ISSN: 1220-3173; E-ISSN: 2457-7618

STUDY OF THE TRANSFER RATE OF AFLATOXIN B1 IN POULTRY MEAT MATURED IN WINE

STUDIUL RATEI DE TRANSFER A AFLATOXINEI B1 ÎN CARNEA DE PASĂREA MATURATĂ ÎN VIN

Ioana Mădălina GEORGESCU[1),*)], N.C. NEGREANU[1)], Elena DUMITRESCU[1)], Veronica Ligia NICOLESCU[1)], O.V. ZVORIȘTEANU[1)], Carmen Daniela PETCU[2),*)], Emilia Ciobotaru PÎRVU[2)]

ABSTRACT

Poultry meat is an important source of nutrients, especially proteins, and is one of the most consumed types of meat in Romania. Due to the high consumption of poultry meat correlated with increasingly modern culinary preparation techniques adapted to international trends, this study had as its main objective the establishment of correlations regarding the rate of transfer of aflatoxin B1 from wine artificially enriched with this mycotoxin in meat from poultry. The study was conducted in an authorised veterinary sanitary and food safety laboratory. To determine the transfer rate of aflatoxin B1 in poultry meat, a set of 6 samples was analysed. These samples were matured in wine enriched with aflatoxin B1 at the level of 10 µg/kg and analysed at the initial time and then at intervals of 7 days and 14 days, respectively. During the entire research period, the samples were kept at a temperature of +4°C. This mycotoxin was chosen for the study because it is proven that it has a toxic effect on the body, being included in toxicity group I by the International Agency for Research on Cancer, substances with a safe carcinogenic effect on the body. Aflatoxin B1 is a mycotoxin produced especially by fungi of the family *Aspergillus flavus* and *Aspergillus parasiticus*, being produced especially under favourable conditions of temperature, humidity, and darkness. The analytical technique used was high-performance liquid chromatography coupled to mass spectrometry (LC-MS/MS), and the results showed a transfer rate of 84.5% at the end of the study of aflatoxin B1 from the wine into the poultry muscle tissue.

Key words: aflatoxin B1, wine, meat, transfer, analyses

REZUMAT

Carnea de pasăre reprezintă o sursă importantă de nutrienți, în special de proteină, fiind unul din tipurile de carne cele mai consumate în România. Datorită consumului ridicat de carne de pasăre, corelat și cu tehnici de preparare culinară din ce în ce mai moderne, adaptate tendințelor internaționale, acest studiu a avut ca obiectiv principal stabilirea de corelații privind rata de transfer a aflatoxinei B1 din vin îmbogățit artificial cu această micotoxină în carnea de pasăre crescută în sistem intensiv. Studiul s-a desfășurat într-un laborator autorizat sanitar veterinar și pentru siguranța alimentelor. Pentru a stabili rata de transfer a aflatoxinei B1 în carnea de pasăre, a fost analizat un set de 6 probe. Aceste probe au fost supuse maturării în vin îmbogățit cu aflatoxina B1 la nivel de 10 µg/kg și analizate la momentul inițial și ulterior la 7 zile și 14 zile. Pe toată perioada de cercetare, probele au fost menținute la temperatura de +4°C. Pentru studiu a fost aleasă această micotoxină deoarece este dovedit faptul că are efect toxic asupra organismului, fiind încadrată în grupa I de toxicitate de către Agenția Internațională de Cercetare a Cancerului, respectiv substanțe cu efect cancerigen sigur asupra organismului. Aflatoxina B1 este o micotoxină produsă în special de fungi din familia *Aspergillus flavus* și *Aspergillus parasiticus.*, fiind produsă în special în condiții favorizante de temperatură, umiditate și întuneric.

Key words: aflatoxin B1, wine, meat, transfer, analyses

Mycotoxins, especially aflatoxin B1 and ochratoxin A, are given special importance in various scientific studies as well as in the legislation in force. The attention paid to the study of these mycotoxins is due to the impact on human health caused by the consumption of foods contaminated with mycotoxins, it being known that aflatoxin B1 causes liver cancer and ochratoxin A causes kidney cancer.Aflatoxin B1 is classified in group I toxicity by the International Agency for Research on Cancer, namely substances with a certain carcinogenic effect on the body (7).

Maximum permitted limits for mycotoxins in wine according to EU Reg. 2023/915 are established only for ochratoxin A and are set at the level of 2µg/kg regardless of the type of wine.

For aflatoxin B1, according to EU Reg. 2023/915, maximum permitted limits are established for a variety of matrices represented by cereals, dried fruits,

1) Sanitary Veterinary and Food Safety Directorate, Bucharest, Romania
2) University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania
*) *Corresponding authors: georgescu.madalina-b@ansvsa.ro, carmen28petcu@gmail.com*

oilseeds, spices, etc. and the value of these limits varies from 2μg/kg to 20μg/kg

For an efficient monitoring of contamination with aflatoxin B1, and not only, at the national level, the Food Safety Surveillance and Control Program is developed, which is approved by Order no. 35/2016 of the President of the National Sanitary,Veterinary and Food Safety Authority, with subsequent amendments and additions. According to this program, which is developed in accordance with the European legislation in force, the type of substances to be monitored (mycotoxins, heavy metals, alkaloids, etc.), the type of sample (including for wine) to be analyzed, as well as the frequency and place of sampling, are provided.

Wine has been consumed since ancient times and lately it has been used in various culinary preparations as the main ingredient in various marinades used to mature different types of meat. Poultry meat consumption is at the top of Romanians' preferences (6) and is correlated with: price accessibility, pleasant organoleptic characteristics, nutritional value, culinary habits and last but not least with the versatility of culinary preparation (1).

MATERIALS AND METHODS

To carry out this study, the analyses were carried out in an authorised sanitary veterinary and food safety laboratory, and the study was carried out on poultry meat artificially contaminated with aflatoxin B1 (2, 4, 5). Thus, for the study, 6 meat samples from intensively reared birds were analysed, and the mycotoxin to be studied was aflatoxin B1. The meat was sliced as if ready to be cooked and placed in a quantity of wine with a known content of aflatoxin B1, respectively a concentration of 10 μg/kg. The meat: wine ratio was 2:1, i.e., 100 g of meat to 50 ml of wine.

The meat samples were analysed using a high-performance technique, namely liquid chromatography coupled with mass spectrometry (LC-MS/MS). This analytical technique is increasingly used because it allows the analysis of a very large range of substances from different classes (mycotoxins, pesticides, antibiotics, food additives, etc.) and brings together two types of separation: the physical separation of compounds, which is achieved by passing the extract through the chromatographic column, and the separation determined by mass spectrometry, which involves the identification of compounds based on their characteristic mass/charge ratio (m/z) (9).

Extraction of aflatoxin B1 from meat samples was performed using a low reagent consumption and increasingly used technique, namely QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction. This extraction is an increasingly used extraction and is, as the acronym suggests, a fast, easy, cheap, efficient, robust, safe method (3, 8).

This extraction involved sample weighing, addition of water and acetonitrile, magnesium sulphate, sodium chloride, intensive homogenisation, centrifugation, supernatant retrieval, filtration, and injection into LC-MS/MS. For the determination of aflatoxin B1 by LC-MS/MS, optimized LC-MS/MS equipment was used for the determination of aflatoxin B1. In this sense, a mixture of aqueous mobile phase (A) and organic mobile phase (B) was used, an optimal gradient to separate the compound of interest and the establishment of the mass/load (m/z) ratio characteristic of aflatoxin B1. Thus, the following parameters were set, as shown in Table 1.

Table 1

Parameters used in liquid chromatography coupled with mass spectrometry (LC-MS/MS)

The compound of interest	Retention Time (min)	Precursor Ion (m/z)	Daughter ion (m/z)	Collision Energy (V)
AB1	8.9	313.05	240.88	38.66
AB1	8.9	313.05	268.88	32.86
AB1	8.9	313.05	269.88	27.99
AB1	8.9	313.05	284.88	23.76

Specific laboratory equipment (analytical balance, homogeniser, centrifuge, and LC-MS/MS equipment) as well as different substances were needed for this study: acetonitrile, methanol, magnesium sulphate, sodium chloride, acetic acid, distilled water, and aflatoxin B1 standard substance. Samples were analysed at time zero, subsequently at 7 days, and at 14 days, accumulating a data set sufficient for statistical processing.

RESULTS AND DISCUSSIONS

The results of the study on the transfer rate of aflatoxin B1 from wine to poultry meat are results obtained strictly under controlled laboratory conditions. Samples analysed at zero moment did not demonstrate aflatoxin B1 contamination. The results obtained after 7 days show an average transfer rate of 65.2%, and after 14 days the results obtained show a transfer rate of 84.1%. The individual results obtained are shown in Table 2.

Table 2

The transfer rate of aflatoxin B1 from wine to poultry meat

Aflatoxin B1	7 days μg/kg	14 days μg/kg
Sample no. 1	6.71	8.71
Sample no. 2	6.85	8.27
Sample no. 3	6.33	8.08
Sample no. 4	6.27	8.34
Sample no. 5	6.59	8.49
Sample no. 6	6.37	8.57
Transfer rate %	65.2%	84.1%

From the statistical processing of the data represented in Table 2, the following emerges:

The results on the 6 series show a very good coefficient of variation, less than 5% both at 7 days and at 14 days, respectively 3.57% (Fig. 1) and 2.69% (Fig. 2).

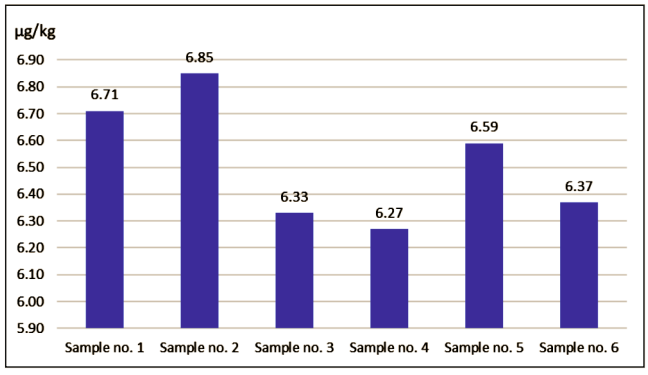

Fig. 1. Aflatoxin B1 transfer rate at 7 days for 6 meat samples from intensively reared birds

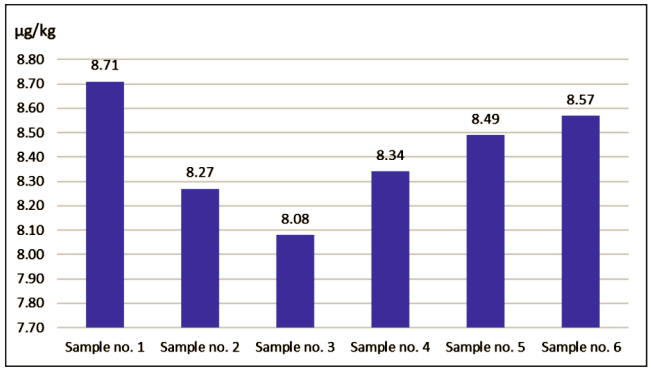

Fig. 2. Aflatoxin B1 transfer rate at 14 days for 6 meat samples from intensively reared birds

The confidence coefficient is very good; respectively, for an accepted level of 95% it is in both cases less than 5% (0.24% at 7 days and 0.23% at 14 days) according to the statistical processing of the data (Table 3).

Table 3
Statistical analysis at 7 and 14 days

	7 days	14 days
Mean	6.52	8.41
Standard Error	0.09504385	0.092267726
Median	6.48	8.415
Standard deviation	0.232808935	0.226008849
Minimum	6.27	8.08
Maximum	6.85	8.71
Confidence level (95.0%)	0.244317993	0.237181741

The study conducted demonstrates that the analysis method is adequate for the intended purpose in the sense that the performance criteria as set out in EU Reg. No. 2023/2782 are respected.

In the event of the presence of aflatoxin B1 in the wine used as a maturation substrate, it should be taken into account that the highest transfer rate of aflatoxin B1 occurs in the first 7 days of maturation.

CONCLUSIONS

Following the study carried out on poultry meat matured in wine contaminated with aflatoxin B1, for 14 days at a temperature of 4C, the following emerges: the highest transfer rate of aflatoxin B1 in poultry meat matured in wine is in the first 7 days of the study of 65.2%; the transfer rate of aflatoxin B1 in poultry meat matured in wine in the 2nd part of the study is 18.9%; the overall average transfer rate at the end of the study period is 84.1%; in the event of the presence of aflatoxin B1 in the wine used as a maturing substrate, it should be taken into account that the highest transfer rate of aflatoxin B1 occurs during the first 7 days of maturing.

As a recommendation, such studies should be continued on other types of meat with the same mycotoxin or other mycotoxin types, as well as in a much more alert dynamic that studies the daily migration rate of mycotoxins from the contaminated substrate (in the present case wine) in different types of meat.

REFERENCES

1. *EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk D., Bignami M., Bodin L., Chipman J.K., del Mazo J., Grasl-Kraupp B., Hogstrand C., Hoogenboom L.R., Leblanc J.C., Nebbia C.S., Nielsen E., Ntzani E., Petersen A., Sand S., Schwerdtle T., Vleminckx C., Marko D., Oswald I.P., Piersma A., Routledge M., Schlatter J., Baert K., Gergelova P., Wallace H.*, (2020), Scientific opinion – Risk assessment of aflatoxins in food. EFSA Journal, 18(3):6040.
2. *FAO/ WHO, Codex Alimentarius*, (2023), General Standard for Contaminants and Toxins in Food and Feed, CXS 193-1995, Available at https://www.fao.org/fao-whocodexalimentarius/sh-proxy/en/?lnk =1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B193-1995%252FCXS_193e.pdf (Accessed: July 28, 2024)
3. *Musarurwa H., Chimuka L., Pakade V.E., Tavengwa N.T.*, (2019). Recent developments and applications of QuEChERS based techniques on food samples during pesticide analysis. J Food Compos Anal, 84: 103314
4. *The European Commission*, (2023), Commission Implementing Regulation (EU) 2023/2782 of 14 December 2023 laying down the methods of sampling and analysis for the control of the levels of mycotoxins in food and repealing Regulation (EC) No 401/ 2006. OJ L, 2023:2782, Available at https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=CELEX:32023R2782 (Accessed: July 28, 2024)
5. *The Council of the European Communities*, (1993), Consolidated text: Council Regulation (EEC) No 315 /93 of 8 February 1993 laying down Community pro-cedures for contaminants in food. OJ L, 37:1, Available at https://eur-lex.europa.eu/legal-content/ EN/TXT/?uri=CELEX%3A01993R0315-20090807 (Accessed: July 28, 2024)
6. *Petcu C.D.,* (2015), Meat quality and technology, (Ed.) Granada Publishing House, Bucharest, Romania
7. *Pitt J.I, Wild C.P, Baan R.A., Gelderblom W.C.A., Miller J.D., Riley R.T., Wu F.*, (2012). Improving public health through mycotoxin control.IARC Science Publ 158:39-51
8. *Rejczak T., Tuzimski T.*, (2015), A review of recent developments and trends in the QuEChERS sample preparation approach. Open Chem, 2015:1
9. *Zhang B., Chen X., Han S.Y., Li M., Ma T.Z., Sheng W.J.*, (2018),Simultaneous Analysis of 20 Mycotoxins in Grapes and Wines from Hexi Corridor Region: Based on QuECh ERS–UHPLC–MS/MS Method. Molecules, 23(8):1926.