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Prevalence of perfluoroalkyl substances in paired batches of precooked and canned bovine meat and their implication on consumer safety

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ABSTRACT

Meat is a highly nutritious food but there is a lot of significant evidence of negative health outcomes related to its excessive consumption, especially for processed one. Among the variety of emerging contaminants of concern for human health, a key role is played by poly- and per-fluoroalkyl substances (PFASs), which show adverse effects in humans who are exposed to them through diet. In the present study, for the first time, 70 paired batches of precooked and canned bovine meat were analysed by Liquid Chromatography coupled to High Resolution Mass Spectrometry to evaluate the presence and concentration of 18 PFASs. These data were used to assess Italian consumers' health risks by performing the PFAS intake evaluation. PFBA and PFOS were detected in the precooked and canned meat samples, with PFBA mean concentration of 0.22 ± 0.36 ng g^{-1}, and <LOQ, respectively, and PFOS mean concentration of <LOQ in both. No PFASs were found in the jelly. The comparison between the PFBA levels in precooked and canned meat showed a significant difference. The PFAS intake evaluation showed an Estimated Daily Intake by far lower than the Tolerable Daily Intake for the average Italian consumer suggested by the European Food Safety Authority.

1. Introduction

There is a complex relationship between meat and healthy nutrition because meat is a highly nutritious food included as part of the dietary guidelines of all European Union (EU) countries (Epha, 2021). On the other hand, there is a lot of significant evidence of negative health outcomes related to the excessive consumption of meat, especially processed ones. Examples are related to coronary heart disease, stroke, and cancers, which have been extensively studied (Bingham, 1999; Micha et al., 2010).

As a part of food control related to the detection of contaminants of great interest, recent concerns are linked to possible chemical contaminations. In fact, among the wide variety of legacy and emerging contaminants of great concern for human health, a key role is played by poly- and per-fluoroalkyl substances, which have become a major issue in the last decades (Tittlemier et al., 2007) (PFASs). These environmentally persistent organic compounds are characterized by the presence of C–F bonds (Buck et al., 2011) and they have been produced since the 1950s for an ample range of applications in industrial processes and consumer goods due to their amphipathic characteristics (Torres et al., 2022). Their properties, such as thermo-resistance, made it possible to analyse these molecules not only in raw foods but also in cooked, pre-cooked, and processed ones (Schaider et al., 2017; Susmann et al., 2019).

Nowadays, there is a great interest in the contribution of PFASs that may occur not only from the environment but also from several pathways, including diet, which represents one of the major possible routes of exposure for humans (Tittlemier et al., 2007; EFSA, 2020). Particularly, for food of animal origin, PFAS contamination may result directly from packaging (Begley et al., 2005) or can be caused by animal exposure to contaminated air, soil, water, and feed (Tittlemier et al., 2007). PFAS's adverse effects on human and animal health underline the importance of investigating their presence in different food matrices, thus this topic has been investigated by many researchers. After oral ingestion, PFASs can accumulate in human blood serum and tissues. Particularly, the immune, endocrine, and reproductive systems are the most affected by PFAS exposure in humans. (Gagliano et al., 2020).

Concerns on the detection of PFASs in meat are related to the most

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recent European Regulation 2022/2388 (EU, 2022) which set limits for perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), and their sum, in bovine animals, pig, and poultry meat. Particularly, the limits set are 0.30, 0.80, 0.20, and 0.20 μg kg^{-1}, for PFOS, PFOA, PFNA, and PFHxS, respectively. Moreover, in these matrices, the limit for the sum of PFOS, PFOA, PFNA, and PFHxS is 1.3 μg kg^{-1}. It is important to note that they are set only for fresh products, meanwhile, there are no limits set for processed products such as canned meat. Thus, to evaluate the safety of this food we referred to the Tolerable Weekly Intake (TWI) of 4.4 ng kg^{-1} bw per week as the sum of these four major PFASs established by the European Food Safety Authority (EFSA) in 2020 (EFSA, 2020). Moreover, there has been increasing concern on the topic because the "International Agency for Research on Cancer" (IARC) categorized recently PFOA as "carcinogenic to humans" (Group 1) as well as PFOS as "possibly carcinogenic to humans" (Group 2B) (Zahm et al., 2024). These decisions were based on substantial evidence of cancer in experimental animals and compelling mechanistic evidence observed in exposed humans for PFOA, while for PFOS there was strong evidence from various tests, including human studies showing epigenetic changes and immunosuppression (Zahm et al., 2024).

According to consumption data, a Crea (Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Council for Agricultural Research and Analysis of Agricultural Economics) report on fresh and processed meat consumption in Italy (Crea) shows that among 4 categories (children, adolescents, adults, and elders) an average of 43 and 27 g/die of fresh and processed meat are consumed, respectively. Detailed information is reported in Table S1 in the supplementary material.

In the scientific literature several studies are reported related to the detection of PFASs in different food matrices such as fish (Chiesa et al., 2022; Valsecchi et al., 2021; Fair et al., 2019; Nobile et al., 2023a; Chiesa et al., 2019), milk (Barbarossa et al., 2014), vegetable (Herzke et al., 2013), game animal (Arioli et al., 2019), and eggs (Nobile et al., 2023 b; Zafeiraki et al., 2016). In this scenario, there is a lack of information about meat, despite its regular consumption, including raw, pre-cooked, and canned meat. Moreover, some studies report the detection of PFASs in canned and processed products (Genualdi et al., 2022; Mohamad Haron, Yoneda, Ahmad and Aziz, 2023), but still less information is present regarding bovine meat and ready-to-eat bovine meat products. To underline the need for data in these matrices, it is interesting to note that the dietary patterns of the Italian citizens have been influenced by the measures implemented to address the COVID-19 pandemic (Nobile et al., 2024). In fact, ready-to-eat products, and canned goods, particularly meat and tuna, have experienced a notable surge in sales, with a 66% and 36% increase, respectively, compared to the period preceding the lockdown (Nobile et al., 2024). Considering the importance of understanding how each food contributes to the daily intake of PFASs, to analyse and exploring potential PFAS contamination in fresh and processed canned meat has become an outstanding need.

For all the previously mentioned reasons, the present study aims to fill the lack in the scientific literature by evaluating, for the first time, the presence and concentration of 18 PFASs in 70 paired batches of pre-cooked and canned (ready-to-eat) bovine meat. In the author's knowledge, this is the first study that assessed consumer health risks related to the PFAS intake that may occur through ready-to-eat canned bovine meat among 4 consumer categories (children, adolescents, adults, and elders, all Italians). The instrumental analysis was performed by High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (HPLC-HRMS), which is the election instrumental method for these analytes in the considered matrices. Moreover, the results and the implications of the study will help to understand whether the canning process may affect the concentration of PFASs in the ready-to-eat product. Particularly, the analysed canned meat is a product consisting of selected lean meat in vegetable jelly and honey. Consequently, in our study, the detection of PFASs has been performed also in the jelly to investigate the possible changes in PFAS concentration that may occur during the industrial process due to its addition.

2. Materials and methods

2.1. Chemical and reagents

Chemical Research 2000 Srl (Rome, Italy) provided: perfluorobutane sulphonic acid (PFBS), PFHxS, PFOS, perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), PFNA, perfluoroundecanoic acid (PFUnDA), perfluorohexadecanoic acid (PFHxDA), perfluoropentanoic acid (PFPeA), PFOA, perfluoroheptanoic acid (PFHpA), PFDA, perfluorododecanoic acid (PFDoA), perfluorooctadenoic acid (PFODA), perfluorootridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) and both 13C-labeled internal standards (ISs) perfluoro [1,2,3,4–13C4] octanesulfonic acid (MPFOS) and perfluoro-[1,2,3,4,5–13C5] nonanoic acid (MPFNA). Merck (Darmstadt, Germany) provided all the analytical LC-MS grade solvents and reagents used. Phenomenex (Torrance, California) provided the cartridges Strata PFAS (WAX/GCB), 200 mg/50 mg/6 mL.

2.2. Sample collection

The total number of samples was 140 (70 pre-cooked bovine meat samples and 70 finished canned products). Particularly, the provenience of the pre-cooked samples was Brazil (60 samples), and France (10 samples). Furthermore, 3 different batches of jelly were analysed, by creating a pool of 10 samples from the same batch. To verify the significance of the sample size the following formula has been used (Naing et al., 2006):

$$N = Z^2 \times [P \times (1\text{-}P)]/D^2$$

Where: Z has the value of 1.96 for a confidence limit of 95%, P is the expected prevalence (set at 0.5 to obtain the highest size value) and D is the precision of the estimate.

The consequent precision value obtained was 12% for both. Given the challenges associated with the availability of both precooked and canned meat, we deemed the outcome acceptable.

2.3. Standard solutions

The stock solutions of perfluoroalkyl substance were prepared at the concentration of 1 mg mL^{-1} in methanol and preserved at −20 °C. The working solutions (10 and 100 ng mL^{-1}) were prepared in methanol daily and kept at 4 °C.

2.4. Extraction protocol

The protocol used in the present study followed the one clearly described by Nobile et al. 2023 b. Concisely, the homogenized samples (5 g) were spiked with internal standards (5 ng g^{-1}). 10 mL of acetonitrile was added, and the samples were vortexed (1 min), sonicated (10 min), and centrifuged (2500×*g*, 4 °C, 10 min). The supernatant was dried and then resuspended in 5 mL of water. Samples were purified by STRATA PFAS cartridges and the extracts were resuspended in 200 μL of mobile phase (20 mM MeOH: ammonium formate (20:80 v/v). The final extracts were centrifuged in an Eppendorf tube and then transferred in a vial for the instrumental analysis.

2.5. UHPLC-HRMS analyses

A Vanquish system (Thermo Fisher Scientific, Waltham, United States) with a Thermo Orbitrap™ Exploris 120 and a heated electrospray ionization (HESI) source was used for the instrumental analysis. Chromatographic separation was achieved using a Raptor ARC-18 column (5

μm, 120 × 2.1 mm) (Restek, Bellefonte, United States). To delay PFASs present in the system, a small Megabond WR C18 column (5 cm, 4.6 mm, i.d. 10 mm) was used before injection. The mobile phases were 20 mM aqueous ammonium formate (A) and methanol (B) at a flow rate of 0.3 mL min^{-1}. The gradient started with 20% B, increased to 95% in 7 min, held until the 10th minute, then returned to initial conditions by the 15th minute.

Detector parameters: capillary temperature at 330 °C, vaporizer at 280 °C, sheath gas at 35 AU, auxiliary gas at 15 AU, and electrospray voltage at 3.50 kV in negative mode. The full scan (FS) acquisition had a resolution of 60,000 FWHM, a scan range of 150–950 m/z, standard automatic gain control (AGC), RF lens at 70%, and an automatic maximum injection time. Parallel reaction monitoring (PRM) mode had a resolution of 15,000 FWHM, standard AGC, automatic maximum injection time, and an isolation window of 1 m/z. Two-step normalized collision energies (10 eV and 70 eV) improved precursor fragmentation. The software used was Xcalibur™ 4.5 (Thermo Fisher Scientific, Waltham, United States).

2.6. Validation of the method

The method validation was performed in accordance with the SANTE 11312/2021 guidelines (SANTE, 2021). Its selectivity was assessed by the injection of extracted pre-cocked and canned blank meat samples. The lack of signal, close to the retention times of the expected PFASs with a signal–to–noise ratio (S/N) < 3, indicated the absence of interferences. The calibration curves were created from 6 calibration points (0, 0.050, 1.0, 3.0, 5.0, 10) by spiking 5g of blank sample with the working solution, each point was done in duplicate. The limit of quantification (LOQ) was obtained from the lowest spiked level (characterized by a recovery range of 70–120%, an RS <20%, and a signal-to-noise ratio of at least 10). The coefficient of variation (CV%), expressing the intraday repeatability, was evaluated across 5 replicates. Meanwhile, the within-day precision was evaluated across 5 replicates prepared and analysed in 3 days, using a one-way analysis of variance (ANOVA). Recovery was evaluated by comparing the concentrations of PFASs spiked before extraction with those inserted at the end of the extraction protocol. The matrix effect percentage was defined by comparing the PFAS peak areas after the extraction of a blank sample and the areas of the standard peaks in a solution mix.

2.7. Statistical analysis

The software "Graphpad Instat 3" (Graphpad Instat Software, San Diego, CA, USA) was used to perform the statistical analyses. Particularly, the Mann-Whitney Test was used to compare data from two populations, since our data were not normally distributed. Significant difference was set at $P < 0.05$.

2.8. Intake evaluation protocol

The following formula was used to calculate the PFAS Estimated Daily Intake (EDI):

$$EDI = C \times DC/BW$$

Where C is the maximum sum of the four main PFASs (PFOA, PFOS, PFNA, PFHxS, which are the ones regulated by the European Regulation 2022/2388 (EU, 2022) found in the analysed canned meat samples and DC is the daily consumption per capita of the product in Italy, and BW is the consumer's body weight. Consumption data are reported in Table S1. Specifically, the Crea data (Crea,) on processed meat consumption illustrated above refer to total consumption of cured meats and canned meats. Since it is not possible to divide the various contributions, a precautionary approach was applied in the present work by considering the total consumption equal to canned meat consumption only. As the same approach, the 50th weight for Italian females in the relevant categories of children (3 years old, 14,5 kg) and adolescents (10 years old, 35 kg) were taken into account when calculating the EDI for these categories. Data were obtained from the report "Italian cross sectional growth charts for height, weight, and BMI (2–20 yr)" (Cacciari et al., 2006). For adults and elders, an average bodyweight of 70 kg was considered.

3. Results and discussion

3.1. Validation parameters

Table 1 shows the obtained values for the validation parameters which satisfy all the requirements set by SANTE 11312/2021 guidelines. The method exhibited notable selectivity, evidenced by a signal-to-noise ratio exceeding 10 from the limit of quantification (LOQ) onwards, and a marked specificity characterized by the absence of interference proximal to the retention times of the considered PFASs. Conformance to identification criteria, including retention time stability in comparison to standard mix solutions, aligned with the considered guidelines. Recoveries spanning from 70% to 120% underscored the commendable efficacy of the extraction and purification protocols. Repeatability and precision, as denoted by coefficients of variation (CVs) ≤20%, met the designated tolerances for these validation parameters. The established LOQs (0.10 ng g^{-1}), underscored the method's heightened sensitivity.

Matrix calibration curves exhibited robust linearity, manifesting a satisfactory fit across the six calibration points, with an R^2 exceeding 0.991 for all PFASs assessed. Furthermore, the observed matrix effect exerted minimal influence (<20%), with percentage changes ranging from 91% to 105%.

3.2. PFAS concentration in pre-cooked and canned bovine meat

Among the 18 investigated molecules, only 2 PFASs were detected in the samples: PFBA and PFOS. The PFBA highest values detected were 0.19 ng g^{-1} (canned meat) and 1.81 ng g^{-1} (pre-cooked meat). Instead, the PFOS highest values detected were lower than LOQ (canned meat) and 0.13 ng g^{-1} (pre-cooked meat). All the results are reported in Table S2 provided in the supplementary material. PFBA was found in 45 and 49 samples for pre-cooked and canned meat, respectively, with mean concentration of 0.22 ± 0.36 ng g^{-1} in precooked meat, meanwhile, in canned meat the mean value was lower than the LOQ. PFOS was found in 27 and 23 samples in precooked and canned meat, respectively, with mean concentrations of <LOQ in both. For a conservative approach, the mean concentrations were calculated considering only the samples in which PFASs have been detected, and as half of the LOQ the samples detected with a PFAS concentration value lower than the LOQ (Nobile et al., 2023b). For the same reason, we considered the mean values instead of the medians because they were higher.

To have a complete idea of the results, Fig. 1 illustrates the number of samples in which PFBA and PFOS have been detected in the analysed precooked and canned samples, focusing the attention on the number of samples in which PFBA and PFOS were found with a value lower than the LOQ, while Fig. 2 reports the boxplot that illustrates the value distribution of PFBA and PFOS for each matrix. For a statistical treatment of the data, to compare the values of PFBA and PFOS detected in precooked meat with the ones detected in canned meat, the Mann-Whitney test was performed, showing that the difference between concentrations of PFBA in the two analysed groups of products was highly significant (two-tailed P value of 0.0402). Meanwhile, the difference between the concentrations of PFOS was not considered significant (two-tailed P value of 0.1061).

None of the 18 investigated PFASs was found in the analysed jelly, and this may justify the significant difference in concentration of PFBA between the precooked and canned meat. In fact, the canned meat is composed of lean meat and jelly thus we may hypothesize that there

Table 1
PFAS compounds and their formula, parent exact mass, recovery and precision.

Compound	Formula	Parent exact mass [m/z]	LOQ (ng g^{-1})	Recovery %	Intra–day CV[a] %	Inter–day CV[a] %
PFBA	C4HF7O2	212.97920	0.10	119	6	19
PFPeA	C5HF9O2	262.97601	0.10	115	13	12
PFBS	C4F9HO3S	298.94299	0.10	107	9	13
PFHxA	C6HF11O2	312.97281	0.10	121	4	10
PFHpA	C7HF13O2	362.96962	0.10	116	5	9
PFHxS	C6F13HO3S	398.93660	0.10	90	6	8
PFOA	C8HF15O2	412.96643	0.10	117	4	8
PFNA	C9HF17O2	462.96323	0.10	91	11	18
PFOS	C8F17HO3S	498.93022	0.10	91	13	17
PFDA	C10HF19O2	512.96004	0.10	75	13	17
PFUnDA	C11HF21O2	562.95684	0.10	75	7	17
PFDS	C10F21HO3S	598.92383	0.10	97	9	13
PFDoA	C12HF23O2	612.95365	0.10	75	8	13
PFTrDA	C13HF25O2	662.95046	0.10	75	5	9
PFTeDA	C14HF27O2	712.94726	0.10	71	17	20
PFHxDA	C16HF31O2	812.94088	0.10	74	19	20
PFODA	C18HF35O2	912.93449	0.10	75	18	20
C6O4	C6F9O6	338.95570	0.10	79	9	13

[a] five replicates.

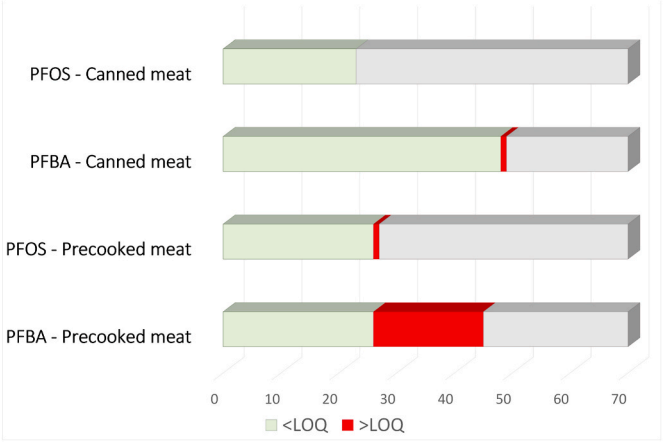

Fig. 1. Number of samples in which PFBA and PFOS have been detected in the analysed precooked and canned samples, on a total number of 70 samples each. In green, the number of samples in which PFASs have been detected with a value lower than the LOQ on the total of each group.

could be a dilution effect operated by the addition of jelly. These novel data and considerations show the necessity of studying and understanding how the different ingredients in the processed foods may contribute to the final concentration of PFASs in the retail products.

According to the scientific literature, PFOS, PFHxS, and PFOA are the main PFASs detected in livestock animal studies (Death et al., 2021) and it is well known that the liver is the target organ for PFASs. Regarding cattle, studies demonstrated that PFAS accumulation is higher in blood, liver, and kidney despite fat and muscle (Death et al., 2021). In fact, measured concentrations of these contaminants in muscle (meat) are generally lower than the ones found in blood and offal (Death et al., 2021). Particularly, a lower accumulation in muscle is demonstrated for shorter-chain PFAS (e.g. PFHxS and PFBS) compared to the long-chain compounds (e.g. PFOS) (Lupton, Huwe, Smith, Dearfield, & Johnston, 2012; Lupton, Huwe, Smith, Dearfield, & Johnston, 2014). The higher occurrence of shorter chain compounds like PFBA in food of animal origin suggests the adoption of alternative compounds in new fluorinated materials. These substitutes, such as PFBA, are used as safer alternatives to longer-chain molecules (Wang et al., 2013). These considerations may justify the detection of only two analytes in the samples, considering that the analysed samples consisted of high quality selected lean meat. As mentioned before, the scientific literature lacks in

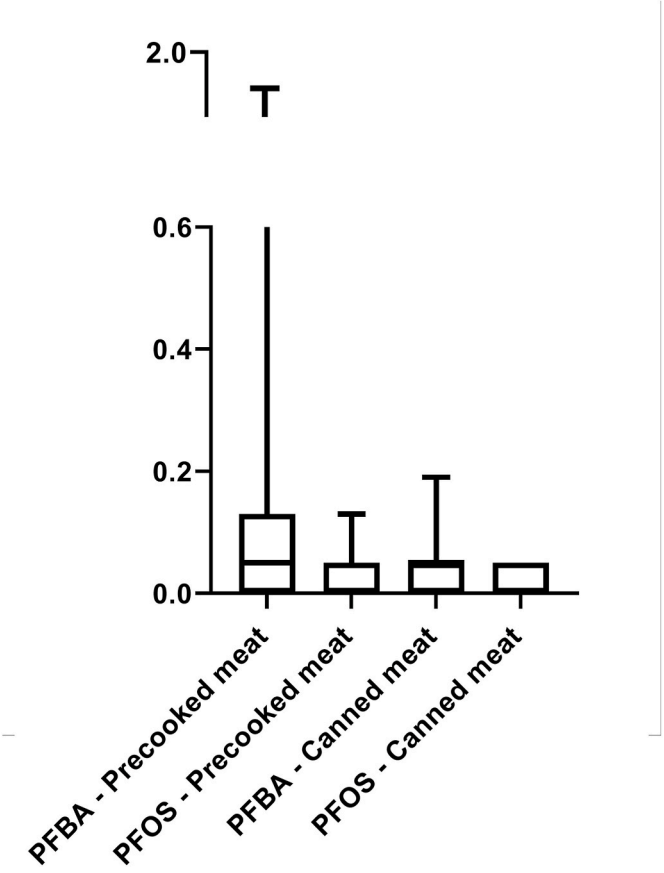

Fig. 2. Distribution values of PFBA and PFOS for each matrix, the values were obtained with the middle-bound approach, thus considering all the samples and a value of half of the LOQ when the analytes are detected in a value < LOQ.

report results regarding the presence of PFASs in meat samples intended for human consumption. Although only two PFASs were detected in the present study, these findings and considerations may suggest to investigate the PFAS presence also in other type of meat products, to deepen the topic and explore potential PFAS accumulation in fatter meat matrices and processed meat products.

3.3. PFAS intake evaluation

Since there are no limits set for processed products, we compared the concentration of PFOS detected in the analysed samples, which is the only one of the four main PFASs found in the samples, with a TDI of 0.63 ng kg^{-1} bw calculated on the 4.4 ng kg^{-1} bw TWI suggested by the EFSA for the sum of four PFAS (Table 2). PFOS was found in the canned samples with values always below the LOQ, as a middle-bound approach a concentration equal to half the LOQ was considered for the intake evaluation, which also corresponds to the maximum found in the samples.

The calculated EDIs were well below the Heath Based Guidance Value suggested by the EFSA for all the considered categories of consumers. Considering that the EDI for children and adolescents was calculated on the lowest bodyweight, the evaluation applies also to male consumers with a higher one.

It is necessary to underline that all the foods and beverages included in our diet, among the several exposure pathways (air, water, and dermal) are the main source of PFASs (Ragnarsdóttir et al., 2024). Thus, these evaluations are fundamental not only to fill a gap in the scientific literature but also to give important information to consumers. It is crucial to understand how each food can contribute to the total PFAS intake, especially regarding ready-to-eat products, such as canned foods, whose sales have increased compared to the last decade.

4. Conclusion

In the present study, to fill the lack of data regarding the detection of PFASs in bovine meat, 18 PFASs were investigated in precooked and canned bovine meat, and in the jelly used to prepare canned meat. Among the 18 investigated molecules, only 2 PFASs were detected in the meat samples (PFBA and PFOS) while no investigated molecules were detected in the jelly. Particularly, PFBA and PFOS were detected in the precooked and canned meat samples, with PFBA mean concentration of 0.22 ± 0.36 ng g^{-1} and <LOQ, respectively, and PFOS mean concentration of <LOQ in both. The comparison between the PFBA levels in precooked and canned meat showed a significant difference that could be related to a dilution effect due to the addition of vegetable jelly. Furthermore, the recent European Commission Regulations (EU, 2022) set maximum levels for the four main PFASs (PFOS, PFOA, PFNA, PFHxS), and their sum in several foodstuffs, including bovine meat, referred to their fresh weight, meanwhile no limits were set for processed products. For this reason, we assessed Italian consumers' health risks in canned bovine meat by performing the PFAS intake evaluation by comparing the exposition with the TWI suggested by the EFSA. These results showed that the consumption of canned meat does not represent a risk for Italian consumers, also accounting for high consumers. In fact, the PFAS intake through canned meat cover less than the 0,5% of the TDI suggested by the EFSA.

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CRediT authorship contribution statement

Maria Nobile: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara Panseri:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dalia Curci:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Luca Maria Chiesa:** Supervision, Resources, Project administration. **Sergio Ghidini:** Supervision, Project administration. **Francesco Arioli:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization.

Table 2
Estimated daily intake of PFOS in canned meat among 4 categories considering the mean, P95th, and P99th consumption percentile. The last column shows the percentage of the TDI covered by the calculated EDI.

EDI canned meat	Mean (ng kg^{-1} b.w. die)	P95 (ng kg^{-1} b.w. die)	P99 (ng kg^{-1} b.w. die)	Percentage of TDI %
Children (3–9 years old)	0,079	0,25	0,31	0,49
Adolescents (10–17 years old)	0,050	0,13	0,21	0,33
Adults (18–64 years old)	0,000020	0,06	0,091	0,14
Elders (65–97 years old)	0,014	0,048	0,071	0,11

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2024.114910.

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