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ORIGINAL PAPER

DIOXINS AND FURANS IDENTIFICATION AND QUANTIFICATION, FROM EGGS OBTAINED IN CONVENTIONAL AND ECOLOGICAL SYSTEMS USING HRGC/HRMS

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Abstract

Dioxins (polychlorinated dibenzo-p-dioxins - PCDD) and furans (polychlorinated dibenzofurans - PCDF) are highly toxic chemical compounds which can accumulate into human and animal bodies, causing many diseases with high risk for consumers health. In this paper are presented the results of the performed experiments for dioxins and furans determination from eggs obtained in conventional and uncertified ecological systems using high resolution gas chromatography coupled to high resolution mass spectrometry. The obtained results, expressed as WHO-PCDD/F-TEQ pg/g fat, revealed that in both cases the values were in the limits set by the Commission Regulation (EU) No. 1259/2011 (below 2,5 pg WHO-PCDD/F-TEQ/g fat).

Keywords: dioxins, furans, eggs, HRGC/HRMS

Introduction

Dioxins and furans are very stable chemicals whose concentration is becoming bigger through the accumulation in the fatty tissues of living organisms that consume contaminated foods. They cannot be reduced through food processing and are not removed from the body once they are ingested.

The native congeners of dioxins and furans are numerous (over 210 compounds) and, in order to assess their toxicological impact on the human body and to facilitate the risk assessment and the regulatory control, it was developed the term of toxic equivalency factors (TEF).

According to US Environmental Protection Agency, the highest levels of dioxins were found in beef, because this animal lives longer and these contaminants can accumulate more in his fat tissues (U.S. EPA [1]). Also, the existence of these compounds in breast milk continues to be a major risk for infants' health (M. SCHUHMACHER & al. [2]).

Dioxins and furans have various deleterious effects on the human body, like: cancer inductions, spontaneous abortions, infertility and internal gland dysfunction, immunosuppressant by direct action on lymphocytes T and B, or indirectly, by affecting the hormones involved in the immune response of the human body, dermal lesions and skin cancer, teratogenic effects (S. OTLES & H. YILDIZ [3]; A.R. SCIALLI [4]).

Dioxins and furans contamination of food is a thorny international problem. Unfortunately, in Europe, there have been identified many cases of food contamination with this class of toxic compounds. A.A. LOVETT & al. [5], studied the degree of contamination with dioxins and furans of eggs and poultry meat samples from farms located in various areas in the UK. The eggs and the poultry meat from a farm located close to a chemical waste incinerator contained high concentrations of dioxins, above the maximum level. Researchers reported the presence of dioxins in the eggs and the poultry meat samples, from various rural and urban areas, apparently unexposed to sources of contamination with these classes of compounds. M. DE VRIES & al. [6], presented the results of a study conducted in EU countries, which showed that the eggs obtained in an organic system had higher concentrations of dioxins and furans, ranged between 0.4-19 pg WHO-PCDD/F-TEQ/g fat, in many cases over maximum level of 3 pg WHO-PCDD/F-TEQ/g fat, according to the European Commission Regulation (EU) No. 1881/2006. In 2010, was reported a contamination with dioxins of pork and poultry, and eggs from several farms in Germany. The contamination source was found to be the oils (contaminated with dioxins) used in the animal feed.

Because of the extreme toxicity of dioxins and furans, at European and international level, particular attention is paid to monitoring the levels of these contaminants, both in the environment (water, air, soil), and in the foods.

This paper presents the results of the experiments undertaken in order to assess the extent of contamination with dioxins and furans of certain eggs obtained in conventional and uncertified ecological systems, using high resolution gas chromatography coupled with high resolution mass spectrometry (HRGC/HRMS), a performance method used for the determination of dioxins and furans in food and environment, as W.A. TELLIARD & al. [7]; M. SIMON & B.J. WAKEFORD [8], demonstrated in their scientific researches.

Materials and methods

In order to determine the concentration of dioxins and furans there were used eggs obtained in conventional system (commercially available) and eggs obtained in uncertified ecological system (purchased from farms).

The fat was extracted from eggs using the following organic solvents: ethylic alcohol (99.7%, v/v) HPLC grade, diethyl ether Pico grade, n-hexane Pico grade. The purification of the extracts was achieved on three columns with different fillings: acid silica gel, aluminium oxide, Florisil, activated with ultrapure water. The purified extract, was concentrated in nitrogen flow, at 40ºC and 5 psi pressure, to a volume of about 20 µL.

The dioxins and furans' congeners separation, detection and quantification, from the purified and concentrated egg extracts, was achieved using a system of two high resolution gas chromatographs coupled to high resolution mass spectrometer, provided with a capillary column of 5% phenyl and 95% dimethylpolysiloxane, 5MS, length of 30 m, diameter of 0.25 mm, thickness film of 0.1 µm. The initial temperature of the GC oven was maintained at 120°C for 2 min, and then increased up to 200°C by 15°C/min, followed by 1,5°C/min to 220°C, and then by 7°C/min to 300°C. The carrier gas used was He of 6.0 p.a., with a flow rate of 0.8 mL/min. The high resolution mass spectrometer was operated under an electron impact mode of 45eV, at a resolution of 10.000 and temperature source of 260ºC. The injected sample volume was 1 µL.

For each of the 17 native congeners of the dioxins and furans, imposed by the current legislation, was achieved a calibration curve, using certified solutions (BCR-614, LGC

Promochem, Wesel, Germany). There were used three types of internal standards: standard for verification of extraction efficiency-S6, quantification standard-S7, recovery standard-S8. The concentrations of each native congener of the dioxins and furans from a sample was multiplied by it's own TEF (Regulation (EU) No. 1259/2011), and then summed in order to obtain the total concentration of dioxins and furans, expressed as toxic equivalents (TEQ).

Results and discussions

According to the results of the experiments, in the case of the egg samples obtained in conventional system and analyzed in 2012, the only detectable native congener was 2378-TCDF. Its concentration varied between 0.236-0.470 pg/g fat. The total concentration of dioxins and furans, expressed as WHO-PCCD/F-TEQ pg/g fat varied between 0.0236-0.0470 pg/g fat, which is below the maximum limit set by the Commission Regulation (EU) No. 1259/2011 (2.5 pg WHO-PCCD/F-TEQ/g fat).

Figure 1 presents the chromatogram of an analyzed egg sample, and Figure 2 presents, the total concentration of dioxins and furans variaton, for the eggs obtained in conventional system and analyzed in 2012.

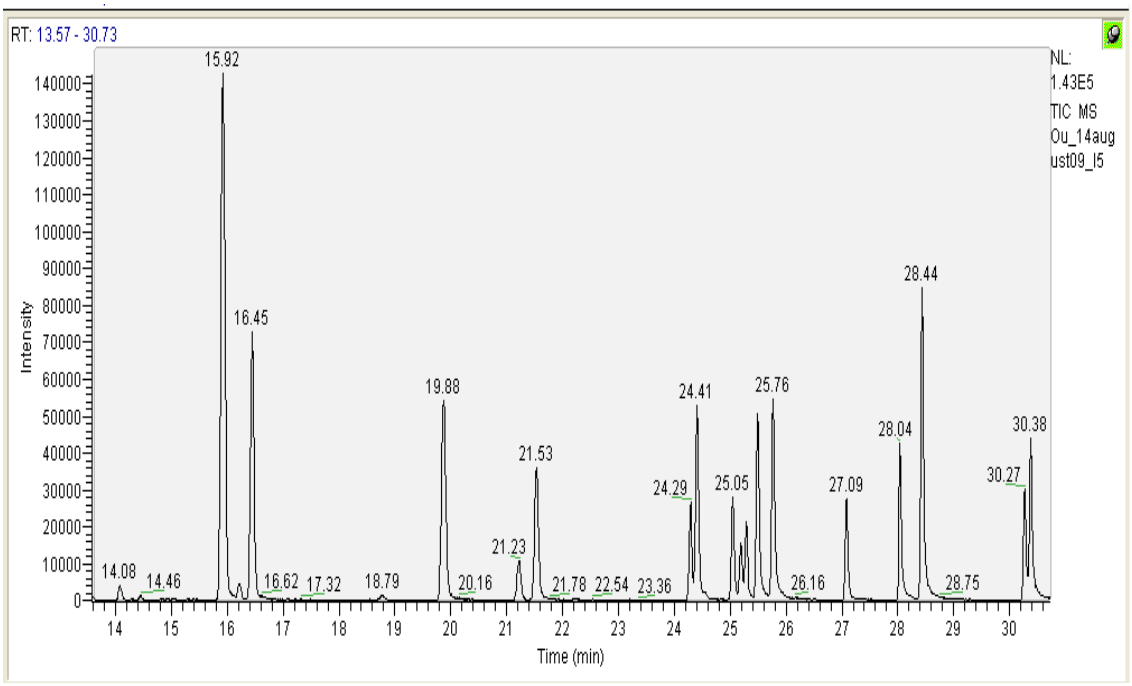

Figure 1. Chromatogram of an analyzed egg sample

The analysis of the eggs obtained in conventional system, analyzed in 2013, revealed the following native native congeners: 2378-TCDF and 12378-PentaCDF. Their concentrations varied within 0.210-0.312 pg/g fat, and respectively 0.294-0.353 pg/g fat.

In the case of these samples, the total concentration of dioxins and furans, expressed as WHO-PCDD/F-TEQ pg/g fat ranged between: 0.00882-0.0312 pg/g fat, being also, under the maximum limits imposed by the legislation (Figure 3).

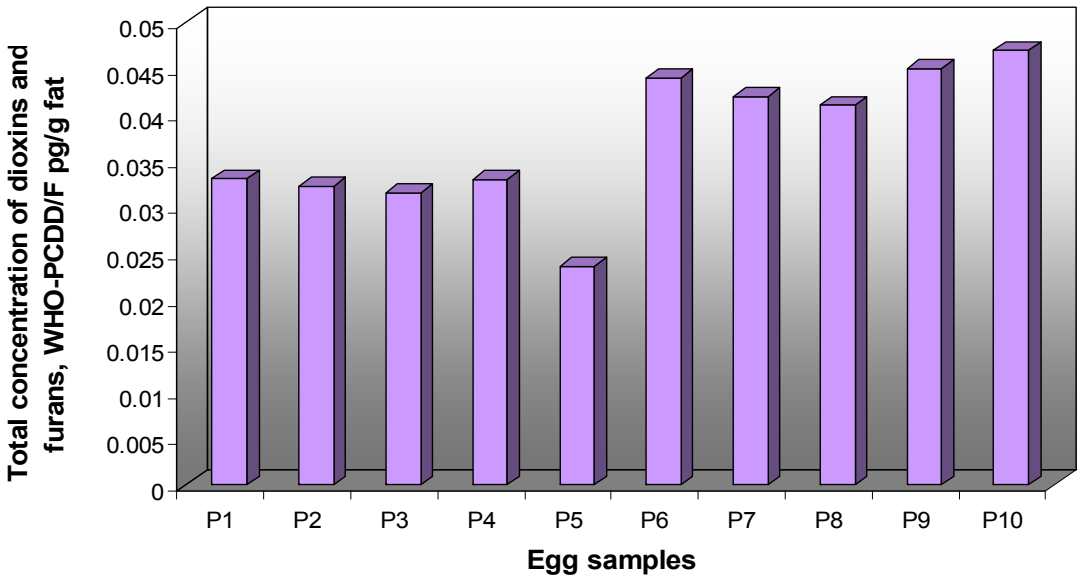

Figure 2. Total concentration of dioxins and furans from eggs obtained in conventional system in 2012

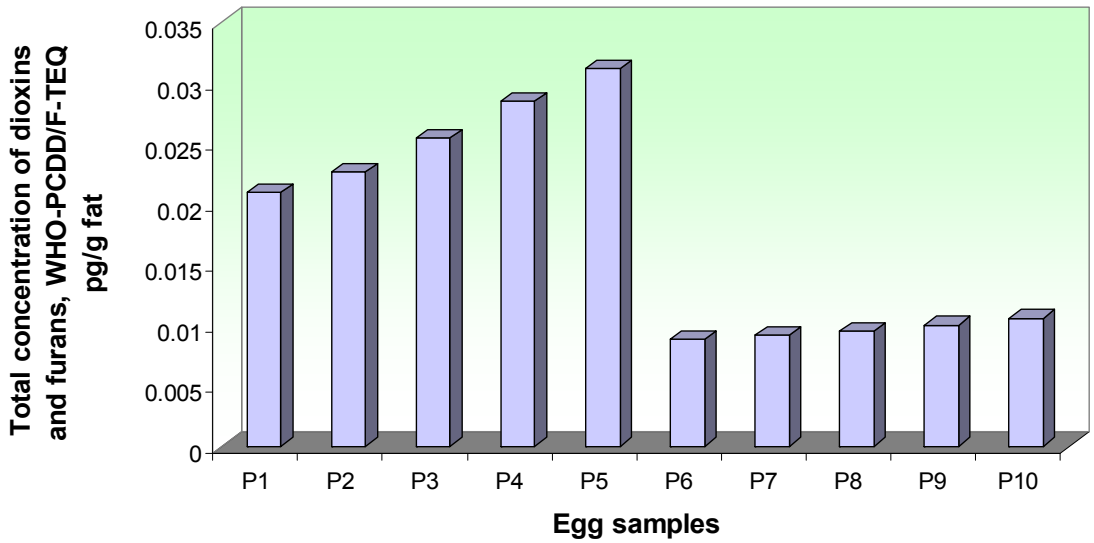

Figure 3. Total concentration of dioxins and furans from eggs obtained in conventional system in 2013

For the eggs obtained in uncertified ecological system, and analyzed in 2012, the following native congeners were identified and quantified: OctaCDD, 2378-TetraCDF, 12378-PentaCDF. Their concentrations ranged between: 0.332-0,455 pg/g fat, 0.243-0.387 pg/g fat and, respectively 0.305-0.438 pg/g fat.

In these samples, the total concentration of dioxins and furans expressed as WHO-PCDD/F-TEQ pg/g fat ranged also, between: 0.03354-0.05197 pg/g fat, which is below the maximum limit allowed by the legislation (Figure 4).

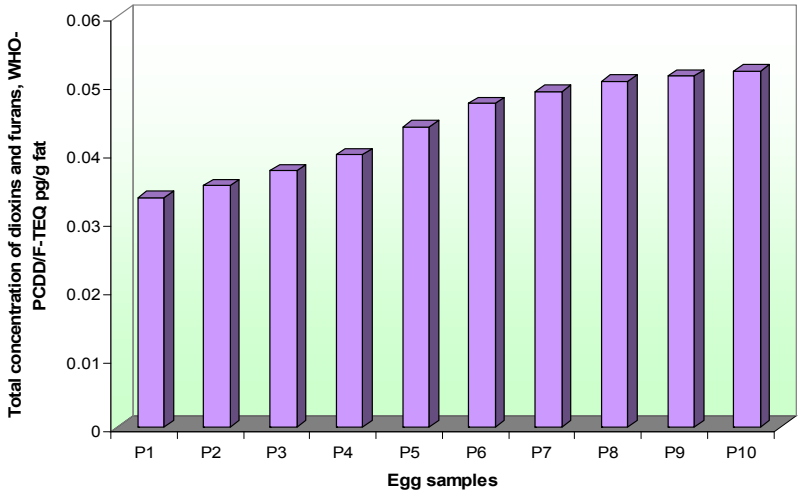

Figure 4. Total concentration of dioxins and furans fromn the eggs obtained in uncertified ecological system in 2012

The analyses carried out in 2013 in order to assess the level of contamination of the eggs in uncertified ecological system, showed that, the following native congeners were identified and quantified: 123789-HexaCDD, 2378-TetraCDF şi 12378-PeCDF. The concentrations of these native congeners of dioxins and furans, ranged between: 0.253-0.415 pg/g fat, 0.263-0.405 pg/g fat and respectively 0.282-0.378 pg/g fat.

The total concentration of dioxins and furans, expressed as WHO-PCDD/F-TEQ pg/g fat, for these samples varied between 0.0295-0.07776 pg/g fat, which is below the maximum limit imposed by legislation (Figure 5).

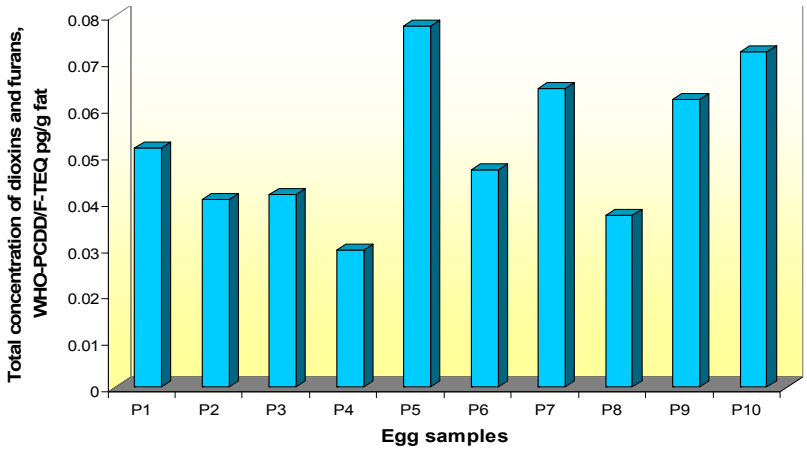

Figure 4. Total concentration of dioxins and furans from the eggsobtained in uncertified ecological system in 2013

In the case of the analyzed egg samples, obtained in conventional and uncertified ecological systems, the detection limit of the dioxins and furans congeners varied between 0.0209-0.3675 pg/g fat and the quantification limit between 0.0354-0.3970 pg/g fat. Therewith, the recovery factors of the internal standards used, ranged between: 81.5%-87.4% in the case of S6 internal standard (used for the efficiency control), 60.2%-92.7% in the case of S7 internal standard (used for quantification and the checking control of the purification and concentration), and 100% in the case of S8 internal standard (used for recovery).

Conclusions

Based on the experiments, it was found that the eggs obtained in conventional system had much lower concentration of dioxins and furans comparing to those obtained in uncertified ecological system, being more sure for the consumers. Also, the total concentration of dioxins and furans for the analyzed eggs (2012, 2013) was located in the limits foreseen by the Commission Regulation (EU) No. 1259/2011 (2.5 pg WHO-PCDD/F-TEQ/g fat). The different results (conventional and uncertified ecological system) may be due to the fact that farmers grow hens in free space and the hens are able to consume contaminated soil, grass, insects etc. However, the practice of burning the crop residues resulting from gardens or fields near the household, is considered the major source of contamination with dioxins and furans of the environment and subsequently of the foods.

Acknowledgements

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