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FORMULATION OF THE HAND CREAM USING EXTRACTED FISH OIL FROM THE VISCERAL STORAGE FAT OF FEMALE PATIN CATFISH (*Pangasius hypopthalmus*)

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ABSTRACT

The fatty acid composition of visceral storage fat, which is a processing waste derived from filleting process, was investigated to evaluate the suitability of the extracted oil from this by-product to be used in the formulation of the hand cream. About 77.64% crude fats (wet basis) could be recovered from this tissue. The extracted oil was characterized by a high level of unsaturated fatty acids (± 50.20%). The total polyunsaturated fatty acids, docosahexaenoic acid (DHA, C22:6 n-3), and eicosapentaenoic (EPA, C20:5 n-3) were about 11.98, 0.82, and 0.91%, respectively. The results of the study indicated that this tissue could be a good source of fish oil. After refining, BHA as an antioxidant was added to the refined fish oil. Different percentages of the refined fish oil including 1%, 2.5% and 5% were used in the formulation of the hand creams. Stability tests were done on the hand creams. Based on the assessment of the physical and chemical properties of the hand creams, different percentages of the refined fish oil were suitable to be used in the hand creams.

***Keywords**: Fatty acid, catfish, fish oil, refining, hand cream.*

INTRODUCTION

Fish and fish oil are good sources of n-3 fatty acids, which exert beneficial effects on human health. Demand for the fish oil increases with population growth. For the production of more fish oil, over fishing caused to depleting fisheries resources, while fish processing by products would be good potential sources [14]. Waste products from fish processing may reach to 55% of the total weight of a live fish [19]. To the best of our knowledge fatty tissue of Patin (*Pangasius spp*) is considered as waste from fish processing in Malaysia. Converting these by-products into fish oil not only may provide health promoting fatty acids but also may be considered for the production of an adding value product. The disposal problem also would diminish. The quality of fish oil can be improved during refining. Refining steps include degumming, neutralizing, bleaching, and deodorizing. The purpose of degumming is to remove soluble and insoluble impurities, and neutralization removes free fatty acids (FFA). Bleaching removes pigments, aldehydes, ketones, trace metals, sulfurous compounds, and soap. Deodorization removes remaining aldehydes, ketones, and FFA, which are responsible for unacceptable odour and flavour [5]. Fish oil is an effective emollient that has high skin penetration ability [24, 12, 16]. Fish oil has interesting enhancement effect in the permeation of drug into the skin [14, 12], and can improve the therapy results for psoriasis and UV- induced erythema [14, 16]. Fish oil has an intrinsic anti inflammatory effect. Inflammation is important in many dermatological disease states [24], cancer [15], autoimmune diseases and atherosclerosis [1].Topical use of fish oil is favourable for the treatment of localized inflammatory diseases such as arthritis rather than using dietary supplementation with fish oil [12].

Over the last years, there has been a great demand by consumer for products made from natural ingredients. The cosmetic industry is one example whereby it encourages the use of natural ingredients in its products. Generally, small amounts of oils or hydro alcoholic extract from plants and less often, animals are being added to the cosmetic products. The skin on the hands can become extremely scaly, dry and inflamed resulting in impaired barrier properties. Hand creams could be applied to rehydrate, smooth and leave a residual non tacky protective film on the skin. In addition to emollients, they can incorporate a healing or soothing agent

into the skin [7]. At the present study, various formulations without the fish oil and containing 1, 2.5, and 5% of fish oil were investigated to evaluate the efficacy and stability of hand cream formulations containing different percentages of refined patin catfish oil.

MATERIALS AND METHODS

Sample preparation: Ten fresh female catfish were obtained from a wholesale market (Pasar Borong, Selangor). Each fish were individually weighed. The fatty tissues were separated from the whole fish. Each tissue was individually weighed, ground, and stored at -18°C until analyzed. Fat were extracted according to the method of Sathivel et al. [18]. Approximately, 200 g of thawed samples were used. Water (water/ ground tissues, 5:1 v/w) was added to mixed samples and the mixture was heated to 70°C for 15 min. Cheesecloth was used for the separation of solid particles from liquid. The remaining solid particles and water were separated from oil by centrifuging at 5000 rpm for 30 min. Two batches were prepared and two crude oil extractions were conducted for each batch.

Esterification of fatty acids: FAMEs were prepared according to the AOCS Official Method 969.33 [2]. Extracted oil from each sample was placed into 50 ml reaction flask, separately. About 4 ml of methanolic sodium hydroxide (2 g of NaOH dissolved in 100 ml of methanol), and 10 boiling chips were added to the flask. The condenser was attached to the flask. Approximately 5 ml of boron trifluoride was added to the mixture and refluxing happened for 12 min. The esterified fatty acids were removed from the mixture by adding 5 ml of heptane and refluxing for 1 min, and then left to cool at room temperature. A saturated solution of NaCl was added steadily and mixed well until the heptane solution containing FAMs reached the neck of the flask. The heptane containing FAMs was recovered and anhydrous sodium sulfate (1.5 g) was used for dehydration. Dry heptane solution was then used for analysis by GC.

Fatty acid analysis by GC: The FAMs were analyzed with a GC Varian, model 3400. Column was DB-23 with the following dimensions: 60 m long, 0.32 mm i.d. with 0.25 µm phase thickness (J&W). One micro litre of sterified fatty acids was injected. Injector temperature was 220°C. The head pressure was set at 2 psi. Carrier gas was Nitrogen, and make up gas was Helium. GC was equipped with flame ionization detector (FID). Detector temperature was 260°C. Temperature program sat at 100°C for 2 min, then 180°C at 10°C/min for 5 min, and at last 220°C at 3°C/min for 10 min. Fatty acids were identified by retention times obtained from the FAMs standards (Sigma Company, St. Louis, MO). Two batches were prepared, each with two extractions and two GC injections per extraction. The fatty acid content was reported as the percentage of W/W of samples.

Proximate analysis:

Lipid content: Fat was extracted according to the method of Bligh and Dyer [6]. About 10 g of each sample was placed in a screw capped test tube and 37.5 ml 1:2 (v/v) $CHCl_3$: MeOH was added and vortex well. Then, 12.5 ml $CHCl_3$ was added and vortex well. Finally, 12.5 ml dH_2O was added and vortex well. Centrifugation of the mixture at 1000rpm for 5 min at room temperature gave two phase system that aqueous phase was at the top and organic phase was at the bottom of tube. Organic phase withdrew through the pasture pipette, carefully. Rotary evaporator was used for the extraction of solvents. The total lipid content was determined gravimetrically (n=2, a=3).

Moisture content: Moisture content was determined by measuring the sample weight loss, by weighing the sample before drying in an oven and after drying in the oven at 105°C, until a constant weight was achieved (n=2, a=3).

Protein content: Total nitrogen was determined by Kjeldahl method [2]. Percentage of protein was calculated as N × 6.25 (n=2, a=3).

Refining procedure: Two batches of crude catfish oil were prepared and two refining procedure were conducted for each batch.

Degumming, degumming was done following the modified method of Dijkstra and Opstal [10]. According to this method around 100 g crude catfish oil was removed from frozen storage, and placed in a 600-ml Beaker. In a temperature-controlled water bath, fish oil was heated to 70 C; then 3 ml of 3% aqueous citric acid solution was added, and thoroughly mixed with fish oil at 70 C for 1 min. Then the oil was cooled to room temperature. Precipitated gum was removed by centrifuging at 5000 rpm for 10 min.

Neutralizing, neutralizing was done following the AOCS Official Method Ca 9b-52 [3]. Sodium hydroxide (12.6 g of 9.5% NaOH solution) was added to the 100 g degummed fish oil and the mixture was heated to 65 C for 30 min with constant stirring with a magnetic stirrer. Then, the mixture was cooled to room temperature. The sample was kept undisturbed for 6 h. After centrifuging at 5000 rpm for 10 min, the oil was decanted from the precipitated soap. Around 50 ml demineralised water was added to wash out any remaining soap. Washing the sample was repeated three times. Impurities and water was removed by centrifuging at 5000 rpm for 10 min.

Bleaching, bleaching was done following the method of Scott and Latshaw [20]. The neutralized oil was heated in a water bath. At 70 °C activated earth (CS Z1077, American Oil Chemists' Society, Champaign, IL, USA) with the amount of 4% (w/w) was added to the oil sample and stirring using a magnetic stirrer was done for 10 min. The activated earth with absorbed impurities was removed by centrifuging at 5000 rpm for 30 min.

Deodorizing, modified laboratory deodorization equipment was prepared according to the method of Baldwin [4]. The deodorization unit was made from two 500-ml round– bottom flasks. The bleached catfish oil (100 ml) was added to the flask and was heated in a water bath to 100 C for 30 min under vacuum. It had one outlet and one inlet. The outlet was connected to a distillation unit and vacuum pump. The inlet was connected to 500-ml round– bottom flasks containing 400 ml water that was heated by a heating system. The temperature was manually controlled. The volatile products were condensed in a cooling system that was installed on the vacuum line and the deodorized oil was collected.

Fish oil specifications:

Peroxide value: Peroxide value was determined following AOCS official method 28.023 [2]. Around 5g of fish oil sample was placed in 250 ml glass Erlenmeyer and 30 ml peroxide solvent 3:2 (v/v) acetic acid: chloroform was added and swirled to dissolve the solution. Around 0.5 ml saturated KI solution was added to the mixture and was let stand with occasional shaking for 1 min. Then, 30 ml H_2O was added. The titration with 0.1 N $Na_2S_2O_4$ was done and the mixture was shacked until yellow colour gone. Around 0.5 ml 1% starch solution was added and titration continued to release I from $CHCl_3$ layer and blue colour disappeared. The experiment was replicated three times for each sample.

Free fatty acid: Free fatty acid content was determined by the titration method according to AOCS official method 28.029 [2], and the results were expressed as mg oleic acid/g oil. The experiment was replicated three times for each sample.

Iodine value: Hanus iodine solution was prepared according to AOCS official method 28.018 [2]. Around 0.5 g fish oil was weighed into 500 ml glass flask and 10 ml $CHCL_3$ was added. Around 25 ml hanus iodine solution was added to the mixture and the flask was placed in dark for 30 min. Then, the mixture was shaken well and 10 ml KI solution was added. After shaking, 100 ml H2O was added to washing down any free I on stopper. Iodine number was determined by the titration method according to AOCS official method 28.019 [2], and the results were expressed as percent by weight of I absorbed. The experiment was replicated three times for each sample.

Saponification value: Saponification value was determined according to AOCS official method 28.026 [2]. About 5 g of fish oil sample was weighed in 300 ml and 50 ml alcoholic KOH solution was added into the flask. The flask was connected with an air condenser and was heated for 30 min using a heating system until the fish oil sample was completely saponified. The sample was cooled and titration was done with 0.5N HCl using phenol phetalein solution. Blank detection was done along with 0.5N HCl on the sample using same pipette for measuring KOH solution. Saponification number was calculated as follows: 28.05(B-S)/g sample, where B is ml 0.5N HCl required by blank detection, and S is ml 0.5NHCl required for sample. Saponification number was expressed as mg KOH/g of oil sample. The experiment was replicated three times for each sample.

Melting point: Melting point was determined according to AOCS official method 28.011 [2]. About 10 mm melted sample was drawn to capillary tube with 1 mm diameter. The end of the tube was sealed with the sample in a small flame, and the tube was placed in a refrigerator overnight (almost 16 hr) at 4°C. Then, the tube was attached to an accurate thermometer and they were suspended in a 600 ml beaker that was half filed with H_2O. For determining of the melting point the temperature of water bath was increased with the ratio of 5°C/min while mixing. Melting point was taken at which the sample became transparent. The experiment was replicated three times for each sample.

Specific gravity: A pycnometer was standardized with H_2O according to AOCS official method 28.003 [2]. Specific gravity was determined according to AOCS official method 28.005 [2] at 40°C by the means of standardized pycnometer. Specific gravity at 25/25 °C was calculated as follows: G= G′+ 0.00064(T- 25°C), where G is specific gravity at 25/25 °C, G′ is specific gravity at T/25 °C, T is temperature at which specific gravity was detected, and 0.00064 is mean correction for 1°C. The experiment was replicated three times for each sample.

Refractive index: A refractometer was standardized with H_2O according to AOCS official method 28.006 [2]. Refractive index was determined by means of abbé refractometer at 40°C according to AOCS official method 28.007 [2]. The experiment was replicated three times for each sample.

Antioxidant addition: About 175 ppm BHA was added to refined catfish oil.

Hand cream production:

For the production of the hand cream, oil phase and water phase were heated up to 75°C, separately. Water phase was added to oil phase and was mixed for 15 minutes. Then, the mixture was cooled while mixing. At 40- 45°C, heat sensitive materials such as perfumes were added. Mixing was continued until the temperature of the product reached to room temperature. Then, the product was filled in special containers and weighed [7].

Table 1: Components in each formulation (%, w/w) under study

components	**Formulation 1% (F1)[a]**	**Formulation 2% (F2)[b]**	**Formulation 3% (F3)[b]**	**Formulation 4% (F4)[b]**
Stearic acid	4.00	4.00	4.00	4.00
Cetearyl alcohol	2.00	2.00	2.00	2.00
Cetyl alcohol	1.00	1.00	1.00	1.00
Glycerin monostearate	1.50	1.50	1.50	1.50
Anhydrous lanolin	1.00	1.00	1.00	1.00
Petrolatum	7.00	7.00	7.00	7.00
Paraffin wax	4.00	4.00	4.00	4.00
Liquid paraffin	22.00	21.00	19.50	17.00
fish oil	0.00	1.00	2.50	5.00
Propyl paraben	0.10	0.10	0.10	0.10
Triethanol amine	0.70	0.70	0.70	0.70
Glycerin	2.00	2.00	2.00	2.00
Perfume	0.30	0.30	0.30	0.30
Methyl paraben	0.20	0.20	0.20	0.20
Water	54.20	54.20	54.20	54.20

[a] F1 Formulation without the fish oil
[b] F2, F3, and F4 containing1%, 2.5% and 5% of fish oil, respectively, whereas liquid paraffin was decreased to 21%, 19.5%, and 17%, respectively.

Stability tests.

Sampling set up. Hand cream samples without the fish oil and containing 1%, 2.5% and 5% fish oil were stored at 4°C in refrigerator, and 37°C and 45°C in different incubator. Efficacy and stability tests were carried out upon the production of the hand cream formulations, after 2 weeks, 1, 2, 3, 4, 5 and 6 months of storage, according to examination schedule suggested by Cannell [8].

Appearance, odour and texture. The study was done following the method of Cannell [8]. Twenty panellists evaluated these properties on five samples of each formulation. Results were recorded in terms of the panellists' satisfaction on a numerical scale of 1. Very poor, 2. Poor, 3. Fair, 4. Good, and 5. Very good.

Efficacy. The study protocol and efficacy perception was done following the method of Gaspar et al. [11]. A total of 60 healthy female subjects 20- 65 years old participated in this study. They divided into 3 groups of 20 volunteers each. They did not apply any products, such as moisturizers and sunscreens, for 2 weeks before and during the investigation. They were washed their hand normally during the test period. Volunteers applied 0.2 g of 2 formulations, twice daily, in the morning and in the evening for the 30 day period of applications. One

group applied the F1 (hand cream formulation without fish oil) in the left hand and the F2 (hand cream formulation containing 1.00% fish oil) in the right hand. The other group applied the F1 and the F3 (hand cream formulation containing 2.50% fish oil) in the left and in the right hand, respectively. The third group applied the F1 in left hand and the F4 (hand cream formulation containing 5.00% fish oil) in the other hand. All formulations assessed and the measurement sites were randomized between volunteers. After the 30 day period of applications of the different hand cream formulations and one week after the end of the treatment, volunteers answered a questionnaire. They were asked about their sense of hand creams qualities on their skins (texture, moisture, smoothness, brightness, and well being perception) and their preference between the one applied in the left hand (F1 formulation without fish oil) and or the one applied in the right hand (F2, F3, and F4 containing various percentages of fish oil). Results were recorded in terms of the volunteers' satisfaction on a numerical scale of 1. Very poor, 2. Poor, 3. Fair, 4. Good, and 5. Very good.

Statistical analysis: All data was analyzed using a SAS program (version 16.0). Wherever was needed, data was transformed to arcsine values before statistical analysis. Analysis of variance (ANOVA) was carried out to compare the means of results at the significance level of $P<0.05$. POST hoc Tukey's test was done to find the significant difference between the means of results.

RESULTS AND DISCUSSIONS

Proximate composition. Protein, fat, and moisture content of fatty tissue are shown in Table 2. The fatty tissue of catfish weighs about 118.09 g, which is approximately 3.63% by weight of a live catfish. Weight of visceral storage fat was relatively higher than that reported in channel catfish (*Ictalurus punctatus*) [19]. Fat content (% wet basis) of fatty tissue was 77.64 %.

Table 2: Protein, Fat, and Moisture content (%, w/w) [a] of fatty tissue of female *P. hypophthalmus*

Catfish parts	**Protein [b] (%)**	**Fat [b] (%)**	**Moisture (%)**
Fatty tissue	1.75	77.64	18.81

[a] An average of six values for each tissue(two batches; and three replications for each batch).
[b] Wet weight basis

Fatty acid profile of crude patin catfish oil. The fatty acid compositions of fatty tissue are shown in Table 3. The major fatty acids present in these tissues were palmitic (C16:0), oleic (C18:1n-9), and linoleic acid (C18:2 n-6). Palmitic acid was dominant among saturated fatty acids, whereas oleic acid was dominant among unsaturated fatty acids. Linoleic acid (C18:2 n-6) was dominant among polyunsaturated fatty acids. The extracted oil from fatty tissue of catfish was characterized by a high level of unsaturated fatty acids. These results are similar to the findings about the fatty acid composition of the muscle of farm raised giant catfish (*Pangasianodon gigas*) and the muscles and viscera of Asian catfish (*Pangasius bocourti*) [9, 21]. The total unsaturated fatty acids accounted for 50.20 %. Docosahexaenoic acid (DHA, C20:5 n-3), eicosapentaenoic acid (EPA, C22:6 n-3), and arachidonic acid (AA, C20:4n-6) of fatty tissue were 0.91, 0.82, and 1.01%, respectively. EPA and arachidonic acid play very important roles in reproduction in female fish [23]. AA is the major precursor of eicosanoids in fish cells, including prostaglandins. In ovary of teleost, prostaglandins type E_2 (PG E_2) stimulate ovarian stroidogenesis [13].

Table 3: Fatty acid composition (%, w/w)[a] of fatty tissue of female *P. hypophthalmus*

Fatty acid	**Fatty tissue (%)**
C12:0	0.60±0.04
C14:0	4.63±0.10
C16:0	32.48±0.35
C18:0	8.63±0.39
C20:0	0.94±0.11
Total amounts of SFA	47.29±0.57
C14:1 n-7	1.02±0.07
C16:1 n-7	3.63±0.21
C18:1 n-9	29.36±0.57
C18:1 n-7	2.91±0.53
C20:1 n-9	1.29±0.02
Total amounts of MUFA	38.22±0.47
C18:2 n-6	5.34±0.19
C18:3 n-3	0.66±0.04
C18:4 n-3	1.65±0.10
C20:4 n-6	1.01±0.13
C20:4 n-3	1.58±0.21
C20:5 n-3	0.91±0.03
C22:6 n-3	0.82±0.07
Total amounts of PUFA	11.98±0.14

[a] Mean values of 8 measurements (two batches, each with two extractions and two injections).

Fatty acid composition of patin catfish refined oil. An average of 200 g fatty tissue was required to produce 100 g of crude oil. For 100 g of crude catfish oil, around 54.25 g of the refined fish oil was produced. The fatty acid compositions of refined catfish oil are shown in Table 4. Fatty acids found in crude and refined catfish oil were C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C18:4, C20:4, C20:5, and C22:6. The total saturated fatty acids amounted to 47.45% of refined oil. Palmitic acid (C16:0) was dominant among saturated fatty acids, accounting for about 68.85% of all saturated fatty acids in the refined oil. The total unsaturated fatty acids amounted to 49.94% and were relatively higher than the amounts of saturated fatty acids in refined catfish oil. Oleic acid (C18:1n-9) was dominant among monounsaturated fatty acids, whereas linoleic acid (C18:2 n-6) was dominant among polyunsaturated fatty acids. Among unsaturated fatty acids, oleic acid, linoleic acid, Docosahexaenoic acid (DHA, C20:5 n-3), and eicosapentaenoic acid (EPA, C22:6 n-3) accounting for about 58.53, 10.65,1.62, and 1.56% of all unsaturated fatty acids in refined catfish oil, respectively. A significant difference was not found in the amount of total saturated fatty acids and monounsaturated fatty acids between the crude and refined oil, whereas the amount of total polyunsaturated fatty acids of crude oil were significantly (α= 0.05) higher than that of refined oil. A significant difference was not found in the amount of DHA between the crude and refined oil, whereas the amount of EPA in the crude fish oil was significantly higher than that in the refined catfish oil.

Table 4: Fatty acid composition (%, w/w)[a] of refined patin catfish oil

Fatty acid	**Refined catfish oil (%)**
C12:0	0.50±0.03
C14:0	4.58±0.13
C16:0	32.67±0.17
C18:0	8.67±0.03
C20:0	1.09±0.02
Total amounts of SFA	47.45±0.08
C14:1 n-7	0.89±0.06
C16:1 n-7	3.89±0.11
C18:1 n-9	29.23±0.37
C18:1 n-7	3.14±0.07
C20:1 n-9	1.21±0.02
Total amounts of MUFA	38.30±0.48
C18:2 n-6	5.32±0.06
C18:3 n-3	0.64±0.03
C18:4 n-3	1.62±0.01
C20:4 n-6	0.94±0.01
C20:4 n-3	1.51±0.04
C20:5 n-3	0.81±0.03
C22:6 n-3	0.78±0.02
Total amounts of PUFA	11.64±0.07

[a] Mean values of 16 measurements (four batches, each with two extractions and two injections).

The n-3 to n-6 ratios of crude and refined catfish oil are shown in Table 5. The total amount of n-6 fatty acids (combined C18:2 n-6, and C20:4 n-6) was relatively higher than the total n-3 fatty acids (combined C18:3 n-3, C18:4 n-3, C20:4 n-3, C20:5 n-3, and C22:6 n-3) in crude and refined catfish oil. The n-3 to n-6 ratios of crude oil was 0.88, whereas the n-3 to n-6 ratios of refined oil was 0.85. The n-3 fatty acids in crude oil were significantly higher than those in refined oil. This may be due to the heat treatments during refining procedure. The n-3 to n-6 ratio of refined patin catfish oil was relatively higher than that reported about the extracted oil from the muscle of Rohu, Malaysian carp, Baung, and African catfish that were 0.18, and 0.17, 0.11, , 0.05 respectively [17].

Table 5: The total n-3 fatty acids, n-6 fatty acids (%, w/w), and n-3/ n-6 ratios of crude and refined catfish oil

tissue	**Total n-3 fatty acids (%)**	**Total n-6 fatty acids (%)**	**n-3/ n-6 ratio**
Crude oil [a]	5.63±0.19	6.35±0.32	0.88
Refined oil [b]	5.38±0.05	6.26±0.05	0.85

[a] Mean values of 8 measurements (two batches, each with two extractions and two injections).
[a] Mean values of 16 measurements (four batches, each with two extractions and two injections).

Refined fish oil specifications. The physical and chemical properties of patin catfish refined oil are shown in Table 6. Melting point of refined catfish oil was about 34.1°C. Melting point of refined patin catfish oil was higher than that reported in channel catfish (*Ictalurus punctatus*) refined oil. This may be due to the chemical structure of fish oil, the amount of total unsaturated fatty acids, and the number of double bonds in unsaturated fatty acid chains [18]. Lipid oxidation was determined by peroxide value which was 1.82 mequiv peroxide/ kg sample. Free fatty acid was about 0.03%. Free fatty acid and peroxide value of fish oil rich in omega-3 fatty should be less than 0.251 and 10.0 mill equivalent/ kg sample, respectively [22].

Table 6: The physical and chemical properties of refined catfish oil [a]

Physical and chemical properties of oil	**Female catfish**
Specific gravity (at 25/25 °C)	0.91±0.00
Refractive index (at 40 °C)	1.46±0.00
Melting point (°C)	34.13 ±0.25
Saponification value (mg KOH/g fat)	191.00±1.41
Iodine value (g^{-1} sample)	62.75±1.71
Free fatty acid (% oleic acid)	0.03±0.00
Peroxide value (mill equivalent/ kg sample)	1.82±0.37

[a] Mean values of 12 measurements (four batches, and each with three measurements).

Appearance, odour, and texture evaluation of hand cream formulations incubated at 45°C are shown in Table 7. All hand cream formulations, with and without the fish oil, were valued as very good formulations. The hand cream formulations without the fish oil and containing 1, 2.5 and 5% of fish oil were evaluated unchanged during 6 months of storage at 4°C in refrigerator and at 37°C in incubator. The appearance, odour, and texture of formulations containing 1 and 2.5% of fish oil were valued very good during the storage at 45°C in incubator as well as the formulation without the fish oil. The hand cream formulation containing 5% of fish oil showed a slight degree of separation at the end of 6 months storage at 45°C in incubator and the value related to the appearance and texture of F4 was relatively less than those related to other formulations. However, a significant difference was not found among the values related to the formulation containing 5% of fish oil and the values related to the appearance and texture of the other formulations.

Table 7: Appearance, odour, and texture evaluation of hand cream formulations incubated at 45°C [a]

Hand cream formulations	**upon production**	**2 weeks**	**1 month**	**2 months**	**3 months**	**4 months**	**5 months**	**6 months**
F1 appearance	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F2 appearance	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F3 appearance	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F4 appearance	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.60±0.54
F1 Odour	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F2 Odour	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F3 Odour	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F4 Odour	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F1 texture	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F2 texture	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F3 texture	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
F4 texture	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.60±0.54

[a] Mean values of 100 measurements (20 panellists, and five samples of each formulation).

Efficacy perception of each hand cream formulation is shown in Table 8. All the formulations, without the fish oil and containing 1, 2.5, and 5% of fish oil, were valued as good to very good hand cream formulations. After 30 days application of various hand cream formulations, values were related to skin texture, moisture, smoothness, brightness, and well being perception of formulation containing 2.5% of fish oil was significantly higher than those related to F 1 formulation, which did not contain fish oil. Skin moisturizing effect of all formulations containing the fish oil was significantly higher than that of formulation without the fish oil.

Table 8: Efficacy perception of each hand cream formulation [a]

Hand cream formulations	Skin texture	Skin moisture	Skin smoothness	Skin brightness	well being perception
Formulation 1[b]	4.40±0.50	4.20±0.41	4.30±0.47	4.50±0.51	4.20±0.41
Formulation 1[c]	4.50±0.51	4.20±0.41	4.30±0.47	4.40±0.50	4.10±0.30
Formulation 2[b]	4.65±0.48	4.85±0.36	4.70±0.47	4.70±0.47	4.70±0.47
Formulation 2[c]	4.55±0.51	4.80±0.41	4.70±0.47	4.70±0.47	4.25±0.44
Formulation 3[b]	4.95±0.22	4.75±0.44	4.70±0.47	4.95±0.22	5.00±0.00
Formulation 3[c]	4.85±0.36	4.80±0.41	4.75±0.44	5.00±0.00	5.00±0.00
Formulation 4[b]	4.65±0.48	4.75±0.44	4.45±0.51	4.65±0.48	4.45±0.51
Formulation 4[c]	4.65±0.48	4.60±0.50	4.55±0.51	4.70±0.47	4.70±0.47

[a] Mean values of 60 measurements for the F1, and mean values of 20 measurements for the F2, F3, and F4.
[b] Measurements were done after 30 days of hand cream application
[c] Measurements were done one week after treatment

The results of the measurements were done one week after the end of the treatment confirmed the previous results which were obtained after 30 days of application of various hand cream formulations. The value related to well being perception of hand cream formulation containing 2.5% of fish oil was significantly higher than that related to the formulation without the fish oil, and the formulation containing1, and 5% of fish oil. However, the values related to skin texture, moisture, smoothness, brightness, and well being perception of all formulations were valued good to very good.

CONCLUSIONS

This investigation has shown that the extracted oil from the fatty tissue of female patin catfish was characterized by a high level of unsaturated fatty acids. However, the n-3 to n-6 ratios of crude and refined oils was less than 1. There were differences in fatty acid profile of crude and refined oil. The total amount of monounsaturated and polyunsaturated fatty acids in refined fish oil were relatively higher than the amount of saturated fatty acids. The physical and chemical properties of refined oil indicated the suitability of patin catfish oil to be used in the formulation of the hand cream. Moreover, the results of the processing procedures that were used to extract and refine catfish oil may provide useful information for oil industry to optimize the unit and produce edible oil from catfish processing wastes. This research has demonstrated various formulation of hand cream without the fish oil and containing 1, 2.5, and 5% of fish oil. A significant change was not observed in appearance, odour, and texture of all formulations during 6 months of storage at different temperatures. The values related to skin texture, moisture, smoothness, brightness, and well being perception of all formulations were valued good to very good. Skin moisturizing effect of formulations containing the fish oil was valued higher than that of formulation without the fish oil. The maximum values were related to well being perception of the hand cream formulation containing 2.5% of fish oil that were evaluated after 30 days of hand cream application, and one week after the end of the treatment.

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