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Determination of ochratoxin A in dry-cured meat products by a HPLC–FLD quantitative method

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Abstract

A fast and sensitive method for the quantification of the mycotoxin ochratoxin A (OTA) in dry-cured meat products has been developed, which does not require a clean-up step, by HPLC with an alkaline mobile phase (pH 9.8). Validation procedures for specificity, trueness, ruggedness, stability, recovery and repeatability were performed. The decision limit (CCα) and the decision capability (CCβ) were calculated at 1.10 and 1.23 μg/kg, respectively. The procedure was applied to representative dehydration levels of dry-cured meat samples.

Keywords: Dry-cured meat products; Ochratoxin A; Validation

1. Introduction

The growth of moulds on the surface of dry-cured meat products during the ripening phases is generally appreciated because their enzymatic activities contribute to the development of the characteristic flavour of these products [1,2]. *Penicillium* spp. and *Aspergillus* spp. are common contaminants of dry-cured meat products [3–6]; within these species, some strains produce mycotoxins in suitable environment and substrate conditions, although it is known that requirements for toxin production are usually more restricted than those for mould growth.

Ochratoxin A (OTA) is a hepatotoxic, immunosuppressive, teratogenic, nephratoxic and nephrocarcinogenic mycotoxin and has been classified as a possible carcinogen to human (Group 2B) by the IARC [7]. Recently, evidences in renal-specific toxicity, epigenetic and genotoxic mechanisms have been reported [8]. OTA has been detected in food and feeds including meat and meat products [9–12]. Its occurrence in meat and meat products can be ascribed to an indirect transmission via the ingestion of OTA-contaminated feed by pigs [13,14] or to direct contamination due to mould growth in the outer layers of meat products. Processing procedures such as ripening have been proved to be ineffective for OTA reduction in meat products [15,16], while frying or boiling could decrease the content of OTA in meat [17].

Whereas several validated official analytical methods for OTA detection have been published for food matrices other than meat, in case of meat products a limited number of analytical procedures are available. The extraction of OTA from meat is commonly performed with acidified chlorinated solvents or ethyl acetate, acetonitrile or mixtures of methanol–sodium bicarbonate [11,12]. OTA extraction from a very proteinaceous food is a critical step for the analysis, due to the high affinity of the analyte for protein [18]. The extraction is commonly followed by a clean-up and concentration procedure by means of immunoaffinity columns [19,20]; detection and quantification are achieved via RP-HPLC–FLD analysis using an acidic mobile phase [11,12,21]. More recently, a chromatographic analysis performed with an alkaline buffer was reported by Dall'Asta et al. [22], achieving a sharp enhancement of OTA fluorescence and allowing the detection of very low mycotoxin amounts.

In this paper, we report a validated, quantitative and confirmatory HPLC–FLD method for the detection of ochratoxin A in dry-cured meat products, based on a first extraction step, a second back-extraction step, followed by a chromatographic analysis using an alkaline mobile phase without any previous clean-up or concentration step. The work was performed

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according to the latest EU criteria for the analysis of residues and contaminants in animal products [23].

2. Experimental

2.1. Chemicals and reagents

Ochratoxin A (solid standard 1 mg), bovine pancreas carboxypeptidase A (50 units/mg protein) and Trizma® base were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents used (LC grade) were obtained from Carlo Erba (Milan, Italy); double distilled water was produced in our laboratory using an Alpha-Q system from Millipore (Marlborough, MA, USA). The 33% ammonia solution and 85% o-phosphoric acid were from Riedel-de-Haen (Seelze, Germany).

α-Ochratoxin (OTα) was prepared by enzymatic hydrolysis using carboxypeptidase A (EC 3.4.17.1) [24]; 100 μl of carboxypeptidase A in 0.04 M Tris–HCl buffer pH 7.5 (50 units/mg protien) were added to an aliquot of 1.5 ml of standard OTA (50 μg/l) in 0.04 M Tris–HCl buffer pH 7.5 in 1 M NaCl. The mixture was incubated for 3 h at 37 °C.

2.2. Standard solutions

The OTA standard solution (200 μg/ml) was obtained by dissolving the solid standard, in toluene–acetic acid (99:1, v/v) and stored at −20 °C. The concentration was checked with a Jasco V550 spectrophotometer calibrated according to AOAC International methods [25]. Further dilutions were prepared in LC grade methanol.

2.3. Sample preparation procedure

In the validation study, 50 independent blank samples of dry-cured pork meat were used. After fat elimination, muscles were minced ($\varnothing_{\text{particles}} \sim 1$ mm) and stored under vacuum at −20 °C. An aliquot of 10 g was extracted with a solution of chloroform (100 ml) acidified with 85% *o*-phosphoric acid solution (0.75 ml) by homogenising the sample with an Ultraturrax (T50 basic IKA®-WERKE, Staufen, Germany) for 2 min. After filtration, an aliquot of the extract (60 ml) was transferred into a separating funnel and extracted twice with 5 ml of buffer 0.2 M Tris–HCl pH 8.5. The upper aqueous phases were carefully collected and mixed. To avoid the growth of OTA-degrading microorganisms in the aqueous phase, a volume of CH_3CN was added to achieve a 0.2 M Tris–HCl: CH_3CN (90:10, v/v) ratio. An aliquot (20 μl) of this solution was analysed by HPLC. According to the stability test (see Table 2 in Section 3), the extracts can be frozen or refrigerated in the dark and analysed within 4 weeks.

2.4. HPLC–FLD conditions

The HPLC analysis was performed using a C18 column (Waters XTerra®, 250 mm × 2.1 mm, 3 μm) on a Waters Alliance 2695 chromatographic system under isocratic conditions at room temperature, with an aqueous NH_3/NH_4Cl (20 mM, pH 9.8):CH_3CN (85:15, v/v) mobile phase; the flow was 0.2 ml/min and the injected volume was 20 μl.

The FLD detection was obtained by means of a Waters 474 Scanning Fluorescence Detector ($\lambda_{ex} = 380$ nm, $\lambda_{em} = 440$ nm; gain = ×100; attenuation = 32; band width = 40 nm). The retention time of the analyte was 20 times the retention time corresponding to the void volume of the column.

No chemical compound was regarded as suitable to be used as internal standard for the ochratoxin A extraction.

2.5. Validation study

An allowed limit of 1 μg/kg (1 ppb) OTA in pork meat and derived products was established by the Italian Ministry of Health since 1999 [26]. A revision of the OTA limit in meat products is expected, according to the risk assessment updating [27]. The validation procedure was performed taking into account the value of 1 μg/kg OTA.

Specificity, ruggedness, stability, recovery, trueness, repeatability, within-laboratory reproducibility, decision limit (CCα), detection capability (CCβ) of the method were calculated. The precision was calculated in terms of within-laboratory coefficient of variation for the repeated analysis of fortified blank samples.

The minimum detectable value or limit of detection (LOD) was estimated from a calibration curve, according to the equation: $\text{LOD} = 3(s_B^2 + s_i^2 + (i/m)^2 s_m)^{1/2}/m$ [28] where m is the slope of the calibration curve, i is the intercept term, s_B, s_i and s_m are the standard errors of the blank response, the intercept term and the slope of the calibration curve, respectively.

Assuming a normal distribution of the estimated quantities, α (error of the first type, i.e. the probability of false negative) = β (error of the second type, i.e. the probability of false negative) = 0.05, the quantification limit was LOQ = 3.04 LOD [29].

The linearity of the analytical response was checked by injecting the calibration standards dissolved in the injection solvent (0.2 M Tris–HCl:CH_3CN = 90:10) over the range 0.5–4.0 μg/kg, using five concentration levels (0.5, 1.0, 1.5, 2.0, 4.0 μg/kg). Using linear regression, the equation for the line which best fits the calibration data was calculated; the calibration curve was used when the correlation coefficient was equal or greater than 0.995. The linearity of the response was checked by means of the relative standard deviation of the average response factor [30]; the response factor for each standard was calculated as the ratio of the peak area to the corresponding analyte concentration. The average response factor is the mean of the response factors of all standards and by assuming a linear response, the relative standard deviation of the average response factor should be less than 10%. The linearity of the method was checked by spiking pooled blank minced samples over the range 0.5–10 μg/kg, using five addition levels (0.5, 1.0, 1.5, 5.0, 10.0 μg/kg) and the regression curve was calculated ($R^2 = 0.991$).

Recovery, repeatability and within-laboratory reproducibility were carried out at three concentration levels, spiking blank minced samples of dry-cured pork meat at 0.5, 1.0 and 1.5 times the current Italian allowed limit (1 μg/kg). Blanks were obtained

by mixing the outer and the inner part of dry-cured pork meat samples previously tested and found negative for OTA.

The spiked samples were prepared by mixing a 10 g aliquot of blank minced sample with 100 μl of OTA standard solution 0.2 M Tris–HCl pH 8.5 at the concentration of 50, 100 and 150 μg/l. After the addition, the samples were allowed to dry for 30 min at room temperature before carrying out the extraction.

2.5.1. Recovery

The recovery of the whole analytical procedure was carried out on three different days by preparing six minced blank sample/day of dry-cured pork meat spiked at 0.5, 1.0 and 1.5 times the reference value (1 ppb). Recovery was calculated as the % mean recovery from the six results at each level (18 samples at all).

2.5.2. Trueness

No certified reference material was available for the trueness assessment of OTA analysis in dry-cured meat products. Repeatability and reproducibility data corrected with the mean recovery were used for trueness determination; trueness (%) was computed as equal to: mean (recovery corrected) concentration of added known amount × 100/added amount.

2.5.3. Repeatability (within-laboratory precision)

Six blank minced samples of dry-cured pork meat were spiked at each of the three fortification levels and analysed three times by the same operator using the same equipment over a 3-week period. Repeatability was given as the mean of the concentrations for three fortification levels determined by the same operator in three different times and the relative standard deviation was computed as %RSD = (standard deviation/mean concentration) × 100.

2.5.4. Reproducibility (within-laboratory reproducibility)

Six blank minced samples of dry-cured pork meat were spiked at each of the three fortification levels and analysed by three operators using the same equipment over a 2-month period. Reproducibility was given as the mean of the concentration of the three fortification levels determined by three operators on the three addition levels and the relative standard deviation was computed as %RSD = (standard deviation/mean concentration) × 100.

2.5.5. Stability

Stability of 1 μg/kg of OTA in 0.2 M Tris–HCl, CH_3CN solution (10%, v/v; the same used in the final analysis for OTA samples and standards) was determined. The freshly prepared solution was analysed, then 40 aliquots were prepared and stored in the dark (at three different temperatures −20 °C, + 4 °C, +20 °C) and in the light (+20 °C). Samples were analysed after 1, 2, 3 and 4 weeks. Stability was computed as (concentration of remaining analyte/concentration of fresh analyte) × 100.

2.5.6. Specificity

The power of discrimination of the method between OTA and the analogues ochratoxin α (OTα) and ochratoxin B (OTB) was checked. A blank minced sample dry-cured pork meat was spiked with an ochratoxin A standard (3 μg/kg) and extracted as described before. A 100 μl OTB standard solution, 200 μg/l in 0.2 M Tris–HCl:CH_3CN (90:10, v/v) and 100 μl OTα solution generated by enzymatic hydrolysis of 50 μg/l of OTA with carboxypeptidase A [24] were added to an aliquot (1.5 ml) of OTA spiked sample extract.

Specificity of the method was assessed by checking that the chromatographic conditions allowed OTA peak to be fully separated from signals corresponding to OTB and OTα. The whole procedure was made in triplicate.

2.5.7. Decision limit (CCα)

Decision limit was estimated by spiking 20 blank minced samples of dry-cured pork meat at the current limit taken as reference value (1 μg/kg). The concentration at this limit plus 1.64 times the corresponding standard deviation equals the decision limit ($\alpha = 5\%$).

2.5.8. Decision capability (CCβ)

Decision capability was estimated by spiking 20 blank minced samples of dry-cured pork meat at the corresponding CCα level. The value of the decision limit plus 1.64 times the corresponding standard deviation equals the decision capability ($\beta = 5\%$).

2.5.9. Ruggedness (minor changes)

The effects of eluent pH variation (HPLC–FLD analysis) and sampling position in dry-cured pork meat products were investigated in order to evaluate their possible influence on OTA analysis. Two samples were taken from dry outer layer and wet core of dry-cured pork muscle, spiked at 1.5 μg/kg and extracted as previously described. At the same time, two buffer pH values corresponding to 9.60 and 10.0 were used in the chromatographic analysis. Each single condition of the assays is shown in the scheme below.

Nominal value	Assayed condition
A	Sample taken from the dry outer layer of dry-cured meat sample
a	Sample taken from the wet core of dry-cured meat sample
B	pH of eluent = 10
b	pH of eluent = 9.6

This results in 2^2 possible combinations, i.e. AB, Ab, aB, ab. The differences of the averages ($D_a = \Sigma A_i - \Sigma a_i$ and $D_b = \Sigma B_i - \Sigma b_i$) were used to compute the standard deviation (SD_i) of the differences [23]. If a factor has an effect, the difference will be larger than the differences of the other factor. If SD_i is smaller than the standard deviation of the within-laboratory reproducibility, the method is regarded as sufficiently robust against the chosen modifications of the selected factors.

2.6. Confirmation by mean of the carboxypeptidase A method

Four blank samples of dry-cured pork meat were spiked at 3 μg/kg and extracted in 0.2 M Tris–HCl pH 8.5; then, the extracts were adjusted at pH 7.5 and divided into two aliquots (1.4 and 1.5 ml respectively). Hundred microliters

of the carboxypeptidase A was added to a 1.4 ml fraction [24]. Both aliquots were incubated at 37 °C for 3 h and analysed by HPLC–FLD. Percent degradation of the OTA peak was calculated and the onset of OTα signal was monitored.

2.7. Analysis of dry-cured meat products from the market

Five dry-cured bone-in hams and five dry-cured, smoked, bone-out hams were purchased in supermarkets in Italy. Samples for OTA analysis were taken from the muscle part, uncovered by skin. From the centre section, a large slice (10 cm thick) was cut. The upper, 1.0 cm thick meat layer was regarded as the outer part, while the deeper layer was the inner part. OTA extraction and analysis were made as described above.

3. Results and discussion

3.1. General considerations on the method

According to the chemical structure of ochratoxin A, it is possible to extract quantitatively the protonated molecule in acidic organic solvents or, when both the carboxylic ($pK_{a_1} = 4.4$) and the phenolic ($pK_{a_2} = 7.1$) moieties are deprotonated in alkaline buffers with low–medium ionic strength (Fig. 1). Moreover, the increased OTA conjugation of the phenate moiety induces a fluorescence enhancement thus improving the sensitivity of the method [22].

The fast method developed for OTA determination here proposed relies on the above-mentioned molecular properties.

The extraction step is a critical point of the method, because is highly influenced by the food matrix. The extraction procedure, adapted from previous procedures used in meat products [19], entails the use of a rather high sample amount (10 g) and of a proper volume of extracting solvent (100 ml of acidic chloroform). The aim of the method was to reduce the number of individual steps while still achieving detection of OTA below 1 μg/kg (Fig. 2). After the first extraction–filtration step, the procedure involves a back-extraction in a small volume of alkaline buffer, in order to get a concentration factor of 1. Filtered samples were directly injected in HPLC system, without a clean-up or other concentration procedure of the sample. Immunoaffinity columns are by the far the most common method for sample clean-up before HPLC analysis, with the aim of cleaning but also of concentrating the sample; however they are quite expensive, time consuming and may be a further source of variation in analyte recovery, due to differences in IAC brand, batch and loaded antigen amount [31]. In our case, as a consequence of the clean-up step removal, the variations in analyte recovery could come from the dry-cured meat composition, extraction/back-extraction steps and slight changes in the eluent composition (μg/kg). The use of an alkaline mobile phase, which is responsible for a 10-fold enhancement of the native ochratoxin A fluorescence, allowed the analyte preconcentration step to be avoided [22]. Under the adopted conditions, OTA eluted in 20 min (Fig. 2); this retention time allowed the specificity requirements of the method to be met and the OTA peak to be fully separated from other substances.

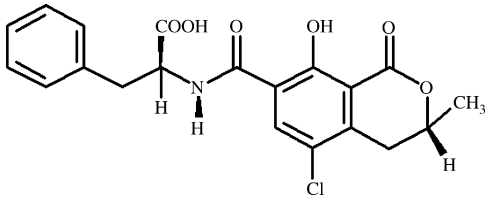

Fig. 1. Chemical structure of ochratoxin A.

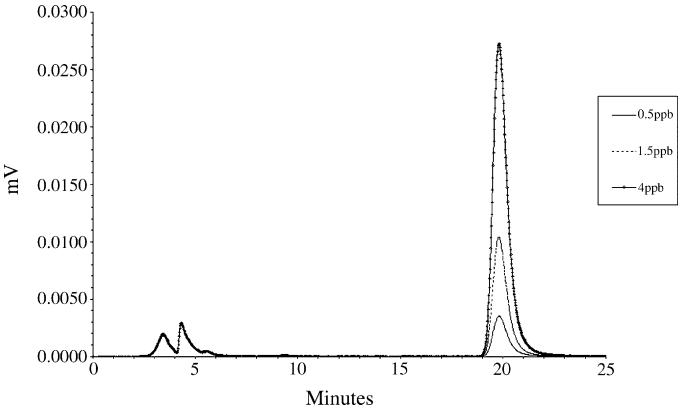

Fig. 2. Chromatogram of an OTA standard solution in 0.2 M Tris–HCl pH 9.8.

Linearity of the analytical response was checked over the range 0.5–4 μg/kg of the calibration curve. Only calibration curves with a correlation coefficient higher than 0.99 and relative standard deviation of the average response factor lower than 15% were used.

LOD and LOQ were calculated from the calibration curve (analyte response) and the chromatographic noise of a blank sample extract (see Section 2) and estimated as 0.02 ng/ml and 0.06 ng/ml, respectively.

The whole analysis, including sample preparation, can be carried out in one and a half hour.

3.2. Validation study

In order to validate the method according to the requirements reported in [23], the following performance parameters were determined: specificity, ruggedness, trueness, stability, recovery, repeatability, within-laboratory reproducibility, decision limit and decision capability. The validation study was carried out with reference to the current limit allowed for OTA in meat and meat products in Italy [26].

Several dry-cured pork meat samples of different origins were analysed to verify the absence of the target analyte and potential interfering compounds; then, 50 blank samples were pooled and used for the validation study. The results are summarised in Table 1. The trueness assessment of the measurements was performed by measuring the recovery data of the added amounts of OTA to the blank matrix. Also in the case of the lowest OTA addi-

Table 1
Validation of the RP-HPLC–FLD method for the determination of ochratoxin A (OTA) in dry-cured pork meat according to [23]

	Spike level (μg/kg)		
Parameter	0.5	1.0	1.5
Recovery (%)	90.5	71.1	67.2
Repeatability conditions			
Mean concentration[a] ± SD	0.43 ± 0.08	0.98 ± 0.06	1.41 ± 0.10
RSD (%)	18.6	6.12	7.09
Trueness[b] (%)	86	98	94
Within-laboratory reproducibility conditions			
Mean concentration[c] ± SD	0.38 ± 0.09	0.96 ± 0.07	1.50 ± 0.16
RSD[c] (%)	23.7	7.29	10.7
Trueness[d]	76	96	100
CCα (ng/ml)		1.10	
CCβ (ng/ml)		1.23	

[a] Mean concentrations (recovery corrected) for three fortification levels determined by the same operator in three different trials.
[b] Determination of trueness under repeatability conditions.
[c] Mean concentrations (recovery corrected) for the three fortification levels determined by three operators.
[d] Determination of trueness under within-laboratory reproducibility conditions.

tion (0.5 μg/kg), the trueness of the method is acceptable because the requirements for the minimum trueness of quantitative methods were met, with reference to mass fraction ≤ 1 μg/kg [23].

The LOD and LOQ of the method have been supplemented by the decision limit and the detection capability. The decision limit is defined as "the concentration at and above which it can be concluded with an error probability of α ($\alpha = 5\%$) that a sample is non-compliant or with statistical certainty of $1 - \alpha$ that the identified analyte content is truly above the permitted limit".

CCα was determined by analysing 20 blank dry-cured pork minced samples fortified with an OTA solution at 1 μg/kg and reported in Table 1. The chromatograms of a blank and of a dry-cured meat sample fortified at 2 μg/kg are compared in Fig. 3.

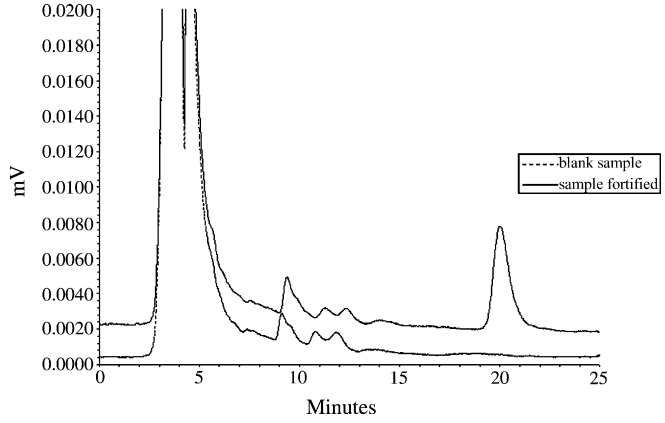

Fig. 3. HPLC elution profiles of a blank and of a dry-cured meat spiked sample (2 μg/kg).

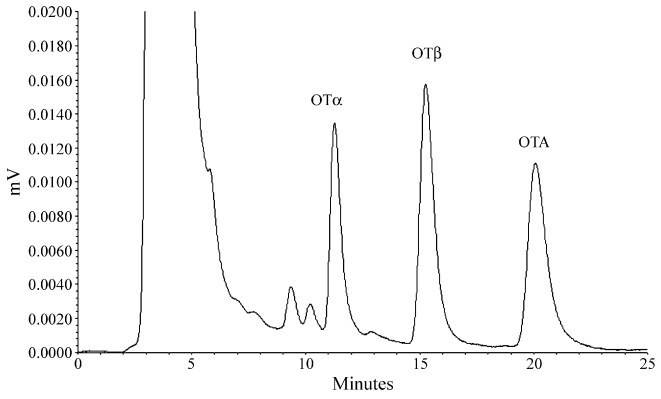

Fig. 4. HPLC separation profile of a dry-cured meat sample spiked with OTA (3 μg/kg), after addition of OTB (final concentration ~12 μg/kg) and OTα formed by enzymatic hydrolysis of 50 μg/kg of OTA.

The detection capability is defined as "the concentration at which the method is able to detect the permitted limit with a statistical certainty of $1 - \beta$ ($\beta = 5\%$)". CCβ was calculated by analysing 20 blank dry-cured pork minced samples spiked at the CCα level and is reported in Table 1.

The specificity of the method was checked for the OTA analogue OTB, generated by OTA dechlorination [32], and for OTα, which may be eventually produced by microbial enzymatic degradation of ochratoxin A [33]. The HPLC–FLD profile of OTA, OTB and OTα is shown in Fig. 4, where it is possible to observe that under the chromatographic conditions adopted, the analytes were fully separated, being the retention times for OTα, OTB and OTA 11.5, 15.5 and 20 min, respectively.

The stability of OTA solutions [1 μg/l in Tris–HCl buffer (0.2 M, pH 8.5):CH_3CN (90:10, v/v)] up to 4 weeks in different storage conditions was investigated. The analyte percentages at different times and conditions are reported in Table 2. As shown in Table 2, the stability of OTA solution is strongly affected by the light, while it is independent from the temperature (from refrigeration to room temperature).

The ruggedness (minor changes) of the method was tested relatively to the variations in sample dryness, mainly due to sampling position (outer layer or inner core of the product) or to different ageing times, and to eventual modifications of the pH of the alkaline mobile phase used in HPLC analysis. The changes of the selected factors are consistent with the dehydration degree of the dry-cured meat products and with slight variations of the buffer pH occurring in laboratory analyses. Ruggedness testing of the method relatively to the assayed changes is reported in Table 3.

Table 2
Stability of an OTA solution (1 μg/l) in 0.2 M Tris–HCl buffer, pH 8.5:CH_3CN = 90:10 (v/v), under the conditions required in [23]

	Dark (−20 °C)	Dark (+4 °C)	Dark (+20 °C)	Light (+20 °C)
1 Week	99.6	99.8	100.1	<LOD
2 Week	99.6	99.7	100.2	<LOD
3 Week	100.5	99.9	100.3	<LOD
4 Week	100.0	99.9	99.9	<LOD

Table 3
Ruggedness testing of the method of determination of OTA in dry-cured pork meat by RP-HPLC–FLD (minor changes)

	Effect of sampling position	Effect of the pH of the mobile phase
Average OTA (A, B)[a] (μg/kg)	0.698	0.614
Average OTA (a, b)[b] (μg/kg)	0.718	0.802
Differences (D_a, D_b)	0.020	0.188
SD of the differences	0.38	

Calculations for ruggedness testing were made according to [23], p. 34. See the 'Section 2 for other details.

[a] Concentration of OTA computed as the average of the values found when the analytical procedure has been applied to very dry portions of pork meat sample (factor A) and eluent pH = 10 (factor B).

[b] Concentration of OTA computed as the average of the values found when the analytical procedure has been applied to wet portions of pork meat sample (factor a) and eluent pH 9.6 (factor b).

A negligible effect was observed as a consequence of the variation in moisture of the dry-cured meat samples. Moreover, the calculated OTA concentration was affected by the ±0.2 unit shift of the pH of the alkaline mobile phase: this strong effect was due to the sensitivity of OTA natural fluorescence to the pH of the eluent [22].

The standard deviation of the differences reported in Table 3 is higher than the standard deviation of the method carried out under within-laboratory reproducibility conditions (Table 1), and the method is not robust against the assayed modification of the pH of the alkaline mobile phase. In conclusion, the method can be applied to wet and dry meat matrices, but the HPLC mobile phase must be carefully buffered at pH 9.8.

3.3. Applicability

Different methods are available to confirm the presence of OTA in food. The method based on the quantitative esterification of the OTA carboxyl group didn't give good results in animal tissue, because it requires clean-up of the sample prior to chemical derivatization [34] and has been regarded as not efficient for the confirmation of low levels of OTA [35]. Another method used to confirm the OTA identity is the enzymatic conversion of OTA into OTα as a result of the cleavage of the amidic bond by carboxypeptidase A [24,36]. In the present study, we applied the method based on the enzymatic conversion of OTA into OTα because this confirmation technique has been regarded as suitable for a meat sample extract obtained without a clean-up step. The degradation of the OTA peak in the extracts of dry-cured pork meat samples spiked at 3 μg/kg in the presence of carboxypeptidase A was 98%, after an incubation step at 37 °C for 3 h.

3.4. Results on commercial samples

The method was applied to 10 dry-cured pork meat products, 5 bone-in dry-cured hams and 5 bone-out smoked hams at the end of the ripening period, analysing separately the outer (10 mm thickness) and the inner parts of the products. Results are reported in Table 4.

Table 4
OTA content detected in the outer part (10-mm thickness) and in the inner sections of dry-cured meat products taken from the market

sample	OTA[a] (μg/kg) inner part	OTA[a] (μg/kg) outer part
Dry-cured ham 1	<LOD	<LOD
Dry-cured ham 2	0.28	0.63
Dry-cured ham 3	<LOD	<LOD
Dry-cured ham 4	<LOD	0.11
Dry-cured ham 5	1.52	7.28
Smoked ham 1	<LOD	<LOD
Smoked ham 2	<LOD	<LOD
Smoked ham 3	<LOD	<LOD
Smoked ham 4	<LOD	6.20
Smoked ham 5	<LOD	5.20

[a] Results were recovery-corrected; LOD = 0.02 ng/ml, LOQ = 0.06 ng/ml.

The OTA detected amounts were higher than LOQ in two inner samples (0.28 and 1.52 μg/kg) and in five outer samples (0.11–7.28 μg/kg), according to few studies carried out on dry-cured meat products; [9,11,12,37] OTA contamination was ascribed to indirect transmission from animals exposed to contaminated feed [11], whereas by some others a direct contamination was supposed, due to the presence of OTA-producing mould strains in the air of ripening rooms or on products surface [9,12,37]. The results obtained for both outer and inner layers of dry-cured and smoked hams showed a higher occurrence of OTA levels exceeding CCβ in the outer layers than in inner samples.

These results are in agreement with a possible direct contamination by OTA-producing mould strains growing on the ham outer part. The HPLC profile of a naturally contaminated dry-cured ham (outer layer) is reported in Fig. 5. It is possible to observe that, besides the peak corresponding to OTA at t_R = 20 min, an other peak corresponding to OTB (t_R = 15 min) is present, as reported in previous works on meat products [9].

The application of this rapid and simple method to several samples, representative of different dry-cured pork meat products, may be a tool to perform an extensive safety evaluation of pork meat products and to obtain more informations about the biological pathway of ochratoxin production in this type of pork meat derivatives.

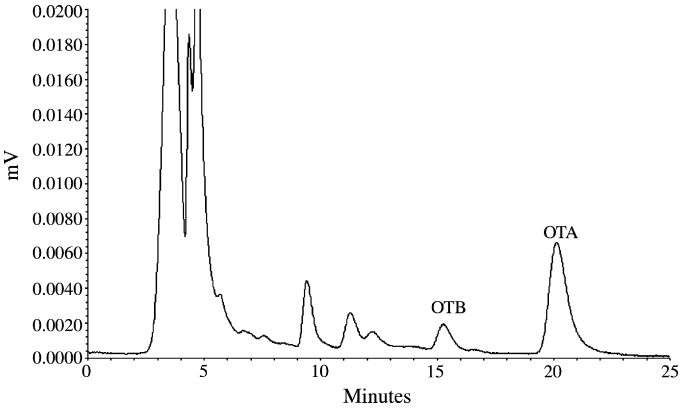

Fig. 5. Chromatogram of naturally contaminated dry-cured ham.

4. Conclusions

A quantitative method has been developed for the determination of OTA contamination in dry-cured meat products at levels below 1 μg/kg, i.e. the current permitted limit in meat and meat products in Italy. The present method does not require any clean-up or concentration step, thanks to the 10-fold OTA fluorescence enhancement obtained by using the alkaline eluent in HPLC. A further advantage is that the final extracts are clean and the HPLC chromatograms show no interferences with OTA derivatives eventually present in cured and fermented meat products like OTB and OTα. The method has been validated according to EU criteria for the confirmatory methods for organic residues and contaminants, and successfully applied in routine quality control for the OTA presence in dry-cured meat products.

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