MSc.THESIS

WORKU DEJENE BEKELE

2024

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

BUDA CAMPUS

INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

MASTER'S THESIS

SCREENING OF PER AND POLY-FLUOROALKYL SUBSTANCES (PFAS) IN FOOD MATRICES IN HUNGARY

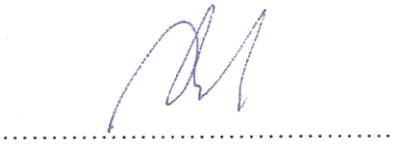
Supervisor: Marczika Andrásné Dr. Sörös Csilla

Department of Chemistry and Analysis

Created By: Worku Dejene Bekele

Budapest, Hungary

2024.4.26



Head of Department

Supervisor

1. **CONTENTS**

2 . LIST OF ABBREVIATION

AA…….....Acetic acid

C-F…….....Carbon-fluorine (C-F) bonds

DLLME…….....Dispersive liquid-liquid microextraction

D-SPE…….....Dispersive solid phase extraction

ECHA ……European chemicals agency

EPA…….....Environmental protection agency

ESI……..Electrospray ionization

FDA …….……..Food and drug administration

HFPO-DA…….…….....Hexafluoropropylene oxide-dimer acid

HPLC …….....High-performance liquid chromatography

HPLC/MS/MS ……...High-performance liquid chromatography-tandem mass spectrometry

IAFNS…….....Institute for the advancement of food and nutrition sciences

IPE……..Ion-pair extraction

LC/MS/MS ……Liquid chromatography/tandem mass spectrometry

LC……...Liquid chromatography

LC- UV ……..Liquid chromatography with ultra violet

LC-MS/MS…….Liquid chromatography-mass spectrometry

LC-QToF-MS…….Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

LLE…….Liquid-liquid extraction

LOD…….....Limit of detection

MeOH….. ……..Methanol

MRM …….Multiple reaction monitoring

OECD……Organization for economic cooperation and development

PAP ……...Polyfluoroalkyl phosphate ester

PFAA …….....Perfluoroalkyl acid

PFAS …….....Per- and polyfluoroalkyl substances

PFAS……..Per- and polyfluoroalkyl substance

PFASA……...Perfluoroalkane sulfonamide

PFASAA …….....Perfluoroalkane sulfonamido acetic acid

PFASE…….....Perfluoroalkane sulfonamide ethanol

PFBA……..Perfluorobutanoic acid

PFBS…….…..Perfluorobutane sulfonic acid

PFCA……..Perfluoroalkyl carboxylic acid

PFDS ……..Perfluorodecane sulfonic acid

PFHpS…….Perfluoroheptane sulfonic acid

PFHxS ……....Perfluorohexane sulfonate

PFOA…….....Perfluorooctanoic acid

PFOPA…….....Perfluorooctyl phosphonic acid

PFOS …….Perfluorooctane sulfonic acid

PFOSA……...Perfluorooctane sulphonamide

PFPA……..Perfluoroalkyl phosphonic acid

PFPS ……..Perfluoropentane sulfonic acid

PFSA……..Perfluoroalkane sulfonic acid

PFTrDS…….…Perfluorotridecane sulfonic acid

PFUnDS…….Perfluoroundecane sulfonic acid

QuEChERS……Quick, Easy, Cheap, Effective, Rugged, and Safe

SPE ……...Solid phase extraction

SPME……Solid-phase microextraction

TWI……..Tolerable weekly intake

UHPLC…….....Ultra high-performance liquid chromatography

UHPLC-MS/MS…...Ultra high-performance liquid chromatography-mass spectroscopy

3. LIST OF TABLE

4. LIST OF FIGURE

5. INTRODUCTION

According to the European Chemical Agency, per and poly-fluoroalkyl substances (PFAS) are a group of synthetic chemicals characterized by carbon atoms linked to each other and bonded to fluorine atoms (Genualdi *et al.*, 2022).

PFASs have a robust, stable backbone in addition to the possibility of terminal carboxylic acids, sulfonic acid, alcohol, phosphate, or amide groups. Because of this structure, PFASs have unique properties such as hydrophilic terminal groups, and lipophilicity, and are chemically stable (Rawn, Ménard, and Feng, 2022).

Compounds classified as polyfluoroalkyls have a partially fluorinated hydrophobic chain, while perfluoroalkyl compounds have a completely fluorinated chain except for the H atom, which would alter the structure of a functional group. Under certain environmental conditions, such as ecological degradation, poly-fluoroalkyl compounds can transform into perfluoroalkyl substances (Carnero *et al.*, 2021a).

The class of synthetic chemicals known as PFAS, is broad and is used extensively in various sectors. These fluorinated compounds exhibit unique properties due to the strong carbon-fluorine bond. Carbon-fluorine (C-F) bonds are powerful in organic chemistry and present in all PFAS. Because of this strong connection, PFAS are resistant to deterioration in the environment and throughout usage. They are useful when heat resistance is essential since they can tolerate high temperatures without degrading. Properties such as the high electronegativity give many PFAS their mutually hydro- and lipophobic (stain-resistant) and surfactant properties and make them thermally and chemically stable (Pasecnaja, 2022)**.**

Certain per- and poly-fluoroalkyl substances can lower surface tension between liquids and solids. Products like firefighting foams, waterproof textiles, and nonstick cookware are impossible without surfactants. Regrettably, the potency of PFAS also plays a role in their environmental persistence. They accumulate in soil, water, and food because of their resistance to natural degradation processes. Persistent flame-retardants PFAS are amenable to long-distance environmental migration. They complicate cleanup operations by contaminating soil, surface water, and groundwater (Carnero *et al.*, 2021b).

The broad range of applications for PFASs is determined by the characteristics of highly fluorinated alkyl chains, including their high stability, heat resistance, surfactant activity, and capacity to repel stains, oils, and water. Highly fluorinated PFASs are used in a variety of products and environments, including paper, cleaning supplies, food packaging, surfactants, and firefighting equipment. The two main processes used to create PFASs are

electrochemical fluorination and telomerization. Whereas electrochemical fluorination yields a mixture of branched and linear isomers, telomerization solely yields materials with a linear alkyl chain. The PFAS compounds exhibit thermodynamic stability and resistance to several mechanisms of degradation, such as oxidation, reduction, hydrolysis, metabolism, microbial degradation, and photolysis, due to their C–F bond strength, which is around 485 kJ mol−1 E. and B. V. and Z. D. Pasecnaja, vol. 2022, vol. 287).

Thus, PFASs are inert chemically, thermally, and physiologically; however, the molecular weight and amount of C–F linkages in a given molecule greatly influence these qualities.Perfluoroalkyl carboxylic acids (PFCAs) such as perfluorooctanoic acid (PFOA) and perfluoroalkyl sulphonic acids (PFSAs) like perfluoro octane sulphonate (PFOS) are the most researched class of long-chain PFASs (Pasecnaja, 2022).

Concerns have been raised about PFAS chemicals because of their environmental stability, ability to bioaccumulate in organisms, and possible negative health impacts. The usage of firefighting foams containing PFAS has resulted in food contamination in communities close to military bases and industrial areas. This is only one example of the noteworthy occurrences of PFAS contamination in the United States. As a result of these occurrences, PFAS pollution in the impacted areas is being addressed through regulations and health concerns (Genualdi et al., 2021).

One of the main ways that people are exposed to PFASs is through food, much like with many other organic compounds that are of concern to the environment. Several researchers have recently measured the amounts of PFASs in food items, and some studies have also evaluated dietary consumption of some of the most well-known PFASs, primarily PFOS and PFOA, as they cause significant food safety problems and affect human health (Domingo and Nadal, 2017)**.** This study aims to screen which foods in Hungary are contaminated with PFAS. By screening a diverse group of food items and analyzing various PFASs, these studies seek to identify contaminated food matrices and formulate corrective measures to safeguard public health.

6. OBJECTIVE

The objective of this study is to Screen PFAS in food matrices in Hungary. In examining the potential contaminations of PFAS within the Hungarian food chain, several critical inquiries arise. These include:

- What are the levels of PFAS contamination in various food matrices commonly consumed in Hungary?

- Are there significant differences in PFAS contamination levels between different types of food products? Are there any specific food items or food categories that consistently show elevated levels of PFAS contamination?
- How do the levels of PFAS contamination in Hungarian food compare to regulatory limits or guidelines established by national or international authorities?

7. LITERATURE REVIEW

7.1 Sources And Pathways of PFAS Contamination of Food

PFAS can enter the food chain through multiple pathways, with environmental contamination being a primary source. Various sources of environmental contamination, including industrial discharges, wastewater treatment plants, landfills, and atmospheric deposition. These sources release PFAS into the environment, where they can accumulate in soil, water bodies, and crops, ultimately leading to bioaccumulation in the food chain. Agricultural practices such as the use of biosolids or irrigation with contaminated water can intensify PFAS contamination in crop foods (Torres & De-la-Torre, 2023).

Moreover, PFAS can also enter the food chain through direct applications in food packaging and processing. Packaging materials treated with PFAS, such as food wrappers and containers, can leach these chemicals into food, particularly fatty and acidic foods that have a higher affinity for PFAS migration. Additionally, PFAS-based compounds are used in food processing equipment to provide non-stick properties, posing another pathway for contamination. The migration of PFAS from packaging or processing equipment into food products has been documented in several studies, highlighting the need for stricter regulations and alternatives to PFAS in food contact materials (Lewis et al., 2022).

Once PFAS contaminate food, they undergo bioaccumulation in animals and humans upon ingestion. Livestock animals can accumulate PFAS from contaminated feed and water, leading to the presence of these chemicals in meat, dairy, and other animal-derived products. Torres emphasize the role of bioaccumulation in amplifying human exposure to PFAS via the consumption of animal-based foods. Furthermore, PFAS has been detected in various seafood types, indicating contamination of aquatic ecosystems and subsequent transfer to humans through seafood consumption(Torres and De-la-Torre, 2023).

Commercial compounds known as fluorotelomer-based polyfluoroalkyl phosphate mono, di, and tri-esters (mono-, di-, and triPAPs, respectively) are used to make paper and board food packaging materials that are grease and water-proof fluorotelomer based compounds are being produced at a faster rate than other highly fluorinated surfactants, such as C8-chemistry perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), which have lately been phased out or reduced in Europe (E. and B. V. and Z. D. Pasecnaja, 2022).

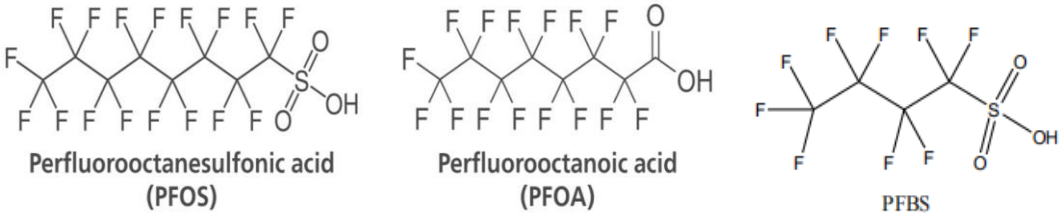


Figure 1:Chemical structure of perfluorobutane sulfonic acid (PFOS), Perflourobutanoic acid (PFBS) and perfluorooctanoic acid (PFOA)

Per- and poly-fluoroalkyl substances, or PFAS, are found in food through various pathways and sources, including industrial emissions, consumer product us--e, and disposal. When different materials containing these compounds are manufactured, applied, and disposed of, PFAS are released into the environment. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are examples of legacy emissions that have historically been connected to product use and manufacturing processes, respectively. Nonetheless, recent initiatives, such as stewardship programs and phaseouts of chemical production, have reduced the amounts of certain PFAS in the environment, demonstrating the effectiveness of concerted action. Aqueous film-forming foam (AFFF) use, manufacturing sites, and wastewater treatment plants are examples of local-scale pollution sources that raise the levels of PFAS in soil, water, and eventually food sources. It is advised that government, business, and academics work together to improve understanding of the effects of PFAS emissions on the environment and to increase transparency. The focus of efforts is also on precursor chemicals, which break down into terminal PFAS. This emphasizes the necessity of thorough monitoring and management plans to reduce PFAS contamination in a variety of environmental compartments (De Silva *et al.*, 2021).

According to Minet, the analysis represents the initial approximation of PFAS flows utilized in food packaging, with 9000 and 940 tonnes annually in the US and Canada, respectively. More than 99 percent of this was polymeric. It is resulting from the estimated 2% of food packaging that intentionally contains PFASs. Even with this low incidence of PFAS usage, there is still a chance that all waste products, including those meant for recycling, landfilling, and litter, could be contaminated (Minet *et al.*, 2022).

Point sources, such as industrial sites, and non-point sources, such as food packaging and contaminated water, are major sources of exposure for humans. Because of the chemical characteristics of PFAS, there is a risk of agricultural contamination through transport processes such as migration to groundwater, surface water overflow, and atmospheric deposition. While European data demonstrate the presence of PFAS in food, the lack of US

studies prevents a thorough risk evaluation and the development of regulatory guidelines (Vorst *et al.*, 2021a).

7.2 Effects Of Polyfluoro Alkyl Substance (PFAS) On Food Safety

There has been a noticeable increase in the attention paid to the safety of food to prevent accidental contamination. The primary emphasis of this attention has been on using PFAS in food (Vorst *et al.*, 2021b).

Because they can enter the food chain through several channels, pervasive and widely used PFAS present serious risks to food safety. Concerns about human exposure are raised by this pollution since PFAS has been linked to harmful health impacts. Furthermore, the stability of PFAS chemicals poses difficulties for food safety oversight and regulation, requiring all-encompassing approaches to reduce risks and protect public health (Vorst *et al.*, 2021a).

A growing amount of focus has been placed on the safety of food from unintended contamination due to health, environmental, behavioral, and regulatory concerns. PFAS, which are utilized in industries to provide oil and moisture resistance, have received a lot of attention. In 2020, a two-day virtual symposium on PFAS in food risk assessment was organized to discuss consumer exposure to PFAS in food, both now and in the future, and health issues. This symposium was hosted by ILSI North America. The seminar also attempted to identify knowledge gaps that may be filled by the Institute's collaborative research methodology around PFAS in food risk assessment (Vorst *et al.*, 2021b).

7.3 Healthy Effect and Toxicology of PFAS Compounds

Many studies have shown that PFAS has detrimental health effects in experimental animals, including hepatotoxicity, developmental toxicity, neurobehavioral toxicity, immunotoxicity, reproductive toxicity, lung toxicity, hormonal effects, and a weak potential for genotoxicity and carcinogenesis. These findings have raised concerns about PFASs and public health (EFSA, 2012).

The study emphasizes how the persistent nature of several PFAS chemicals raises concerns about human health, even if they provide desired characteristics like oil and water repellence in food packaging materials. In particular, long-chain PFAS chemicals have been connected to several health hazards because of their extended half-lives in the human body (Carnero *et al.*, 2021b).

According to toxicological evaluations, exposure to some PFAS compounds, especially the long-chain ones, can have a major negative impact on a person's immunological, endocrine,

and reproductive systems. Toxicological assessment of PFAS chemicals is further complicated by the vast variation in their chemical structure and behavior (Fenton *et al.*, 2021). There are still debates over acceptable exposure levels and the suitability of the regulatory frameworks in place, even though efforts to mitigate the exposure to perfluorochemicals are becoming more and more focused (Pasecnaja, Bartkevics, and Zacs, 2022).

The scientific opinion on the risk of exposure to persistent PFASs on human health was published by EFSA in September 2020. The organization also set a tolerable weekly intake (TWI) of 4.4 ng kg−1 body weight for the total of the four priority PFASs (Σ4PFASs), which are PFOA, PFNA, PFHxS, and PFOS. Out of all the PFASs for which the exposure was assessed, these four PFASs accounted for around half of the total. These substances exhibit comparable lengthy half-lives, accumulation patterns, and toxicokinetic characteristics in humans. The selection of the combination technique in the risk evaluation was based on these parallels in chemical characteristics and consequences (Pasecnaja, 2022).

According to studies, exposure to PFAS may affect fetal development and immune system performance and may raise the chance of developing specific cancers. These health hazards emphasize how crucial it is to control and monitor the amounts of PFAS in food and the environment to safeguard human health (Genualdi *et al.*, 2021).

Numerous PFASs are known to be harmful to human health and to be environmentally persistent organic pollutants. One's diet is believed to be the primary way that PFASs are exposed (EFSA, 2012). PFAS chemicals are present in food sources like fish, meat, and dairy products as well as in a variety of environmental media. Concerns regarding human exposure and possible health consequences are raised by the persistent and extensive usage of PFAS, which has resulted in its presence in the environment and food chain (Carnero et al.,2021b).

The existence of PFAS compounds in the environment and their possible effects on human health emphasize the necessity of ongoing regulation, monitoring, and remediation efforts to safeguard the environment and public health from these persistent chemicals' harmful effects (Genualdi *et al.*, 2021).

Knowing the present dietary intakes of PFASs is crucial to preventing health hazards from exposure to food, as is the case with any potentially harmful environmental pollutant. Food organizations should be aware of this issue because many countries still have very little information available (Domingo and Nadal, 2017).

7.4 International Regulations of PFAS In Foods

For food safety and compliance, it is crucial to accurately determine the presence of PFAS residues in virgin and recycled fiber feedstocks used to produce food-contact packaging materials. Several national and international food safety and environmental authorities are developing new limits on allowable levels and zero-tolerance prohibitions of PFAS in direct contact with food items. Such acceptable limits should ideally be determined via toxicological and risk assessment research. Unfortunately, neither a single organization nor a joint effort has committed the funds necessary to conduct a thorough toxicological analysis of all 5,000 distinct PFAS compounds (Curtzwiler *et al.*, 2021).

The Food and Drug Administration (FDA) in the United States (US) maintains a list of per- and poly-fluoroalkyl substances (PFASs) that are permitted for use in food packaging; however, details regarding the actual use of these PFASs in food packaging production, as well as whether manufacturers are utilizing non-approved PFASs, are not readily available. There is no public list of approved PFASs or regulatory control of PFAS use in food packaging. As a party to the Stockholm Convention, Canada, however, imposed limitations on the use, sale, and importation of PFOS and products containing PFOS in 2008, with certain exceptions. Similarly, in 2012, restrictions were placed on PFOA, its precursors, or long-chain or LC-PFCAs, and their salts. Recently, the Canadian parliament was presented with a proposed legislation that tightens these already stringent exemptions even further. This agreement was brought about by fresh research indicating substantial possible human health concerns from long-term food consumption. Furthermore, a recent report from the Organisation for Economic Cooperation and Development (OECD) has advocated further regulation of these chemicals in food packaging (Minet *et al.*, 2022).

PFAS have been found by the European Union in several food categories, mainly in meat and fish, with seafood having more significant quantities of the chemicals. States have varied regulation limits for per- and polyfluoroalkyl substances (PFAS), and the EPA has set a lifetime health advisory level for both in drinking water. The USDA regularly tests meat, poultry, and egg products for PFAS to guarantee food safety through FSIS (Vorst *et al.*, 2021b).

Producers of perfluoropolyether and other PFASs, such as shorter-chain homologs, have mostly replaced long-chain PFASs after the 2006 agreement by North American and

European producers. As firm as long-chain PFASs, short-chain alternatives are even more challenging to remove from water treatment systems. Because they can degrade into non-polymeric, persistent, and mobile PFASs and contain non-polymeric PFAS impurities, polymeric PFASs most notably side chain fluorinated polymers threaten human health and the environment. The potential for PFASs used in food packaging to pollute the environment and waste stream. It also offers a standard to measure the success of local efforts to restrict the use of PFASs in food packaging (Minet *et al.*, 2022).

PFAS regulation in food is a growing global problem. Research has indicated the presence of PFAS in a range of food products. To guarantee food safety, efforts are being conducted to evaluate the amounts of PFAS in various food sources. Seafood, meats, eggs, milk, and dairy products have all been identified by the European Food Safety Authority as essential sources of PFAS exposure in the human diet. The FDA has conducted studies on the amounts of PFAS in milk, seafood, and composite food items bought in the US. To safeguard the public's health, regulations and guidelines are being developed to track and manage the levels of PFAS in food products (Genualdi *et al.*, 2021).

7.5 Analytical Methods for PFAS Determination

The techniques of gas chromatography and LC with UV detection are not appropriate for the investigation of PFASs because they are not particularly volatile and lack appropriate chromophores in their structures. Most of the techniques involve the extraction and purification of the target molecule from the components of the sample matrix, and then instrumental analysis uses liquid chromatography (LC) in conjunction with quadrupole tandem mass spectrometry (LC-MS/MS) and electrospray ionization (ESI). Different extraction procedures are utilised to isolate PFASs from the food matrix depending on the kind of food product. The most popular ones are ion-pair extraction (IPE), liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), dispersive solid phase extraction (dSPE), and dispersive liquid-liquid microextraction (DLLME). Solvents with a medium polarity, like methanol or acetonitrile, are used for extraction. Weak anion exchange SPE is commonly used to isolate PFAS components. Analytical chemistry uses the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample preparation technique extensively, especially for analyzing pesticides and other pollutants in food samples. This method, which was created in 2003 by Anastassiades and associates, consists of two primary steps: extraction and cleanup. utilising a combination of acetonitrile and a buffering salt, such as magnesium sulfate, analytes are extracted from the

sample matrix in the extraction step. Interfering chemicals are then removed through a partitioning step utilising dispersive solid-phase extraction (d-SPE) sorbents. Due to its many benefits, such as affordability, speed, and ease of use, laboratories all over the world favour this streamlined approach.

Various QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique may be utilized for further sample extract clean-up. These intricate cleanup techniques guarantee the successful elimination of co-extractives and the decrease of matrix effects (Pasecnaja, 2022).

Several analytical techniques, including high-resolution mass spectrometry, ultra-performance liquid chromatography-mass spectrometry, and liquid chromatography-tandem mass spectrometry (LC-MS/MS), can be used to determine the presence of PFAS in food. With these techniques, PFAS chemicals in food samples may be precisely and sensitively quantified, allowing for the evaluation of contamination levels and any health hazards (Genualdi *et al.*, 2021).

The preferred technique for the quantitative analysis of PFASs is the internal standardization-isotope dilution quantification approach, which guarantees a high level of precision and quality control. Given the richness of the PFAS family, it is strongly advised to expand the list of isotopically labeled representatives that are currently commercially available, even if a large range of PFCA and PFSA major representatives are covered by these surrogates (Pasecnaja, 2022).

The efficacy of the procedures was assessed by proficiency test schemes and interlaboratory comparison studies, demonstrating high accuracy and precision, low detection limits, and strong linearity. Analyte concentration measurements were made more accurate by adjusting for matrix effects using performance and surrogate standards, which were employed in recovery studies to evaluate accuracy and precision for various analytes in diverse food matrices (Rawn, Ménard, and Feng, 2022).

Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QToF-MS) was used for the accurate identification of different PAPs and their intermediate and end degradation product. This technique makes accurate identification and measurement of PFAS and its precursors possible. However, because some precursors might not be included in the study, using LC-QqQ-MS/MS may not be able to detect all PFASs. Furthermore, the extraction method such as concentrated ultrasonic solid-liquid extraction may have limits in

reproducibility and efficiency. More research is required to create more thorough techniques to identify a greater variety of PFASs and their precursors in food samples (Zabaleta *et al.*, 2017).

The total diet study by the FDA was used to identify and measure PFAS in processed foods. The techniques used were extraction, solid-phase extraction, and mass spectrometry analysis using liquid chromatography. It was especially highlighted that high-resolution mass spectrometry might be used to confirm the presence of PFAS in food samples. The research team took a multidisciplinary approach to analyzing PFAS in food matrices by involving experts from the FDA in analytical chemistry and food safety. During method optimization, issues, including chromatography related false positives and interference from cholic acids, were addressed, highlighting the precision of the analytical procedure. Liquid chromatography-mass spectrometry (LC-MS/MS) analysis was effective for analyzing a diverse set of food matrices for PFAS, highlighting the importance of utilizing a combination of techniques for comprehensive detection and quantification of these contaminants in food samples (Genualdi *et al.*, 2021).

Numerous analytical techniques have been created to identify and measure PFAS in food-related matrixes. PAPs in environmental samples were analyzed using LC/MS/MS. The approach involved separating the mono-, di-, and triPAPs on a column and using 1-MP to improve resolution. More precise quantification was ensured by addressing matrix effects using matrix-matched calibration curves. For accurate quantification, labeled internal standards were essential, and the technique demonstrated low detection and quantification limits for several PAPs. Furthermore, it was discovered that a clean-up and fractionation phase employing a WAX SPE approach was practical. Another study created a method for using UPLC/MS/MS to analyze food samples for polyfluoroalkyl phosphate esters (PAPs). It found mono-, di-, and triPAPs in all the food packaging materials examined, with diPAPs being the most common type of chemical. Food samples were analyzed for perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl phosphonic acids (PAPs). PAPs were found to be precursor chemicals of PFCAs. The study highlighted the variety of PFCA sources, indicating that food consumption of PFCAs is a more critical exposure pathway. These investigations highlight how critical it is to use cutting-edge analytical techniques to determine the quantities of PFAS in food matrices precisely and comprehend possible exposure pathways (Gebbink *et al.*, 2013).

7.6 Trends Or Patterns in PFAS Contamination Levels in Different Types Of Foods

Research has found that different food matrices contain different PFAS. Food samples contained polyfluoroalkyl phosphate esters (PAPs) and perfluoroalkyl carboxylic acids (PFCAs). Low amounts of PFCAs, including PFHpA, PFOA, and PFNA, were observed in food samples. From a PFAS perspective, some food matrices have been highlighted as critical; examples include food packaging materials like popcorn bags, where PFCAs have been found regularly.PAPs are recognized as PFCA precursor molecules, and it has been shown that they degrade into PFCAs in various systems, suggesting that eating certain foods may be an indirect way for people to be exposed to PFCAs. PFAS have been found in food matrices overall, and their presence in food packaging materials and specific food items especially emphasizes the need for ongoing regulation and monitoring to reduce human exposure to these potentially hazardous substances (Gebbink *et al.*, 2013).

PFAS can alter the environment and give rise to new PFAS chemicals. Polyfluoroalkyl phosphates (PAPs), for instance, are precursors that can degrade into intermediate compounds like FTCAs and FTUCAs (fluoroalkyl unsaturated carboxylic acids), which can then break down into PFCAs. Additionally, PFAS can bioaccumulate in the food chain, leading to higher levels in animal-based products. Overall, the presence of PFAS in various food matrices, their transformations into different compounds, and the sources of contamination highlight the need for continued monitoring and regulation to mitigate potential health risks associated with PFAS exposure through food consumption (Zabaleta *et al.*, 2017).

PFAS can change the environment and produce a variety of transformation products. These substances can build up in living things, such as fish, and eventually spread to people when they eat contaminated food. Fish, shellfish, pizza, chicken nuggets, and other items that come into touch with PFAS containing grease-proofing chemicals may be included in these matrices. To guarantee food safety and safeguard the public's health from possible exposure to these persistent pollutants, it is necessary to monitor the levels of PFAS in these matrices (Rawn, Ménard and Feng, 2022).

7.7 Occurrence Of PFAS In Food

Quantified results for the following PFASs found in food were obtained: PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, PFDS, and FOSA.When food products from the European market were considered, fish, meat, and eggs were the main sources of PFOS. Meat, meat products, and particularly offal, displayed the highest amounts of PFOA, which accounted for the majority of the PFOA consumption. Fish is a significant source of PFOAs even though research from Europe found that their quantities varied (Pasecnaja, Bartkevics and Zacs, 2022).

Fish and seafood are the food product category where PFASs have been investigated the most out of all the other categories. This food product group was shown to have rather high quantities of PFASs, with fish consumption being one of the main sources of PFOS and PFOA intake. PFOA and PFOS were the primary components among the assessed PFASs in the fish and shellfish group, with PFOS predominating over PFOA, according to samples taken from European nations. Numerous factors, including the origin, feeding sources, habitat, and the worldwide spectrum of PFAS pollution, influenced the measured levels in fish and other marine species. It is necessary to have a better understanding of the specific dynamics of contamination of various fish species and marine products, possibly through production chain monitoring. Given that PFAS levels in fish liver are substantially greater than in muscle, care should be taken in determining how much of these meals are consumed (Kotthoff *et al.*, 2015).

While EFSA indicated that meat and meat products were significant contributors to chronic human exposure, it may be concluded that PFAS contamination of meat samples was everywhere and highly variable among European countries. It should be noted that PFAS levels were found to be higher in offal samples across all countries than in muscle samples, which is consistent with the distinct patterns of PFAS bioaccumulation and biodistribution in animal animals, pork, beef, and chicken. According to research that is currently available, plants can absorb PFASs from soil or water. The sorption of PFAS molecules to natural organic matter is linked to the functional groups and chain length of the molecules. These findings explain the higher prevalence of PFOA, PFHpA, and PFHxA in food samples originating from vegetables. potato, spinach (Pasecnaja, 2022).

Europe is the region that has the most research papers about PFAS consumption through diet. European nations continue to top the list, with France and Italy among the nations offering a sizable number of studies. Comparably, there has been a notable surge in publications reported in Asia, albeit limited to China and South Korea. Conversely, it is important to draw attention to how little data is available in other parts of the world, such as South America and Africa, and how little data is available in North America (Domingo and Nadal, 2017).

7.8 Risk Assessment and Management Strategies

The evaluation and handling of PFAS provide a complex problem in the field of food safety. Because of their durability and possible health dangers, PFAS have gained a lot of attention in consumer products and industrial operations. To address PFAS contamination in food, a thorough risk assessment approach that incorporates toxicological, epidemiology, and exposure data is needed. PFAS in food management strategies cover a range of food supply chain phases, from manufacturing to consumption, and include actions including regulation, mitigation, and monitoring. In addition, the development of interdisciplinary cooperation between scientists, policymakers, and stakeholders is essential to the creation of successful risk communication plans and the enforcement of strong regulatory frameworks that protect the public's health and the integrity of the food supply (Vorst *et al.*, 2021a).

Determining the exposure pathways, food processing, packaging, and environmental pollution is essential to developing efficient management plans. Establishing regulatory thresholds and implementing comprehensive monitoring programs are crucial measures in reducing the dangers associated with per and polyfluoroalkyl substances (PFAS) in the food chain. Additionally, encouraging openness and communication among interested parties helps to make well informed decisions and makes it easier to put preventative measures into action. In the end, a coordinated effort is necessary to protect the public's health and guarantee the food system's integrity in the face of PFAS contamination (Phelps *et al.*, 2024).

The risk assessment and management measures surrounding the presence of PFAS in food are a hot topic in today's environmental discourse and require careful consideration and methodical review. managing the complexities of PFAS contamination in the food chain requires a comprehensive strategy that includes toxicological analyses, comprehensive exposure assessments, and regulatory frameworks. The literature emphasizes the need for proactive risk mitigation strategies and strict regulatory measures to protect public health and environmental integrity in the face of growing PFAS challenges. It also emphasizes the

significance of interdisciplinary collaboration and technological advancements (Brown *et al.*, 2020).

Thorough risk assessment frameworks make it easier to evaluate PFAS ingestion related exposure pathways, toxicity profiles, and possible health effects. Simultaneously, to set strict guidelines, track pollution levels, and carry out helpful actions, efficient management plans require cooperation between industries, regulatory authorities, and the scientific community. Stakeholders can work to protect public health and lower the negative impacts of PFAS in the food supply chain by combining strong risk assessment procedures with proactive management programs (Guelfo *et al.*, 2018).

Additionally, the authors emphasis that to protect public health and guarantee the integrity of the food supply chain, strict regulatory measures and proactive risk management strategies are needed. The novel study by Schrenk not only clarifies the distinctions surrounding PFAS in food, but also offers stakeholders, regulators, and policymakers a road map for effectively and diligently addressing this issue (Schrenk *et al.*, 2020).

The research highlights the necessity of strict regulatory frameworks and strong monitoring mechanisms to reduce potential health dangers linked with PFAS contamination in food through a thorough examination. By utilizing sophisticated analytical methods and risk assessment approaches, the research promotes anticipatory actions to locate contamination sources, evaluate exposure routes, and set acceptable thresholds. It also highlights how crucial it is for stakeholders government organizations, business leaders, and researchers to work together to create efficient mitigation plans and guarantee consumer safety. This literature adds to the continuing discussion on protecting public health from emerging pollutants PFAS by combining scientific evidence with policy initiatives (Pasecnaja, Bartkevics and Zacs, 2022).

7.9 Challenges And Future Directions

The need for standardized procedures is the most significant of these obstacles since it is necessary to guarantee uniformity and dependability across a range of research. Simultaneously, the lack of thorough data-sharing protocols hold up group development and the creation of resilient risk assessment models. Furthermore, there is still a lack of good risk communication techniques, which makes it difficult to tell stakeholders and the general public of important information. It is critical to address these complex issues to strengthen our knowledge of PFAS contamination in food, guarantee improved regulatory measures, and protect public health (Rodriguez *et al.*, 2020).

The most important of these difficulties is the requirement for standardized techniques, which is essential to guarantee the precision and comparability of outcomes between research and geographical areas. Furthermore, the lack of data-sharing systems is a significant barrier to mitigation efforts and thorough understanding. It will be extremely difficult to determine the complete level of PFAS contamination in food without strong structures for cooperative data exchange. Furthermore, while underdeveloped, effective risk communication tactics are crucial for communicating the possible health risks linked to PFAS exposure through food consumption. Protecting public health and guiding evidence-based policies and interventions in the area of PFAS contamination in food requires addressing these issues and closing these knowledge gaps (Cheng *et al.*, 2022).

Due to the prevalence of PFAS chemicals, immediate action is required. To reduce exposure and protect public health, addressing these difficulties calls for interdisciplinary teamwork, sophisticated detection techniques, and strict laws. Prospective future directions include implementing cutting-edge technologies for the elimination of PFAS and embracing sustainable methods in food production. To provide a route toward safer food systems for future generations, legislators, researchers, and food business operators must work together to navigate the complexity of PFAS contamination in food (Rodriguez *et al.*, 2020).

For the scientific community, determining the extent and managing PFAS contamination in food matrices presents several difficulties and knowledge gaps. The most important of these difficulties is the urgent need for standardized techniques to identify PFAS reliably and accurately in a variety of food types since differences in matrices might affect detection limits and precision. Furthermore, efforts to assemble a complete understanding of PFAS exposure pathways and related dangers are hampered by the absence of robust data-sharing channels. Effective risk communication techniques are also necessary to close the knowledge gap between the general public and scientific discoveries, facilitating well-informed decision-making. The development of novel analytical techniques to improve detection sensitivity, investigating the effects of processing methods on PFAS concentrations, and clarifying the mechanisms of PFAS uptake and accumulation in food should be the top priorities for future research efforts. Furthermore, to incorporate research from environmental science, toxicology, and food safety to improve our understanding of PFAS contamination in food and reduce related dangers, interdisciplinary collaborations are essential (Bell *et al.*, 2021).

8. MATERIALS AND METHODS

8.1 Materials

8.1.1 Chemicals

The analysis process used chemicals such as isopropanol, distilled water, surrogate standard, internal standard, acetonitrile, formic acid, salt (magnesium sulfate and sodium chloride), graphitized carbon black, and methanol (MeOH) were purchased from the same company as was written in the diploma thesis of Majercsik, 2020. Multi-PFAS Analyte Primary Dilution Standard mix solution (18 analytes in 1.2ml methanol containing 4% water, 2mg/L for each) was purchased from Kromat Kft. and is available in the Hungarian University of Agriculture and Life Science, analytical chemistry laboratory.

8.1.2 Apparatus

Tools including cutting knife, paper bag, chopper machine, refrigerator, black plastic boats, 15ml centrifuge tubes, mass balance, spoon, tray, 50ml centrifuge tubes, cellulose-acetate syringe filter, centrifuge and UHPLC-MS/MS instrument, zorbax eclipse plus C18 RRHD chromatographic column with the particle size of 1.8µm and with a dimension of 2.1 x 50mm were purchased from the same company as was written in the diploma thesis of Majercsik, 2020 and employed in the study.

8.2 Methods

In this study, we adopted the QuECHERSs (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample preparation technique coupled with UHPLC-MS/MS. EU regulations regarding QuECHERSs are extensive and are designed to ensure the safety of food products and protect human health and the environment. The regulation of pesticide residues, including quenchers used in pesticide analysis, falls under various EU directives and regulations, such as Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin. QuECHERSs are substances used in analytical chemistry to prevent unwanted reactions or stabilize samples during analysis. The method involves several critical steps, including sample collection, homogenization, extraction, and concentration.

8.2.1 Sample Collection and Homogenization

Eight food samples representing different categories (meat, fish, and plant-based cereals) were purchased from a Lidl supermarket in Budapest, Hungary. These samples were

transported to the Hungarian University of Agriculture and Life Science (Buda campus) Analytical Chemistry Laboratory. The samples were milled using a chopper machine to ensure uniformity. Homogenized samples were placed in small plastic bags and stored in a deep fridge for one week.

Figure 2: Samples selected for the screening of PFAS

8.2.2 Quechers Sample Preparation

Sample weighing and standard Addition: Each sample (5g) was measured into a 50ml container. A 10µl aliquot of the surrogate standard (SUR STD) was added to each sample. A surrogate standard such as HFPO-DA(13C13), PFDA(13C9), PFHxA(13C6), and NETFOSAA(D5), is introduced into a sample during the analytical process to assess the accuracy and precision of the method.

Hydration: To prevent excessive dryness, 10 ml of water was added to each sample.

Solvent Extraction: A mixture of 10 ml acetonitrile (ACN) and formic acid (HCOOH) was added to each sample. The samples were shaken for 1 minute to facilitate extraction. ACN helps extract PFAS compounds from the sample. Formic acid was added to defeat the ionization of the compounds and by this, to participate in the organic phase.

Salting-Out Step: following this 6g of magnesium sulphate ($MgSO_4$) and 1.5g of sodium chloride (NaCl) were added to each sample. The samples were shaken for 5 min by using a hand and centrifuged by centrifuge for 5 minutes using a centrifuge. $MgSO_4$ and NaCl remove water from the sample, promoting phase separation. They assist in partitioning PFAS compounds between the aqueous and organic phases.

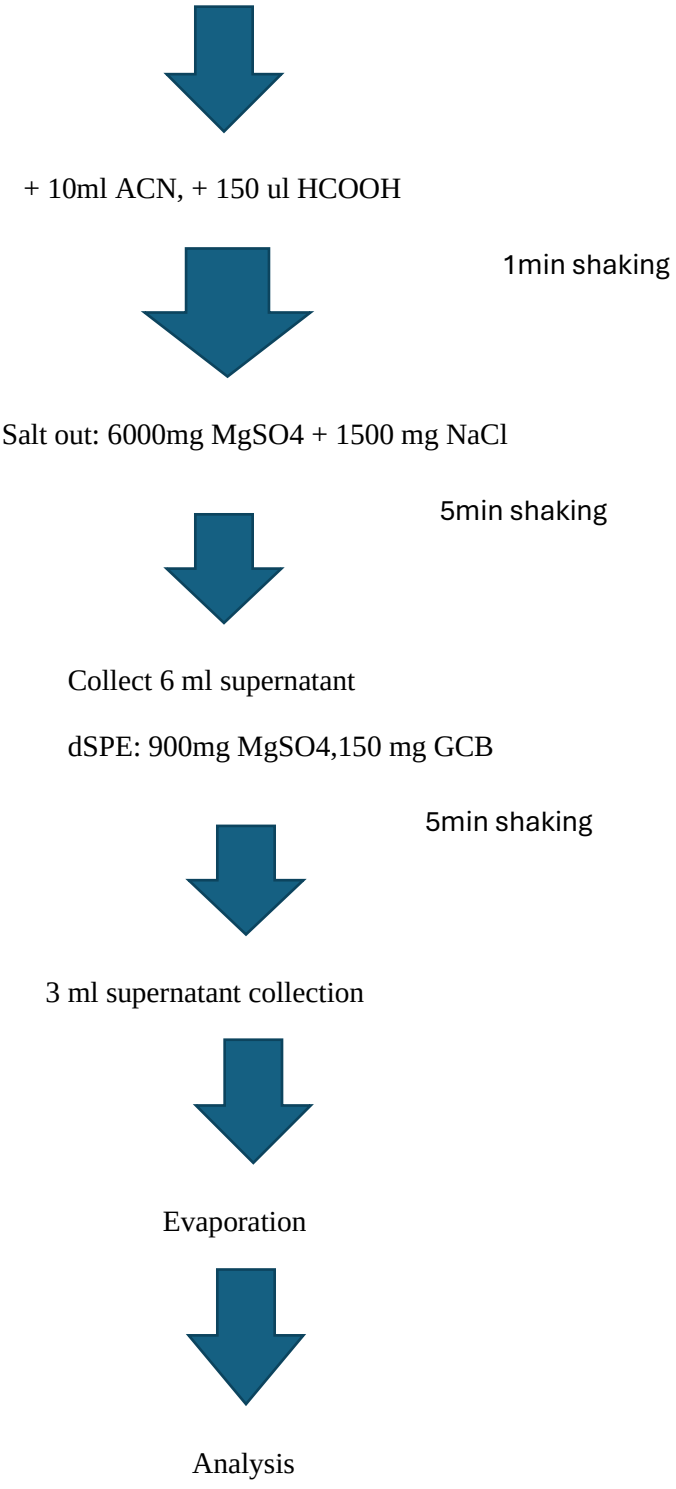
Dispersive Solid-Phase Extraction (dSPE): afterward,6 ml of supernatant was collected from each sample and 900mg of $MgSO_4$ and 150mg of graphitized carbon black (GCB) were added. The mixture was shaken for 5 minutes using a centrifuge. GCB is frequently used for matrix cleanup. It efficiently eliminates matrix interferences that may compromise the robustness of the approach and quantitation. we can get cleaner extracts and improve the precision of PFAS by employing GCB.

Concentration and Evaporation:3ml of supernatant was transferred to a 5ml Eppendorf tube. The samples were evaporated. Evaporation concentrates the sample for subsequent analysis.

After evaporation, the samples were resolved in 495µl of MeOH (4% water). An internal standard (5µl) was added to each sample before analysis to improving the accuracy and precision of the analysis. MeOH reconstitutes the sample for injection into the UHPLC-MS/MS system.

Filtration and Vial Preparation: Each sample was filtered through a nylon filter into a polypropylene (PP) HPLC vial and the prepared vials were ready for subsequent analysis.The QuECHERSs method, combined with UHPLC, provides an efficient and reliable approach for PFAS analysis in food samples.

A 5g sample was weighed,10ul of SUR STD added to each sample and then 10ml water was added.



8.2.3 Preparation of Calibration Solutions

Calibration Solutions Preparation: Calibration solutions were prepared using the isotope-labelled internal standard method, and a six-point calibration line was established. All the solutions were prepared into Eppendorf. Primary dilution solution is a solution used to dilute the stock solutions of analytes and internal standards. The analytes PDS (containing the PFAS analytes) and the IS PDS (containing the internal standard) are both prepared using the primary dilution solution. The primary dilution allows to creation of working solutions with appropriate concentrations for calibration and sample analysis. A working solution was prepared from 25µl of surrogate primary dilution solution (SUR PDS), and 25µl of analytes primary dilution solution (PDS)+450 ml of MeOH(4%water). Six-point calibration lines were prepared into Eppendorf (Genualdi *et al.*, 2022) . During the preparation and analysis of the sample, surrogate standards were incorporated into the sample matrix. Surrogate standards were used to monitor all aspects of the analytical process, such as instrument performance, matrix effects, and extraction efficiency. We can evaluate the analytical method's accuracy and dependability by assessing the surrogate recovery.

8.2.4 Preparation of Mobile Phase

The mobile phase comprised 4mM ammonium-HCO_3 and 0.01% acetic acid (AA), prepared by diluting stock eluent (100mM filtered stock ammonium-HCO3) with water. It was prepared by putting 8ml of stock eluent into 200ml of the volumetric flask, diluted into 50ml with water, then 20ul acetic acid was added and the line was filled until 200ml and it was filled to eluent bottle A. The stock solution was prepared by weighing in 796mg salt in a 100 volumetric flask, filtered, and filled to the line.

8.2.5 Measurement

Calibration points were measured alongside samples, and the internal standard method was used for calculations. Surrogated recovery of all samples was calculated for accuracy assessment.

8.2.6 Analysis Criteria

Qualitative and quantitative analyses are two distinct branches of the analytical process. Finding the existence of target analytes is the main focus of qualitative analysis. To ensure a noticeable difference, this entails comparing the analyte's retention time with the standard's average retention time. The area ratio of the two MRMs transition must be quite similar. For

a complete identification, the mass transition should surpass the detection limit. On the other hand, quantitative analysis focuses on figuring out how much of the analytes in the sample are concentrated. To determine the amount of PFAS chemicals present, this calculation evaluates both the analyte and surrogate concentration and the surrogate recovery. It is possible to realize the composition and concentration of the sample by combining qualitative and quantitative methods.

9. RESULT AND DISCUSSION

9.1 Identification of PFAS In Food Samples

Perfluoro butane sulfonic acid (PFBS) was successfully detected in spinach using UHPLC-MS/MS analysis. It is the only analyte that met the criteria which is mentioned in the materials and method section whereas others were below the detection limit. To evaluate the possible health hazards connected to PFAS exposure in food samples, this information is essential.

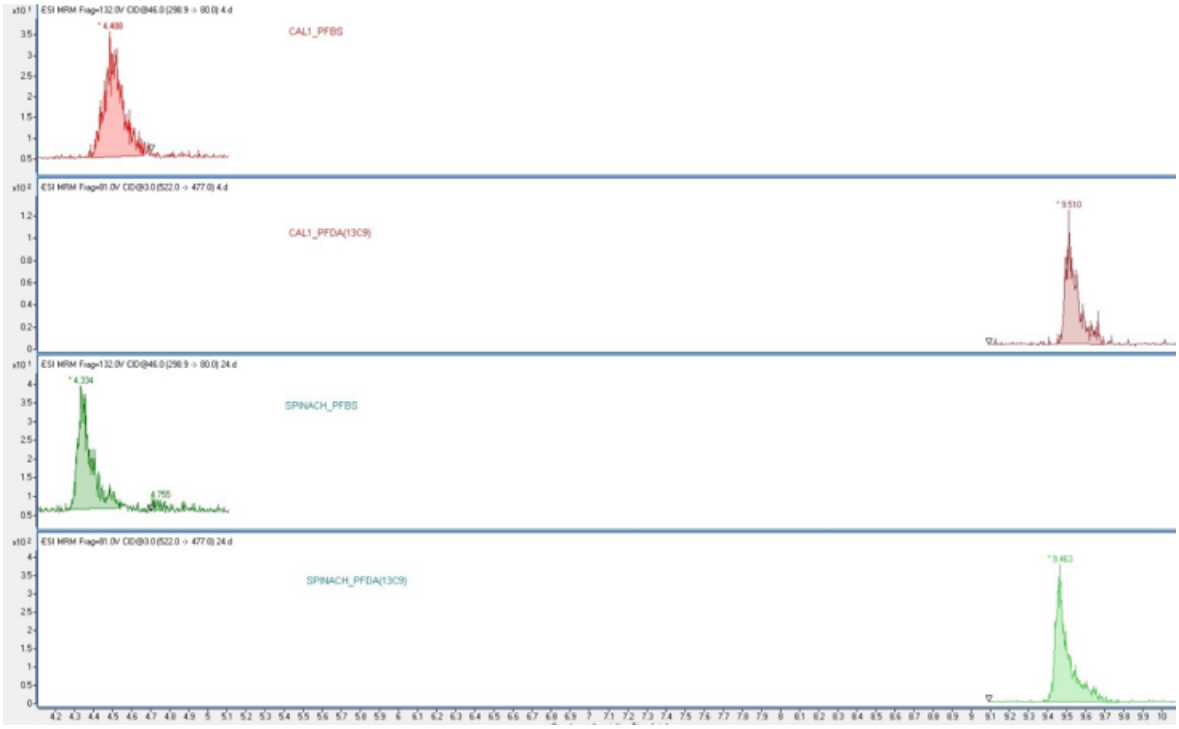


Figure 3:PFBS, PFDA(13C9) and their calibrations Concentrations chromatogram in spinach

As shown above on the chromatogram, the retention time of PFBS calibration which is 4.4 is closer to the retention time of Sinach PFBS which is 4.3. This indicates the presence of PFBS in spinach according to the criteria set in the methodology. the first peak indicates the chromatogram of PFBS calibration while the lower green peaks indicate the chromatogram of PFBS in spinach and PFBS standard chromatogram respectively.

9.2 Quantitative Analysis Of PFAS Compounds In Food Samples

The abundant nature of PFAS and the possible health hazards that come with exposure have led to a great deal of attention being paid to them. This study used both calibration curves

and surrogate standard approaches in quantitative analysis to determine the amounts of PFAS contamination in different food matrices.

PFBS concentrations were quantified using a calibration curve approach, in which the concentration in each sample was calculated by applying the equation derived from the curve. The amount of PFBS in spinach was found to be 1.57ng/ml, indicating that this sample had a quantifiable degree of contamination. Other food samples, on the other hand, had PFBS values below the limit of detection (LOD), indicating little to no contamination in those samples.

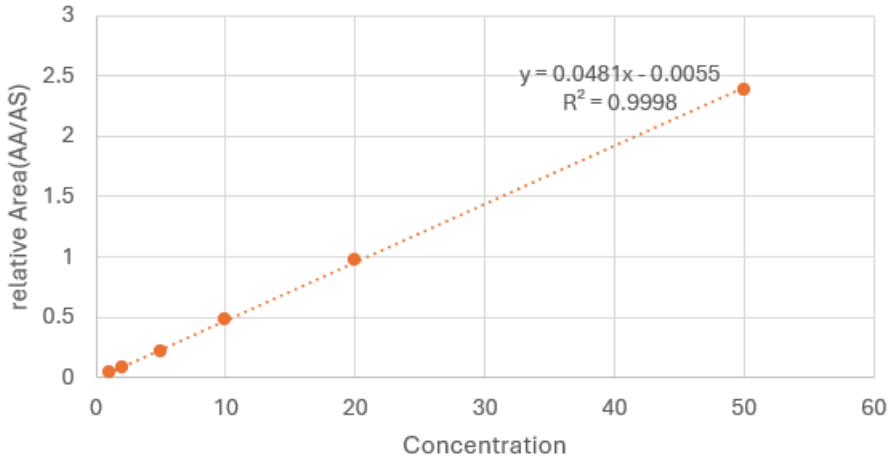


Figure 4 : PFBS calibration curve

The food samples were mixed with surrogate substances, before the examination. It is used as an indication to evaluate the precision and dependability of the analytical techniques used to identify PFAS. Additional information is obtained by carefully analyzing the surrogate component concentrations in each food sample. These values are used as benchmarks to assess the analytical method's accuracy and guarantee the consistency of the findings for PFBS concentrations.

A thorough evaluation of the levels of PFAS contamination can be achieved by comparing the quantities of surrogate chemicals in various food samples. Some samples, including potato fries, popcorn, tuna, beef, chicken, pig, and salmon, had PFBS amounts below the limit of detection (LOD), but spinach had a detectable concentration of the PFBS. This indicates that different food categories have varying amounts of contamination, with spinach showing the highest level of contamination among the examined samples.

Table 1: surrogates and PFBS concentration in all sample

***Concentration in* ng/ml**					
Sample	NETFOSA(D5) Concentration	PFDA(13C9)Concentration	PFHxA(13C6) Concentration	HFPODA(13C13) Concentration	PFBS Concentration
Spinach	17.97	4.15	2.29	0.92	1.57
Potato fries	11.65	5.78	4.49	4.02	LOD
Popcorn	22.32	5.57	5.03	4.01	LOD
Tuna	36.35	7.57	4.37	1.91	LOD
Beef	25.24	7.25	5.11	5.16	LOD
Chicken	26.08	6.00	3.50	2.56	LOD
Pork	24.47	5.62	3.12	2.84	LOD
Salmon	39.53	7.58	3.69	1.84	LOD

Furthermore, the robustness and dependability of the results are increased when surrogate standards and calibration curves are used in quantitative analysis. Target compound quantification is made possible via calibration curves, which offer a mathematical link between analyte concentration and instrument response. Surrogate standards, on the other hand, serve as internal references and make ongoing method validation.

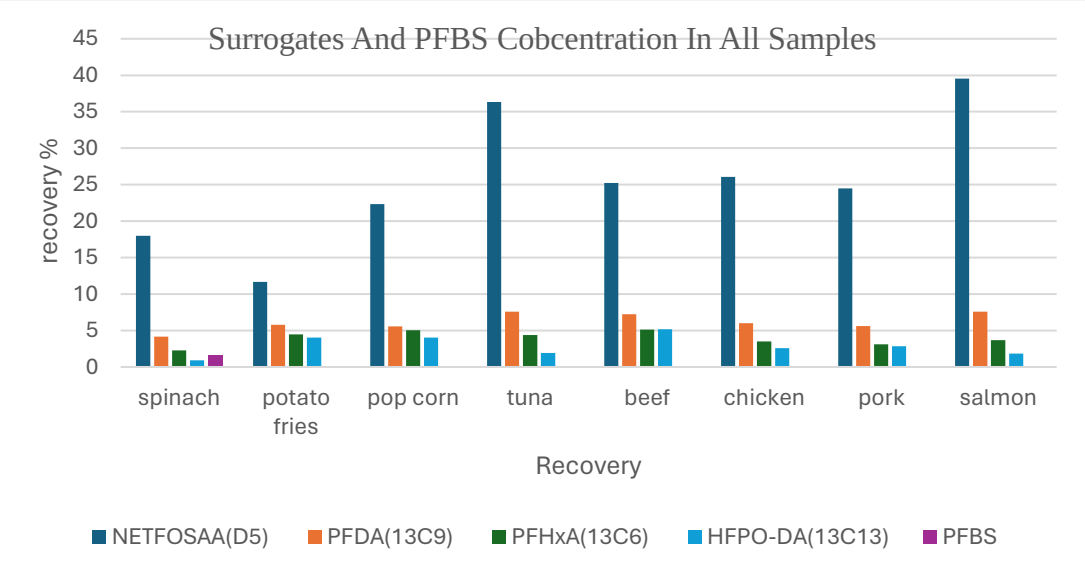


Figure 5 Surrogates and PFBS Concentration In All Samples

The results highlight how important it is to screen PFAS levels in food to protect consumers and inform policy decisions meant to reduce any possible health hazards related to PFAS exposure. To fully understand the amount of PFAS contamination in the food supply chain

and its consequences for environmental sustainability and public health, more investigation and monitoring are necessary.

9.3 Surrogates Recovery Percentage

Reliability and accuracy of analytical methods are critical in the rigorous field of food sample analysis for PFAS. Important indications and surrogate recovery percentages provide information about the precision and dependability of The degree to which a surrogate chemical, which imitates the behavior of target analytes, is recovered throughout the analytical process is known as surrogate recovery. The concentrations of surrogates in all food samples were quantified using the equation from the calibration curve. All surrogates in all samples are calculated and put in the table below.

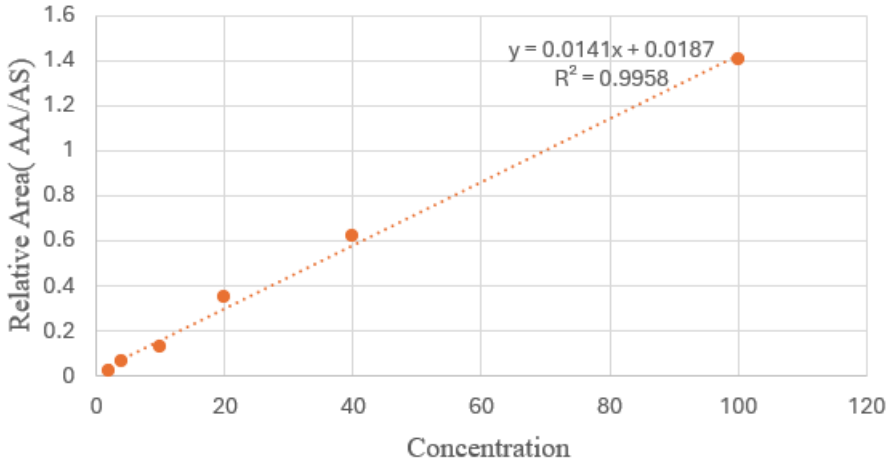


Figure 6 : N-EtFOSAA (D5) calibration curve

Notable conclusions may be drawn by examining the surrogate recovery percentages concerning the effective recovery range (70–130%). Although most recovery concentrations are within this range, certain observations show variances, either falling below or above the predicted threshold. These variations need to be closely examined because they could indicate problems with the analytical procedure itself, including lost chemicals during analysis or insufficient extraction.

Table 2: Surrogate Recovery percentage

Sample	NETFOSA(D5) REC.%	PFDA(13C9) REC.%	PFHxA(13C6) REC.%	HFPO-DA(13C13) REC.%
Spinach	38.9	14.4	69.5	84.4
potato fries	65.7	64.1	80.5	57.6
Popcorn	85	66.6	93	103.2
Tuna	74	43.1	123.3	154.2
Beef	98	108.6	125.4	110.1
Chicken	65.1	57.8	103.6	110.8
Pork	64.1	49	98.7	108.9
Salmon	81.7	46.2	148	188

The supplied surrogate recovery statistics, which are displayed in tabular and graphical forms, provide a thorough summary of the accuracy and consistency of PFAS measurement in food samples. Every sample, ranging from pig to spinach, has distinct surrogate recovery patterns that are indicative of the complex matrix effects and methodological details included in the analysis.

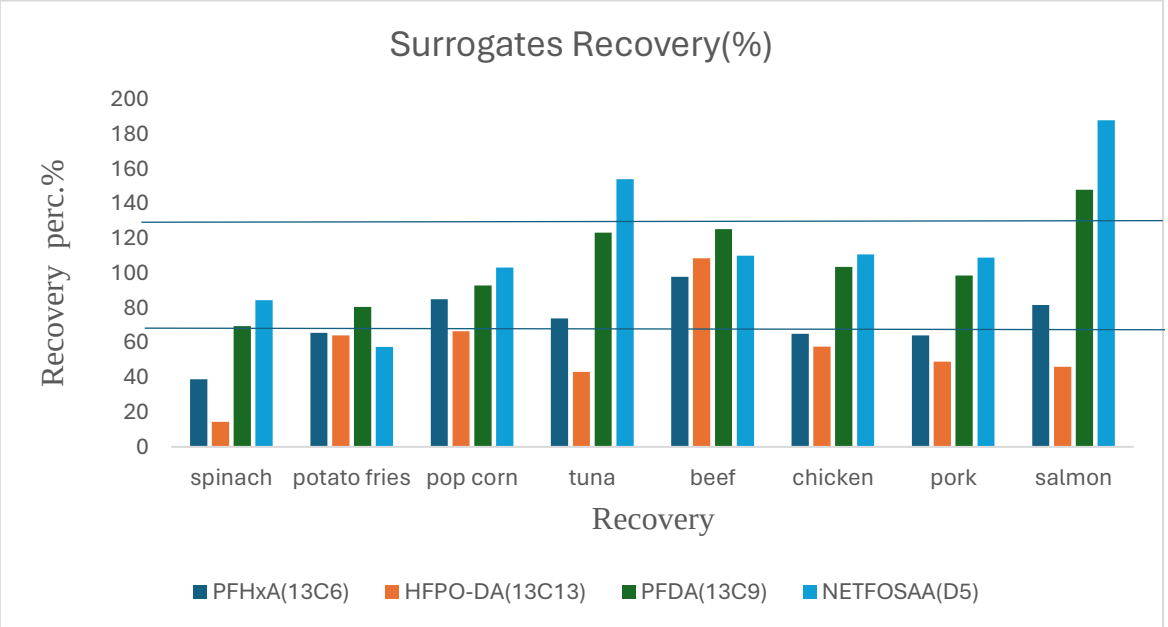


Figure 7: Surrogates Recovery(%) bar chart

Surrogate recovery percentages that are too high or too low may point to issues with the analytical process. Excessive recovery percentages, particularly those considerably higher than 130%, may indicate possible problems like contamination, the presence of substances that interfere and cause analyte concentrations to be overestimated, matrix components that

affect extraction efficiency or instrument response and cause inflated recovery values, and systematic errors in the analytical method that result in inaccurate recovery determination.
On the other hand, low recovery percentages especially those under 70% may point to inadequate or incomplete analyte extraction from the sample matrix as a result of problems with the extraction solvent or other circumstances. Low recovery values arise from matrix elements or chemical interactions that inhibit the recovery of the surrogate substance or loss of analytes during sample preparation or analysis stages.
To sum up, the assessment of surrogate recovery percentages offers precious information about the precision and dependability of PFAS analysis in food samples. Through a close examination of these measures and the resolution of possible sources of variability, scientists can improve the accuracy of analytical techniques, which will strengthen confidence in the evaluation of PFAS contamination levels and provide guidance for regulatory actions intended to reduce related risks.

9.4 Discussion of Findings

In the screening of PFAS in different food matrices in Hungary we used European-regulated methodology and internal standards and also high-performance equipment. Therefore the method used was nearly suitable for the measurement. The concentrations of PFBS found in spinach having the highest concentration among the samples tested highlight the complex issues related to PFAS contamination in food. These results call for a detailed examination of the sources of PFAS contamination in food and the mechanisms by which these substances enter the food chain. To effectively minimize the presence of PFAS in food items and apply control measures to protect consumer health, one must have a thorough understanding of the origins and mechanisms of PFAS contamination.

The observable variation in surrogate recovery percentages across various dietary samples and surrogate substances offers important insights into the complexities of PFAS measurement. This variability highlights the need for improving sample preparation and analysis procedures to improve measurement consistency and reliability as it raises the possibility of challenges in precisely characterizing PFAS levels. To ensure accurate quantification of PFAS pollutants in food matrices, optimization efforts might be required. Regulatory bodies are encouraged to think about setting rules and guidelines for PFAS levels in food products in response to these findings. To reduce the possible health hazards connected to PFAS exposure through food intake, such actions are crucial. To protect public

health and promote food safety, robust regulatory frameworks can offer a framework for tracking and managing PFAS contamination throughout the food supply chain.

The detection of PFBS in food samples, emphasizes how important it is to address PFAS contamination in the food matrices. Using thorough inquiries into the origins of contamination, refinement of analytical techniques, and establishment of strict regulatory actions, we can reduce the dangers connected to exposure to PFAS and protect the public's health. These initiatives are crucial to safeguarding the integrity and safety of our food supply as well as the welfare of consumers everywhere.

10. CONCLUSION

In conclusion, the presence of these persistent chemicals in the food supply chain has been provided by the Hungary food sample contamination investigation including PFAS levels. In particular, the finding that PFBS contamination has been found in a Spinach food sample emphasizes how vital it is to address PFAS exposure through food consumption to safeguard public health. By addressing challenges in quantifying PFAS levels accurately and implementing targeted interventions, we can ensure the safety of the food supply chain and minimize potential health risks associated with PFAS.

This revised section integrates the analysis of surrogate recovery percentages into the discussion of findings, providing a comprehensive assessment of PFAS contamination in food samples and the challenges associated with quantifying PFAS levels accurately. The complexities of PFAS analysis are clarified by this comprehensive methodology, which also highlights the necessity of ongoing improvement in analytical techniques to guarantee the validity of assessment results

10.1 Recommendations and Future Directions

Based on this study, it is recommended that regulatory agencies establish guidelines and standards for PFAS levels in food products to ensure consumer safety. Continued research and supervision of PFAS contamination in food are essential to monitor trends over time and assess the effectiveness of control measures. Sustained research endeavors are essential for tracking patterns of PFAS contamination over time and evaluating the efficacy of mitigation strategies. Governmental organizations, academic institutions, and business partners working together can make it easier to gather completed evidence-based decision-making. Finding the precise sources of PFAS contamination in the food supply chain should be the

top priority for future research. To stop additional pollution and reduce exposure hazards, customized solutions can be devised by identifying the sources of contamination.

More research is needed to determine the possible health effects of PFAS exposure from food consumption. Insights into the relationship between PFAS exposure and unfavorable health outcomes can be gained through long-term epidemiological studies, which can help direct risk assessment and management tactics. Prioritizing efforts should be made to optimize analytical procedures for PFAS-level quantification in food samples. The improvement of assessment outputs' validity and regulatory compliance depends on research and development projects that strive to improve the accuracy and dependability of analytical methodologies.

11. SUMMARY

The research aimed to assess the contamination of different foods with PFAS in Hungary, focusing on qualitative identification and quantitative analysis of PFAS compounds, as well as the evaluation of surrogate recovery percentages to ensure the accuracy and reliability of analytical methods.

The study effectively identified PFBS in spinach using ultrahigh-pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), which is a European standard and high-performance equipment and it is nearly suitable for assessment demonstrating the effectiveness of the method. By identifying PFAS chemicals in food samples, this approach helped set the stage for additional analysis. The study used calibration curves and surrogate standard approaches to quantify the levels of PFAS contamination in various food matrices. Using calibration curves, the quantities of PFBS in the tested samples were determined; the sample with the highest content was spinach. Additionally, the dependability and accuracy of the analytical method were assessed using surrogate standards, demonstrating that the recovery percentages of various food samples and surrogates varied.

To reduce potential health hazards, it is critical to address PFAS contamination in the food supply chain. This is highlighted by the detection of PFBS in food samples. Different food kinds of food matrices were assessed and PFBS was detected in Spinach, which highlights the complexity of PFAS contamination and needs more research into the pathways and sources of contamination. To protect consumer safety, regulatory bodies are recommended to set standards for PFAS levels in food products. To determine precise sources of contamination and assess the health hazards connected to PFAS exposure, more study is necessary. Also to improve measurement accuracy and reliability, optimizing analytical techniques for PFAS quantification in food samples should be given top priority.

To safeguard food safety and the public's health, the study's conclusion highlights the need to continuously assess for PFAS contamination in food samples. Accurately assessing PFAS levels and putting targeted actions in place can help to protect the integrity of the food supply chain and reduce the health concerns that could arise from exposure to PFAS.

The research offers important insights into screening of PFAS contamination in food matrices in Hungary through a careful approach that includes qualitative and quantitative analyses, as well as an assessment of surrogate recovery percentages. This shows the way for future efforts to mitigate risks and ensure consumer safety.

12. ACKNOWLEDGMENTS

I am strongly grateful to God for giving me the strength and resilience to direct the challenges encountered along my academic journey. My heartfelt appreciation extends to my esteemed supervisor, Marczika Andrásné Dr.Sörös Csilla, whose unwavering guidance and encouragement have been instrumental in shaping the trajectory of my research endeavors. Special recognition is extended to the Food and Agriculture Organization (FAO) and the Hungarian Ministry of Agriculture for their invaluable financial support, which has facilitated my academic pursuits. Additionally, I wish to acknowledge the Hungarian University of Agriculture and Life Sciences and all my respected teachers for their enduring dedication to fostering my intellectual growth. To my cherished friends, your unwavering support has been a source of strength and inspiration throughout this journey. Finally, to all those whose contributions may not be mentioned, your presence and influence have left an enduring mark on my journey, for which I am deeply grateful.

14. REFERENCE

Bell, E.M. *et al.* (2021) 'Exposure, health effects, sensing, and remediation of the emerging PFAS contaminants – Scientific challenges and potential research directions', *Science of the* Total Environment. Elsevier B.V. Available at:https://doi.org/10.1016/j.scitotenv.2021.146399.

Brown, J.B. *et al.* (2020) 'Assessing Human Health Risks from Per- And Polyfluoroalkyl Substance (PFAS)-Impacted Vegetable Consumption: A Tiered Modeling Approach', *Environmental Science and Technology*, 54(23), pp. 15202–15214. Available at: https://doi.org/10.1021/acs.est.0c03411.

Carnero, A.R. *et al.* (2021a) 'Presence of perfluoroalkyl and polyfluoroalkyl substances (Pfas) in food contact materials (fcm) and its migration to food', *Foods*. MDPI AG. Available at: https://doi.org/10.3390/foods10071443.

Carnero, A.R. *et al.* (2021b) 'Presence of perfluoroalkyl and polyfluoroalkyl substances (Pfas) in food contact materials (fcm) and its migration to food', *Foods*. MDPI AG. Available at: https://doi.org/10.3390/foods10071443.

Cheng, B. *et al.* (2022) 'Per- and polyfluoroalkyl substances exposure science: current knowledge, information needs, future directions', *International Journal of Environmental Science and Technology*. Institute for Ionics, pp. 10393–10408. Available at: https://doi.org/10.1007/s13762-021-03710-7.

Curtzwiler, G.W. *et al.* (2021) 'Significance of Perfluoroalkyl Substances (PFAS) in Food Packaging', *Integrated Environmental Assessment and Management*, 17(1), pp. 7–12. Available at: https://doi.org/10.1002/ieam.4346.

Domingo, J.L. and Nadal, M. (2017) 'Per- and polyfluoroalkyl substances (PFASs) in food and human dietary intake: A review of the recent scientific literature', *Journal of Agricultural and Food Chemistry*. American Chemical Society, pp. 533–543. Available at: https://doi.org/10.1021/acs.jafc.6b04683.

EFSA (2012) 'Perfluoroalkylated substances in food: occurrence and dietary exposure', *EFSA Journal*, 10(6). Available at: https://doi.org/10.2903/j.efsa.2012.2743.

Fenton, S.E. *et al.* (2021) 'Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research', *Environmental Toxicology and Chemistry*. Wiley Blackwell, pp. 606–630. Available at: https://doi.org/10.1002/etc.4890.

Gebbink, W.A. *et al.* (2013) 'Polyfluoroalkyl phosphate esters and perfluoroalkyl carboxylic acids in target food samples and packaging-method development and screening', *Environmental Science*

and Pollution Research, 20(11), pp. 7949–7958. Available at: https://doi.org/10.1007/s11356-013-1596-y.

Genualdi, S. *et al.* (2021) 'Analysis of per- and poly-fluoroalkyl substances (PFAS) in processed foods from FDA's Total Diet Study'. Available at: https://doi.org/10.1007/s00216-021-03610-2/Published.

Genualdi, S. *et al.* (2022) 'Analysis of per- and poly-fluoroalkyl substances (PFAS) in processed foods from FDA's Total Diet Study', *Analytical and Bioanalytical Chemistry*, 414(3), pp. 1189–1199. Available at: https://doi.org/10.1007/s00216-021-03610-2.

Guelfo, J.L. *et al.* (2018) 'Evaluation and management strategies for per-and polyfluoroalkyl substances (PFASs) in drinking water aquifers: Perspectives from impacted U.S. northeast communities', *Environmental Health Perspectives*. Public Health Services, US Dept of Health and Human Services. Available at: https://doi.org/10.1289/EHP2727.

Kotthoff, M. *et al.* (2015) 'Perfluoroalkyl and polyfluoroalkyl substances in consumer products', *Environmental Science and Pollution Research*, 22(19), pp. 14546–14559. Available at: https://doi.org/10.1007/s11356-015-4202-7.

Lewis, A.J. *et al.* (2022) 'Exposure pathways and bioaccumulation of per- and polyfluoroalkyl substances in freshwater aquatic ecosystems: Key considerations', *Science of the Total Environment*. Elsevier B.V. Available at: https://doi.org/10.1016/j.scitotenv.2022.153561.

Minet, L. *et al.* (2022) 'Use and release of per- and polyfluoroalkyl substances (PFASs) in consumer food packaging in U.S. and Canada', *Environmental Science: Processes and Impacts*, 24(11), pp. 2032–2042. Available at: https://doi.org/10.1039/d2em00166g.

Pasecnaja, E. and B.V. and Z.D. (2022) 'Occurrence of selected per- and polyfluorinated alkyl substances (PFASs) in food available on the European market – A review on levels and human exposure assessment - ScienceDirect', *Chemosphere*, 287.

Pasecnaja, E., Bartkevics, V. and Zacs, D. (2022) 'Occurrence of selected per- and polyfluorinated alkyl substances (PFASs) in food available on the European market – A review on levels and human exposure assessment', *Chemosphere*. Elsevier Ltd. Available at: https://doi.org/10.1016/j.chemosphere.2021.132378.

Phelps, D.W. *et al.* (2024) 'Per- and Polyfluoroalkyl Substances in Food Packaging: Migration, Toxicity, and Management Strategies', *Environmental Science & Technology*, 58(13), pp. 5670–5684. Available at: https://doi.org/10.1021/acs.est.3c03702.

Rawn, D.F.K., Ménard, C. and Feng, S.Y. (2022) 'Method development and evaluation for the determination of perfluoroalkyl and polyfluoroalkyl substances in multiple food matrices', *Food Additives and Contaminants - Part A*, 39(4), pp. 752–776. Available at: https://doi.org/10.1080/19440049.2021.2020913.

Rodriguez, K.L. *et al.* (2020) 'Recent developments of PFAS-detecting sensors and future direction: A review', *Micromachines*. MDPI AG. Available at: https://doi.org/10.3390/mi11070667.

Schrenk, D. *et al.* (2020) 'Risk to human health related to the presence of perfluoroalkyl substances in food', *EFSA Journal*, 18(9). Available at: https://doi.org/10.2903/j.efsa.2020.6223.

De Silva, A.O. *et al.* (2021) 'PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding', *Environmental Toxicology and Chemistry*. Wiley Blackwell, pp. 631–657. Available at: https://doi.org/10.1002/etc.4935.

Torres, F.G. and De-la-Torre, G.E. (2023) 'Per- and polyfluoroalkyl substances (PFASs) in consumable species and food products', *Journal of Food Science and Technology*. Springer, pp. 2319–2336. Available at: https://doi.org/10.1007/s13197-022-05545-7.

Vorst, K.L. *et al.* (2021a) 'Risk assessment of per- and polyfluoroalkyl substances (PFAS) in food: Symposium proceedings', in *Trends in Food Science and Technology*. Elsevier Ltd, pp. 1203–1211. Available at: https://doi.org/10.1016/j.tifs.2021.05.038.

Vorst, K.L. *et al.* (2021b) 'Risk assessment of per- and polyfluoroalkyl substances (PFAS) in food: Symposium proceedings', in *Trends in Food Science and Technology*. Elsevier Ltd, pp. 1203–1211. Available at: https://doi.org/10.1016/j.tifs.2021.05.038.

Zabaleta, I. *et al.* (2017) 'Screening and identification of per- and polyfluoroalkyl substances in microwave popcorn bags', *Food Chemistry*, 230, pp. 497–506. Available at: https://doi.org/10.1016/j.foodchem.2017.03.074.

STATEMENT ON CONSULTATION PRACTICES

As a supervisor of Worku Dejene Bekele (NEPTUN ID: SIC8BH) ID), I here declare that the final essay/thesis/master's thesis/portfolio[1] has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules. I recommend/don't recommend[2] the final essay/thesis/master's thesis/portfolio to be defended in a final exam.

The document contains state secrets or professional secrets: yes no*[3]

Place and date: Budapest,April 26,2024

Internal supervisor

[1] Please select applicable and delete non-applicable.

[2] Please underline applicable. [3] Please underline applicable.

REQUEST FOR CONFIDENTIALITY

I,the undersigned Worku Dejene Bekele (Neptune code: SIC8BH) student at MSc in food safety and Quality Engineering programme request that my thesis / diploma thesis[3] titled Screening Of Polyfluoro Alky Substance (PFAS) In Food Matrices In Hungary (Dr. Sörös Csilla Marczika Andrásné) be encrypted by applying point c) of Section 95 (5) of the Study and Examination Regulations of the Hungarian University of Agriculture and Life Sciences (hereinafter referred to as 'SER'). I understand that if my request is approved, the encryption of the thesis / diploma thesis will cover 5 years following the successful defense, in accordance with point c) of Section 95 (5) of SER.

Done at: Budapest, April 26,2024

Student's signature

I, the undersigned[4]Dr. Sörös Csilla Marczika Andrásné, proffessor, as the representative of Hungarian University of Agriculture and Life Science,1118,Budapest villanyi utca 29-43.request that the thesis / diploma thesis[5] titled Screening Of Polyfluoro Alky Substance (PFAS) In Food Matrices In Hungary made by Worku Dejene Bekele (Neptun code:SIC8BH) by using the data provided by the Hungarian University of Agriculture and Life Sciences be encrypted.

Done at:Budapest, April 26,2024

Representative's signature

I APPROVE / REJECT the request for confidentiality.

Reasons for rejection: _____

_____

__

Done at: Budapest, April 26,2024 _____ _

Programme leader's signature

[3] Choose and keep the appropriate one, delete the other.

[4] The additional request of the business partner is not obligatory, if it is not requested, it can be deleted from the form.

[5] Choose and keep the appropriate one, delete the other.

footnotes from the form before printing.

DECLARATION

on authenticity and public assess of final essay/thesis/master's thesis/portfolio[6]

Student's name: Worku Dejene Bekele
Student's Neptun ID: SIC8BH
Title of the document: Screening Of Polyfluoro Alky Substance (PFAS) In Food Matrices In Hungary.
Year of publication: 2024
Department: Chemistry and Analysis

I declare that the submitted final essay/thesis/master's thesis/portfolio[7] is my own, original individual creation. Any parts taken from an another author's work are clearly marked, and listed in the table of contents.

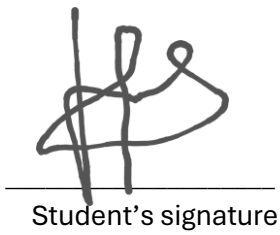
If the statements above are not true, I acknowledge that the Final examination board excludes me from participation in the final exam, and I am only allowed to take final exam if I submit another final essay/thesis/master's thesis/portfolio.

Viewing and printing my submitted work in a PDF format is permitted. However, the modification of my submitted work shall not be permitted.

I acknowledge that the rules on Intellectual Property Management of Hungarian University of Agriculture and Life Sciences shall apply to my work as an intellectulal property.

I acknowledge that the electric version of my work is uploaded to the repository sytem of the Hungarian University of Agriculture and Life Sciences.

Place and date:Budapest, April 26,2024



Student's signature

[6] Please select the one that applies, and delete the other types.
[7] Please select the one that applies, and delete the other types.