Contamination of Microplastics in Retail *Paratapes undulatus* Clams from Fresh Markets in Nakhon Pathom Province, Thailand

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Abstract

Microplastic contamination in food is a growing problem in the modern world. Clams, in particular, are consumed whole and are particularly susceptible to contamination entering the body. In 120 clams, microplastic contamination was investigated. Clam tissues were digested with 30% hydrogen peroxide and 10% potassium hydroxide. A total of 1,001 microplastic items were found. There was a statistically significant difference between the weights of clam tissue and microplastic ($\chi^2 = 36.945$, $df = 5$, $p = 0.000$; $\chi^2 = 35.842$, $df = 5$, $p = 0.000$). The most prevalent microplastic shapes, at 38% and 36%, were identified as fragment and film microplastics. The most common microplastic color was white or transparent (29%), whereas the majority (44%) of the microplastics were less than 100 µm. A Fourier transform infrared spectrometer (FTIR) was used to confirm 99 microplastics that were chosen at random and amounted to approximately 10% of the total microplastics. PET (polyethylene terephthalate), BEHP (bis(2-ethylhexyl) phthalate), PP (polypropylene), CA (cellulose acetate), PVAc (polyvinyl acetate), PMMA (poly (methyl methacrylate), and EC (ethyl cellulose) are examples of plastic materials. According to the study's findings, the *Paratapes undulatus* sold in Thailand's fresh market was heavily contaminated with microplastics. As a result, the findings of this study can be utilized to inform future research on assessing exposure to microplastics and the health risks associated with consuming contaminated bivalves commonly consumed in Thailand.

Keywords: Microplastics, *Paratapes undulatus*, FTIR**,** human health

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Introduction

Microplastics (plastic particles smaller than 5 mm) (Frias et al., 2019) have been identified as one of the most concerning contaminants in marine and coastal habitats worldwide (Harding, 2016; Shahul Hamid et al., 2018). According to reports, between 4.8 and 12.7 million metric tons of plastic trash from 192 coastal nations had entered marine habitats by 2010 (Jambeck et al., 2015), making plastic debris the most prevalent type of marine litter, accounting for three-quarters of marine waste (Harding, 2016). Worryingly, this number is expected to rise substantially in the coming years, with plastic items expected to reach 33 billion tons by 2050 (Harding, 2016). Because of the persistence and abundance of plastic waste in the ecosystem, larger plastics have broken down into secondary microplastics, resulting in a huge number of microplastics in all coastal and marine environments, including estuaries, mangroves, lagoons, bays, and deep-sea areas (Hitchcock et al., 2019; Nor & Obbard, 2014; Bayo et al., 2019; Falahudin et al., 2020; Van Cauwenberghe et al., 2013). Additionally, due to product discharges containing microplastics after use or accidents in plastic transportation, primary microplastics—plastics made for a specific purpose (e.g., plastic pellets for drugs and cosmetics products)—are a major source of pollution in the environment (Pandey et al., 2022). Microplastics have been spread to all levels of trophic organisms in the food web due to their extensive availability in all coastal and marine ecosystems. According to Gall et al. (2015), plastic accounts for 92% of all contacts between individual creatures and marine litter, affecting these species through ingestion, entanglement, and habitat disruption. The major effects of microplastics on aquatic habitats include physical damage, atypical behavior patterns, obstacles in the nutrient cycle, cytotoxicity and genotoxicity, and a rise in mortality (Pandey et al., 2022).

The bivalve mollusks are filter feeders and belong to species that are most vulnerable to microplastic contamination due to their feeding habits, as they ingest suspended particles from the water column (Setälä et al., 2016; Ward et al., 2019; Li et al., 2019). These microplastics can potentially have a wide range of deleterious impacts on bivalves, including affecting filtering function and reproduction systems, causing genotoxicity, and producing indirect consequences related to modifying the structure of habitat and food supplies (Zhang et al., 2020). In Asian green

mussels (*Perna viridis*), exposure to polyvinyl chloride (PVC) raised mortality rates, whereas in Pacific oysters (*Crassostrea gigas*), exposure to polystyrene (PS) affected sperm motility, egg production, and oocyte quality. (Pandey et al., 2022). Various organisms, including invertebrates, fish, birds, and mammals, rely on bivalves for food (Waser, 2018). As a result, these faunas can move microplastics up the food chain to higher-trophic species. More significantly, bivalves are a significant source of sustenance for humans; their yearly production exceeds 15 million tons, or 14% of all marine production worldwide (Smaal, 2019). Because humans consume all of the tissues of bivalves, unlike other seafood species, bivalves may be a significant source of microplastic for humans (Zhang et al., 2020). Microplastics have been found in a variety of economically popular bivalves, such as clams, scallops, oysters, and mussels, throughout natural and aquaculture habitats (Qu et al., 2018; Naji et al., 2018; Phuong et al., 2018; Davidson et al., 2016; Li et al., 2016; Van Cauwenberghe & Janssen, 2014). Microplastic concentration in bivalves is commonly between 10^{-1} and 10^{1} items g^{-1} (Cho et al., 2019; Jin-Feng et al., 2018; Renzi et al., 2018). In Canadian bivalve species, however, microplastic amounts up to 657.5 items g^{-1} have been reported (Murphy, 2018). Van Cauwenberghe & Janssen (2014) estimate that Europeans with an average mollusk intake of 11.8–72.1 g cap^{-1} day^{-1} may consume up to 1,800–11,000 microplastics per year, which can have serious consequences for human health.

Bivalve mollusks are a popular type of seafood in Thailand, among locals and tourists. The government intends clams, in particular, to be one of Thailand's main seafood exports to nations worldwide. However, data on the level of microplastic contamination in edible bivalves is currently scarce, despite the fact that microplastics have been identified in quite significant concentrations across the country throughout freshwater systems and coastal areas (Strady et al., 2021; Tran-Nguyen et al., 2020). As a result, microplastics are highly likely to accumulate in living organisms and negatively affect consumer health. The presence and characteristics of microplastics in the clam *Paratapes undulatus*, a common edible bivalve mollusk in Thailand, were investigated in this study.

A wide range of acids (Van Cauwenberghe & Janssen, 2014), enzymes (Catarino et al., 2017), alkalis (Rochman et al., 2015), and oxidizing agents, such as H_2O_2 (Li et al., 2015), are frequently used in the digestion of bivalve tissue. Numerous steps in certain digestion procedures raise the risk of airborne contaminants getting into the sample. As a result, this study determined the most appropriate approach for breaking down bivalve tissues and extracting microplastics by contrasting the KOH and H_2O_2 approaches used in previous research. The study describes a method for the extraction and quantification of microplastics from clams. This study will add to the understanding of microplastic contamination in Thai biota as well as the implications for humans, including dietary exposure, in order to determine the potential threat of contaminated seafood.

Materials and methods

1. Sample collection and preparation

In the Gulf of Thailand and the Andaman Sea, the clam *P. undulatus* (Born, 1778) supports Thailand's largest shellfishery. In general, the harvest areas for clams are consolidated mud substrates within 3 to 7 kilometers of the shoreline. Shellfish grounds can be found off the coasts of various coastal provinces and are collected all year. Clams are typically acquired for export as canned products. A total of 120 individuals of *P. undulatus* (approximately 1 kilogram) were purchased at the fresh market in Nakhon Pathom Province for an investigation of microplastics (MPs). These clams were harvested from Samut Prakarn Province. All *P. undulatus* samples were rinsed with filtered distilled water. The shell lengths of *P. undulatus* were also measured. *P. undulatus* samples were separated from their shells prior to tissue processing, and the soft tissue of each sample was weighed separately. The tissue from each sample was preserved in the freezer until it was time to be tested.

In this investigation, the clam tissue digestion studies were separated into two groups: H_2O_2 and KOH. Each group had three replications, each of which had 20 individual clams.

2. Microplastic isolation from the soft tissue of *P. undulatus*

Clam tissue digestion was carried out, according to Ehlers et al. (2019). To break down the soft tissue, each individual was put down in a labeled 100-mL Erlenmeyer flask that had already been cleaned, and approximately 20 mL of 30% H_2O_2 was poured into each flask. Each flask was wrapped in parafilm. It was then boiled at 60 °C for three hours in a shaken water bath at 150 rpm, or until all of the organic stuff was digested. The blanks were checked in parallel for the presence of MPs. In the other group, 20 mL of 10% KOH was introduced to each flask containing clam soft tissue at the same time.

Microplastics (MPs) were recovered from a dissolved organic matter solution via flotation with potassium formate (HCO2K) (Zhang et al., 2016). 99% HCO2K was added to each sample in a glass separating funnel until the

concentration reached 1.6 g/mL. The samples were then kept at room temperature for a minimum of three hours. A layer of MPs emerged when the less dense particles separated from the saturated solution, resulting in the undissolved organic residues and inorganic materials sinking to the bottom of the glass containers. Subsequently, the samples were filtered using a pressure filtering system and a nylon membrane filter (Whatman, Kent, UK; pore size, 0.45 µm; diameter, 47 mm). After being filtered, each membrane was put on a sterile Petri dish, covered with aluminum foil, and allowed to dry for two days at 50 °C in a drying cabinet.

3. Microscopic examination of MPs

The prevalence and characteristics of MPs in filtered filters were determined using a stereomicroscope equipped with a camera and image analysis software (Leica EZ4E, Germany). The microplastic particle size was measured using the LAS X software, and the length was calculated from the longest side. A visual inspection was also done to identify expected MPs based on morphological characteristics such as color and shape (Hidalgo-Ruz et al., 2012). The kinds of microplastic particles were classified as fiber, spherical, film (a thin and small layer), and fragment (part of a larger plastic item) (Su et al., 2016; 2018). MPs were divided into four groups, with L representing the length of the longest diameter: first ($L \leq 100$ µm); second ($100 < L \leq 250$ µm); third ($250 < L \leq 500$ µm); and fourth ($L > 500$ µm).

4. Polymer type identification

To determine the polymer types, a representative number of MPs from each morphotype were randomly picked and evaluated with a PerkinElmer Spectrum-Fourier transform infrared spectrometer (FTIR) in attenuated total reflection (ATR) mode. The MPs chosen reflected the most common types of visually observed particles in all samples. A Hyperion 2000 FTIR microscope (Bruker Daltonik, Billerica, MA, USA) with a mercury-cadmium telluride detector was used to manually analyze 99 particles from soft tissue at wave numbers ranging from 4000 to 600 cm^{-1}, with 32 co-added scans and a spectrum resolution of 4 cm^{-1}. The Bruker spectrum library was used to compare polymer types and functional group characterizations. Based on the spectrum analysis, a matching level of spectra with a quality index of ≥0.7 was considered acceptable (Woodall et al., 2014).

5. Quality control

To prevent airborne microplastic contamination, non-plastic equipment such as cotton lab coats and nitrile or latex gloves was worn throughout the experimental procedures. In addition, glassware and metal tools were utilized in the lab to evaluate the samples. During filtering and sieving, any potential contaminants were discovered. Air exposure was kept to a minimum in order to reduce microplastic loss.

6. Statistical analyses

Microplastics in clam tissue were measured individually based on type, color, and size. Pooled samples (20 specimens, 6 replicates) were used to calculate the average amount of MPs per gram of wet weight and individual clam tissue. Furthermore, for each replication, the Chi-square test of independence was used to assess microplastic shape, color, and size. All statistical tests were run on Statistica 20.0, a computer program.

Results and Discussion

1. Abundance of Microplastics

The shell length of the 120 individual *Paratapes undulatus* was 4.32 ± 0.38 cm, and the wet tissue weight was 1.82 ± 0.44 g. Microplastic contamination in all *P. undulatus* was detected in a total of 1,001 suspected microplastic items (Table 1). *P. undulatus* had a microplastic abundance of 4.88 ± 1.65 items g^{-1} (8.34 ± 3.87 items $individual^{-1}$). Our results confirmed the presence of MPs in all of the clams analyzed.

In experiment H_2O_2_2, the average microplastics were detected at 13.70 ± 12.25 items $individual^{-1}$ (7.61 ± 5.61 items g^{-1}), followed by experiment H_2O_2_3, where microplastics were found at 12.90 ± 5.60 items $individual^{-1}$ (8.41 ± 4.56 items g^{-1}), KOH_1 discovered 7.00 ± 5.71 items $individual^{-1}$ (3.85 ± 3.34 items g^{-1}), H_2O_2_1 detected 6.15 ± 7.90 items $individual^{-1}$ (3.18 ± 3.99 items g^{-1}), KOH_2 found 5.1 5± 1.90 items $individual^{-1}$ (3.20 ± 1.56 items g^{-1}), and KOH_3 found 5.15 ± 2.08 items $individual^{-1}$ (3.02 ± 1.48 items g^{-1}) (Fig. 1, Table 1). The weights of clam tissue and microplastic digested by hydrogen peroxide and potassium hydroxide differed statistically ($\chi^2 = 36.945$, $df = 5$, $p = 0.000$; $\chi^2 = 35.842$, $df = 5$, $p = 0.000$) (Fig. 1).

Table 1 The weight of clam flesh and the number of microplastics (MPs) found after digesting clam tissue with hydrogen peroxide and potassium hydroxide.

30% H_2O_2_1			30%H_2O_2_2			30%H_2O_2_3			10% KOH_1			10%KOH_2			10%KOH_3	
No.	Edible tissue (g)	MPs (item)	No.	Edible tissue (g)	MPs (item)	No.	Edible tissue (g)	MPs (item)	No.	Edible tissue (g)	MPs (item)	No.	Edible tissue (g)	MPs (item)	No.	Edible tissue (g)	MPs (item)
1	2.01	30	1	1.61	24	1	1.97	18	1	2.94	6	1	1.74	5	1	1.99	6
2	1.97	9	2	2.46	41	2	1.49	15	2	1.93	7	2	1.23	7	2	1.90	6
3	1.56	3	3	1.40	13	3	1.13	12	3	1.36	7	3	2.13	6	3	1.32	9
4	1.80	16	4	1.10	12	4	1.06	6	4	1.82	8	4	3.50	1	4	1.60	2
5	2.19	19	5	1.76	7	5	1.51	14	5	1.72	28	5	1.56	9	5	2.03	6
6	2.16	5	6	1.72	3	6	0.96	15	6	1.48	7	6	1.87	6	6	1.69	2
7	2.11	1	7	2.05	8	7	1.30	11	7	2.15	7	7	1.28	7	7	1.47	4
8	1.53	3	8	1.50	6	8	1.89	13	8	1.91	11	8	2.16	4	8	1.55	5
9	2.56	1	9	2.10	5	9	1.45	20	9	2.72	9	9	1.63	5	9	1.75	2
10	1.45	4	10	2.70	44	10	1.99	15	10	1.69	2	10	1.51	6	10	1.85	4
11	2.14	2	11	1.70	7	11	1.38	29	11	1.81	12	11	1.36	7	11	2.67	6
12	2.35	1	12	1.63	5	12	2.05	13	12	1.87	6	12	2.07	4	12	1.37	4
13	2.04	10	13	1.71	33	13	1.75	12	13	1.78	7	13	1.94	7	13	1.48	6
14	2.65	1	14	1.74	9	14	2.87	7	14	1.39	5	14	1.56	5	14	1.51	7
15	1.91	1	15	1.77	3	15	1.72	5	15	2.10	5	15	1.73	2	15	1.68	10
16	3.12	2	16	1.70	17	16	1.44	16	16	1.20	3	16	1.31	5	16	1.86	4
17	2.14	1	17	1.53	7	17	2.56	10	17	1.99	3	17	1.90	3	17	2.26	6
18	1.70	12	18	2.26	9	18	1.98	13	18	1.79	3	18	1.61	4	18	2.36	4
19	1.74	1	19	1.74	7	19	1.32	9	19	2.35	2	19	1.38	6	19	1.34	5
20	1.26	1	20	1.14	14	20	1.59	5	20	2.15	2	20	2.35	4	20	1.82	5
sum	40.38	123		35.32	274		33.41	258		38.13	140		35.80	103		35.46	103
mean	2.02	6.15		1.77	13.70		1.67	12.90		1.91	7.00		1.79	5.15		1.77	5.15
SD	0.44	7.90		0.39	12.25		0.48	5.60		0.43	5.71		0.51	1.90		0.36	2.08

Note: H_2O_2_1 = digesting clam tissue with hydrogen peroxide in the first replication; H_2O_2_2 = digesting clam tissue with hydrogen peroxide in the second replication; H_2O_2_3 = digesting clam tissue with hydrogen peroxide in the third replication; KOH_1 = digesting clam tissue with potassium hydroxide in the first replication; KOH_1 = digesting clam tissue with potassium hydroxide in the second replication; KOH_1 = digesting clam tissue with potassium hydroxide in the third replication.

The average amount of microplastics ingested by clams in this study (8.34 ± 3.87 items individual^{-1} or 4.88 ± 1.65 items g^{-1}) is higher than values reported in coastal waters of China (1.5 to 7.6 items individual^{-1}) (Li et al., 2016) and the UK (1.1–6.4 items individual^{-1}) (Li et al., 2018), as well as in Giglio Island, Italy (1–2 items individual^{-1}) (Avio et al., 2017) and the French Atlantic coast (0.6 ± 0.6 items individual^{-1}) (Phuong et al., 2018). According to the reports studied, the Gulf of Thailand has significant microplastic contamination (Imasha & Babel, 2023; Srikrajang & Prommi, 2021). However, of the total debris items reported in the UK mussel study (Li et al., 2018), only 50% were microplastics. The microplastic ingestion values of our study are much higher than those of Vandermeersch et al. (2015) in the estuaries of Portugal, Italy, and Spain; De Witte et al. (2014) in the Belgian coast; and Van Cauwenberghe et al. (2015) in the French, Belgian, and Dutch North Sea coasts. The previous three studies provided results per weight of complete mussel tissues, which may explain the observed differences from our results, which are calculated per weight of gills and digestive tract and include higher microplastic quantities among mussel tissues (Tsangaris et al., 2015). Moreover, previous investigations utilized pooled samples and reported mean values for all individuals studied, whereas we computed the number of microplastics on a wet-weight basis, considering only those with microplastics. Our findings are not comparable to other studies that show microplastic ingestion in mussels on a dry weight basis (Leslie et al., 2017; Karlsson et al., 2017). Most mussel studies do not reveal the frequency of ingested microplastics, which is likely due to the use of pooled animal samples for microplastic extraction. Microplastic occurrence rates in mussels in our study (100%) are higher than those reported by Avio et al. (2017) in Giglio Island, Italy (10–36%), who employed individual mussels for microplastic extraction in a similar manner. The variations that were observed could be related to the use of different mussel sampling strategies; for example, in our research, we collected both wild and cultured clams, whereas Avio et al. (2017) implanted mussels at two depths (surface and bottom) for four weeks.

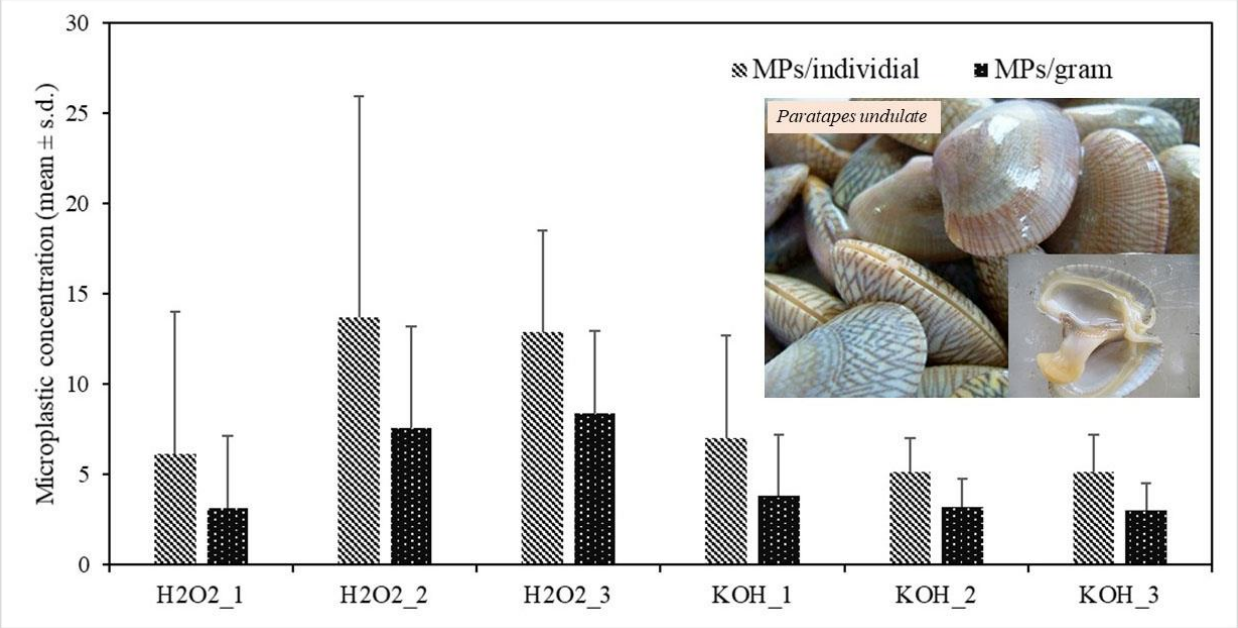

Fig. 1 Mean concentrations of microplastics found in *Paratapes undulatus* clams after digesting clam tissue with hydrogen peroxide and potassium hydroxide.

2. Microplastic shape, size, and color

On average, each sample contained eight microplastic items. In the present study, the most prevalent microplastics were fragments, followed by films and fibers. Approximately 38% of the microplastics obtained from *P. undulatus* soft tissues were fragment-shaped. Microplastics in the film shape accounted for 36% of the total, whereas microplastics in the fiber shape accounted for 26% of the total (Fig. 2a). The microplastic shape in clam tissue degraded by hydrogen peroxide and potassium hydroxide differed statistically in fragment, fiber, and film (χ^2 = 25.126, *df* = 5, p = 0.000; χ^2 = 12.739, *df* = 5, p = 0.000; χ^2 = 70.222, *df* = 5, p = 0.000, respectively) (Fig. 3). Mechanical and UV radiation are likely to break down fragments from larger plastic objects (e.g., plastic containers, furniture, and toys) (Horton et al., 2017). Thus, this study's higher percentage of fragments indicates that MPs found in the soft

tissue are mainly secondary microplastics. In this investigation, the composition of ingested microplastics in market clams revealed higher levels of fragments than fibers and film. Similarly, Phuong et al. (2018) found that most microplastics in mussels from the French Atlantic coast were fragments, reaching 82%, greater than our result (38% fragments). However, most mussel investigations (De Witte et al., 2014; Li et al., 2016) show that fibers predominate fragments. Different sources and waste management practices could explain the variation in shape categories of ingested microplastics among market clams and other places (Rochman et al., 2015). Poor waste management methods, both on land and on the shoreline, where tourism and leisure activities are intense, are among the litter inputs in the Gulf of Thailand, where the clams were harvested. This may result in large amounts of plastic inputs into the sea (e.g., plastic bags, bottles, and cups) that can degrade into microplastic fragments. Furthermore, poorly managed plastic can fragment more quickly on land and enter the sea as microplastics (Kalogerakis et al., 2017). Plastic particles were found in similar proportions in ingested microplastics across species, habitats, and sampling sites, indicating widespread distribution in the study area.

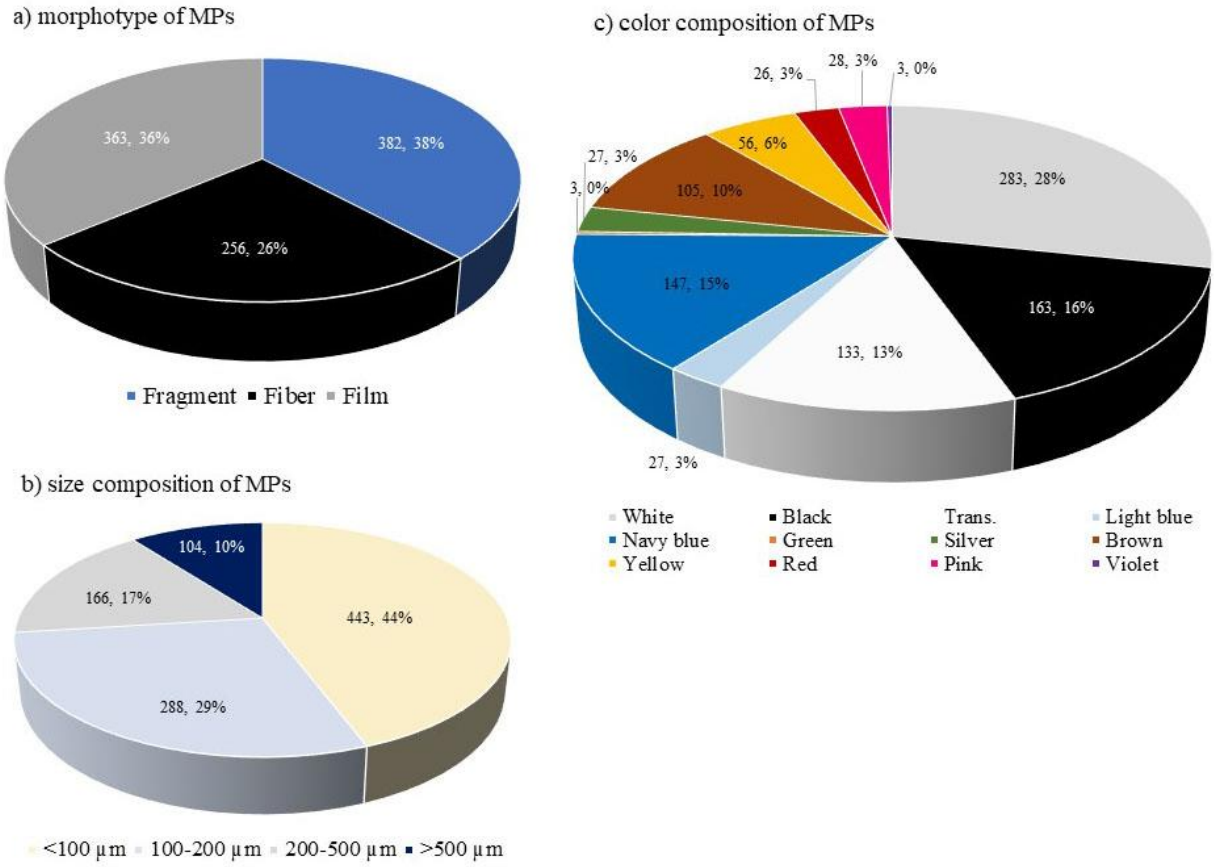

Fig. 2 Composition of microplastic (MPs) found in clam tissue; a) morphotype; b) size; c) color.

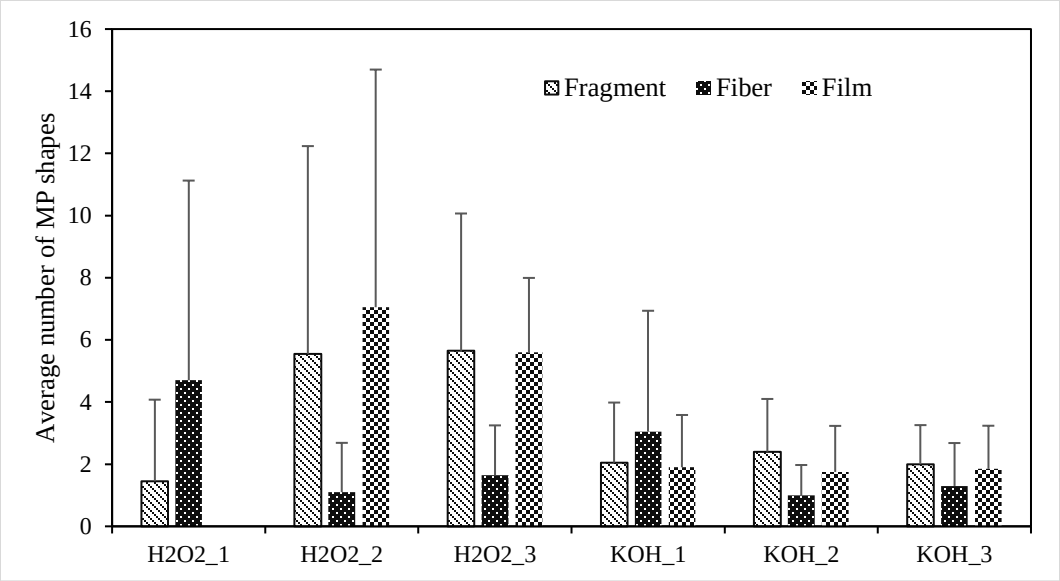

Fig. 3 Mean concentrations of microplastic shapes found in *Paratapes undulatus* after digesting clam tissue with hydrogen peroxide and potassium hydroxide.

Microplastics were divided into four sizes based on the largest dimension: < 100 µm, 100–250 µm, 250–500 µm, > 500 µm. Microplastic particles with a diameter of less than 100 µm (44%) were the most abundant, followed by those with a diameter of 100–250 µm (29%) and 250–500 µm (17%). Particles larger than 500 µm (10%) were less common (Fig. 2b). The microplastic size in clam tissue degraded by hydrogen peroxide and potassium hydroxide found that all sizes (<100 µm, 100-200 µm, 200-500 µm, and >500 µm) differed statistically (χ^2 = 53.501, *df* = 5, p = 0.000; χ^2 = 27.885, *df* = 5, p = 0.000; χ^2 = 16.701, *df* = 5, p = 0.000; χ^2 = 19.203, *df* = 5, p = 0.000) (Fig. 4). In terms of microplastic size, particles less than 100 µm were the most common, followed by particles 100–250 µm. Microplastics with diameters smaller than 0.1 mm, on the other hand, may have been underestimated, as recovery rates decline with particle size (Avio et al., 2015). Suaria et al. (2016) found that the existence of particles with a diameter of less than 0.5 mm conforms to the size distribution of floating microplastics in Mediterranean waterways. Because of their filter-feeding approach, the clams ingested more microplastics of smaller sizes (less than 0.1 mm, 0.1 mm–0.5 mm).

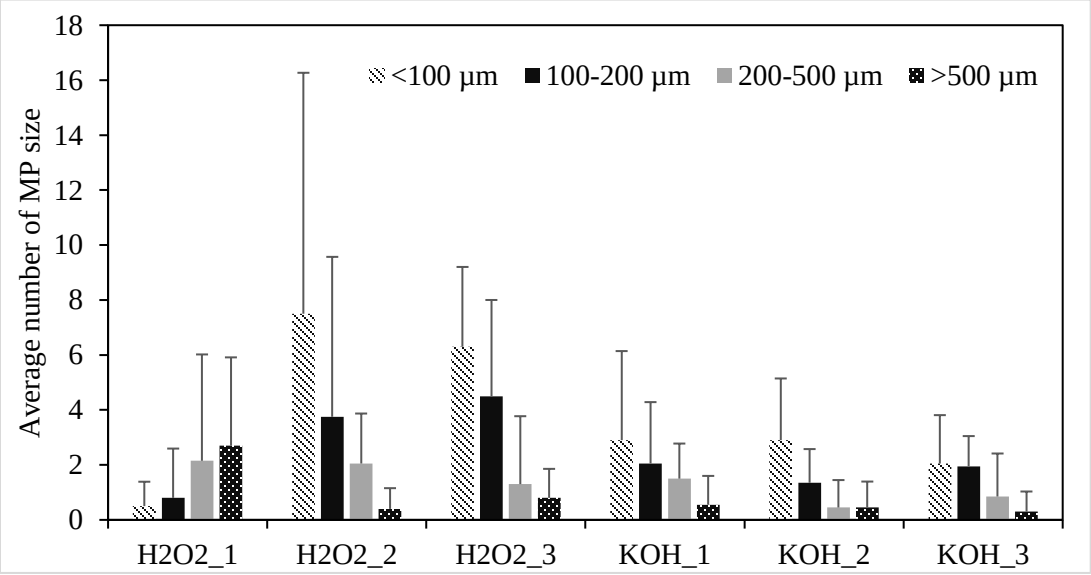

Fig. 4 Mean concentrations of microplastic sizes found in *Paratapes undulatus* after digesting clam tissue with hydrogen peroxide and potassium hydroxide.

According to Figs. 2a and 4, the size ranges of less than 100 µm and 100–250 µm were the most common classes for *P. undulatus*, representing 44% and 29%, respectively. Microplastics that are larger than 500 µm exhibit 10% of *P. undulatus*. The feeding mode is hypothesized to influence the size range of digested microplastics. Small-size microplastics are a significant component of marine animal ingestion because they make plastic particles extensively accessible to various biota in both benthic and pelagic environments. Smaller particles appear to be more likely to be swallowed, resulting in stomach blockage, physiological consequences, chemical transfer, and trophic transfer, whereas larger particles are more likely to entangle, preventing marine animals from swimming, filtering, or catching their prey (Cole et al., 2011; Lehtiniemi et al., 2018; Lusher, 2015). Some feeding processes do not allow them to differentiate between prey and anthropogenic materials in some situations, while others may mistake it for food and consume the plastic directly (Lusher, 2015).

Twelve distinct hues of microplastic particles were found (Fig. 2c): white, black, transparent, light blue, navy blue, green, silver, brown, yellow, red, pink, and violet. The most prevalent colors of microplastics in clam samples were white (28%), black (16%), navy blue (15%), and brown (10%); some were yellow (6%), light blue (3%), red (3%), pink (3%), silver (3%), and a few were green (0.3%) and violet (0.3%) (Fig. 2c). The color of microplastic in clam tissue degraded by hydrogen peroxide and potassium hydroxide found that the white, black, transparent, silver, brown, pink, and violet differed statistically ($\chi^2 = 19.057$, $df = 5$, $p = 0.002$; $\chi^2 = 13.043$, $df = 5$, $p = 0.023$; $\chi^2 = 29.728$, $df = 5$, $p = 0.000$; $\chi^2 = 11.756$, $df = 5$, $p = 0.038$; $\chi^2 = 23.777$, $df = 5$, $p = 0.000$; $\chi^2 = 24.170$, $df = 5$, $p = 0.000$; $\chi^2 = 15.256$, $df = 5$, $p = 0.009$,) whereas the light blue, navy blue, green, yellow, and red, did not differ statistically ($\chi^2 = 5.545$, $df = 5$, $p = 0.353$; $\chi^2 = 8.629$, $df = 5$, $p = 0.125$; $\chi^2 = 10.084$, $df = 5$, $p = 0.073$; $\chi^2 = 7.098$, $df = 5$, $p = 0.213$; $\chi^2 = 2.807$, $df = 5$, $p = 0.730$) (Fig. 5).

The primary source determines the color of microplastics; however, they can be altered by UV radiation, weathering, and microbial deterioration (Zhang et al., 2020). The colors of microplastics found in this study are similar to those found in earlier studies, where white, black, and blue are the most prevalent in fishing sector equipment such as fishing nets and ground ropes (Martin et al., 2017). According to previous research, these marine organisms prefer dark-colored microplastics because they are attracted to their color resemblance to their natural prey or to their prey, increasing the risk of ingestion (de Sá et al., 2015; Duncan et al., 2019; Ory et al., 2017).

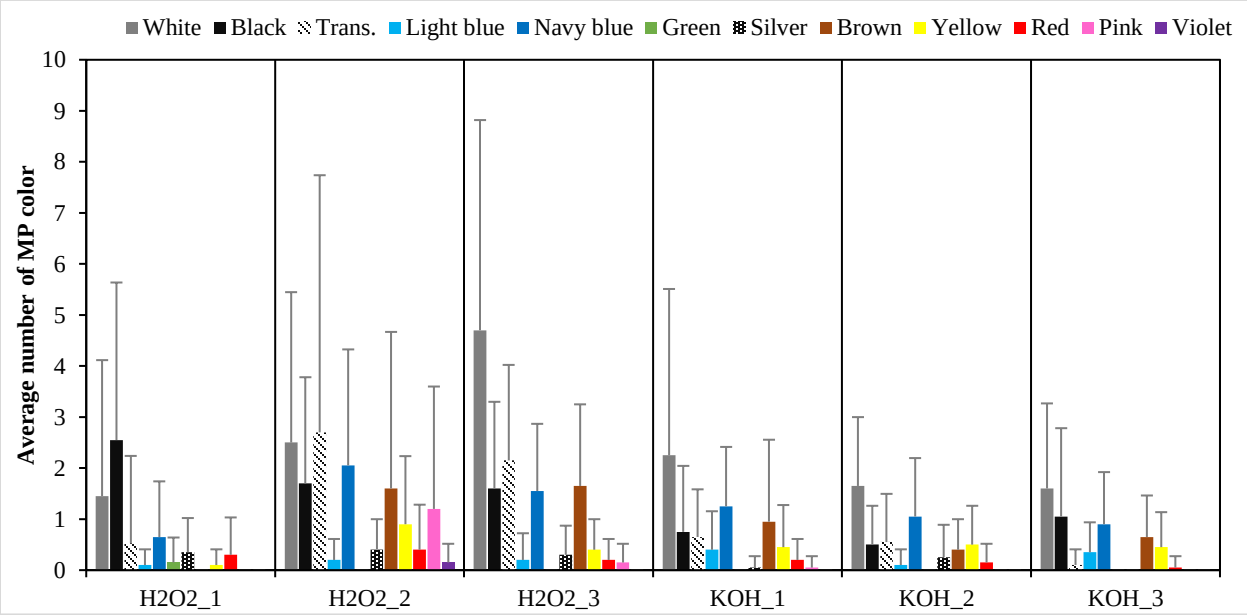

Fig. 5 Mean concentrations of microplastic colors found in *Paratapes undulatus* after digesting clam tissue with hydrogen peroxide and potassium hydroxide.

2. Microplastics' chemical composition

From the 1,001 suspected MP subsamples, FTIR analysis was employed to validate a representative number of samples (9.89%, 99 plastic items) (Table 2). The following polymer types were identified: cellulose acetate butyrate (CAB), polyethylene terephthalate (PET), bis (2-ethylhexyl) phthalate (DEHP), polypropylene (PP), cellulose acetate (CA), polyvinyl acetate (PVAc), poly (methyl methacrylate) (PMMA), and ethyl cellulose (EC) (Table 2, Fig. 6).

CAB (31.31%) was the most prevalent polymer type in the clams studied, followed by PET (9.09%). CAB is a widely used adhesive and additive in coating processes for a wide range of substrates, including plastics, textiles, metals, and wood. PET is commonly used in the production of fabrics, ropes, plastic bottles, plastic bags, and food containers (Qiu et al., 2015; Liu et al., 2020). According to the findings, anthropogenic trash may be a significant source of microplastics in the area where clams are harvested. PET is denser than water; it is more likely to sink and, hence, be ingested by benthic creatures. This is consistent with our results, which show that clams ingest PET.

High-molecular-weight phthalates, such as bis (2-ethylhexyl) phthalate (DEHP), are utilized in various flexible PVC daily-use products, including food packaging and home furnishings. This polymer is also found in mussels (*Mytilus galloprovincialis*) (Rios-Fuster et al., 2022).

Table 2 Polymer of microplastics identified by FTIR.

Description	Number	Percentage (%)
Total particle measured (random selection)	99	100[a]
Total polymer identified	58	58.58[b]
Cellulose acetate butyrate (CAB)	31	31.31[c]
Polyethylene terephthalate (PET)	9	9.09[c]
Bis (2-ethylhexyl) phthalate (DEHP)	7	7.07[c]
Polypropylene (PP)	5	5.05[c]
Cellulose acetate (CA)	3	3.03[c]
Polyvinyl acetate (PVAc)	1	1.01[c]
Poly (methyl methacrylate) (PMMA)	1	1.01[c]
Ethyl cellulose (EC)	1	1.01[c]
Total non-plastic particle	41	41.42

Remark:
[a]Percentage of analyzed MP particles.
[b]Percentage of polymers in analyzed MP particles.
[c]Percentage of MP polymer type.

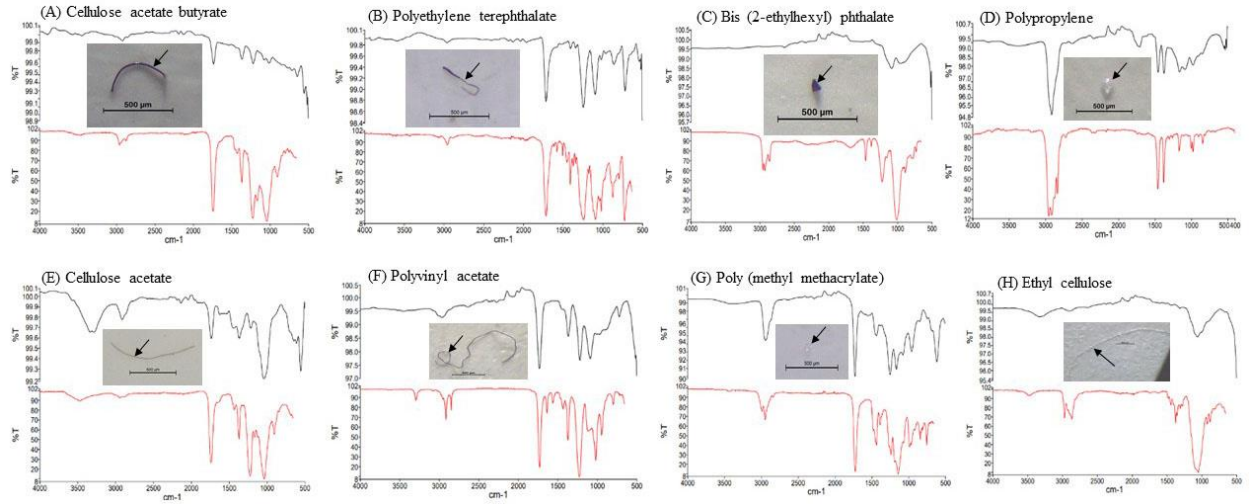

Fig. 6 FT-IR spectra of the representative microplastic found in clam tissue samples. Possible types are identified according to the peak position of the spectra. (A) cellulose acetate butyrate; (B) polyethylene terephthalate; (C) bis (2-ethylhexyl) phthalate; (D) polypropylene; (E) cellulose acetate; (F) polyvinyl acetate; (G) poly (methyl methacrylate); (H) ethyl cellulose.

PMMA, sometimes known as acrylic glass, is a popular choice in the automotive and maritime transportation industries due to its more effective impact and ultraviolet resistance. The Gulf of Thailand, where the pier is located, is well-known for being one of Thailand's busiest shipping lanes due to its sea-based industries, such as shipping, which can obtain an abundance of plastics from commercial fisheries areas due to normal wear and tear of fishing gear, as well as commercial shipping and offshore industries derived from plastic abrasive powder and paint flakes.

Moreover, as a result of nearby commercial and industrial expansion, rising urbanization has resulted in microplastic contamination in the Gulf of Thailand. In the presence of oxygen, PMMA can undergo thermal oxidative deterioration, resulting in decreased stiffness and tensile strength, causing it to become breakable and eventually begin to develop a crack (Manoukian et al., 2019; Odli, 2020). Denser PMMA sinks to the seafloor and is ingested by filter feeders.

According to FTIR, 41% of the suspected microplastic particles in this analysis were non-microplastic particles. These microplastics have the potential to be confused with organic matter and other anthropogenic particles. Algae and cellulose fibers are sometimes misidentified as plastics because they break readily and contain a variety of cell layouts. In some cases, chitinous compounds that are glossy and potentially similar to plastics are not completely removed following filtration (Lusher et al., 2020). Aside from that, the centers of spherical organic particles can resemble plastic films, which can deceive our eyes. At times, salt and sand fragments may resemble plastic. Natural polymers such as wool, linen, and cotton can also be confused for microplastics during microscopic analysis due to air microfiber pollution.

3. Microplastic extraction methods

Microplastics in marine species are detected by microscopically analyzing tissues or gastrointestinal contents directly (Lusher et al., 2013) or after extraction by a digestion treatment (Miller et al., 2017). Currently, a variety of digestion methods, such as acid digestion (Van Cauwenberghe & Janssen, 2014; Vandermeersch et al., 2015), KOH digestion (Foekema et al., 2013), hypochlorite digestion (Collard et al., 2015), hydrogen peroxide digestion (Mathalon & Hill, 2014), and protease digestion (Cole et al., 2014), are being used to break down organic matter and make it easier to detect plastic particles. The most effective methods are those that break down tissues without damaging microplastics. In the current study, the detection of microplastics in the contents of clam tissue was made achievable by hydrogen peroxide digestion. Several studies have recently used this digesting approach for discovering microplastics in fish and mussels (Li et al., 2016; Avio et al., 2017; Güven et al., 2017). According to Tsangaris et al. (2015), preliminary testing utilizing hydrogen peroxide on virgin microplastics in powder form (PE, PP, PS, and PET) revealed no changes in the appearance of plastic particles. PE, PP, PVC, PS, and PET particles in the 0.3–1 mm size class had average recovery rates of 86%, which is within the range of recovery rates reported for microplastics following tissue sample digestion with hydrogen peroxide (70–95%) (Miller et al., 2017).

Although the gastrointestinal tracts of fish are frequently examined for microplastics, the entire body of small invertebrates, including mussels, shrimp, and lugworms, is utilized to extract microplastics (Devriese et al., 2015; Li et al., 2015; Van Cauwenberghe et al., 2015; Phuong et al., 2018). In this research, whole calm tissue showed a larger number of microplastics per gram of tissue (4.88 ± 1.65 items g^{-1}) than whole mussel tissues of *Paratapes undulatus* (2.17 ± 0.43 to 2.38 ± 1.28 items g^{-1}) collected in Vietnam (Tran-Nguyen et al., 2023). This method can potentially detect microplastics, especially if the number of microplastics is low. This approach is also well-suited for hydrogen peroxide digestion because the time required for digestion is mostly determined by the amount of tissue to be digested (Li et al., 2015). Figure 7 depicts the organic matter-containing residue of clam tissue degraded by potassium hydroxide.

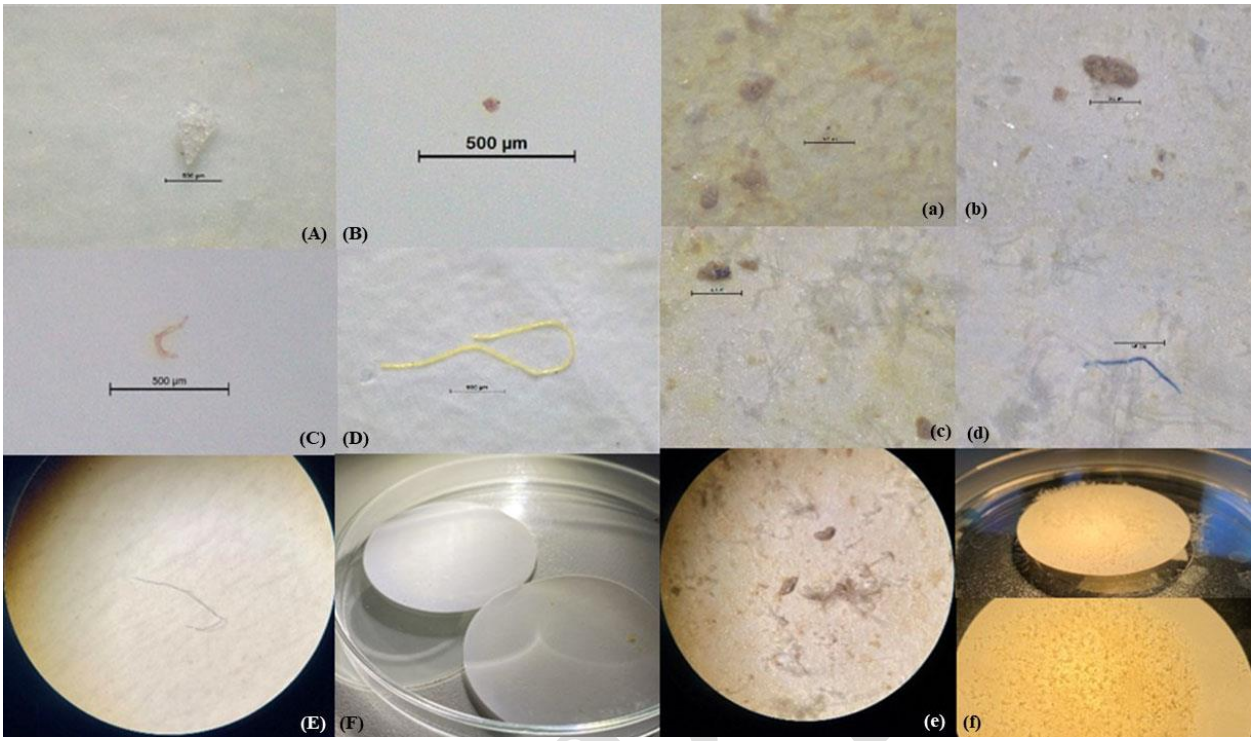

Fig. 7 Microplastic items (A-F) found in *Paratapes undulatus* after digesting clam tissue with 30% hydrogen peroxide and 10% potassium hydroxide (a-f).

Conclusion

The presence of microplastics in clams from the fresh market, where the clams were harvested from the Gulf of Thailand, indicates the ubiquitous presence of microplastics in Thai and worldwide biota. The weights of clam tissue and microplastic differed statistically significantly. The two most prevalent polymer types of microplastics identified in *P. undulatus* were polyethylene terephthalate and cellulose acetate butyrate. The quantity of microplastic discovered was consistent with earlier studies. The microplastics found in clam tissue were mostly fragments with a high percentage of sizes ranging from less than 100 µm to 200 µm, which is similar to the microplastic properties found in estuary waters. According to our results, approximately 1,011 items of microplastic per kilogram per person may enter Thai consumers' bodies from clam consumption, raising concerns about food safety and human health. It is suggested that more research be undertaken to better understand the key factors influencing microplastic abundance in clam species in order to provide effective solutions for reducing microplastic accumulation within clams.

Acknowledgements

This research is funded by Kasetsart University through the Biological Science Program in collaboration with the Faculty of Liberal Arts and Science.

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