2020)

[(b)]: with respect to $BDML_{10}$ of 14.5 µg OTA/kg body weight per day for the combined incidence of renal adenomas and carcinomas in rats as a reference point for the characterisation of the risk of neoplastic effects (value recommended by EFSA, 2020).

[(c)]: corresponds to the limit of quantification of the analytical methodology used in the analysis of OTA in this work.

[(d)]: corresponds to half of the detection limit of the analytical methodology used in the analysis of OTA in this work.

IV. CONCLUSION

Level of OTA was below the limit of detection (1 µg/kg) in all the 65 commercial dry-fermented sausages analysed. With the reference values used, the safety margins established by the MoE are met in almost all cases. Only extreme daily consumption of these meat products (P_{95}; 97.5 g/day) with an OTA concentration equivalent to the LoQ (3 µg/kg) compromise the MoE. Additionally, it is important to note that many consumers remove the skin/casing from dry-fermented sausages before consumption, which further reduces the potential public health risk.

ACKNOWLEDGEMENTS

Project funded through Operation 16.01.01 (cooperation for innovation) of the PDR of Catalonia 2014-2022 and by TECQUAL (2021 SGR 00461) and SEQUSAL (2021 SGR 00468) and the CERCA Programme from Generalitat de Catalunya.

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