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MYCOTOXIN ASSESMENT IN MEAT PRODUCTS: CHALLENGES FOR OCHRATOXIN A QUANTIFICATION IN DRY-CURED HAM

Quinteros, G.[2]; Martín, B.[1]; Serra, X.[1]; Castellari, M.[2*]; Guàrdia, M.D.[1]; Bover-Cid, S.[2]

[1] IRTA, Food Quality and Technology, Finca Camps i Armet, 17121 Monells, Catalonia, Spain

[2] IRTA, Food Safety and Functionality, Finca Camps i Armet, 17121 Monells, Catalonia, Spain

*Corresponding author email: massimo.castellari@irta.cat

I. INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi, and their presence in dry-cured meat products has become an increasing concern due to their potential health risks. Contamination can occur through various pathways, including the use of contaminated spices and other raw materials, the growth of mold on the surface of dry-cured meat products, and carry-over effects from farm animals exposed to contaminated feed [1] [2]. Such exposure can lead to residual contamination in meat products, posing a risk to human health [2].

Several studies have reported varying levels of mycotoxin contamination across different regions, highlighting the importance of continuous monitoring. The issue is complex and demands comprehensive research alongside stringent control measures to mitigate health risks. Ongoing efforts in monitoring, regulation, and prevention are essential to safeguard consumer health. Consequently, accurate determination of mycotoxin concentrations in meat products is crucial for understanding contamination levels and implementing effective control strategies [1].

II. MATERIALS AND METHODS

Samples: One blank sample and two OTA-positive samples of dry-cured ham were obtained from an experimental trial carried out by inoculating *Penicillium nordicum* CECT2320 in "Serrano" dry-cured hams (samples containing the *Biceps femoris*, *Gluteus medius*, *Gluteus maximus* and *Tensor fasciae latae* muscles). One blank sample (OTA< LOD, see Table 1) and two OTA-positive samples (250 g each) were homogenized using a domestic mincer (Robot Coupe R2) under dry ice and stored at –80 ºC until analysis. Two different extraction/purification strategies (LLE+SPE cartridge & immunoaffinity column - Figure 1) and two chromatographic setups (UPLC-MS/MS & HPLC-FLD) were used to analyse both blank and OTA positive samples.

Chromatographic analyses (Figure 1): UPLC-MS/MS analyses were performed with an Acquity Premier UPLC coupled with a Xevo-TQ-S tandem quadrupole mass spectrometry detector (Waters). The chromatographic separation was carried out employing a Cortecs Premier C18 1.6 µm (2.1 x 100 mm) column (Waters). A gradient elution was based on a mixture of Methanol, Water, Ammonium Acetate, Acetic Acid and Formic Acid. OTA identification was done based on retention time and ratio of specific MRM transitions (m/z 404.11->239.05; m/z 404.11->221.03). For quantification, a solvent calibration standard curve with isotope labelled internal standard (OTA $^{13}C_{20}$) was employed [3]. HPLC-FLD consisted of an Agilent HPLC serial 1200 (Agilent Technologies) equipped with a column Luna 5 µm C18 100 Å (150 x 4.6 mm, Phenomenex). Isocratic elution was carried out with a mixture of ACN, Methanol, Waters and Acetic Acid. OTA identification was done based on retention time and specific excitation/emission λ (333 nm/460 nm). Quantification was done was done with matrix matched calibration standard curve following IAC provider application [4]. Validation parameters (Trueness, Precision, LOD, LOQ) were estimated following SANTE 2021 criteria [5].

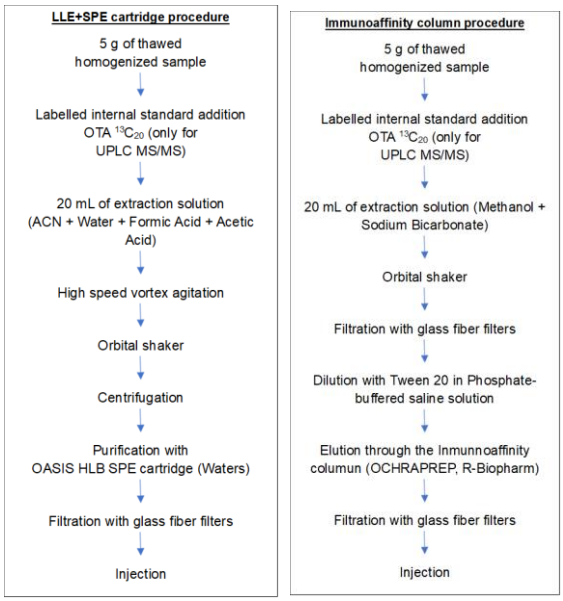


Figure 1. Extraction and purification strategies. Liquid-liquid extraction + Solid Phase Extraction LLE+SPE (*left*), Immunoaffinity column (*right*).

RESULTS AND DISCUSSION

Table 1 shows the results of the protocol validation and OTA quantification with the different analytical procedures. All the strategies fulfil the requirements established by SANTE 2021 for Trueness (range 70-120%) and Precision (≤20%), with LOD ranging from 0.1 ppb to 2.9 ppb depending on the extraction and detection procedure. Overall, samples from LLE+SPE purification showed lower OTA levels than those purified with IAC. At the same time, OTA was quantified at lower concentration in the same extracts when analysed by UPLC MS/MS.

Table 1. Results of validation and OTA quantification with the different analytical procedures (mean ± std.dev. from 3 independent assays).

	LLE+SPE		IAC	
	UPLC MS/MS	HPLC-FLD	UPLC MS/MS	HPLC-FLD
Trueness (%)	100.2±13.2	112.1±13.6	118.4±5.2	117.9±15.7
Precision (RSDr, %)	13.1	12.1	4.4	13.4
LOD (ppb)	0.1	2.9	0.7	1.9
LOQ (ppb)	0.3	8.8	2.1	5.7
OTA+ Sample 1 (ppb)	119.4±2.9	375.3±4.7	295.6±0.6	381.3±0.3
OTA+ Sample 2 (ppb)	346.5±10.3	853.2±38.8	792.0±19.5	1512.9±2.3

III. CONCLUSION

Despite meeting in-house validation criteria, different analytical procedures yielded significantly variable results for OTA levels in dry-cured ham samples, depending on the extraction/purification and chromatographic conditions applied. This variability highlights the difficulties that laboratories could face to provide reliable results for this matrix, which is currently not subjected to official regulation in EU, and the need for harmonized & tailored analytical criteria. To address these challenges and ensure reliable results, further research and targeted interlaboratory proficiency tests are essential.

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