

REFERENCE MATERIALS FOR MICROPLASTICS: KEY TO STANDARDIZATION AND VALIDATION OF METHODS IN FOOD AND THE ENVIRONMENT

Jozef Čapla¹, Peter Zajác¹, Jozef Čurlej¹, Martina Fikselová¹, Lukáš Hleba²

Address(es): Titul(s) Firstname Surname of the corresponding author

¹ Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.

² Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.

*Corresponding author: jozef.curlej@uniag.sk

<https://doi.org/10.55251/jmbfs.13092>

ARTICLE INFO

Received 21. 7. 2025
Revised 25. 2. 2026
Accepted 6. 3. 2026
Published 1. 4. 2026

Regular article



ABSTRACT

The increasing presence of microplastics in food products has attracted significant scientific and public concern due to their potential health risks. However, defining and accurately measuring microplastics remains a critical challenge, primarily due to their diverse size distribution and material properties. To address this issue, the development and application of reference materials (RMs) have emerged as essential tools for enhancing the reliability and reproducibility of analytical methods.

This article explores the pivotal role of RMs in microplastic analysis within the food sector. It reviews existing examples, such as polyethylene terephthalate (PET) particles used for homogeneity and stability testing, and innovative approaches like soda tablets and capsules containing microplastics designed for laboratory intercomparisons. These developments highlight the potential of RMs in standardizing methodologies, validating detection techniques, and improving data comparability across laboratories.

The utilization of RMs is particularly critical for validating analytical methods to detect microplastics in food products. Standardization through RMs enhances analytical precision and lays the groundwork for establishing harmonized analytical approaches for microplastic testing following European Union (EU) regulations. Despite these advancements, challenges persist, including the limited availability of certified reference materials (CRMs) tailored explicitly to food matrices. Future research should focus on developing CRMs that address these specific needs and ensuring their incorporation into regulatory frameworks.

By bridging the gap between scientific innovation and practical application, this work underscores the necessity of RMs as a cornerstone for advancing the analytical landscape of microplastic detection in the food sector. Addressing these gaps will enable the food industry to achieve harmonized and reliable microplastic monitoring and control.

Keywords: microplastics, reference materials, certified reference materials, food analysis, standardization, analytical methods, EU regulations

INTRODUCTION

Microplastics in food chains are becoming an increasingly significant threat, attracting the attention of experts and the general public. These particles, originating from the degradation of plastics or environmental contamination, are commonly found in a wide range of foods, such as seafood, salt, water, and even plant-based products (Tambo Magni, 2023; Garrido Gamarro and Costanzo, 2022; Cowger et al., 2020; Dehaut et al., 2023). Recent studies have highlighted that microplastics can also enter food products through atmospheric deposition and soil contamination. Pironti et al. (2021) suggest that microplastics can migrate from agricultural soils into crops, raising concerns about their presence in plant-based foods. Furthermore, airborne microplastics have been detected in food production environments, increasing the risk of contamination during processing and packaging.

According to Tambo Magni (2023), microplastics are not just environmental pollutants but also pose potential risks to human health. From a biological perspective, microplastics can act as carriers of toxic substances that subsequently enter the human body through food consumption. Studies focusing on the health risks of microplastics suggest that their accumulation in tissues may lead to oxidative stress, inflammatory reactions, and even endocrine disruptions (Zhang, 2024; Tsochatzis et al., 2024; Mason et al., 2018). Additionally, there is a growing body of research investigating their possible neurotoxic and reproductive effects (Dehaut et al., 2023; Yee et al., 2021).

Measuring and analyzing microplastics in food presents numerous challenges. These particles occur in various size ranges, from nanoparticles to macro-sized particles, and exhibit various material compositions (Kye et al., 2023; Leslie and Depledge, 2020; Sharma and Chatterjee, 2017). The size, shape, and composition variability complicates their detection and quantification, leading to inconsistencies in data reported across different publications (Cowger et al., 2020; Wiesinger et al., 2021; O'Connor et al., 2019).

A unified definition of microplastics is still lacking, hindering comparisons between studies and research fields (Prata et al., 2019). Analytical methods, such as Raman spectroscopy and Fourier-transform infrared spectroscopy (FTIR), are

commonly employed to identify the material composition of microplastics. However, their accuracy and specificity remain limited (Zhang, 2024; Dehaut et al., 2023). Thus, the development of reference materials that can serve as standards for measuring and analyzing microplastics is crucial.

The growing attention to this issue is evidenced by the increasing number of studies and publications over the past decade, reflecting the need for a coordinated scientific and regulatory approach (Cowger et al., 2020). With heightened awareness of the environmental and health risks associated with microplastics, there is an urgent need to improve analytical tools and methodologies to assess better their impact on food chains (Garrido Gamarro and Costanzo, 2022; Tsochatzis et al., 2024). This review is based on literature published between 2017 and 2025, retrieved from databases such as Scopus and Web of Science using the keywords microplastics, reference materials, food analysis, and standardization. The reviewed publications were critically compared based on analytical scope, detection limits, and validation parameters, emphasizing harmonization with ISO and EU regulatory frameworks. Special attention was given to identifying methodological gaps relevant to reference material (RM) development and interlaboratory comparability.

MICROPLASTIC CONTAMINATION IN FOOD PRODUCTS

Microplastic contamination in food has become a growing concern, with evidence of its presence across a wide range of food categories. Studies have shown that microplastics can enter food products through packaging materials, processing equipment, and environmental exposure. The occurrence and potential risks vary depending on the food matrix. In meat and dairy products, microplastics have been detected in milk, cheese, and processed meats as a result of migration from packaging and filtration systems used during processing. Reported contamination levels in dairy products can reach up to 14 MP/L, primarily due to membrane filters (Da Costa Filho et al., 2021). Vacuum-packed meat samples have shown the presence of plastic-derived compounds such as diisooctyl phthalate and polyethylene glycol, which migrate from packaging films depending on factors including fat content and storage time (Sanches Silva et al., 2007). Recent studies

further indicate that microplastics can bind to biological tissues, particularly in protein- and lipid-rich matrices. Adsorption to muscle fibers and fat globules has been observed in meat and fish, while protein-polymer interactions may stabilize microplastic particles in dairy systems. Thermal processing such as pasteurization, cooking, or frying can enhance particle migration by disrupting matrix integrity and releasing bound microplastics into the surrounding medium. This highlights the importance of evaluating both matrix-bound and migratory fractions in food safety assessments.

Microplastics are also prevalent in beverages. Synthetic polymer contamination is widespread in bottled water, with concentrations reported as high as 1,410 MP/L in PET-bottled samples (Schymanski et al., 2018). Beer has also been shown to contain microplastics, likely introduced during processing or via atmospheric deposition (OBmann et al., 2018). Fruits and vegetables may accumulate microplastics through contaminated irrigation water or soil, raising concerns about their occurrence in fresh produce (Oliveri Conti et al., 2020). Seafood represents another significant exposure pathway; marine species such as fish and shellfish are particularly susceptible to microplastic ingestion, with concentrations reaching up to 34.9 MP/g in fish muscle tissue (Collard et al., 2017). Bivalves such as mussels and oysters frequently accumulate microplastics due to their filter-feeding mechanisms (Cho et al., 2019).

Given the variability among food matrices, factors such as fat content, processing methods, and packaging materials strongly influence microplastic retention and potential human exposure. Analytical detection remains challenging due to interference from food components, underscoring the need for improved and standardized methodologies to ensure accurate quantification (Geyer et al., 2017).

THE NEED FOR STANDARDIZATION

The absence of standardized reference materials for microplastic analysis has led to significant inconsistencies across studies, complicating data comparison and impeding scientific progress. To mitigate these issues, multiple research institutions and organizations have developed specialized reference materials designed to accurately detect and quantify microplastics (Dehaut et al., 2023). A

crucial aspect of standardization is ensuring comparability between laboratories and across different regulatory frameworks. Recent findings indicate that microplastics' impact on human health and the environment can only be fully assessed if detection methodologies are aligned and validated using reference materials (Kumar et al., 2024). Establishing universally accepted standards would facilitate comparability and reproducibility, ultimately enhancing the reliability of microplastic research.

CERTIFIED REFERENCE MATERIALS (CRMS)

Certified reference materials (CRMs) ensure accuracy, precision, and reproducibility in microplastic analysis. To be effective, CRMs must adhere to key criteria, including homogeneity, stability, defined particle size distribution, and well-characterized chemical composition (Dehaut et al., 2023). Recent efforts by companies such as Chiron AS have led to the development of standardized microplastic reference materials across a range of polymers, including polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), and polycarbonate (PC), all within a controlled size range of 50–300 μm (Dehaut et al., 2023). However, new advancements include CRMs covering nano-sized microplastics (<50 μm), which are crucial for assessing cellular uptake and potential bioaccumulation (Wiesinger et al., 2021). Studies show that the availability of CRMs with a broad range of polymer compositions and particle sizes significantly enhances analytical accuracy. These CRMs play a pivotal role in interlaboratory comparison studies, validating analytical methodologies and improving measurement reliability (Cowger et al., 2020). The density and molecular weight of polymer types significantly influence their behavior in food matrices and migration potential. For instance, low-density polyethylene (LDPE, 0.91–0.94 g/cm^3) tends to float and shows lower sorption of hydrophobic compounds compared to denser polymers such as PET (1.38 g/cm^3) or PVC (1.40 g/cm^3), which may accumulate more readily in fatty foods or adhere to processing surfaces.

Integrated framework for microplastic RM development and standardization



Figure 1 Integrated framework for microplastic RM development and standardization

The conceptual diagram illustrates the stepwise progression from the preparation of reference materials (RMs) to their certification (CRMs), harmonization with ISO 24187, and practical application in ISO 17025-accredited food laboratories. The framework emphasizes continuous quality improvement, linking research, validation, and regulatory implementation to ensure reproducible and standardized microplastic analysis.

In practical terms, the implementation of CRMs in food testing laboratories directly enhances the reliability of microplastic analysis under ISO 17025 quality management systems. Using certified materials for calibration, recovery experiments, and interlaboratory comparisons improves traceability and measurement uncertainty estimation. This practice strengthens method validation, supports accreditation compliance, and facilitates transparent quality assurance (QA/QC) procedures for laboratories involved in microplastic monitoring in food and environmental samples.

STANDARDIZED MICROPLASTIC MATRICES

To simulate real-world conditions, microplastic reference materials are incorporated into diverse environmental matrices such as water, sediments, biota, and food samples (Garrido Gamarro and Costanzo, 2022). Recent studies also emphasize the importance of reference materials in atmospheric samples and

wastewater, contributing significantly to food contamination through deposition and irrigation. Integrating reference materials into these matrices allows for the evaluation of analytical methods under realistic contamination scenarios, ultimately contributing to the robustness and applicability of detection techniques. However, challenges remain in differentiating microplastics from natural particulates and biological contaminants, necessitating further refinement of analytical protocols (Prata et al., 2019).

Analytical techniques and challenges

Despite notable advancements in developing microplastic reference materials, several analytical challenges continue to hinder precise quantification and identification. One of the most critical limitations lies in the detection of nanoparticles smaller than 100 nm, where current spectroscopic and thermal methods lack sufficient spatial resolution and sensitivity (Tsochatzis et al., 2024). Chemical differentiation between microplastics and organic or biopolymeric particles remains complex, particularly when analyzing food matrices that contain natural proteins, polysaccharides, or lipid residues (Prata et al., 2019). Matrix interferences caused by fats, proteins, and other organic compounds can obscure spectral features and reduce analytical accuracy (Garrido Gamarro and Costanzo, 2022). Furthermore, spectral overlap between synthetic polymers and

naturally occurring organic materials often results in false positives during FTIR or Raman identification, highlighting the need for improved data preprocessing, spectral libraries, and standardized validation protocols (Cowger et al., 2020).

Common analytical methods

In addition to instrumental spectroscopic methods, several classical approaches have been applied for the preliminary screening of microplastics, including optical microscopy, density separation, and filtration-based gravimetric determination. Although these methods lack chemical specificity, they remain essential for sample preconcentration and size fractionation prior to advanced spectroscopic analysis. Their simplicity and low cost make them suitable for routine monitoring, especially in laboratories without access to high-resolution instrumentation. Among the instrumental methods, Fourier-Transform Infrared Spectroscopy (FTIR), Raman Spectroscopy, Pyrolysis–Gas Chromatography–Mass Spectrometry (Py-GC/MS), and Thermogravimetric Analysis (TGA) are the most commonly employed techniques for detecting and characterizing microplastics (Tsochatzis et al., 2024). Each of these analytical approaches provides complementary information—FTIR and Raman spectroscopy enable polymer identification based on molecular vibrations, while Py-GC/MS and TGA support quantitative assessment through thermal decomposition and mass spectral analysis. Together, these methods form the analytical foundation for microplastic detection and polymer characterization in environmental and food matrices. Recent advances include hyperspectral imaging and AI-driven spectral libraries to improve classification accuracy (Leslie and Depledge 2020). Each method presents distinct advantages and limitations, underscoring the necessity for standardized reference materials to ensure accurate validation and reproducibility. Comparative evaluation of these methods indicates that no single analytical technique can fully characterize the diverse size and compositional range of microplastics. FTIR spectroscopy remains the most accessible tool for qualitative polymer identification above 10 μm, while Raman spectroscopy provides higher spatial resolution and enables detection of pigmented or colored particles but suffers from fluorescence interference. Py-GC/MS offers direct mass-based quantification yet lacks information on particle morphology or number. Therefore, multi-technique workflows combining spectroscopic and thermal approaches are essential for obtaining complementary data. Method selection should depend on the matrix type, target particle size, and required detection limit, and all methods should be verified or calibrated using appropriate CRMs to ensure traceability and comparability under ISO 17025 and ISO 24187 frameworks.

Regulatory perspectives and future directions

Multiple international organizations are actively contributing to the establishment of standardized guidelines for microplastic analysis. The International Organization for Standardization (ISO) has released ISO 24187:2023, which provides harmonized methodologies for sampling, sample preparation, and measurement of microplastics across various environmental and food matrices (Cowger et al., 2020). The Food and Agriculture Organization (FAO) has developed complementary guidelines that emphasize the importance of robust monitoring strategies and harmonized analytical procedures for food commodities (Garrido Gamarro and Costanzo, 2022). Concurrently, the European Food Safety Authority (EFSA) is conducting systematic assessments of potential health risks associated with dietary exposure to microplastics (Tsochatzis et al., 2024). The European Chemicals Agency (ECHA) has also advanced regulatory action by proposing restrictions on the intentional release of microplastics within the REACH framework (ECHA, 2023). Collectively, these initiatives reflect a global effort to harmonize analytical practices, enhance data comparability, and strengthen regulatory mechanisms addressing microplastic contamination in food and the environment.

Future directions

Future research should focus on advancing detection and quantification techniques for microplastics through the integration of high-resolution mass spectrometry, hyperspectral imaging, and AI-assisted data processing, which together can improve polymer specificity and reduce false-positive identification. Another priority is the strengthening of standardization frameworks by promoting the widespread adoption of internationally recognized certified reference materials (CRMs) across different environmental and food matrices to ensure analytical comparability and traceability. In parallel, toxicological research should be expanded to investigate the bioavailability, accumulation, and long-term health effects of microplastics in humans and animals. Collectively, these directions represent the essential next steps toward harmonized analytical methodologies and comprehensive risk assessment of microplastics in food systems (Tsochatzis et al., 2024).

LEGISLATIVE FRAMEWORK FOR MICROPLASTICS IN THE EUROPEAN UNION

Microplastics in food: Regulatory landscape in the EU

Currently, there is no specific EU legislation directly regulating the presence of microplastics in food. However, microplastics are considered contaminants, which fall under general food safety regulations. According to **Regulation (EC) No. 178/2002**, also known as the General Food Law, food products placed on the EU market must not pose a risk to human health or be unsuitable for consumption (Rautio, 2023). Although there are no defined limits for microplastic content in food, the European Food Safety Authority (EFSA) is actively monitoring the issue and assessing potential risks. Future regulations may establish maximum allowable concentrations based on emerging scientific evidence. In addition, the EU is addressing microplastic pollution at the source, which indirectly impacts food safety. In 2024, the European Parliament approved a regulation to ban certain single-use plastic packaging by 2030, including small condiment sachets, to reduce excessive plastic use and prevent further microplastic contamination in the environment (**Regulation (EU) 2025/40**).

General EU regulations on microplastics

Growing concerns over the accumulation of microplastics in the environment and their potential impact on human health have prompted the European Union to implement several regulatory measures. One key legislative step has been the inclusion of microplastics as a restricted substance under the REACH regulation (registration, evaluation, authorization, and restriction of chemicals) (Rautio, 2023).

Table 1 Overview of key EU legislation and initiatives related to microplastics

Legislation / Initiative	Year	Scope / Area of Application	Relevance for Microplastics
Regulation (EC) No 178/2002	2002	General food law establishing food safety principles and EFSA	Defines microplastics as potential food contaminants subject to general safety requirements
Regulation (EU) 2023/2055 (REACH amendment)	2023	Restriction on intentionally added microplastics in products	Prohibits microplastic particles > 0.01% w/w in various consumer and industrial products
Regulation (EU) 2025/40 on packaging and packaging waste	2025	Reduction of single-use plastics and packaging waste	Indirectly reduces microplastic pollution from packaging degradation
ECHA proposal under REACH	2023	Registration, evaluation, authorization, and restriction of chemicals	Introduces phased ban on microplastic use in cosmetics, agriculture, and sports fields
EFSA scientific assessment	2024 – ongoing	Food safety and toxicological risk assessment	Evaluates health risks associated with dietary exposure to micro- and nanoplastics
ISO 24187:2023	2023	Standardized principles for sampling and measurement of microplastics	Harmonizes terminology, size classification, and analytical procedures
Plastics strategy and circular economy action plan	2018 – 2024	EU framework for plastic reduction and recycling	Supports research and policy measures to minimize secondary microplastic release

Together, these legislative and strategic measures provide the regulatory foundation for harmonizing analytical standards such as ISO 24187:2023 and for developing future food-related microplastic limits.

According to **Commission Regulation (EU) 2023/2055**, the sale of intentionally added microplastics is prohibited. This restriction applies to materials containing microplastic particles in concentrations exceeding 0.01% by weight. The ban is being phased across various industries, including cosmetics, agriculture, and sports. For example, by 2027, the restriction will apply to rinse-off cosmetic products, while by 2031, it will extend to plant protection products and granular infill used on synthetic sports surfaces. While current regulations primarily target

primary microplastics, efforts are also underway to address secondary microplastics resulting from the breakdown of larger plastic items. These initiatives are being pursued through the Plastics Strategy and the Circular Economy Action Plan (Rautio, 2023).

For better clarity, the main EU legal and policy instruments related to microplastic regulation are summarized in Table 1, highlighting their scope and significance for food and environmental safety.

ISO STANDARDS FOR MICROPLASTIC ANALYSIS: STANDARDIZING METHODS AND CLASSIFICATION

In September 2023, the International Organization for Standardization (ISO) released ISO 24187:2023, titled “Principles for the analysis of microplastics in the environment”. This standard provides a framework for analyzing microplastics in various environmental matrices. It includes unique classifications for particle sizes, recommended sampling equipment, and standardized approaches for sample preparation and analysis. The standard aims to improve consistency and comparability of results across laboratories by defining minimum requirements, although it does not address monitoring activities.

ISO 24187:2023 defines microplastics as solid plastic particles insoluble in water, with at least one dimension between 1 µm and 1,000 µm. It also introduces the “large microplastics” category for particles measuring between 1 mm and 5 mm. This distinction contrasts with typical definitions used by European and American environmental agencies, which often classify microplastics as particles smaller than 5 mm without additional subdivisions.

Categorization of particles into size ranges

ISO 24187:2023 introduces a classification system that divides microplastic particles into seven distinct size ranges, detailing the average particle size, mass, and number of particles per 14.13 mg for each class. This classification facilitates more accurate impact assessments by considering the differences between samples containing numerous small particles versus a few larger ones, which might have an equivalent mass but vary significantly in particle count. ISO 24187:2023 can also provide a valuable basis for analyzing microplastics in food samples, as research indicates that microplastics are present in a wide range of foods, including seafood, beverages, fruits, and vegetables (Kumar et al., 2024). The classification of particle size ranges defined in ISO 24187:2023 is summarized in Table 2, which provides a quantitative overview of representative size intervals and their corresponding mass and particle counts.

Table 2 Particle size classification in ISO 24187:2023

Particle size range (µm)	Average particle size (µm)	Mass of average-sized spherical particle with a density of 1 (mg)	Number of particles per 14.13 mg
1 to < 5	3	1.4×10^{-8}	1.0×10^9
5 to < 10	7.5	2.2×10^{-7}	6.4×10^7
10 to < 50	30	1.4×10^{-5}	1.0×10^6
50 to < 100	75	2.2×10^{-4}	6.4×10^4
100 to < 500	300	0.014	1.0×10^3
500 to < 1,000	750	0.22	6.4×10^1
1,000–5,000*	3,000	14	1

* Particles in this size range are classified as “large microplastics”.

ISO 24187:2023 highlights the importance of selecting the most appropriate analytical method for microplastic testing according to the specific objectives of the analysis. These objectives typically involve identifying the types of polymers present in the sample, determining the total number of particles together with their distribution across different size ranges and polymer categories, and quantifying the mass fraction of microplastics relative to the overall sample. In addition to these quantitative parameters, qualitative information—such as particle shape, surface morphology, and the presence of additives—provides valuable insight into the origin, degradation state, and potential behavior of microplastics within food and environmental matrices. The analytical methods most commonly specified in ISO 24187:2023, together with their information output, detection limits, and key advantages or limitations, are presented in Table 3.

Table 3 Overview of selected microplastic analysis methods specified in ISO 24187:2023

Method	Obtained information	Smallest detectable particle size	Advantages	Disadvantages
Raman spectroscopy	Number of particles per size range and polymer type	~ 1 µm	+ Detects a wide variety of plastics + No interference from water	- No direct quantification - Fluorescence from inorganic materials
Pyrolysis-gas chromatography-mass spectrometry (py-GC/MS)	Mass fraction by polymer type	~ 0.4 µm**	+ Direct quantification	- No particle size information - Limited capability to detect different polymers
Fourier transform infrared spectroscopy (FTIR)*	Number of particles by size range and polymer type	~ 5 to 10 µm	+ Detects a wide variety of plastics + No fluorescence from inorganic materials	- No direct quantification - Interference from water

* The standard also mentions ATR-FTIR and FPA-FTIR as possible FTIR setups

** The method does not directly detect individual particles, but filtering can be used to measure the total mass of plastic particles belonging to certain size ranges. In theory, there is no lower limit for particle size, but in practice, filters with a minimum pore size of 0.4 µm are used during sample preparation.

The techniques previously mentioned represent the most commonly utilized methods in commercial microplastic laboratories. However, ISO 24187:2023 also acknowledges additional analytical techniques, such as Differential Scanning Calorimetry (DSC), Near-Infrared Spectroscopy (NIR), Quantum Cascade Laser Infrared Spectroscopy (QCL-IR), Thermal Extraction and Desorption-Gas Chromatography-Mass Spectrometry (TED-GC-MS), and visual particle sorting using microscopy. Recent studies emphasize the need for standardized methodologies to improve comparability between microplastic analyses, particularly given the variations in polymer composition, particle size, and environmental contamination sources (Garrido Gamarro and Costanzo, 2022). In recent years, analytical development has shifted toward automated and hybrid technologies that increase throughput and reduce human error. Automated µFTIR imaging and Laser Direct Infrared (LDIR) chemical imaging systems enable high-resolution in-situ mapping and polymer classification with minimal sample handling, supporting large-scale microplastic monitoring. Moreover, coupling vibrational spectroscopy with chromatographic and mass-spectrometric

approaches, such as LC-MS/MS or Py-GC/MS-MS, facilitates simultaneous identification of polymers and additive compounds. These advances represent a general trend toward miniaturization, automation, and integrated analytical workflows for real-time microplastic assessment in complex food and environmental matrices.

Sampling and sample preparation principles

ISO 24187 specifies several essential guidelines for microplastic sampling and sample preparation to ensure representativeness and analytical reliability. The collected sample volume must be sufficient to provide a representative mass or number of particles, and in environments where microplastic concentrations are expected to be low, larger sample volumes are recommended. Filtration procedures should comply with the size ranges defined in the standard, and filters must be free of plastic materials to prevent cross-contamination. To preserve the physical integrity of plastic particles, preparatory steps such as drying, milling, or grinding

should be performed carefully; drying temperatures, for example, should not exceed 40°C to avoid polymer deformation or degradation. ISO 24187 also encourages the adaptation of relevant existing standards—such as ISO 5667 for water, sediment, and sludge, ISO 23611 for soil, and ISO 16000 for air—to meet the specific requirements of microplastic analysis, ensuring consistency across environmental and food-testing laboratories.

Microplastic testing under ISO 24187:2023

Measurlabs offers microplastic testing services utilizing methods aligned with ISO 24187, such as Raman spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR), and pyrolysis-GC/MS. These methods can analyze microplastics in environmental samples, consumer products, food, biological matrices, and other specialized contexts. Recent studies indicate that microplastics have been detected in a variety of food products, including seafood, bottled water, salt, and processed foods, with concentrations varying depending on the food matrix and processing methods (Kumar et al., 2024). Testing plans can be tailored to include compliance with legal requirements, such as the recent REACH restrictions on microplastics. Detailed descriptions of the sample and analysis objectives are recommended for inquiries or quotes to ensure accurate and timely service.

ADVANCEMENTS IN REFERENCE MATERIALS FOR MICROPLASTIC ANALYSIS: STANDARDIZATION, CHALLENGES, AND INNOVATIONS

The detection and quantification of microplastics in environmental and food samples have become critical research areas due to growing concerns about their potential impacts on ecosystems and human health. To ensure the accuracy and comparability of analytical methods across laboratories, the development of reliable reference materials (RMs) is essential. Building on previous developments by institutions such as Chiron AS and the Norwegian Institute for Water Research (NIVA), recent innovations have expanded beyond traditional solid microplastic reference materials to include liquid-based formats. These new materials aim to improve homogeneity, simulate environmental matrices, and enhance method validation. Harmonization efforts under initiatives such as EUROqCHARM further contribute to standardizing RM production across laboratories (Tambo Magni, 2023). The increasing need for validated reference materials has been highlighted in large-scale interlaboratory comparison (ILC) studies, demonstrating the significant variability in microplastic analysis across laboratories.

The role of reference materials in microplastic analysis

Reference materials are pivotal in validating and standardizing analytical techniques used to detect microplastics. Given the heterogeneous nature of microplastics—encompassing a variety of polymers, shapes, and sizes—standardized RMs are necessary to ensure reproducibility and comparability between laboratories (Martínez-Francés et al., 2023). Interlaboratory comparison (ILC) studies have revealed significant variability in the quantification and identification of microplastics. RMs serve as quality assurance (QA/QC) tools, facilitating spiking experiments where known quantities of microplastics are added to sample matrices to assess recovery efficiency (Martínez-Francés et al., 2023). The WEPAL-QUASIMEME study found that while laboratories could accurately identify polymer types, quantification varied widely, emphasizing the urgent need for harmonization.

Recent developments in reference materials

Solid-based reference materials

Traditional approaches to RMs have focused on solid forms, such as dissolvable gelatin capsules containing polymers like polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene (PE), and polystyrene (PS) in various size fractions (50–1000 µm). Soda tablets have also been utilized as carriers for microplastic RMs in interlaboratory comparison exercises, demonstrating reliability in these applications (Martínez-Francés et al., 2023).

The ZeptoMetrix MicroPRefs® tablets contain standardized microplastic particles to validate particle counting in microscopy and vibrational spectroscopy. These tablets dissolve in water, releasing known microplastic quantities, and are crucial for laboratory calibrations (ZeptoMetrix Corporation, 2024).

Liquid-based reference materials

Innovations have extended to the development of liquid-based RMs, which offer advantages in specific analytical contexts. For instance, MicroplasticSolution has introduced a liquid form of RMs designed to facilitate more uniform dispersion of microplastic particles within sample matrices. This approach aims to improve the accuracy of spiking experiments and method validation by reducing issues related to particle aggregation and sedimentation (MicroplasticSolution, 2025).

The liquid RMs are engineered to mimic environmental conditions more closely, providing a more representative assessment of analytical methods used for

microplastic detection. This development addresses some of the limitations associated with solid-based RMs, particularly in achieving a homogeneous distribution of microplastics in test samples (MicroplasticSolution, 2025).

Preparation of environmentally relevant reference materials

Recent research has focused on developing RMs that closely mimic the characteristics of environmental microplastics. A study by Tambo Magni (2023) introduced a method for preparing RMs using natural water matrices to better simulate environmental conditions. This approach involves collecting environmental water samples, spiking them with known quantities of microplastics, and homogenizing the mixture to create RMs that reflect the complexity of natural samples.

Similarly, Chiron AS has been developing PRefs® reference materials, including microplastic tablets and neat PE, PP, PET, PS, PVC, and PC powders, with size fractions ranging from 50 to 300 µm. These materials are undergoing certification for inclusion in proficiency testing programs (Tambo Magni, 2023).

Challenges in the development of microplastic reference materials

Producing environmentally relevant RMs

Most commercially available RMs consist of uniform microplastics, such as spherical beads or pellets, which do not fully replicate the complexity of microplastics in real-world environmental and food matrices. Environmental microplastics are heterogeneous in size, shape, and polymer composition, making their detection and quantification more challenging (Martínez-Francés et al., 2023). This issue has also been highlighted in recent research, emphasizing the need to develop more representative microplastic reference materials to improve interlaboratory comparability and quality assurance (Dehaut et al., 2023). To improve environmental relevance, recent efforts have focused on cryogenic milling and sonication techniques, which generate microplastic particles with more realistic size distributions and morphologies. These methods produce fragments that appear closer to degraded plastic pollutants found in nature, enhancing the applicability of RMs for analytical validation (Martínez-Francés et al., 2023). Additionally, alternative preparation methods, such as encapsulating microplastic fragments in gelatin-based capsules, have been proposed to facilitate handling and reproducibility in laboratory settings (Dehaut et al., 2023).

Contamination and loss of particles

Microplastics have low mass and are susceptible to static electricity, making accurate dosing into sample matrices difficult. Additionally, particles may adhere to laboratory containers, leading to sample loss and underestimation during analysis (Martínez-Francés et al., 2023). These issues complicate the validation of microplastic recovery experiments and introduce uncertainties in measuring concentration levels in food and environmental samples. One study suggests implementing stringent contamination control measures, such as procedural blanks and clean-air environments, to mitigate these risks.

Lack of validated methods for small microplastics (<50 µm)

Most currently available RMs are suitable for microplastic particles above 50 µm, whereas methods for smaller particles remain inconsistent. Further research is required to optimize RMs for nano- and sub-50 µm microplastics, as their analytical variability is too high for reliable QA/QC applications (Martínez-Francés et al., 2023). The development of standardized methodologies for detecting smaller microplastics is essential, given their increased prevalence in food and environmental samples (Cowger et al., 2020). Recent advancements in micro-Raman spectroscopy and advanced filtration techniques are promising approaches for addressing this gap (Garrido Gamarro and Costanzo, 2022).

CONCLUSION

Microplastic contamination in food chains represents a pressing environmental and public health concern, necessitating robust analytical methodologies and standardized reference materials (RMs) to ensure accurate detection and quantification. Despite significant progress, challenges such as inconsistent data reporting, the lack of certified reference materials (CRMs) tailored for food matrices, and limitations in current analytical techniques continue to hinder research comparability and regulatory efforts. The development and implementation of CRMs play a pivotal role in addressing these challenges by improving method validation, enhancing interlaboratory comparability, and fostering regulatory integration. Recent advancements, including the incorporation of RMs into diverse environmental and food matrices, underscore their potential to standardize microplastic detection techniques and mitigate inconsistencies in analytical outcomes. However, gaps remain in the availability of CRMs that accurately reflect the diversity of microplastics in real-world food samples. Emerging research highlights the need for CRMs that account for microplastic degradation products and interactions with food components such as lipids and proteins, which can alter analytical results. Furthermore, developing microplastic

standards that include realistic environmental contaminants, such as persistent organic pollutants (POPs) and heavy metals, is essential for improving toxicological risk assessments. Future research must focus on refining CRM production, optimizing analytical methodologies for detecting smaller microplastic particles, and integrating these advancements into regulatory frameworks such as ISO standards and EU food safety directives.

A critical aspect of future developments will be the expansion of international collaborations aimed at harmonizing microplastic monitoring efforts. Initiatives such as EUROqCHARM and the OECD's work on plastic pollution illustrate the importance of global cooperation in standardizing methodologies and defining regulatory thresholds. Adopting internationally recognized standards, including ISO 24187:2023, will be essential in harmonizing methods and improving the reliability of microplastic monitoring. Addressing these challenges requires a coordinated effort between researchers, policymakers, and industry stakeholders to develop a comprehensive regulatory framework. By advancing methodological standardization and expanding the availability of high-quality RMs, the scientific community can enhance the reliability of microplastic analysis, inform risk assessments, and support evidence-based policy decisions to mitigate microplastics' impact on human health and the environment. Future research should prioritize the development of certified reference materials (CRMs) specifically designed for complex food and plant-based matrices, where natural polymers and pigments interfere with spectroscopic identification. Particular emphasis should be placed on nano- and sub-50 µm fractions to improve method validation and quantification accuracy at lower particle sizes. These research priorities would bridge the current analytical gap and support harmonized risk assessment of micro- and nanoplastics in diverse food systems. From an environmental perspective, the development of sustainable analytical workflows represents an essential step toward reducing laboratory waste and solvent use. Applying the principles of green analytical chemistry in microplastic analysis—including miniaturization, automation, and solvent-free extraction—can minimize ecological impact while maintaining analytical reliability. Integrating such eco-efficient approaches into future CRM development and food testing will align microplastic research with broader sustainability objectives and EU Green Deal policies.

Acknowledgements: This work was supported by the Slovak Research and Development Agency under grant: the Contract no. APVV-23-0036.

REFERENCES

- Collard, F., Gilbert, B., Compère, P., Eppe, G., Das, K., Jauniaux, T., & Parmentier, E. (2017). Microplastics in livers of European anchovies (*Engraulis encrasicolus*, L.). *Environmental Pollution*, 229, 1000–1005. <https://doi.org/10.1016/j.envpol.2017.07.089>
- Cowger, W., Booth, A. M., Hamilton, B. M., et al. (2020). Reporting guidelines to increase the reproducibility and comparability of research on microplastics. *Applied Spectroscopy*, 74(9), 1066–1077. <https://doi.org/10.1177/0003702820930292>
- Da Costa Filho, P.A., Andrey, D., Eriksen, B., et al. (2021). Detection and characterization of small-sized microplastics (≥ 5 µm) in milk products. *Scientific Reports*, 11(1), 23458. <https://doi.org/10.1038/s41598-021-03458-7>
- Dehaut, A., Himber, C., Colin, M., and Duflos, G. (2023). Think positive: Proposal of a simple method to create reference materials in the frame of microplastics research. *MethodsX*, 10, 102030. <https://doi.org/10.1016/j.mex.2023.102030>
- ECHA (2023). Proposal for restricting microplastics under REACH regulation. *European Chemicals Agency, Technical Report*. Retrieved from <https://echa.europa.eu/hot-topics/microplastics>
- European Commission (2023). Protecting the environment and public health: Commission Adopts Measures to Restrict Intentionally Added Microplastics. Available at: https://ec.europa.eu/commission/presscorner/detail/en/ip_23_4581
- European Commission. (2023). *Commission Regulation (EU) 2023/2055 of 25 September 2023 amending Regulation (EU) No 10/2011 as regards the use of certain substances in plastic materials and articles intended to come into contact with food*. Official Journal of the European Union, L 155, 12–18. Available at <https://eur-lex.europa.eu/eli/reg/2023/2055/oj>
- Garrido Gamarro, E., and Costanzo, V. (2022). Microplastics in food commodities – A food safety review on human exposure through dietary sources. *Food Safety and Quality Series No. 18, FAO*. <https://doi.org/10.4060/cc2392en>
- Geyer, R., Jambeck, J.R., Law, K.L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, 3(7), e1700782. <https://doi.org/10.1126/sciadv.1700782>
- Cho, Y., Shim, W.J., Jang, M., Han, G.M., Hong, S.H. (2019). Abundance and characteristics of microplastics in market bivalves from South Korea. *Environmental Pollution*, 245, 1107–1116. <https://doi.org/10.1016/j.envpol.2018.11.091>
- International Organization for Standardization (ISO). (2023). *ISO 24187:2023 – Microplastics – Guidelines for sampling, preparation, and measurement techniques*. Geneva: ISO. Available at: <https://www.iso.org/standard/78033.html>
- International Organization for Standardization (ISO). (2023). *ISO 5667 – Water quality – Sampling*. Geneva: ISO. Available at: <https://www.iso.org/standard/55452.html>
- International Organization for Standardization (ISO). (2023). *ISO 23611 – Soil quality – Sampling of soil invertebrates*. Geneva: ISO. Available at: <https://www.iso.org/standard/72744.html>
- International Organization for Standardization (ISO). (2023). *ISO 16000 – Indoor air – Sampling and analysis of volatile organic compounds*. Geneva: ISO. Available at: <https://www.iso.org/standard/73522.html>
- Jacob, O., Stefaniak, E. A., Seghers, J., La Spina, R., Schirinzi, G. F., Chatzipanagis, K., Held, A., Emteborg, H., Koeber, R., Elsner, M., Ivleva, N. P. (2024). Towards a reference material for microplastics' number concentration—case study of PET in water using Raman microspectroscopy. *Analytical and Bioanalytical Chemistry*, 416 (12), 3045–3058. Springer Science and Business Media LLC. <https://doi.org/10.1007/s00216-024-05251-7>
- Kumar, V., Sharma, N., Umesh, M., Gupta, P., Sharma, P., Basheer, T., et al. (2024). Microplastics in food: Occurrence, toxicity, green analytical detection methods and future challenges. *Green Analytical Chemistry*, 11, 100152. <https://doi.org/10.1016/j.greeac.2024.100152>
- Kye, H., Kim, J., Ju, S., Lee, J., Lim, C., and Yoon, Y. (2023). Microplastics in water systems: A review of their impacts on the environment and their potential hazards. *Heliyon*, 9, e14359. <https://doi.org/10.1016/j.heliyon.2023.e14359>
- Leslie, H. A., Depledge, M. H. (2020). Where is the evidence that human exposure to microplastics is safe? *Environment International*, 142, 105807. <https://doi.org/10.1016/j.envint.2020.105807>
- Martínez-Francés, E., van Bavel, B., Hurley, R., Nizzetto, L., Pakhomova, S., Buenaventura, N. T. and Lusher, A. (2023). Innovative reference materials for method validation in microplastic analysis including interlaboratory comparison exercises. *Analytical and Bioanalytical Chemistry*, 415(2907–2919). <https://doi.org/10.1007/s00216-023-04636-4>
- Mason, S. A., Welch, V. G., Neratko, J. (2018). Synthetic polymer contamination in bottled water. *Frontiers in Chemistry*, 6, 407. <https://doi.org/10.3389/fchem.2018.00407>
- MicroplasticSolution (2025). *Microplastic reference materials*. Retrieved January 29, 2025, from <https://www.microplasticsolution.com/microplastic-reference-materials>
- O'Connor, J. D., Mahon, A. M., Ramsperger, A. F. R. M., Trotter, B., Redondo-Hasselerharm, P. E., Koelmans, A. A., Lally, H. T., & Murphy, S. (2019). Microplastics in freshwater biota: A critical review of isolation, characterization, and assessment methods. *Global Challenges*, 4, 6. <https://doi.org/10.1002/gch2.201800118>
- Oliveri Conti, G., Ferrante, M., Banni, M., Favara, C., Nicolosi, I., Cristaldi, A., Fiore, M., Zuccarello, P. (2020). Micro- and nano-plastics in edible fruit and vegetables. The first diet risks assessment for the general population. *Environmental Research*, 187, 109677. <https://doi.org/10.1016/j.envres.2020.109677>
- Oßmann, B.E., Sarau, G., Holtmannspötter, H., Pischetsrieder, M., Christiansen, S.H., Dicke, W. (2018). Small-sized microplastics and pigmented particles in bottled mineral water. *Water Research*, 141, 307–316. <https://doi.org/10.1016/j.watres.2018.05.027>
- Pironti, C., Ricciardi, M., Motta, O., Miele, Y., Proto, A., and Montano, L. (2021). Microplastics in the environment: Intake through the food web, human exposure and toxicological effects. *Toxics*, 9(9), 224. <https://doi.org/10.3390/toxics9090224>
- Prata, J. C., Silva, A. L. P., da Costa, J. P., Mouneyrac, C., Walker, T. R., Duarte, A. C., Rocha-Santos, T. (2019). Solutions and integrated strategies for the control and mitigation of plastic and microplastic pollution. *International Journal of Environmental Research and Public Health*, 16(13), 2411. <https://doi.org/10.3390/ijerph16132411>
- Rautio, A. (2023). Microplastic regulations in the EU – how to ensure compliance? *Measurlabs*. Available from: <https://measurlabs.com/blog/microplastic-regulations-and-testing-in-the-eu/>
- Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities, L 31, 1.2.2002, pp. 1–24. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32002R0178>
- Regulation (EU) 2025/40 of the European Parliament and of the Council of 19 December 2024 on packaging and packaging waste, amending Regulation (EU) 2019/1020 and Directive (EU) 2019/904, and repealing Directive 94/62/EC. Available at: <https://eur-lex.europa.eu/eli/reg/2025/40/oj/eng>
- Sanchez Silva, A., Cruz, J.M., Sendón García, R., Franz, R., Paseiro Losada, P. (2007). Kinetic migration studies from packaging films into meat products. *Meat Science*, 77(2), 291–296. <https://doi.org/10.1016/j.meatsci.2007.03.009>
- Sharma, S. and Chatterjee, S. (2017). Microplastic pollution, a threat to marine ecosystem and human health: A short review. *Environmental Science and Pollution Research*, 24, 21530–21547. <https://doi.org/10.1007/s11356-017-9910-8>
- Schymanski, D., Goldbeck, C., Humpf, H.U., Fürst, P. (2018). Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles

- from different packaging into mineral water. *Water Research*, 129, 154–162. <https://doi.org/10.1016/j.watres.2017.11.011>
- Tambo Magni, M.-L. (2023). Reference materials for microplastic analysis. EUROqCHARM Final Conference. https://ilvo.vlaanderen.be/uploads/documents/04_Reference-materials-for-microplastic-analysis_Marie-Louise-Tambo-Magni.pdf
- Tsochatzis, E. D., Gika, H., Theodoridis, G., Maragou, N., Thomaidis, N., Corredig, M. (2024). Microplastics and nanoplastics: Exposure and toxicological effects require important analysis considerations. *Heliyon*, 10, e32261. <https://doi.org/10.1016/j.heliyon.2024.e32261>
- Wiesinger, H., Wang, Z., Hellweg, S. (2021). Deep dive into plastic monomers, additives, and processing aids. *Environmental Science & Technology*, 55 (13), 9339–9351, American Chemical Society (ACS). <https://doi.org/10.1021/acs.est.1c00976>
- Yee, M. S.-L., Hii, L.-W., Looi, C. K., Lim, W.-M., Wong, S.-F., Kok, Y.-Y., Tan, B.-K., Wong, C.-Y., Leong, C.-O. (2021). Impact of microplastics and nanoplastics on human health. *Nanomaterials*, 11(2), 496, <https://doi.org/10.3390/nano11020496>
- ZeptoMetrix Corporation (2024). *FAQ-Microplastics*, 2024 (Rev. 3). Accessed: 03-02-2025. Available at: <https://web-resources-prod.zeptometrix.com/Product+Literature/Analytical+Product+Literature/FAQ-MICROPLASTICS-2024-REV3test.pdf>
- Zhang, L., Xiao, R., Jin, T., Pan, X., Fransen, K. A., Alsaiani, S. K., Lau, A., He, R., Han, J., Pedretti, B. J., Yeo, J. Y., Yang, X., Olsen, B. D., Alexander-Katz, A., Smith, Z. P., Langer, R., & Jaklenec, A. (2024). Degradable poly(β -amino ester) microparticles for cleansing products and food fortification. *Nature Chemical Engineering* 2 (1), 77–89 <https://doi.org/10.1038/s44286-024-00151-0>