Preliminary monitoring of the presence of perfluoroalkyl substances in Italian eggs from different breeding systems

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Abstract

Perfluoroalkylated substances (PFASs) are a wide cluster of fluorinated molecules largely engaged industrially and commercially for many purposes. Because of the strength of the fluorine-carbon bond, PFASs show a firm tenacity against thermal degradation, hydrolysis, photolysis and biodegradation. On the other hand, such chemical stability gives them persistent environmental pollutant feature. In 2012, EFSA published a scientific report on PFASs in food, mentioning their adverse effects on health. Based on observational studies evidences, EFSA has recommended a tolerable daily intake (TDI) for the two most known PFASs, i.e. PFOS 150 ng/kg b.w./day and PFOA 1500 ng/kg b.w./day. The aim of this study was to monitor, for the first time, the level of contamination of PFASs in chicken eggs laid in Northern Italy. The eggs were collected from different rearing systems, in order to search a correlation between this variable and the contamination of PFASs. In this study four PFASs [perfluoro-n-nonanoic acid (PFNA), perfluoro-n-octanoic acid (PFOA), sodium perfluoro-1-hexanesulfonate (PFHxS) and sodium perfluoro-1-octanesulfonate (PFOS)] were analyzed by liquid chromatography-tandem mass spectrometer (LC-MS/MS). 132 eggs were analyzed, split up in 11 groups according to the geographical origin and rearing system. Results accord with literature data available for chicken eggs: almost all the samples show a PFASs contamination level under the limit of quantification (LOQ) of 0.25 ng/mL. No significant difference results from the rearing system, attesting an equal distribution and a concentration of PFASs detectable under the limit of quantification.

Introduction

In the last six decades a wide cluster of fluorinated molecules called with the generic name of perfluoroalkylated substances (PFASs) was largely engaged industrially and commercially for many purposes because of their specific technical and chemical properties (Buck et al., 2011). The acronym PFAS involves all the molecules in which each hydrogen-carbon bond is replaced with a much stronger fluorine-carbon bond. This chemical difference gives these substances unique robustness characteristics, take on strong endurance against thermal degradation, hydrolysis, photolysis and biodegradation. For the same reasons they are persistent environmental pollutants in groundwater as well as in soil, and through these sources they can enter the food chain (Lau et al., 2004; Brüning et al., 2017). Moreover PFAS are also amphiphilic substances, so they have been widely used as surfactants and for stain-resistant coatings, packaging materials, fire-extinguishing fluids, textiles, carpets, paper, furniture, floor polishing agents, cleaning agents, varnish, polish, photograph paper and insecticides (3M Company, 1999; Lindstrom et al., 2011). In 2012, EFSA, the European Food Safety Authority, published a scientific report on PFAS in food, mentioning their adverse effects on health based on several studies on experimental animals, i.e. hepatotoxicity, developmental and reproductive toxicity, neurobehavioral toxicity, immunotoxicity, lung toxicity and endocrine disruptors (EFSA, 2012). For this reason, EFSA has recommended a tolerable daily intake (TDI) for the two most known PFAS, i.e. PFOS 150 ng/kg b.w./day and PFOA 1500 ng/kg b.w./day, and additionally recommended to the scientific community to collect more data on PFAS levels in food (EFSA, 2012).

Therefore, except for PFAS industrial workers, diet is considered the main exposure route to PFAS (Jain, 2018). For this reason, many scientific papers have been published with the purpose to evaluate the PFAS level contamination in different food commodities. From literature it emerged that fish and seafood in general are the most contaminated food categories (EFSA, 2012; Vestergren et al., 2012; Hlouskova et al., 2013; Barbarossa et al., 2016; Jain et al., 2017). In a recent study, it was evidenced that there is a PFAS level affinity within foodstuffs that is showed in the following order: fish and shellfish > eggs and meat products > milk products and beverages > vegetables (Jain et al., 2017). This trend is explainable by their protein affinity, the high bioaccumulation potential and biomagnification effect of these resistant pollutant in the food chain (Jain et al., 2017).

Chicken eggs play an important role in human diet, due to their content of high biological value proteins combined with low cost and lack of religious and ethical restrictions. In Italy their consumption is relevant, and is about 215 eggs per person per year (Unaitalia, 2017) corresponding to about 13.5 kg per person per year. A part of the consumption of these eggs occurs indirectly with the intake of foods, such as egg pasta, sweets, biscuits and bakery products. Despite this scenario, information on their contamination by PFAS is still very low. Only few scientific studies have been carried out on the contamination of chicken eggs (D’Hollander et al., 2011; Zafeiraki et al., 2016), while most of the scientific works concern the monitoring of PFAS in wild bird eggs (Miller et al., 2015; Letcher et al., 2015) or other wild animal species related to environmental biomonitoring studies. Otherwise some studies report PFAS levels in different food items amongst which also egg, but in a limited number, considering a wide foodstuff range (Guerranti et al., 2013; Hlouskova et al., 2013; Jain, 2018; Jian et al., 2017; Vestergren et al., 2012). In Italy, a few scientific works on the monitoring of PFAS have been conducted on food matrices different from chicken eggs (Farabegoli et al., 2012).
2013; Barbarossa et al., 2014), so there are currently no studies on PFAS monitoring in chicken eggs.

The aim of this study was to monitor, for the first time, the contamination of PFASs in commercial Italian chicken eggs, obtained from different hen rearing systems. In this study the four PFAS more frequently studied were considered: perfluoro-n-nonanoic acid (PFNA), perfluoro-octanoic acid (PFOA), sodium perfluoro-1-hexanesulfonate (PFHxS) and sodium perfluoro-1-octanesulfonate (PFOS) by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

Materials and Methods

Sample collection

The egg samples were collected from commercial laying hen farms in 2017. Sampling was based on the following variables: rearing system (organic, aviary system, battery cage and barn) and geographical origin of the eggs. A total of 132 eggs were collected, divided into 11 groups based on the variables listed in Table 1. After sampling, eggs were boiled and the yolks were separated from egg white. Four pools (containing three homogenized yolks) were created for each group, for a total of 44 samples analyzed. The pools were stored at -20°C until the analysis.

Reagents and chemicals

The PFNA, PFOA, PFHxS and PFOS standards and their respective labeled standards (IS), perfluoro-n-nonanoic acid (M-PFNA), perfluoro-octanoic acid (M-PFOA), perfluoro-1-hexane (M-PFHxS), sodium perfluoro-1-(1,2,3,4-13C4) octanesulfonate (M-PFOS) were purchased from Wellington (Guelph, Ontario, Canada). An appropriate amount of each labeled standard was combined and diluted with methanol to obtain a “IS working solution” at a concentration of 50 ng/mL. The same procedure was used to prepare a “PFASs working solution”.

Methanol, ammonium acetate, acetonitrile and formic acid were all of mass spectrometry grade and were all from Fluka (Milford, MA, USA). Hydrochloric acid (37%) was obtained via a Human Power I lab water purification system (Human Corp., Seoul, South Korea).

Analytical conditions

PFASs in commercial Italian chicken eggs, samples were extracted adopting to the method of Zafeiraki et al. (2016). The procedure was carried using polypropylene (PP) materials, to avoid the interaction with glass.

Briefly, 50 μL of “IS working solution” was added at 1 g of homogenized yolk for quantification. Later, the sample was digested by an alkaline solution (2 mL of sodium hydroxide 200 mM) and homogenized by Ultra Turrax for 1 min. After that, 10 mL of methanol were added for extraction and then agitated by magnetic stirrer for 30 minutes. Afterwards 150 μL of HCl (37%) were added and the sample centrifugated for 10 minutes at 10,000 rpm, then the supernatant was transferred into a tube containing 25 mL of ultrapure water. The extract was purified by SPE Oasis WAX (Weak Anionic eXchange) cartridges. The cartridge was conditioned with 4 mL of methanol and 4 mL of water before sample loading. After a wash by 4 mL of 25 mM sodium acetate buffer (adjusted to pH 4 with hydrochloric acid), the elution was obtained by 2 mL of 2% ammonium hydroxide solution in acetonitrile. Finally, the eluate was dried by gentle N2 flow at 45 °C and redissolved with 300 μL of 20 mM ammonium acetate : methanol (90 : 10) and then transferred into a vial for UPLC-MS/MS analysis.

A calibration curve was prepared with 1 g of blank yolk which was spiked with appropriate amounts of the “PFASs working solution” to obtain 5 levels of concentration (0.5, 1, 5, 10 ng/g) and 50 μL of the internal standard IS PFASs working solution (50 ng/mL). In addition, quality control (QC) samples at three concentrations (0.5, 2 and 5 ng/g) were used to monitor the performance of the method.

Limits of quantification (LOQs) and limits of detection (LODs) of the method, defined as the concentrations providing a chromatographic signal with a signal-to-noise (S/N) ratio equal to 10 and 3 respectively, were 0.25 ng/g and 0.1 ng/g for all analytes.

Table 1. Eggs sampling based on different rearing systems and different geographical origin.

| Group | N° pool | Rearing system | Geographical origin |
|-------|---------|----------------|---------------------|
| A     | 4       | Barn           | Pavia               |
| B     | 4       | Organic        | Verona              |
| C     | 4       | Battery cage   | Forlì-Cesena        |
| D     | 4       | Barn           | Bologna             |
| E     | 4       | Battery cage   | Forlì-Cesena        |
| F     | 4       | Aviary system  | Ravenna             |
| G     | 4       | Aviary system  | Ravenna             |
| H     | 4       | Organic        | Bologna             |
| I     | 4       | Battery cage   | Romagna             |
| J     | 4       | Organic        | Romagna             |
| M     | 4       | Barn           | Romagna             |

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Results and Discussion

PFASs were determined in the yolk, because in literature earlier works reported that PFASs are primarily found in the egg yolk than in the egg white (Zafeiraki et al., 2016).

In all samples the level of each analyte was under the LOQ of 0.25 ng/mL, except for two samples belonging to group A, obtained from barn, that showed contamination of PFOS in one sample and of PFHxS in the other, both at the level of 0.4 ng/g. Levels of PFOA, PFNA and PFHxS, between the LOD (0.1 ng/g) and LOQ, were found in the 15.9% of sample (Table 3). PFOS was detected only in one sample, but at quantifiable level, while in many works PFOS is the most frequently found PFAS and the most abundant (Zafeiraki et al., 2016).

This very low uniform distribution of PFASs in commercial eggs is in accordance with the few data reported in literature, in particular with Zafeiraki’s work on Greek and Netherlands commercial eggs. Furthermore, the low contamination of commercial eggs is in contrast to the high levels found in home produced eggs as reported in literature (D’Hollander et al., 2011; Zafeiraki et al., 2016).

The origin of the sampled eggs depending on the rearing system was the following: 27% organic, 27% battery cage, 27% barn and 19% aviary system. Regarding the rearing system of hen eggs production, the levels of contamination were so limited, that no difference emerged.

As far as the geographical origin is concerned, the irregular sampling does not allow to draw a correct correlation between this variable and results.

Conclusions

In this preliminary study, commercially Italian chicken eggs were investigated for the first time in order to monitor their level of PFASs contamination analyzing the four most widespread substances (PFOS, PFOA, PFNA, PFHxS). Data show a very low and uniform contamination in commercial eggs regardless the rearing system and origin. Only two samples show a quantifiable contamination of PFOS and of PFHxS.

Considering the very low level of PFAS contamination measured in the present study and the TDI established, it could be concluded that the consumption of Italian commercial eggs does not pose a high risk for consumers.

| Group | Pool | PFOA | PFHxS | PFOS | PFNA |
|-------|------|------|-------|------|------|
| A     | 1    | traces | -     | 0.4 ng/g | - |
|       | 2    | traces | -     | 0.4 ng/g | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| B     | 1    | -     | -     | -     | - |
|       | 2    | traces | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| C     | 1    | -     | -     | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| D     | 1    | -     | -     | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | traces |
| E     | 1    | -     | -     | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| F     | 1    | -     | -     | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| G     | 1    | -     | -     | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| H     | 1    | -     | -     | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| I     | 1    | traces | traces | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| L     | 1    | -     | -     | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |
| M     | 1    | -     | -     | -     | - |
|       | 2    | -     | -     | -     | - |
|       | 3    | -     | -     | -     | - |
|       | 4    | -     | -     | -     | - |

Table 2. Mass spectrometry parameters for the selected PFASs.

| Compound | Precursorion (m/z) | Productions (m/z) | Cone Voltage (kV) | Collision Energy (eV) |
|----------|-------------------|------------------|-------------------|-----------------------|
| PFOS     | 498.50            | 99.10            | 50                | 35                    |
| PFHxS    | 398.60            | 99.10            | 50                | 40                    |
| PFNA     | 462.50            | 419.00           | 12                | 10                    |
| PFOA     | 412.60            | 368.90           | 12                | 9                     |
| M-PFOS   | 502.50            | 99.10            | 50                | 35                    |
| M-PFHxS  | 402.60            | 103.10           | 55                | 32                    |
| M-PFNA   | 467.50            | 423.00           | 12                | 10                    |
| M-PFOA   | 416.50            | 372.00           | 12                | 10                    |

Table 3. Results of PFASs contamination level in analyzed hen eggs. Trace: value between limit of detection (LOD) and limit of quantification (LOQ).
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