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Original Scientific Paper

Cyclopiazonic acid in meat products originating from different production practices

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ABSTRACT

The consumption of dry-fermented and dry-cured meat products is widespread, primarily due to their desirable sensory characteristics and nutritional value. However, the intake of these products may pose health risks associated with contamination by mycotoxins. One of the mycotoxins that can be present in meat products is cyclopiazonic acid (CPA). The present study aimed to compare the occurrence of CPA in the same types of meat products originating from different production practices. Traditional and industrial practices are characterized by different production conditions, including temperature, humidity, and seasonal variations, which, in traditional production, are often not strictly controlled. Therefore, comparing mycotoxin contamination in meat products with regard to production practices is important for food safety. The results revealed a notable difference in CPA prevalence, with a markedly higher occurrence in traditionally produced meat products (21.7%) compared to those from industrial production (2.9%). Among the traditional products, CPA levels ranged from 6.1 µg/kg to 335.5 µg/kg. In contrast, CPA was detected in only two industrial meat products, with levels of 2.7 µg/kg in a sausage and 17.6 µg/kg in a *Pečenica*. These findings suggest that industrially produced meat products are generally safer for consumers with respect to CPA contamination. Nevertheless, despite the low incidence observed in industrial products, ongoing surveillance is necessary, particularly given the current lack of established regulatory limits for CPA in all foodstuffs.

1. Introduction

Cyclopiazonic acid (CPA) is a mycotoxin produced by various moulds belonging to the genera *Aspergillus* and *Penicillium*. Numerous studies have confirmed that *Aspergillus flavus*, *Aspergillus tamarii*, and *Aspergillus versicolor* are capable of synthesizing this toxin as well as *Penicillium griseofulvum, Penicillium camemberti* and *Penicillium commune* (*Burdock and Flamm*, 2000; *Zadravec et al.*, 2023). Historically, CPA has been associated with cases of poisoning in both humans and animals in India, where contaminated millet was identified as the source, and strains of *A. flavus* and *A. tamarii* were isolated through microbiological analysis. CPA belongs to the category of potentially dangerous mycotoxins that can cause damage to the liver, the spleen, the pancreas, the kidneys, the salivary glands, the myocardium and the skeletal muscles. Toxic effects of CPA are most pronounced in the digestive organs, the muscles, the liver and the spleen, although the symptoms vary depending on animal breed, age and sex, mycotoxin uptake, dose and duration of exposure (*Ostry et al.*, 2018).

To date, CPA has been detected in a wide range of plant-based food products, including peanuts,

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corn, figs, rice, wheat and tomatoes, as well as in animal-derived products, such as cheese, milk, and ham (*Oliveira et al.*, 2006; *da Motta and Valente Soares*, 2010; *Fernández Pinto et al*., 2010*; Ostry et al*., 2018; *Peromingo et al*., 2018; *Lešić et al*., 2022; *Andrade et al*., 2025; *Lešić et al*., 2025). Over the past five years, several studies have investigated the presence of CPA in various types of dried meat products, reporting high incidence rates and levels reaching up to 550 µg/kg (*Andrade et al*., 2025). Notably, most of these studies have focused on meat products produced in households, where manufacturing conditions are often unregulated and influenced by seasonal factors (i.e., temperature and relative humidity). In contrast, data on CPA occurrence in industrially produced meat products characterized by standardized and controlled conditions remain limited. Despite industrial production being controlled, including conditions in production facilities, and not being subject to seasonal variations, it is still important to assess the potential contamination of these products for consumer protection. Although significant progress has been made in research on CPA, regulatory limits for its presence in various food products have yet to be established. Given these observations, the present study aims to give an overview of the occurrence of CPA in some types of meat products originating from different production systems and to compare the levels of this mycotoxin in meat products originating from traditional versus industrial production systems.

2. Materials and methods

2.1. Meat products

For one year, the same types of meat products were collected from family households (n = 83) and the commercial market (n = 69). Sampling from family households was conducted at 23 locations, while commercial meat products originated from 14 medium- to large-scale meat processing industries. The collected meat products were dry-fermented sausages, *Kulen*, bacon/pancetta, dry-cured hams, dry rack and *Pečenica/Ombolo/Zarebnjak*, each with an approximate weight of 500 g. The distribution of the meat products by product type and origin is detailed in Table 1 and Table 2. Following collection, samples of the meat products were homogenized using a Grindomix GM 200 laboratory mill (Retsch, Germany) and subsequently stored at -20 °C pending mycotoxin analysis.

2.2. Chemicals and standards

The analytical standard for cyclopiazonic acid (CPA; CAS No. 18172-33-3) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All solvents, including acetic acid, methanol and acetonitrile, were procured from Honeywell (Charlotte, NC, USA). Zinc acetate dihydrate (CAS No. 5970-45-6), potassium hexacyanoferrate (II) trihydrate (CAS No. 14459-95-1) and ammonium acetate (CAS No. 631-61-8) were also sourced from Sigma-Aldrich. QuEChERS extraction packets (4.0 g of $MgSO_4$, 1.0 g of NaCl, 1.0 g of sodium citrate tribasic dihydrate, and 0.5 g of sodium citrate dibasic sesquihydrate) along with syringe filters equipped with PTFE membranes (4 mm diameter, 0.2 µm pore size) were supplied by Phenomenex (Torrance, CA, USA). Captiva EMR–Lipid cartridges (3 mL) used for lipid removal were obtained from Agilent Technologies (Santa Clara, CA, USA). Carrez I solution (10.6 g of potassium hexacyanoferrate (II) trihydrate per 100 mL of deionized water) and Carrez II solution (21.9 g of zinc acetate dihydrate in 32 mL of acetic acid and diluting the solution with distilled water to a final volume of 100 mL.

2.3. Sample preparation

The sample preparation was described earlier by *Vulić et al*. (2021). Briefly, 2 g of homogenized sample was weighed, upon which 5 mL of 25 % acetic acid was added and samples were shaken on a vertical vortex shaker. Afterwards, 5 mL of organic solvent (acetonitrile) was added, and samples were extracted for 30 minutes on a head-over-head shaker (Multi RS-60, Biosan, Riga, Latvia). After the extraction, Carrez solution was added to precipitate proteins and fats, followed by the addition of QuEChERS salts, and samples were centrifuged at 5,000 rpm for 15 minutes at room temperature (Universal 320R, Hettich, Kirchlengern, Germany). Clear extracts obtained after centrifugation were passed through lipid removal columns and then filtered through a PTFE filter directly into a vial.

2.4. Instrumental analysis

A HPLC (1260 series, Agilent Technologies, Santa Clara, CA, USA) consisting of a degasser, a binary pump, an autosampler and a column compartment was coupled with a triple quadrupole mass spectrometer equipped with an electrospray ionization

(ESI) source (6410 QQQ, Agilent Technologies, Santa Clara, CA, USA). The analytical C 18 HPLC (Gemini, 150 × 4.6 mm, 5 µm particle size, 110 Å) was from Phenomenex (Torrance, CA, USA). The instrumental conditions, including chromatographic conditions as well as mass spectrometer set up, were described earlier (*Vulić et al*., 2021; *Lešić et al*., 2022)

2.5. Method validation

The calibration curve spanning from 0.5 to 10 ng/mL showed good linearity with the regression coefficient of R^2=0.998, but also in the 0.5 to 20 ng/mL range (R^2=0.996). The limit of detection (LOD) calculated from the calibration curve was 0.43 ng/mL, which corresponds to a value of 2.17 µg/kg in the meat product matrix given the sample dilution factor of 5. The limit of quantification (LOQ) calculated from LOD was 1.43 ng/mL, which corresponded to 7.15 µg/kg in the meat product matrix. The recovery of the method was assessed at two concentration levels, selected based on the established limits of detection (LOD) and quantification (LOQ). The mean recovery rates were 95.5% at 3 µg/kg and 102.1% at 10 µg/kg.

3. Results and discussion

In this study, CPA was detected in meat products taken from family households with traditional production, often characterized by uncontrolled processing conditions, in all product types except dry-cured ham with an overall occurrence rate of 21.7%. The distribution of traditionally produced meat products and descriptive statistics related to CPA levels are presented in Table 1. Among the traditionally produced meat products, the highest occurrence was observed in dry-cured neck (44.4%), followed by *Kulen* (25.0%), dry-fermented sausages (24.0%), *Pečenica/Ombolo* (20.0%), and bacon/pancetta (10.5%). As previously noted, production conditions such as ripening temperature and relative humidity, along with product-specific properties such as pH and water activity, influence the growth of surface moulds both desirable and undesirable (*Asefa et al*., 2011; *Mediani et al.*, 2022). Furthermore, the production characteristics of each meat product, particularly the duration of the ripening process, also play a critical role in mould proliferation and mycotoxin synthesis. Based on the characteristics of the products, a higher CPA occurrence rate might be expected in dry-cured hams due to their extended ripening period, which can last up to six months. This extended maturation theoretically allows extended period for mould growth and subsequent mycotoxin production, compared to products with shorter production cycles. However, in this study, CPA was not detected in dry-cured hams, which may be attributable to unfavourable environmental conditions for mould development during the production period.

The mean levels of CPA in the analyzed traditionally produced meat products ranged from 6 µg/kg in *Pečenica/Ombolo/Zarebnjak* to 74.1 µg/kg in dry-fermented sausages. The highest individual level of CPA (335.5 µg/kg) was also observed within the dry-fermented sausage category. When compared with previous studies (*Vulić et al*., 2021; *Lešić et al.*, 2021; *Lešić et al*., 2022), the pattern of CPA occurrence was largely consistent across most dry-fermented and dry-cured meat products, except for dry-cured hams.

Table 1. Cyclopiazonic acid level in different types of meat products originating from traditional practice

Origin	CPA	Dry-fermented sausages	*Kulen*	Bacon/ pancetta	Dry-cured ham	Dry rack	*Pečenica/ Ombolo/ Zarebnjak*
Traditional	N	25	16	19	13	9	10
	n+	6	4	2	/	4	2
	n %	24.0	25.0	10.5	/	44.4	20.0
	Min (µg/kg)	5.5	6.5	7.2	na	6.3	6.1
	Max (µg/kg)	335.5	27.3	9.0	na	44.3	6.8.
	Mean of positives (µg/kg) (µg/kg)	74.1	17.7	8.5	na	21.2	6.4
	Median (µg/kg)	26.8	18.6	8.5	na	17.2	6.4

Legend: CPA—cyclopiazonic acid.; n – number of analyzed meat products; n+ - number of meat products above LOQ; n % - percentage of meat products above LOQ; na—not applicable

In particular, Lešić et al. (2025) reported CPA levels in dry-cured hams ranging from 4.3 to 6.2 µg/kg. Similarly, *Peromingo et al.* (2018) detected CPA levels ranging from 36.1 to 540.1 µg/kg. These substantial differences in reported levels may be attributed to variations in production technologies/parameters of dry-cured hams as well as differing environmental conditions between production regions of Spain and Croatia. Notably, CPA was not detected in dry-cured hams in the present study. When considered alongside previous findings, this absence may be ascribed to environmental conditions during the current study period that were less conducive to mould growth and mycotoxin production.

In contrast to the traditionally produced meat products, the overall occurrence rate of CPA in industrially produced meats was notably lower, at just 2.9 %. The distribution of the industrial meat product group by the category and the descriptive statistics for CPA levels are presented in Table 2.

Table 2. Cyclopiazonic acid level in different type of meat products originating from industrial production

Origin	CPA	Dry-fermented sausages	*Kulen*	Bacon/ pancetta	Dry-cured ham	Dry rack	*Pečenica/ Ombolo/ Zarebnjak*
Industry	N	21	9	11	9	9	10
	n+	1	/	/	/	/	1
	n %	4.8	/	/	/	/	10.0
	Min (µg/kg)	2.7	Na	na	na	na	17.6
	Max (µg/kg)	2.7	Na	na	na	na	17.6
	Mean of positives (µg/kg)	2.7	Na	na	na	na	17.6
	Median (µg/kg)	2.7	Na	na	na	na	17.6

Legend: CPA—cyclopiazonic acid.; n – number of analyzed meat products; n+ - number of meat products above LOQ; n % - percentage of meat products above LOQ; na—not applicable

CPA was detected in only two industrial meat products: one dry-fermented sausage with a level of 2.7 µg/kg, and one *Pečenica/Ombolo* with a level of 17.6 µg/kg. These meat products originated from two different producers, indicating that the observed contamination cannot be attributed to poor practices at a single facility.

These findings suggest that, with few exceptions, industrial production is generally characterized by good manufacturing practices with control of crucial parameters, such as temperature and relative humidity. This observation is consistent with our previous study (*Lešić et al*., 2022), which focused specifically on *Kulen* produced using both traditional and industrial practices. Notably, in both the current and previous studies, CPA was not detected in any of the *Kulen* originating from industrial production.

When comparing the incidence of CPA occurrence in the same type of meat product originating from different production practices, it can be observed that traditional practices pose a higher risk of product contamination. As mentioned earlier, the possible reasons include inadequate temperature control, humidity and seasonal variations, as addressed by *Lešić et al*. (2022). Regarding the industrial meat products, the lack of information about potential issues during the production process prevents us concluding anything about the causes of CPA occurrence. Since CPA was detected in only two products from the industrial group, statistical comparison for traditional and industrial products was not possible. Further research, including detailed information about the production process, is necessary to explain the occurrence of CPA in industrial meat products.

The European Union (EU) has established regulatory limits for mycotoxins across various food categories. In the case of meat and meat products, maximum permitted levels (MPLs) for ochratoxin A have been defined by several EU Member States. However, for other mycotoxins, including cyclopiazonic acid (CPA), specific regulatory thresholds have not yet been established. Consequently, the findings of this study cannot be directly assessed against current legislation. Nevertheless, the results provide important data that may inform future risk assessments and contribute to the development of regulatory frameworks.

4. Conclusion

Traditional production of dry-fermented and dry-cured meat products, often conducted under unregulated and seasonally variable conditions, can been associated with the occurrence of CPA, especially at the notably higher concentrations seen. In contrast, industrial production, typically characterized by standardized and controlled processing conditions, exhibited a markedly lower incidence of CPA contamination. Due to the limited number of CPA positive findings in industrial meat products, a direct statistical comparison between traditional and industrial production systems was not feasible. Despite the low occurrence of CPA in industrial products, continued monitoring remains essential, particularly in the absence of established regulatory limits for this mycotoxin.

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References

Andrade, M. J., Delgado, J., Rodríguez, M., Núñez, F., Córdoba, J. J., & Peromingo, B. (2025). Mycotoxins in meat products, *Meat Science*, 227, Article 109850. https://doi.org/10.1016/j.meatsci.2025.109850.

Asefa, D. T., Kure, C. F., Gjerde, R. O., Langsrud, S., Omer, M. K., Nesbakken, T., & Skaar, I. (2011). A HACCP plan for mycotoxigenic hazards associated with dry-cured meat production processes. *Food Control*, 22, 831–837. https://doi.org/10.1016/j.foodcont.2010.09.014.

Burdock, G. A. & Flamm, W. G. (2000). Safety Assessment of the Mycotoxin Cyclopiazonic Acid. *International Journal of Toxicology*, 19(2), 195–218. https://doi.org/10.1080/10915810050074964.

da Motta, S. & Valente Soares, L. M. (2000). Simultaneous determination of tenuazonic and cyclopiazonic acids in tomato products. *Food Chemistry,* 71(1), 111–116. https://doi.org/https://doi.org/10.1016/S0308-8146(00)00040-6.

Fernández Pinto, V., Patriarca, A., Locani, O., & Vaamonde, G. (2001). Natural co-occurrence of aflatoxin and cyclopiazonic acid in peanuts grown in Argentina. *Food Additives & Contaminants,* 18(11), 1017–1020. https://doi.org/10.1080/02652030110057125.

Lešić, T., Pleadin, J., Kudumija, N., Tomašković, D., & Vulić, A. (2025). Mycotoxin Residues in Chicken Breast Muscle and Liver. *Foods,* 14, Article 14122017. https://doi.org/10.3390/foods14122017.

Lešić, T., Vulić, A., Vahčić, N., Šarkanj, B., Hengl, B., Kos, I., Polak, T., Kudumija, N., & Pleadin, J. (2022). The Occurrence of Five Unregulated Mycotoxins Most Important for Traditional Dry-Cured Meat Products. *Toxins*, 14, 14070476. https://doi.org/10.3390/toxins14070476.

Mediani A, H. S. Hamezah, F. A. Jam, N. F. Mahadi, S. X. Y. Chan, E.R. Rohani, N. H. Che Lah, K. Azlan, N. A. Khairul Annuar, N. A. F. Azman, H. Bunawan, M. N. Sarian, N. Kamal & F. Abas (2022). A comprehensive review of drying meat products and the associated effects and changes. *Frontiers in Nutrition*, 9, 1057366. https://doi.org/10.3389/fnut.2022.1057366.

Ostry, V., Toman, J., Grosse, Y., & Malir, F. (2018). Cyclopiazonic acid: 50th anniversary of its discovery. *World Mycotoxin Journal,* 11(1), 135–148. https://doi.org/10.3920/WMJ2017.2243.

Peromingo, B., Rodríguez, M., Núñez, F., Silva, A., & Rodríguez, A. (2018). Sensitive determination of cyclopiazonic acid in dry-cured ham using a QuEChERS method and UHPLC–MS/MS. *Food Chemistry,* 263, 275–282. https://doi.org/https://doi.org/10.1016/j.foodchem.2018.04.126.

Vulić, A., Lešić, T., Kudumija, N., Zadravec, M., Kiš, M., Vahčić, N., & Pleadin, J. (2021). The development of LC-MS/MS method of determination of cyclopiazonic acid in dry-fermented meat products. *Food Control,* 123, 107814. https://doi.org/10.1016/j.foodcont.2020.107814.

Zadravec, M., Lešić, T., Brnić, D., Pleadin, J., Kraak, B., Jakopović, Ž., Perković, I., Vahčić, N. Jaki Tkalec, V., & Houbraken, J. (2023). Regional distribution and diversity of Aspergillus and Penicillium species on Croatian traditional meat products. *International Journal of Food Microbiology,* 406, 1–11. https://doi.org/10.1016/j.ijfoodmicro.2023.110404.

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