



UNIVERSITI PUTRA MALAYSIA

***ISOLATION AND CHARACTERIZATION OF MICROPLASTICS IN
MARINE FOODSTUFF***

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**ISOLATION AND CHARACTERIZATION OF MICROPLASTICS IN MARINE
FOODSTUFF**

By

ABOLFAZL GOLIESKARDI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Master of Science**

October 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of Science

ISOLATION AND CHARACTERIZATION OF MICROPLASTICS IN MARINE FOODSTUFF

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ABOLFAZL GOLIESKARDI

October 2017

Chairman: Ali Karami Varnamkhasti, PhD
Faculty: Medicine and Health Sciences

Due to the persistence and ubiquity of plastic debris in the marine environment, these contaminants have become one of the major threats to the ecosystem. Disposed plastic debris may never degrade but instead breakdown to smaller particles, termed microplastic (MP). Microplastics have been previously detected in a wide array of organisms, however, MP loads in processed seafood products such as canned sardine and saltwater products such as commercial salt originating from various regions of the world has never been investigated. To extract MPs from organisms, an efficient digesting solution is required. In an attempt to select an optimum digesting solution, the efficiency of different oxidative agents (NaClO or H₂O₂), bases (NaOH or KOH), and acids [HCl or HNO₃; concentrated and diluted (5%)] in digesting fish tissues at room temperature (RT, 25 °C), 40, 50, or 60 °C was assessed and those treatments that were efficient in digesting the biological materials (>95%) were evaluated for their compatibility with eight major plastic polymers (assessed through recovery rate, Raman spectroscopy analysis, and morphological changes). Following the selection of appropriate solution, the MP loads in 20 brands of canned sardine originating from 13 countries were assessed. In addition, the presence of MPs in 17 brands of commercial salts originating from 8 countries has been investigated. Among the tested solutions, NaClO, NaOH, and diluted acids did not efficiently digest the biological matrices. The H₂O₂ treatment only at 50 °C resulted in satisfactory digestion efficiency, although it decreased the recovery rate of nylon-6 and nylon-66 and changed the colour of polyethylene terephthalate fragments. Concentrated HCl and HNO₃ treatments at RT fully digested the fish tissues, but also had a destructive impact on most polymers, particularly nylon-6 and nylon-66. Potassium hydroxide solution was able to fully eliminate the biological matrices at all temperatures. However, at 50 and 60 °C, it reduced the recovery rate of polyethylene terephthalate and polyvinyl chloride, and changed the colour of nylon-66. According to our results, treating biological materials with 10% KOH solution and incubating at 40 °C was time and cost-effective, efficient in

digesting biological materials, and had no impact on the integrity of the plastic polymers. Study on canned sardine has shown that plastic particles were absent in 16 brands while between 1 and 3 plastic particles per brand were found in the other 4 brands. Out of 21 extracted particles, 28.5% were plastic polymers, 42.8% were additives, 4.7% were non-plastic, and 23.8% were unidentified. The most abundant plastic polymers were polypropylene (33.3%) and polyethylene terephthalate (33.3%). The analysis of commercial salts for MP loads has revealed that MPs were absent in one brand while others contained between 1 and 10 MPs/Kg of salt. Out of the 72 extracted particles, 41.6% were plastic polymers, 23.6% were pigments, 5.50% were amorphous carbon, and 29.1% remained unidentified. The most common plastic polymers were polypropylene (40.0%) and polyethylene (33.3%). Although this study presents a low level of anthropogenic particles intake from the canned sardine (1 to 5 anthropogenic particles per individual per annum) and salts (maximum 37 particles per individual per annum) which warrants negligible health impacts on consumers, however, due to the progressive fragmentation of MPs in the environment, the number of MPs in these products tend to increase. As such, it is highly recommended that the quantification of MPs in foodstuffs to be included as one of the components of food safety management system.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGASINGAN DAN PENCIRIANMIKROPLASTIK DALAM MARIN BAHAN MAKANAN

Oleh

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Disebabkan oleh kehadiran saki-baki plastik dalam persekitaran marin, bahan pencemar ini telah menjadi salah satu ancaman utama kepada ekosistem. Saki-baki plastik yang terbuang tidak akan luput, tetapi sebaliknya akan berpecah kepada partikel-partikel kecil dipanggil mikroplastik (MP). Mikroplastik telah dikesan di dalam pelbagai jenis organisma, namun demikian beban MP dalam produk makanan laut yang diproses sardin dalam tin dan produk air masin seperti garam komersil yang berasal dari pelbagai kawasan di dunia belum pernah dikaji lagi. Untuk mengasingkan MP dari organisma, satu larutan yang menghadam diperlukan. Dalam usaha memilih kaedah penghadaman yang optima, keberkesanan agen oksidatif (NaClO or H_2O_2), bes-bes (NaOH or KOH) dan asid-asid [HCl or HNO_3 ; pekat atau cair (5%)] dalam tisu ikan yang menghadam pada suhu bilik (RT, 25 °C), 40, 50, atau 60 °C dinilai dan rawatan-rawatan yang berkesan dalam menghadam bahan-bahan biologi (>95%) dinilai untuk menguji keberkesanannya dengan lapan polimer plastik utama (dinilai melalui kadar pemulihan, analisis spektroskopi Raman dan perubahan morfologi). Berikutan pilihan larutan yang sesuai, beban MP dalam 20 jenama sardin dalam tin berasal dari 13 buah negara telah dikaji. Tambahan pula, kehadiran MP dalam 17 jenama garam komersial yang berasal dari 8 negara telah dikaji. Dalam kalangan larutan yang diuji, NaClO , NaOH , dan asid cair tidak menghadam matriks penghadaman dengan berkesan. Rawatan H_2O_2 hanya pada 50 °C membawa kepada penghadaman yang baik, walaupun ia mengurangkan kadar pemulihan nilon-6 dan nilon-66 dan mengubah warna serpihan *polyethylene terephthalate*. Rawatan HCl dan HNO_3 pada suhu bilik menghadam penuh tisu ikan tetapi ia juga memberi kesan buruk kepada kebanyakan polimer, terutamanya nilon-6 dan nilon-66. Larutan potasium hidroksida berjaya membuang terus matriks biologi pada semua suhu. Namun demikian, pada suhu 50 dan 60 °C, ia mengurangkan kadar pemulihan polyethylene terephthalate dan polyvinyl chloride, dan mengubah warna nilon-66. Mengikut keputusannya, merawat bahan biologikal larutan 10% KOH dan

inkubasi pada 40 °C menjimatkan masa dan murah, berkesan dalam menghadam bahan biologi, dan tidak memberi apa-apa impak kepada integriti polimer plastik. Kajian ke atas sardin dalam tin menunjukkan bahawa partikel plastik tidak ada dalam 16 jenama di mana di antara 1 dan 3 partikel plastik dijumpai dalam setiap 4 jenama yang lain. Daripada 21 partikel yang diekstrak, 28.5% adalah polimer plastik, 42.8% adalah bahan penambah, 4.7% bukan plastik, dan 23.8% tidak dikenalpasti. Polimer plastik terbanyak adalah polypropylene (33.3%) dan polyethylene terephthalate (33.3%). Analisis garam komersial untuk beban MP menunjukkan bahawa MP tidak ada dalam satu jenama sementara yang lain mengandungi 1 dan 10 MPs/Kg garam. Daripada 72 partikel yang diekstrak, 41.6% adalah polimer plastik, 23.6% adalah pigmen, 5.50% karbon amorfus, dan 29.1% masih tidak dapat dikenalpasti. Polimer plastik yang paling lazim adalah polypropylene (40.0%) dan polyethylene (33.3%). Walaupun kajian ini membawa kepada aras rendah pengambilan partikel antropogenik dari sardin dalam tin (1 kepada 5 partikel antropogenik setiap individu setahun) dan garam (maksima 37 partikel setiap individu setahun) yang memberi impak yang buruk kepada kesihatan ke atas pengguna, walaubagaimanapun, disebabkan oleh perpecahan progresif MP dalam persekitaran, bilangan MP dalam produk-produk ini semakin meningkat. Oleh itu, saranan terbaik adalah mengkuantifikasi MP dalam barang makanan untuk dimasukkan sekali sebagai salah satu komponen sistem pengurusan keselamatan makanan.

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I certify that an Examination Committee has met on 5 October 2017 to conduct the final examination of Abolfazl Golieskardi on his thesis entitled “Isolation and Characterization of Microplastics in Marine Foodstuff” in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science..

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LIST OF ABBREVIATIONS

BPA	Bisphenol A
DDT	Dichlorodiphenyltrichloroethane
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
H ₂ O ₂	Hydrogen peroxide
HCL	Hydrochloric acid
HDPE	High-density polyethylene
HNO ₃	Hydrochloric acid
HPLC	High-performance liquid chromatography
KOH	Potassium hydroxide
LDPE	Low-density polyethylene
M-Cell	Microfold cells
Min	Minutes
MP	Microplastic
NaCl	Sodium chloride
NaClO	Sodium hypochlorite
NaOH	Sodium hydroxide
NaI	Sodium iodide
NPCG	North Pacific Central Gyre
NY6	Nylon-6
NY66	Nylon-66
PAH	Polynuclear Aromatic Hydrocarbon
PCB	Polychlorinated biphenyl
PET	Polyethylene terephthalate
POP	Persistent Organic Pollutants
PP	Polypropylene

PS	Polystyrene
Pyr-GC-MS	Pyrolysis–gas chromatography–mass spectrometry
ROS	Reactive oxygen species
Rpm	Rotate per minute
SEM	Scanning Electron Microscope
USA	United States of America
Nm	Nanometer
μm	Micrometer
mm	Millimeter
MPs/Kg	Microplastics per kilogram
MP/m^3	Microplastic per cubic meter
mL	Milliliter
L	Liter
g/mL	Gram per milliliter
g/cm^3	Gram per cubic centimeter
Kg/L	Kilogram per liter
g	gram
Kg	Kilogram
w/v	Weight over volume
v/v	Volume over volume
h	Hour
$^{\circ}\text{C}$	Degrees Celsius

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Aquatic bodies are exposed to various anthropogenic pollutions. Anthropogenic pollution comes in different forms such as solid waste, noise, chemical, and biological. Plastic debris has been a major environmental concern since 1940s and a main subject of scientific studies (Thompson et al., 2009). There is now supporting evidence describing ecological, economical, and social impacts associated with plastic debris in the aquatic bodies worldwide.

Plastics have become an indispensable part of human life due to their low cost, versatility, and corrosion resistance properties (Thompson et al., 2009). Since the mass plastic production in the 1940s (Cole et al., 2011), the annual global demand for plastics has been increasing and is currently standing at 322 million tons (PlasticsEurope, 2016). Many plastics are either directly disposed to the natural environment or by passing through the wastewater treatment process and end up in aquatic bodies (Andrady, 2011). As such, plastics may pose a serious threat from an ecological point of view. It was reported that there are more than 5 trillion plastic items with the net weight of over 250,000 tons are currently floating at the surface of oceans (Eriksen et al., 2014) whereby 5 to 13 million tons of plastic waste are added to this amount on an annual basis (Jambeck et al., 2015). To accelerate the breakdown process of plastic debris in the environment, biodegradable plastic polymers have been developed (O'Brine and Thompson, 2010). Their development was proposed to replace the conventional plastic polymers. However, some of the degradable materials may fragment into microscopic particles and may not degrade any quicker than the conventional plastic polymers (Barnes et al., 2009; O'Brine and Thompson, 2010).

The future input of plastic waste tend to increase due to the high production rate (PlasticsEurope, 2016) and shifting in demographic in favor of immigration to coastal areas (Ribic et al., 2010). The origin of MPs in the environment can be divided into primary and secondary sources. Primary MPs are those manufactured to be of microscopic size (Cole et al., 2011). For example, microbeads employed in cosmetic industries (Fendall and Sewell, 2009) may be directly disposed into the marine environment by passing through the wastewater treatment. Secondary MPs are the result of the breakdown of larger plastic debris into the smaller particles in the size range of 1 to 1000 μm (Karami et al., 2016b).

Microplastics are widely detected in many saltwater and freshwater ecosystems such as Singapore's coastal water (Ng and Obbard, 2006) and Laurentian Great Lakes (Eriksen

et al., 2013). The accumulation of MPs in saltwater bodies may be concerning as saltwater is used in the production of abiotic products such as salt. Therefore, MPs may also be collected by the saltpans along with saltwater for the salt manufacturing. Furthermore, due to their abundance and small size, they are readily ingested by the aquatic organisms such as fish which may be caught for human consumption (Figure 1.1).

The vast distribution of MPs coupled with their small size will make them bioavailable to a wide array of aquatic organisms. For example, MPs were detected in seafood products cultured for human consumption such as fish (Rochman et al., 2015) and mussels (Van Cauwenberghe and Janssen, 2014). However, MP loads in the processed seafood products such as canned sardine which are widely consumed globally and the edible part of fish have never been assessed which is of great importance as MPs have shown the ability to translocate to different organs of organisms (e.g., Lu et al., 2016).

Microplastics may elicit their harmful effect through different pathways. Many potentially harmful additives such as bisphenol A (BPA) and phthalates are incorporated during manufacturing of plastics which are known to disrupt endocrine system (Halden, 2010). Furthermore, owing to their lipophilic characteristic, MPs will likely absorb these harmful chemical compounds such as heavy metals and pesticides (Rios et al., 2010) from the marine environment and will subsequently release them when ingested (Bakir et al., 2014). Microplastics may also directly cause micro-injuries in aquatic organisms such as blockage of the digestive system, internal abrasion, and ulceration (Wright et al., 2013b).

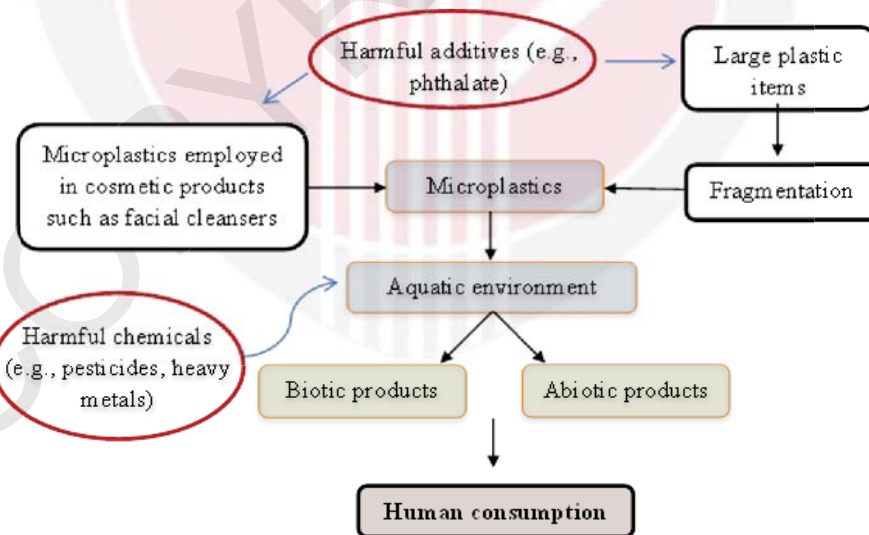


Figure 1.1: Transfer of MPs into human food web

So far, researchers have employed various protocols to extract MPs from biological matrices. Some studies have relied on visual speculation of digestive system under a microscope to estimate the MP loads (Lusher et al., 2013). However, this method cannot

be implemented when investigating the presence of MPs in many other aquatic organisms such as mussels. In addition, the number of MPs may be underestimated as the MPs embedded in tissues may not be detected. As such, it was evident that to investigate the presence of MPs in biological tissue, a digestion phase is required to degrade the organic matters without impacting the plastic polymers.

1.2 Problem Statement

To date, a number of digesting agents were employed to remove the biological materials namely, acids (Van Cauwenberghe and Janssen, 2014), bases (Foekema et al., 2013), oxidative agents (Nuelle et al., 2014), and enzyme (Cole et al., 2014). Although the digesting solutions employed by researchers appeared to be somewhat successful in the isolation of MPs from organisms, the information on their performance in degrading organic matters and more importantly, their corrosiveness towards plastic polymers across different temperature are not sufficient.

To date, there are many speculations on the MP contamination of the aquatic environment which led to the investigation of MP loads in the seafood products. However, no study was conducted to assess the MP loads in processed seafood products such as canned sardines that are widely known and directly consumed without prior cleaning process.

Despite the detection of MPs in various seafood products, information on the presence of MPs in abiotic saltwater products which could contain contaminants from the water is scares. Yang et al. (2015) have conducted the only available study on the abiotic products and have reported a high level of MPs (up to 681 MPs/Kg) in Chinese brands of commercial salt samples. However, the chemical composition of every extracted particle was not identified, but the characterization was based on categorizing the particles according to their morphology and identifying the representative particles. Nonetheless, employing the visual identification as standalone in an attempt to identify particles is not a reliable procedure as the number of anthropogenic debris could be significantly overestimated. Furthermore, there is currently no report on the presence of MPs in table salts originating from other parts of the world with a potential 6 billion consumers, not to mention billions of other organisms such as cattle that need to consume salt regularly (Martin and Ward, 1973).

1.3 Significance of the Study

A method has been developed on how to isolate MPs from aquatic organisms based on density separation. This study will provide a benchmark on MP isolation in aquatic organisms.

This thesis has been focused on MP isolation in canned sardine. Previous studies on MPs in fish were limited to MP isolation from alimentary tract of fish species. However, this could not highlight the transfer of MPs into human food web as the digestive tract is usually not consumed.

An experiment was conducted to investigate the MP contamination in the Chinese salt samples. No study was done to estimate the MP loads in the commercial salts manufactured by other regions of the world with billions of potential consumers.

1.4 Objectives of the Study

The general aim of this research was to investigate the transfer of MPs into human food web at a global scale and furthermore, to estimate a preliminary dietary exposure to MPs. This aim was attained through completion of the following objectives:

1. To develop a protocol to efficiently digest biological materials without affecting the polymer integrity.
2. To isolate and characterize MPs in various brands of canned sardine, originating from different countries.
3. To isolate and characterize MPs in different brands of commercial table salts, manufactured by various countries.

1.5 Research Hypotheses

1. The developed protocol will efficiently digest organic matters without any impact on the polymer integrity.
2. Canned sardines manufactured by different countries are contaminated with MPs.
3. Commercial table salts originating from different countries are contaminated with MPs.

1.6 Novelties

This is the first study to:

1. Investigate MP loads in any processed seafood products.
2. Investigate the MP loads in table salts manufactured by different countries.

1.7 Organization of Thesis

This thesis is organized into seven chapters as follows:

Chapter one: This chapter summarizes the general introduction, problem statement, significance of the study, objectives of the study, research hypotheses, and novelties.

Chapter two: This review chapter explains the plastic debris and their impact on the environment and reviews the previous field studies on plastic accumulation in the environment, MP translocation in organs, biological effects of MPs, and potential hazard associated with MPs.

Chapter three: This chapter explains the methods developed and followed in isolation and characterization of MPs in canned sardines and commercial table salts.

Chapter four: This chapter is the published research article on developing a protocol to isolate MPs in fish.

Chapter five: This chapter is a submitted manuscript on MP isolation in canned sardine.

Chapter six: This chapter is the published research article on MP isolation in commercial salts.

Chapter seven: The last chapter provides summary, conclusion, and recommendation for future studies.

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