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RESEARCH ARTICLE

Microplastic contamination of packaged spirulina products

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Abstract

Microplastic (MP) contamination in commercially sold spirulina products has not been previously investigated. In this study, 29 spirulina samples in various packaging types were purchased from different brands and origins to assess the presence of MPs. Microplastic analysis was conducted using microscopic and μ-Raman techniques. To ascertain whether the content is indeed spirulina and make a comparison with the MP level, C-Phycocyanin levels were also analyzed. A total of 251 MP-like particles were observed. Out of the 29 examined packaged spirulina brands, 26 showed potential MPs upon visual inspection, with 35 particles confirmed as MPs (73% of the analyzed particles). The mean abundance of MPs was estimated at 13.77 ± 2.45 MPs/100 g dw. Powdered spirulina had a higher but not statistically significant MP abundance (17.34 ± 4.22 MPs/100 g dw) compared to capsule/tablet forms (10.43 ± 2.45 MPs/100 g dw). Fragments accounted for 38.3% while fibers constituted 61.7% of the identified MPs, with sizes ranging from 0.07 to 2.15 mm for fragments and 0.19 to 5.691 mm for fibers. The color distribution of MPs in spirulina samples was predominantly blue (52.8%), followed by black (25.4%), white (10.9%), and others (10.9%). Ten synthetic polymers and cellulose were identified through μ-Raman analysis, with polypropylene (31.6%) and polystyrene (8.3%) being the most prevalent. The correlation between C-Phycocyanin and MPs concentrations, was not found statistically significant. The abundance and composition of MPs were found to be influenced by packaging and processing stages. Identifying potential sources of MPs in spirulina products and evaluating their risks to human health is crucial.

Keywords Plastic pollution · *Spirulina* · Food contamination · Food packaging · Food safety

Introduction

Global plastic production has experienced continuous growth, reaching approximately 390 million tons in 2021 (PlasticEurope 2022). This extensive production has made plastics a ubiquitous environmental pollutant, posing a significant threat to various organisms. The long-lasting nature of plastic exacerbates its negative environmental and economic consequences, further compounding the issue. Plastic contaminants can be categorized based on size, with megaplastic (> 100 mm), macroplastic (> 20 mm), mesoplastic (20–5 mm), microplastic (5 mm–1 μm), and nanoplastics (< 1 μm) forming different types of plastic pollutants (Masura et al. 2015; Gesamp 2019). The global concern regarding microplastics (MPs) has been increasing due to their emergence as environmental pollutants (Mihai et al. 2022). Research conducted in recent years has revealed the widespread distribution of MPs in various environments, including sea surface water, water columns, marine sediments, lakes, rivers, polar glaciers, soil, groundwater and the

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Highlights
- Packaged spirulina were estimated to have an average 13.77 ± 2.45 MPs/100 g.
- Powdered spirulina had a higher MP abundance compared to capsule/tablet forms.
- Fragments accounted for 38.3% while fibers constituted 61.7%.
- Ten polymers and cellulose were identified, and PP and PS was the most prevalent.

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atmosphere (Aytan et al. 2016; Güven et al. 2017; Gündoğdu et al. 2023a, b; Nava et al. 2023).

MPs in various media are widespread at the entry points to the food chain (Akoueson et al., 2020). Aquatic food products, such as table salt (Gündoğdu 2018; Masiá et al. 2022), fish (Blankson et al. 2022; Menéndez et al. 2022), mussels (Gedik and Eryaşar 2020), jellifying algae (Bilbao-Kareaga et al. 2023) and seaweeds (Li et al. 2020), serve as the primary pathway for MPs to enter the food chain. Furthermore, Kedzierski et al. (2020) examined the contamination of meat with MPs through its packaging, while Mason et al. (2018) investigated the release of MPs from bottled water. Hernandez et al. (2019) conducted a study on the impact of temperature on the release of microplastics into beverages, and Li et al. (2020) demonstrated the presence of MP contamination in Nori seaweed. Nonetheless, MPs can penetrate the bodies of various organisms, notably humans, via direct consumption (Kashfi et al. 2022), leakage from packaging into food (Sobhani et al. 2020), and even exposure via plastic cutting boards (Habib et al. 2022). In the context of food safety, the direct contact between food and plastic packaging requires attention (Fadare et al. 2020). The primary cause of MPs from plastic take-out containers is their exposure to the atmosphere during food production and MPs leachion out from the container's interior due to slight mechanical agitation, structural fragility, and rough surfaces (Du et al. 2020). It is crucial to account for containers pollution by airborne MPs during the manufacturing process, which are expected to endure when consuming takeaway food (Jadhav et al. 2021). Du et al. (2020) estimated that on average, individuals who consume take-out food unwittingly ingest approximately 2977 MPs each year. Additionally, those who consume plastic-packaged food 4–7 times per week may be exposed to 12–203 MPs each week that are released from the containers (Du et al. 2020).

The widespread use of plastic in packaging further amplifies the risk of MP exposure, as MPs can migrate from the packaging to food. Materials such as plastic-coated aluminium or paper, composite papers with multiple layers, and plastic are commonly used for food preservation, including packaging of seafood.

Packaged aquatic foods, primarily associated with fish and invertebrates, have gradually increased production capacity over time. This includes the cultivation of seaweed and algae, albeit to a lesser extent. Seaweed, in particular, has become a promising product in the seafood industry, with the global commercial seaweed market projected to grow from $15.01 billion in 2021 to $24.92 billion in 2028 (Vintage Market Research 2023). The brown, green, and red seaweed production reached 35.5 million tonnes in 2019, with steady growth since 2015. Asia dominates seaweed production, accounting for approximately 97% of the world's seafood production (Vintage Market Research 2023). Among the algae, spirulina stands out as an important species and has a significant share in the seaweed/algae market.

Spirulina, a type of microalgae, is the oldest living plant on Earth, dating back approximately 3.6 billion years. It played a vital role in creating the oxygen atmosphere necessary for life. Spirulina, specifically the cyanobacterial speciesspirulina is grown for its high protein content. Recognized as one of the most nutritious and concentrated foods, spirulina contains antioxidants, phytonutrients, probiotics, and nutraceuticals. It has gained popularity for its therapeutic uses and has been declared the best food for the future by the United Nations (Fernandes et al. 2023; Markou et al. 2023).

The spirulina market is expected to reach $1.10 billion by 2030, with a compound annual growth rate (CAGR) of 9.4% from 2023 to 2030 (Vintage Market Research, 2023). The increasing demand for health and wellness products, dietary supplements, natural food colors, vegetarianism, and the environmental impact of spirulina cultivation are driving market growth (FAO 2021; Vintage Market Research 2023). Strict regulations on synthetic colors and flavors, the preference for spirulina-based products, its use in aquaculture, and investments from natural-color manufacturers are additional factors contributing to market expansion (Vintage Market Research 2023).

Moreover, new applications and the demand for C-Phycocyanin (a pigment derived from spirulina), spirulina from bio-refineries, and fresh/frozen spirulina are expected to create significant opportunities for market growth (Thevarajah et al. 2022). In terms of product type, powdered spirulina is projected to dominate the market in 2023, driven by its use in nutraceuticals, food and beverage products, vegan diets, and cosmetic formulations. The business channel segment is expected to hold the largest market share in 2023, as manufacturers purchase bulk spirulina directly for further product development in various industries such as nutraceuticals, food and beverages, and animal feed (Vintage Market Research 2023).

Humans consume spirulina as fresh, frozen, dry powder, capsules and tablets. All of these types sold in the packages. spirulina products is commonly packaged in plastic packages made of polyethylene (PE). The commercial forms of spirulina being sold in plastic packaging can be one of the sources of MP contamination. Additionally, if the water in the systems used for spirulina cultivation is not filtered for MPs and similar particles before use, the harvested spirulina can also be contaminated with MPs. Kosuth et al. (2018) reported that almost all (81%) of the tap water samples they sampled worldwide had anthropogenic particulate contamination well above the background levels detected in deionised blanks. Therefore, it is probable that water intended for

spirulina cultivation and not subjected to special filtration also contains MP. MPs can contaminate the commercially sold spirulina during processing and packaging as well (EFSA 2016). Compared to naturally produced foods, processed foods are more prone to contamination with MPs during food production and packaging processes (Kutralam-Muniasamy et al. 2020; Kwon et al. 2020; Li et al. 2020). Furthermore, MPs can contaminate the respective product when plastic packaging is opened by consumers, regardless of the method used (Sobhani et al. 2020).

To the best of our knowledge, no study has been conducted on the presence of MPs in commercially available spirulina products. Due to the lack of specific precautions against MP contamination during both production and packaging of spirulina products, and the use of plastic materials in the production processes, the potential for MP contamination is highly plausible. In order to test this hypothesis, a range of branded and sourced capsule/tablet or powder-form spirulina products, packaged in various types of containers, were examined to investigate contamination with MPs. This study represents the first investigation into MP contamination in commercially available packaged spirulina products.

Material and methods

Collection of samples

In this study, 29 spirulina samples (powdered and capsule/tablet) sold in commercially available packaging made from various materials (including glass, rubber, paper-coated plastic, etc.) were purchased in May 2022 (Table 1). Emphasis was placed on obtaining samples from well-known brands and commonly used packaging formats. Details of the samples, including brand, packaging type, weight and product type, were recorded and presented in Table 1. It has been determined that 14 of the sampled packaged spirulina brands are in powder form, and 15 of them are sold in capsule/tablet form. Additionally, it has been found that the packaging used includes ziplock plastic, plastic-capped glass, aluminum-capped glass, plastic bottle, or plastic bag packaging (Table 1).

C-Phycocyanin analysis

C-Phycocyanin is commonly used as an indicator to assess the quality of spirulina products and to verify their authenticity. In this study, the analysis of C-Phycocyanin was conducted to determine whether the products were indeed spirulina and to understand the correlation between C-Phycocyanin levels and the concentration of MPs. This correlation was investigated to address the question of whether there is a relationship between the authenticity of spirulina products and the presence of MPs. Selling products as spirulina when they are not genuine poses risks to food safety and also raises the possibility of containing various contaminants. C-Phycocyanin is the blue colored pigment obtained from *Spirulina platensis* is an excellent source of C-Phycocyanin in this regard. C-Phycocyanin can constitute 20% of the dry weight of spirulina (Jaouen et al. 1999; Vonshak 1997). Due to its C-Phycocyanin properties, it is widely used in the food, cosmetics and pharmaceutical industries (Herrera et al. 1989; Silveira et al. 2007). In addition, it is used in these areas due to its antioxidant and anti-cancer properties (Romay et al. 2003; Eriksen 2008).

For C-Phycocyanin analysis, 100 ml of $NaNO_3$ ($15gNaNO_3/L$) is added to 1 g of sample. It is kept in the shaker for 2 h. 5 ml of the samples are taken and centrifuged at 3500–5000 rpm for 5 min. The supernatant is read in the spectrophotometer at 620 nm. The amount of C-Phycocyanin (µg/ml) is calculated from the readings using the equation below (Boussiba and Richmond 1979).

$$\mathrm{C-Phycocyanin}(\mu g/mL) = OD(620nm) \times 137$$

where; OD is optical density; 137 is a factor determined from absorption coefficient for spirulina (Boussiba and Richmond 1979).

MP extraction

The extraction of MPs from packaged spirulina was carried out following the methodology outlined by Gündoğdu and Köşker (2023). Initially, 50 g of sample (in both type) were weighed from each package, and placed in a clean beaker. However, in some brands, the total sample weight was less than 50 g, so the entire sample was used for analysis in those cases. A solution consisting of 30% KOH and NaClO was prepared for the digestion of organic material. This solution was made by combining 700 mL of Milli-Q water (Millipore, Bedford, MA, USA), 150 mL of saturated KOH solution (1,120 g/L), and 150 mL of NaClO with 14% active chlorine (Gündoğdu and Köşker 2023). Subsequently, 250 mL of this prepared solution was added to each beaker containing the samples. The beakers were again covered with aluminum foil and kept at a temperature of 60 °C for one week to allow complete digestion of the organic materials. Once all the organic material was dissolved, the solution was transferred to a separation funnel, and 500 mL of NaI solution (5 M, density of 1.6 g/mL) was added to the samples. After waiting for one day to allow for density separation, any settled material was removed. The supernatant was then transferred to a separate sterile beaker and filtered through a filter paper

Table 1 Information about collected *Spirulina* products from the Turkish market

Sample No	Brand code	Type of package	Weight	Type of product	Weight of analyzed sample	Notes
1	S1	Ziploc Craft	50 g	Powder	Whole Package	
2	S2	Glass with Aluminum cap	40,2 g	Capsule	Whole Package	
3	S3	Glass with Aluminum cap	45 g	Capsule	Whole Package	
4	S4	Ziploc plastic	100 g	Powder	50 g	Moisture Absorbers was in the package
5	S5	Plastic bottle with plastic cap	63 g	Capsule	50 g	Cotton and Moisture Absorbers were in the package
6	S6	Glass with Aluminum cap	80 g	Powder	50 g	Moisture Absorbers was in the package
7	S7	Plastic bottle with plastic cap	74 g	Capsule	50 g	Moisture Absorbers was in the package
8	S8	Ziploc plastic	50 g	Powder	Whole Package	
9	S9	Glass with Plastic cap	39,6 g	Capsule	Whole Package	Moisture Absorbers was in the package
10	S10	Plastic	50 g	Powder	Whole Package	
11	S11	Glass with Plastic cap	40 g	Capsule	Whole Package	Moisture Absorbers was in the package
12	S12	Plastic bottle with plastic cap	54 g	Capsule	Whole Package	
13	S13	Ziploc Plastic	100 g	Powder	50 g	
14	S14	Glass with Aluminum cap	59,4 g	Capsule	Whole Package	Cotton was in the package
15	S15	Ziploc Craft	85 g	Powder	50 g	
16	S16	Glass with Aluminum cap	100 g	Capsule	50 g	Moisture Absorbers was in the package
17	S17	Ziploc Craft	100 g	Powder	50 g	
18	S18	Plastic	50 g	Powder	Whole Package	
19	S19	Plastic bottle with plastic cap	100 g	Powder	50 g	
20	S20	Glass with Aluminum cap	44,4 g	Capsule	50 g	Moisture Absorbers was in the package
21	S21	Ziploc plastic	100 g	Powder	50 g	
22	S22	Plastic bottle with plastic cap	30 g	Capsule	Whole Package	
23	S23	Plastic bottle with plastic cap	36 g	Capsule	Whole Package	Moisture Absorbers was in the package
24	S24	Glass with Aluminum cap	43,2 g	Capsule	Whole Package	
25	S25	Glass with Aluminum cap	60 g	Capsule	50 g	Moisture Absorbers was in the package
26	S26	Plastic	50 g	Powder	Whole Package	
27	S27	Plastic with Aluminum cap	35 g	Capsule	Whole Package	
28	S28	Ziploc plastic	100 g	Powder	50 g	
29	S29	Ziploc plastic	100 g	Powder	50 g	

(single packaged and free from particles) with a pore size of 0.45 µm. The filter papers containing the particles were placed in clean petri dishes and set aside for microscopic and spectroscopic analyses.

To examine the shapes (fiber/filament and fragment) and colors of the MP-like particles, a camera (EOS 450D, Canon Co., Tokyo, Japan) was used in conjunction with a stereo microscope (SZX16, Olympus Co., Tokyo, Japan). The size of the MPs was analyzed using ImageJ v1.52 s software (http://imagej.nih.gov/ij).

µ-Raman analysis

The MP-like particles were examined using a confocal Raman microscopy system (inVia Qontor, Renishaw, Gloucestershire, UK) equipped with 532 nm and 785 nm lasers. The particles were focused at 50 × magnification using a Leica microscope, and two accumulation scans of 10 s each were taken with a variable grating setting between 600 l/mm and 1,200 l/mm, and a spectrum width of 300–3,200. The obtained spectra were compared to the

ST-Japan MPs Library, and a match of 70% or higher was considered as the basis for determining the polymer type (Gündoğdu and Köşker 2023; Song et al. 2021).

QA/QC procedure

The method that Gwinnett and Miller (2021) recommended was implemented to minimize procedural contamination. These methods included wearing non-synthetic polymer clothing, cleaning all equipment and surfaces prior to use, avoiding the use of plastic equipment, and working in controlled air environments (Gwinnett and Miller 2021). Additionally, controls and blanks were taken during the sampling stage to assess contamination levels. In this study, the aforementioned preventive and measurement methods were followed, as Gwinnett and Miller (2021) mentioned. To avoid potential contamination, all equipment was thoroughly washed three times with microfiltered water and subjected to an acetone bath before and after (Beer et al. 2018). The equipment was stored in a closed cabinet throughout the study. All solutions used were filtered using GF/C Whatman filter paper with a pore size of 1.2 µm prior to use. All analyses were performed inside a closed laminar flow cabinet (Class-4, Esco Technologies Inc.), and aluminum foil was used to cover the equipment during all procedures. A control group was prepared in triplicate to detect any potential contamination. The same procedures applied to the sample analysis were also carried out for the control group with three replicates.

Statistical analysis

The MP level was reported as MPs/100 g dw. In order to assess whether the number of MPs followed a normal distribution, the Kolmogorov–Smirnov and Shapiro–Wilk tests were utilized. If necessary, a logarithmic transformation was applied as an appropriate data transformation method. To determine the difference in MP levels among different product type, an independent sample t-test were applied. Furthermore, a Pearson correlation analysis was performed to examine the relationship between the C-Phycocyanin level of the samples and the abundance of MPs. All statistical analyses were carried out using the SPSS v22 (IBM Co., Armonk, NY, USA) and Tableau v10.2 (Tableau Software LLC., Mountain View, CA, USA), with a significance level set at $p < 0.05$.

Results

As a result of visual counting of MPs, a total of 251 particles were counted. This number was then corrected using the numbers and ratios obtained by both control and µ-Raman analysis, and the overall MP concentration was recalculated. In one of the three petri dish replicates designated as the control group, a blue-colored fiber-like particle was detected. This suggests the presence of background contamination at a rate of 0.33 MPs/sample resulting from the working environment and analysis procedures. Consequently, these numbers were subtracted from the overall mean.

Out of the 29 examined packaged spirulina brands, 26 showed potential MPs upon visual inspection. Subsequently, all filters underwent µ-Raman analysis to confirm the previously detected MPs and identify the types of polymers. During this analysis, 48 randomly selected particles were examined, and 35 were confirmed as MPs (accounting for 73% of the analyzed particles). This percentage was then utilized as a correction factor for the total particle count (Table 2). After excluding the verified non-MP particles, the mean abundance was estimated at 13.77 ± 2.45 MPs /100 g dw (Table 2).

Based on the product type, the MPs were determined as 17.34 ± 4.22 MPs/100 g dw in powder form and 10.43 ± 2.45 MPs/100 g dw in capsule form. Despite the apparent difference in MP concentration, no statistically significant differences were found between different product types (t-test, $p > 0.05$). In terms of particle shape, the distribution of fragments accounted for 38.3% while fibers constituted 61.7% (Fig. 1 upper panel). The size of the particles varied, ranging from 0.07 to 2.15 mm for the fragments and from 0.19 to 5.69 mm for the fibers (Fig. 1, lower panel; Data Appendix). Analyzing the color of MPs in spirulina samples, the percentages were as follows: blue (52.8%), black (25.4%), white (10.9%), and others (10.9%) (Fig. 1 middle panel).

During the microscopic examination, a total of 251 MP-like particles were counted, including replicates. Out of these, 48 randomly selected particles were subjected to µ-Raman analysis, which accounted for approximately 19% of the total particles. Using µ-Raman analysis, 10 synthetic polymers and cellulose were identified and documented in Table 3. The most prevalent polymers found were polypropylene (PP) (31.6%), polystyrene (PS) (8.3%), followed by PE (8.3%), and polyester (PES) (7.8%) (Fig. 2). C-phycocyanin levels of the samples were presented at Fig. 3, Table 4 and Data Appendix.

The samples varied in both color and quantity. Based on the results, we found that brand 28 had the highest C-Phycocyanin content, with a percentage of 3.49, following brand 24 (3.44%), brand 9 (3.43%), and brand 22 (3.41%) respectively. On the contrary, brand 29 had a very low C-Phycocyanin content, nearly non-existent at 0.18%.

Based on the Pearson correlation analysis conducted to determine the correlation between C-Phycocyanin and MPs concentrations, a negative but statistically not significant relationship between the two variables has been identified ($p > 0.05$, $r = -0.11$).

Table 2 The mean and the corrected mean concentration of MPs per 100 g of spirulina sample and C-Phycocyanin levels according to brand and product type

Type of product	Brand	C-Phycocyanin (µg/mL)	MPs count	Corrected MPs in the weighted sample	Weight of the sample	Corrected MPs in 100 g
Capsule	2	289.41	5	3.41	40.2	8.5
	3	125.01	6	4.13	45	9.2
	5	191.39	6	4.13	50	8.3
	7	114.81	1	0.49	50	1.0
	9	343.25	8	5.59	39.6	14.1
	11	191.66	17	12.16	40	30.4
	12	337.23	9	6.32	54	11.7
	14	83.50	2	1.22	59.4	2.1
	16	239.41	7	4.86	50	9.7
	20	338.12	0	0.00	44.4	0.0
	22	341.68	11	7.78	30	25.9
	23	203.65	0	0.00	36	0.0
	24	344.08	12	8.51	43.2	19.7
	25	98.02	0	0.00	50	0.0
	27	336.54	8	5.59	35	16.0
	Mean	238.52	6.13	4.28	44.45	10.43
Powder	1	166.59	7	4.86	50	9.7
	4	202.28	2	1.22	50	2.4
	6	227.56	11	7.78	50	15.6
	8	256.74	2	1.22	5	24.4
	10	248.04	3	1.95	50	3.9
	13	339.28	8	5.59	50	11.2
	15	303.25	5	3.41	50	6.8
	17	137.27	8	5.59	50	11.2
	18	206.05	13	9.24	50	18.5
	19	328.46	7	4.86	50	9.7
	21	162.48	14	9.97	50	19.9
	26	328.73	24	17.26	50	34.5
	28	349.76	8	5.59	50	11.2
	29	18.08	44	31.84	50	63.7
	Mean	233.90	11.14	7.88	46.79	17.34
Total Mean		236.29	8.55	6.02	45.58	13.77

Discussion

Spirulina, a microalgae of great commercial importance, is cultivated worldwide (Ahda et al. 2023). While it thrives in hot climates, it can also be grown in colder regions. Spirulina production commonly occurs in pools and photobioreactor systems (Lupatini et al. 2017). Open system production is typically carried out in plastic pools and ponds (Ragaza et al. 2020). Unfortunately, the chemicals utilized during production often have low purity, negatively impacting all aspects of cultivation (Soni et al. 2017). The use of such chemicals not only compromises product quality but can also introduces various types of plastics (such as plastic sacks and boxes) into the production systems. Consequently, these plastics find their way into the culture along with the chemicals. Additionally, harvesting methods employed in underdeveloped countries often involve rudimentary systems that utilize rags with specific mesh openings or nylon sieves. Consequently, plastic materials can contaminate the harvested product (Chen et al. 2021). Furthermore, some countries or businesses opt for sun drying, utilizing materials like cloth or plastic, which can also introduce plastics into the product. Airborne MPs can contaminate the drying process during sun exposure (Wu et al. 2023). Lastly, due to its affordability, the practice of storing dried products in plastic bags further suggests the potential for plastic contamination.

In this study, we examined the presence of MP contamination in a selection of 29 commercially packaged spirulina brands. Out of the samples analyzed, MPs were detected in 26 of them (89.6%). Specifically, all spirulina products sold as powder ($n = 14$) contained MPs, while

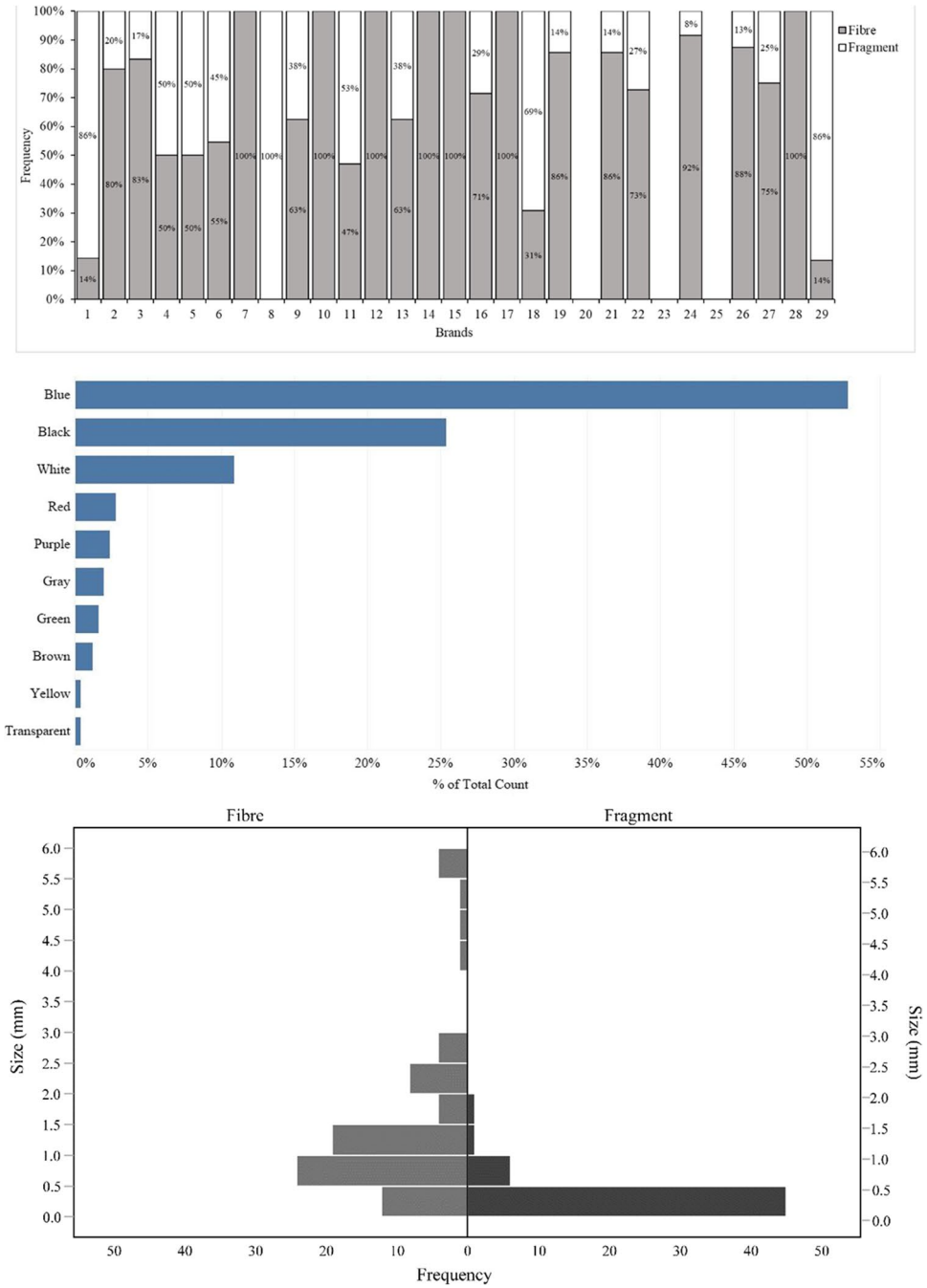


Fig. 1 Shapes (upper panel), color (middle panel), and size distribution (lower panel) of identified MPs in spirulina samples

80% ($n = 13$) of the products sold in tablet or capsule form ($n = 15$) were found to be contaminated as well. The detection rate of MPs is lower compared to what has been reported in seaweed nori (95.8%) (Li et al. 2020), canned fish (100%) (Gündoğdu and Köşker 2023), commercial salts (94.1%) (Karami et al. 2017), and table salts (100%) (Gündoğdu 2018). However, it is higher than the rates reported for canned sprats and sardines (20%) (Karami et al. 2018). These differences can be attributed to variations in analytical methods, food sources, and food processing procedures.

The purchased samples examined in this study were in two different forms: capsule/tablet and powder. Statistical analysis did not reveal a significant difference in the number of MPs between the two product types ($p > 0.05$). However, despite the lack of statistical significance, the observed variations in the presence and quantity of MPs may be attributed to food processing procedures, growing conditions, and the packaging methods employed. Commercial cultivation of spirulina is typically conducted in transparent plastic tubes or shallow synthetic ponds with mechanical impellers for culture mixing (Pulz 2001). The ponds are typically

Table 3 The chemical composition of particles

Shape	Polymer	Percentage
Fiber	Cellulose	2.08%
	NA	16.67%
	Polyester	4.17%
	Polyethylene	2.08%
	Polymethylmethacrylate	2.08%
	Polypropylene	6.25%
	Polystyrene	6.25%
	Polyvinylidene chloride	4.17%
Fragment	Acrylnitril-Butadien-Styrol-Copolymer	2.08%
	NA	10.42%
	Nylon-6	2.08%
	Polyester	2.08%
	Polyethylene	6.25%
	Polyolefine	2.08%
	Polypropylene	25.00%
	Polystyrene	2.08%
	Polyvinylidene chloride	2.08%
	Styrene-acrylonitrile	2.08%
Total	Acrylnitril-Butadien-Styrol-Copolymer	2.08%
	Cellulose	2.08%
	NA	27.08%
	Nylon-6	2.08%
	Polyester	6.25%
	Polyethylene	8.33%
	Polymethylmethacrylate	2.08%
	Polyolefine	2.08%
	Polypropylene	31.25%
	Polystyrene	8.33%
	Polyvinylidene chloride	6.25%
	Styrene-acrylonitrile	2.08%

constructed from materials such as concrete, PVC, or other forms of plastic (Belay 2008). The ponds should be left exposed to the sun or covered with transparent polyethylene covers as needed. It is worth noting ponds in large-scale aquaculture are typically open. Foreign substances may contaminate the product during the production phase due to the open nature of the ponds (Belay 2008). Additionally, the lack of measures to prevent plastic contamination during the harvesting of the spirulina increases the risk of MP contamination. It is conceivable that this contamination can be linked to various factors, including the water utilised in cultivation, pond material, MP concentration in the ambient air in the packaging environment, the packaging material and even the way in which the packaging is opened, so that it is quite possible that there are differences and similarities in terms of contamination in different types of product. In order to gain a comprehensive understanding of this issue, further detailed studies are necessary to assess MP pollution at every stage, from the aquaculture environment to the final product obtained by the end user.

The abundance of MPs in commercially packaged spirulina ranged from 1 to 63.7 MPs/100 g dw, with an average of 13.77 ± 2.45 MPs/100 g dw. While there is a lack of previous studies specifically examining MPs in spirulina, these findings can be compared with the levels of MPs found in other packaged products, despite methodological and product type differences. However, it should be noted that this assessment is solely intended to improve our understanding of MP contamination in packaged products and does not involve direct comparison. The concentration of MPs in processed foods varied significantly, ranging from 1.68 MPs/kg to 10 640 MPs/kg. Dessì et al. (2021) analyzed 52 store-bought rice samples, and MPs with different polymer types and shape were found in all of them. PE was present in all samples, with concentrations ranging from 45 to 317 µg/g dw. PP was found in 40% of the samples, with a maximum concentration of 105 µg/g dw. PES was in 6% of the samples, with the highest concentration being 17 µg/g dw. In another study, Kedzierski et al. (2020) examined the chemical composition and quantity of extruded-PS MPs in packaged meats. The study revealed that MPs were present in meat products at 4.0 to 18.7 MPs/kg levels. Gündoğdu and Köşker (2023) investigated the presence of MPs in 33 different canned fish brands sold in the Turkish markets, and they reported that all the samples had at least one MPs particle. Plenty of research is available that exemplifies the presence of MPs in packaged food (Jadhav et al. 2021; Vitali et al. 2022; Mamun et al. 2023). These studies collectively demonstrate that MP in packaged foods can originate from packaging materials, production processes, and pollution at the source of the packaged product. The concentration of MPs detected in previous studies on packaged foods differs from the amounts found in this study. These variations can be attributed to disparities in analytical methods, food sources, and food processing procedures.

Various studies in the literature reveal the presence of different MPs in packaged foods, such as PP, PET, PVC, PA, PS, and others (Jadhav et al. 2021; Lin et al. 2022; Vitali et al. 2022; Mamun et al. 2023). The occurrence of these polymers in foods implies that packaging plastics can be a major source (Fadare et al. 2020; Kedzierski et al. 2020; Gündoğdu and Köşker 2023). These polymers and additives can affect the human body (Nobre et al. 2015). Multiple studies have revealed the harmful impact of MP exposure on various polymer types (Brouwer et al. 2022; Prata et al. 2020; Udovicki et al. 2022). These effects encompass the size-dependent behavior of plastic particles, the transfer of additives and absorbed chemical pollutants, disturbance of the gut microbiome, the induction of oxidative stress, and the escalation of inflammatory response (Barboza et al. 2018; De-la-Torre 2020). Therefore, the presence of microplastics

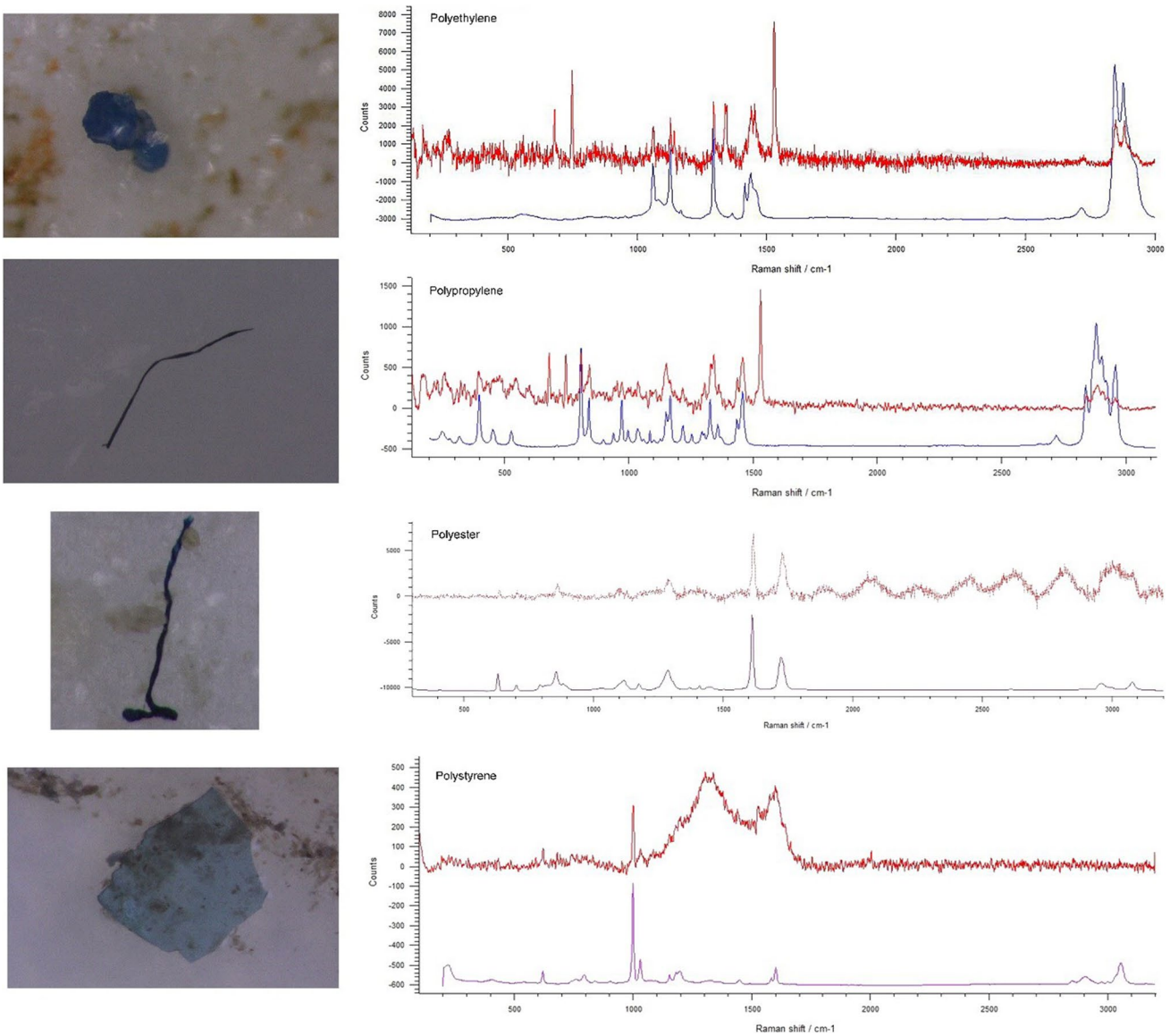


Fig. 2 Photographs and μ-Raman spectrum of different types of microplastics by polymers a) polyethylene, b) polypropylene, c) polyester, and d) polystyrene extracted from the packaged *Spirulina* products

(MPs) in food products has the potential to create a risk for consumers (De-la-Torre 2020). To mitigate such occurrences, addressing multiple factors is crucial. These factors comprise minimizing the use of plastic packaging in food products, implementing stricter regulations to limit and reduce the chemicals used in these materials, and reducing the overall production of plastics as outlined in the UN Plastics Treaty Initiative (Bergmann et al. 2022; Dey et al. 2022).

In Europe, approximately 8,000 substances are used in food packaging and other materials in contact with food (FCMs), and out of these, 388 different chemicals have been classified as the most harmful chemicals according to the Criteria of the EU Chemicals Strategy for Sustainability due to their carcinogenic, mutagenic, reproductive toxic, persistent, bioaccumulative, and/or endocrine-disrupting properties (Zero Waste Europe 2023; Zimmermann et al. 2022). More importantly, many of these chemicals can migrate from food packaging, thus becoming a significant source of contamination in food and, ultimately, in the consumer's body. Food packaging is one of the main sources of exposure to endocrine disruptors, such as bisphenol (BPA) and phthalates, in children and adults, with an increasing number of hazardous chemicals found in human blood and body tissues. Numerous chemicals that come into contact with food have been associated with harmful effects on human health (Zero Waste Europe 2023). This study indicates that the most commonly found plastics align with plastics commonly used in food packaging and the most

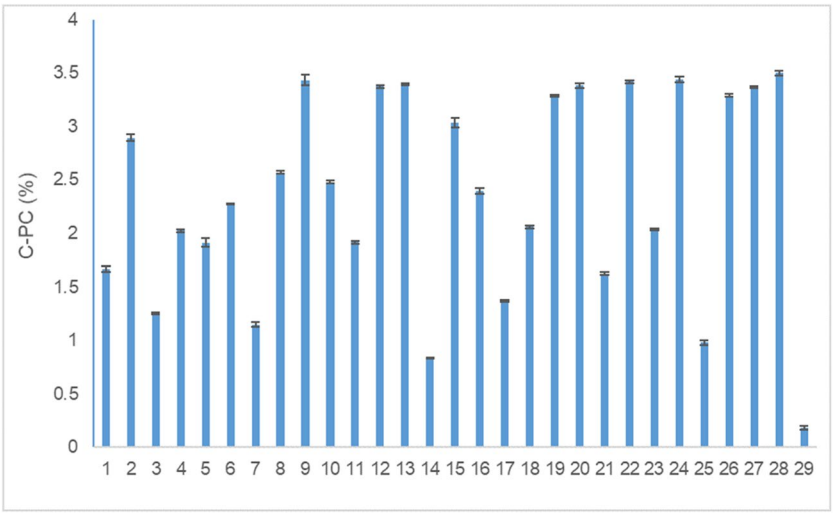


Fig. 3 C–C-Phycocyanin levels of the samples (error bars represent standard deviation)

Table 4 C-Phycocyanin levels of the samples (PC µg/ml and PC %)

Brand	C-Phycocyanin (µg/mL)	PC (%)
1	166.592 ± 2.7	1.66 ± 0.02
2	289.4125 ± 2.8	2.89 ± 0.02
3	125.0125 ± 0.8	1.25 ± 0.009
4	202.2805 ± 1.6	2.02 ± 0.01
5	191.389 ± 3.6	1.91 ± 0.03
6	227.557 ± 0.5	2.27 ± 0.006
7	114.806 ± 2.1	1.14 ± 0.02
8	256.738 ± 1.3	2.26 ± 0.01
9	343.2535 ± 4.7	3.43 ± 0.04
10	248.0385 ± 1.6	2.48 ± 0.016
11	191.663 ± 1.5	1.91 ± 0.01
12	337.2255 ± 1	3.37 ± 0.01
13	339.2805 ± 1	3.39 ± 0.01
14	83.5015 ± 0.09	0.83 ± 0.001
15	303.2495 ± 4.7	3.03 ± 0.04
16	239.4075 ± 2.6	2.39 ± 0.02
17	137.274 ± 0.9	1.37 ± 0.01
18	206.048 ± 1.6	2.06 ± 0.01
19	328.4575 ± 1	3.28 ± 0.01
20	338.116 ± 1.9	3.38 ± 0.02
21	162.482 ± 1.3	1.62 ± 0.01
22	341.678 ± 1.7	3.41 ± 0.02
23	203.6505 ± 1	2.03 ± 0.01
24	344.0755 ± 2.6	3.44 ± 0.02
25	98.0235 ± 2.2	0.98 ± 0.02
26	328.7315 ± 1.4	3.28 ± 0.01
27	336.5405 ± 0.6	3.36 ± 0.007
28	349.761 ± 2.5	2.49 ± 0.02
29	18.084 ± 1.7	0.18 ± 0.02

Experimental values were expressed as mean ± standard deviation

produced plastics (Muhib et al. 2023; PlasticEurope 2022). For example, the PP, which was detected in the highest proportions in this study, can be used for packaging purposes. Specifically, large woven bags made of PP are widely used for transporting various packaged foods, including spirulina, in large quantities. The other most common polymers found in this study is PE which is also commonly used in food packaging (Raj et al. 2004). Similarly, PS is used for packaging purposes and contains hazardous chemicals. Thus, the polymer types of the plastics found in this study are consistent with previous studies and with commonly produced plastics. Consequently, this underscores the requirement for further extensive investigations to evaluate the risks, and to consider MPs concerning food safety, encompassing their chemical components and physical particles.

It should be noted that the quantity of C-Phycocyanin can vary significantly across different studies, influenced by production conditions and the quality of chemicals used in the growth media. For instance, Oğuz et al. (2011) conducted a study where spirulina was cultured in open system ponds in Çukurova during three seasons. They discovered the highest C-Phycocyanin content in the autumn season (332.7 ± 1 µg/mL), with similar results in the summer (323.4 ± 1 µg/mL) and spring (327.5 ± 2 µg/mL) seasons. In another study conducted by Maulana et al. (2023), spirulina was cultured in two different media (Cultured with Liquid Organic and NPK Fertilizers), resulting in C-Phycocyanin amounts of 0.148 and 0.097 mg/L, respectively. Furthermore, Walter et al. (2011) investigated the effect of different light sources (white, red, yellow, and blue) on *S. platensis* spirulina culture. They found the highest C-Phycocyanin content of 0.237 mg/mL under white light conditions. These findings illustrate that C-Phycocyanin content can vary widely depending on the growth conditions and media used in spirulina cultivation. C-Phycocyanin, the primary accessory photosynthetic blue pigment found in spirulina, constitutes

up to 20% of its dry weight (Jaouen et al. 1999; Vonshak 1997). The C-Phycocyanin levels of brand 29 were found to be significantly low. Additionally, the high concentration of MPs detected in the same brand is raising concerns regarding the production and preparation conditions. The presence of MPs and brownish colour instead of green are indicating a lack of authenticity of brand 29 as genuine spirulina.

The contamination of food and beverage products with plastic has been suggested to result from various stages of production and processing. These stages encompass the fragmentation of plastic tools employed during production and processing, such as those used in agricultural fields and tanks. Additionally, contamination can occur through water used for production and workspace cleaning, as well as in the general facility area where processing takes place. Other potential sources include the work area and airborne fallout, which may contain synthetic fibers found in employee uniforms (EFSA 2016; Shruti et al. 2020; Dessì et al. 2021; Gündoğdu and Köşker 2023). According to EFSA (2016), plastic contamination is expected to rise during processing. Li et al. (2020) and Gündoğdu and Köşker (2023) provide evidence supporting this claim and propose that if adequate precautions are not taken to eliminate MP contamination, the processing phase could introduce a comparatively higher amount of MPs into the products.

Conclusion

Our findings demonstrate that MPs are commonly present in spirulina's final commercial products, and their abundance and composition are influenced by the packaging and processing stage. It is crucial to carefully identify the potential sources of MPs in these food products and evaluate their potential risks to human health in future research. Further detailed investigations and comprehensive monitoring studies should be conducted to understand the interaction between packaged convenience foods and dietary supplements with MPs, revealing their presence more comprehensively. Measures should be taken to minimize MP contamination originating from materials in contact with food and food production and processing processes. The detailed exploration of this aspect should be prioritized in future studies.

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Authors contributions S. Tutaroğlu: Conceptualization; Sampling; Formal Analysis; Investigation; Methodology; Writing—original draft; Writing—review & editing, L. Uslu: Conceptualization; Sampling; Methodology; Project administration; Resources; Writing—original draft; Writing—review & editing, S. Gündoğdu: Conceptualization; Data curation; Formal Analysis; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; Writing—original draft; Writing—review & editing.

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Data Availability Data is provided as a supplementary file.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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