HPLC DETERMINATION OF OCHRATOXIN A IN BREAD AND CORN FLOUR

DETERMINAREA OCHRATOXINEI A DIN PÂINE ŞI MĂLAI PRIN METODA HPLC

BULEA Delia[1], ŞPAC A. F.[1], DORNEANU V.[1]
e-mail: dbulea@yahoo.com

Abstract. *Ochratoxin A is a mycotoxin produced by different species of Aspergillus and Penicillinium fungi. Ochratoxin A has been found in peanuts, cereals, coffee, bread, flour, corn, peas, beans, beer, wine. The aim of this paper is to determine ochratoxin A in bread and corn flour. The samples purchased from markets and agro - food markets were processed and then analyzed by high performance liquid chromatography (HPLC) with fluorescence detection.*
Key words: ochratoxin A, bread, corn flour, HPLC

Rezumat. *Ochratoxina A este o micotoxină produsă de diferite specii de fungi din genurile Aspergillus şi Penicillinium. Ochratoxina A a fost identificată în alune, cereale, cafea, pâine, făină, mălai, mazăre, fasole, bere, vin. Scopul lucrării este de a determina ochratoxina A din probe de pâine şi mălai. Probele achiziţionate din reţeaua comercială şi pieţe agro-alimentare au fost prelucrate şi apoi supuse analizei prin cromatografie de lichide de înaltă performanţă (HPLC) cu detecţie în fluorescenţă.*
Cuvinte cheie: ochratoxina A, pâine, mălai, HPLC

INTRODUCTION

Ochratoxin A (fig. 1) is a toxic metabolite produced by species of *Aspergillus* genus in tropical and subtropical areas and species of *Penicillium* genus in temperate zones (Eskola M., 2002). For the first time, ochratoxin A was isolated from cultures of *Aspergillus ochraceus* by Van der Merwe et all. in 1965 while testing the toxin ability of fungal strains isolated from cereals and vegetables (Van der Merwe K.J. et all., 1965).

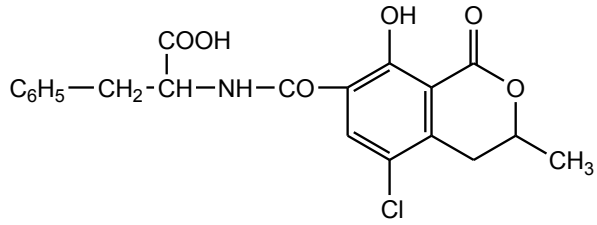

Fig.1 – Chemical structure of ochratoxin A

[1] University of Medicine and Pharmacy "Gr. T. Popa" Iaşi

Penicillium verrucosum is frequently isolated from cereal samples while *Aspergillus ochraceus* contaminates green coffee beans, spices, cocoa beans, soybeans and peanuts (Kuiper-Goodman T. et al., 1987).

Experimental studies demonstrated toxic effects of ochratoxin A: immunotoxic (Harvey R. B. et al., 1992), nephrotoxic (Vrabcheva T. et al., 2004), teratogenic (Wangikar P.B. et al., 2005). Due to the carcinogen action, shown on experimental animals, ochratoxin A has been included by the International Agency for Research on Cancer (1993) among the possible carcinogenic substances - 2B (IARC, 1993).

The aim of this study is to determine the content of ochratoxin A in two categories of food, bread and corn flour, given the high consumption of these foods by the population of Romania and reporting data obtained with the regulations set by the EU regarding the maximum limits permitted. Thus, the European legislation sets the maximum limits for ochratoxin A: 5 μg / kg for cereals and 3 μg / kg for cereal product (Commission Regulation, 2006).

MATERIAL AND METHOD

Reagents:

- Ochratoxina A from *Aspergillus ochraceus* (Sigma);
- Methanol Chromasolv ® min 99,9% (Sigma-Aldrich) ;
- Acetonitrile R Chromasolv ® min 99,8% (Riedel-de Haën);
- Glacial acid acetic 100% (Sigma-Aldrich);
- Purified water (resistivity 18,2 MΩ);
- Analytical balance Adam;
- Magnetic stirrer type AG-3;
- Filter Millipore 45 μm;
- Liquid chromatograph type HP 1090 Series II, equipped with a fluorescence detector type HP 1046 A;
- Phenomenex column, type Luna C18(2) 100Å (150 x 4,6 mm, 5μm).

Working procedure:

Analyzed samples (bread, corn flour) are subject to liquid-liquid extraction in acid medium to separate the ochratoxin A. Thus, 20 g of sample is shaken for 30 minutes with a magnetic stirrer in 100 mL mixture of chloroform and 10 mL phosphoric acid 0.1 M solution; filtered through the quantitative filter paper and the organic phase is evaporated to dryness. The residue is restarted with a volume of 0,5 mL methanol and subjected to analysis by high performance liquid chromatography (Langseth W. et al., 1989; Muscarella M. et al., 2004).

HPLC analysis was performed on high performance liquid chromatograph type HP 1090 Series II equipped with fluorescence detector type HP 1046 A. The analysis was performed at a Phenomenex column, Luna C18(2) 100Å (150 x 4,6 mm, 5μm) with a mobile phase formed by a mixture of acetonitrile : water : acetic acid (99 : 99 : 2), a flow of 0.7 mL/min; in the column compartment temperature was set at 25° C. For detection, the wavelenght of excitation was 228 nm and for emision was 423 nm.

The method was validated by establishing the linearity on concetration range 6.25 - 50 ng / mL (fig. 2) (regression line equation is Peak area = 0.6339 x concentration + 6.7353), the system precision (RSD = 0.9645 %, n = 10, RSD = relative standard deviation, n = number of determinations), method precision (RSD = 2.4975%, n = 9, where RSD = relative standard deviation, n = number of determinations), accuracy (mean

recovery 100.1%), limit of detection (LD = 1.6 ng/mL) and limit of quantification (LQ = 4.6ng /mL).

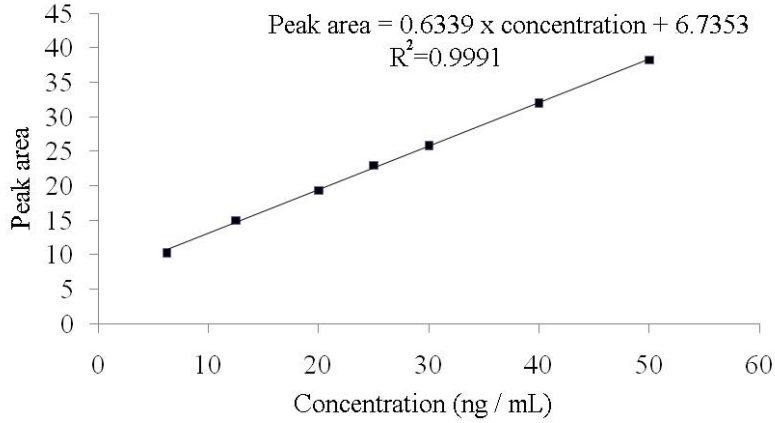

Fig. 2 – The calibration curve for ochratoxin A

The validated HPLC method was applied with good results in the determination of ochratoxin A in bread and corn flour samples.

Bread samples were purchased from commercial network of the Iasi area. We have analyzed 20 samples of bread of various kinds, from different manufacturers: 12 samples of white bread, 3 samples of graham bread, a sample of black bran bread, a rye black bread sample, 2 samples of rye bread and a sample wholemeal bread.

Samples of corn flour were purchased from open markets in the town of Paşcani (Iasi county), from private producers. 20 samples were analyzed.

RESULTS AND DISCUSSIONS

As a result of the analysis by high performance liquid chromatography chromatogram, the chromatograms that were obtained were processed and then the area of the peaks corresponding to ochratoxin A was established. The identification of ochratoxin A peak was done according to the retention time.

Figures 3 show a chromatogram obtained from the analysis of bread samples.

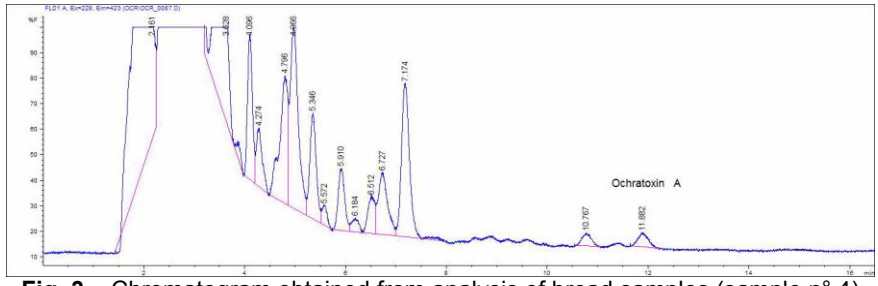

Fig. 3 – Chromatogram obtained from analysis of bread samples (sample n° 4)

Figure 4 show a chromatogram obtained from the analysis of corn samples.

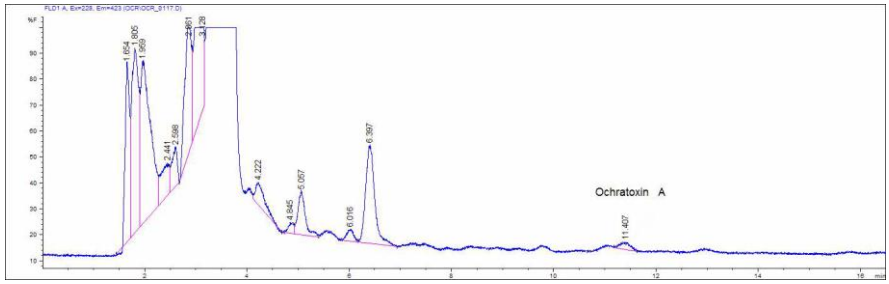

Fig. 4 - Chromatograms obtained from analysis of corn flour samples (sample n° 14)

Using the regression line equation (Area peak = 0.6339 x concentration + 6.7353) ochratoxin A content in bread and maize flour samples was calculated. The values obtained are shown in the tables below (table 1 and table 2).

Table 1

The content of ochratoxin A in bread samples

N° sample	Sample name	Ochratoxin A (µg/Kg)
1.	Sliced graham bread	1,13
2.	Sliced graham bread	2,67
3.	Sliced graham bread	absent
4.	Sliced black bran bread	1,46
5.	Sliced black rye bread	absent
6.	Sliced rye bread	absent
7.	Sliced wholemeal bread	1,45
8.	Sliced white bread	absent
9.	Sliced white bread	trace
10.	Sliced white bread	trace
11.	Sliced white bread	1,20
12.	White bread	trace
13.	Sliced white bread	1,74
14.	Rye bread for sandwich	trace
15.	White bread for sandwich	absent
16.	White bread	trace
17.	Sliced white bread	1,15
18.	White bread	trace
19.	White bread	1,99
20.	Sliced white bread	1,46

Table2

The content of ochratoxin A in corn flour samples

N° sample	Ochratoxin A (µg/Kg)	N° sample	Ochratoxin A (µg/Kg)
1.	trace	11.	trace
2.	absent	12.	1,21
3.	trace	13.	9,08
4.	1,73	14.	2,41
5.	absent	15.	trace
6.	trace	16.	1,07
7.	1,69	17.	absent
8.	1,39	18.	1,13
9.	1,56	19.	trace
10.	1,45	20.	1,38

The analysis of the data obtained from the determination of ochratoxin A in the 20 bread samples has determined that no sample contains a higher level of ochratoxin A than the maximum permitted by applicable law (3 µg/kg); ochratoxin A is present, but within limits, in 45% of the samples, traced in 30% of the samples and absent in 25% of them (fig.5).

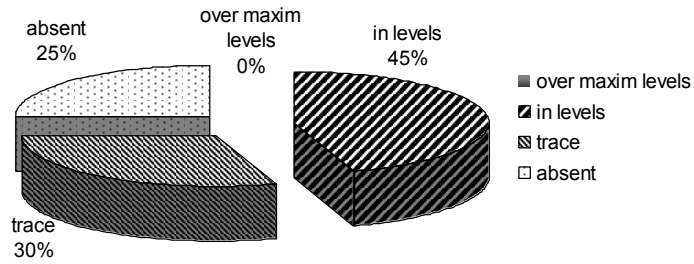

Fig. 5 -The content of ochratoxin A in bread samples

In corn flour samples ochratoxin A is present in a single sample in excess of the maximum level allowed by applicable law (5 µg/kg); in 50% of samples, ochratoxin A is within the maximum allowed limits, in 30% as trace and in 15% of the samples it is absent (fig. 6).

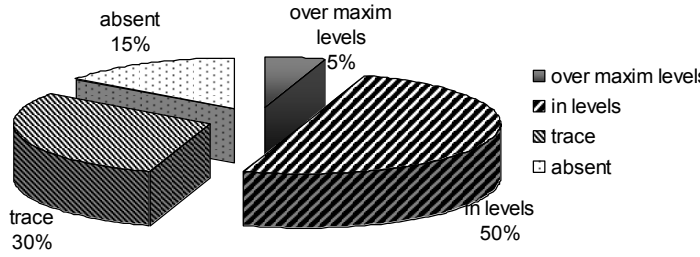

Fig. 6 - The content of ochratoxin A in corn flour samples

CONCLUSIONS

1. A validated HPLC method was applied for determination of ochratoxin A in bread and corn flour samples.

2. This paper contributes to the evaluation of the ochratoxin A present in agricultural products commercialized in open markets (maize flour) and food stores (bread) in the county of Iasi.

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