UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF OIL FROM LIVER AND VISCERAL FATS OF PATIN (Pangasianodon hypophthalmus SAUVAGE) AND ITS USE IN HAND CREAM PREPARATION

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By

SAMANA SHABANIKAKROODI

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy.

CHARACTERIZATION OF OIL FROM LIVER AND VISCERAL FATS OF PATIN (*Pangasianodon hypophthalmus* SAUVAGE) AND ITS USE IN HAND CREAM PREPARATION

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June 2014

Chairman: Annie Christianus, PhD

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The visceral fat and liver of patin, *Pangasianodon hypophthalmus*, are considered as waste from fish processing. Converting these byproducts into fish oil not only provide a good source of health promoting fatty acids but also can be used for the production of value added products.

The proximate and fatty acid compositions of the visceral storage fat and liver of patin were determined. Fat content of the visceral fatty tissue of female and male patin were 77.64 and 73.23%, respectively, whereas fat content of the liver of female and male were 11.71 and 9.59%, respectively. The total amounts of monounsaturated and polyunsaturated fatty acids of the liver of female and male catfish were 51.03 and 50.55 %, respectively, whereas in fatty tissue of female and male were 50.46 and 50.31%, respectively.

Refining was done on the extracted oil from byproducts of patin to produce a more pure and stable product. The effect of refining procedure on the fatty acid composition of oil from various refining steps and the quality of the final product were investigated. Fatty acids found in crude and refined patin oil were C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C18:4, C20:0, C20:1, C20:4, C20:5, and C22:6. The amount of saturated fatty acids of refined oil were significantly ($P < 0.05$) higher than that of crude oil. The total amounts of monounsaturated fatty acids of oil from different refining steps were not significantly ($P > 0.05$) different, whereas the total amount of polyunsaturated fatty acids of refined oil was significantly ($P < 0.05$) lower than that of crude oil. The eicosapentaenoic acid, docosahexaenoic acid, and n-3 to n-6 ratio of

refined oil were 0.83, 0.79%, and 1.01, respectively. Analysis of the physical and chemical properties of refined oil indicated the suitability of patin oil to be used in the formulation of hand cream.

In the present study, the efficacy and stability of various hand cream formulations using 0, 1.0, 2.5 and 5.0% of fish oil were investigated and compared. The appearance, odor, texture, and pH of formulations containing 1 and 2.5% of fish oil remained stable in very good qualities during the storage, whereas the related values to the appearance and texture of formulation containing 5.0% of fish oil at the end of 6 months of storage at 45°C were significantly ($P < 0.05$) lower than other formulations and previous evaluations of this formulation. Weight loss and microbial contamination were not observed in all formulations under study. Efficacy of preservative system was proved through preservative challenge tests. Efficacy test indicated that the formulations containing fish oil were preferred by volunteers rather than the one without the fish oil. Addition of fish oil significantly ($P < 0.05$) increased the moisturizing effects of hand creams. The formulation containing 2.5% of fish oil obtained the best related values in all investigated items including skin texture, moisture, smoothness, brightness, and well being perception.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia untuk memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN MINYAK DARI HATI DAN LEMAK VISCERA PATIN (*Pangasianodon hypophthalmus* SAUVAGE) DAN PENGGUNAANNYA DALAM PENYEDIAAN KRIM TANGAN

Oleh

SAMANA SHABANIKAKROODI

Jun 2014

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Simpanan lemak viscera dan hati patin, *Pangasianodon hypophthalmus* merupakan sisa dari pemprosesan ikan. Penukaran bahan sampingan ini kepada minyak ikan bukan sahaja sebagai sumber asid lemak yang baik untuk kesihatan tetapi boleh digunakan untuk penghasilan produk yang boleh ditambah nilai.

Komposisi proksimat dan asid lemak simpanan lemak viscera dan hati patin telah ditentukan. Kandungan lemak tisu lemak viscera patin betina dan jantan adalah 77.64 dan 73.23%, masing-masingnya. Manakala kandungan lemak hati betina dan jantan adalah 11.71 dan 9.59%, setiap satunya. Jumlah kandungan asid lemak mono tak tepu dan poli tak tepu hati betina dan jantan adalah 51.03 dan 50.55 %, setiap satunya, manakala tisu lemak betina dan jantan adalah 50.46 dan 50.31%, masing-masingnya.

Penulenan dijalankan ke atas minyak yang diekstrak dari bahan sampingan patin untuk menghasilkan produk yang lebih tulen dan stabil. Kesan prosedur penulenan ke atas komposisi asid lemak minyak dari berbagai peringkat penulenan dan kualiti produk yang terhasil dikaji. Asid lemak yang terdapat dalam minyak patin tanpa dan dengan penulenan adalah C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C18:4, C20:0, C20:1, C20:4, C20:5, dan C22:6. Kandungan asid lemak tepu dalam minyak yang telah ditulenkan ketara lebih tinggi ($P < 0.05$) benbanding minyak mentah. Jumlah kandungan asid lemak mono tak tepu dari minyak yang terhasil dari peringkat penulenan yang berbeza tidak menunjukkan perbezaan yang ketara ($P > 0.05$), manakala jumlah kandungan asid lemak poli tak tepu minyak yang ditulenkan ketara lebih rendah ($P < 0.05$) berbanding dengan minyak mentah. Asid eikosapentaenoik,

dokosahexaenoik, dan nisbah n-3 kepada n-6 minyak yang ditulenkan adalah 0.83, 0.79%, dan 1.01, masing-masingnya. Analisis ciri fizikal dan kimia minyak yang ditulenkan menunjukkan kesesuaian minyak patin untuk digunakan dalam formulasi krim tangan.

Dalam kajian ini, keberkesanan dan kestabilan beberapa formulasi krim tangan menggunakan 0, 1.0, 2.5 dan 5.0% minyak ikan dikaji dan dibandingkan. Rupabentuk, bau, tekstur, dan pH formulasi yang mengandungi 1 dan 2.5% minyak ikan ternyata stabil dengan kualiti yang baik semasa tempoh penyimpanan, manakala nilai yang berkaitan dengan rupabentuk dan tekstur formulasi yang mengandungi 5% minyak ikan pada akhir tempoh 6 bulan simpanan pada 45°C ketara menurun ($P < 0.05$) berbanding dengan yang formulasi yang lain dan dari formulasi yang telah dinilai sebelumnya. Tidak terdapat sebarang kehilangan berat dan pencemaran mikrobial diperhatikan dalam kajian ini. Keberkesanan sistem pengawetan terbukti melalui kajian cabaran pengawetan. Kajian keberkesanan menunjukkan bahawa formulasi yang mengandungi minyak ikan menjadi pilihan sukarelawan berbanding dengan yang tidak meengandungi minyak ikan. Penambahan minyak ikan meningkatkan kesan pelembapan krim tangan dengan ketaranya ($P < 0.05$). Formulasi yang mengandungi 2.5% minyak ikan mendapat nilai terbaik diantara semua bahan yang dikaji termasuk tekstur kulit, lembapan, kelembutan, kecerahan, dan persepsi keselesaan.

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

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DECLARATION

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
AOCS	American Oil Chemists' Society
ATCC	American Type Culture Collection
BHA	Butylated Hydroxy Anisole
CFU	Colony Forming Units
DHA	Docosahexaenoic Acid
EFA	Essential Fatty acids
EPA	Eicosopentaenoic Acid
F1	Hand cream Formulation without fish oil
F2	Hand cream Formulation containing 1.00% fish oil
F3	Hand cream Formulation containing 2.50% fish oil
F4	Hand cream Formulation containing 5.00% fish oil
FAME	Fatty Acid Methyl Esters
FAO	Food and Agriculture Organization of the United Nations
FFA	Free Fatty Acids
GC	Gas Chromatography
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
SFA	Saturated Fatty Acid
USFA	Unsaturated Fatty Acid
U.S. FDA	U.S. Food and Drug Administration

CHAPTER 1

INTRODUCTION

Biotechnologies are those technologies in which living organisms or parts of them are used to produce large quantities of products which are useful for human (Barbarisi, 2011). According to this definition of biotechnology and to fulfill a study in the field of aquatic biotechnology, extraction and refining of oil from byproducts of patin (*Pangasianodon hypophthalmus*) was considered to be applied in a formulation of a cosmetic hand cream. Production of hand cream using fish oil extracted from by-products can add value to these wastes and provide health promoting advantages for human skin.

According to Department Of Fisheries Malaysia (2010) patin is the second largest cultured fish species in Malaysia. Patin was chosen for this study because it is easier to harvest large amount of by-products from patin since they are being sold at big size (> 2.8 kg/fish). However, African catfish (*Clarias gariepinus*) is the first largest cultured fish species but they are being sold in smaller sizes of around < 0.2 kg/fish which means too small amount of by-products per fish which is not very suitable for this study. The big size of patin catfish is obvious on the measuring ruler in Figure 1.1.

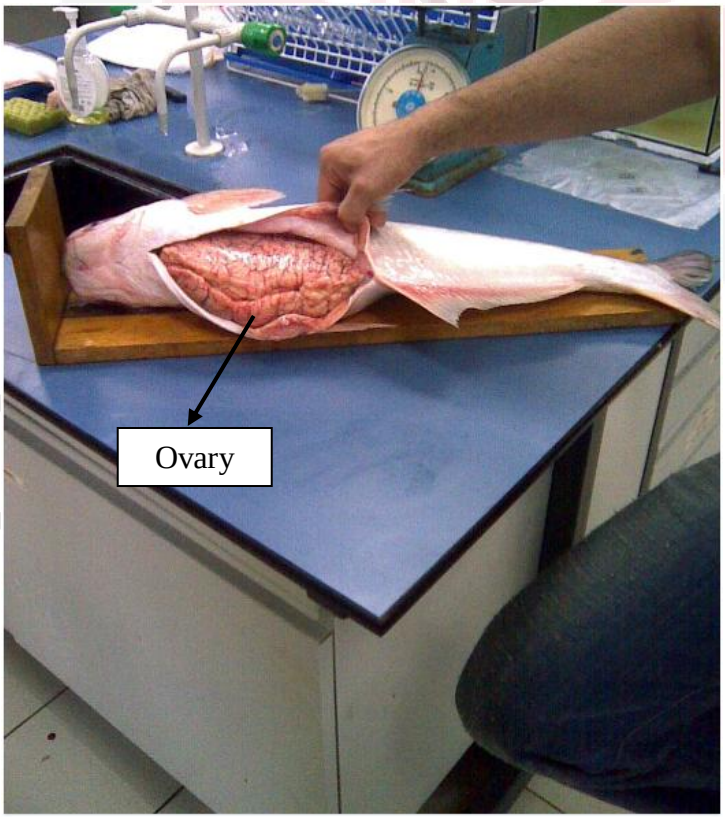


Figure 1.1. Patin catfish on the measuring ruler

In the present study, application of fish oil in a formulation of hand cream was considered because application of fish oil is beneficial for human skin (Heard *et al.*, 2003; Puglia *et al.*, 2005; and Zulfakar *et al.*, 2010) and Mero Cosmetic (Tehran, Iran) is interested on production of hand cream using fish oil and provided all the materials and equipments which made this study possible.

1.1 Problem statement and justification

- The visceral storage fat and liver of patin are discarded. Disposal of these wastes incurs cost and environmental pollution. Therefore, finding a usage for them can decrease environmental pollution caused by fish waste.

- Because of well-known health benefits of fish oil there is an increased interest in fish oil consumption, while fish resources are restricted. Therefore, finding new sources are important for on growing human population. Fish byproducts can be considered as valuable available sources.

- The visceral storage fat and liver accumulate fat. Therefore, they are potential sources of extractable fish oil. Evaluating the fatty acid composition of fish oil extracted from by-products of patin can provide useful information and emphasize their values for food, pharmaceutical, and cosmetic industries which are eager to use fish oil in their products.

- Fish oil extracted from fish wastes is economically important as it can be obtain in very cheap price which is favorable for industries and final consumers.

- Crude fish oil is a mixture of several compounds which decrease the quality and shelf life of oil. Therefore, it is suitable to be refined. Unfortunately, a few researches have been conducted to provide data on the effect of refining on the valuable fatty acid composition of fish oil. Thus, this study considered to evaluate the fatty acid composition of refined patin catfish oil after extraction, degumming, neutralizing, bleaching, and deodorizing to provide a better understanding of their changes. However, refining can affect the fatty acid composition of fish oil but it is still is one of the most common methods for removing and decreasing impurities (such as free fatty acids, lipid oxidation products, odor, and colorant compound) from fish oil (Young, 1982). Results of this research are important for oil industry interested in refined patin catfish oil production and scholars who are interested in modification of refining processes.

- There are a few examples of available cosmetic skin care products containing fish oil in the market but it is too hard to find a scholar study presenting data on formula and procedure suitable for production of these kinds of products. Therefore, this study can provide vital information on choosing hand cream

ingredients, their suitable amounts, and processes for formulation of hand cream which can contain fish oil and can be stable and effective.

- Generally, small amounts of fish oil are used in cosmetic products as compared to topical therapeutic products (Abamba, 1993). It is important to provide data which indicate the efficacy of these small amounts. Therefore, in this study comparison of hand cream without the fish oil was conducted to the hand cream containing fish oil.

- Unfortunately, there is a lack of data for determining the suitable and functional amounts of fish oil in a cosmetic skin care product and specifically in hand cream. This study attempted to find the suitable amount of fish oil which does not adversely affect the stability of hand cream during storage and to provide convincing evidence of effectiveness of that amount.

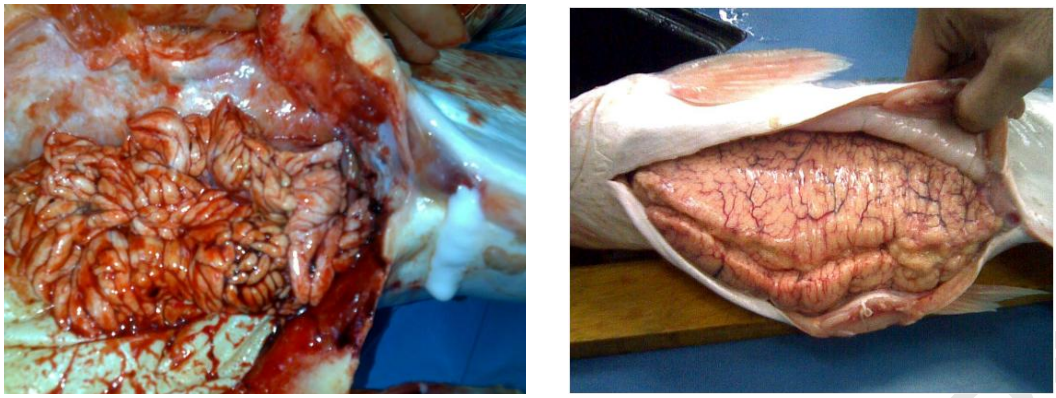
1.2 Specific objectives

The objectives of this study were divided into two main categories:

1. Patin catfish oil

1.1 Extracting and evaluating the fatty acid composition of liver and fatty tissue of female and male and combination of both

(At the place that patin is filleted and during filleting processes, because of big size of patin catfish it is practical and easy to separate female and male as well as their byproducts. Obvious differences in the gonad of female and male are shown in Figure 1.2. Moreover, without scientific data it is hard to predict whether the males and females have similar fatty acid composition or not. Moreover, for cosmetic and fish oil industries, providing information on male and female byproducts separately and combination of them is important to provide a better understanding of fatty acid composition of final fish oil product and its variation based on row materials which are used).



a b

Figure 1.2. Gonad of male (a) and female (b) patin catfish

1.2 Degumming, neutralizing, bleaching, and deodorizing the patin catfish oil and evaluating the fatty acid composition of oil at each step

1.3 Evaluating the physical and chemical properties of refined oil

1.4 Evaluating the antibacterial efficacy of crude and refined patin catfish oil through disk diffusion test, agar well diffusion test, and broth dilution test against *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16404)

2. Hand cream

2.1 Formulating and producing hand cream using various amounts of refined patin catfish oil

2.2 Evaluating the appearance, odor, texture, pH, weight loss, preservative efficacy, bacterial, fungal, and molds growth of various formulations of hand cream during six months of storage at 4, 25, 37, and 45°C

2.3 Evaluating various hand cream formulations through the *in vivo* dermal irritation test and clinical observations of skin reactions of mice

2.4 Evaluating various hand cream formulations through human study

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