

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

Elisa Ghelli,¹ Maria Teresa Tondo,¹
Elisa Zironi,¹ Giampiero Pagliuca,¹
Federico Sirri,² Teresa Gazzotti¹

¹Department of Veterinary Medical Sciences and ²Department of Food Science and Technology, University of Bologna, Ozzano dell'Emilia, Italy

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 (PFAS) 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; Bräunig *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; Jian *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 (Jian *et al.*, 2017). This trend is explainable by their protein affinity, the high bioaccumulation potential and bio-

Correspondence: Elisa Ghelli, Laboratory of Analytical Bio-Agroalimentary Chemistry (CABA-Lab), Department of Veterinary Sciences, Alma Mater Studiorum - University of Bologna, via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy.
Tel. +39.340.4092343.
E-mail: elisa.ghelli2@unibo.it

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magnification effect of these resistant pollutant in the food chain (Jian *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 (Unitalia, 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.*,

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 system. In this study the four PFAS more frequently studied were considered: perfluoro-n-nonanoic acid (PFNA), perfluoro-n-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- (1,2,3,4,5-¹³C₅) nonanoic acid (M-PFNA), perfluoro-n- (1,2,3,4-¹³C₄) octanoic acid (M-PFOA), perfluoro-1-hexane (¹⁸O₂) sulfonate (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 (Honeywell). Sodium hydroxide pellets (>98%) were from Fluka (Honeywell), sodium acetate and ammonium hydroxide (33%) were both from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (37%) was from Carlo Erba Reagents (Cornaredo, MI, Italy). The solid-phase extraction (SPE) cartridges were Oasis WAX 3 cc, 60 mg

(Waters Corp., Milford, MA, USA). Ultrapure water (18.2 MΩ/cm) was obtained via a Human Power I lab water purification system (Human Corp., Seoul, South Korea).

Sample preparation

Samples were extracted adopting to the method of Zafeiraki (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 minute. 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, 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.

Analytical conditions

The separation was achieved by an Acquity ultra-performance liquid chromatographic system consisting of a binary pump, solvent degasser, autosampler and column heater fitted with a Waters BEH C18 column (1.7 µm, 2.1×50 mm) equipped with a guard column (Waters Corporation, Milford, MA, USA). In order to avoid contamination by LC system an isolator column for PFAS (2.1×50 mm) (Waters Corporation, Milford, MA, USA) was used for all the analysis. The mobile phase consisted of 20 mM ammonium acetate aqueous solution (A) and methanol (B); the chromatographic gradient was operated at a flow rate of 0.4 mL/min starting from 0 min: 90% A, 10% B; 1,50 min: 90% A, 10% B; 2,50 min 20%A, 80% B, 4 min 20% A, 80% B; 4,50 min: 90% A, 10% B; 6 min: 90% A, 10% B. The mass spectrometer was a Quattro Premiere XE, a triple quadrupole instrument equipped with an ESCI™ Multi-Mode Ionization Source (Waters Corporation).

The whole analysis was performed in ESI- mode using multiple reaction monitoring (MRM). The monitored transitions for each analyte were reported in Table 2.

The capillary voltage was 2.0 kV, extractor voltage 2.00 V, source temperature 150°C, and desolvation temperature 450°C. Nitrogen was used as desolvation gas 700 L/hr and cone gas 100 L/hr, whereas the collision gas was argon. Data acquisition processing was performed using Mass Lynx 4.1 software (Waters Corp.).

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
L	4	Organic	Romagna
M	4	Barn	Romagna

Results and Discussion

PFAS were determined in the yolk, because in literature earlier works reported that PFAS 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.

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
		80.20	50	40
PFHxS	398.60	99.10	52	35
		80.20	52	38
PFNA	462.50	419.00	12	10
		219.10	12	16
PFOA	412.60	368.90	12	9
		169.00	12	17
M-PFOS	502.50	99.10	50	35
		80.20	50	40
M-PFHxS	402.60	103.10	55	32
		84.20	55	35
M-PFNA	467.50	423.00	12	10
		219.10	12	16
M-PFOA	416.50	372.00	12	10
		169.00	12	17

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

Group	Pool	PFOA	PFHxS	PFOS	PFNA
A	1	traces	-	0.4 ng/g	-
	2	traces	traces	-	-
	3	-	0.4 ng/g	-	-
	4	-	-	-	-
B	1	-	-	-	-
	2	traces	-	-	-
	3	-	-	-	-
	4	-	traces	-	-
C	1	-	-	-	-
	2	-	-	-	traces
	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	-	-	-	-
	2	traces	traces	-	-
	3	-	-	-	-
	4	-	-	-	-
L	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
M	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-

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