Study of fibrous microplastic and natural microfiber levels in branded milk samples from Italy

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Abstract

As far as we know, there is no evidence regarding the microfiber (MF) occurrence and abundance in branded milk samples from Italy. Therefore, a total of 20 milk samples from 5 brands were collected and analyzed using a digestion step with hydrogen peroxide, followed by filtration. Natural and synthetic MFs were classified according to the evaluation of surface morphology (*i.e.*, shape and texture), followed by chemical identification using Fourier Transform Infrared (FTIR) Microspectroscopy. Results revealed the occurrence of MFs in 67.5% of the analyzed samples and showed variability ranging between 1 and 27 particles/100 mL with an overall average of 3.85 MFs/100 mL. The FTIR analyses confirmed the presence of polyethylene, polyester, acrylic, and cellulosic MFs.

According to the literature, the contamination of milk may occur at various stages along the production chain. The blood-milk barrier would prevent MFs from being transferred across the mammary gland into the milk. The highest MF levels found in ultra-high temperature skimmed milk of some brands may indicate that the more complex the processing of milk, the more MFs it contains. However, due to the different MF types and polymers, an unambiguous conclusion on MF sources cannot be made. MFs could be shed from the filters used in the milk processing factories and the protective clothing for workers. Therefore, the MF contamination should be properly investigated along the entire supply chain, identifying the sources of contamination and implementing control strategies and mitigation measures.

Introduction

In recent years, microplastics (MPs) have received considerable attention from governments, non-governmental organizations, the scientific community, and the media as emerging pollutants (Kaseke *et al*., 2023). These particles, generally defined as plastic debris that are <5 mm, are classified as primary or secondary MPs based on their source of release into the environment (Dong *et al*., 2023). MPs have been widely found in the marine environment, and many edible fish and shellfish with microfibers (MFs) being the predominant form (Santonicola *et al*., 2023; Yang *et al*., 2023). In the aquatic ecosystem, MPs have been shown to be transferred along the food chain from algae to zooplankton and then to fish, leading to human exposure (Santonicola *et al*., 2020).

Many MPs have also been observed in the air and in the soil, which may result in the accumulation in plants with a potential risk of transfer along the food chain (Kwak *et al*., 2022; Dong *et al*., 2023).

Updated information refers to the presence of MPs in a wider variety of foods intended for human consumption (Rubio-

Armendáriz *et al*., 2022). In addition to environmental pollution, industrial processes may accidentally contribute to MP contamination (Diaz-Basantes *et al*., 2020).

Milk and dairy products play a key role in the human diet throughout life because of their high nutritional value as a source of essential amino acids and bioactive molecules. Although milk intake has declined in the Western world during the last decades, recent evidence promotes the consumption of cow's milk and its derivatives as part of a healthy Mediterranean diet (Marangoni *et al*., 2019).

Considering the importance of milk in human nutrition, the occurrence of MPs in this widely consumed food has received growing attention (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). The possible risks of MP contamination could derive from the friction of plastic components during the different stages of milk production and processing, poor cleaning procedures, the surrounding environment, water supply conditions, and inadequate handling (Diaz-Basantes *et al*., 2020; Kutralam-Muniasamy *et al*., 2020). However, although the available information shows the prevalence of fibrous MPs in milk samples (Kutralam-Muniasamy *et al*., 2020; Basaran *et al*., 2023; Chakraborty *et al*., 2024; Rbaibi Zipak *et al*., 2024), the extent of natural and artificial MF pollution has not yet been assessed.

Human MP exposure *via* milk consumption varies among studies, from 6.60 to 35-80 particles/day (Kiruba *et al*., 2022; Lin *et al*., 2022). Following oral exposure, MPs in the gastrointestinal tract may reach different parts of the body, where they may have both physical and chemical effects. The potential mechanism of action of MPs may vary based on the particle size. MPs with sizes ≤150 µm can cross the mucosal barrier, while those <1.5 µm can reach deeper tissues and cause damage to different organs (Kaseke *et al*., 2023; Rbaibi Zipak *et al*., 2023). In the case of MFs, the fragmentation inside the organisms after ingestion should not be ignored, given that these particles are thin and may break into smaller pieces and then be transported to other organs (Bai *et al*., 2022). The potential risks of MPs and MFs may be greater for infants (Zhang *et al*., 2023). However, information on the exposure *via* the consumption of commonly consumed foods like milk is also scarce, due to the difficulty of isolating and quantifying these particles in such a complex food matrix (Da Costa Filho *et al*., 2021).

The current study aimed to evaluate the abundance, distribution, and characteristics of MFs in branded milk samples from Italy. To the best of our knowledge, no study has been conducted on Italian milk samples, and in this preliminary survey, we decided to point our attention on MF contamination considering that: i) previous investigations on milk revealed that synthetic MFs were dominant accounting for >90% of total MPs (Kutralam-Muniasamy *et al*., 2020; Basaran *et al*., 2023); ii) researchers are encouraged to evaluate MFs separately from other MPs to make more consistent comparisons across different investigations, and the categorization based on the particle origin (Avio *et al*., 2020); iii) no study included the evaluation of natural/artificial MF contamination in milk, despite their abundance in environmental samples, and the documented harmful effects to the exposed organisms (Liu *et al*., 2023).

Materials and Methods

Milk sampling

Five different brands were selected and, for each of them, samples of skimmed, semi-skimmed, and whole ultra-high temperature (UHT) processed milk were purchased. For brands A and D, samples of semi-skimmed and whole pasteurized milk were purchased (Table 1). The choice of pasteurized and UHT milk samples for each brand was based on market availability. As reported by Basaran *et al.* (2023), for each type of milk, two samples with different batch numbers were purchased and analyzed considering the standardized milk production process, and, therefore, the selected samples were representative of the overall production.

The majority of milk samples were packed in 500 mL recyclable Tetrapak aseptic cartons, which are 75% paper; the rest portions are aluminum and polyethylene. Only two pasteurized samples were packed in polyethylene bottles. Pasteurized samples were kept unopened at +4°C in the refrigerator until the analyses were carried out before the expiration date (Basaran *et al*., 2023).

Table 1. Characteristics of analyzed milk samples.

Brand	UHT processed milk	Pasteurized milk
A	Whole milk (3.6% fat) Half fat milk (1.6% fat) Skimmed milk (0.1 %fat)	Whole milk (3.6% fat)* Half fat milk (1.6% fat)*
B	Whole milk (3.6% fat) Half fat milk (1.5% fat) Half fat lactose-free milk (1.5% fat) Skimmed milk (0.1% fat)	
C	Whole milk (3.6% fat) Half fat milk (1.6% fat) Skimmed milk (0% fat)	
D	Whole milk (3.7% fat) Skimmed milk (0.1% fat) Half fat milk (1.6% fat)	Whole milk (3.7% fat) Half fat milk (1.6% fat)
E	Whole milk (3.6% fat) Half fat milk (1.6% fat) Skimmed milk (0.05% fat)	

For each milk type, two samples with different batch numbers were analyzed. *Packed in polyethylene bottles. UHT, ultra-high temperature.

Microfiber isolation and visual identification

At the time of the analysis, each sample was mixed by inverting the bottle, and 100 mL was transferred into a precleaned Erlenmeyer beaker. A dilution 1:1 with 100 mL of prefiltered distilled water (0.45 µm pore size cellulose acetate filters, Sartorius Stedim Biotech, Gottingen, Germany) was prepared and 40 mL of H_2O_2 (30 vol%; Carlo Erba, Val De Reuil, France) were added, in a ratio of H_2O_2 to milk sample of 1:2.5 (v/v), to allow degradation by organic matter oxidation (Diaz-Basantes *et al*., 2020). Then, the samples were stored at 40°C for 48 hours. Previous investigations showed that cold milk cannot pass through the filters easily, and warm milk may end up blocking filters with size ranges of 0.22, 0.45, and 5 µm (Kutralam-Muniasamy *et al*., 2020). Therefore, after digestion, vacuum filtration of warm milk (40°C) was carried out through membranes of 8 µm pore size (cellulose nitrate filters, Sartorius Stedim Biotech, Gottingen, Germany).

Following the digestion and filtering processes, MFs recovered on the filters were observed under a stereomicroscope (M205C; Leica, Wetzlar, Germany) with a magnification of 0.78-16×. Micrographs were taken for each MF, and then they were processed using ImageJ software (release 1.43 u, NIH, Bethesda, MD, USA) to determine size distribution and analyzed to discriminate between natural/artificial and synthetic MFs based on the typical morphological features of the textile fibers. Synthetic MFs usually show a smooth and shiny surface, cylindrical cross-section, uniform diameter, and solid edges, while MFs have a flattened and curly shape, and twisting and irregular frayed terminations are of natural origin (Stanton *et al*., 2019; Zhu *et al*., 2019; Rodríguez-Romeu *et al*., 2020; Volgare *et al*., 2022; Santonicola *et al*., 2024). Regenerated MFs (*i.e.*, viscose/rayon) present regular surfaces with dark longitudinal regular stripes, and a serrated cross-section (Liu *et al*., 2023). In addition, MFs were counted and categorized based on their color. The MF quantification in the milk samples was obtained by subtracting the number of MFs counted in the blank samples. To confirm the results obtained by morphological identification, the chemical analysis of some MFs was performed.

Chemical composition identification

For the chemical identification, five samples (one for each brand) containing MFs representative of each morphological pattern, identified through the visual approach, were selected. MFs recovered on cellulose filters were transferred to MakroPor silicon filters (Thermo Fisher Scientific, Waltham, MA, USA) by washing using filtered distilled water. A subset of MFs, corresponding to 26% of the total isolated particles, were analyzed using the Fourier Transform Infrared (FTIR) Microspectroscopy (Nicolet iN10 MX, Thermo Fisher Scientific, Waltham, MA, USA) and the obtained spectra were matched with spectral libraries to define the polymer (Basaran *et al*., 2023). FTIR spectra were acquired in transmission mode, averaging 64 scans with a resolution of 4 cm-1. Thermo Scientific OMNIC™ Spectra™ 2.0 software (Waltham, MA, USA) was used for the analysis of the acquired spectra, and a match of more than 70% was considered for MF identification.

Contamination prevention

Strict precautions were taken to minimize airborne particle contamination during milk analysis. The milk samples were recovered and treated under a laminar flow hood, all doors and windows were closed, and the entrances and exits to the laboratory were limited during MF extraction. Nitrile gloves and laboratory 100% cot-

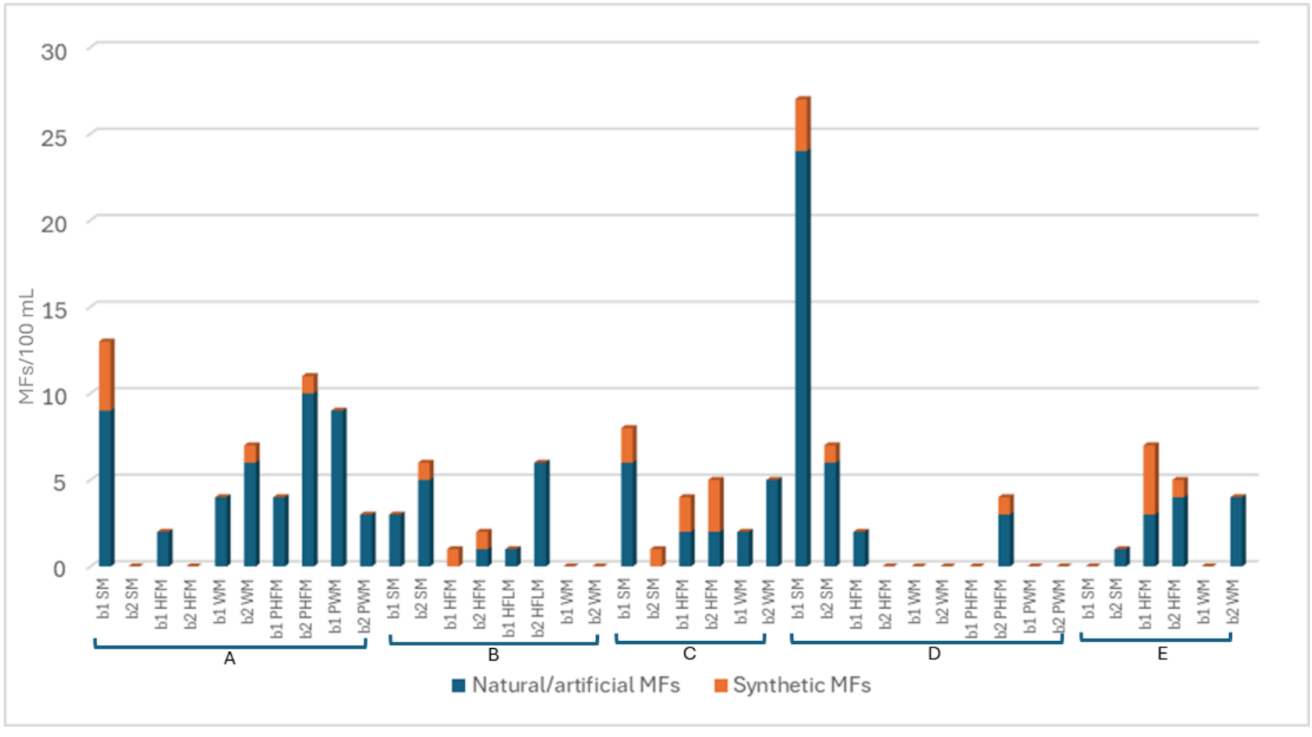

Figure 1. Microfiber abundance in milk samples from different Italian brands (A-E). b1, batch 1; b2, batch 2; MFs, microfibers; SM, ultra-high temperature (UHT) skimmed milk; HFM, half fat milk; WM, whole milk; PHFM, pasteurized half fat milk; PWM, pasteurized whole milk; HFLM, half fat lactose-free milk.

ton coats were worn. The distilled water used in the study was filtered through a 0.45 µm cellulose acetate membrane filter before being used. The work surfaces and apparatus, and glass containers were cleaned and rinsed three times with filtered water. Before filtration, each filter was observed under the microscope to detect MF contamination. During the filtration process, the top of the filtration funnel was covered with aluminum foil, and the glass Petri dishes used for the storage of filter membranes were washed with filtered distilled water before use. Blank samples containing 100 mL of H_2O_2 were analyzed. No contamination was detected. Blank controls, obtained by adding 100 mL of filtered water to the beaker without adding milk, were analyzed following the same procedure as samples to eliminate any additional contamination that may have occurred in the experimental process.

Statistical analysis

Statistical analysis of the data was carried out by using SPSS® Statistics software (IBM, Armonk, NY, USA). The data were tested for normality by using a Shapiro-Wilk test and for homogeneity of variance by using Levene's test. One-way analysis of variance (ANOVA) was performed to assess any significant differences among the data acquired. Kruskal-Wallis or Mann-Whitney U tests were performed when data did not comply with the assumption of homogeneity of variances. Pearson correlation was performed to assess significant correlations. A 5% significance level was used for all statistical tests.

Results

Microscopical inspection of filters revealed that MFs were detected in 67.5% of milk samples. A total of 154 MFs were counted with an average concentration of 3.85 particles/100 mL. In the contaminated samples, the MF abundance was in the range of 1-27 particles/100 mL: more than half of the samples (53.5%) showed a MF abundance of 1-4 particles/100 mL, 32.14% and 10.71% of milk samples exhibited MF levels of 5-8 and 10-13 particles/100 mL, respectively; only one sample showed a number of 27 particles/100 mL. Natural/artificial MFs, classified according to the evaluation of fiber morphologies, were the most numerous, accounting for 84% (Figure 1).

No significant difference was detected among MF levels in the different brands (Kruskal-Wallis, p=0.309), and among UHT and pasteurized milk of the brands A [ANOVA, F(1.8)=0.659, p=0.440] and D (Mann-Whitney, p=0.476). Results showed no significant differences between MF levels in the pasteurized samples packed in Tetrapak cartons and polyethylene bottles.

The highest MF levels were found in UHT skimmed milk. For brand B, a negative correlation between the number of MFs and milk fat content was found [Pearson, r(1.6)= - 0.874, p=0.023]. In addition, for brand D, a significant difference was observed among whole and skimmed milk for the total MFs (Kruskal-Wallis, p=0.010), and synthetic (Kruskal-Wallis, p=0.013) and natural (Kruskal-Wallis, p=0.010) MF levels.

The results of MF proportion based on colors and sizes are represented in Figure 2. The most common colors of MFs (both natural and synthetic) were blue (32%), black (30%), and clear (16%).

The average length of synthetic MFs was determined to be 1066.33 µm (range 124.821-4537.195), while natural MFs showed a mean length of 1076.302 µm (range 127.567-4821.884). MFs longer than 5000 µm were not included in the present study, because they did not fall within the range of MF definition. MFs in the range 350-1000 µm (37%) were dominant, followed by the size 1000-2000 µm (26%); only a minor number of MFs were larger in size.

The FTIR analyses performed on a sub-sample of MFs allowed us to identify MFs as cellulose/regenerated cellulose (90%), polyethylene (5%), acrylic (3%), and polyester (2%). According to the literature, natural and regenerated cellulosic MFs were grouped since the distinction by FTIR techniques is difficult, especially when processing environmentally degraded polymers (Santonicola *et al*., 2024). For clarity, in Figure 3, the micrographs and corresponding FTIR spectra of some selected MFs found in the analyzed samples are reported.

Discussion

The results of this preliminary study provide evidence that milk was contaminated by MFs. In general, the occurrence of MPs has been detected in both raw and processed milk, with the prevalence of fibrous particles (Kutralam-Muniasamy *et al*., 2020; Basaran *et al*., 2023; Chakraborty *et al*., 2024; Rbaibi Zipak *et al*., 2024).

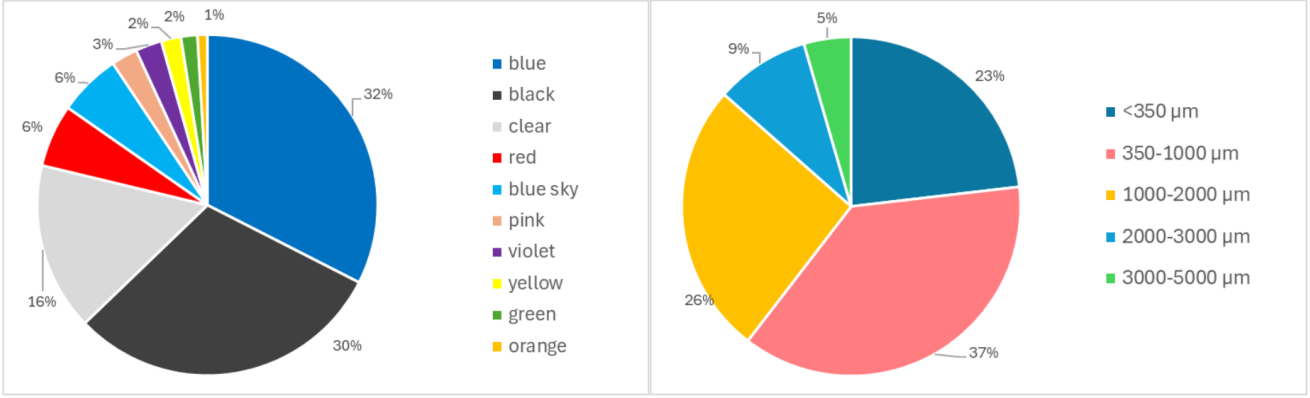

Figure 2. Color and size distribution of microfibers detected in milk samples from Italy.

As reported in the literature, the levels of MPs in raw milk from Turkey were between 84.2 and 128 particles/L, with an average of 92.1 MPs/L, and fibers represented 52.40% of the total particles (Rbaibi Zipak *et al*., 2024). Kutralam-Muniasamy *et al.* (2020) and Basaran *et al.* (2023) found a mean number of MPs in branded milk samples of ~6 particles/L, among which MFs accounted for >90% of total MPs. In detail, the mean level of fibrous MPs in milk samples was 17.8 particles/L, but all natural polymers (a total of 171 particles) were excluded from the data analysis (Basaran *et al*., 2023). The mean level of MFs found in this study was consistent with the results of Diaz-Basantes *et al*. (2020), but lower than those detected by Chakraborty *et al*. (2024) in milk samples from Bangladesh (182.27±55.13 MPs/L, of which 81% were MFs). The available data are difficult to compare since the levels of milk contamination are highly variable among different studies, also due to the differences in methodologies applied during the sample analyses. Kutralam-Muniasamy *et al.* (2020) used only a warm filtration step, while other research prepared the samples by a digestion step, which included the use of a combination of enzymes and strong bases (Da Costa Filho *et al*., 2021), and oxidative agents such as hydrogen peroxide (Diaz-Basantes *et al*., 2020). While analyzing milk samples, a series of quality assurance and quality control measures lowering contamination of the method blanks is pivotal. However, these measures may differ among studies (Diaz-Basantes *et al*., 2020; Kutralam-Muniasamy *et al*., 2020; Da Costa Filho *et al*., 2021). Moreover, researchers filtered the samples using membranes with different pore sizes; apart from that, some studies consider only low-fat milk samples because lipids slow down the filtration process (Diaz-Basantes *et al*., 2020). Furthermore, no information on natural/artificial MFs was available, despite the occurrence of these particles being observed in the analyzed samples (Da Costa Filho *et al*., 2021).

Differences between investigations may also be related to the type of milk, the characteristics of the processing facility, and the geographic locations of the raw material (Basaran *et al*., 2023). Based on these limited data, and according to the obtained results, it was observed that the number of MPs and MFs tended to increase in processed milk, and the more complex the processing and packaging process of milk, the more particles they contain (Kutralam-Muniasamy *et al*., 2020; Da Costa Filho *et al*., 2021). The contamination of milk may occur at various stages along the production chain, from the farm to processing and packaging (Kaseke *et al*., 2023). Studies have shown that MPs enter the animals' bodies through many ways, such as during inhalation and feeding of cows, and may pass blood circulation and accumulate in various tissues. However, there is a lack of evidence that MPs can accumulate in the mammary (Dong *et al*., 2023). On the other hand, MPs may come from the milking parlor environment or the milking machine (Da Costa Filho *et al*., 2021). Due to the different types of polymers and the different MF sources, an unambiguous conclusion cannot be drawn. The filters that are used in the milk processing factories could be a source of MP and MF contamination due to the physical cracking of the membrane under continuous use with time (Kutralam-Muniasamy *et al*., 2020). These filters can be made of a mixture of natural and synthetic polymers (cellulose, cotton, viscose, and polyester) (Da Costa Filho *et al*., 2021), most of which were detected in the current investigation. There is clear evidence of widespread MF contamination in the air,

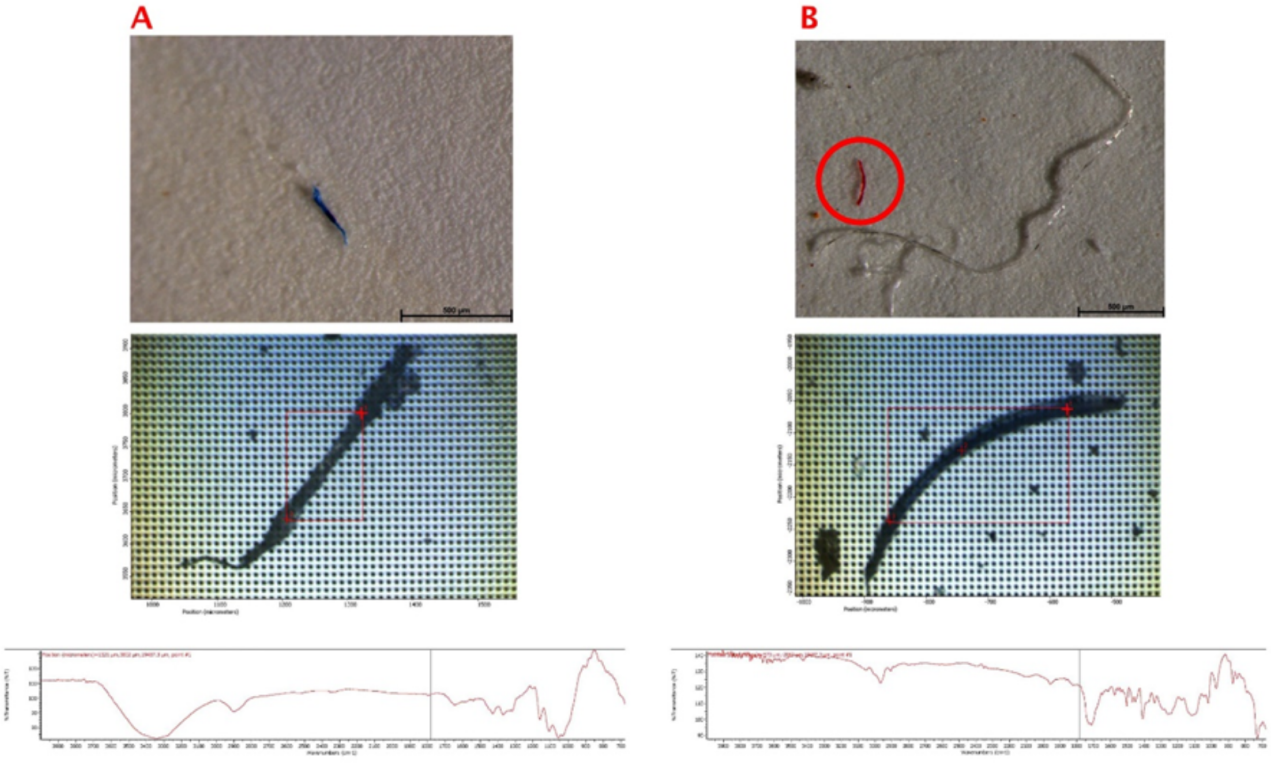

Figure 3. Optical microscope image of cellulosic (**A**) and polyester (**B**) microfibers identified based on the typical morphological features, and Fourier transform infrared spectroscopy.

both in indoor and outdoor environments, with the prevalence of natural/artificial MFs, which may be deposited by atmospheric fallout (De Falco *et al*., 2020). In addition, polyethylene MFs found in the analyzed samples may be shed from protective clothing for workers, hygiene caps, and masks (Rbaibi Zipak *et al*., 2023; Zhang *et al*., 2023).

According to the obtained results, blue-colored fibers were found to be predominant (Kutralam-Muniasamy *et al*., 2020). The polymeric filters used in milk factories may be colored to help identify white spots of mastitic milk (Da Costa Filho *et al*., 2021). Moreover, considering that MFs could also come from textile items and farming equipment (such as mats, curtains, cloths, and rope), their color may vary among investigations (Chakraborty *et al*., 2024).

The sizes of MFs detected in the examined milk samples are consistent with the literature (Basaran *et al*., 2023). The prevalence of MFs in the size range of 350-1000 µm, on the one hand, may reassure consumers as particles larger than 150 µm are not normally absorbed by the intestine; on the other hand, MFs are thin particles and can fragment into smaller pieces after ingestion (Bai *et al*., 2022; Dong *et al*., 2023). Moreover, larger particles could also lead to local inflammation and affect intestinal microbes (Kaseke *et al*., 2023).

Considering the role of milk and milk products in the human diet from birth throughout life, it is extremely important to ensure food safety (Basaran *et al*., 2023). Nevertheless, the availability of data on the impact of MPs, and even more for MFs, from the food supply chain to human health remains limited, and the research is still in its infancy.

The obtained results face a future challenge, that is evaluating the levels of MF contamination along the entire milk supply chain, from the farm to the packaging of processed milk, to properly identify the sources of contamination and implement control strategies and mitigation measures.

Conclusions

The results of this study revealed the occurrence of fibrous MPs and natural MFs in Italian milk samples. Growing evidence suggests that milk contamination may occur at various stages along the production chain, and the more complex the processing of milk, the more MPs it contains. The number and types of MFs detected in milk may vary depending on many variables such as the milking environment and conditions, and the characteristics of the processing facility. Considering that the level of MF exposure resulting from milk consumption may differ according to consumption habits and age, children could be more exposed. The prevalence of MFs in the size range of 350-1000 µm detected in the current study, on the one hand, may reassure consumers as these larger particles are not normally absorbed by the intestine; on the other hand, it must be taken into account that MFs may break into smaller pieces after the ingestion and then be transported to other organs. Therefore, more research is needed, and the entire milk production chain should be reviewed to detect the process steps that may cause MP and MF contamination in milk to implement mitigation and control measures.

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