Italian Journal of Food Safety

https://www.pagepressjournals.org/index.php/ijfs/index

eISSN 2239-7132

Publisher's Disclaimer. E-publishing ahead of print is increasingly important for the rapid dissemination of science. The Early Access service lets users access peer-reviewed articles well before print/regular issue publication, significantly reducing the time it takes for critical findings to reach the research community.
These articles are searchable and citable by their DOI (Digital Object Identifier).

The **Italian Journal of Food Safety** is, therefore, E-publishing PDF files of an early version of manuscripts that have undergone a regular peer review and have been accepted for publication, but have not been through the copyediting, typesetting, pagination, and proofreading processes, which may lead to differences between this version and the final one.

The final version of the manuscript will then appear in a regular issue of the journal.

The E-publishing of this PDF file has been approved by the authors.

Ital J Food Saf 2026 [Online ahead of print]

Please cite this article as:

Santonicola S, Volgare M, Cocca M, et al. **Analytical protocol assessment for microplastic and microfiber isolation in milk: a preliminary study of contamination in raw milk samples**. *Ital J Food Saf* doi:10.4081/ijfs.2026.14780

Submitted: 26-12-2025
Accepted: 02-02-2026

© the Author(s), 2026
Licensee PAGEPress, Italy

Note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries should be directed to the corresponding author for the article.
All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Analytical protocol assessment for microplastic and microfiber isolation in milk: a preliminary study of contamination in raw milk samples

Serena Santonicola,[1,2] Michela Volgare,[3] Mariacristina Cocca,[2]
Franca Rossi,[1] Gennaro Raimo,[1] Giampaolo Colavita[1]

[1]Department of Medicine and Health Sciences "V. Tiberio", University of Molise, Campobasso; [2]Institute of Polymer, Composites and Biomaterials, National Research Council of Italy, Pozzuoli (NA); [3]Department of Chemical Engineering, Materials, and Industrial Production, University of Naples Federico II, Naples, Italy

Correspondence: Serena Santonicola, Department of Medicine and Health Sciences "V. Tiberio", University of Molise, Campobasso, Italy.
E-mail: serena.santonicola@unimol.it

Key words: microplastics, microfibers, raw milk contamination, recovery experiments, analytical method accuracy.

Contributions: Serena Santonicola: writing original draft, investigation, data curation. Michela Volgare: data curation, statistical analyses. Mariacristina Cocca, Franca Rossi, Gennaro Raimo, Giampaolo Colavita: manuscript writing and editing. Mariacristina Cocca, Giampaolo Colavita: supervision, and conceptualization. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare that they have no competing interests.

Ethics approval and consent to participate: not applicable.

Availability of data and materials: the datasets used and/or analyzed during the current study are available upon reasonable request from the corresponding author.

Conference presentation: this paper was presented at the XXXIV National Conference of the Italian Association of Veterinary Food Hygienists (AIVI), Parma, September 10-12, 2025.

Abstract
Microplastics (MPs) and microfibers (MFs) are emerging contaminants in food, with milk representing a critical concern due to its importance in human nutrition, particularly for vulnerable populations. This study aimed to optimize an analytical protocol for isolating MPs and MFs in milk samples and to assess contamination levels in raw milk collected at farms. The digestion method employed hydrogen peroxide (30% v/v) at optimized temperatures (40°C for commercial milk and 50°C for raw milk), followed by vacuum filtration. Method accuracy was evaluated through recovery experiments using spiked reference materials, and then the extraction protocol was applied to raw milk samples from mechanical milking. Results revealed average concentrations ranging from 1.3±0.5 to 5.5±5.0 MPs and synthetic MFs per 100 mL, with natural MFs accounting for 47% of total particles detected. Chemical characterization through Fourier transform infrared spectroscopy identified polypropylene (41%), polyester (6%), and polyvinyl chloride (6%) as the predominant polymer types, suggesting contamination from milking equipment and textile materials. No significant differences in contamination levels were observed across sampling days or among individual cows, indicating systematic contamination along the production chain. This study provides a validated analytical approach for monitoring MP contamination in milk and establishes baseline data for raw milk contamination at farm level, supporting the development of mitigation strategies along the dairy supply chain.

Introduction
Microplastics (MPs), defined as plastic particles smaller than 5 mm, are widespread across aquatic, atmospheric, and terrestrial ecosystems, with the potential to contaminate the trophic chain and ultimately reach humans through food consumption (Dong *et al.*, 2023). Recent studies have demonstrated the presence of MPs not only in seafood products but also in various food matrices, including milk (Mamun *et al*., 2023; Diaz-Basantes *et al*., 2020; Kutralam-Muniasamy *et al*., 2020; Kiruba *et al*., 2022; Basaran *et al*., 2023; Kaseke *et al*., 2023; Zhang *et al*., 2023; Chakraborty *et al*., 2024; Rbaibi Zipak *et al*., 2024; Santonicola *et al*., 2025a).
Food consumption is the primary route of human exposure to MPs (Rahman *et al*., 2021; Corte Pause *et al*., 2024; Kim *et al*., 2022). This issue raises concerns about the impact on human health, mainly for vulnerable populations such as children aged 0-3 years (Mamun *et al*., 2023; Banica *et al*., 2024). The additives that are added to plastic may interfere with hormones and lead to disease at such young ages (Banica *et al*., 2024). These findings are particularly concerning, considering that bovine milk is used for infant formula production (Caba-Flores *et al*., 2023), and yogurt is also among the first recommended complementary foods to be added to breast milk in infant feeding (Rbaibi Zipak *et al*., 2022).
The contamination of food chains may derive from two main sources: the environment and/or food production processes, and through contact with packaging materials (Kadac-Czapska *et al*., 2023). In detail, MP contamination may occur at multiple points, such as processing, treatment, distribution, and final preparation stages of food products (Toussaint *et al*., 2019). Da Costa *et al*. (2021) evaluated the presence of MPs in raw milk collected at the milking parlor and in commercial liquid and powdered milk, demonstrating that the number of plastic particles increased from farm to processed milk. Also, Italian-branded milk samples showed the highest levels of fibrous MPs in ultrahigh-temperature skimmed milk, indicating that the more complex the processing of milk, the more MPs it contains (Santonicola *et al*., 2025a). Although this provides useful insights into the accumulation of plastic particles along the milk production chain, further investigation is needed to identify which specific points contribute directly and significantly to milk and dairy products contamination.
At the farm, the occurrence of cellulose fragments and microfibers (MFs) in raw milk obtained by manual milking has been attributed to the wipes used for cleaning the udder before milking and clothing worn by farm personnel (Santonicola *et al.*, 2025b). In addition, MPs could be introduced during milking from the plastic components of milking machines (Santonicola *et al*., 2025a). Therefore, to address the issue of MP contamination, the entire milk production chain should be

reviewed to better understand the levels of MPs already present in the raw product and those which are introduced during processing and packaging (Corte Pause *et al*., 2024; Kaseke *et al*., 2023; Santonicola *et al*., 2025a). This information may help to implement mitigation and control measures that are likely to change the content of MPs in milk and dairy products (Di Fiore *et al*., 2023).
Milk represents an extremely complex food matrix for MP analysis (Kwon *et al*., 2020; Banica *et al*., 2024). Moreover, differences in size cutoff, instruments used for identifying MPs, and related analytical protocols among research make it difficult to assess the true plastic load in milk and the evaluation of human exposure levels (Di Fiore *et al*., 2023; Corte Pause *et al*., 2024).
The standardization of methods applied for quantifying MPs in foods is an urgent requirement, and recovery of analytical methods for MP isolation should be validated using spiked reference materials in test matrices (Kim *et al*., 2022; Kadac-Czapska *et al*., 2023). Among the most common MP shapes found in food, fragment reference materials are relatively easy to produce through physical grinding, while MFs are more difficult to use during recovery tests due to potential intertwining between them (Pirc *et al*., 2016; Kim *et al*., 2022).
In this study, we assessed an analytical method for isolating and identifying MPs and both natural and synthetic MFs in milk samples. In the context of a supply chain study, we proposed an extraction protocol suitable for raw milk and heat-treated milk samples. Through a review of existing methods, the most appropriate pretreatment was selected and applied to commercial milk samples (Santonicola *et al*., 2025a) and then adapted for the analyses of raw milk (current study). For the evaluation of the extraction efficiency, reference materials of three different types [low-density polyethylene (LDPE) fragments, polyethylene terephthalate (PET) MFs, and artificial cellulose MFs] were spiked into milk samples and analyzed to obtain recovery rates. Subsequently, the validated method was applied to raw milk samples collected through mechanical milking to provide useful data on MP contamination at the first point of the dairy production chain.

Materials and Methods

Materials and chemicals

Hydrogen peroxide solution, 30% was provided by Carlo Erba (Val De Reuil, France). For the filtration of the samples and solutions, cellulose nitrate (pore size 8 µm) and acetate (pore size 0.45 µm) filters (Sartorius Stedim Biotech, Gottingen, Germany) were used, respectively. The filtration system was supplied by Advantec (Dublin, CA, USA). Macroporous silicon filters with 5 µm pore size, MakroPor, were purchased from Thermo Fisher Scientific (Massachusetts, USA).

Optimization of a digestion method for raw milk samples

Adequate sample preparation is essential for reliable quantification of plastic particles in food matrices. Pre-treatment protocols should optimize extraction yields and maintain the integrity of isolated particles throughout the analytical process (Kadac-Czapska *et al*., 2023). The appropriate digestion protocol for analyzing commercial milk samples, with different fat concentrations, was established through previous trials (Santonicola *et al*., 2025a). In brief, each sample was homogenized by gentle inversion of the containers prior to analysis, and a 100 mL aliquot was transferred to a pre-cleaned Erlenmeyer flask. Milk was then diluted at a 1:1 ratio using 100 mL of pre-filtered distilled water, followed by the addition of 40 mL H_2O_2 30% (v/v) to facilitate the degradation of organic material through oxidation (Kwon *et al*., 2020). In particular, hydrogen peroxide was chosen for sample digestion since it effectively removes organic components without altering the integrity of plastic particles (Tagg *et al*., 2017).
Subsequently, the samples were incubated at 40°C for a 48-hour period, since at lower temperatures, fat globules in milk migrate to the surface, remaining undigested and unable to pass through filters (Banica *et al*., 2024). In fact, refrigerated milk presents filtration challenges, while warm milk may end up blocking filters with the size ranges of 0.22, 0.45, and 5 µm (Kutralam-Muniasamy *et al*., 2020). Consequently, following the digestion process, the milk samples, maintained at 40°C, underwent vacuum filtration using membranes with an 8 µm pore diameter. For this purpose, cellulose

nitrate membranes were selected as they are less prone to fragmentation, while the choice of pore size of membranes was also correlated with the analytical technique used for identification, since the sensitivity of Fourier transform infrared microspectroscopy (μ-FTIR) reaches particles with a size of 10 μm (Bai *et al*., 2022; Kadac-Czapska *et al*., 2023).
The pre-treatment protocol was subsequently tested on raw milk samples. Since raw milk does not undergo fat globule homogenization, unlike commercial milk (Postelmans *et al*., 2020), the incubation temperature was raised to 50 °C for 48 h to achieve a rapid filtration without requiring multiple filter membranes per sample. Incubation temperatures between 50 and 70°C are sufficiently mild to preserve particle integrity and are routinely used for extracting thermoplastic polymers [*e.g*., polyethylene (PE), polypropylene (PP), polystyrene (PS), and PET], which constitute the predominant MP types in food (Kwon *et al*., 2020).

Assessment of extraction efficiency
Method accuracy was evaluated through recovery experiments, where known amounts of plastic particles were spiked into milk samples prior to digestion. In addition to the evaluation of extraction efficiency, potential particle alterations were also considered (including morphological changes and color modifications).
For a more thorough assessment, different polymer types need to be evaluated through spiking experiments in food matrices (Kadac-Czapska *et al*., 2023). Moreover, testing different particle shapes is essential, with particular attention required for MF recovery from food, as fibers can be lost more easily than other particles during digestion and filtration (Kwon *et al*., 2020).
The reference materials (PET, cellulose, and LDPE) were subjected to cryogenic grinding in a Retsch mill (Retsch Mulino Ultra Centrifugo ZM 300, Haan, Germany), using a sieve with 1 mm openings and subsequently with 0.80 mm openings to obtain a fine powder (MF length 170±134 μm; MP length 228±91 μm). According to Kim *et al.* (2022), each milk sample was spiked with a known number of MPs and MFs (sample 1: n.4 sky blue LDPE fragments, sample 2: n. 4 blue PET MFs, sample 3: n.4 green cellulosic MFs). The samples were analyzed in triplicate (R1-R3, Figure 1). Cellulose-based MFs were included in recovery experiments since these particles were the most prevalent contaminants found in commercial milk samples (Santonicola *et al*., 2025a).
Standard particles were carefully handled with tweezers and added individually to each sample. All samples were processed following the optimized digestion methodology. Each batch included at least one negative blank for quality control.

Identification of microplastics and microfibers isolated in spiked samples.
MP identification and characterization involve the analysis of particle size, morphology, color, and number, followed by the identification of polymer composition. Visual inspection serves as a preliminary screening step, which is subsequently confirmed through μ-FTIR spectroscopy (Kadac-Czapska *et al*., 2023). Particles retained on filters were examined using a light microscope (M205C; Leica, Wetzlar, Germany) at magnification ranging from 0.78× to 16×. Spiked particles were recognized based on their distinctive color and morphological features. Microscopy images were captured for each particle and subsequently analyzed with ImageJ software (release 1.43 u, NIH, Bethesda, MD, USA) to determine size distributions. For chemical characterization, particles were carefully transferred onto MakroPor silicon filters using distilled water and subjected to FTIR microscopy analysis (Nicolet iMX10, Thermo Fisher Scientific). The resulting spectra were compared with the FTIR library of the device to define the polymer type. FTIR measurements were performed in transmission mode with 64 averaged scans at a spectral resolution of 4 cm^{-1}. Spectral data were processed using OMNIC™ Spectra Software, and polymer identification was accepted when library matches exceeded 70% similarity.

Application of optimized method to raw milk samples collected at farm

Raw milk samples from mechanical milking were collected at a dairy farm located in Sant'Elia a Pianisi (Campobasso, Molise, Italy), housing 25 lactating Holstein-Friesian cows with an average milk production of 28 liters per day. The farm produces high-quality milk with protein and fat contents of 3.6% and 3.8%, respectively. Animals were fed with on-farm forage and commercial concentrates and maintained under tie-stall housing conditions. Previous samplings at the same farm were carried through manual milking out in order to identify contamination sources other than the milking machine (Santonicola et al., 2025b).

In the current study, sampling was conducted over 3 weeks, with one sampling per week. Three cows were selected, and milk from the same animals was sampled throughout the study. A total of 9 samples were obtained from the evening milking. During sampling, open Petri dishes containing cellulose filters, moistened with filtered distilled water, were used to assess environmental contamination. Samples were subsequently transported to the laboratory and analyzed following the digestion and identification protocols for MPs and MFs described in *Optimization of a digestion method for raw milk samples* and *Identification of microplastics and microfibers isolated in spiked samples* Sections.

Contamination prevention

Throughout milk analysis, rigorous contamination control measures were implemented to prevent airborne particles from contaminating the samples. The analyses were conducted within a laminar flow cabinet with all windows and doors sealed and restricted laboratory access. Personnel wore nitrile gloves and 100% cotton laboratory coats. Distilled water underwent pre-filtration through 0.45 μm cellulose acetate membranes. Work surfaces, equipment, and glassware were cleaned and triple-rinsed with filtered water prior to use. Each filter membrane was microscopically examined before filtration to verify the absence of contamination. During the filtration, aluminum foil covered the funnel, and filter membranes were stored in sealed, unused glass Petri dishes. Procedural blanks, consisting of 100 ml of filtered water, were analyzed alongside samples to account for potential procedural contamination.

Statistical analysis

Non-parametric statistical analyses were conducted using IBM SPSS Statistics software, version 23.0 (IBM, Chicago, IL, USA). The Kruskal-Wallis (KW) test was applied to compare MP and MF content across sampling days and among individual cows, as well as to assess differences in particle length. A significant level of $\alpha = 0.05$ was adopted for all tests.

Results

Method assessment

Visual inspection of the recovered reference materials revealed no signs of degradation or chromatic changes in any of the samples analyzed. The average recovery rates of reference materials in the validation were reported in Table 1. Total recovery rates were 102.77% and 91.6% for heat-treated and raw milk, respectively. In some cases, a greater number of reference particles than initially spiked were detected. Two possible reasons include potential intertwining between MFs when they were spiked into the samples or confusion with particles already present in the test matrix (Kim *et al*., 2022).

It should be noted that the dimensions of the recovered particles were considerably larger than those initially specified for the reference materials. Larger particles were deliberately selected for the recovery experiments to facilitate counting, manipulation, and spiking procedures.

FTIR analysis confirmed the polymeric composition of the recovered particles.

Microplastic and microfiber levels in raw milk samples

In the analyzed raw milk samples, the average number of MPs (including synthetic MFs) was 1.3±0.5 and 5.5±5.0 particles/100 mL, depending on the sampling day. A considerable number of natural MFs was also detected, averaging 5.5±5.7 MF/100 mL in positive samples.

No significant differences were observed in MP (KW–p=0.467) or MF (natural and synthetic) content (KW–p=0.948) across different sampling days; moreover, when comparing individual cows, no significant differences were found in either MP (KW – p=0.808) or MF content (KW – p=0.229) in milk samples.

The particles exhibited the following mean dimensions: natural MFs 1090.6 ± 810.1 μm, synthetic MFs 1168.5 ± 1260.1 μm, and fragments 321.5 ± 226.3 μm (Figure 2). Natural MFs showed significant differences in length depending on the sampling day (KW – p=0.006).

Regarding color distribution, MP fragments were mainly transparent (47%), while MFs (natural and synthetic) were predominantly black (48%) and blue (18%; Figure 3).

Chemical characterization revealed that 53% of particles were MPs and synthetic MFs, primarily composed of PP (41%, Figure 4A), polyester (PES; 6%), and polyvinyl chloride (PVC; 6%, Figure 4B), while 47% were natural/artificial cellulosic MFs (Figure 4C).

Discussion

Optimization of analytical method

One of the main routes of human exposure to MPs is food consumption; therefore, the quantitative analysis of these particles in most consumed foods is important (Kim *et al*., 2022; Kadac-Czapska *et al*., 2023). Discrepancies in methodological approaches have emerged, as well as in the expression of results, highlighting the urgent need for harmonization of analytical methods and the necessity of the validation of applied analytical methods (Kim *et al*., 2022; Di Fiore *et al*., 2023).

In this study, we optimized a pretreatment method for raw and commercial milk samples within a farm-to-fork perspective and verified the analytical method through recovery tests using particles with different compositions and morphologies. For sample digestion, solvents and temperatures, known in the literature not to damage the polymers most frequently found in food, were applied (Tagg *et al*., 2017; Kwon *et al*., 2020). The results demonstrated the integrity of the particles in terms of shape and color. The recovery rate obtained, in accordance with the literature, can be considered satisfactory as it exceeds 90% (Kadac-Czapska *et al*., 2023). However, in the case of MFs, recovery rates occasionally exceed 100%; this could be attributed to the tendency of these particles to entangle when collected with tweezers during spiking (Kim *et al*., 2022). To minimize procedural errors, the operators selected the longest and most easily manipulable MFs. Nevertheless, considering that the smallest particle size recovered in the spiking experiments was 93.5 μm, it is evident that further efforts are needed to improve the method and include smaller particles, which predominantly constitute a health risk to humans. Current knowledge about MP toxicity in humans remains limited. Evidence suggests that particles smaller than 150 μm may pass the gastrointestinal barrier, while MPs around 20 μm can penetrate cellular membranes, build up in organs, trigger cellular damage, and cause immune reactions through inhalation or ingestion pathways (Kannan and Vimalkumar, 2021; Rbaibi Zipak *et al*., 2022).

Despite some limitations, the proposed method demonstrates high versatility as it can be successfully applied to both raw and heat-treated milk simply by modifying the incubation temperature of the samples. These characteristics enable efficient monitoring of contamination throughout the entire dairy production chain, from raw material reception to final product distribution, thus providing a comprehensive quality assurance approach that supports food safety objectives.

Raw milk contamination

The results obtained in the present study revealed an average concentration ranging from 1.3 ± 0.5 to 5.5 ± 5.0 MPs (including synthetic MFs) per 100 mL in raw milk samples collected through mechanical milking. Kutralam-Muniasamy *et al*. (2020) detected MPs in commercially branded milk

samples at a mean concentration of 6.5 particles/L, while Diaz-Basantes *et al*. (2020) reported 40 MPs/L in skim milk from Ecuador. More recently, Basaran *et al*. (2023) found a mean number of 6 particles/L in Turkish branded milk, and Chakraborty *et al*. (2024) reported an average of 182.27 particles/L in Bangladesh commercial milk samples. This discrepancy may reflect differences in milking practices, farm management protocols, and local variations in contamination sources. In addition, it is important to consider the additional sources of contamination that may affect commercial milk after milking (Santonicola *et al*., 2025a).

A considerable presence of natural MFs was also detected, accounting for 47% of the total identified particles. This proportion is consistent with previous investigations, where the percentage of MFs in various food items, including milk, exceeded 50% (Kwon *et al*., 2020; Kutralam-Muniasamy *et al*., 2020; Basaran *et al*., 2023; Chakraborty *et al*., 2024; Rbaibi Zipak *et al.*, 2024; Santonicola *et al*., 2025a). The predominance of these particles represents a critical aspect from a toxicological perspective, since they are considered more hazardous than spherical particles, as they may cause toxic effects at lower doses (Kwon *et al*., 2020; Banica *et al*., 2024; Santonicola *et al*., 2023).

The absence of significant differences across sampling days (KW–p=0.467 for MPs; p=0.948 for MFs) and among individual cows (KW–p=0.808 for MPs; p=0.229 for MFs) suggests a relatively constant and systematic contamination along the production chain. However, it should be acknowledged that the limited sample size (n=9 samples from 3 cows over 3 weeks) may reduce the statistical power to detect subtle differences, particularly considering the high variability observed in contamination levels. MPs and MFs could be transmitted to raw milk via room air during milking and different components of the milking machine (Da Costa Filho *et al*., 2021; Di Fiore *et al*., 2023; Banica *et al*., 2024; Rbaibi Zipak *et al*., 2024). In detail, the milking unit removes milk from the udder, then milk flows from teat cups through short tubes to the claw and through long tubes to the receiver (Reinemann, 2019). Critical contamination points may include vibrating vacuum teat cups attached around the udder, milking buckets, and tubes (Rbaibi Zipak *et al*., 2022; Banica *et al*., 2024). Water and detergent residues used to clean tankers, valves, and pipes may pose an additional risk to the MP load (Rbaibi Zipak *et al*., 2024). Different factors may contribute to the variability observed in MP and MF levels, e.g., airborne particle deposition during milking can vary considerably, as well as the release of particles from plastic components of milking equipment may not be uniform across milking sessions due to different factors, such as mechanical stress during operation and cleaning procedures.

Color distribution provides further valuable insights into potential contamination origins. MP fragments were mainly transparent (47%), while MFs were predominantly black (48%) and blue (18%). The prevalence of colored fibers is compatible with textile origins, suggesting a significant contribution from workers' clothing who operate and handle the raw milk, textile materials used for udder cleaning and sanitization and farming equipment, such as mats and curtains (Zhang *et al*., 2023; Chakraborty *et al*., 2024; Santonicola *et al*., 2025a).

The mean dimensions recorded for isolated particles (natural MFs: 1090.6±810.1 μm; synthetic MFs: 1168.5±1260.1 μm; fragments: 321.5±226.3 μm) were consistent with the dimensional range reported by Rbaibi Zipak *et al*. (2022), who found that 50% of particles in raw milk samples ranged between 1000 and 5000 μm, with a median of 913.5 μm. The significant variability in natural MF dimensions across sampling days (KW–p=0.006) may reflect differences in farm management practices or environmental conditions during milking operations.

FTIR analysis revealed that among the analyzed particles, MPs were composed of PP (41%), PES (6%), and PVC (6%), while the remaining particles were identified as natural/artificial cellulosic MFs. As mentioned before, some milking machine components are made of various types of plastic approved for sanitary applications (Reinemann, 2019). The prevalence of PP in our samples confirms that milking equipment primarily contributes to milk contamination (Kwon *et al.*, 2020). The presence of PES and PVC may be traced back to various sources throughout the farm environment, in addition to the milking process (Di Fiore *et al*., 2023).

The significant presence of natural/artificial cellulosic MFs enhances the role of textile materials as an important contamination source (Zhang *et al*., 2023; Chakraborty *et al*., 2024; Santonicola *et al*., 2025a,b). In addition, the introduction of "unplanned air" in milking machines could significantly contribute to milk contamination, especially regarding MFs, through airborne fallout of textile particles present in the environment (Reinemann, 2019). This would explain why environmental blanks remained relatively clean while milk contained numerous fibers.

Conclusions

In this study, an analytical protocol for the isolation and identification of MPs and MFs in milk samples was optimized by achieving satisfactory recovery rates exceeding 91% for various polymer types and particle morphologies. The versatility of this approach, requiring only minor temperature adjustments between raw and heat-treated milk, enables comprehensive monitoring throughout the entire dairy production chain. However, several limitations remain. The minimum particle size recovered (approximately 100 μm) highlights the need for further methodological refinements to capture smaller particles, which pose greater health risks. Additionally, challenges in handling MFs during recovery experiments underscore the complexity of achieving precise quantification for these particle types.

The application of the extraction method to raw milk revealed MP and MF contamination in the samples collected from mechanical milking. The predominance of PP among identified polymers strongly suggests milking equipment as a primary contamination source, while the prevalence of colored fibers indicates significant contributions from textile materials used in farm operations and worker clothing. The findings emphasize the urgency of implementing contamination control measures at the farm level, including the implementation of scheduled maintenance programs for milking equipment components, and the establishment of good practices to minimize textile fiber shedding.

Future research should focus on: i) improving analytical methods to reliably detect and quantify particles below 100 μm; ii) conducting comprehensive supply chain studies to identify specific contamination points from farm to consumer; iii) investigating mitigation strategies in reducing MP loads. Moreover, standardization of analytical protocols and harmonization of reporting data across studies remain critical priorities for enabling accurate risk assessment and establishing regulatory frameworks for MPs contamination in food products.

References

Bai CL, Liu LY, Hu YB, Zeng EY, Guo Y, 2022. Microplastics: a review of analytical methods, occurrence and characteristics in food, and potential toxicities to biota. Sci Total Environ 806:150263.

Banica AL, Radulescu C, Dulama ID, Bucurica IA, Stirbescu RM, Stanescu SG, 2024. Microplastics, polycyclic aromatic hydrocarbons, and heavy metals in milk: analyses and induced health risk assessment. Foods 13:3069.

Basaran B, Özçifçi Z, Akcay HT, Aytan Ü, 2023. Microplastics in branded milk: dietary exposure and risk assessment. J Food Compos Anal 123:105611.

Caba-Flores MD, Martínez-Valenzuela C, Cardenas-Tueme M, Camacho-Morales A, 2023. Micro problems with macro consequences: accumulation of persistent organic pollutants and microplastics in human breast milk and in human milk substitutes. Environ Sci Pollut Res 30:95139-54.

Chakraborty TK, Hasan MJ, Netema BN, Rayhan MA, Asif SMH, Biswas A, Hasibuzzaman M, 2024. Microplastics in the commercially available branded milk in Bangladesh: An emerging threat for human health. J Hazard Mater 477:135374.

Corte Pause F, Urli S, Crociati M, Stradaioli G, Baufeld A, 2024. Connecting the dots: livestock animals as missing links in the chain of microplastic contamination and human health. Animals 14:350.

Da Costa Filho PA, Andrey D, Eriksen B, Peixoto RP, Carreres BM, Ambühl ME, Poitevin E, 2021. Detection and characterization of small-sized microplastics (≥ 5 μm) in milk products. Sci Rep 11:24046.

Di Fiore C, Carriera F, Russo MV, Avino P, 2023. Are microplastics a macro issue? A review on the sources of contamination, analytical challenges and impact on human health of microplastics in food. Foods 12:3915.

Diaz-Basantes MF, Conesa JA, Fullana A, 2020. Microplastics in honey, beer, milk and refreshments in Ecuador as emerging contaminants. Sustainability 12:5514.

Dong X, Liu X, Hou Q, Wang Z, 2023. From natural environment to animal tissues: a review of microplastics (nanoplastics) translocation and hazards studies. Sci Total Environ 855:158686.

Kadac-Czapska K, Trzebiatowska PJ, Knez E, Zaleska-Medynska A, Grembecka M, 2023. Microplastics in food-a critical approach to definition, sample preparation, and characterisation. Food Chem 418:135985.

Kannan K, Vimalkumar K, 2021. A review of human exposure to microplastics and insights into microplastics as obesogens. Front Endocrinol 12:724989.

Kaseke T, Lujic T, Cirkovic Velickovic T, 2023. Nano-and microplastics migration from plastic food packaging into dairy products: impact on nutrient digestion, absorption, and metabolism. Foods 12:3043.

Kim J, Pham DT, Park HJ, Chae MY, Lee SH, Hong S, Kwon JH, 2022. Development and validation of analytical methods for detecting and identifying microplastics in salts, soy sauce, and salted pollock roe. J Food Compos Anal 114:104856.

Kiruba R, Preethi M, Aganasteen R, Rithick RM, Hannah TC, Monica P, Naseera BI, 2022. Identification of microplastics as emerging contaminant in branded milk of Tamil Nadu State, India. Asian J Biol Life Sci 11:181.

Kutralam-Muniasamy G, Pérez-Guevara F, Elizalde-Martínez I, Shruti VC, 2020. Branded milks–are they immune from microplastics contamination? Sci Total Environ 714:136823.

Kwon JH, Kim JW, Pham TD, Tarafdar A, Hong S, Chun SH, Jung J, 2020. Microplastics in food: a review on analytical methods and challenges. Int J Environ Res Public Health 17:6710.

Mamun AA, Prasetya TAE, Dewi IR, Ahmad M, 2023. Microplastics in human food chains: food becoming a threat to health safety. Sci Total Environ 858:159834.

Pirc U, Vidmar M, Mozer A, Kržan AJ, 2016. Emissions of microplastic fibers from microfiber fleece during domestic washing. Environ Sci Pollut Res 23:22206-11.

Postelmans A, Aernouts B, Jordens J, Van Gerven T, Saeys W, 2020. Milk homogenization monitoring: Fat globule size estimation from scattering spectra of milk. Innov Food Sci Emerg Technol 60:102311.

Rahman A, Sarkar A, Yadav OP, Achari G, Slobodnik J, 2021. Potential human health risks due to environmental exposure to nano- and microplastics and knowledge gaps: a scoping review. Sci Total Environ 757:143872.

Rbaibi Zipak S, Muratoğlu K, Büyükünal S, 2022. Evaluation of microplastic presence in yogurt production process. Kafkas Univ Vet Fak Derg 28:633-41.

Rbaibi Zipak S, Muratoglu K, Buyukunal SK, 2024. Microplastics in raw milk samples from the Marmara region in Turkey. J Verbrauch Lebensm 19:175-82.

Reinemann, 2019. Milking Machines and Milking Parlors. In: Kutz M, ed. Handbook of farm, dairy and food machinery engineering. Academic Press, Cambridge, MA, USA, pp 177-99.

Santonicola S, Volgare M, Cocca M, Colavita G, 2025a. Study of fibrous microplastic and natural microfiber levels in branded milk samples from Italy. Ital J Food Saf 14:13523.

Santonicola S, Volgare M, Cocca M, Colavita G, 2025b. Preliminary investigation of microplastic and microfiber contamination in raw milk from Italy: implications for food safety. In: Gentile G, Cocca M, Capuano D, Olivieri F, Avolio R, Castaldo R, Bemporad E, Errico ME, eds. Proceedings of the 4th International Conference on Microplastic Pollution in the Mediterranean Sea, 2025 Sep 19-22, Ischia, Italy. Springer Nature Switzerland.

Santonicola S, Volgare M, Cocca M, Dorigato G, Giaccone V, Colavita G, 2023. Impact of fibrous microplastic pollution on commercial seafood and consumer health: a review. Animals 13:1736.

Tagg AS, Harrison JP, Ju-Nam Y, Sapp M, Bradley EL, Sinclair CJ, Ojeda JJ, 2017. Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater. Chem Commun 53:372-5.

Toussaint B, Raffael B, Angers-Loustau A, Gilliland D, Kestens V, Petrillo M, Van den Eede G, 2019. Review of micro-and nanoplastic contamination in the food chain. Food Addit Contam Part A 36:639-73.

Zhang Q, Liu L, Jiang Y, Zhang Y, Fan Y, Rao W, Qian X, 2023. Microplastics in infant milk powder. Environ Pollut 323:121225.

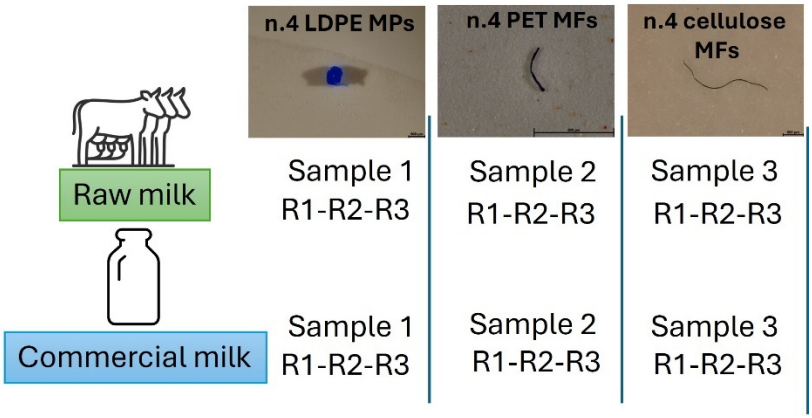

Figure 1. Recovery experiment design showing triplicate sample preparation (R1-R3) with spiked microplastic (MP) and microfiber (MF) standards.

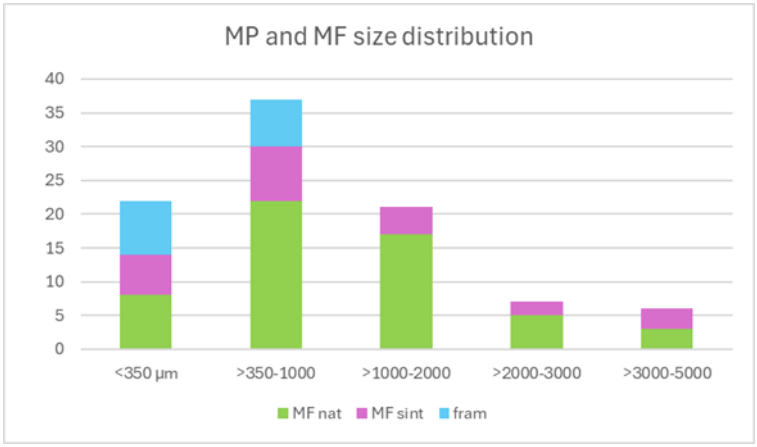

Figure 2. Size distribution of microplastics (MPs) and microfibers (MFs) detected in raw milk samples.

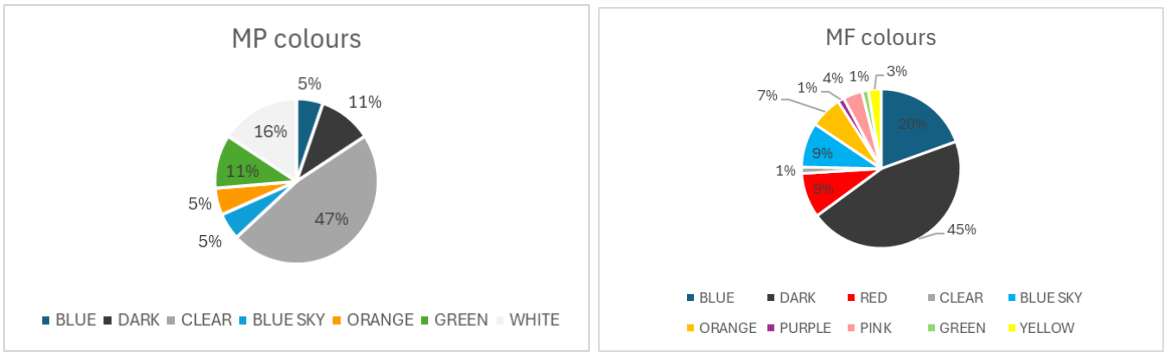

Figure 3. Color distribution of microplastics (MPs) and microfibers (MFs)found in raw milk samples.

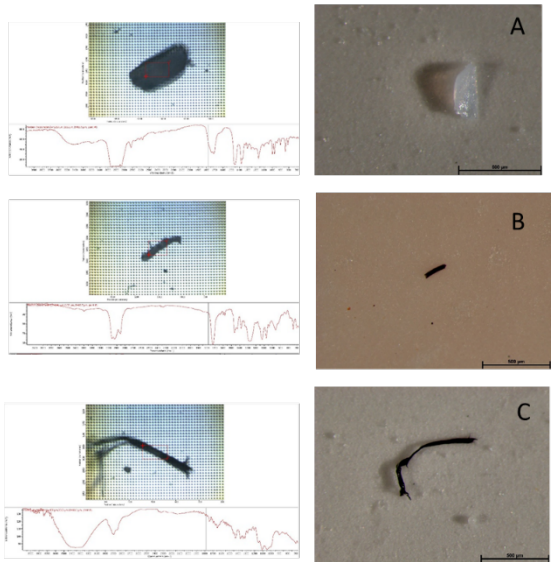

Figure 4. Microscopic characterization and chemical identification of microplastics from raw milk samples. (A-C) Optical/stereomicroscope images showing representative particles isolated from raw milk; corresponding FTIR spectra confirming polymer composition, with characteristic absorption peaks identifying the particles as PP (A), PVC (B), and cellulose (C).

Table 1. Recovery rates and particle sizes of spiked reference materials.

Reference materials	Recovery in commercial milk	Recovery in raw milk	Length of recovered particles (µm range)	Mean length of recovered particles ± SD
PET MFs	116.6%	91.6%	95.3-2302.7	564.5±573.8
Cellulose MFs	91.6%	91.6%	142.8-4925.6	3176.3±1261.2
LDPE fragments	100%	91.6%	423.1-1068.5	738.44±144.8

SD, standard deviation; PET, polyethylene terephthalate; MF, microfiber; LDPE, low-density polyethylene.