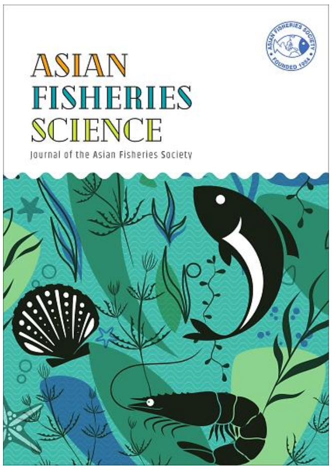


# Volatile Chemical Profiling and Microplastic Inspection of Fish Pastes from Balayan, Batangas, Philippines

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ISSN: 0116-6514
E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2020.33.3.002

## Abstract

Fermented fish pastes (Bagoong) are one of the most commonly used liquid condiments among Asian countries, wherein the production of fish pastes may vary from one Asian country to another. In the Philippines, Balayan is one of the municipalities in the province of Batangas that is popular for its Bagoong Balayan. Chemical profiling from volatile organic compounds (VOCs) can be used to determine aroma-inducing compounds that are specific to this local variant and for quality assessment for food safety. In the meantime, the emerging pollution in the marine environment from persistent organic pollutants (POPs) and microplastics are alarming and threats to food safety. These marine-derived commodities, such as fish pastes may therefore harbour these kinds of pollutants. In this study, Bagoong Balayan samples were subjected to solid-phase microextraction coupled with gas chromatography-mass spectrometry. A total of 29 compounds were detected that passed the minimum match factor of 80 and 14 of them were common in all collected fish paste samples. Some of these compounds were also reported to be in fish paste samples produced in other Asian countries. However, five of them were observed to be found only in Bagoong Balayan, namely 1-octen-3-ol, 1-octen-3-one, 2-nonanone, tridecane, and 2,6,10,14-tetramethylpentadecane. No traces of POPs were found in Bagoong Balayan samples. The presence of microplastics was seen in all of the samples after centrifugation, vacuum filtration, and inspection using a microscope. Most of the microplastics that are present appeared to be fibrous in structure and coloured red or blue.

**Keywords:** volatile organic compounds (VOCs), solid phase microextraction, gas chromatography-mass spectrometry, persistent organic pollutants (POPs)

## Introduction

Fermented fish pastes are one of the most widely used liquid condiments in Asian countries due to its distinctive flavour, texture, and aroma. In the Philippines, fermented fish or shrimp pastes are locally known as Bagoong. It is used as a key cooking ingredient and condiment in Philippine cuisine. It is prepared by mixing salt to shrimp, Caridea or to varieties of locally available fish such as Anchovy, Engraulidae known as anchovies and Mackerel scad, *Decapterus macarellus* (Cuvier, 1833). These are then fermented inside jars for a maximum of 6 months before finally packing them in glass bottles to be sold to consumers (Batangas Philippines, 2012). During fermentation, biochemical reactions take place, which gives rise to the distinct features of the fish paste.

One of the most popular variants of fish pastes in the Philippines can be found in the province of Batangas at a municipality called Balayan (Fig. 1), hence the name Bagoong Balayan. Being near the bay, most residents in Balayan have engaged in the small-scale production of fish pastes as one of their most common sources of income (Department of the Interior and Local Government, 2016). This product is not only popular in Balayan but also throughout the entire Philippines and are being exported to other countries as well. Bagoong Balayan is distinct from other local variants because it is more fluid and less viscous as opposed to another famous local variant made from shrimp, known as Bagoong alamang, which appears as a solid oily paste. However, being mostly a backyard industry, its production may lack the technical capability of implementing rigorous quality control measures and standardised protocols for food safety. Part of this should be ascertained that the fish pastes produced contain no contaminants and pollutants that might be harmful to human health.


Fig. 1. Location of Balayan Bay relative to Balayan, Batangas (OpenStreetMap Contributors, 2020). Image generated using QGIS mapping software (QGIS Development Team, 2019).

Possible pollutants to be observed in fish paste samples are the persistent organic pollutants (POPs). These toxic organic compounds are usually hydrophobic and lipophilic, which resist chemical, biological, and photolytic degradation. They have been known to be present in seawater and can bioaccumulate in the lipids of living organisms that explain the persistence of these chemicals in the food chain. The most prevalent POPs are industrial by-products and pesticides such as polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dichloro-diphenyl-trichloroethane (DDT) (Wenning and Martello, 2014; Huang et al., 2015; UN Environment, 2015).

Along the coast of Balayan Bay, two coal-fired thermal power plants, one cement production plant, and a steel fabrication plant are running their operations. These industries might unintentionally emit POPs that would possibly end up in the seawater. Cement production and coal-fired thermal power plants may emit by-products such as PCDDs, PCDFs, PCBs, and polycyclic aromatic hydrocarbons (PAHs) (Meij and te Winkel, 2007; van Loo, 2008; Rodrigues, 2012). These mentioned POPs together with polychlorinated naphthalenes (PCNs) and polybrominated diphenyl ethers (PBDEs) are also known to be emitted from steel fabrication plants (Odabasi et al., 2010).

Another concern about marine pollution is the prevalence of plastic wastes because they are prone to be broken down into smaller particles called microplastics. Microplastics in the marine environment are defined as minuscule plastic debris in the form of fibres, fragments, beads/pellets or granules (Thompson et al., 2004; Cole et al., 2011) that are less than 5 mm in size as standardised by the National Oceanic and Atmospheric Administration (NOAA) (Arthur et al., 2009; Thevenon et al., 2014; Van Sebille et al., 2015). Several studies have verified the presence of microplastics in the marine environment, organisms, and commodities derived from it. Anchovies are filter-feeders and they can ingest microplastics from contaminated seawater. Also, they would often mistake these microplastics as their prey because some might possess the smell of food (Lusher et al., 2017; Savoca et al., 2017). Samples of Asian green mussels, *Perna viridis* (Linnaeus, 1758), cultured in Bacoor Bay, Cavite, Philippines were found to contain microplastics (Argamino and Janairo, 2016). According to Li et al. (2019), mussels were used as bioindicators for coastal pollution since the presence of microplastics in them can be correlated to the human activity near the coastal area. Microplastics were also found in the guts of amphipods from deep-sea locations including the Mariana Trench (Jamieson et al., 2019). Aside from this, sea salts, lake salts, and rock salts that are commercially available in China, tested positive for the presence of microplastics with sea salts having the highest concentration of microplastic (Yang et al., 2015). Moreover, East Asian seas surrounding entire Japan were found to contain 1,720,000 pieces of microplastics per square kilometre (Isobe et al., 2015). These microplastics can absorb POPs and they can leech other toxic chemicals that are used in the manufacture of plastic to the environment.

Since producing fish pastes, such as Bagoong Balayan, involves fermenting the whole fish itself, these kinds of marine-derived commodities may be contaminated with POPs and microplastics. In this study, the aroma-inducing compounds, as well as any POPs that might be present in Bagoong Balayan samples, were determined using solid-phase microextraction/gas chromatography-mass spectrometry (SPME/GC-MS). The presence of microplastics was also verified using microscopic analysis after centrifugation and filtration and then followed by a confirmatory hot needle test. Tests using other local variants of Bagoong, such as Bagoong alamang, were not included in this study due to their solid character and thus it requires performing a wet acid digestion technique for it to be suitable for filtration.

## Materials and Methods

### *Analysis of volatile compounds using SPME/GC-MS*

Ten different brands of non-FDA approved fish pastes were bought from the wet market and nearby commercial areas in Balayan, Batangas, the Philippines in September 2018. Before obtaining samples for analysis, the bottles containing the fish pastes were shaken thoroughly to achieve homogeneity. All of the ten fish paste samples are dark brown, as shown in Figure 2.

Fig. 2. A sample of the fish paste from Balayan, Batangas.

Amber flasks (60 mL capacity) were thoroughly cleaned and rinsed with acetone. They were placed in an oven for 30 min at 150 °C to dry. Five millilitres (5 mL) from each of the samples were transferred individually to their respective amber flasks. The mouth of the flasks was then covered with aluminium foil which was secured with parafilm. Each sample was heated at 70 °C for 5 min on a hot plate. Heating to this temperature ensured an efficient extraction of the volatiles (Silva et al., 2017). A small hole was created on the aluminium foil covering the mouth of the flask where the needle containing the SPME fibre will be inserted. The fibre was then exposed to the heated fish paste sample for 3 min.

After extraction through SPME, the fibre with the adsorbed compounds is then introduced into the Shimadzu GCMS-QP2020 (Japan) equipped with an SH-Rxi-5Sil MS capillary column with the following dimensions: 30 m × 0.25 mm ID × 0.25 µm (df). Installed at the injector port are straight/SPME inlet liner for SPME injection and Merlin Microseal Septa general-purpose kit from Restek (USA). The oven temperature was programmed at 50 °C for 5 min with an increased rate of 10 °C/10 min until it reached the final temperature of 200 °C. The mass spectrometer conditions were as follows: interface temperature at 250 °C, electron impact mode, the acquisition is set to scan mode. Only compounds with molecular weight ranging from 35 $m.z^{-1}$ to 500 $m.z^{-1}$ were recognised by the mass spectrometer. The volatile compounds were then identified with the aid of NIST 2017 Mass Spectral Library and Wiley Registry 11$^{th}$ Edition. All GC-MS analyses were done in triplicate.

## *Determination of the presence of microplastics*

The method to determine the presence of microplastics was adapted from Argamino and Janairo (2016). All glassware used was thoroughly cleaned and rinsed with filtered deionised water before using. Approximately 45 mL from each sample was centrifuged at 15,000 rpm for 5 min. The supernatants obtained were then transferred in an Erlenmeyer flask and covered with parafilm for storage. A blank which only contains distilled water was also prepared in the same manner.

Afterwards, the samples were vacuum filtered using a Buchner funnel and cheesecloth to initially separate unwanted macroscopic particles. The filtrates that were obtained were again vacuum filtered using a Whatman filter paper grade 1 (11 µm pore-size). Finally, the resulting filtrates were vacuum filtered for the last time using a Whatman filter paper grade 5 (2.5 µm pore-size). Immediately after, the filter papers used were stored in individual Petri dishes and left to dry while covered to avoid possible contamination. The dried filter papers were examined under a dissecting microscope at 400× magnification to detect the presence of microplastics. To further confirm if the detected microscopic particle is made of plastic, a hot needle test was also conducted. Under the presence of a hot needle, materials made of plastic will either melt or change in its structure unlike cellular or organic materials which would only char.

# Results

## *Analysis of volatile compounds using SPME/GC-MS*

Detected compounds were tabulated based on its peak area in each sample afterwards and compared if there are any that are common in all 10 fish paste samples. Compounds with a match factor less than 80 were disregarded. The common compounds among each of the samples were identified as to how it contributed to the distinctive aroma of fish paste and compared to existing literature regarding the inspection of volatile compounds in fish paste produced in other Asian countries.

A total of 355 chemical compounds were detected from all of the samples after performing peak area analysis of the chromatograms produced by each sample. Figures 3a and b show the overlaid chromatograms of the fish paste samples A to J.

After careful assessment of each peak, further reduction of the peaks that has a match factor of 80 and above were considered to be relevant peaks. Any compound below the match factor of 80 was removed from the list. A total of 29 compounds were then listed after as presented in Table 1. Stein (1999) and Hübschmann (2015) note that compound identification by library search that gives a match factor of 80 and above are considered to provide a high degree of confidence in identification. Compound identification of the GC-MS analysis was based on library search. Two libraries were used to identify the peaks namely Wiley Registry 11$^{th}$ Edition and NIST 2017 mass spectral library. These two libraries are the most comprehensive mass spectral library available (Wiley Registry, 2017). Confidence in

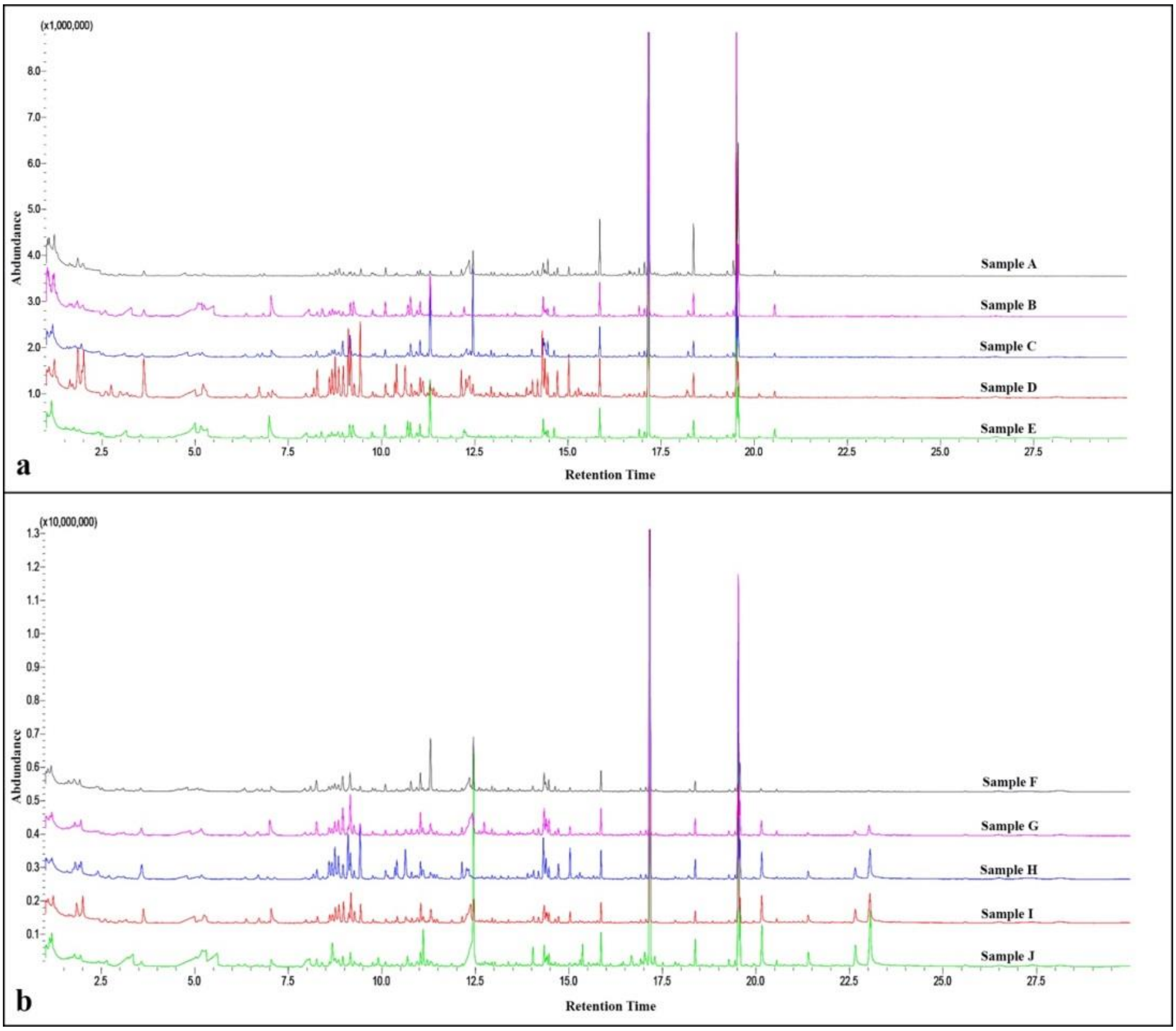



Fig. 3. Overlaid chromatograms of fish paste samples (a) A to E and (b) F to J.

the library search matches was based on library quality and its comprehensiveness, citations in present materials and if it is known to exist with an assigned registry number (Stein, 2012).

Finally, only 14 compounds were observed to be present in all 10 samples of fish paste collected from Balayan, Batangas. Figure 4 lists these compounds together with their corresponding average relative abundance.

## Determination of the presence of microplastics

The microplastics observed are mostly fibrous in structure and some are in the form of pellets and fragments. The images, description, and the number of particles of the microplastics obtained in each sample are shown in Table 2. All scale bars are 250 μm (0.25 mm). After performing the hot needle test, most of the microplastics detected in the filtered fish paste samples were completely melted especially the fragments and pellets. On the other hand, the fibrous microplastics detected have been either completely melted or broke into smaller fragments and a change in colour was observed.

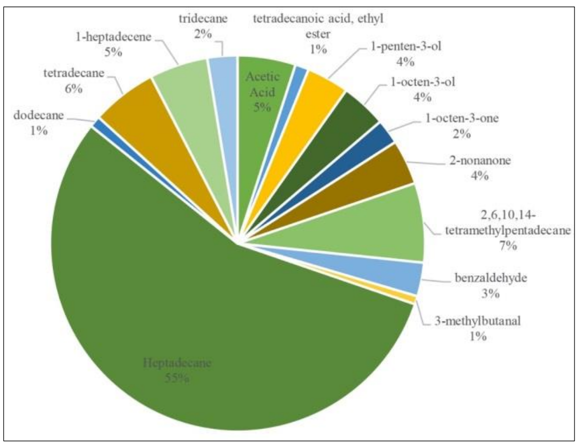



Fig. 4. Average relative abundance of the 14 volatile organic compounds (VOCs) common in all of the fish paste samples.

Table 1. Volatile compounds detected in the fish paste samples.

| Volatile compounds | Relative abundance (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J |
| Acids | | | | | | | | | | |
| acetic acid | 0.75 | 0.93 | 1.52 | 2.12 | 0.78 | 2.08 | 0.92 | 0.65 | 0.88 | 1.16 |
| benzoic acid | 1.16 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Alcohols | | | | | | | | | | |
| 1-penten-3-ol | 0.67 | 0.48 | 0.10 | 0.18 | 2.71 | 0.97 | 0.76 | 1.20 | 1.31 | 0.23 |
| 1-octen-3-ol | 0.27 | 0.33 | 0.58 | 0.32 | 1.80 | 0.83 | 1.07 | 2.16 | 1.32 | 0.57 |
| Aldehydes | | | | | | | | | | |
| 3-methylbutanal | 0.15 | 0.29 | 0.14 | 0.11 | 0.46 | 0.15 | 0.08 | 0.07 | 0.16 | 0.05 |
| hexanal | ND | ND | ND | 2.46 | ND | ND | ND | ND | ND | ND |
| (E)-oct-2-enal | ND | ND | ND | 1.18 | ND | ND | ND | ND | ND | ND |
| 2,6-nonadienal, (E,Z)- | ND | ND | ND | 0.84 | ND | ND | ND | 0.76 | ND | ND |
| benzeneacetaldehyde | ND | ND | ND | ND | ND | 0.61 | ND | ND | ND | ND |
| 5-isopropenyl-2-methylcyclopent-1-enecarboxaldehyde | ND | 1.94 | 4.82 | 4.82 | 3.38 | 4.53 | ND | ND | ND | ND |
| 2,4-heptadienal, (E,E)- | 0.28 | ND | ND | 2.78 | ND | ND | ND | 2.78 | 0.76 | ND |
| benzaldehyde | 0.13 | 0.19 | 0.53 | 0.17 | 1.27 | 1.42 | 1.17 | 0.66 | 0.60 | 0.34 |
| Alkanes and alkenes | | | | | | | | | | |
| (3E)-2,7-dimethyl-1,3,7-octatriene | ND | ND | ND | 0.95 | ND | ND | ND | 0.78 | ND | ND |
| 2,6,10,14-tetramethylpentadecane | 0.18 | 2.58 | 2.11 | 2.33 | 0.98 | 1.78 | 1.61 | 1.50 | 1.40 | 1.77 |
| heptadecane | 64.29 | 0.93 | 4.44 | 12.04 | 0.74 | 3.91 | 12.58 | 12.46 | 7.79 | 11.42 |
| dodecane | 0.11 | 0.09 | 0.38 | 0.13 | 0.29 | 0.37 | 0.32 | 0.15 | 0.31 | 0.15 |
| tetradecane | 1.87 | 1.28 | 1.52 | 1.40 | 1.28 | 1.32 | 1.26 | 1.21 | 1.09 | 1.08 |
| 1-heptadecene | 0.52 | 0.26 | 0.25 | 0.23 | 9.55 | 0.22 | 0.20 | 0.18 | 0.26 | 0.20 |
| tridecane | 0.60 | 0.34 | 0.81 | 0.37 | 0.74 | 0.72 | 0.97 | 0.54 | 0.60 | 0.41 |
| Esters | | | | | | | | | | |
| tetradecanonic acid, ethyl ester | 0.23 | 0.58 | 0.25 | 0.50 | 0.23 | 0.19 | 0.24 | 0.09 | 0.26 | 0.21 |
| benzoic acid, ethyl ester | 0.96 | ND | 5.18 | 5.18 | ND | 4.01 | ND | ND | 1.66 | 1.48 |
| Ketones | | | | | | | | | | |
| 1-octen-3-one | 0.17 | 0.24 | 0.24 | 0.22 | 1.15 | 0.41 | 0.50 | 1.16 | 0.75 | 0.22 |
| 7'-oxaspiro[cyclopropane-1,4'-tricyclo[3.3.1.0(6,8)]nonan-2'-one] | ND | ND | ND | 2.90 | ND | ND | ND | 2.60 | ND | ND |
| 2-nonadecanone | 0.68 | ND | ND | ND | ND | ND | ND | ND | ND | 0.81 |
| 3-undecen-2-one | ND | ND | ND | 1.35 | ND | ND | ND | 1.34 | 0.63 | ND |
| 3,5-octadien-2-one | ND | ND | ND | 1.83 | ND | ND | ND | 2.30 | ND | ND |
| 2-nonanone | 0.21 | 0.69 | 1.13 | 0.89 | 0.73 | 1.48 | 1.30 | 0.85 | 1.29 | 0.55 |
| Heterocyclic compounds | | | | | | | | | | |
| pyrazine, 3-ethyl-2,5-dimethyl- | ND | ND | ND | ND | ND | 0.93 | ND | ND | ND | ND |
| 2-pentyl-furan | ND | ND | ND | 1.30 | ND | 2.04 | 2.07 | ND | 1.58 | ND |

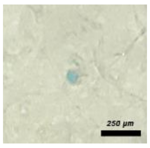
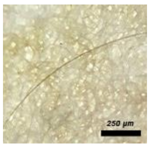
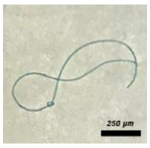
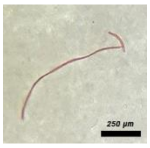
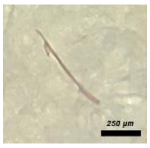
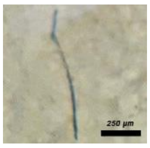
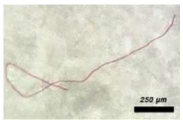
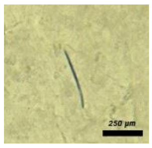
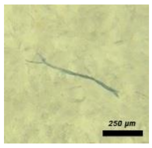
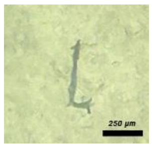
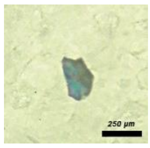
ND = Not detected.

## Discussion

Comparing the results with previous studies using fish paste samples from other Asian countries, it was observed that acetic acid, 1-penten-3-ol, and 3-methylbutanal were also the predominant aroma-inducing compounds in both Thai and Korean fish paste (Pham et al., 2008). Similar with Bagoong Balayan, Vietnamese fish paste called "nuoc mam" as well as Japanese fish paste called "shottsuru" were also identified to contain acetic acid, benzaldehyde, and dodecane (Sanceda et al., 1986). Also, a Chinese fish paste known as "yu lu" was discovered to contain similar volatile compounds including acetic acid, benzaldehyde, tetradecanoic acid ethyl ester, 1-heptadecene, and heptadecane (Jiang et al., 2011). Malaysian traditional fish paste called "budu" was again found to contain acetic acid, benzaldehyde, tetradecane, and 3-methylbutanal (Mohamed et al., 2012).

The prevalence of acetic acid in these fermented fish pastes is due to the presence of acetic acid bacteria called Acetobacter, which produces acetic acid as a metabolic product (Johnston and Gaas, 2006). Also, 1-penten-3-ol gives off a pungent horseradish odour (Bedoukian Research Inc., 2016). In comparison, 3-methylbutanal contributes to a malty scent (Zhou et al., 2002). Benzaldehyde has a bitter almond odour (National Center for Biotechnology Information, 2020a) while tetradecanoic acid ethyl ester provides a waxy odour (Food and Agriculture Organization of the United Nations, 2020). Heptadecane, dodecane and tetradecane are all saturated hydrocarbons that provide fuel or gasoline-like odour (National Center for Biotechnology Information, 2020b). In contrast, 1-heptadecene has been identified to be found in daisy and in safflower (FooDB, 2019a).

Table 2. Summary of the microplastic particles present from each of the 10 fish paste samples.

| Sample | Appearance viewed from a dissecting microscope at 400× magnification | Description (colour and morphology) | Number of microplastic particles present |
|---|---|---|---|
| A |  | Blue pellet | 2 |
| |  | Dark blue fragment | |
| B |  | Black fibre | 1 |
| C |  | Blue fibre | 2 |
| |  | Red fibre | |
| D |  | Red fibre | 1 |
| E |  | Blue fibre | 1 |
| F |  | Red fibre | 1 |
| G |  | Blue fibre | 1 |
| H |  | Blue fibre | 1 |
| I |  | Blue fibre | 1 |
| J |  | Blue fibre Greenish biofouled fragment | 1 |
| Mean of microplastic particles per 45 mL sample (n = 10) | | | 1.2 |

Among all the 14 detected volatile compounds that were confirmed to be responsible for the fish paste samples' aroma, only five of these were not reported in any other fish paste produced in other Asian countries. These compounds include 1-octen-3-ol, 1-octen-3-one, 2-nonanone, tridecane, and 2,6,10,14-tetramethylpentadecane. 1-octen-3-ol, is commonly found in herbs and spices which gives off a typical mushroom scent (Chemical Entities of Biological Interest, 2015). 1-octen-3-one are commonly found in fruits and they contribute to the aroma produced by metallic objects and blood (Human Metabolome Database, 2012). On the other hand, 2-nonanone contributes to a sweet, earthy odour. Tridecane is commonly found in food such as lemon balm, lime, safflower (FooDB, 2019b). Pristane or 2,6,10,14-tetramethylpentadecane is an odourless oily compound usually found in fish liver (National Center for Biotechnology Information, 2020c). No compounds in the volatiles of Bagoong Balayan were found to belong to the 12 initial POPs according to the 2008 Stockholm convention (Stockholm Convention, UN Environment Programme, 2008).

The detection of the microplastic particles in the samples was guided by considering the following criteria: no cellular or organic structures are visible, it does not taper towards the ends and has a threedimensional, it will be examined with extra care and under a microscope with higher magnification if the colour of the particles is not obvious (Norén, 2007). Upon detection of microplastic particles from the samples, these were then characterised based on their appearance and size. Microplastics were categorised based on their morphology and colour (Crawford and Quinn, 2016).

Each of the ten 45 mL samples of Bagoong Balayan was found to have at least one particle of microplastic. On average, there are about 1.2 particles per 45 mL of fish paste. All of the microplastics seen are blue or red. And again, the majority of them are fibrous. As fish pastes are manufactured through fermentation, bacterial growth is present in the samples. Microplastics are commonly homogeneously-coloured. However, under the presence of bacteria, the possibility of biofouling, the accumulation of bacteria on moist surfaces, is inevitable. One of the microplastics detected (Table 2, sample J) was observed to have undergone biofouling. After the hot needle test, it underwent complete melting and colour change from blue to black (Fig. 5).

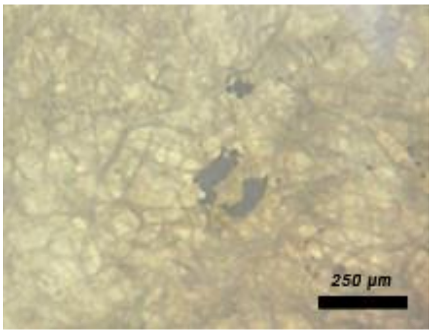



Fig. 5. Biofouled microplastic fragment from sample J after the hot needle test.

These fish paste samples have been verified to be contaminated with microplastics. These contaminants may either be from marine pollution that is mainly due to the effect of improper waste disposal, or from equipment or tools used in the manufacture of the fish pastes that may be of plastic material that is old and has been repeatedly used over time which makes it very susceptible to degradation.

## Conclusion

In the assessment for the chemical compounds responsible for the aroma of the fish paste samples collected from Balayan, Batangas, a total of 355 chemical compounds were detected after performing SPME coupled with GC-MS. Among these, only 14 compounds were discovered to be common among all 10 fish paste samples which are acetic acid, tetradecanoic acid ethyl ester, 1-penten-3-ol, 1-octen-3-ol, 1-octen-3-one, 2-nonanone, benzaldehyde, 3-methylbutanal, tridecane, heptadecane, dodecane, tetradecane, 1-heptadecene, and 2,6,10,14-tetramethylpentadecane. Most of these compounds are known to contribute to the distinctive aroma of fermented fish paste.

The compounds acetic acid, benzaldehyde, and 3-methylbutanal that are in fish paste samples in other Asian countries such as Korea, Vietnam, Japan, Thailand, China, and Malaysia are also present in Bagoong Balayan. However, five compounds were observed to be present only in Bagoong Balayan and these are 1-octen-3-ol, 1-octen-3-one, 2-nonanone, tridecane, and 2,6,10,14-tetramethylpentadecane. These five compounds may be responsible for the distinctive aroma and taste of the popular fish paste produced in Balayan, Batangas which makes it unique and different from other fish paste products available in other Asian countries. In addition, no POPs were detected in the Bagoong Balayan.

Microplastics were also found to be present in all of the samples of Bagoong Balayan. There are about an average of 1.2 particles of microplastic per 45 mL of fish paste. This is a possible indication of microplastic pollution in the Balayan bay area and nearby bodies of water. Another probable cause is the manufacturers' usage of plastic equipment to produce their Bagoong Balayan. If the usage of plastic materials is inevitable, it should immediately be replaced once there are signs of tear and degradation.

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