From Coop to Carton: A Study of PFAS in Backyard & Store-Bought Eggs Using Automation and LC-MS/MS

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INTRODUCTION

Many consumers enjoy eating eggs from local backyard chickens. People often believe these eggs are healthier yet may be unknowingly exposing themselves to PFAS contamination from sources such as the environment, bedding, food, and drinking water of chickens. This study compares PFAS concentrations in store-bought cage-free eggs and cage-free eggs from backyard chickens, which may be exposed to a broader range of environmental factors and dietary variations due to free-range access and consuming kitchen scraps.

A workflow for the analysis of whole egg (a dense, proteinaceous, and fatty matrix) is presented, utilizing automated sample preparation to reduce analyst involvement, minimize variability, and improve robustness when working with this challenging matrix. The automated sample extraction process takes less than 15 minutes per sample, and the automated solid-phase extraction (SPE) system can process up to 8 samples simultaneously in under 70 minutes. This method also uses dual-phase Oasis™ GCB/WAX for PFAS Analysis Cartridges. The graphitized carbon black (GCB) and weak anion exchange (WAX) SPE cartridges clean up challenging samples to ensure precise and repeatable results across samples.

Ultimately, this study aims to provide a clearer understanding of PFAS contamination in both commercially and locally sourced eggs, contributing insights into food safety through the creation of a consistent and efficient automated method for extracting PFAS from the challenging matrix of whole raw egg.

METHODS

Sample Preparation

1. Add 2.5g CEM eCleanUP (Q-Matrix) and 2g homogeneous egg mixture to assembled Q-Cup containing a PFAS Q-Disc stack
2. Spike samples respectively with EIS and Native PFAS.
   Sample types: Native spiked eggs, native-free eggs, method blanks, system blanks
3. Place Q-Cups in rack with 50mL polypropylene conical collection tubes, and slide rack into place in the CEM EDGE PFAS
   Start method for egg extraction: 1 cycle 5 minute with 0.02M NaOH and 2nd cycle with 5 min 0.02M NaOH
4. Concentrate collection to 2.5mL (40°C bath with nitrogen)
   Reconstitute to 50mL with LC/MS grade Reagent Water, vortex, check pH < 6
5. Load 50mL sample on Promochrom SPE-03 with Waters Oasis GCB/WAX for PFAS Cartridges with 15mL polypropylene collection tubes
   Run 1633 50mL Method with 5mL elute
6. To eluted sample: add 25µL Acetic acid, spike NIS, vortex
7. Aliquot 500µL of sample in polypropylene vials and load on Waters Xevo TQ Absolute for analysis

Figure 1. Sample Preparation Procedure

LC-MS/MS Conditions

MS System: Xevo™ TQ Absolute Mass Spectrometer
Software: waters_connect for Quantitation
Ionization Mode: ESI-
Capillary Voltage: 0.5 kV
Desolvation Temp: 350°C
Desolvation Gas Flow: 900 L/hr
Cone Gas Flow: 150 L/hr
Source Temperature: 100°C

LC System: ACQUITY™ Premier System with BSM, FTN and fitted with PFAS Kit
Isolator Column: Atlantis™ Premier BEH™ C18 AX, 2.1 x 50 mm, 5.0 µm Column
Analytical Column: Atlantis Premier BEH C18 AX, 1.7 µm; 2.1mm x 50 mm
Column Temp: 35°C
Sample Temp: 10°C
Injection Volume: 2 µl
Flow Rate: 0.3 mL/min
Mobile Phase A: Water + 2 mM ammonium acetate
Mobile Phase B: Acetonitrile + 2 mM ammonium acetate

Gradient:

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0	0.3	95	5	initial
0.5	0.3	75	25	6
3	0.3	50	50	6
6.5	0.3	15	85	6
7	0.3	5	95	6
8.5	0.3	5	95	6
9	0.3	95	5	6
11	0.3	95	5	6

RESULTS

PFAS Concentrations in Un-spiked Eggs

The heat map uses color intensity to indicate PFAS concentration levels. ND indicates levels not detected. Of the 45 compounds analyzed, 24 were detected in at least one sample demonstrating a diverse PFAS profile in eggs. Backyard eggs generally contained more compounds at higher concentrations compared to store-bought eggs, likely due to greater environmental exposure (roaming, soil, diet, water, bedding). Precursors such as FOSA and FTCA were only detected in grocery store eggs suggesting potential legacy exposure.

PFAS Recovery in Eggs

Percent recovery pf each PFAS Spiked into both backyard and grocery store eggs. Most compounds showed acceptable recoveries within the 70-130% range, demonstrating broad method application across a diverse range of PFAS. The four compounds of regulatory focus (PFOS, PFNA, PFOA, and PFHxS all fell within this range supporting the robustness of the method for compounds under regulation. The complex whole egg matrix did not significantly compromise recoveries.

Compound	Grocery Store Eggs	Backyard 1 Eggs	Backyard 2 Eggs	Backyard Eggs % Recovery	Grocery Store Eggs % Recovery
PFBA	0.49	0.70	1.02	112	99
PFPeA	0.08	0.09	0.24	108	96
PFHxA	0.04	0.06	0.09	117	95
PFHpA	0.10	0.10	ND	110	99
PFOA	ND	0.11	ND	97	106
PFNA	ND	0.11	ND	104	98
PFDA	0.03	0.13	0.14	92	85
PFUnDA	0.07	0.25	0.09	112	100
PFDoDA	0.05	0.45	0.07	117	103
PFTriDA	ND	0.55	0.12	137	122
PFTreDA	ND	0.58	0.09	112	107
PFBS	ND	ND	ND	121	109
PFPeS	ND	ND	ND	117	109
PFHxS	ND	0.07	0.03	103	100
PFHpS	ND	ND	ND	118	115
PFOS	ND	1.91	0.39	119	96
PFNS	ND	ND	ND	96	88
PFDS	ND	ND	ND	79	77
PFDoDS	ND	ND	ND	48	56
PFEESA	ND	ND	ND	117	103
PFMPA	ND	ND	ND	101	92
PFMBA	ND	ND	ND	114	101
GenX	ND	ND	ND	111	104
ADONA	ND	ND	ND	189	172
FOSA	0.09	ND	ND	107	91
NMeFOSA	ND	0.02	ND	105	99
NEtFOSA	ND	ND	ND	121	107
N-MeFOSAA	ND	ND	ND	109	99
N-EtFOSAA	ND	0.01	ND	114	99
NMeFOSE	0.10	0.13	ND	107	99
NEtFOSE	ND	ND	ND	102	95
NFDHA	0.04	0.04	ND	64	56
9Cl-PF3ONS	ND	ND	ND	115	101
11Cl-PF3OUdS	ND	ND	ND	88	84
4:2 FTS	ND	ND	ND	126	122
6:2 FTS	0.64	ND	ND	100	77
8:2 FTS	ND	ND	ND	103	92
3:3 FTCA	0.24	0.33	0.15	98	86
5:3 FTCA	ND	ND	ND	148	121
7:3 FTCA	ND	ND	ND	124	111
PFTrDS	ND	ND	ND	--	--
PFTreDA	ND	0.58	0.09	112	107
PFTriDA	ND	0.55	0.12	137	122
PFUnDA	0.07	0.25	0.09	112	100
PFUnDS	ND	ND	ND	--	--

Highest Concentration
Lowest Concentration

Table 1. Concentrations in ng/g of PFAS detected in eggs and percent recovery of each PFAS

DISCUSSION

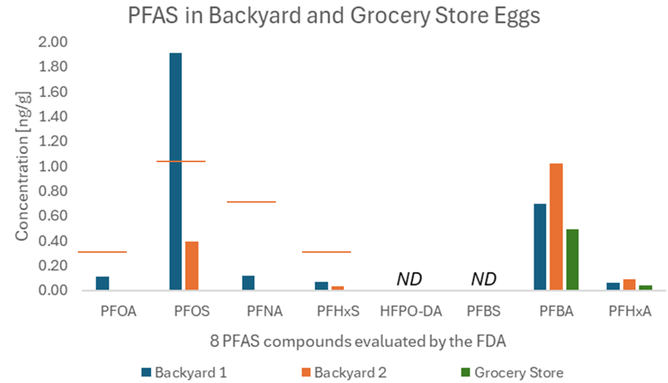

Figure 2. Eight PFAS compounds evaluated by the FDA, with four compounds regulated by the EU. Orange line represents maximum ng/g for PFAS in eggs.
EU regulated levels of PFAS are found in the Official Journal of the European Union. 8.12.2022.
FDA evaluated compounds are found in "Analytical Results of Testing Food for PFAS from Environmental Contamination"

PFAS Evaluated and Regulated in Food

PFOA, PFOS, PFNA, and PFHxS in eggs are regulated by the EU. PFOA, PFOS, PFNA, PFHxS, HFPO-DA (GenX) PFBS, PFBA, and PFHxA in food are evaluated by the FDA, though there are no current regulations.

After blank correction, grocery store eggs did not have detectable levels of the 4 EU regulated compounds but had detectable levels of FDA evaluated PFHxA and PFBA. Backyard eggs had greater total PFAS, with variation from one backyard to another, suggesting environmental factors like location may influence PFAS accumulation in eggs. One backyard with historical use of firefighting foam (known PFOS exposure) had higher levels of PFOS, suggesting free-ranging chickens can be exposed to historical PFAS contamination

CONCLUSIONS

The CEM EDGE and PromoChrom SPE-03 systems, combined with the dual phase Oasis GCB/WAX for PFAS cartridges, enabled efficient and reproducible extraction of PFAS from the challenging matrix of whole egg. Automation of the sample preparation and SPE steps delivered consistency across replicates and reduced overall method time. The workflow enabled the evaluation of PFAS compounds actively regulated by the European Union. This method demonstrates that even difficult food matrices like whole egg can be prepared easily and reliably for PFAS analysis, supporting broader applications in food testing.

Analysis revealed that PFOS and other PFAS were consistently higher in backyard chicken eggs compared to grocery store eggs, likely due to greater and differing environmental exposure from increased roaming space and dietary variation. PFAS precursors like FOSA were higher in grocery store eggs, likely due to potential legacy exposure. These findings highlight both the robustness of the automated method and the importance of monitoring PFAS contamination in non-commercial food sources. As awareness and monitoring of PFAS in food grows, utilization of this method offers a robust, high-confidence solution with limited user interaction -- ideal for researchers and laboratories seeking to expand testing capabilities that meet and outperform regulated methods as well as future-proof workflows for emerging PFAS compounds.

Debbie, collaborator

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