UNIVERSITI PUTRA MALAYSIA

COMPARISON AND DETERMINATION OF PROPERTIES OF OIL EXTRACTED USING SOXHLET AND SUPERCRITICAL FLUID FROM NILE TILAPIA (Oreochromis niloticus Linnaeus) PROCESSING WASTE

MASOUMEH KHAZAALI

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By

MASOUMEH KHAZAALI

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

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DEDICATION

In the name of Allah, Most Gracious, Most Merciful
This thesis is dedicated to:

My caring and devoted parents who are always giving me unlimited courage, passion and support
and
My sister and brothers for their moral support and love

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

COMPARISON AND DETERMINATION OF PROPERTIES OF OIL EXTRACTED USING SOXHLET AND SUPERCRITICAL FLUID FROM NILE TILAPIA (*Oreochromis niloticus* Linnaeus) PROCESSING WASTE

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MASOUMEH KHAZAALI

June 2016

Chairman : Professor Jamilah binti Bakar, PhD
Faculty : Food Science and Technology

Fish oil contains the important omega-3 polyunsaturated fatty acids (PUFAs), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). EPA and DHA extracted from fish oil are reported to have beneficial effects to human health which resulted in a world-wide demand. There are already several methods available for extracting fish oil, with varying degrees of yield and quality. Supercritical fluid carbon dioxide extraction (SFE-CO_2) is an emerging process for the extraction of edible oils containing labile components like PUFAs. Therefore, the objectives of this study were (i) to extract and compare the distribution of oil and physicochemical properties of tilapia oil extracted from the viscera, head, skin and flesh using the soxhlet and supercritical fluid methods; (ii) to optimize the yield and extracted EPA and DHA from the identified part using supercritical fluid extraction (SFE-CO_2) technique. The results from the soxhlet method revealed that the distribution of oil was significantly ($p < 0.05$) different among the head, viscera, skin and flesh which is as expected. The highest oil yield was obtained from the viscera (51.81%) followed by the head (30.12%), skin (16.88%) and flesh (6.78%). The chemical properties of head, viscera, flesh and skin oil were 4.37-9.87 meq O_2/kg, 116.94-125.65 g I_2/100 g, 4.49-9.86 meq/kg and 13.23-25.08 meq/kg for PV, IV, p-AnV and TOTOX value, respectively. Although the average fatty acids composition of the viscera, head, skin and flesh samples were similar, the highest amounts of SFAs (49.74 g/100 g oil), MUFAs (24.40 g/100 g oil) and PUFAs (22.14 g/100 g oil) were found in the flesh, viscera and head, respectively. The optimization of oil yield, extraction of EPA and DHA based on central composite design with pressure (20-40 MPa), temperature (35-75 ℃), flow rate (2.5-4.5 mL.min^{-1}) and extraction time (2-4 h) from the viscera was determined. The highest oil yield (40.02% on dry weight basis), EPA (0.46 g/100 g oil) and DHA (1.53 g/100g oil) was obtained at 40 MPa, 57.22 ℃, 3.4 mL.min^{-1} and 3.23 h with non-significant ($p > 0.05$) lack of fit and high R^2 ($p > 0.90$). The viscera oil from supercritical fluid extraction (SFE-CO_2) had lower total oxidation value (15.3 meq/kg) and higher amount of PUFAs composition (28.71 g/100 g oil) than oil obtained from soxhlet method (25.08 meq/kg and 17.32

g/100 g oil for TOTOX value and PUFAs, respectively). Hence, tilapia visceral oil is better extracted using supercritical fluid extraction method.

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

PERBANDINGAN DAN PENENTUAN CIRI-CIRI MINYAK EKSTRAKSI DARIPADA SISA IKAN NILE TILAPIA (*Oreochromis niloticus linnaeus)* MELALUI KAEDAH SOXHLET DAN FLUIDA SUPERKRITIK

Oleh

MASOUMEH KHAZAALI

Jun 2016

Pengerusi : Professor Jamilah binti Bakar, PhD
Fakulti : Sains dan Teknology Makanan

Minyak ikan mengandungi asid lemak omega-3 politaktepu (PUFAs), asid eikosapentaenoik (EPA) dan asid dokosaheksaenoik (DHA) yang penting. EPA dan DHA yang diekstrak daripada minyak ikan dilaporkan bermanfaat kepada kesihatan manusia. Oleh sebab demikian, ia mendapat permintaan yang tinggi dari serata dunia. Terdapat beberapa kaedah yang telah digunakan untuk mengekstrak minyak ikan, dengan kadar pengeluaran dan mutu yang berlainan. Ekstraksi fluida superkritik CO_2 (SFE-CO_2) adalah satu proses yang baru muncul untuk pengekstrakan minyak makan yang mengandungi komponen labil seperti PUFA. Tujuan kajian ini adalah untuk (i) mengekstrak dan membandingkan taburan minyak dan sifat fisiokimia minyak tilapia yang diekstrak daripada viscera, kepala, kulit dan isi, menggunakan kaedah soxhlet dan kaedah SFE-CO_2; (ii) mengoptimumkan pengeluaran minyak dan pengekstrakan EPA dan DHA daripada bahagian ikan terpilih menggunakan kaedah SFE-CO_2. Seperti yang dijangkakan, hasil kajinan kaedah soxhlet menunjukkan bahawa taburan minyak berbeza dengan ketara ($p<0.05$) antara kepala, viscera, kulit dan isi ikan. Kandungan minyak paling banyak didapati daripada viscera (51.81%), diikuti dengan kepala (30.12%), kulit (16.88%) dan isi ikan (6.78%). Sifat-sifat kimia minyak daripada kepala, viscera, isi dan kulit ikan adalah 4.37-9.87 meq O_2/kg (nilai peroksida), 116.94-125.65 g I_2/100 g (nilai iodine), 4.49-9.86 meq/kg (p-anisidine) and 13.23-25.08 meq/kg (nilai TOTOX) masing-masing. Walaupun purata kandungan asid lemak daripada viscera, kepala, kulit dan isi ikan adalah hampir sama, kandungan tertinggi SFA (49.74 g/100 g minyak), MUFA (24.40 g/100 g minyak) dan PUFA (22.14 g/100 g minyak) diperoleh daripada bahagian kulit, viscera dan kepala ikan. Pengoptimuman hasil minyak, pengekstrakan EPA dan DHA daripada viscera ikan telah ditentukan dengan tekanan (20-40 MPa), suhu (35-75 ℃), kadar aliran (2.5-4.5 $mL.min^{-1}$) dan masa pengekstrakan (2-4 jam) berdasarkan reka bentuk komposit pusat. Hasil tertinggi minyak (40.02% berasaskan berat kering), EPA (0.46 g /100 g minyak) dan DHA (1.53 g /100g minyak) telah diperolehi pada 40 MPa, 57.22 ℃, 3.4 mL.min-1 dan 3.23 jam dengan "lack of fit" yang tidak signifikan ($p>0.05$) dan R^2 yang tinggi ($p>0.90$). Minyak viscera daripada pengekstrakan fluida superkritik SFE-CO_2

mempunyai jumlah nilai pengoksidaan yang lebih rendah (15.3 meq/kg) dan komposisi PUFA yang lebih tinggi (28.71 g/100 g minyak) berbanding minyak yang diperolehi daripada kaedah soxhlet (25.08 meq/kg dan 17.32 g/100 g minyak untuk nilai TOTOX dan PUFA masing-masing). Oleh itu, minyak visceral tilapia adalah lebih baik diekstrak menggunakan kaedah pengekstrakan fluida.

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I certify that a Thesis Examination Committee has met on 20 June 2016 to conduct the final examination of Masoumeh Khazaali on her thesis entitled "Comparison and Determination of Properties of Oil Extracted using Soxhlet and Supercritical Fluid from Nile Tilapia (*Oreochromis niloticus* Linnaeus) Processing Waste" in accordance with the 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.

Members of the Thesis Examination Committee were as follows:

Tan Chin Ping, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Abdulkarim Sabo Mohammed, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Mohamed Elwathig Saeed Mirghani, PhD
Associate Professor
International Islamic University Malaysia
Malaysia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 September 2016

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

Jamilah Bt. Bakar, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Jinap Selamat, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

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BUJANG KIM HUAT, PhD
Professor and Dean
Dean School of Graduate Studies
Universiti Putra Malaysia

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Signature: ______________________________

Name of Chairman of Supervisory Committee: Professor Dr.Jamilah Bt. Bakar

Signature: ______________________________

Name of Member of Supervisory Committee: Professor Dr.Jinap Selamat

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
AIs	Adequate Intakes
ALA	Alpha Linolenic Acid
ANOVA	Analysis of Variance
AnV	Anisidine Value
CCD	Central Composite Design
CCL	Carbon Chain Length
CHD	Coronary Heart Disease
CSE	Conventional Soxhlet Extraction
CVD	Cardiovascular Diseases
DF	Degrees of Freedom
DHA	Docosahexaenoic Acid
DOE	Design of Experiment
EFAs	Essential Fatty Acids
EP	European Pharmacopeia
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acids
FPH	Fish Protein Hydrolysate
GC	Gas Chromatography
GC-FID	Gas Chromatography Flame Ionization Detector
GC-MS	Gas Chromatography Mass Spectrometry
GIFT	Genetically Improved Farmed Tilapia
GOED	Global Organization for EPA and DHA
HCl	Hydrochloric Acid
HDL	High Density Lipoprotein
IOM	Institute Of Medicine
ISSFAL	International Society for the study of Fatty Acids and Lipids

IV	Iodine value
KI	Potassium Iodide
LA	Linoleic Acid
LCPUFA	Long Chain Polyunsaturated Fatty Acid
LDL	Low Density Lipoprotein
MI	Myocardium Infarction
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acids
PV	Peroxide value
RSM	Response Surface Methodology
SC-CO_2	Supercritical Carbon Dioxide
SCFAs	Short Chain Fatty Acids
SD	Standard Deviation
SFA	Saturated Fatty Acid
SFE	Supercritical Fluid Extraction
SS	Sum of Squared
TOTOX	Total Oxidation
US FDA	United States Food and Drug Administration
VLDL	Very low Density Lipoprotein

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CHAPTER 1

INTRODUCTION

1.1 Background

Fish and fishery products are excellent sources of essential fatty acids (EFAs), protein, vitamins and minerals; with all of these being crucial to a healthy diet (Gokoglu et al., 2004). Extensive worldwide farming of fish and shellfish has increased significantly and the number of fish harvested through aquaculture is actually the same as those harvested from the wild (FAO, 2010). The main species groups in aquaculture are freshwater and marine fishes, molluscs and crustaceans. The supply of marine fish is predicted to show low growth and the prospects of increasing the production of these fishes are on the pessimistic side (Vannuccini, 2005). However, the potential for enhanced supply is exceedingly good for freshwater fish because of the contribution of aquaculture production (Mohamad et al., 2010). In recent years, the production of freshwater fish has been on the rise in Malaysia. The most common species of freshwater fish being cultivated are catfish (*Clarias nieuhofii*), tilapia (*Oreochromis niloticus)* and patin (*Pangasianodon hypophthalmus*) (Silva & Turchini, 2009). The Nile Tilapia (*Oreochromis niloticus*) is one of the most significant and popular choices of fish species in fish farming (Santos et al., 2013). The popularity of this variety of fish in aquaculture is due to its rapid growth, high resistance to very poor water quality and diseases. In fact, tilapia is now being produced in over 100 countries (Yones et al., 2013). Among all the continents, Asia has seen the most rapid increase in tilapia aquaculture production.

During fish processing, a large amount of waste which include viscera, head, blood, skin and scale are usually discarded (Potaros et al., 2009). These by-products could be used as the raw materials for fish oil extraction. Unfortunately, this is not being practiced at present. Several studies have shown that fatty acids (FAs) from fish oils, particularly two of the constituent FAs, which are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may have a beneficial role in promoting good human health (Russo, 2009; Domingo et al., 2007; Kris-Etherton, 2003). These ω-3 FAs are necessary for normal growth and development in the human body. Furthermore, the ω-3 FAs are also associated with reduced risks of cardiovascular diseases, diabetes mellitus, arthritis, hypertension, cancer and a wide range of inflammatory and autoimmune disorders (Lopez-Huertas, 2010). The EPA and DHA can be obtained directly from their natural sources or extracted and purified to form fish oil supplement. At present, fish oil is commercially extracted from marine species.

In the area of food technology, an extraction process is used for the following: 1) to recover essential and important compounds, 2) to isolate desired components, such as antioxidants, from food sources and 3) to remove any contaminant and undesirable compounds, such as cholesterol and heavy metals (mercury or copper). In recent times, supercritical fluid technology has emerged as an extraction method for heat-labile valuable compounds like EPA and DHA. It reduces the use of toxic organic solvents and does not require the application of high temperatures, thus preventing the oxidation of valuable compounds such as omega-3 FAs (Moret et al., 2000). The supercritical fluid technology, which usually utilizes carbon dioxide (CO_2) as solvent, has many advantages such as low critical pressure and temperature for extraction. In addition, the final extracts are usually free of contaminants and impurities, and the CO_2 used can be easily removed from the extracts (Sarker et al., 2012).

1.2 Problem Statement and Hypothesis

The main concern of a fish factory is the management of a large amount of fish wastes during the processing of fish products (Potaros et al., 2009). In reality, these by-products can be used as the raw materials for fish oil extraction. In extracting fish oils, several methods have been reported with varying extract yields and qualities (Maqsood et al., 2012). Hitherto, the general focus has been on oil extraction technologies which can achieve maximum oil yield. However, in recent times, there has been an emergence of new technologies which can achieve maximum oil yield and at the same time, minimize the presence of undesirable impurities in the final extract (Amusan, 2008). The conventional methods for fish oil extraction such as rendering and Soxhlet extraction have disadvantages such as requiring high temperatures and long processing times, which can lead to the decomposition or degradation of the thermally-labile compounds, and the rapid oxidation of these compounds may have negative health effects on human (Lee et al., 2012). On the other hand, the use of supercritical fluid CO_2 extraction (SFE-CO_2) has attracted substantial attention because it is a safe and environmental-friendly method. The quality of oil extracted by using SFE-CO_2 is also better due to the milder operation conditions involved.

1.3 Objectives

The overall objectives of this research were to determine and compare the quality of viscera oil extracted by using SFE-CO_2 and conventional methods, and to optimize the extraction of EPA and DHA using SFE-CO_2. The specific objectives of this study were:

1) To extract and compare the distribution of oil and physicochemical properties of tilapia oil extracted from the viscera, head, skin and flesh using the soxhlet and supercritical fluid methods
2) To optimize the yield and extracted EPA and DHA from the identified part using supercritical fluid extraction (SFE-CO_2) technique.

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