UNIVERSITI PUTRA MALAYSIA

QUALITY, THERMAL BEHAVIOR AND FATTY ACID COMPOSITION OF LIPID EXTRACTED FROM SARDINE AND TUNA WASTES

ALI KHODDAMI

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QUALITY, THERMAL BEHAVIOR AND FATTY ACID COMPOSITION OF LIPID EXTRACTED FROM SARDINE AND TUNA WASTES

By

ALI KHODDAMI

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

Oct 2009
To my beloved family

Father, Mother, and sisters
QUALITY, THERMAL BEHAVIOR AND FATTY ACID COMPOSITION OF LIPID EXTRACTED FROM SARDINE AND TUNA WASTES

By

ALI KHODDAMI

Oct 2009

Chairman : Dr. Azis Ariffin, PhD

Faculty : Food Science and Technology

Fish lipid contains long-chain n-3 (Omega-3) PUFA, particularly EPA (eicosapentaenoic acid, C20:5) and DHA (docosahexaenoic acid, C22:6). Consumption of these PUFAs has been perceived to be important in human nutrition, health, and disease prevention. In this context, there is significant demand for fish lipid. Currently, fish lipid is extracted from fish muscle or liver of herring, mackerel and cod. Sardine and tuna, which are important industrial fishes, produce substantial amount of wastes. The waste of Sardinella lemuru and Euthynnus affinis consist of head, intestine and liver. Therefore the target of the study was to extract the n-3 essential fatty acid rich lipid from the waste with modified Kinsella extraction method by using chloroform-methanol (toxic solvent) and hexane-acetone extraction method by using hexane-acetone (less toxic solvent) and establish the physico-chemical properties of the lipid with a view to use as nutritional supplement or other prospective applications. The yield of extraction, free fatty acid content (FFA), peroxide value (PV), anisidine value (AV),
saponification value (SV), iodine value (IV) and lipid composition (neutral and polar lipid) of the extracted lipid from these two fish species wastes (head, intestine and liver) were determined. Thermal behavior (cooling and melting points) and fatty acid composition of the respected lipid were also evaluated.

The yield of lipid extraction of *S. lemuru* liver showed the highest value than head and intestine in both extraction methods. *E. affinis* head lipid yield indicated significant difference (*P < 0.05*) with other lipids abstracted from intestine and liver in both extraction methods.

Among different lipid sources (head, intestine and liver), the free fatty acid, peroxide value and anisidine value significantly increased (*P < 0.05*) from head to liver. This increase was observed in all lipid samples extracted by hexane-acetone. The saponification value of the waste lipid samples were in the range of 108 – 197 but significant increases were observed in waste lipid extracted by hexane-acetone. The highest iodine value was found in head lipid in both fish species with significant changes (*P < 0.05*) with other waste lipid samples in both extraction methods. Higher polarity of solvent used for lipid extraction (chloroform-methanol) increased the extracted polar lipid in fish waste lipid than lower polarity solvents (hexane-acetone). Fifteen fatty acids (FA) were determined from all waste samples except sardine intestine. The major fatty acid were: palmitic (C16:0), oleic (C18:1) and docosahexaenoic (C22:6) acids. The fish waste lipids showed similar fatty acid composition but the proportion of the fatty acids differ. Among different lipid sources, highest concentration of PUFA especially n-3 fatty acids were detected in head lipid
samples. The concentration of respective PUFA was in lower content in lipid extracted by hexane-acetone. The n6 / n3 fatty acid ratio of the respective head, liver and intestine lipid samples showed values lower than 1. Differential scanning calorimetry results for fish waste lipid samples indicated that higher unsaturation in lipid sample showed lower cooling and melting temperature.
Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KUALITI, CIRI TERMA DAN KOMPOSISI ASID LEMAK DARI LEMAK YANG DIEKSTRAK DARIPADA BAHAN BUANGAN IKAN SARDIN DAN TUNA

Oleh

ALI KHODDAMI

Oct 2009

Pengerusi: Dr. Azis Ariffin, PhD

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Minyak ikan mengandungi asid lemak politaktepu (PUFA) rantai panjang n-3 (Omega-3), terutamanya EPA (asid eikosapentaenoik) dan DHA (asid dokosaheksaenoik). Pengambilan PUFA penting terhadap nutrisi, kesihatan dan pencegahan penyakit pada manusia. Dalam hal ini, permintaan terhadap minyak ikan sangat tinggi. Pada masa kini, minyak ikan biasanya diekstrak daripada otot ikan atau hati ikan hering, ikan pelata dan ikan kod. Sardin (Sardinella lemuru) dan tuna (Euthynnus affinis) yang merupakan tangkapan penting dalam industri ikan, menghasilkan banyak bahan buangan. Bahan buangan ini terdiri daripada kepala, usus dan hati ikan. Oleh sebab itu, maklumat utama kajian ini adalah untuk mengekstrak minyak yang kaya dengan asid lemak perlu n-3 daripada bahan buangan ikan tersebut dengan menggunakan cara pengekstrakan yang
berlainan serta melaporkan ciri-ciri fizikokimia minyak tersebut dari segi kesesuaiannya sebagai bahan makanan tambahan atau untuk penggunaannya pada bidang lain. Dua cara pengekstrakan minyak ikan tersebut ialah kaedah pengubahsuaian Kinsella (melibatkan penggunaan pelarut bertoksik iaitu kloroform-metanol) dan kaedah heksana-aseton (melibatkan penggunaan pelarut tidak bertoksik iaitu heksana-aseton).

Kajian ini bertujuan untuk menentukan hasil pengekstrakan, kandungan asid lemak bebas (FFA), nilai peroksida (PV), nilai anisidin (AV), nilai saponifikasi (SV), nilai iodin (IV) dan komposisi lemak (neutral dan polar). Selain itu, kelakuhan terma (titik penyejukan dan pencairan) dan komposisi asid lemak turut dikaji.

Kedua-dua cara pengekstrakan minyak menunjukkan hati ikan S. lemuru menghasilkan kandungan lipid yang lebih tinggi berbanding kepala dan ususnya. Manakala kandungan minyak kepala ikan E. affinis menunjukkan perbezaan yang ketara (p<0.05) berbanding usus dan hati untuk kedua-dua cara pengekstrakan minyak. Kandungan asid lemak, nilai peroksida dan nilai anisidin minyak bahan buangan ikan mengalami peningkatan yang ketara (p<0.05) daripada kepala ke hati ikan. Peningkatan ini berlaku kepada semua minyak yang diektrak dengan kaedah heksane-aseton. Nilai SV berada dalam lingkungan (108-197) tetapi peningkatan ketara diperhatikan untuk minyak yang diekstrak dengan kaedah heksana-aseton. Minyak yang diekstrak daripada kepala untuk kedua-dua spesies ikan mempunyai nilai IV paling tinggi dan berbeza secara bererti (p<0.05) berbanding minyak daripada bahagian lain untuk kedua-dua cara pengekstrakan minyak. Pengekstrakan minyak dengan menggunakan pelarut berpolar...
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Finally, my deepest gratitude goes to my family, father, mother and sisters, for being so supportive and helpful. Thank you, thank you and love you all.
I certify that an Examination Committee has met on 20 October, to conduct the final examination of Ali Khoddami on his thesis entitled “Quality, Thermal Behavior and Fatty Acid Composition of Lipid Extracted from Sardine and Tuna Wastes” in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(106)] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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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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Date: 14 January 2010
DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

_______________________
ALI KHODDAMI
Date: 29 June 2009
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HAM       Hexane-Acetone extraction Method
HSD       Honestly Significant Different
HUFA      High Unsaturated Fatty Acid
id        Internal Diameter
IV        Iodine value
kg        Kilogram
KI        Potassium Iodide
LA        Linoleic Acid
Rº        Alkyl Radical
ROOº      Peroxyl Radical
ROOH      Hydroperoxides
LPC       Lysophosphatidyl Choline
M         Molar
m         Meter
meq       Milliequivalents
mg        Milligram
min       Minute
MKM       Modified Kinsella extraction Method
MMT       Million Metric Tones
mL        Milliliter
mm        Millimeter
m.p       Melting point
MUFA      Monounsaturated Fatty Acid
N Normality
n-3 Omega-3
n-6 Omega-6
ND Not Detected
NL Neutral Lipid
NO. Number
ºC degree centigrade
ºOH Hydroxyl Radical
PC Phosphatidyl Choline
PE Phosphatidyl Ethanolamine
PI Phosphatidyl Inositol
PL Polar Lipid
PORIM Palm Oil Research Institute of Malaysia
PS Phosphatidyl Serine
PUFA Polyunsaturated Fatty Acid
PV Peroxide Value
sec Second
SFA Saturated Fatty Acid
SFE Super Critical Fluid Extraction
SPE Sphingomyelin
SPSS Statistic Package for Social Science
SV Saponification Value
TAG Triacylglycerole
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CHAPTER I
INTRODUCTION

Lipid consists of chemical combinations of glycerol with certain fatty acids. They are insoluble in water, soluble in organic solvent and may serve as food to supply the body’s calorie. The dietary lipids are mostly present in or are supplied from living organisms such as vegetable seeds (soy bean, cottonseed, sunflower and corn), oil-bearing fruits and nuts (palm, palm kernel and olive) followed by land animal and fish.

Fish are cold-blooded animals with scaly streamlined bodies. Fish play essential roles in the world as, animal feed, fertilizer and food. They are special in the human food chain due to their nutritive components such as protein, vitamins A, B and D, minerals namely calcium, phosphorous, iodine and lipids.

Fish lipid composition differs from that of land animal lipids or vegetable oils. It is generally composed of triacylglycerol (TAG), phospholipids and sterols in major and in minor quantity including the metabolic products of the latter components with some remarkable lipids such as glycolipids. The fish lipid is different from land animal lipids and vegetable oils due to the large quantity of two distinct n-3 fatty acids including eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) that cannot be synthesized by human body (Jittrepotch et al., 2006). n-3 fatty acids intake through fish lipid, can improve human health by abating or curing diseases such as coronary heart problems, stroke, kidney disorders, arthritis, diabetes arrhythmias,
hypertension and cancer (Shahidi et al., 2004; Pepping, 1999; Von Schacky et al., 1999; Daviglus et al., 1997; and Christensen et al., 1996). Fish lipid also improves visual function (Birch et al., 2000); DHA remarkably demonstrates an essential effect on human brain growth and development (Horrocks et al., 1999).

Fish lipid is mainly stored in fish body in the subcutaneous tissue, belly flap, mesenteric tissue, head, muscle tissue and liver (Ackman, 1994). Fish lipid are primarily extracted from meat and liver but due to the growing human population and the demand for fresh or canned fish meat, new sources for lipid extraction have been proposed.

Each year a large amount of total marine capture is disposed as processing waste namely intestine, fin, skeleton, head and skin. Approximately, for each tonne of fish captured, an equivalent mass of fish material is discarded either as waste or as a low value by-product. There is some opportunity for acquiring more value from fish waste. Fish processing supplies this opportunity for utilizing wastes to supplement needs for animal feed, fertilizer, pet food, fish silage, chitin and chitosan. Fish waste can also be utilized in the production of fish lipid, which has more benefit over the last (refer to pet food, fertilizer) mentioned products (Choudhury and Bublitz, 1996; Choudhury and Gogoi, 1995).

Several methods have been used to extract lipid from fish, such as solvent extraction method and super critical fluid extraction method. Among these extraction methods,