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Research article

Microplastics contamination in the most popular brands of Iranian sausages and evaluation of its human exposure

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ABSTRACT

Microplastics (MPs) pollution represents a nascent environmental contaminant that has recently infiltrated human life and the food chain. The primary objective of this study was to investigate the presence of MPs in different brands of Iranian sausages. Qualitative and quantitative analyses of MPs particles were conducted using stereo- and fluorescent microscopy, FT-IR (Fourier-transform infrared spectroscopy), and SEM-EDS (Scanning electron microscopy-energy dispersive X-ray spectroscopy) techniques. Samples were collected from the most commonly consumed sausage brands in Iranian markets. The findings showed that the various sausage brands contained an average abundance of 25.7 ± 21.68 (range 10–70) and 55.45 ± 45.5 (range 10–175) particles/kg based on optical and fluorescent microscopy analyses, respectively. Predominantly, MPs were identified in fiber form (77–89 %), with a smaller proportion present in fragmented form (11–23 %). Polymer analysis using FT-IR identified polyethylene (PE) and polystyrene (PS) as the primary constituents. Furthermore, the estimated annual intake (EAI) of MPs was calculated at 804 and 3517 particles/kg bw/year for adults and children, respectively, based on optical microscopy observations. In comparison, fluorescent microscopy indicated an intake of 1734 and 7589 particles/kg bw/year for the respective age groups. These results emphasize the potential of MPs contamination to penetrate into different food products including sausages through processing routes, which can threaten human health.

1. Introduction

The escalation in plastic production and consumption over the past five decades can be attributed to its inherent characteristics, including lightweight, durability, cost-effectiveness, high power-to-weight ratio, and low thermal conductivity [1]. Typically, plastics do not biodegrade entirely but fragment into smaller particles known as microplastics (MPs) under the influence of ultraviolet (UV) light, mechanical forces, and relatively low temperatures. MPs encompass plastic particles ranging from a few micrometers to 500 μm in size [2]. MPs originate from various sources such as fine plastic particles in industrial processes, municipal and industrial wastewater, laundry activities, personal care products like facial scrubs and cosmetics, food packaging, synthetic fabrics, and synthetic fibers

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[3]. Reports indicate the accumulation of MPs in marine environments, oceans, beaches, soils, sediments, and freshwater systems. Consequently, the pervasive contamination of MPs poses a potential risk of entering the food chain and ultimately affecting human consumption [4].

Research has elucidated the extent and implications of human exposure to MPs. Studies have investigated the presence of MPs in fish and have shown high concentrations of MPs in their systems [5]. Fine particles and plastic fibers have been detected in tap water and various bottled water sources globally [6]. Furthermore, studies have documented the presence of MPs in sea salt, beer, and honey [7–9], along with reports of contamination in numerous commercial fish and shellfish species [5,10].

MPs have been identified as substances that can detrimentally affect aquatic digestion, potentially leading to issues such as intestinal obstruction, animal malnutrition, and altered energy intake [11]. Ingestion of MPs can lead to a range of issues, including obstructed digestive tracts, inflammation, organ damage, and the accumulation of toxic chemicals in the body. Furthermore, MPs can disrupt the immune system, trigger detrimental immune responses, and interfere with hormone signaling pathways [12–14].

Studies have indicated that the excessive consumption of plastics by aquatic organisms may result in reduced intake of essential nutrients, consequently impacting the energy levels and reproductive capabilities of these organisms [15,16]. Baalkhivar et al. (2018) [17] documented the presence of MPs in various fish species inhabiting diverse habitats off the coast of the Saudi Red Sea. Similarly, Yang et al. (2015) [18] detected MPs in 15 different brands of Chinese commercial salts sourced from lakes, mines, and coastal seas. These findings underscore the critical need for further research on MPs in the food chain, beyond seafood, as processed foods, including ready-made meals, can also be susceptible to contamination during processing and packaging, thereby increasing human exposure to MPs [19–22].

Meat products are widely consumed globally due to their nutritional value, economic significance, and the diverse range of processing facilities available. Consequently, ensuring the safety and quality of meat products has become imperative with the rise in consumption rates [23]. The human exposure to MPs through food consumption is a growing concern, particularly in countries where sausages are a significant part of the diet.

In Iran, sausages from various brands are prevalent in the market and are among the most favored meat products in the country [24]. Therefore, guaranteeing the safety, quality, and appropriate packaging of these products is paramount. While existing research predominantly focuses on MPs contamination in aquatic environments [5,10,25], seafood [26], and select processed foods [27], there exists a notable research gap concerning the presence and implications of MPs in meat products, particularly in popular items like sausages. The potential contamination of Iranian sausages with MPs and its associated impact on human exposure remain largely unexplored. This research gap presents an opportunity to investigate a important aspect of food safety and public health that has not received widespread attention. Therefore, the primary objective of this study is to determine the presence of MPs in renowned Iranian sausage brands and to evaluate the potential human exposure to MPs by quantifying the levels of MPs in these food items and estimating the annual consumption amount resulting from the ingestion of microplastic-contaminated sausages.

2. Materials and methods

2.1. Sample collection

In the initial phase, seven prominent and extensively utilized sausage brands were identified within the Iranian market. Subsequently, six samples weighing approximately 100 g each were randomly procured from various locations in the market for each brand, resulting in a total of 42 samples. These samples were assigned unique codes (A-G), carefully stored in a temperature-controlled container, and then transported to the laboratory for analysis.

2.2. Sample preparation

10 g of each sausage sample were precisely sectioned using a laboratory-grade steel knife and subsequently transferred into individual 250 mL Erlenmeyer flasks. Following this, 100 mL of a 10 % potassium hydroxide (KOH) solution from Merck, Germany, prepared at a ratio of 1:10 wt/volume, was introduced into each flask to facilitate the digestion of meat and other organic components within the sausages. The samples were then subjected to a 24-h incubation period at 60 °C. In instances where complete digestion was not achieved within the designated time frame, a 35 % hydrogen peroxide (H_2O_2) solution from Merck, Germany, was utilized as an additional digesting agent. Upon completion of the incubation process, 150 mL of filtered 10 % sodium iodide (NaI) solution (6:10 w/v) from Merck, Germany, was added to each flask to separate MPs from the dissolved solution via flotation. Consequently, the samples underwent filtration using a Buchner funnel flask kit equipped with a vacuum pump and a glass fiber filter (Grade 50/A, 1.2 μm pore size, 47 mm diameter). Subsequently, the filters were promptly transferred onto clean, covered Petri dishes to undergo thorough drying before further analysis.

2.3. Quantification and qualification of MPs

After sample preparation, morphological characterization and quantitative detection of MPs were conducted using a stereomicroscope (Olympus SZX16, Olympus K.K., Japan) and a fluorescent microscope (Axio Scope A1, Zeiss, Jena, Germany) in conjunction with Nile Red (NR) (Sigma-Aldrich, St Louis, MO) staining. All analyses were performed in triplicate. The MPs were categorized based on size ranges utilizing Feret's diameter measurement. Additionally, classification was done based on color (red, blue, green, black, and white/transparent) and shape (fiber and fragment). A hot needle test was employed to differentiate MPs particles from other potential crystal structures like chemical particles and salt; MPs particles exhibit curling or melting upon contact with the hot needle, distinguishing them from non-plastic materials [28,29].

Surface analysis of the MPs particles was carried out using a scanning electron microscope (SEM/Hitachi SU3500, Bruker Nano GmbH Berlin, Germany) and energy dispersive X-ray spectroscopy (EDS/Bruker, Germany) to examine surface features and surface composition signatures, respectively. Furthermore, Fourier-transform infrared spectroscopy (FT-IR/PerkinElmer, USA) was utilized to identify the types of polymers present. Comparison of the obtained spectra with existing data [30] allowed for the determination of polymer types.

2.4. Quality assurance and control

To prevent contamination, all equipment utilized in the laboratory analysis underwent a thorough cleaning process involving three washes with distilled water followed by drying under a fume hood. When not in use, the apparatuses were promptly covered with aluminum caps. Lab personnel adhered to strict protocols by wearing 100 % cotton lab coats and nitrile gloves throughout all laboratory procedures. Before and after each task, all surfaces and tools were meticulously wiped down with 70 % ethanol. The use of plastic materials was prohibited during the entire sample analysis process. For air quality control, a 100 ml volume of distilled water was placed in an open glass container near the laboratory equipment during the analysis of sausage samples. This blank sample served to capture any airborne MPs particles that could potentially impact the test accuracy. Additionally, 100 ml of 10 % KOH, 100 ml of 35 % H_2O_2, and 100 ml of 10 % NaI were employed to ensure the quality and purity of the samples. Following the filtration of the raw distilled water, the distilled water used as an air sample, as well as the KOH, H_2O_2, and NaI solutions, and subsequent examination (as detailed in section 2.3), no MPs were detected on the filter's surface.

2.5. Exposure assessment

The estimated annual intake (EAI) serves as a metric for evaluating the quantity of a substance, specifically MPs, that an individual is projected to consume annually through their diet. The EAI for MPs from sausage consumption among the Iranian population was determined using Equation (1).

$$\text{EAI (particles / kg bw / year)} = \frac{\text{IR} \times \text{C}}{\text{bw}} \times 365 \tag{1}$$

where [IR] represents the annual sausage consumption rate per capita (6 kg per year), [bw] is the body weight (70 kg for adults and 16 kg for children), and [C] denotes the number of MPs in Sausage (particles/kg).

3. Results & discussion

3.1. Quantification of MPs

The microscopic inspections showed varying quantities of MPs across all samples of sausage brands. Fig. 1 illustrates that each of the seven brands exhibited contamination with MPs, ranging from a minimum of 10 particles/kg to a maximum of 175 particles/kg. Notably, fluorescence microscopy indicated a significantly higher MPs content compared to optical microscopy. The optical analysis detected an average of 25.7 ± 21.68 particles/kg in the sausage brands, while the fluorescence microscopy method showed an average of 55.45 ± 45.5 particles/kg.

Literature has documented the presence of MPs in various food items, including table salt, fish, shellfish, canned foods, processed foods, and bottled water, with concentrations ranging from 1 to 5.4×10^7 particles/L or kg [4,31]. For instance, Karimi et al. (2018) [20] identified 1–3 MPs per brand, encompassing both micro and mesoplastics, in canned fish. Additionally, Makhdoumi et al. (2021)

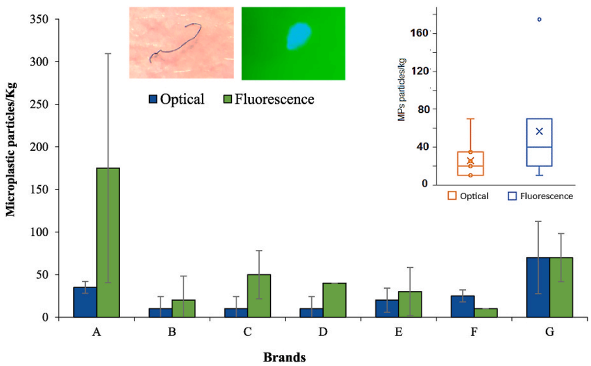


Fig. 1. Optical and fluorescence counting of MPs in the different sausage brands (particles/kg).

[32] reported an average contamination of bottled water by MPs at approximately 8.5 ± 10.2 particles/L. Another study highlighted the contamination of vinegar from Iranian brands, with optical and fluorescence methods detecting around 16.64 ± 6.34 and 51.35 ± 20.73 particles/L, respectively [33].

The discrepancy in MPs distribution between optical and fluorescent methods can be attributed to the limitations of optical analysis, errors in particle detection, and the transparency of MPs. Staining methods have proven to be a stable, rapid, reliable, and valuable approach for quantifying MPs in environmental samples [34]. However, research by Stanton et al. (2019) [35] cautioned against relying solely on non-routine methods, as they may lead to an overestimation of MPs and a higher error rate.

Various factors influence the variation in the abundance of MPs among different sausage brands, including the age and frequency of use of packaging containers, mechanical stresses during material preparation, packaging material composition, and packaging methods [10]. The detection methods employed also play a important role in the identification and quantification of MPs [32]. Mechanisms such as packaging, transport, distribution, and mechanical stresses can introduce small and large MPs particles into sausages [32]. Moreover, the use of softeners in packaging materials and during processing can facilitate the entry of MPs into the sausage product [4].

3.2. *Qualification of MPs*

3.2.1. *Shape of MPs*

The majority of MPs were fiber-shaped in both the stereo- (89 %) (Fig. 2 a) and fluorescence microscopy (77 %) (Fig. 2 b) methods. Fiber-shaped MPs are commonly found in various products due to their prevalence in manufacturing processes, packaging materials, and environmental contamination [33]. The structural characteristics and widespread distribution of fiber-shaped MPs in manufacturing, packaging, and environmental matrices contribute to their significant representation in detection studies across diverse sample types [22,33]. Makhdoumi et al. (2021) [32] suggested that some fragmentary particles may originate from the manufacturing or packing processes of products. In their study on contaminated bottled water with MPs, they found a significant presence of fragment-shaped MPs (93 %). Additionally, Kown et al. (2020) [4] observed that fiber structures are present in various food products statistically over 50 %, aligning with the findings of this research.

Photographic images of microplastics under stereo- (Fig. 3 a-g) and fluorescence microscopy (Fig. 3 h-m) highlight the common occurrence of fragment and fiber-shaped particles. The diversity in MPs shapes can be attributed to multiple factors. Firstly, the production and disposal of plastic materials result in the fragmentation of larger plastic items into smaller particles over time, influenced by environmental factors such as UV radiation and mechanical stress. These particles can contaminate diverse sources, including food and water, contributing to their widespread presence. Additionally, the utilization of plastic packaging in food processing and packaging industries heightens the risk of MPs contamination in consumables [10].

3.2.2. *SEM-EDS*

Scanning Electron Microscopy (SEM) is a powerful tool that offers high-magnification images of targeted samples, enabling a detailed 3D observation of MPs morphology. In Fig. 4 a-c, MPs are visualized at magnifications of 100, 300, and 500 μm, revealing distinct features such as fractures, crushing, and crumpling. These alterations in the MPs' structure are attributed to various processing stages and the preparation of sausages, indicating changes that occur as MPs age and detach from their sources.

The aging process of MPs holds significant implications. Aged MPs exhibit a more porous surface with functional groups compared to their original plastic form, enhancing their capacity to adsorb pollutants and consequently intensifying adverse effects [36]. Literature has documented instances of exposure to aged MPs, highlighting that aging can elevate neurotoxicity levels beyond those of the pristine MPs [37]. These findings underscore the importance of understanding the physico-chemical characteristics of MPs, as they can potentially instigate specific disorders in both animal and human cells [13].

Moreover, the combination of SEM with Energy Dispersive X-ray Spectroscopy (EDS) offers additional insights into the elemental composition of MPs. Typically, plastics consist of additives like sulfur (S), hydrogen (H), silicon (Si), chlorine, carbon, and oxygen [38]. The EDS analysis, as illustrated in Fig. 4 d, confirms the presence of carbon in the MPs' composition, along with the detection of rare elements. These rare elements may originate from plastic additives or be absorbed from the surrounding environment, further emphasizing the complexity of MPs' composition and their interaction with the ecosystem [25].

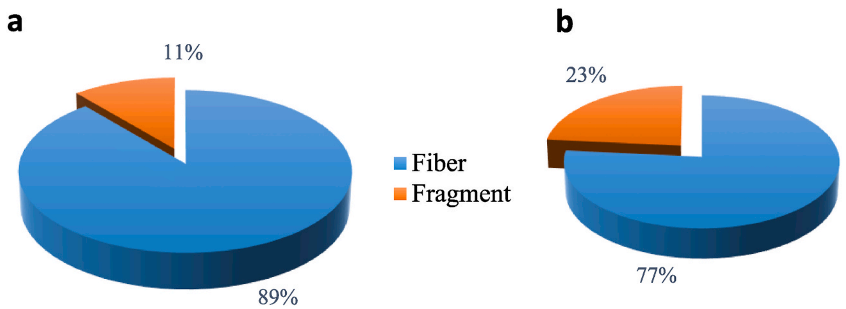


Fig. 2. Distribution of MPs identified in sausages based on shape, under stereo- (a) and fluorescence (b) microscopy.

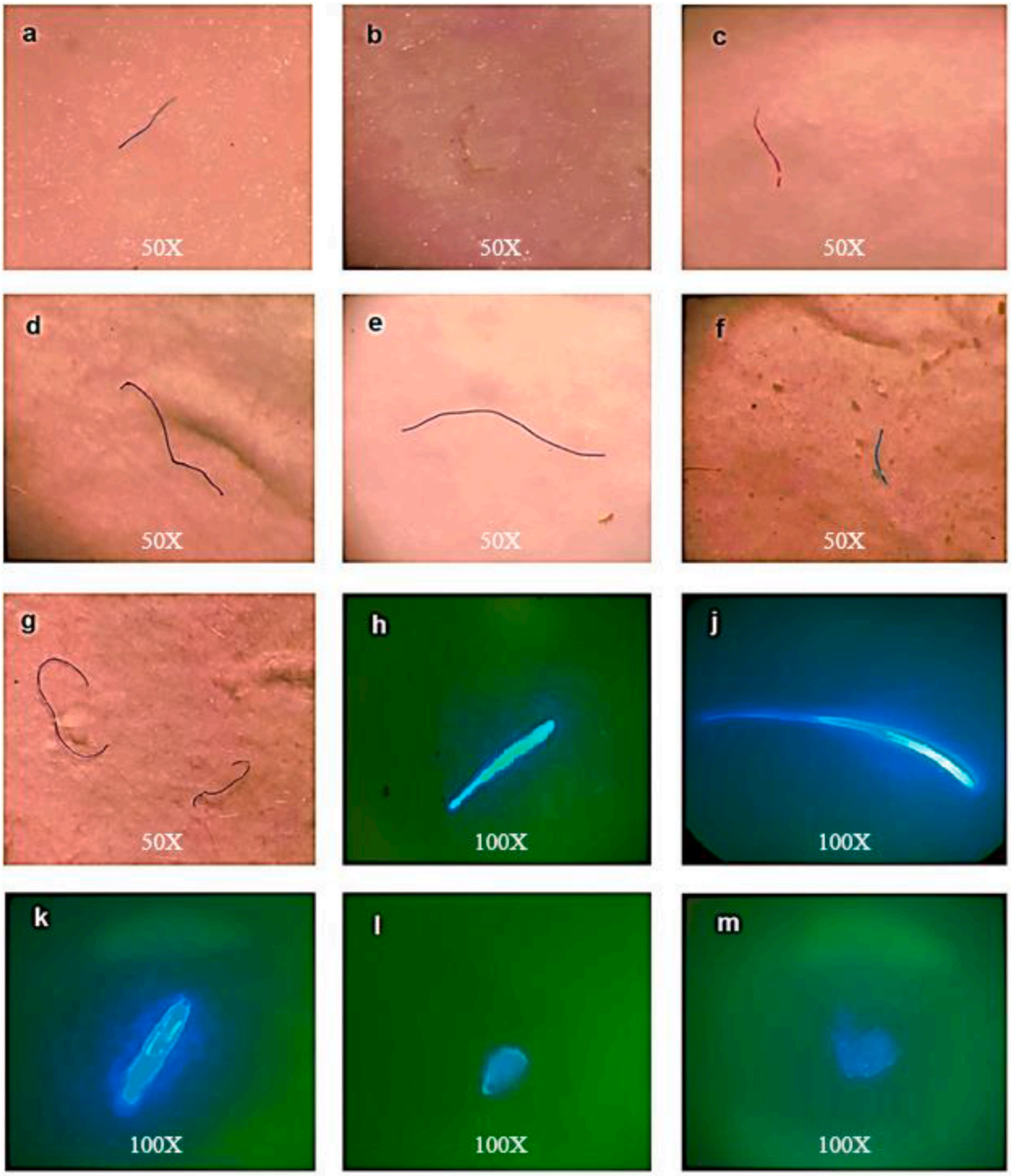


Fig. 3. Photographs of MPs identified in sausages, under stereo- (a–g) and fluorescence (h–m) microscope.

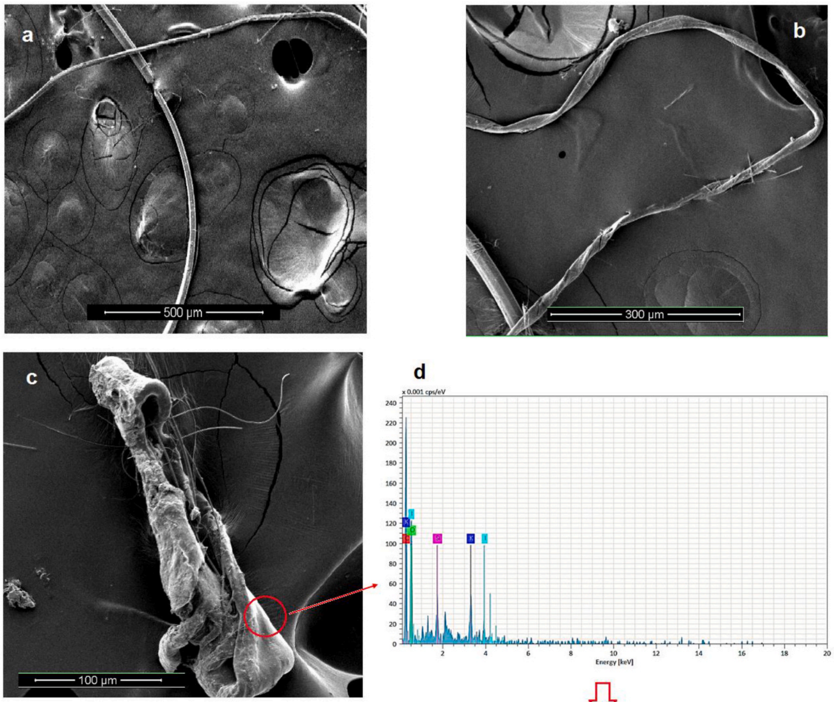
3.2.3. Color of MPs

MPs exhibit a variety of colors, reflecting their diverse origins and intended uses [22]. The results presented in Fig. 5 show that MPs are commonly found in black (46 %) white/transparent (28 %), blue (14 %), green (7 %) and red (4 %) colors. This prevalence of specific colors suggests potential sources and pathways of MPs contamination during production processes.

Understanding the color of MPs is important, especially concerning interactions with aquatic organisms that may mistakenly ingest these particles. The predominant colors of MPs can also serve as indicators of contamination by other pollutants, such as organic matter, as observed in yellow and black MPs [39]. Moreover, colors can offer insights into the polymer type, with propylene particles typically appearing transparent, while polyethylene (PE) and low-density polyethylene (LDPE) are commonly white and matte, respectively [39]. In a recent study by Azizi et al. (2021) [40], an analysis of existing literature emphasized that the majority of MPs are characterized by fibrous shapes, composed of PE polymers, and exhibit black coloring.

3.2.4. Size of MPs

Different stages of sausage processing in the food industry, such as grinding, mixing, and stuffing the meat in the sausage casing,



Element	At. No.	Line s.	Netto	Mass [%]	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	abs. error [%] (2 sigma)	abs. error [%] (3 sigma)	rel. error [%] (1 sigma)	rel. error [%] (2 sigma)	rel. error [%] (3 sigma)
Carbon	6	K-Serie	289	29.32	47.21	57.80	9.86	19.72	29.58	33.63	67.26	100.88
Oxygen	8	K-Serie	188	26.46	42.59	39.15	10.38	20.76	31.14	39.24	78.48	117.72
Potassium	19	K-Serie	105	2.63	4.24	1.59	0.31	0.63	0.94	11.91	23.82	35.73
Iodine	53	L-Serie	53	2.53	4.07	0.47	0.40	0.80	1.20	15.75	31.50	47.24
Silicon	14	K-Serie	72	1.17	1.88	0.99	0.19	0.38	0.57	16.38	32.77	49.15
			Sum	62.11	100.00	100.00						

Fig. 4. SEM-EDS analysis of MPs identified in sausages (a, b and c: Surface morphology of MPs. d: Elemental composition of the surface of MPs).

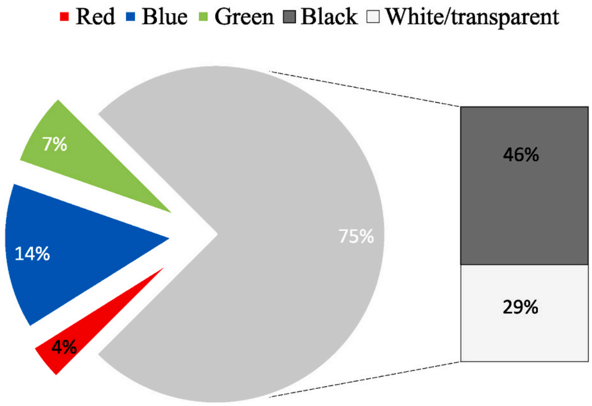


Fig. 5. Color distribution of MPs in the sausage samples.

can introduce MPs of various sizes into the final product. The size distribution of MPs is presented in Fig. 6. The majority of MPs ranged between 1 and 500 μm, while particles between 1500 and 3000 μm constituted a smaller portion of the distribution. Given that the characteristics of MPs can directly impact their toxic effects on metabolism, neurotransmission, immunity, and reproduction, certain properties like size are important. For instance, particles smaller than 5 μm are more likely to trigger oxidative stress, liver inflammation, genotoxicity, and carcinogenicity [40]. Although the adverse effects of MPs in the human gastrointestinal tract are not entirely clear, Walczak et al. (2015) [41] showed that particle size is a critical factor influencing the absorption efficiency of MPs in the gastrointestinal, alveolar, and dermal epithelium. Stock et al. (2019) [42] highlighted that larger MPs (ranging from 0.1 to 5 mm) could pose environmental concerns, while particles smaller than 150 μm raise worries due to their bioavailability to humans.

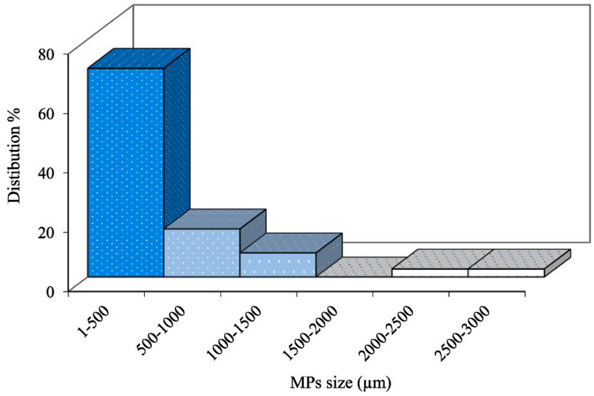


Fig. 6. Size distribution (µm) of MPs in sausage samples.

3.2.5. The type of MPs polymer

In this study to determine the specific polymers present in samples, FT-IR analysis was utilized (Fig. 7 a and b), which showed the presence of PE and polystyrene (PS). These polymers are frequently used as raw materials in producing bags, covers, and various other

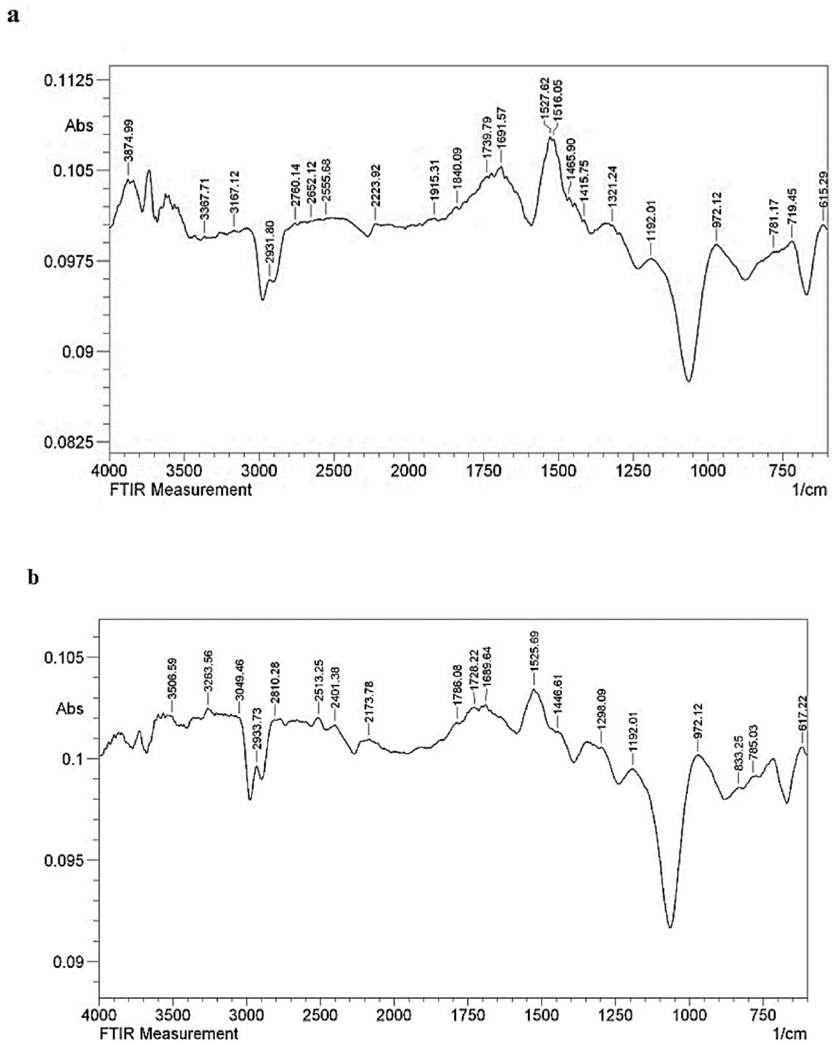


Fig. 7. FT-IR Spectra of some selected samples (a and b).

products. The type of MPs is significant as each type can have distinct adverse effects on human health. For instance, studies have shown that the accumulation of polyvinyl chloride (PVC) particles in the blood was documented by Volkheimer (1975) [43]. Han et al. (2020) [44] identified that PVC and acrylonitrile butadiene styrene (ABS) may trigger immune responses. PS and PE have been associated with various health issues such as alterations in lipid profiles and gut microbiota, impacts on energy metabolism, liver damage, interference with ATP synthesis, among others [45]. Hwang et al. (2019) [46] demonstrated that propylene (PP) particles exhibit low cytotoxicity in human-derived cells, stimulate the immune system, and potentially enhance hypersensitivity reactions. Additionally, polyamide (PA) MPs were found to weakly inhibit musculoskeletal growth in zebrafish larvae [47].

3.3. *Intake of MPs by consuming sausages*

The presence of MPs in Iranian sausages raises concerns due to the potential health risks associated with ingesting these particles. MPs have the capacity to obstruct digestive tracts, resulting in decreased feeding efficiency and the accumulation of these particles in tissues, leading to inflammation and organ damage [48]. Moreover, MPs can absorb and concentrate toxic chemicals from the environment, which may be released upon ingestion, causing toxic effects. Additionally, the plastic particles themselves may harbor harmful additives or chemicals that enhance their toxicity [13]. MPs can instigate oxidative stress in organisms, generating reactive oxygen species (ROS) that harm cells and tissues [13,49]. Furthermore, MPs can disrupt the immune system of organisms, triggering detrimental immune responses. Some MPs also contain chemicals that function as endocrine disruptors, interfering with hormone signaling pathways in organisms [13,50,51].

Estimating the amount of MPs entering the human body through the consumption of sausages can be crucial for future evaluations and decisions related to the consumption of this product. This information can lead to improvements in the production process in factories by reducing the amount of MPs present. It is essential to inform supervisors and health policymakers in each country about this issue. In Iran, where sausages have a significant presence in fast food and sandwich shops, this matter holds particular importance for public health.

According to available statistics, each Iranian consumes an average of 6 kg of sausages per year. Considering the frequency of MPs found in sausages, the annual intake of MPs through sausage consumption has been calculated for Iranians and presented in Table 1. The result showed that the estimated annual intake (EAI) of MPs, according to optical microscope method, for children and adults, was 3517 and 804 particles/kg bw/day, respectively. When using the Fluorescence microscope method, the EAI for children and adults was higher, at 7589 and 1734 particles/kg bw/day, respectively.

The presence of MPs in Iranian sausages is causing concern because of the potential health risks associated with eating these particles. MPs are known to have a number of negative health effects, such as obstructing digestive tracts, causing inflammation and organ damage, and potentially triggering oxidative stress, immune system disruptions, and endocrine disruptions [12–14]. Our study's findings on the estimated annual intake of MPs through sausage consumption, particularly the higher levels detected using fluorescence microscopy, highlight the need for further research on the long-term health implications of MPs exposure. The findings are significant because they show that the consumption of sausages in Iran may be leading to the intake of MPs, which can have negative health effects.

Comparing these findings with a previous study in Iran, the maximum EAI for MPs from salt consumption was approximately 3552 and 15540 particles/kg bw/day for children and adults, respectively. Similarly, for sugar consumption, the estimated EAI was 5408 and 23660 particles/kg bw/day for children and adults, respectively [22].

4. Conclusion

The research findings on MPs contamination in Iranian sausages yield significant insights into the presence, quantification, characteristics, and potential dietary intake of MPs in these food products. The study showed varying quantities of MPs across all sampled sausage brands. Utilizing fluorescence microscopy demonstrated a higher MPs content compared to optical microscopy, highlighting the critical role of detection methods in accurately assessing contamination levels. Moreover, the estimated annual intake of MPs through sausage consumption is significant, with potential health risks associated with ingesting these particles. The research also investigated the morphological characteristics of MPs through SEM-EDS analysis, along with exploring their color, shape and size distribution. These findings emphasize the necessity for further research on MPs contamination in food products, particularly sausages, to strengthen food safety protocols and safeguard public health. Continued research is essential to deepen our understanding of the sources, distribution, and effects of MPs in food products. Future studies should focus on long-term health implications, bioaccumulation pathways, and mitigation strategies to address this emerging environmental and public health concern.

Ethical consideration

Ethical issues (Including plagiarism, Informed Consent, mis-conduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors. The project was found to be in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran (IR.NIMAD.REC.1400.075).

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Table 1
EAI of MPs through sausage consumption for children and adults.

Method of detection	Average abundance of MPs (particles/kg)	EAI (particles/kg bw/year)	
		Children	Adult
Optical	25.7	3517	804
Fluorescence	55.45	7589	1734

National Institute for Medical Research Development (NIMAD), Tehran, Iran.

Data availability statement

All relevant data are included in this document.

CRediT authorship contribution statement

Meghdad Pirsaheb: Project administration, Conceptualization. **Monireh Nouri:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Tooraj Massahi:** Writing – review & editing, Software, Methodology, Formal analysis. **Pouran Makhdoumi:** Methodology, Conceptualization. **Negin Azadi Baban:** Visualization, Resources, Data curation. **Hooshyar Hossini:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition.

Declaration of competing 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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