EXTRACTION, CHARACTERIZATION, AND MICROENCAPSULATION OF PATIN [*Pangasianodon hypophthalmus* (Sauvage, 1878)] FISH OIL

By

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

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Chair : **Tan Chin Ping, PhD**
Faculty : **Food Science and Technology**

Patin fish (*Pangasianodon hypophthalmus*) is a freshwater fish that gains much attention nowadays due to its abundance of monounsaturated fatty acids and a considerable amount of polyunsaturated fatty acids in its oil, particularly omega-3 and omega-6 fatty acids. The enzyme-assisted oil extraction appears to be a more environmental friendly, safer, and lower energy consumption method compared to the conventional oil extraction techniques. This study aimed to optimize the enzymatic extraction conditions of patin fish oil using the Box-Behnken design. The crude patin fish oil (PFO) extracted using the optimized extraction parameters were subsequently refined and characterized. The effects of alcalase enzyme concentration (0.5, 1.5, and 2.5%), hydrolysis temperature (45, 50, and 55 ºC), and duration (1, 2, and 3 h) towards the yield and quality of PFO were evaluated. The highest PFO yield (23.91%) with a low peroxide value (4.33 mEq/kg oil) was obtained from patin fish flesh including its viscera and extracted by using 2.46% enzymes with 1 h incubation time at 50.4 ºC.

The crude PFO extracted using the optimized extraction parameters was subjected to further chemical refining in order to obtain the refined PFO. The refined PFO exhibited a low free fatty acid content (0.75%) and peroxide value (5.49 meq O_2/kg oil), as well as a high saponification value (215 mg KOH/g oil). Besides, the refined PFO consisted of 48.65% saturated fatty acids, 39.98% monounsaturated fatty acids, and 11.37% polyunsaturated fatty acids, with n-3 and n-6 fatty acids contributing up to 2.65% and 8.72% of the total polyunsaturated fatty acids, respectively.

The microencapsulation of PFO would be able to mask the fishy odor as well as for ease of application, especially for fortification purposes in various food products. This study investigated the influence of spray drying inlet air temperatures and core-to-wall ratios towards the physicochemical characteristics of the PFO microcapsules. Maltodextrin was employed as the primary wall material, and the PFO microencapsulation was performed using the pilot-scale spray dryer. Two main parameters, namely the inlet air temperature (160 – 180 ºC) and core-to-wall ratio (1:2 – 1:4) were varied. The full characterization (eg: moisture content, water activity, encapsulation efficiency, solubility, hygroscopicity, density properties, flowability, and powder morphology) was conducted on the PFO microcapsules. The concentration of the wall material and the spray-drying inlet air temperature significantly ($p < 0.05$) impacted the encapsulation efficiency and the solubility of the PFO microcapsules. Besides, the increasing maltodextrin concentration significantly ($p < 0.05$) decreased the hygroscopicity and enhanced the PFO microcapsules' flowability. The highest encapsulation efficiency (91.54%) was achieved when the PFO was encapsulated using a core-to-wall ratio of 1:4 at 180 ºC. Furthermore, the PFO microcapsules produced using the optimized spray-drying parameters and formulation were found to be spherical and exhibited smooth surfaces. The high microencapsulation efficiency and good surface morphology of PFO microcapsules demonstrated a high stability of the PFO microcapsules produced using the optimized conditions.

The findings justified the feasibility of PFO microcapsules being used for nutritional fortification purposes in food products or as a dietary supplement. The utilization of freshwater fish as an alternative source for fish oil production could contribute to more sustainable aquaculture growth and development.

Keywords : patin fish; freshwater; enzymatic extraction; microencapsulation

SDG: GOAL 2: Zero hunger; GOAL 3: Good health and well-being; GOAL 12: Responsible consumption and production; GOAL 14: Life below water.

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

PENGEKSTRAKAN, PENCIRIAN DAN MIKROENKAPSULASI MINYAK IKAN PATIN [*Pangasianodon hypophthalmus* (Sauvage, 1878)]

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Ikan patin ((*Pangasianodon hypophthalmus*) adalah ikan air tawar yang mendapat perhatian meluas hari ini kerana terdapat banyak komposisi asid lemak mono tak tepu dan sejumlah besar asid lemak poli tak tepu didalam minyaknya, terutama asid lemak omega-3 dan omega-6. Pengekstrakan minyak menggunakan enzim merupakan kaedah pengekstarakan yang lebih mesra alam, selamat dan penggunaan tenaga yang rendah berbanding dengan kaedah pengekstarakan minyak secara konvensional. Matlamat penyelidikan ini adalah untuk mengkaji tahap optimum bagi parameter yang terlibat dalam pengekstrakkan enzim dengan menggunakan reka bentuk Box-Behnken. Minyak ikan patin mentah yang telah diekstrak menggunakan parameter pengekstarakn yang dioptimumkan kemudiannya menjalani proses penulenan dan dicirikan. Kesan kepekatan enzim alcalase (0.5, 1.5 and 2.5%), suhu pengekstrakan (45, 50 and 55 ºC) dan masa (1, 2 and 3 j) terhadap hasil dan kualiti minyak ikan patin telah dinilai. Hasil minyak ikan patin tertinggi (23.91%) dengan nilai peroksida rendah (4.33 mEq/kg oil) diperoleh dengan menggunakan kepekatan enzim sebanyak 2.46% selama 1 j masa pengekstarakan pada suhu 50.4 ºC. Minyak ikan patin mentah yang telah diekstrak menggunakan parameter pengekstarakan yang dioptimumkan seterusnya ditulenkan untuk mendapatkan minyak ikan patin tulen. Minyak ikan patin tulen menunjukkan kandungan asid lemak bebas (0.75%) dan nilai peroksida (5.49 meq O_2/kg oil) yang rendah, serta nilai saponifikasi yang tinggi (215 mg KOH/g oil). Selain itu, minyak ikan patin tulen mengandungi 48.65% asid lemak tepu, 39.98% asid lemak mono tak tepu, dan 11.37% asid lemak poli tak tepu, dengan asid lemak n-3 dan n-6 menyumbang kepada 2.65% dan 8.72% daripada keseluruhan jumlah asid lemak poli tak tepu.

Mikroenkapsulasi minyak ikan patin dapat melitup bau hanyir ikan serta memudahkan penggunaan terutamanya untuk tujuan penguatan ke dalam pelbagai produk makanan. Penyelidikan ini mengkaji pengaruh suhu udara masuk bagi pengeringan sembur dan nisbah *core-to-wall* terhadap ciri-ciri fizikokimia mikrokapsul minyak ikan patin. Maltodekstrin telah digunakan sebagai bahan dinding utama, dan mikroenkapsulasi

minyak ikan patin dijalankan dengan menggunakan pengering sembur skala perintis (*pilot-scale*). Dua parameter utama, iaitu suhu udara masuk (160 – 180 ºC) dan nisbah *core-to-wall* (1:2 – 1:4) telah dipelbagaikan. Pencirian penuh (contoh: kandungan lembapan, aktiviti air, kecekapan enkapsulasi, keterlarutan, higroskopi, sifat ketumpatan, kebolehaliran dan morfologi serbuk) telah dijalankan pada mikrokapsul minyak ikan patin. Kepekatan bahan dinding dan suhu udara masuk pengeringan sembur memberi kesan yang ketara (p < 0.05) ke atas kecekapan pengkapsulan dan keterlarutan mikrokapsul minyak ikan patin. Selain itu, peningkatan kepekatan jumlah maltodextrin mengurangkan dengan ketara (p < 0.05) nilai higroskopi dan meningkatkan kebolehaliran mikrokapsul minyak ikan patin. Kecekapan pengkapsulan (91.54%) dicapai apabila minyak ikan patin dikapsulkan menggunakan nisbah *core-to-wall* 1:4 pada suhu 180 ºC. Tambahan pula, mikrokapsul minyak ikan patin yang dihasilkan menggunakan parameter pengeringan semburan dan formulasi yang telah dioptimumkan didapati mempunyai morfologi permukaan yang licin dan berbentuk sfera. Kecekapan mikroenkapsulasi dan morfologi permukaan yang baik bagi mikrokapsul minyak ikan patin menunjukkan bahawa ianya mempunyai kestabilan yang tinggi.

Dapatan penyelidikan ini mewajarkan bahawa keupayaan mikrokapsul minyak ikan patin untuk digunakan dalam tujuan fortifikasi dalam produk makanan atau sebagai bahan makanan tambahan. Ini menunjukkan bahawa penggunaan ikan air tawar sebagai sumber alternatif bagi penghasilan minyak ikan boleh menyumbang kepada pertumbuhan dan pembangunan akuakultur yang lebih mampan.

Kata kunci: ikan patin, air tawar, pengekstrakan enzimatik, mikroenkapsulasi

SDG: MATLAMAT 2: Kelaparan sifar; MATLAMAT 3: Kesihatan dan kesejahteraan yang baik; MATLAMAT 12: Tanggungjawab terhadap penggunaan dan pengeluaran lestari; MATLAMAT 14: Kehidupan di dalam air.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AI	Acidity index
AV	Anisidine value
ANOVA	Analysis of variance
AOCS	American Oil Chemists' Society
BBD	Box-Behnken Design
CI	Carr index
DE	Dextrose equivalent
DHA	Docosahexaenoic acid
EE	Encapsulation efficiency
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
GC-FID	Gas chromatography-flame ionization detector
HR	Hausner ratio
IV	Iodine value
LC	Long chain
MAE	Microwave-assisted extraction
MUFA	Monounsaturated fatty acids
n-3	Omega-3 fatty acids
n-6	Omega-6 fatty acids
O/W	Oil in water
pH	Potential of hydrogen
PTFE	Polytetrafluoroethylene
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
R^2	Coefficient of determination
RSM	Response surface methodology
SC-CO_2	Supercritical carbon dioxide
SEM	Scanning electron microscopy
SFA	Saturated fatty acids
SFE	Supercritical fluid extraction
SV	Saponification value

CHAPTER 1

INTRODUCTION

1.1 Background of study

Fish oils are often extracted from the fish's fat tissues and their by-products. Typically, the long-chain unsaturated fatty acids included in fish oils make them more complex than those found in oils extracted from land animals or plants (Abdulkadir et al., 2010). Fish oils are unique due to the number and types of unsaturated fatty acids they contain (Adeniyi & Bawa, 2006). Polyunsaturated fatty acids (PUFAs) notably eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be responsible for the health advantages associated with fish oil intake. Omega-3 fatty acids found in fish have been shown to lower cholesterol and lower the risk of cardiovascular diseases (Nurul Izzah et al., 2016).

The growing demand for fish oil as a food supplement due to its well-documented health advantages makes fish lipids a novel and high-potential economic commodity. Hitherto, the market estimation implies that marine resources are getting scarcer due to the increasing demand for PUFA (Šimat et al., 2019). The recent research indicates that freshwater fish can be a potential alternative resource for fish oil production, as it can be obtained at a lower cost compared to marine fish resources. Furthermore, viscera storage fat is present throughout the body of freshwater fish, primarily in its internal organs, and the liver stores fat. Due to the health-promoting effects, various research works have been conducted to incorporate fish oil in food, pharmaceutical, and cosmetic products (Venugopalan et al., 2021).

1.2 Problem statements

The aquaculture sector has recorded a production of 411,782 metric tonnes which is valued at RM3.304 billion and equivalent to 22% of the national fisheries production (*Department of Fisheries Malaysia*, 2019). Freshwater aquaculture commodities contributed 25% to the total production with RM780 million worth. Being the second-largest freshwater fish species in Malaysia, patin (*Pangasianodon hypophthalmus*) fish were selected for this study because it was relatively easy to harvest and contained high values of its muscle and by-products for oil extraction owing to its huge market size. The disposal of the fatty deposits in the liver and visceral fat tissues is actually expensive and harmful to the ecosystem. In 2016, world fish production was around 171 million tons, of which 88% was destined for human consumption and the rest was used for non-food products (FAO,2018). Approximately, 76 million tons of fish waste including viscera were generated in that year. These residues of the fish industry contain approximately 40-65% of oil and repurposing these fish waste can aids in reducing environmental contamination. In comparison to the African catfish (*Clarias gariepinus)* which is the largest farmed fish species, the average size of these fish sold commercially is only 0.2 kg/fish, meaning that they contain a negligible amount of muscle and its by-products per fish and therefore are not ideal for large-scale fish oil production (Shabanikakroodi, 2014).

There are various oil extraction techniques, for instance using solvent, direct steaming, supercritical fluid, vacuum distillation, hydraulic pressing, and microwave-assisted extraction. All the aforementioned methods require the usage of either toxic solvents, high temperature, high pressure, or moisture reduction of the sample prior to oil extraction. Environmental concerns have led to an increasing interest in using enzymatic extraction as the alternative method for extracting lipids from fish. This method employs commercial and low-cost food-grade neutral proteases (Qi-Yuan et al., 2016). The bacterial protease, alcalase which has been used in this study has been reported to be one of the most effective proteases for extracting fish oil and other protein hydrolysates of fish at temperatures between 50 and 70 ºC (See et al., 2011).

After the fish oil enzymatic extraction, the collected crude fish oils require further refining in order to meet the oil quality standards for human consumption (Crexi et al., 2010). The chemical refining technique which includes degumming, neutralization, washing, bleaching, and deodorization is often used to enhance fish oil's qualities by eliminating the impurities (Menegazzo et al., 2014). Furthermore, deodorization is a crucial stage in the refining process which requires monitoring as the high temperature may initiate the breakdown of long-chain fatty acids. Therefore, temperature control is essential during the deodorization of crude fish oil to avoid PUFA degradation.

Furthermore, the major challenge of fish oil incorporation into food applications is the unpleasant fishy odour and taste. Microencapsulation can be a good strategy to mask the fishy odour and taste of the fish oil, as well as delay PUFA oxidation. Microencapsulation is also one of the most suitable approach in order to achieve stability, storage and also delivery of the product (Hashim et al., 2021). Among the microencapsulation techniques, spray drying appears to be the most popular approach for microencapsulation of oils and flavours (Koç et al., 2015) owing to the low operating cost and high production yield compared to the other methods. The careful consideration of the wall material used for the spray drying process is vital to protect the core components from oxidation and other chemical reactions (Chew et al., 2018).

The most commonly used wall material in the spray drying process is maltodextrin because it is inexpensive and has high solubility, neutral flavor and low viscosity at high concentrations; however, it has no emulsifying properties (Ferraz et al., 2022). The effects of different core-to-wall ratios and spray-drying inlet air temperatures on the patin fish oil microcapsules were investigated. The physical parameters such as encapsulation efficiency, hygroscopicity, and morphological properties were analyzed to evaluate the quality of the spray-dried patin fish oil microcapsules.

1.3 Objectives

The objectives of this study were:

1. To optimize the enzymatic extraction parameters in enhancing the patin fish oil yield and quality using response surface methodology (RSM)
2. To optimize the formulation and spray-drying inlet air temperature in the production of patin fish oil microcapsules

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