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The Invisible Threat: Assessing Microplastic Contamination in Beef and Its Implications for Food Safety

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ABSTRACT

Microplastics (MPs) have been widely found in various food products cultivated on land, including meat. This study focuses on detecting the presence of MPs in beef available at traditional markets, specialty meat shops, and retail stores in the city of Semarang. The samples were digested using a combination of KOH solution, hydrogen peroxide, Fenton's reagent, and identified using micro-FTIR. All samples were analyzed in duplicate. MPs were found in all beef samples. The highest concentration of MPs was found in beef from the traditional market ($2.57\times10^6\pm3.35\times10^6$ MPs/kg WB), followed by samples taken from the meat shop ($7.51\times10^5\pm1.58\times10^5$ MPs/kg WB), and supermarket ($6.78\times10^5\pm2.41\times10^5$ MPs/kg WB). Samples from traditional markets contained predominantly polyethylene (PE, 94.1%), while those from supermarkets and meat shops were mainly polyvinyl chloride (PVC), at 47.5% and 38.8% respectively. Polyvinyl alcohol (PVAL) was often found in supermarket samples (27.1%), and ethylene vinyl alcohol (EVOH) was commonly observed in meat shop samples (20.4%). Most of the MPs in fragment form were frequently found in supermarket and meat shop samples (62.5% and 65.9%, respectively), while foam MPs dominated traditional market samples (62.3%). Film MPs were also common in supermarket (25.3%) and traditional market (26.6%) samples. Most MPs across all samples measured >100-≤300 µm (33.4% to 37.7%). The intake of MPs from beef consumption for the Indonesian population is considered high, ranging from 1,866,750 MP/person/year to 6,733,400 MP/person/year based on national beef consumption data. Therefore, MP contamination still needs to be monitored to prevent food safety issues.

HIGHTLIGHTS

- MPs in beef from traditional markets are three times higher than in other markets.
- PVC, PET, EVOH, and PVAL are the dominant types of polymers found in beef samples.
- MP intake from beef consumption among Indonesians is considered high.

1. INTRODUCTION

Microplastics (MPs) are tiny plastic particles, ranging from 1 to 5,000 µm, (GESAMP, 2019), originating from primary sources, intentionally produced for industrial or commercial use, and secondary sources formed by the breakdown of larger plastic debris through environmental processes (Andrady, 2017). MPs are commonly found in the air, water, and soil due to various human activities, such as improper disposal of plastic waste, industrial processes, fishing and maritime activities, the use of personal products and synthetic textiles, transportation and urban runoff, and improper wastewater treatment (Sharma and Chatterjee, 2017; Lundebye et al., 2022). Once present in the environment, MPs can enter both aquatic and terrestrial food chains and contaminate

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numerous food and beverage products consumed by humans (Gündogdu et al., 2023; Prata and Dias-Pereira, 2023; Sewwandi et al., 2023).

It is possible for MPs to enter the human body, with evidence of their presence found in feces (Luqman et al., 2021), the colon (Ibrahim et al., 2020), the placenta (Ragusa et al., 2021), blood (Leslie et al., 2022), lungs (Jenner et al., 2022), and breast milk (Ragusa et al., 2022). Concerns about the potential health impacts of MPs include inflammation, oxidative stress, and toxicity, as demonstrated in both in vitro and in vivo studies (Emenike et al., 2023; Mattioda et al., 2023). However, our understanding of the risks posed by MPs to human health remains limited, particularly regarding realistic human exposure (Zhao et al., 2024).

Studies have identified MPs in seafood (Hantoro et al., 2019), freshwater fish (Li et al., 2018), various food products like salt, honey, and beer (Peixoto et al., 2019; Toussaint et al., 2019), drinking water (Kirstein et al., 2016; Oßmann, 2021), poultry including chicken meat, eggs (up to 12 particles per egg) (Liu et al., 2022; Zhao et al., 2024), and livestock (Chen et al., 2023; Bahrani et al., 2024), indicating their widespread presence. A study in Iran reported an average of 0.14 and 0.13 particles/g in cattle and goats, respectively, with the highest concentration in beef tissue at 0.19 particles/g, mainly comprising nylon and fiber polymers (Bahrani et al., 2024). Likewise, Visentin et al. (2024) demonstrated the occurrence of MPs in beef hamburgers in Italy. Furthermore, analysis on top sirloin steaks from a USA supermarket showed 25±38 MP particles/serving (Milne et al., 2024). These findings provide the initial evidence of MPs in beef.

Research on MP contamination in livestock meat, particularly beef, in Indonesia is limited, despite its importance as a protein source. Although beef consumption in Indonesia, is relatively low (approximately 2.62 kg/capita/year in 2022, compared to the world average of 6.4 kg/capita/year), it has been increasing over the past five years (Chafid, 2024).

MPs enter livestock through the environment, water, and feed. Some low-income populations in Indonesia rear cattle near or inside municipal garbage disposal sites due to limited grazing areas, increasing the risk of MP exposure. MPs have also been detected in meat, with post-slaughter contamination linked to the use of plastic cutting boards during preparation for customers (Habib et al., 2022a). Habib et al. (2022a) further demonstrated that plastic cutting boards are a source of MPs contamination in raw cut fish and livestock, and that washing cutting boards before meat preparation increases MP release into wastewater sinks.

Thus, further research is needed to understand the extent of MP contamination in beef and its potential impact on consumer health. This study aims to investigate the occurrence of MPs in beef from traditional markets, specialty meat shops, and retail stores in Semarang.

2. METHODOLOGY

2.1 Sampling and sample preparation

2.1.1 Study area

This study was conducted in Semarang City, Central Java Province, and focused on butchers from a traditional market, a meat shop, and a supermarket who met specific criteria: the beef must be sourced from Semarang City and consist of sirloin and brisket.

2.2.2 Sample collection and preparation

A total of 1 kg sample consisting of each 500 g of brisket and sirloin cuts were collected from each location and wrapped in aluminum foil before being transported to the laboratory. A disinfected cooler box (wiped with 70% ethanol) was used to transport the samples to the laboratory. For each location, those cuts were divided into smaller pieces before being ground and homogenized. The homogenized sample was weighed at 5 grams, wrapped in aluminum foil, placed in glass containers, labeled, and stored in a freezer at -18°C. As for the digestion process, samples were removed from the freezer and thawed until they reached room temperature.

2.2 Microplastics detection

2.2.1 Quality assurance of analysis

Before sample analysis, laboratory quality assurance was performed in accordance with Dehaut et al. (2019). All equipment and work surfaces were sterilized with 70% ethanol. The floor and working areas were vacuumed, and an air purifier was turned on during the analysis. To prevent external microplastic contamination, only non-plastic equipment such as glass, stainless steel, and wood were employed. The entire operation was carried out while wearing laboratory coats and nitrile gloves. Each glassware was washed and rinsed with ethanol and aquabidest, then wrapped in aluminum foil and dried in the oven. The solutions were initially filtered using Whatman filter paper. Moreover, blank analysis and air contamination control checks were conducted to correct the results of MPs analysis on the samples.

2.2.2 Sample digestion

The method for identifying microplastics in beef was derived from Milne et al. (2024). Five grams of homogenized sample were digested in a 10% KOH solution and incubated at 40°C for 24 hours. The materials were then filtered with Whatman filter paper 541. In addition, 120 mL of 30% H_2O_2 solution was added to the sample and left for another 24 hours to remove any organic matter. The digestion process continued with wet peroxide oxidation (WPO), which used iron sulfate ($Fe(II)SO_4$) as a catalyst in conjunction with 30% H_2O_2 at a 1:5 ratio. To keep the temperature below 50°C and reduce microplastic loss, an ice bath was used. The sample was filtered through PTFE filter paper, before the identification step, and placed in a Petri plate and dried.

2.2.3 Characterization and identification of MPs

The characterizations were based on quantity, form, and size. The shapes were classified as fragments, fibers, films, foams, and pellets. The types of microplastic polymers were evaluated using micro-FTIR (IR Tracer100 & AIM 9000, Shimadzu) in the wavenumber range of 4,000-400 cm^{-1}, resolution of 4 cm^{-1}, utilizing reflection mode and %transmission measurement mode. A total of 50 scans were completed. From the total surface area of the filter paper in contact with the sample, 5% of the area was taken for identification. Sixteen points, each measuring 2 × 2 mm^2 on the membrane filter surface, were randomly selected for analysis. The number of particles found was extrapolated to cover the entire surface area of the membrane filter.

Polyamide (PA) was used as a reference material to ensure the accuracy of the spectral observations. The spectra from the samples were compared to known plastic polymer spectra from many reference spectral libraries from Shimadzu. To determine the types of polymers present, the examined spectrum data were matched to the reference database using a matching threshold of more than 65% (Corami et al., 2020).

To determine the shape and size of MP particles identified from micro-FTIR analysis results, the program ImageJ Version 1.54 was used. The image analysis was conducted through a series of systematic procedures. Initially, microplastic (MP) images obtained from the micro-FTIR instrument were imported into the ImageJ software for further analysis. Scale calibration was then performed to ensure accurate measurement. In cases where particle images appeared unclear, brightness and contrast adjustments were applied to enhance visual clarity. The particle size was subsequently determined based on the axial diameter, defined as the maximum linear distance measured from one end of the particle to the opposite end. The morphology of microplastic particles was determined by comparing visual images with shape classification criteria reported in previous studies (Rochman et al., 2019; Lusher et al., 2020).

2.3 Data analysis

Microplastic concentrations were presented as mean value data (MPs/kg, WB). The MP concentration in the samples was corrected using the average of blank controls and airborne contaminants found in the laboratory. The characteristics of MPs, including shape, size, and type of polymer, are presented in the graphs.

3. RESULTS AND DISCUSSION

3.1 MP concentration in the beef samples

The average concentration of MPs in beef obtained (MPs/kg, WB) is displayed in Table 1. MPs were detected in all analyzed samples, confirming their ubiquitous presence in markets. Beef from the traditional market exhibited the highest concentration ($2.57\times10^6\pm3.35\times10^6$ MPs/kg, WB), followed by the meat shop ($7.51\times10^5\pm1.58\times10^5$ MPs/kg WB) and the supermarket ($6.78\times10^5\pm2.41\times10^5$ MPs/kg WB). However, statistical analysis using the Kruskal-Wallis test revealed no significant differences among the markets (χ^2=2.000, df=2, p=0.368), indicating relatively similar contamination levels across sampling sites. The observed variations in mean values may be associated with differences in post-slaughter handling practices. Field observations show that meat sold in traditional market is transported from slaughterhouses in plastic sacks and displayed on plastic trays or wrapped in plastic. Meanwhile, meat from the supermarket and the meat shop is transported in plastic but displayed differently: hung on hooks over ceramic tables or placed on melamine trays and wrapped in cling wrap. Furthermore, the highly open condition of traditional market increases the likelihood of microplastic contamination from the surrounding environment.

The findings of this study are significantly higher than the MP levels found in beef hamburgers produced in Italy, where the contamination density ranges from 200 to 30,000 MP/kg of sample (Visentin et al., 2024). Compared to the highest MP density in beef burger samples, the contamination level in fresh beef from this study is 24 to 86 times higher.

Table 1. The concentration of MPs in beef obtained from different markets

Location	MP concentration ((MP/kg, WB)
Supermarket	$6.78\times10^5\pm2.41\times10^5$
Meat shop	$7.51\times10^5\pm1.58\times10^5$
Traditional market	$2.57\times10^6\pm3.35\times10^6$

Compared to MP findings in other types of meat measured in France—such as 1.1±1.9 and 10.8±6.0 MP/kg in packaged meat (Kedzierski et al., 2020) and 0.03±0.04 to 1.19±0.72 MP/g in meat cut on plastic cutting board (Habib et al., 2022a)—this study also shows significantly higher concentrations. This significant difference may be attributed to variations in identification methods, such as the hit quality index used, and the extrapolation method employed in this study, which could have led to an overestimation of MP concentrations. Although microscopic observations revealed an even distribution of MP particles on the membrane filter, this indicates a high density of MPs in the beef samples. Additionally, differences in location may also play a role.

MP contamination in beef can start from cattle production on farms and extend through to market sales (Velebit et al., 2023). MPs can enter the meat supply chain primarily through livestock feed. If cows ingest contaminated water, feed, or fodder, MPs can accumulate in their tissues. Additionally, the use of plastic containers and packaging during beef handling and display contributes to MP contamination.

3.2 The characteristics of MPs in the beef samples

Figure 1 shows that the dominant particles in beef samples from the traditional market were polyethylene (PE, 94.1%). In contrast, samples from the supermarket and the meat shop were predominantly composed of polyvinyl chloride (PVC), at 47.5% and 38.8% respectively. The type of polymer polyvinyl alcohol (PVAL) was frequently found in samples from the supermarket (27.1%, while ethylene vinyl alcohol (EVOH) was commonly found in samples from the meat shop (20.4%). The diversity of MP polymer types in meat samples is much less than in the supermarket and the meat shop. The infrared spectra of the most dominant MPs are presented in Figure 2.

To identify the identic functional group of polymers, FTIR spectra was recorded. Figure 2 represents the functional group of two most dominant polymer types which were found in beef samples. Characteristic of PE are shown at peak 1,465 cm^{-1} which represents to bending vibration of $C\text{-}H_2$ and 2,924 cm^{-1} of C-H stretching vibration. Peak at 599 cm^{-1} is attributed to stretching vibration of C-Cl from PVC spectra. The spectra also has the same signal at 2,908 cm^{-1} as C-H stretching vibration.

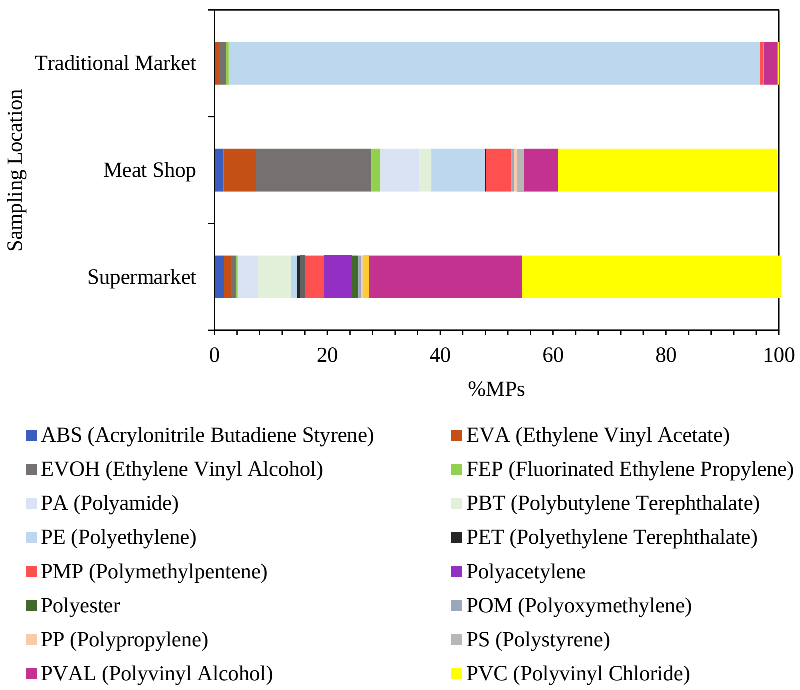

Figure 1. Polymer type of MPs found in beef samples

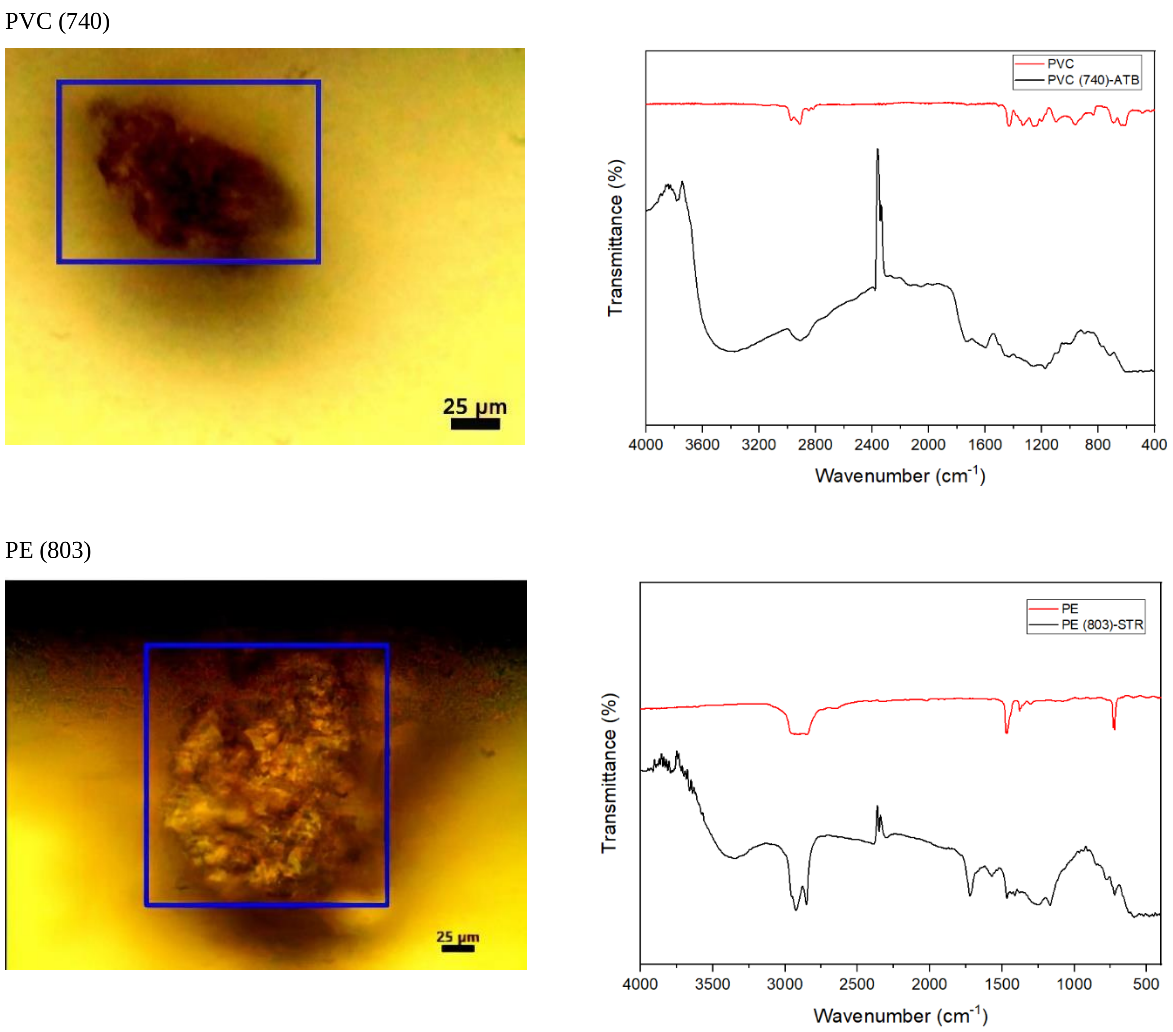

Figure 2. The shape and size of the most abundant identified MPs in beef samples (PVC and PE) analyzed with ImageJ and the FTIR spectra of detected MP particles.

The PE plastic found in the meat samples, particularly prevalent in traditional markets, is most likely from the plastic wrapping bags commonly used in these markets and throughout the beef supply chain. The study by Katsara et al. (2022) also found that the most common plastic in cured beef product samples was LDPE. The presence of PE has also been reported in chicken meat, as demonstrated by Habib et al. (2022a), who identified PE originating from cutting boards. This study demonstrates that LDPE from plastic packaging can be transferred to meat products during distribution and storage. PE could also originate from cling wrap, which is commonly used in supermarkets. However, the PE polymer found in beef samples from supermarkets is relatively low compared to other samples.

The detection of PVC in beef is supported by previous findings from Van Der Veen et al. (2022), who successfully identified PVC in several meat samples. PVC is predominantly found in meat samples from the meat shop and the supermarket. PVC with the addition of plasticizers is commonly used as food packaging material (Carlos et al., 2018), which, in this case, is likely a source of MP exposure to beef. PVAL is frequently detected in beef samples from the supermarket. PVAL is widely used in food packaging because it is biodegradable, non-toxic, has excellent film-forming capabilities, good absorbency, ready availability, and low processing costs (Channa et al., 2022; Uysal-Unalan et al., 2024). EVOH is commonly found in beef samples from the meat shop. EVOH is a plastic polymer commonly used in food packaging due to its exceptional barrier resistance to gases, aroma, water hydrocarbon permeation and chemical resistance (Luzi et al., 2020; Uysal-Unalan et al., 2024), making it ideal for preserving food with 70% crystalline structure. The presence of hydroxyl groups make EVOH is frequently used as a copolymer with hydrophobic materials as a good barrier coating system. Blending this material with compounds or monomers will ease the process of decomposition (Tyagi et al., 2021).

Figure 3 shows that fragments were frequently found in samples from supermarkets and meat shops (62.5% and 65.9%, respectively), while MPs in foam form were prevalent in beef samples from traditional markets (62.3%). MPs in film form were also commonly found in samples from supermarkets (25.3%) and traditional markets (26.6%). Fragments were also found to be the most prevalent form in beef hamburgers, and the other MP forms were fibers and beads (Visentin et al., 2024). The cutting board used by markets contributed to the generation of the fragment shape of MPs (Habib et al., 2022a). It was estimated that every gram of cut meat will release 2.2 mg of plastic (Habib et al., 2022b). Considering that fragment form of MPs has been detected in supermarket samples, the source of this shape can come from the usage of cutting boards as the result of field observation during this research. Research on MPs in meat or meat products is very limited, and previous studies have not found the presence of MPs in the form of foam.

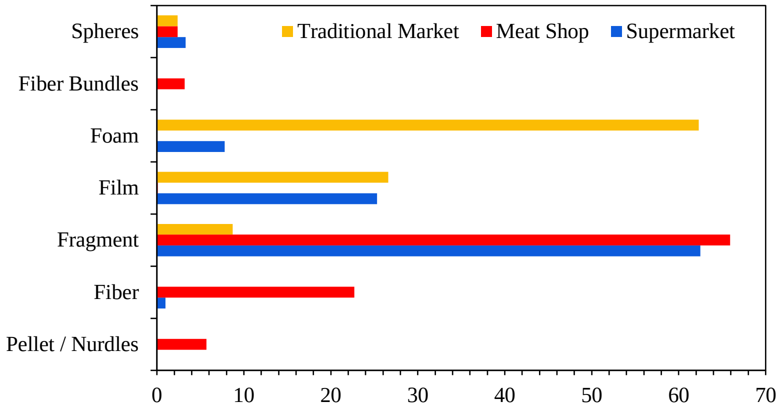

Figure 3. The morphotypes of MPs found in beef samples

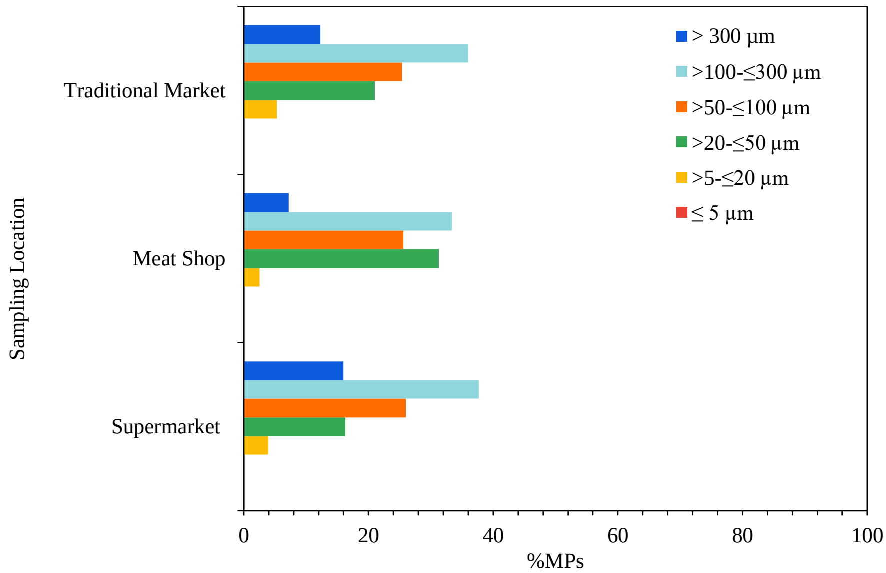

Figure 4. The size of MPs found in beef samples

The most common size of MPs found in all samples was >100-≤300 μm, followed by size range >50-≤100 μm and >20-≤50 μm (Figure 4). Particles smaller than 20 μm and larger than 300 μm were found in lower proportions in all beef samples. A similar range of MP sizes has also been found in other related studies (Kedzierski et al., 2020). Meanwhile, the study by

Visentin et al. (2024) showed that the most common polymers were in the size range of 51-100 µm.

3.3 Implications for food safety

Beef available in markets, meat shops, and retail stores shows high levels of MP contamination. Consequently, beef becomes a significant pathway for human exposure to MPs. Although beef consumption in Indonesia is relatively low compared to other countries, the high contamination levels can still result in substantial MP intake.

MP contamination in beef may pose health risks, given that beef and its products are essential sources of protein and other nutrients in the Indonesian diet. However, assessing the potential health risks of MPs in beef requires an understanding of the extent of human exposure through its consumption. Based on the average beef consumption in Indonesia in 2022, which was approximately 2.62 kg/capita/year (Chafid, 2024), the annual intake of MPs could range from 1,866,750 MPs/capita/year to 6,733,400 MPs/capita/year. This intake level is much higher compared to the annual MP intake through green mussel consumption, which range from 218,400-775,180 MPs/capita/year in Indonesia (Irnidayanti et al., 2023). It's worth noting that green mussels are known to be one of the seafood products with high levels of MP contamination. However, as determined by this study, MP exposure from beef consumption is still lower than intake from drinking water, which can reach 12,273,490 MPs/capita/year (Rubio-Armendariz et al., 2022).

Even though the concentration of MPs in beef is very high, it does not necessarily imply a greater health risk, as factors such as the shape, size, and type of polymer of MPs also play a role (Hantoro et al., 2024). The smaller-sized MPs showed greater toxicity reactions in in-vitro studies (Pelegrini et al., 2023). Furthermore, another study indicated that toxicity is influenced by the composition of polymer monomers (Yuan et al., 2022).

MP contamination in meat can be influenced by various stages along the supply chain, including production on farms, slaughtering, transportation, and distribution to different types of market. Therefore, implementing good practices throughout the food supply chain is essential to reduce the likelihood of MPs entering beef. The use of various plastic equipment and plastic packaging needs to be minimized throughout the food supply chain. Consumers should also avoid using plastic cutting boards, as their use has been shown to contribute to the entry of MPs into meat (Habib et al., 2022a). Despite the health effects of MPs on humans are not yet fully understood, precautionary measures suggest avoiding MP intake and highlight the importance of proper beef handling to reduce risks.

4. CONCLUSION

The high levels of MP contamination detected in beef from markets in Semarang highlight a potential route of MP exposure to consumers. Although the contamination observed in this study is most likely linked to post-slaughter handling practices, such as displaying, cutting, weighing, and packaging, the possibility of pre-slaughter contamination cannot be ruled out, as it was beyond the study's scope. Variations in MP concentrations and characteristics among market types suggest that handling practices along the supply chain, from slaughter to retail, may influence the degree of MP contamination in beef available to the consumers.

Future research should focus on elucidating the fate and dynamics of microplastic contamination across pre- and post-slaughter stages of the meat supply chain. Considering the potential health risks associated with MP-contaminated beef, it is crucial to broaden research on human exposure pathways and the toxicity of MPs. There is an urgent need for mitigation measures in beef handling and processing to lower human exposure risks. Future studies should also focus on improving risk assessments and creating effective strategies to reduce MP contamination along the beef supply chain and safeguard public health.

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AUTHOR CONTRIBUTIONS

Conceptualization, Hantoro I, Widianarko B; Methodology, Hantoro I, Harumi M, Ardanareswari K, Soedarini B, Widianarko B; Validation, Hantoro I; Formal Analysis, Hantoro I and Widianarko B; Investigation, Hantoro I, Harumi M, Ardanareswari K, Soedarini B, Widianarko B; Data Curation Hantoro I, Harumi M, Ardanareswari K, Soedarini B, Widianarko B; Writing-Original Draft Preparation, Hantoro I, Harumi M, Ardanareswari K; Writing-Review and Editing, Soedarini B, Widianarko B; Visualization, Hantoro I; Supervision, Widianarko B; Project Administration, Hantoro I.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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