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Dioxins and furans eggs contamination evaluated using high resolution gas chromatography coupled to high resolution mass spectrometry

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Abstract

Dioxins (PCDDs) and furans (PCDFs) are a group of chemical substances with highly toxic potential, which can accumulate in food commodities, through the food chain. In this paper are presented the results of the experiments conducted in order to determine dioxins and furans in egg samples, using high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC/HRMS). There had been analyzed egg samples from the market, through the following steps: fat extraction, purification and concentration of the extract, separation, identification and quantification of the PCDD/F compounds. The concentrations of each dioxin and furan native congener, from each egg sample, were multiplied by their respective Toxic Equivalency Factor (TEF), as established by the World Health Organisation (WHO) and listed in the Commission Regulation (EU) No 252/2012, and then summed to give the total concentration of dioxins expressed as Toxic Equivalents (TEQs), in pg/g fat. In the case of the analyzed samples, the sum of dioxins (WHO-PCDD/F-TEQ) variated between 0.0236 to 0.047 pg/g fat, which is below the maximum allowed level by the Commission Regulation (EU) No 252/2012**.**

Keywords: dioxins, furans, eggs, HRGC-HRMS

1. Introduction

Dioxins (polychlorinated dibenzo-*p*-dioxins - PCDD) and furans (polychlorinated dibenzofurans - PCDF) represent a goup of chemical substances with high toxicological potential, which are persistent within environment and which can be accumulated within organisms through food chain. Decomposition of dioxins in the environment is extremely slow, so that, dioxins can be accumulated on food chain, animals having in their bodies (through bioaccumulation) higher concentrations (hundred and thousand times) than plants, water and soil [7].

Dioxins have 75 congeners, of which 7 are the most toxically, and furans have 135 congeners with variable toxicity. Among these, the compound with the highest toxicity is: 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (2,3,7,8-TCDD) [1].

Dioxins and furans are by-products resulting from: chemical reactions (carbo-chemical processes, treatment of wood with pentachloro-phenol, paper bleaching, etc.), combustion processes (wood and wood waste combustion, combustion of oil and coal), incineration of waste and crop residues in the field, large fires involving large quantities of materials containing chlorine [6, 8, 11].

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Accumulation of dioxins in the human body is produced, in particular, by eating contaminated food (more than 90% of cases). Inhalation of contaminated air and absorption in the dermis are minor sources for contamination of the human body with dioxins and furans [9,4,5].

Taking into consideration high toxic potential of dioxins and furans (skin lesions and skin cancer, liver cancer, genital cancer, lung cancer, teratogenic effects, etc.), specialists in the field have developed performance analytical methods for determination of these contaminants in food and the environment (water, air, soil).

High resolution gas chromatography coupled to high resolution mass spectrometry (HRGC/HRMS) is a performance analytical method used for determination of dioxins and furans in food and environment.

In this paper are presented results of the performed researches for dioxins and furans determination in eggs, through *high resolution gas chromatography coupled with high resolution mass spectrometry*. There were analyzed egg samples from the market.

2. Materials and methods

In It was determined contamination degree with dioxins and furans of eggs, through high resolution gas chromatography coupled with high resolution mass spectrometry, within the performed experiments. There were analyzed egg samples from the market, following the steps: preparation of test sample, fat extraction, extract clean-up and concentration, separation, identification and quantification of different PCDD/F compounds.

Fat extraction from eggs was achieved in much more steps with organic solvents (ethyl alcohol HPLC grade (99.7%, v/v), diethyl ether pico grade, n-hexane pico grade). Extracts clean-up was achieved on multiple columns, using different absorption materials (acid silica gel, aluminium oxide, florisil, activated with ultrapure water). Concentration of cleaned extract was achieved under nitrogen flow, at 40ºC and 5 psi pressure, about 15-18 minutes. Separation, identification and quantification of different PCDD/F compounds were achieved with a complex equipment: System of two high resolution gas chromatographs coupled to high resolution mass spectrometer (Capillary column - 5% phenyl - 95% dimethylpolysiloxane, 5MS, L = 30 m, di = 0.25 mm, thickness film = 0.1 µm; Carrier gas = He 6.0, High resolution mass spectrometer - Ionization type = EI+; Ionization energy = 45 eV; Resolution = 10,000; Source temperature = 260ºC).

In order to achieve calibration curves of the 17 native congeners of dioxins and furans from the analyzed egg samples, there were used standard solutions S1, S2, S3, S4, S5 (solutions certified BCR-614, LGC Promochem, Wesel, Germany). Also, there were used the following internal standards: standards for verification of extraction efficiency-S6, quantification standards-S7, recovery standards-S8.

3.Results and discussions

To quantify the total concentration of dioxins and furans, expressed as *toxic equivalents (TEQ), in pg/g fat* (according to the European Commission Regulation (EU) 1259/2011), calibration curves were made for the 17 native congeners of these contaminants: 2378-TetraCDD, 12378-PentaCDD, 123678-HexaCDD, 123789-HexaCDD, 123478-HexaCDD, 1234678-HeptaCDD, OctaCDD, 2378-TetraCDF, 23478-PentaCDF, 12378-PentaCDF, 1234678-HeptaCDF, 123789-HexaCDF, 123478-HexaCDF, 234678-HexaCDF, 123678-HexaCDF, 1234789-HeptaCDF, OctaCDF. Calibration curve is used for to calculate relative response factor for each congener of interest. Relative response factors are used with ^{13}C-labeled dioxins and furans congeners, which are added in sample, in order to determine mass of native congeners of interest through isotope dilution.

Relative response factor for each congener *i*, is defined and calculated by equation (1):

$$rrf_i = \frac{A_i(^{12}C)}{A_i(^{13}C)} \cdot \frac{c_i(^{13}C)}{c_i(^{12}C)} \quad (1)$$

where:
rrf_i-relative response factor, response factor of the native congener *i* relative to the ^{13}C-labeled congener*i*
$A_i(^{12}C)$ - the area of the native congener *i*
$A_i(^{13}C)$ - the area of the ^{13}C-labeled congener *i*
$c_i(^{12}C)$ - concentration of the native congener *i*, in calibration solution
$c_i(^{13}C)$ - concentration of the ^{13}C-labeled congener *i*, in calibration solution

In figures 1, 2, 3, 4, 5 are presented the calibration curves of 5 natives congeners of dioxins and furans.

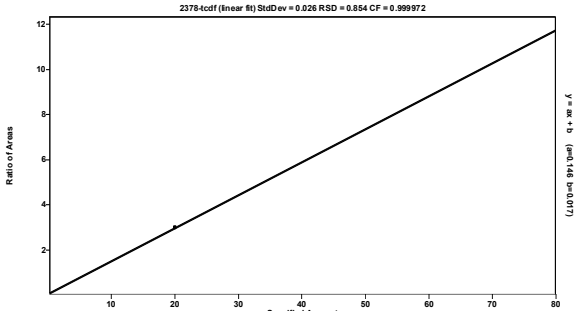

Figure 1. Calibration curve for 2378-TetraCDF

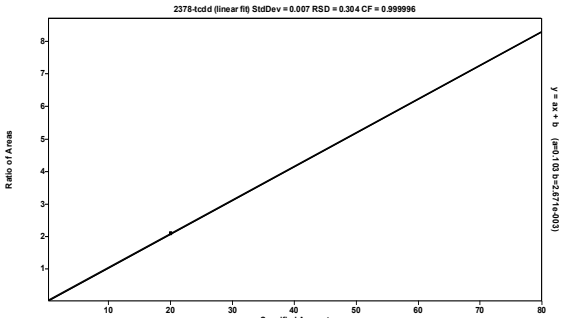

Figure 2. Calibration curve for 2378-TetraCDD

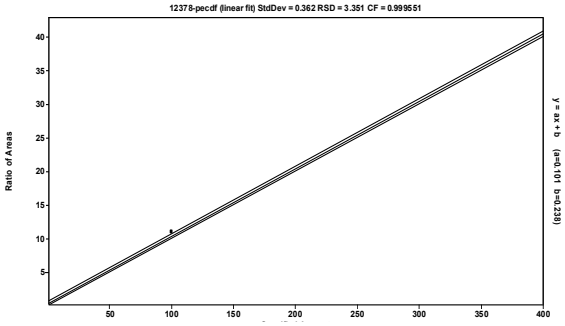

Figure 3. Calibration curve for 12378-PentaCDF

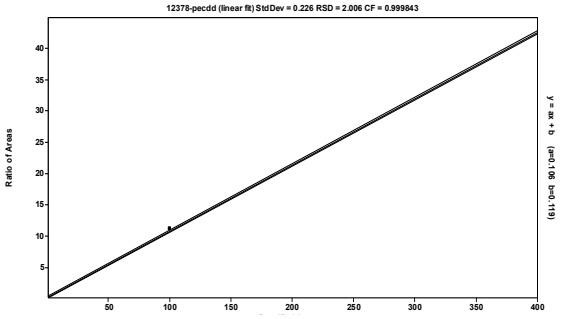

Figure 4. Calibration curve for 12378-PentaCDD

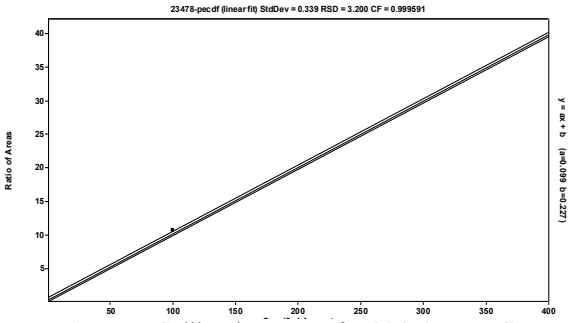

Figure 5. Calibration curve for 23478-PentaCDF

Concentration of the congener *i* in sample is calculated using the equation (2):

$$c_i(^{12}C) = \frac{A_i(^{12}C)}{A_i(^{13}C)} \cdot \frac{c_i(^{13}C)}{rrf_i} \quad (2)$$

where:

rrf_i - relative response factor, response factor of the native congener *i* relative to the ^{13}C-labeled congener *i*

$A_i(^{12}C)$ - the area of the native congener *i*

$A_i(^{13}C)$ - the area of the ^{13}C-labeled congener *i*

$c_i(^{12}C)$ - concentration of the native congener *i*, in sample

$c_i(^{13}C)$ - concentration of the ^{13}C-labeled congener *i*, in sample

Recoveries of the internal standards are calculated using the equation (3):

$$R_i = \frac{A_{i(E)}}{A_{(R)}} \cdot \frac{c_{(R)}}{rrf_i} \cdot \frac{100}{c_{i(E)}} \quad (3)$$

where:

R_i - recovery of the internal standard *i*, in percentages

rrf_i - relative response factor, response factor of the internal standard *i* relative to the ^{13}C-labeled recovery standard

$A_{(R)}$ - the area of the recovery standard

$A_{i(E)}$ - the area of the internal standard *i*

$c_{(R)}$ - concentration of the recovery standard

$c_{i(E)}$ - concentration of the internal standard *i*

Concentrations of the native congeners of dioxins and furans are corrected with recoveries.

$$c_{i\,R}(^{12}C) = \frac{c_i(^{12}C)}{R_i} \cdot 100 \qquad (4)$$

where:
R_i - recovery of the internal standard *i*, in percentages
c_i (^{12}C) - concentration of the native congener *i* in sample
$c_{i,R}$(^{12}C) - concentration of the native congener *i* in sample, corrected with recovery

Since food samples typically contain complex mixtures of different dioxin congeners, it was developed *the concept of toxic equivalency factors (TEF)*, to facilitate their risk assessment on human body. The toxic equivalency factors (TEF) for human risk assessment, were established by experts in the field, based on the conclusions of the World Health Organisation, held in Stockholm, Sweden, on 15-18 of June 1997.

The concentrations of each dioxin and furan native congener, from each egg sample, were multiplied by their respective Toxic Equivalency Factor (TEF), as established by the World Health Organisation (WHO) and listed in the Commission Regulation (EU) No 252/2012, and then summed to give the total concentration of dioxin compounds expressed as Toxic Equivalents (TEQ), in pg/g fat.

In the case of the 11 analyzed samples, the total amount of dioxins (WHO-PCDD/F-TEQ) variated between 0.0236 to 0.047 pg/g fat, which is below the maximum allowed level by the Commission Regulation (EU) No 1259/2011 (2,5 WHO-PCDD/F-TEQ pg/g fat).

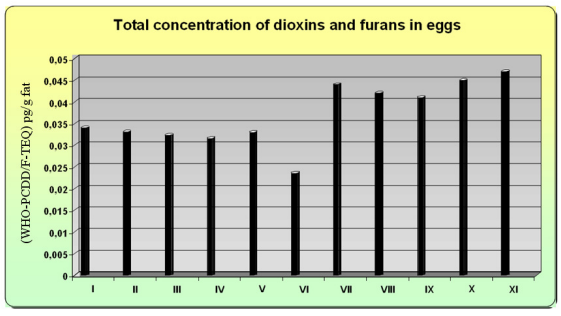

Figure 6. Total concentration of dioxins and furans in egg samples

Method used for assessment of the contamination degree with dioxins and furans of eggs has high sensitivity. Limit of detection of the native congeners of dioxins and furans varied in the range 0.0231 to 0.3734 pg/g fat.

The minimum value of this range is the limit of detection of the 1234678-HpCDF congener, and the maximum one represents the limit of detection of the OCDF congener.

The limit of quantification of the native congeners of dioxins and furans was in the range 0.0924 - 1.4936 pg/g fat. The minimum value of this range is the limit of quantification of the 1234678-HpCDF congener, and the maximum one represents the limit of quantification of the OCDF congener.

In the case of the all native congeners of dioxins and furans, limit of detection was calculated at a ratio signal/noise S/N = 2.5, and limit of quantification at a ratio signal/noise S/N = 10.

In the case of the analyzed egg samples for determination of concentration of dioxins and furans, the average recoveries of the used internal standards are in the following ranges:

- 80% - 89% (in the case of the internal standards used for verification of extraction efficiency - S6)
- 57% - 97% (in the case of the quantification standards used for verification of clean-up and concentration - S7)
- 100% (in the case of the recovery standards - S8)

4. Conclusion

In this paper are presented the performed experiments for determination of dioxins and furans in eggs, using high resolution gas chromatography coupled to high resolution mass spectrometry. In the case of those 11 egg samples analyzed, the only native congener detectable was 2378-TCDF, of which concentration was in the range 0.24 - 0.47 pg/g fat. The total concentration of dioxins and furans, expressed in toxic equivalents (WHO-PCDD/F-TEQ) was in the range: 0.0236 - 0.047 pg/g fat, being under the maximum level according to Commission Regulation (EU) No 1259/2011. Within the performed experiments, the limit of detection of the native congeners of dioxins and furans was in the range 0.0231 - 0.3734 pg/g fat. The minimum value of this range is the limit of detection of the 1234678-HpCDF congener, and the maximum one represents the limit of detection of the OCDF congener. The limit of quantification of the native congeners of dioxins and furans was in the range 0.0924 - 1.4936 pg/g fat. The minimum value of this range is the limit of quantification of the 1234678-HpCDF congener,

and the maximum one represents the limit of quantification of the OCDF congener.

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Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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