Received: 17 May 2022 | Revised: 3 August 2022 | Accepted: 18 August 2022

DOI: 10.1111/1750-3841.16326

FOOD CHEMISTRY

Investigation of ochratoxin A in commercial cheeses and pork meat products by liquid chromatography–tandem mass spectrometry

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Funding information
Italian Ministry of Health

Abstract

Ochratoxin A (OTA) is a mycotoxin produced by several species of *Aspergillus* and *Penicillium* and commonly detected in a wide range of foodstuffs. The purpose of this work was to monitor the presence of OTA in cheeses and pork meat products. A simple and accurate "dilute and shoot" method with no need of immunoaffinity column and isotopic labeled internal standard, by liquid chromatography–tandem mass spectrometry, was validated in accordance with the criteria set out in Commission Regulation (EC) No. 401/2006. The method showed good linearity in solvent and in matrix ($R^2 \geq 0.995$), limit of detection was 0.2 µg/kg for cheese and 0.3 µg/kg for pork meat products, limit of quantification was fixed at 1 µg/kg, and recovery was estimated at two different concentration levels (1 and 5 µg/kg) and ranged from 75% to 101%. The interday and intraday laboratory precisions were lower than 7%. The matrix effect, the recovery of the extraction process, and the overall process efficiency were evaluated. No significant ME was observed in the two matrices considered. This method was applied to the analysis of 75 samples, coming from official controls implemented by the Lazio Region (Central Italy). In one sample of dry-cured ham, the concentration found (69.3 µg/kg) was well above the guidance value recommended by the Italian Ministry of Health (1 µg/kg). These data together with the detection of OTA in three grated cheeses suggest the importance of monitoring these products. Considering the high dietary intake of these matrices, especially among vulnerable populations, further research should be devoted to estimate exposure and risk assessment for OTA.

KEYWORDS
analytical methods, food safety, mass spectrometry, mycotoxins

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1 | INTRODUCTION

Ochratoxin A (OTA) is a common mycotoxin produced by secondary metabolism of several species belonging to the *Aspergillus* and *Penicillium genera* (El Khoury & Atoui, 2010). Several studies have reported its numerous toxic effects (Cimbalo et al., 2020; Malir et al., 2016), and it was classified by the International Agency for Research on Cancer as a possible carcinogen to humans belonging to Group 2B (IARC, 1993).

Dietary intake accounts for the main human exposure to OTA (Malir et al., 2016). Indeed, given its peculiar characteristics of high stability and resistance to acidity and high temperatures, once a food is contaminated by ochratoxin it is difficult to totally eliminate the molecule (El Khoury & Atoui, 2010). The importance of this mycotoxin is reflected in the wide range of commodities that may become contaminated (Duarte et al., 2012). In particular, the latest European Food Safety Authority (EFSA) scientific report showed that the most important contributors to chronic dietary exposure to OTA were "Preserved meat," "Cheese," and "Grains and grain-based products" (EFSA Panel on Contaminants in the Food Chain [CONTAM] et al., 2020). With regard to meat and meat products, contamination can occur indirectly through animals fed with contaminated feedstuffs (Bertuzzi et al., 2013; Ostry et al., 2013), even though the effects of OTA differ among species (Battacone et al., 2010). Pigs are notoriously sensitive to OTA accumulation. Or contamination may occur directly by OTA produced by fungi growing on meat products during ripening (Bertuzzi et al., 2013); Cimbalo et al., 2020.

Penicillium spp. make up the main part of the mycobiota of some products such as salami, ham, as well as several types of cheeses. Their actions contribute to the ripening of the product through enzymatic activities, to the organoleptic properties of the product, to antagonist action against other undesirable microorganisms, and to protecting the surface of the product thereby preventing it from becoming rancid. In most other cases, fungal contamination leads to unwanted cheese spoilage. However, in some cases the presence of mold on food should be avoided for several reasons, first and foremost, the risk of mycotoxin production (Chávez et al., 2011; Pattono et al., 2013).

At European Union level, Commission Regulation (EC) No. 1881/2006 (European Union, 2006a) sets maximum levels for OTA in various foodstuffs, but no maximum limits have been identified for meat, meat products, and dairy products. However, some countries have implemented maximum limits for OTA, such as Denmark (10 μg/kg in pig kidney), Estonia (10 μg/kg in pig liver), Romania (5 μg/kg in pig kidney, liver, and meat), and Slovakia (5 μg/kg in meat and 5 μg/kg in milk) (Tolosa et al., 2020). Regarding Italy, the Ministry of Health has set a guideline value of 1 μg/kg for pork meat and derived products since 1999 (Italian Ministry of Health, 1999).

Pork meat in Italy is one of the largest agri-food chains. Processing tends to focus on production of protected designations of origin (PDO) and protected geographical indication (PGI) cured meats (Bonazzi et al., 2021). Furthermore, among the EU Member States, Italy is the third most important producer of cheese (Eurostat [https://ec.europa.eu/eurostat]). In 2021, European Union-27 per capita cheese consumption was 20.44 kg, higher than other countries worldwide (CLAL-Il mercato del latte [https://www.clal.it]). Given the economic importance of these sectors and the high consumption of these products, especially among the infant and prepubertal population, it is vital to evaluate the risk assessment of OTA in these commodities, having regard to the available data in Altafini et al. (2021) investigating the presence of OTA in cheeses. For this reason, the aim of this work was to validate a simple and quick method, avoiding the use of immunoaffinity columns (IACs), suitable for the determination of OTA in both cheeses and pork meat products by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Furthermore, the method was used to examine 75 samples coming from official controls implemented by the Lazio Region (Central Italy).

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

LC gradient-grade acetonitrile and methanol were obtained from Merck KGaA (Darmstadt, Germany). *n*-Hexane and ammonium acetate were obtained from Carlo Erba® Reagents s.r.l (Cormoredo, Milan, Italy) and acetic acid from VWR International (Radnor, PA, USA).

Ultrapure water was produced by a Millipore Milli-Q system (Millipore, Bedford, MA, USA).

Analytical reference standard of OTA (100 μg/ml) was achieved from Pribolab (Biopolis Rd, Singapore) and was stored at −20°C. Working solutions were obtained by dissolving the appropriate amount of OTA standard solution in methanol and were prepared daily.

Extraction solution was prepared just before use by mixing H_2O:CH_3CN (20:80 [v/v]) acidified with 1% of CH_3COOH.

2.2 | LC tandem mass spectrometer apparatus

LC–MS/MS analysis was performed on a Perkin Elmer micro M200 series pump connected with a triple

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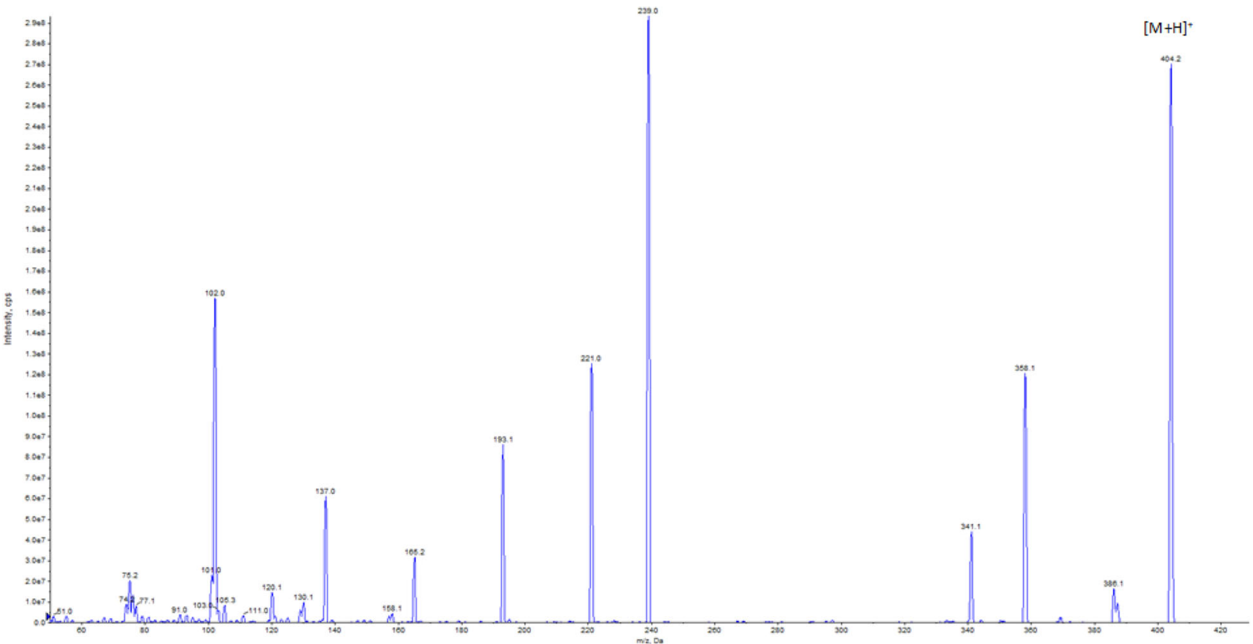

FIGURE 1 Electrospray mass spectrum of OTA (ESI+)

quadrupole mass spectrometer from AB Sciex® 6500+ (Sciex, Framingham, MA, USA) using a Phenomenex® Gemini C_{18} column (3 µm 150 × 3 mm ID) (Phenomenex, Torrance, CA, USA).

Mobile phases were as follows: CH_3OH:H_2O:CH_3COOH (1:98.9:0.1 [v/v]) with 5 mM ammonium acetate as mobile phase A, and CH_3OH:H_2O:CH_3COOH (99.7:0.2:0.1 [v/v]) with 5 mM ammonium acetate as mobile phase B. Elution profile was as follows: 0–2 min 10% B, 2–7 min 90% B, 7–11 min 90% B, 11–11.1 min 90% B, and 11.1–15 min 10% B at a flow rate of 0.5 ml/min. Injected volume was 5 µl.

Electrospray Ionization (ESI) conditions were as follow: capillary voltage, 5.5 kV; curtain gas (CUR), 20; collision gas (CAD), 9; source temperature (TEM), 500°C; ion source gas (GS1 and GS2), 30.

The data acquisition and processing were performed using Analyst® 1.6.3 software and MultiQuant® 3.0.2 software from AB Sciex®. Quantitation was carried out in positive ion mode with multiple reaction monitoring (MRM).

Direct infusion of OTA standard (0.5 µg/ml) was carried out to optimize the MRM transition. The most intense daughter ions were chosen from the electrospray mass spectrum (ESI+) obtained (Figure 1). The following transitions were monitored: 404 ◊ 239 (quantifier transition) and 404 ◊ 102 (qualifier transition). Dwell time was set to 200 ms, collision energy for quantifier to 30, and collision energy for qualifier to 80.

2.3 | Sampling

Samples were from official control belonging to the Regional Plan for the monitoring of OTA in foods of animal origin implemented by the Lazio Region (Italy). Seventy-five samples (45 pork products and 30 cheeses) commercially sold in Lazio and belonging to different brands were analyzed between January 2019 and October 2021. They include salami ($n = 15$), dry cured ham ($n = 18$), cooked sausage ($n = 1$), *Capocollo* ($n = 1$), cooked ham ($n = 1$), *Mortadella* ($n = 1$), *Bacon* ($n = 1$), *Guanciale* ($n = 2$), spicy salami ($n = 2$), dried meat ($n = 1$), *Cotechino* ($n = 1$), cured sausage ($n = 1$), *Caciotta* cheese ($n = 4$), blue cheese ($n = 1$), *Provolone* ($n = 1$), *Provola* ($n = 1$), whole hard cheese ($n = 9$), and grated hard cheese ($n = 14$).

At arrival, samples were immediately stored at −20°C until processing.

2.4 | Extraction procedure

Starting from the method developed by Biancardi et al. (2013) on the cheese matrix, some modifications were made, and the optimization of the procedure was extended also to different pork by-products. Five grams of homogenized sample was weighed into a 50-ml centrifuge tube and 30 ml of extraction solution was added in the cheese samples and 20 ml in pork meat product samples. After being placed for 30 min in a horizontal shaker and after

centrifugation at 4000 rpm, 4°C for 10 min, a portion of the supernatant was degreased with the same volume of *n*-hexane, vortexed, and again centrifuged. The upper phase was removed and the aqueous layer transferred to 2 ml vial and injected in LC–MS/MS.

2.5 | Method validation

2.5.1 | Evaluation of signal suppression/enhancement, matrix effect

Matrix interference is a drawback of practically all the instrumental techniques and unfortunately also for LC–MSMS, where the effects more frequently lead to ion suppression phenomena induced by the presence in the matrix of volatile compounds. Therefore, the evaluation of matrix effect (ME) should be included in the validation process of a new method (Gosetti et al., 2010).

In MS analysis, matrix effect can be determined by comparing (a) the instrument response for calibrators injected directly in mobile phase, (b) the same amount of compound added to pre-extracted samples, and (c) the same amount of compound added to matrix before extraction (Annesley, 2003).

The percentages of matrix effect (ME %), the recovery of the extraction process (RE %), and the overall process efficiency (PE %) are calculated as follows:

$$\mathrm{ME}\,(\%) = B/A \times 100, \quad (1)$$

$$\mathrm{RE}\,(\%) = C/B \times 100, \quad (2)$$

$$\mathrm{PE}\,(\%) = (\mathrm{ME} \cdot \mathrm{RE})\,/100. \quad (3)$$

where A is the peak area of the standard solution, B the peak area of the standard spiked after extraction, and C is the peak area of the standard spiked before extraction (Matuszewski et al., 2003).

The matrix effect can also be determined by comparing the slopes of the neat standard calibration curves and matrix standard calibration curves. In the absence of matrix effect, the slopes in the two diagrams correspond to each other within the experimental error deviation (Gosetti et al., 2010).

2.5.2 | Method performance parameters

The method was validated in accordance with Commission Regulation (EC) No. 401/2006 (European Union, 2006b) and UNI CEI EN ISO/IEC 17025:2018 (UNI, 2018).

Specificity, selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy (recovery percentage), precision (CV %), intraday and interday precision (CV %), and accuracy were assessed.

Specificity and selectivity were evaluated by examining 20 different blank samples of different type of cheese and pork by-products and compared with those spiked with OTA at 1 μg/kg.

LOD is the lowest concentration that can be detected from the background noise and a minimum signal to noise (S/N) ratio of 3 is required. LOQ is the lowest concentration that can be quantitatively determined by an analytic procedure.

Linearity was evaluated by the calibration curves prepared in solvent, spiked samples, and spiked extract at five concentration levels of 0.125, 0.250, 0.500, 1.000, and 2.000 ng/ml corresponding to 0.75, 1.5, 3.0, 6.0, and 12.0 μg/kg of OTA in cheese and 0.5, 1.0, 2.0, 4.0, and 8.0 μg/kg of OTA in pork by-products, respectively. Quantitation was performed by plotting peak areas versus the corresponding concentration values. The regression equation was calculated by the minimal square method.

Accuracy and precision were determined in terms of recovery and repeatability (CV %) using blank samples spiked at two different levels (1 and 5 μg/kg). Each level was analyzed with eight independent replicates. The acceptable range for recovery of OTA in samples spiked between 1 and 10 μg/kg is 70%–120% and the acceptable CV % for the same range has to be less or equal to 20%.

Uncertainty of measurements for OTA was calculated using a metrological approach and as per the statistical procedure of UNICHIM 179/2 and compared with that calculated using the reference formula of Regulation (EC) No. 401/2006 (European Union, 2006b).

Intraday and interday precision and accuracy were evaluated by analyzing 12 independent replicates for each concentration (1 and 5 μg/kg) in the same day (intraday precision) and six replicates on three different days (interday precision). Precision was expressed as CV % of the replicate measurements; acceptable CV % should be less or equal to 20%.

3 | RESULTS AND DISCUSSION

3.1 | Method performance evaluation

In this work, matrix effects in cheese and pork by-products were evaluated by comparing the mean of the areas of three independent sets of neat solution OTA standard at five different concentration levels of 0.125, 0.250, 0.500, 1.000, and 2.000 ng/ml, with the mean of the areas of OTA

TABLE 1 Signal suppression/enhancement evaluation for cheese and pork meat products matrices

Nominal concentration (ng/ml)	Pork meat products			Cheese			CV %		
								Pork meat products	Cheese
	ME %	RE %	PE %	ME %	RE %	PE %	Solve	ANTE	ANTE
0.125	112	91	102	105	77	81	8.0	11.1	5.5
0.250	117	88	103	105	89	93	1.2	3.2	8.8
0.500	122	84	102	118	90	106	3.2	1.9	2.9
1.000	117	89	104	113	93	104	2.2	0.9	0.6
2.000	109	92	106	113	95	107	0.6	0.2	0.3

Abbreviations: SOLV, solvent; ANTE, before extraction; CV%, coefficient of variation percentage.

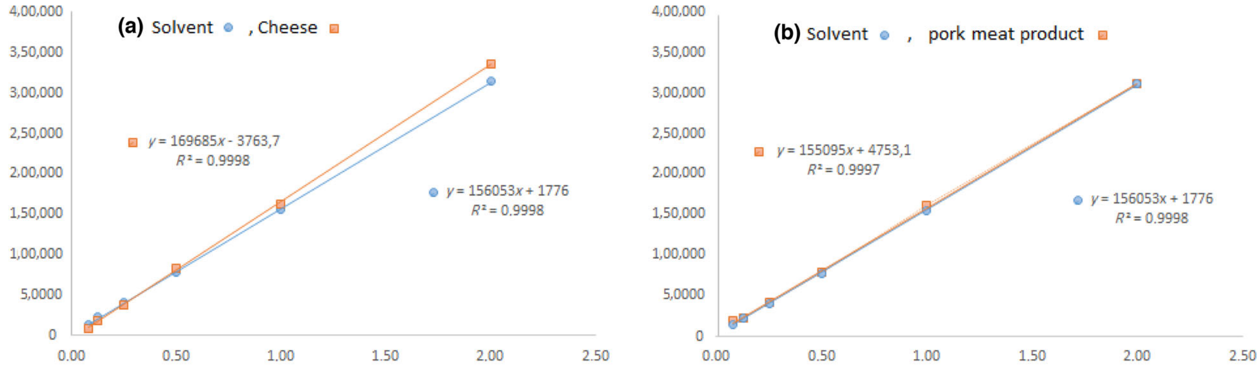

FIGURE 2 Comparison of neat standard calibration curve and matrix standard curves, added before extraction procedure: (a) cheese and (b) pork meat products

in spiked samples after and before extraction at the same concentrations.

The absolute matrix effects were calculated using Equation (1); the "true" recovery values (RE %), not intersected by the matrix effect, were determined by Equation (2); and the values that represent the overall process efficiency (PE %) were obtained by Equation (3). The results are reported in Table 1. ME % values ranged from 109% to 122% in the case of pork meat products with an average value of 115%, while 105%–118% in the case of cheese with an average value of 111%. A value within 85%–115% indicates that there are no significant matrix effects, lower than 85% indicates ion suppression, while higher than 115% indicates ion enhancement (He et al., 2018). The mean values of RE % for both matrices were 89% and the performance of the entire process PE % was 103% for pork meat products and 98% for cheese.

In addition, slopes of curves in neat standard solution and in matrix, obtained by interpolating the mean of the areas of three independent sets of analyses against the nominal concentration, were compared (Figure 2). The equation of the neat standard calibration curve was $y = 156{,}053x + 1776$, and $y = 169{,}685x - 3763.7$ and $y = 155{,}095x + 4753.1$ for cheese matrix and pork product before extraction, respectively. The percentage differences, 9% for cheese matrix and 1% for pork product, were in accordance with the result obtained with the data reported in Table 1. Moreover, a difference in the slopes of the calibration curves of ±20% indicates the absence of matrix effect, as the variation may be due to repeatability (Ferrer et al., 2011).

From the same dataset, at each given concentration, it was possible to determine the relative matrix effect, expressed as CV %. The variability of CV % of peak areas of OTA in cheese added to the already extracted sample ranged from 0.3% to 8.8% and from 0.2% to 11.1% for OTA in pork meat product and seemed to be comparable to the range values obtained with those of OTA injected in solvent (0.6%–8.0%), as shown in Table 1. Data pointed out that the relative matrix effect was practically negligible; therefore, quantitative analysis was carried out using an external calibration curve in solvent for both matrices considered.

In Figure 3, chromatograms of (A) blank and (B) spiked grated cheese sample at 1 μg/kg are reported. In Figure 4, chromatograms of (a) blank and (b) spiked sample at

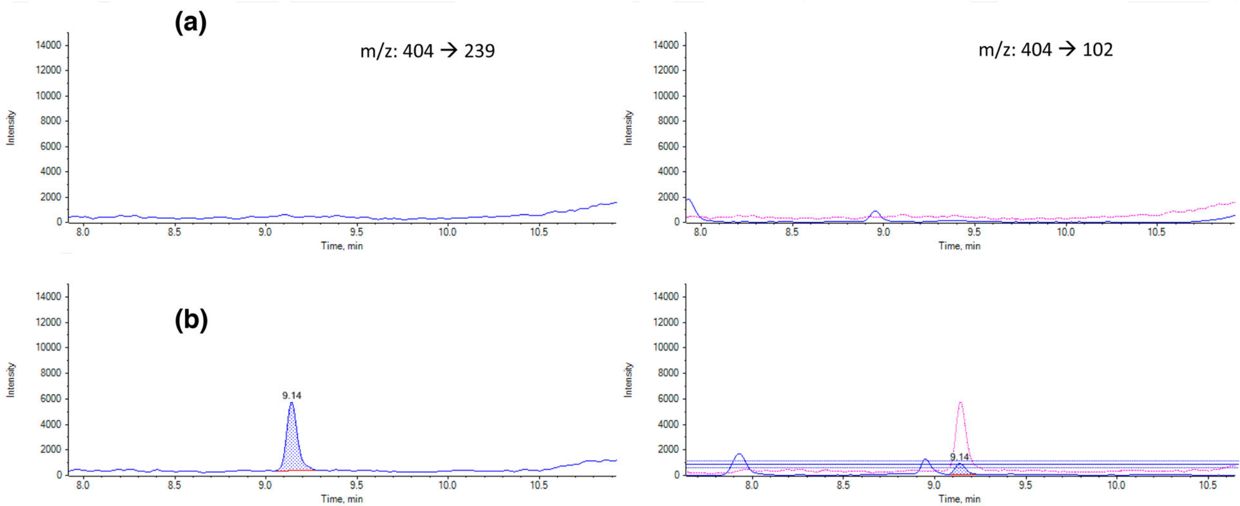

FIGURE 3 (a) Chromatogram of a blank cheese. (b) Chromatogram of a blank cheese spiked at 1 μg/kg

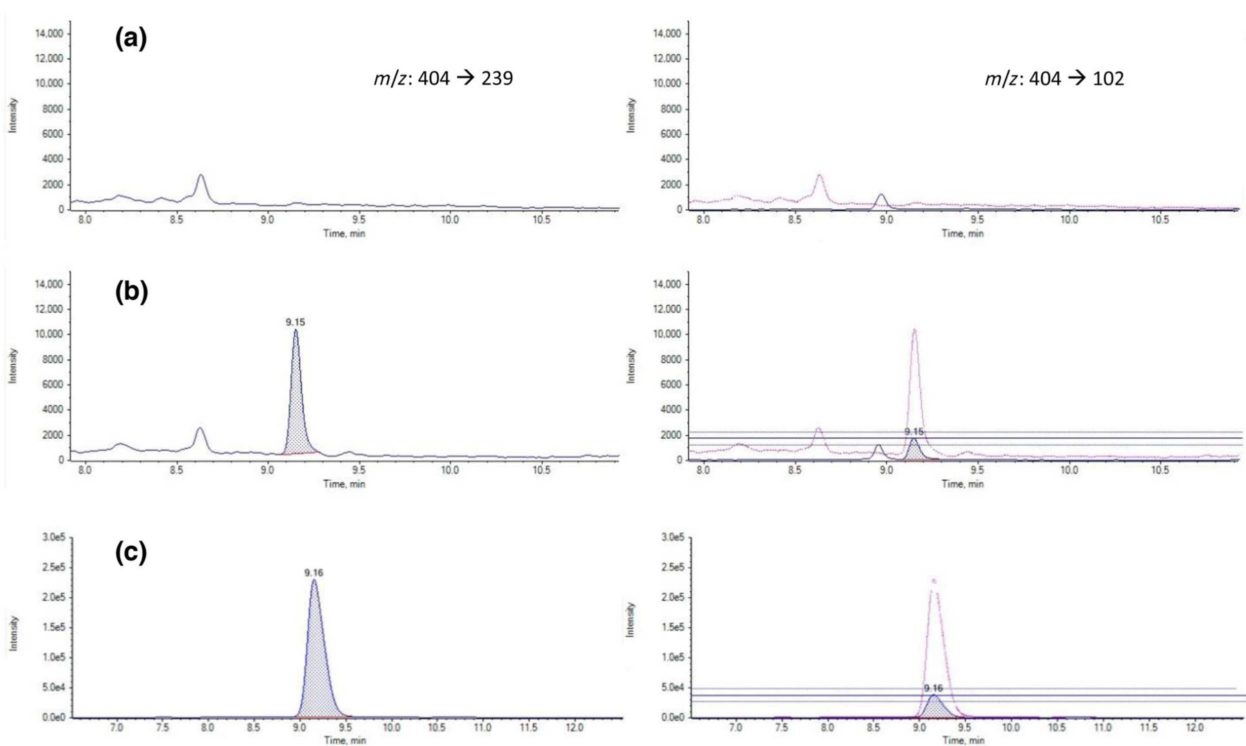

FIGURE 4 (a) Chromatogram of a blank pork meat product. (b) Chromatogram of a blank pork meat product spiked at 1 μg/kg. (c) Chromatogram of a naturally contaminated dry-cured ham

1 μg/kg and (C) a naturally contaminated dry-cured ham sample are reported. The blank samples showed that no significant interfering peaks were detected at the OTA retention time in both monitored transitions.

All the calibration curves showed good linearity within a concentration range of 0.125–2.000 ng/ml and the correlation coefficient (R^2) was always greater than 0.995.

Accuracy and precision were tested at two different levels and results are shown in Table 2. Mean recoveries were 75% for OTA spiked at 1 μg/kg and 100% for 5 μg/kg in cheese matrix, while 91% and 101% for pork meat products, respectively. LOQ was fixed and verified at 1 μg/kg.

LOD was verified by performing independent analyses of blanks and was 0.2 μg/kg for cheese and 0.3 μg/kg for

TABLE 2 Validation performance criteria

Levels (μg/kg)	Matrix Cheese Mean recovery (%)	CV %	Relative uncertainty[a]	Relative uncertainty[b]	Pork meat products Mean recovery (%)	CV %	Relative uncertainty[a]	Relative uncertainty[b]
1 μg/kg (LOQ)	75	19	14%	25%	91	5	12%	22%
5 μg/kg	100	1	20%	20%	101	2	15%	20%

[a]Metrological Approach.
[b]Maximum Standard Uncertainty 401/2006 (European Union, 2006b).

TABLE 3 Intraday and interaday accuracy and precision for OTA

Levels (μg/kg)	Matrix Cheese Accuracy (%)	Precision (%) Intraday ($n = 12$)	Interday ($n = 18$)[a]	Pork meat products Accuracy (%)	Precision (%) Intraday ($n = 12$)	Interday ($n = 18$)[a]
1 μg/kg (LOQ)	108.8	3.61	6.51	105.3	5.26	5.86
5 μg/kg	101.3	3.00	3.01	95.9	4.19	6.95

[a]Six replicates for three separate days.

pork meat products. All the blank samples spiked were confirmative with an S/N > 3.

In both cases (cheese and pork by-products), the measurement uncertainties obtained using metrological approach were equal or lower than that calculated according to Commission Regulation (EC) No. 401/2006 (European Union, 2006b) and shown in Table 2.

CV % for intraday and interday precision for cheese and pork meat products was lower than 7%, as reported in Table 3; the accuracy was evaluated and ranged from 95.9% to 108.8%.

The accuracy, precision, and intraday and interday repeatability values obtained in this study were comparable to those reported in the literature from the same spiked levels and similar matrices. The average recovery reported in the literature for 1 μg/kg fortification level for cheeses ranged between 84% and 99% with CV % of intraday repeatability between 3% and 11%. (Altafini et al., 2021; Biancardi et al., 2013; Dall'Asta et al., 2008; Sakin et al., 2018). For pork meat products, the average recovery ranged between 80% and 85% with CV % of intraday repeatability ranging between 3% and 14% (Chiavaro et al., 2002; Pleadin et al., 2015; Roncada et al., 2020; Tangni et al., 2021).

Seventy-five commercial samples were analyzed in the 3-year period 2019–2021, which include 30 of cheese products at various levels of maturation, from fresh to aged (40%), and 45 of different pork-derived products (60%). A blank sample (cheese or pork meat product) and two replicates of the same blank spiked at 1 μg/kg were used for each analytical session. In the case of positive screening, the sample was subjected to confirmatory analysis in duplicate and quantified with a curve in the solvent. Samples outside the calibration range were properly diluted to evaluate the concentration of OTA. The "dilute and shoot" approach used in this study permitted numerous samples to be analyzed in a short time and was cost-effective. In fact, it does not involve the use of IACs or stable isotope-labeled internal standard to tackle matrix effects.

3.2 | Cheese samples

No detected level (>LOD) was obtained in 26 samples of cheeses out of 30 analyzed, as reported in Table 4.

In three samples (10%), which belonged to the category of grated hard cheeses, the concentration of the mycotoxin was above our LOQ (1 μg/kg) with levels of 1.14, 1.52, and 4.7 μg/kg; in another case, it was lower than LOQ. Data collected are in agreement with those reported in the recent study conducted by Altafini et al. (2021), in which seven out of the 84 different types of cheeses analyzed, with OTA values ranging between 1.3 and 22.4 μg/kg, were grated hard cheeses. Moreover, Biancardi et al. (2013), in six out of 40 commercially grated cheeses analyzed, detected OTA at concentrations ranging from 1.62 to 54.07 μg/kg. The crust of ripened cheeses can be particularly contaminated with OTA. Therefore, grated cheeses, where the rind is included in the final product, are the main contributors to OTA

TABLE 4 Number of samples per type and occurrence of ochratoxin A

Pork meat products	Ochratoxin A Number of samples/ presences > LOQ	Number of samples/LOD < presences < LOQ
Salami	15/0	15/0
Dry cured-ham	18/1	18/3
Cooked sausage	1/0	1/0
Capocollo	1/0	1/0
Cooked ham	1/0	1/0
Mortadella	1/0	1/0
Bacon	1/0	1/0
Guanciale	2/0	2/0
Spicy salami	2/0	2/0
Dried meat	1/0	1/0
Cotechino	1/0	1/0
Cured sausage	1/0	1/0
Total	45/1	45/3
Cheeses	**Ochratoxin A Number of samples/ presences > LOQ**	**Number of samples/LOD < presences < LOQ**
Caciotta	4/0	4/0
Blue cheese	1/0	1/0
Provolone	1/0	1/0
Provola	1/0	1/0
Whole grated hard	14/3	14/1
Hard cheese	9/0	9/0
Total	30/3	30/1

exposure (EFSA Panel on Contaminants in the Food Chain [CONTAM] et al., 2020). OTA contamination rises from the surface through environmental cross-contamination (Biancardi et al., 2013). The control of fungal spoilage is still an important challenge for industrialists and modern practices to prevent or limit the incidence of contamination in dairy products. To this end, traditional methods can be implemented such as the use of efficient aeration systems, cleaning and disinfection procedures, heat treatment, and reduction of water activity by brining, refrigeration, and modified atmosphere packaging (Garnier et al., 2017).

Other authors reported the occurrence of OTA in other types of cheese samples. In an Italian study, 32 traditional handmade semihard cheeses were examined and OTA was detected in six samples, with amounts ranging between 18.4 and 146.0 μg/kg in the interior and between 1.0 and 262.2 μg/kg in the rind. In this case, the high levels found may be due to the handmade cheeses having been processed in uncontrolled environmental conditions (Pattono et al., 2013).

Dall'Asta et al. (2008) have reported for the first time the occurrence of OTA in different commercial samples of blue mold ripened cheeses with different levels (0.25–3.0 μg/kg). In a study conducted in Turkey, OTA was determined in 28 samples of Sürk cheese samples with concentration ranging from 0.058 to 5.04 μg/kg (Sakin et al., 2018). Therefore, the results of the studies in the literature reveal the importance of further monitoring cheese products to assess OTA incidence, with a special emphasis on the grated cheeses that repeatedly showed to be more prone to OTA contamination.

3.3 | Pork meat products samples

Regarding pork by-products in this study, 45 different types of samples were analyzed. Notably, in 41 samples, OTA was not detected as shown in Table 4. In three dry-cured hams, OTA levels found were between LOD and LOQ. Only a dry-cured ham was found to be contaminated with OTA at high levels (69.3 μg/kg) (about 70 times higher than the maximum value recommended by the Italian Ministry of Health). The relative chromatogram is reported in Figure 4c. This sample generated a report to the RASFF (the Rapid Alert System for Food and Feed). Specifically, it was a PGI Italian ham sold in slices and packaged. Dry-cured hams are produced starting from the thighs of pigs, which undergo basic technological treatments, and are stored in plants placed suitable for ripening (Battilani et al., 2007). The traditional technology for the production of dry-cured ham consists of salting, post-salting, and dry-ripening. The last stage can last from 2–3 months to 2–3 years. Increasing ripening times leads to higher enzymatic degradation, improves taste and flavor, and thereby increases the quality of the final product (Petrova et al., 2015). Reducing drying times could result in a reduction of the drying facilities, capital, and labor but could create some safety issues including mold growth (Arnau et al., 2007). Battilani et al. (2007) reported that *Penicillium nordicum* are commonly present in ham manufacturing plants and air, and ham surface contamination proved to be greater in the ripening rooms, where higher temperatures were recorded (Battilani et al., 2007).

The occurrence of OTA in ham was reported in several studies in the recent literature. An Italian survey conducted on 106 fresh pork and pork meat products reported that OTA contamination, among the samples analyzed, is a phenomenon that particularly concerns dry-cured ham. Levels of OTA > 1.0 μg/kg, ranging from 1.03 to 28.42 μg/kg, were found in five out of 30 dry-cured ham (Pietri et al., 2006). In a study performed on 410 samples of Croatian

traditional pork meat products, OTA concentrations found in the fermented sausages and hams were around five to 10 times higher than the maximal recommended level (Pleadin et al., 2015). In another study, the maximum contamination level in ham samples was 2.3 μg/kg (Chiavaro et al., 2002).

Dall'Asta et al. (2010) and Bertuzzi et al. (2013) evaluated the direct and indirect contamination routes in different pork products. Both studies reported significant direct contamination in dry-cured hams. OTA contamination in this matrix is probably due to the environmental conditions in ripening plants, where *Penicillium* mold could contaminate ham and produce OTA (Dall'Asta et al., 2010), and due to the long curing time and to the fact that ham is not protected by casing (Bertuzzi et al., 2013). Unlike in the present study, OTA contamination was reported in other pork meat products (Altafini et al., 2019; Kudumija et al., 2020; Merla et al., 2018; Roncada et al., 2020). In a study conducted in Belgium, an LC–MS/MS method was developed for the quantification of OTA in meat products and applied to kidney, liver, and black sausages. The data showed OTA contamination in kidney samples with mean levels of 0.22 ± 0.25 μg/kg. In addition, in this work the dietary exposure of OTA of consumers was estimated (Tangni et al., 2021).

4 | CONCLUSIONS

The latest EFSA report on the contamination and risk assessment of OTA in food emphasized preserved meet and cheese matrices as sources of dietary exposure to OTA for the whole population and especially for the infant and prepubertal population. Therefore, the purpose of this work was to investigate the presence of OTA in cheese and pork meat products, based on data from official controls. In particular, the validated method, starting from an already existing one with some modifications, proved to be suitable for the routine analysis of different products belonging to these two types of matrices.

Furthermore, the simple and accurate method proved to be suitable for the confirmatory and quantitative analysis of OTA. Despite the small number of samples per type, preliminary data from the analysis of 75 samples highlighted that occurrence of OTA is quite low and occasional in the matrices considered. However, the high level found in a sample of raw ham and the detection of mycotoxin in concentrations above our LOQ (1 μg/kg) in grated cheeses posed a possible risk to the health of consumers, given their high consumption in the daily diet. Accordingly, the data reported could help in further understanding the matrices belonging to these two commodities most commonly subject to possible OTA contamination. It is thus necessary to increase the number of samples to be analyzed in order to implement the statistics and possibly to draw further conclusions. Furthermore, it would be desirable to investigate distribution of OTA within the matrix.

These monitoring data could be useful crucially for a more accurate risk assessment and further and dedicated studies in this area should be developed, especially to assess exposure and consequent potential risk in vulnerable populations.

AUTHOR CONTRIBUTIONS

Daniela Delfino: data curation, investigation; methodology; validation; writing – original draft. **Dario Lucchetti**: data curation; investigation; methodology; validation; writing – original draft. **Tabita Mauti**: investigation; validation. **Marta Mancuso**: investigation; visualization; **Katia Russo**: conceptualization; project administration; writing – review & editing.

ACKNOWLEDGMENTS

We thank the Italian Ministry of Health for research support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Delfino, D., Lucchetti, D., Mauti, T., Mancuso, M., Di Giustino, P., Triolone, D., Vaccari, S., Bonanni, R. C., Neri, B., & Russo, K. (2022). Investigation of ochratoxin A in commercial cheeses and pork meat products by liquid chromatography–tandem mass spectrometry. *Journal of Food Science*, *87,* 4465–4475. https://doi.org/10.1111/1750-3841.16326