Research Article

Identification of Microplastics as Emerging Contaminant in Branded Milk of Tamil Nadu State, India

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ABSTRACT

Nowadays, human exposure to microplastic particles through the contamination of foods may be a risk factor for human health. Microplastic particle contamination is highly intended in the food chain by consuming food products packed in plastic bags. In this study, around 13 brands (16 samples) of milk were collected from different parts of Tamil Nadu state, South India, and analysed. Results showed the presence of microplastic particles (polyethylene, polypropylene, and polyacrylamide) of size <500 µm. The result confirmed that the variability in the total number of microplastics per litre ranged from 164-427. The percentages of the microplastics in the tested samples were polyethylene (PE)-3.75%, polypropylene (PP)-6.61%, and polyacrylamide (PA) -2.67 %. Typical shapes like fibres, fragments, and pellets of different colours such as pink, purple, and blue were observed. The pellets range between 19 and 82 in number. The FTIR spectrum confirms the presence of polyethylene, polypropylene, and polyacrylamide types of microplastic composition. Our results showed that consumption of microparticles is estimated to be 35–80 microparticles per day per person. This study provides a basic outline of microplastic contamination in branded milk samples, and the findings may have implications for understanding the negative effects of microplastics in the ecological food chain.

Keywords: Microplastics, Milk, Polyethylene (PE), Polypropylene (PP), FTIR, Plastic Contaminants.

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INTRODUCTION

The use of plastic materials and corresponding creation has increased as the demand for plastic has increased. Approximately 8300 million metric tonnes of plastic have been produced since 1950. Asia contributes about 50% of plastic production globally.[1] Plastic packaging in food benefits transport, storage, preservation, protection from oxygen, water vapour, microorganisms and also helps maintain the desired temperature.[2] It is estimated that around 350 million tonnes of plastic are produced per year, and only 32% of the demand is fulfilled by recycling.[3] Plastic contamination gained attention owing to its serious environmental issues among the public and scientific communities.[4] Plastics have become an ubiquitous contaminant, and when they degrade, they deform into microplastics, also known as MPs, which have a size of less than 5 mm.[5] Primary MPs are virgin resin pellets that are produced commercially for their applications in sandblasting media during the production of cosmetics, personal care products, toothpaste, hand soaps, and facial scrubs. They are known as primary MPs.[6-9] Secondary MPs are formed by the breaking down of large plastic litter into fibres, fragments, and flakes of the desired size.[10]

This is of great concern about potential human health since MPs have become an integral contaminant of plastic food packaging. In fact, MPs have been identified in human foods such as salt,[11-12] sugar,[13] sea fish,[14] mineral and drinking water[15] Growing body evidence

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suggested the presence of MPs in digestive tracts and human stools because of MPs contaminated food consumption.[16-17] A recent study demonstrated human intake of microplastics via plastic food packages ranged from 203 to 332 particles per person per day.[18] Increasing evidence reveals the fact that MPs are getting established in the food chain by transferring themselves by all means. Microplastics are mainly made up of polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), and nylon. Of these, polyethylene, polyethylene terephthalate, and nylon are degradable, while polypropylene and polymethyl methacrylate are non-degradable.

Milk composites calories, proteins, carbohydrates, fat, saturated fat, cholesterol, Vitamin A, Vitamin C, foliate, calcium, magnesium, zinc, phosphorus, iron[19] (Hanna Górska, 2019). Milk is a part of a globalised food product that gives sustained income to smallholder livestock.[20] Milk varieties that have been developed include low-fat milk, low-calorie milk, fortified milk, skimmed milk (fat-free or non-fat milk), lactose-free milks, standardised milk, and high-fat, high-protein milk. diversification of milk quality causes significant changes in nutrient intake.[21-23] In recent decades, technological development in the production process, from the farms to the dairy industry, intense milk processing, poor cleaning procedures equipment, the surrounding environment, inadequate handling of milk, and plastic-based packaging materials influence the milk components and increase the possibility of contamination of MPs.[24] In India, the most common method of packaging milk is by packing milk in pouches made up of polyethylene and polypropylene. In the laboratory-based trials, microplastics concentrations, higher than environmentally relevant concentrations, displayed potential adverse effects on marine organisms due to the range of incorporated additives, metals, and biological load they carry.[25-29] In addition, there is growing evidence of the impacts of plastic-based packaging materials on microplastic contamination in various foods and beverages.[30-32] As a result, there is a need to analyse food and beverage products. In the present study, we chose 16 brands of milk samples available in Tamil Nadu, India in order to investigate the occurrence of MP contamination in branded milk samples. Common microplastics found in cosmetics,[33] as well as microplastic fibres, are derived from synthetic fabrics such as polyester and polyamide.[34,35] The main focus of this study is to identify the abundance of MPs contamination in milk samples and determine the nature, size, colour, shape, and chemical composition of MPs. We have analysed that there is a contamination of the MPs in the branded samples and there is a consumption of MPs per person per day.

MATERIALS AND METHODS

Sampling Strategy

Milk products are chosen based on distribution availability in super, local markets, retail shops, and based on domestic consumption. Accordingly, 13 brands (4 global, 1 national, 7 private, and 1 local brand) were chosen in this study. About 16 samples were purchased from supermarkets and local stores located in the state. They are available as pasteurized packaged milk at local markets and stores. Most of the samples were packed with films made of polyethylene, other than polyethylene, PP also used in the manufacture of milk bags. Milk samples were stored at 6°C until processed for microplastic extraction.

Sample Treatment and Microplastics Extraction

Customary filtration technique was applied for microplastics extraction.[36-37] Each sample was initially mixed by inverting the milk pouches and then transferred to a precleaned Erlenmeyer beaker. Filtration was performed using Whatman filter grade 1 (pore size of 11 µm) filters. Milk components such as fat and casein do not pass-through pores when cold, so milk (1 litre) is heated and filtered for better filtration. Warm milk samples were filtered with Whatman filters using a vacuum pump set to about 0.5 bar pressure.[38-39] All milk samples were handled with extreme caution to maintain a warm temperature throughout the filtration process, preventing clogging of any solid particles before they reached the bottom of the filtering unit. Filters become clogged and blocked in the case of fat-rich milk; at that point, new filters were used for the remaining volume of samples. Triplicates were prepared from the milk packets of the same brands. After complete filtration, filters were carefully transferred to petri dishes using metal tweezers and kept for drying in a hot air oven at 40°C. Dried samples were stored and subjected to further analysis.

Sample Analyses

Visual Characterization and Identification of Microplastics

The completely air-dried filters were observed under the Nikon epifluorescence microscope H6000L with 10×, 40× and 100× magnification with its inbuilt software for size and scale determination. Microplastics

were identified with reference to the microscopical instructions from Masura *et al.* (2015).[40] The observed objects were considered to be microplastics only after rubbing and pressing with metal tweezers, and those were not broken like organic materials. The identified microplastics were categorised based on colour, shape, and nature.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Microplastics were identified microscopically, though their chemical nature was not determined. So, the samples were further subjected to FTIR spectrometer analysis. All the samples were recorded in an absorbance range spectrum between 4,000-600 cm with a 4 cm^{-1} resolution and 16 scans. The acquired result spectra were compared with reference spectra of polystyrene, polyethylene, polypropylene, and also with polyacrylamide.

Estimated Per Capita Consumption of Microplastics

The per capita consumption of the microplastic particles was calculated using the litre of the milk sample and the average milk consumption rate of the persons.

The following equation is used to identify the number of microparticles per person and per day,

$$N_t = N_{MP} / L^* M_t$$

Where, N_t is the number of microparticles per person and per day,

N_{MP} is the number of microparticles and L is the litre of the milk of sample,

M_t is the litre of milk consumed per unit of time and per person (189 ml /d/person).

By using the excel student and the fisher test, the equality of group averages was measured.

Contamination prevention

During the analysis of milk samples, caution was taken to ensure the evasion of contamination of microplastics from laboratory conditions and that no airborne microparticles were contaminating milk samples. Sample extraction was performed inside a restricted area where no foot traffic is permitted. In particular, all the samples were kept inside the turned off fume hood throughout the filtration. Experimental research areas were maintained with quality standards like using 70% ethanol for cleaning work areas, containers, beakers and filtering units were rinsed with distilled water before conducting experiments, and new petri dishes were used for storing purposes. Moreover, MilliQ water was taken as a blank sample to show no microplastic contamination on the filter. Our results showed no self-contamination of microplastics under laboratory conditions.

RESULTS

The present study shows the occurrence of microparticles in all the branded milk samples analysed. This shows the prevalence of contamination in milk and consumption of MPs among the people of Tamil Nadu state, India.

Visual characterization and identification of microplastics

Blank filters were analysed before extraction. Microplastics like particles were found and photographed. They are shown in Figure 1 a, b. 13 brands of 16 samples were collected around the areas of Tamil Nadu state, India, and they were named Sample 1-16. Microscopic images of microplastics were shown in Figure 1. MPs were observed in all the branded milk samples. A complete characterization analysis is depicted in Table 1. The number of fibres in all the samples was between 48-159 and the pellet range was 19-82 as mentioned in Table 1. The microplastics were counted in all the milk samples and it was found that, Sample 15 was found to have the highest abundance of microplastics per litre, 427 ±2 MPs per litre. The total number of MPs in the milk samples ranges from 164-512. The lowest concentration was observed in sample 12 at about 164 ± 2 MPs per litre. Different shapes, like fibres, fragments, and pellets, were observed. The most prominent form of MPs observed was a fragment, and subsequently, fibres were also present.

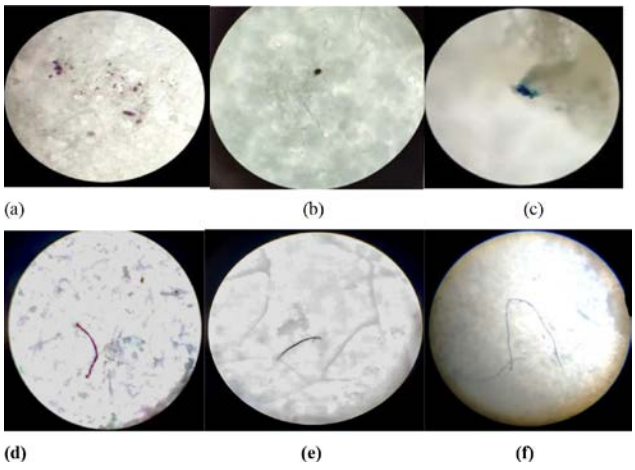

Figure 1: Microscopical photographs of Microplastics observed in branded milk samples. a–b Particles observed in blank filters. c-f images represent fibre particles. c- blue, d-pink, e-purple, f-blue.

Table 1: This table represent the number of microplastics found across the range of the sample per litre.

Milk Samples	Total Number of MPs Per Litre	Micro plastics Size % <500 µm	Fibre	Fragment	Pellet
SAMPLE-1	214	64	64	118	32
SAMPLE-2	296	76	71	183	42
SAMPLE-3	338	79	97	192	49
SAMPLE-4	425	93	127	226	72
SAMPLE-5	261	84	63	172	26
SAMPLE-6	324	96	84	198	42
SAMPLE-7	374	84	97	215	62
SAMPLE-8	207	67	56	107	44
SAMPLE-9	192	74	51	112	29
SAMPLE-10	348	89	92	203	53
SAMPLE-11	206	73	57	123	26
SAMPLE-12	164	67	48	97	19
SAMPLE-13	233	84	64	131	38
SAMPLE-14	295	79	73	173	49
SAMPLE-15	427	86	136	219	72
SAMPLE-16	512	91	159	271	82

Most of the observed MPs were blue in colour. Purple and pink fragments and fibres were also seen. The samples were further subjected to FTIR analysis.

Out of the samples analysed, the abundance of MPs varied from 150-400 MPs per litre. This is less when compared to MPs in other plastic packed food products. Milk contaminated with microplastic particles poses unknown risks to both adults and children. As per the result, the microparticle fragments have a dimension ranges from 97-271 and the fibres are in a range of 48-159.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR provides information on the basis of the chemical composition and physical state of the whole sample. The composition of MPs revealed the presence of polyacrylamide, polyethylene and polypropylene as the main contaminants. Figure 2a, b, and c denote the FTIR spectrum of MPs that corresponds to the chemical composition of PA, PE, and PP milk samples. From Table 2, the amount of polyethylene (PE) present in the sample is identified as 21 with a percentage of 3.75 percent, followed by polypropylene (PP), polyacrylamide (PA) and other particles as 37 (6.61%), 15 (2.67%) and

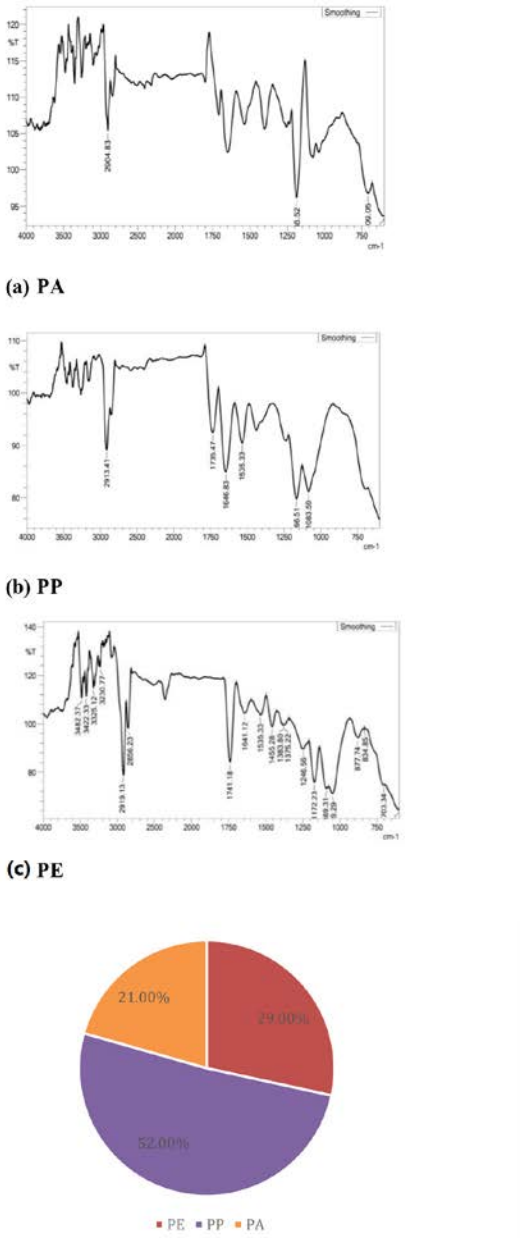

Figure 2a, b, c: Shows the FTIR spectrum of the microplastic particles in the Branded milk samples. The peak confirms the presence of polyethylene (PE), polyacrylamide (PA) and polypropylene (PP).

442 (78.9%) respectively. A total of 560 particles were examined. A maximum of 72 microplastics were found in the milk sample. The number of microplastics identified as polypropylene, polyethylene, or polyacrylamide in the filter sample is 51.

Estimation of consumption of microplastics

The per capita consumption of microplastic particles per person per day is calculated using the formula, $N_t = N_{MP} / m^*M_t$ The samples were chosen based on the number of MPs identified and the consumption

Table 2: Microparticles analysed with its relative proportion and distribution of Synthetic polymers found in branded milk samples (PP-polypropylene, PE-polyethylene, PA-polyacrylamide).

	Micro Particles (Number)	Micro Particles (%)
Polyethylene	21	3.75
Polypropylene	37	6.61
Poly-acrylamide	15	2.67
Other Particles	442	78.9
Total Particles analysed	560	
Microplastic Particles	72	12.8
Micro plastics identified as PP or PE or PA	51	70.8

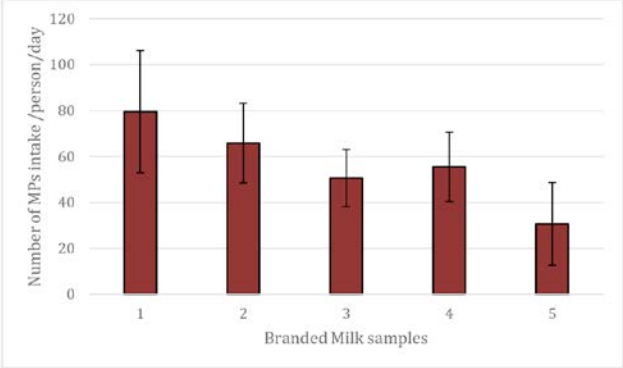

Figure 3: The chart describes the amount of intake of microplastic particles per person through milk samples in one day.

of milk brands in the state of west Tamil Nadu, India. An approximate prediction of the intake of MPs per person, per day has been made. The values are processed and the result is shown in Figure 3.

We have taken the five branded milk samples (sample 1, sample 2, sample 3, sample 4, sample 5) which is highly circulating in Tamil Nadu state. The number of MPs intaking per person per day ranges between 30.6 and 79.5. In samples 1, 2, 3, 4, and 5, the approximate number of MPs consumed per person/day is 30.6, 55.5, 50.6, 65.8, and 79.5, respectively.

DISCUSSION

The amount of microplastics identified in packed meat, salt, honey, and beer is relatively high when compared to the microplastics found in milk.[42-43] Different methods are adapted to extract microplastics, hence it is not easy to compare the number of microparticles in food products.[44-45] The size of the particles identified in this study is consistent with previous reports, but the amount of microplastics varies depending on the processing technology of the milk and nature of packaging materials.[23]

Different types of microplastics have been reported in various studies, with fibres being more prevalent in solid food products packed with LDPE, whereas fragments are more prevalent in this study than fibre, which possibly due to the liquid nature of milk.[6,8] The packaging material determines the colour of the microplastics in most cases; packaged meat contaminated with microplastics has the same colour as the packaging materials; as a result, colours (Blue, Purple, Pink) of microplastics were observed as a contaminant in this study.[2] FTIR spectra confirm the presence of synthetic polymers PP, PE and PA which coincides with previous studies.[39]

This global recognition of the scale and potential impacts of plastic pollution is resulting in increased calls for research by regional and national funding bodies. For example, Canada has recently stated its intentions to create a regulatory framework for plastics by declaring them to be a toxic substance.[46-47] Apart from microplastics, heavy metals are also identified in milk products, i.e., whey powder, as a toxic contaminant in Iran.[46] In the case of other food products, packaging influences the quality, so biological preservative Bacteriocin is used along with packaging film to extend the shelf life of food products.[47]

The greater number of atmospheric contributions to the contamination of microplastics in food products is clearly studied. Processing equipment and packaging materials are the main sources of contamination, whereas anthropogenic activities do not directly influence the amount of microplastics contamination. Identifying the key sources of contamination in milk is a tedious process. As such, processing of milk includes filtration units, the pipeline used to transfer, and sometimes the adulteration of water into the milk. This is a major source of contamination.[41] Microplastics can come from a variety of sources, including fragments of large pieces being released directly as tiny particles.[45] Our results coincide with the existing studies about the amount of microplastic contamination in accordance with the packaging materials.

CONCLUSION AND SUMMARY

While MP pollution research is rapidly progressing, it is still heavily skewed toward reducing the contamination of packaged foods.. There have been studies that have shown microplastics in several domestic food products,

however there are still many gaps in the knowledge. Our study confirms the presence of microplastics in branded milk samples from Tamil Nadu, India. Microscopic analysis demonstrated the presence of microplastics, and FTIR analyses showed the composition of microplastics were identified as polyethylene, poly propylene, and polyacrylamide. In this study, the amount of microplastics consumed per person per day has been estimated. This study's findings presented a description of microplastic contamination in milk and added vital information to the field's current research. Furthermore, the presence of microplastics in food products intended for human consumption necessitates the implementation of preventative measures to limit both direct and indirect releases along the supply chain. Plastics, on the other hand, cannot be completely eliminated; instead, they are broken down or recycled. the contamination of microplastics is an alarming event for the global environment as it increases the consumption of microplastics by humans.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MP: Microplastics; **PE:** polyethylene; **PP:** polypropylene; **PA:** polyacrylamide; **FTIR:** Fourier transform infrared spectroscopy, **LDPE:** Low Density Polyethylene.

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