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RESEARCH ARTICLE

Evaluation of Microplastic Presence in Yogurt Production Process

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Abstract: Plastics, which have made our lives easier since their invention and have found a wide range of applications because they offer numerous solution alternatives, are currently being investigated as a potential food safety risk. Microplastics (MPs) are defined as plastic waste particles smaller than 5 mm in size. Microplastics are commonly consumed orally, and their presence in various foods has been reported. The purpose of this study was to investigate the presence of MPs in yogurt production steps. The study's samples were drawn from a medium-sized national yogurt producing facility in İstanbul. Initially, samples were subjected to artificial digestion. They were subsequently filtered by a vacuum pump. Suspicious MP in the filters were examined with a binocular microscope and classified based on their size, color, and shape. Finally, SEM and ATR-FTIR techniques were utilized to characterize MPs. According to the results, the filters of twelve process steps/sampling locations contained a total of 171 microplastic particles. MPs were prevalent within the range of 20 to 580 particles L^{-1}. The concentration of MPs in raw milk and yogurt containers were found extremely high. To evaluate the level of risk associated with MP and to reduce MP contamination at plants of varying sizes, interdisciplinary research is required.

Keywords: *Food pollution, Microparticles, Microplastics, Polymer particles, Yogurt*

Yoğurt Üretim Sürecinde Mikroplastik Varlığının Değerlendirilmesi

Öz: Buluşlarından bu yana hayatımızı kolaylaştıran ve çok sayıda çözüm alternatifi sunduğu için geniş bir uygulama alanı bulan plastikler, günümüzde potansiyel bir gıda güvenliği riski olarak araştırılmaktadır. Mikroplastikler (MP'ler), boyutu 5 mm'den küçük plastik atık parçacıkları olarak tanımlanmaktadır. MP'ler genellikle ağız yoluyla organizmaya alınırlar ve çeşitli gıdalarda da bulundukları rapor edilmiştir. Bu çalışmanın amacı, yoğurt üretim basamaklarında mikroplastik partiküllerin varlığını araştırmaktır. Araştırmanın örnekleri İstanbul'da faaliyet gösteren orta ölçekli bir ulusal yoğurt üretim tesisinden alınmıştır. Toplanan örnekler ilk önce yapay sindirime tabi tutulmuştur. Daha sonra bir vakum pompası ile filtrasyon işlemi uygulanmıştır. Filtrelerdeki şüpheli MP'ler, binoküler mikroskopla incelenmiş, boyutlarına, renklerine ve şekillerine göre sınıflandırılmıştır. Son olarak, SEM ve ATR-FITR teknikleri kullanılarak MP'ler karakterize edilmiştir. Sonuçlara göre, on iki işlem aşamasının/örnekleme lokasyonunun filtrelerinin toplam 171 mikroplastik parçacık içerdiği tespit edilmiştir. Mikroplastikler, 20 ila 580 partikül L^{-1} aralığında bulunmuştur. Çiğ süt ve yoğurt kaplarındaki mikroplastik konsantrasyonunun son derece yüksek olduğu görülmüştür. Farklı ölçeklerde yoğurt üretimi yapan tesislerde MP risk seviyesini ortaya koyacak, MP kontaminasyonunu önleyecek veya asgari seviyeye indirebilecek tedbirlerle ilgili interdisipliner çalışmalar yapılması gereklidir.

Anahtar sözcükler: *Gıda kirliliği, Mikropartiküller, Mikroplastik, Polimer parçacıkları, Yoğurt*

INTRODUCTION

Plastics, which have made our lives easier since the day they were invented and have found a wide variety of applications because they offer abundant solution alternatives, are now being investigated as a potential food safety risk. Plastics have become a major source of problems for the environment, animal health, and human health, with their waste and residues spreading uncontrollably into the environment during their production, use, and disposal after use [1,2]. Microplastics (MPs) are defined as plastic waste particles smaller than 5 mm in size [2,3]. Based on their origin, MPs are classified as primary or secondary material. The secondary groups of MPs are microparticles that are the result of environmental degradation and whose base material is not MPs and that

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are subsequently formed by mechanical tearing, abrasion, and the fragmentation of larger plastic objects or related debris [4].

In scientific studies, the harmful effects of MPs on the human body have been reported. In addition to being a physical hazard, MPs can serve as carriers for other chemicals with known toxicity, such as environmental pollutants and plastic additives [5-7]. MPs have been shown to cross the placental barrier, which is known to be permeable to numerous toxic substances [6]. These dangers fall into four major categories: gastrointestinal toxicity, liver toxicity, neurotoxicity, and reproductive toxicity [2]. MPs also prepare the environment for biofilm formation and allow pathogenic microorganisms to enter the body, which is a significant potential threat [8].

Microplastics are commonly consumed orally, and their presence in various foods has been reported. Research has focused on foods of marine origin, including invertebrates, crustaceans and fish [9,10], microplastics have also been found in table salt [11,12]; sugar [13]; beer [12]; water [12,14-16], soft drinks [17], honey [13,18], and broilers [19,20]. There are two major microplastic sources. The granular raw material used to mold new plastic products and polymer microparticles added to cosmetics as exfoliants and abrasives are the primary sources. Microparticles formed by abrasion access environmental water systems, inevitably causing secondary pollution and increasing the dangers for humans [21]. The secondary source reflects the deterioration of larger plastics [22]. Numerous MPs, which are environmental contaminants of public concern, can accumulate in the food chain. Yogurt plays a significant role in global nutrition strategies and is recommended for all age groups. Moreover, yogurt is essential as the first recommended complementary food to be added to breast milk in the complementary feeding of infants after the sixth month [23].

The potential presence of MP contamination in yogurt production may pose a significant public health risk. The purpose of this study was to investigate the presence and the source of MP particles in yogurt production steps.

Material and Methods

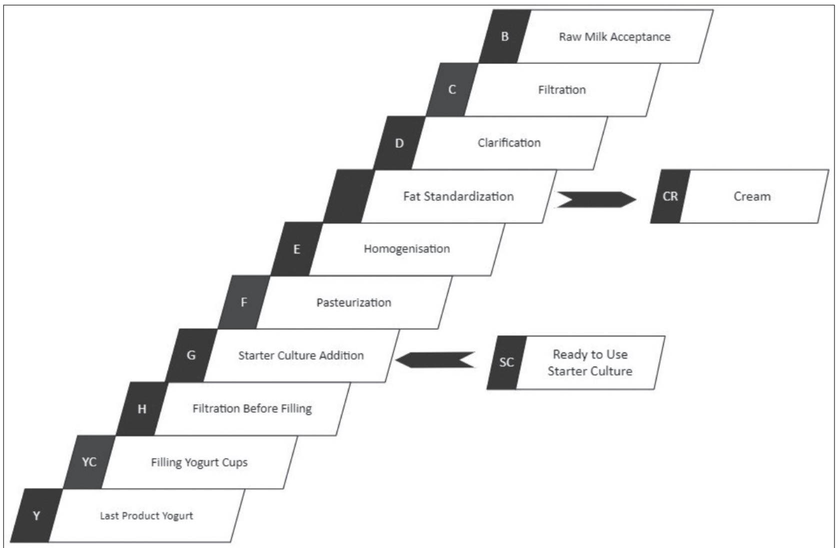
Sampling

The study's samples were drawn from a medium-sized national yogurt producing facility in İstanbul. In this facility, which routinely continues to produce "set type yogurt", process steps have been determined and critical control points and hazard types have been marked on the workflow chart according to the food safety management system applied in the enterprise. On a randomly selected production day, parallel sampling was performed at enterprise locations and equipment where MP contamination was expected during the process. Before accepting milk, the bulk tank's wall, which is the first point of contact with raw milk on the CIP (Clean-in-place) process band, was rinsed with MP-free ultra-pure water, and 100 mL of this water was obtained as a sample. 100 mL-g samples were taken from the phases of raw milk acceptance, filtration, clarification, separated cream, pasteurization, starter culture addition, filtration before filling, and ready-to-eat last product. Furthermore, samples were taken from the starter culture and the empty yogurt buckets, which were added to the process line later *(Fig. 1)*. A 100 mL sample of ready-to-use starter culture was taken for starter culture. The empty yogurt buckets were filled with 100 mL of MP-free water and brought to the laboratory with all of the sealed samples for analysis.

Analyse Safety

Plastic-free materials have been selected for all sampling and analysis consumables and equipment. The solutions used in the analysis, including ultrapure water, multi-

Fig 1. Process steps/sampling locations of yogurt production



enzymatic detergent, ethylenediaminetetraacetic acid disodium salt (EDTA), and tetramethylammonium hydrate, were analyzed for the presence of MP. Before use, empty sample bottles were rinsed with MP-free water and subjected to microscopic examination. All filtration processes were performed in a laminar flow cabinet to prevent MP contamination by airborne particles.

Positive control samples were prepared by adding Polypropylene (PP), Polystyrene (PS), polyethylene (PE), Polyvinyl Chloride (PVC), Linear low-density polyethylene (LLDPE), Thermoplastic elastomers (TPE) to ultrapure water were included in the study to control the analysis. Air and ultrapure water blank samples were examined under a microscope, and it was determined that there was no contamination caused by the analysis method.

Artificial Digestion

In our study, the methodology proposed by Costa Filho et al.[4], Kutralam-Muniasamy et al.[24], and Diaz-Basantes et al.[22] was implemented. Prior to analysis, each milk, cream, and yogurt sample were transferred to an erlenmeyer flask that had been cleaned with MP-free ultrapure water. Due to their density, yogurt and cream were difficult to filter through. In a glass bottle, 25 mL of a yogurt/cream sample was combined with 40 mL of MP-free ultrapure water by vigorously shaking. In contrast, milk samples did not require this step. The samples were then mixed for two minutes at 40°C after 2 mL of a multi-enzymatic detergent was added. Then, 10 mL of sodium ethylenediamine tetra acetate was added and stirred for an additional three minutes at a temperature of 40°C. Finally, 30 mL of tetramethylammonium hydrate was added, and the mixture was incubated at 40°C for 24 hours. After removing yogurt samples from the incubator, they were filtered immediately.

Sample Filtration

Every piece of filtration equipment was washed with MP-free ultrapure water before and after each sample filtration. Filtration was performed at a pressure of approximately 0.5 bar using glass microfiber filters (Whatman, Grade GF/B circles, 47 mm) with a pore size of 1 μm via a vacuum pump [12,13,15].

Microscopic Analysis and Visual Imagination

Filters were carefully transferred to glass petri dishes by using metal tweezers. They were air-dried at room temperature and subsequently stored for analysis. Filter papers were examined using a binocular biological microscope (Olympos CX31) with a camera (Canon A640) attached at a magnification of 4x. Filter papers were examined under the binocular biological microscope (Olympos CX31) with a camera (Canon A640) attached at magnification level 4x. Particles believed to be MPs were photographed using Kameram Software 1.3.0.8 (Mikrosistem, Turkey). The particles were measured and sorted by color, shape, and size using IC Measure (The Imaging Source®, 2.0.0.286, Germany).

SEM and FTIR Analysis

The morphological characterization and elemental composition of the particles that give a polymer image detected and marked by the light microscope were performed in high vacuum pressure mode and at different magnifications operating at an acceleration voltage of 10.00 kV in the secondary electron and backscattering modes using scanning electron microscopy (SEM, JSM-7001F, Jeol). The material chemical composition of microplastics was identified using FTIR spectroscopy (Agilent Cary 630). The results were evaluated using Agilent Polymer Handheld ATR Library, Agilent Elastomer Oring and Seal Handheld ATR Library and Agilent ATR General Library.

Calculation of Microplastic Ingestion by Humans

The recommended daily amount of yogurt (dairy product) for adults is three servings (1 serving = 240 mL)., while children, adolescents, pregnant-breastfeeding women, and postmenopausal women should consume two to four servings. These groups consume 720 mL and 480-960 mL, respectively, per day [23]. The total number of microplastic particles consumed orally is the basis for our risk assessment. This evaluation does not contain any toxicokinetic components.

$$\text{EDI} = \frac{\text{W x C}}{100}$$

EDI = Estimated daily intake of MPs with yogurt

W = Recommended amount of yogurt (mL/day)

C = Microplastic concentration (particle number/100 mL)

Statistical Evaluation

Descriptive statistics were used to summarize the characteristics (number of MPs, length of MPs) and distribution of the dataset by SPSS 21.0.

Results

In our research, glass microfiber filters were examined through a microscope. To prevent erroneous assessments, the optical properties of the glass fiber filters have been thoroughly examined. After this procedure, the evaluation of the filters commenced. The properties of microplastic particles, including their color, shape, and number, are detailed in *Table 1*. Except for the ready-to-use starter culture filtration, MP was detected in all of the observed filters. Examples of microscopic images of typical micro-

***Table 1.** MPs descriptive statistical data at all steps*			
Process Step/ Sampling Location	***Total MP Number/100 mL**	**MPs' Size Dispersion in Samples (μm)**	
		Range (mean±sd)	**Median**
A	2	179-1256 (717.50±761.55)	717.50
B	10	32-3697 (1248.60±1357.80)	913.50
C	8	68-4933 (1685.50±1950.76)	765.50
D	9	96-2533 (1036.33±734.70)	915.00
E	9	114-4994 (973.78±1563.28)	373
CR	9	103-956 (313.56±253.79)	239
F	13	36-3747 (1280.23±1263.22)	605
SC	0	-	-
G	16	9-2675 (380.38±653.42)	155
H	9	30-1688 (284.13±407.37)	152.50
YC	58	10-2913 (345.21±554.82)	128.50
Y	28	31-4946 (702.71±1200.11)	109

**MPs greater than 5000 μm are not evaluated.*
***A:** Bulk tank, **B:** Raw Milk Acceptance, **C:** Filtration, **D:** Clarification, **E:** Homogenisation, **CR:** Cream, **F:** Pasteurization, **SC:** Ready to Use Starter Culture, **G:** Starter Culture Addition, **H:** Filtration Before Filling, **YC:** Filling Yogurt Cups, **Y:** Last Product Yogurt*

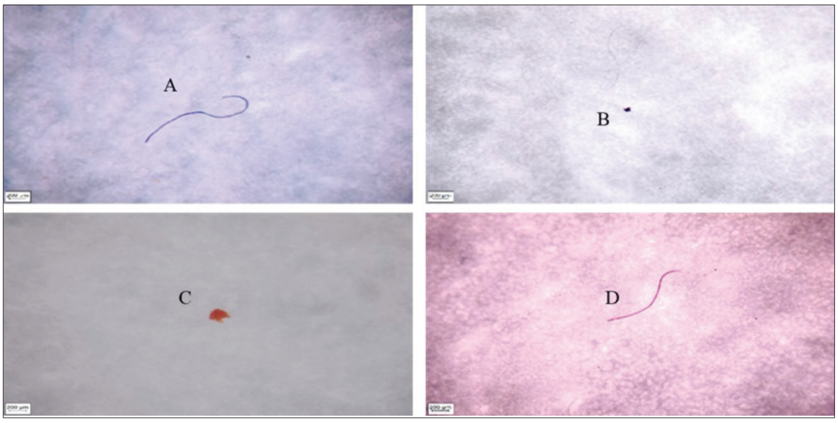


Fig 2. Image of microplastics in binocular biological microscope

A: Ethylene propylene fibre (1974 μm), **B:** Neoprene fragment (96 μm), **C:** Polytetrafluoroethylene fragment (275 μm), **D:** Polyacrilamide fibre (1394 μm)

plastics collected from process steps and sampling locations are shown in *Fig. 2*. The results of microplastic ratios based on colors, shapes and sizes are shown in *Table 1, Table 2, Fig. 3* and *Fig. 4*.

The filters of twelve process steps/sampling locations contained a total of 171 microplastic particles. In the range of 20 to 580 particles L^{-1}, microplastics were abundant. Example process step YC had the most microplastics in its filter (580 particles L^{-1}) and process step A contained 20 particles L^{-1} of microplastics at the lowest concentration.

Black, blue, brown, gray, green, orange, pink, red, purple, reddish brown, and transparent white were among the many hues exhibited by the microplastics described.

IC Measure software was used to determine the size distribution of microplastics, with fibres measured along their true length and parts measured along their longest dimensions. Of the total microplastics detected, 1-150 μm (43.27%) were dominated by microplastics *(Table 2)*. Using SEM, the surface morphologies of representative microplastics were observed, and the outcomes are depicted in *Fig. 5*.

Table 2. MPs size dispersion at all process step/sampling location

Process Step/ Sampling Location	MPs' Size Categorisation (µm)					
	1 - 10 n (%)	10.1 - 50 n (%)	50.1 - 150 n (%)	150.1 - 500 n (%)	500.1 - 1000 n (%)	1000.1 - 5000 n (%)
A	0	0	0	1 (50.00%)	0	1 (50.00%)
B	0	1 (10.00%)	3 (30.00%)	1 (10.00%)	0	5 (50.00%)
C	0	0	4 (50.00%)	0	0	4 (50.00%)
D	0	0	1 (11.11%)	1 (11.11%)	4 (44.44%)	3 (33.33%)
E	0	0	2 (22.22%)	4 (44.44%)	1 (11.11%)	2 (22.22%)
CR	0	0	1 (11.11%)	7 (77.77%)	1 (11.11%)	0
F	0	1 (7.69%)	2 (15.38%)	2 (15.38%)	2 (15.38%)	6 (46.15%)
SC	0	0	0	0	0	0
G	1 (6.25%)	2 (12.50%)	5 (31.25%)	5 (31.25%)	2 (12.50%)	1 (6.25%)
H	0	1 (11.11%)	3 (33.33%)	4 (44.44%)	0	1 (11.11%)
YC	1 (1.72%)	6 (10.34%)	24 (41.38%)	16 (27.59%)	6 (10.34%)	5 (8.62%)
Y	0	4 (14.29%)	12 (42.86%)	4 (14.29%)	3 (10.71%)	5 (17.86%)

***A:** Bulk tank, **B:** Raw Milk Acceptance, **C:** Filtration, **D:** Clarification, **E:** Homogenisation, **CR:** Cream, **F:** Pasteurization, **SC:** Ready to Use Starter Culture, **G:** Starter Culture Addition, **H:** Filtration Before Filling, **YC:** Filling Yogurt Cups, **Y:** Last Product Yogurt*

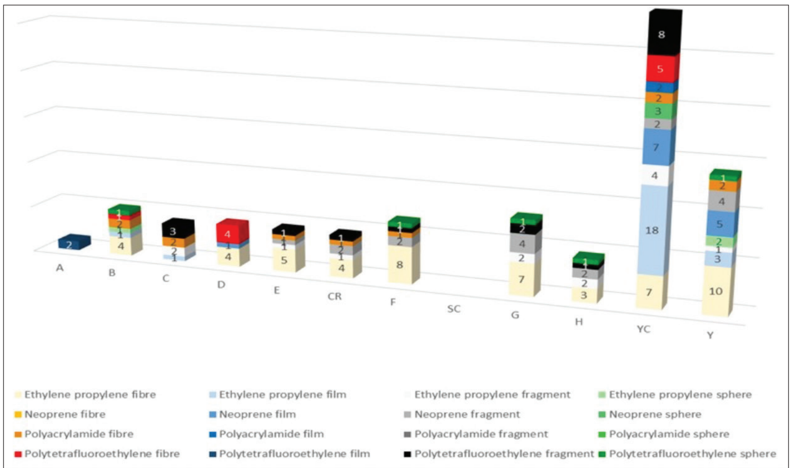


Fig 3. MPs shape and polymer type dispersion at all process step/sampling location (n)

The results of the ATR-FTIR analysis of the microplastic particles' chemical composition are depicted in *Fig. 6.* In the samples, four different types of microplastics were identified: ethylene propylene, neoprene, polyacrylamide, and polytetrafluoroethylene. Ethylene propylene was the most common type of microplastic found in the specimens.

Microplastic risk assessment in yogurt was calculated according to the consumption amounts recommended in Turkish Nutrition Guide [23]. Considering the age groups and important life stages, the consumption amounts given in the Turkish Nutrition Guide were evaluated as 201.60±14.40 MP ingestion/day in adult individuals, and children, adolescents, pregnant-breastfeeding women, and post-menopausal women 134.40±9.6 - 268.8±19.2 MP ingestion/day.

Discussion

In this study, the number, type, size, color, and shape of microplastics found in the yogurt manufacturing process were evaluated. Microplastic contamination may pose a

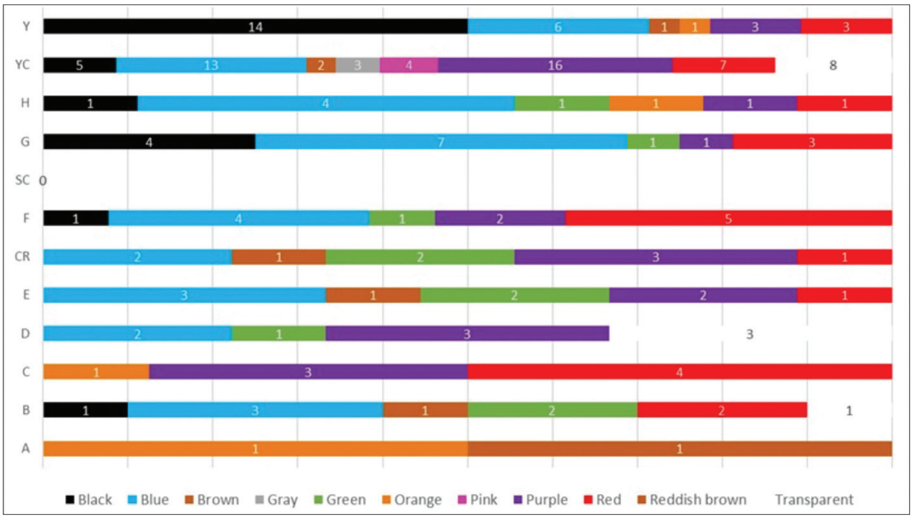
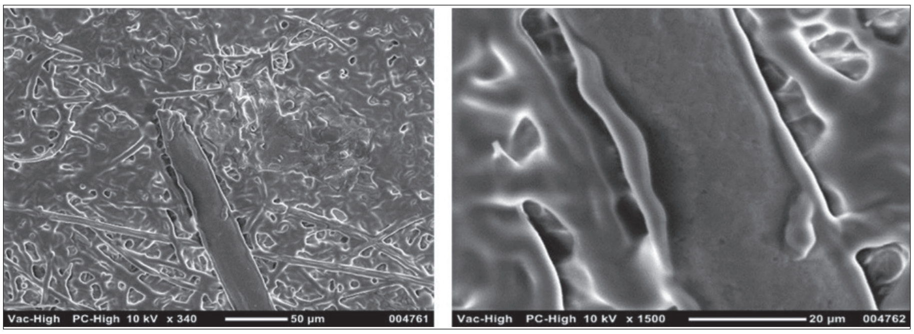


Fig 4. MPs color dispersion at all process step/sampling location (n)

Fig 5. SEM image of microplastics



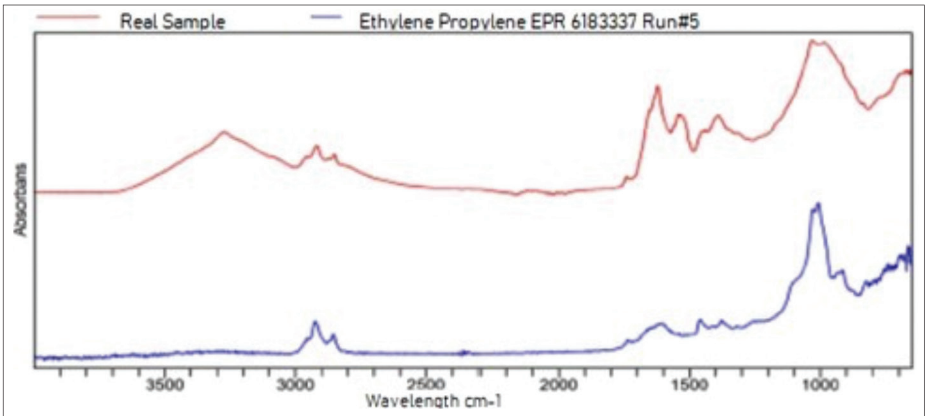


Fig 6. Identification of microplastic in ATR-FTIR

risk to human health, as demonstrated by the findings of our study indicating that microplastics are prevalent in the production process steps of yogurt. Despite the presence of microplastics at each process step and sampling location, the amounts of microplastics varied considerably between process steps *(Table 1)*.

The majority of analyzed samples (83%) contained an abundance of 80 particles L^{-1} microplastics; 20 particles of L^{-1} microplastic were found in one sample (8.33%); and microplastic could not be detected in one sample (8.33%). According to our research results, the concentration of microplastics in raw milk and yogurt containers was extremely high.

There were no comprehensive studies on the presence of microplastics in raw milk. However, when dairy cattle operations and milk logistics operations in Turkey are analyzed, numerous potential microplastic contamination risk points are anticipated. There are processes that must be regulated when a step-by-step backward flow chart is drawn from the moment milk is accepted by the enterprise. Plastic pipes and valves used to transport milk from tankers can be a significant source of contamination for MP. Tankers transporting milk from milk collection points to yogurt manufacturing facilities can be considered a risk factor. Water and detergent residues used to clean tankers, valves, and pipes may pose a risk to the MP load.

Moreover, rubber pipes themselves can be a source of contamination.

Refrigerated collection tanks at milk collection points, churns used to transport milk to milk collection points, milking buckets or automatic milking units with vibrating vacuum rubber-coated teat cups attached around the udder are additional potential contamination points. Cloths, gloves, plastic equipment, as well as detergents, disinfectants, and water used at every stage of cleaning, can contaminate plastic [25,26].

Another possibility is that MPs could be present in the udder and contaminate the milk. No study was found on this subject. Nevertheless, in mammals, plastics smaller than 0.1 µm can cross the blood-brain barrier and the placenta [27]; similarly, in a study with inhalation and translocation of MPs, 10 µm particles were reported to be absorbed from the alveolar epithelium [28,29].

The gaskets and filtration units located between the pipes through which the milk circulates in the enterprise are also the points that need to be checked. The increased permeability of filtration units over time could facilitate the migration of microplastics into dairy products. The milk accepted by the enterprise is ultrafiltered to reduce its microbial load and physical contamination and to prevent its transportation into yogurt [30,31]. Keeping organic materials, mineral substances, and colloids on the surface and/or pores of the filter can increase its pore size and its MP permeability if the milk filters are not cleaned and/or replaced on a regularly [32].

The microplastics' composition provides hints about their origin. Due to their hydrolytic stability, low and high pH stability, and excellent flow rates, sulfone family polymers are generally used as ultrafiltration and microfiltration membranes in the food and dairy industry [33-35]. Ethylene propylene, detected in our study, is used for air and water tightness in closed circuit systems, whereas neoprene is used as an oil tightness gasket and polyacrylamide is a polymer that is utilized in filtration processes [36]. Politetrafloroetilen (PTFE) are the most ubiquitous in the environment, and their presence in milk samples may be due to environmental contamination, the milking process (a series of macro, micro, and ultrafiltration using polymeric membranes), and packaging conditioning from farms to dairy processing facilities [22,24].

MP size determines the efficiency of uptake through the gastrointestinal, alveolar, and dermal epithelium. It has been reported that >90% of ingested MPs, especially those larger than 150 µm, are eliminated in the faeces. However, particles with a size range of 0.1-10 µm can cross the blood-brain barrier and placenta. Particles 150 µm can cross the gastrointestinal epithelium. Endocytosis allows particles 2.5 µm in size to enter the systemic circulation [37]. The risk assessment of MPs in food products should focus not only on the effects of MPs themselves, but also on the effects of the chemical pollutants that MPs absorb. MPs, which can adsorb the majority of pollutants such as bisphenol A, phthalates, and some brominated flame retardants, which are endocrine disruptors and can cause serious health problems, are able to absorb these contaminants [38].

In our study, we observed MPs of various hues, including black, blue, brown, gray, green, orange, pink, red, purple, reddish brown, and transparent white. While fibres are blue, green, red, pink, purple, brown, black, and transparent; fragments are blue, green, red, pink, orange, purple, brown, gray, black, and transparent white; films are black, blue, brown, gray, orange, pink, purple, red, and reddish brown; and spheres were found to be red, green, blue, black, and orange. Color is an essential feature for visually distinguishing the chemical composition of MPs [39]. In our study, FTIR analysis revealed that black and blue MPs were ethylene propylene and neoprene; brown, red, and reddish-brown MPs were PTFE and polyacrylamide; gray MPs were neoprene; green and purple MPs were ethylene propylene; and orange, pink, and clear white MPs were PTFE. It should not be forgotten that different color pigments can be added to polymer mixtures during the production of polymers. It should also be considered that the colors detected with a microscope may be the result of the color pigments and additives used in the manufacturing of plastic packaging [40]. Consequently, FTIR verification is necessary for a conclusive diagnosis.

A risk assessment was carried out by considering the data on ready-to-eat yogurt, which is the final product of the process. The risk assessment for microplastics provides the total number of microplastics consumed. It does not indicate how much of the microplastics ingested are excreted in feaces. It excludes the rate of translocation from the intestinal epithelium [41]. It may be subject to MP absorption of particles smaller than 1.5 µm; it has been reported that larger particles can be taken into the organism via endocytosis and phagocytosis [37,41-43]. This study evaluated the physical destruction of microplastics within the body. It excludes the microbial and toxicological risks adsorbing to the plastic's content or surface. Regarding the potential toxic effects of MPs on humans, little is known. MPs 20 µm in size can reportedly penetrate biological membranes, accumulate in tissues, cause cytotoxicity, and elicit immune responses when inhaled or ingested [37].

In our study, according to Turkey Nutrition Guide [23], considering the yogurt consumption of adults and children, adolescents, pregnant-breastfeeding women, post-menopausal women, the number of microplastic particles that could be included in the evaluation groups was found to be approximately 73.500-98.000, respectively.

Considering these results, it is thought that the risk should be evaluated carefully. Cox et al.[44] estimated the total intake of MPs utilizing 402 data sets from 26 studies. According to the authors, the annual microplastics consumption ranges from 74.000 to 121.000 particles, depending on the person's age and size. The annual MP intake per capita is estimated to range from 39.000 to 52.000 items, including 37-1.000 from sea salt, 4.000 from tap water, and 11.000 from shellfish.

The average daily consumption of PP MPs by infants is estimated to be 1.580.000 particles per capita in the range of 14.600-4.550.000 particles, depending on the region. The average value corresponds to approximately 3000 times the total adult consumption of MPs from water, food and air (up to 600 particles per day for adults) [45].

In conclusion, analyses were conducted on raw materials, semi-finished materials, finished products, intermediate products, and starter cultures gathered from the production line of a national medium-sized yogurt manufacturer. The results indicated the microplastic particle source of the collected samples. This study's findings may provide a clear indication of the contamination risk associated with this product. When investigating microplastic concentrations in yogurt and other dairy products, it is necessary to collect additional information on the contamination of raw milk with plastic residues. Microplastic contamination in yogurt buckets is quite severe. Before filling, precautions must be taken to prevent contamination. Considering the potential health risks posed by microplastics, food research must be intensified.

Availability of Data and Materials

The datasets and analyzed during the current study available from the corresponding author (K. Muratoglu) on reasonable request.

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Competing Interests

The authors declared that there is no competing interests.

Ethical Approval

N/A

Authors Contributions

SRZ, KM, and SKB planned and designed the experiment. SRZ collected the samples and performed the experiment. KM and SKB analyzed the data, wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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