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Some Physical, Chemical and Sensory Properties of Fish Oil Extracted from Fish Wastes by Physical and Chemical Methods

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Abstract: This study was carried out to assess some physical, chemical and sensory properties of fish waste oil extracted by two different methods: physical (boiling) and chemical (acid fermentation). Samples of common carp viscera were collected from local markets in the city of Basrah, which included viscera of the common carp *Cyprinus carpio*. The most prominent and significant difference was the extraction yield which amounted to 57.14% vs 35.71% (vol./wt.) for the physical and chemical methods, respectively. Other physical properties: refraction index, specific weight, specific gravity, viscosity, cloud point and pour point, Were superior in physical method of the physical method. Measured chemical indices (free fatty acids FFA, fatty acid profile, peroxide value, iodine number and Thiobarbituric acid value TBA) indicated some variability between oil types, the most obvious was FFA which increased significantly from 0.107 to 0.205% for physical and chemical methods, respectively. The sensory tests (color and odor) were in favor of oil extracted with the physical method characterized by a light amber color and a mild fishy flavor.

Keywords: Acid fermentation, Boiling, Common carp, Oil extraction, Fish viscera

Introduction

Although production-wise aquaculture has surpassed wild capture fisheries, feed input in aquaculture still heavily depends on wild capture fisheries. In 2014, 16.9% (15.8 million tons) of whole wild capture landings were reduced to fish meal and fish oil. Fish meal and fish oil are the most nutritious and most easily digestible feed ingredients for farmed fishes (FAO, 2016), which is the main reason why approximately 70% of all globally produced fish meal and fish oil is still used in aquacultures as feed (Tacon & Metian, 2015).

The global production of wild fisheries and aquaculture reached 190.9 million tons of fishes and crustaceans (FAO, 2016). Handling and cleaning of fishes leave large amounts of waste matter, the most important of which are internal viscera, heads and skins of some species, which are characterized by their high nutritional value (Arvanitoyannis & Kassaveti, 2008; Al-Noor, 2014). Release of large amounts of fish wastes and secondary products from their clean-up and preparation

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processes and fish product canning plants causes two main problems; the loss of large amounts of nutrients such as proteins, fats, and mineral salts, as well as contributing to environmental and health pollution and damage with an economic loss of its disposal (Ben Rebah & Miled, 2012). On the other hand, fish wastes are of high economic and nutritional value because they contain minerals from 0.8-2%, fat up to 25%, and protein between 15-30% (Ghaly et al., 2013). These wastes are recovered by converting into some by-products, including fish meal and fish oil, which are still known to be the most nutritious and most digestible ingredients for feeding farmed fishes (FAO, 2016).

Fish oil consists of three major categories; saturated and monounsaturated fatty acids (MUFAS) and polyunsaturated fatty acids (PUFAS) (ώ-3). The latter is essential for animals that cannot pre-synthesize them from an external source. Fish oils are the main source of long-chain polyunsaturated fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DAH), particularly important for healthy embryonic growth in vertebrates (Spalvins & Blumberga, 2018).

Human consumption patterns of fish oil are divided into four sections as healthy food ingredients, as commodities for food factories, as pharmaceutical ingredients, or as components of animal feed (Dunbar et al., 2014). Fish oil is considered a vital nutrient in aquaculture, and oil recovery from fermented fish silage can contribute to the production of low-cost fish oil, an alternative to conventional fish oil in aquaculture (Regost et al., 2003).

The most common extraction method for fish oil production includes three basic steps; cooking at high temperatures (85-95 °C), press, and centrifugation (Shepherd & Bachis, 2014). Abd El-Rahman et al. (2018) indicated that the oil extracted by wet extraction method has high yields and optimal physical and chemical properties for use in various industries. So, the extraction method has an effect on the physical and fatty acid properties of fish oil (Nazir et al., 2017). Because of the existence of large amounts of fish wastes, the present study was aimed at maximizing the use of such wastes for the production of fish oil and evaluating its quality by analyzing their most significant chemical, physical and sensory properties.

Materials and Methods

This study was conducted from September 2019 to March 2020. Fish wastes were obtained from the local markets of Basrah city as a by-product of fish sale and cleaning practices. Wastes included the internal viscera of marketed common carp. Upon arrival at the laboratory, samples were chopped by using an electric meat grinder. The chemical content of fish wastes was estimated using the methodology set out in A.O.A.C. (2000) and the moisture content by drying at a temperature of 105 °C until weight stabilization. Protein content was assessed by calculating the amount of nitrogen × 6.25 after digestion by the micro-Kjeldahl method. A soxhlet extraction method for intermittent extraction was applied to assess lipid content by using cyclohexane as a solvent for six hours. Ash content was estimated by

muffling at a temperature of 525 °C for 14 hours. Carbohydrates in samples were calculated by using the following equation:

Carbohydrates (%) = 100 - (Moisture% + Ash% + Protein% + Fat %)

Oil Extraction

Chemical Method (Acid Fermentation)

Fish oil was extracted by acid fermentation as indicated by Al-Kanaani (2014). A weight of 1000 grams of viscera was chopped in a plastic container and mixed with 100 ml of 5% acetic acid and 3% citric acid with 100 ml of distilled water, mixing well for 15 minutes and transferring into the 2l polyethylene bags which eventually sealed tightly taking into account the volume of gases resulting from fermentation. Samples were incubated at 40-45 ºC and from 4-7 days with daily continuous shaking. After complete fermentation, the mixture was centrifuged for 10 minutes at 3000 rpm. After centrifugation, the mixture was separated into three notable layers; the top (oil layer), middle semi-rigid layer (protein-rich) and the bottom (water and minerals). The oil layer was transferred into a separating funnel for further purification. The lower layer was discarded and purified oil was preserved into dark glass vials and refrigerated for further testing.

Physical Method

Fish oil is prepared according to the wet (boiling) extraction method as described by Vidotti et al. (2011). A weight of 1000 g chopped viscera was placed into a cooking bowl with 1000 ml of distilled water and cooked at 100 °C for 50 minutes. The mixture was left for one day before it was separated into two layers. The upper oil layer was collected and placed into a centrifuge to eliminate suspended impurities and obtain crude oil which was further purified by using the separating funnel method.

Yield Value

The yield value was expressed as a percentage of the oil extracted from a known weight of fish wastes and calculated as follows:

Yield value (%) = Extracted fish oil/ Weight of sample x 100

Chemical Quality Tests

Iodine value had been estimated by the Hanes method mentioned in A.O.A.C. (2000). Peroxide value was assessed according to the method indicated in Pearson (1976) where the results were expressed in milliequivalent (meq) of peroxide per kilogram of oil. The acid value was estimated according to the method mentioned in Pearson (1976). The saponification number was estimated according to the method reported in Pearson (1976) for the oils extracted. Thiobarbituric acid (TBA) value was estimated according to the method indicated in Pearson (1976). Total fatty acids were estimated based on Stoffel et al. (1959), while fatty acid profile was assessed by using GC-MS chromatography (Bako et al., 2017).

Physical Analysis

The refractive index was measured according to the guide of A.O.A.C. (2000). The density of oils as well as the qualitative weight were determined according to the methods mentioned in Pearson (1976) by using a density bottle. The ASTM D445 has been used, which is the standard method, to estimate the kinematic viscosity of transparent and non-transparent liquids, and the dynamic viscosity was calculated by the equation stated by Sathe & Salunkne (1981) as:

$$v = r \times t$$

Where v = viscosity, r = route length and t = time.

The cloud point and pour point were estimated according to Regost et al. (2003).

Sensory Tests

Sensory tests (odor, color and general acceptance) were performed according to the oil sensory evaluation form (Pearson, 1976).

Statistical Methods

All data were expressed as the mean of three replicates ± standard deviation. The statistical package of SPSS (Ver. 20) was used to analyze data sets. Differences between means were calculated by the LSD method.

Results

Yield Value

The yield value of the oil extracted by the physical method was 57.14%, which is greater than the value of the chemical method (35.71%). Statistical analysis showed that differences were significant ($p \leq 0.05$).

Chemical Composition of Fish Wastes

Table 1 demonstrates the chemical composition from fish wastes used in the study, where the moisture was 68.74%, protein 18.32%, fat 7.11% and ash 5.85%.

Chemical Properties

Table 2 demonstrates the chemical properties of oils extracted from fish wastes. A variation could be observed in the values of the qualitative characteristics and chemical oils derived from methods, characterized by oil extracted the way the physical qualities of good spirits and a difference of moral from the oil obtained by way of chemical and the highest iodine value was 94.43 g/100 g oil for the oil produced by the physical method while the value was 85.16 g/100 g oil in the fermentation oil sample. As for the chemical properties, there was a variation in the values of the chemical properties, which is the value of peroxide (1.8 and 2 meq/kg oil). The value of free fatty acids was 0.107% and 0.205% and the saponification value was 187 and 168.5 mg/g oil. The thiobarbituric acid value was 3.88 and 5.52 mg malaldehyde/kg for the oil extracted by physical and chemical methods, respectively, and the total fatty acid composition and the percentage were as shown in table 3 and 4.

Table 1: Chemical composition of fish wastes.

Ingredients	Percentage
Moisture	68.74
Protein	18.32
Fat	7.11
Ash	5.85

Table 2: Chemical properties of physically and chemically extracted fish waste oils.

Chemical properties	Physical extraction	Chemical extraction
Iodine value (g/100 g oil)	94.43±2.881	85.16±2.011
Peroxide value (meq/kg oil)	1.8±0.179	2±0.264*
Free fatty acids value (%)	0.107±0.006	0.205±0.0134*
Saponification value (mg/g oil)	187±1.069	168.5±1.732
Thiobarbituric acid (TBA) mg Malonaldehyde/kg	3.88±0.030	5.52 ±0.485*

Values are mean of triplicates ± S.D., * significant at p≤0.05 (rows).

Table 3: Fatty acid profiles for both extracted methods of fish waste oils.

Fatty acid	Extraction method	
	Physical	Chemical
C12:0	0.78	0.54
C14:0	6.96	6.48
C16:0	18.01	19.44
C17:0	1.94	1.38
C18:0	3.12	5.63
C20:0	0.33	1.09
C22:0	0.35	0.46
C14:1 w5	0.59	0.97
C16:1 w7	9.35	9.88
C17:1 w7	2.78	3.88
C18:1 w9	21.8	19.55
C20:1 w9	2.66	1.89
C24:1 w9	2.48	4.38
C18:2 w6	10.11	8.41
C18:3 w3	8.64	6.14
C20:3 w3	2.94	2.14
C20:4 w6	1.87	1.84
C20:5 w3 (EPA)	3.41	4.11
C22:6 w3 (DHA)	1.78	1.69

Table 4: Percentages of saturated and unsaturated fatty acids (%) in fish waste oils.

Fatty acid group	Physical extraction	Chemical extraction
Saturated SFA	31.49±5.921	35.02±6.325*
Mono-un Saturated MUFA	39.66±7.324	40.55±6.384
Poly- un Saturated PUFA	28.75±3.318	24.33±2.499*

Values are mean of triplicates ± S.D., * significant at p≤0.05 (rows).

Physical Properties

Table 5 demonstrates the physical properties of oils extracted from fish wastes. The value of the refractive index was similar in the two forms of oil: 1.472744 in the physical and 1.471564 in the chemical extracted oil. Viscosity values were 34.14 in the physical extracted oil and 39.21 for chemical extracted oil. Cloud point value was lower in chemical (8.84 °C) than in physical extracted oil (5.44 °C). The pour point was higher in physical (-5.8 °C) in comparison with chemical oil (-3.6 °C).

Table 5: Physical properties of fish oils by both extracted methods.

Physical properties	Physical method	Chemical method
Refractive index	1.472±0.180	1.471±0.0037
Specific gravity (g/ml)	1.100±0.891	1.121±0.914
Specific weight (g)	1.078±0.878	0.901±0.616
Viscosity (cm^2/sec)	34.14±0.858	39.21±0.916*
Cloud point (˚C)	5.44±4.3	8.84±7.1*
Pour point (˚C)	-5.8±0.152	-3.6±0.173*

* Significant at $p \leq 0.05$ (rows).

Sensory properties

Table 6 illustrates the sensory tests for oils extracted from fish wastes by the two methods. The physically extracted oil was characterized by better colour and odor in comparison with chemically extracted oil which has a darker colour and more fishy acid flavour. General acceptance was clearly different between tow oil types where physically extracted oil was characterized by more acceptability.

Table 6: Sensory tests for oil extracted from fish wastes.

Type of sample	Sensory tests		General acceptance
	Color	Odor	
Physical method	light amber	light fish	good
Chemical method	yellowish-brown	acidic fish	fair

Discussion

Previous studies have been conducted to investigate the utilization of fish processing wastes by converting them into more valuable products that directly or indirectly contribute to human consumption (Al-Hussainy, 2007). The results showed that oil extracted from common carp wastes is of high quality, which makes it suitable for use in most of the involved industries. The extraction method saffects oil yield where the physical method (wet extraction) includes curing of protein in fish waste- water mixture, and this process eventually separates oil easily from the solids in the mixture. The differences in the yield value of the oil could reflect the type of waste sample and its condition before physical extraction and temperature profile during extraction (Nazir et al., 2017).

The fatty acid profile according to the results of GC-MS indicated that the most potent fatty acid chains were found in the range of C12 and C22 which in turn determines the intended functional properties of the extracted oil. The extraction method may have variable impacts on the content and quality of the three major fatty acid groups: SFA, MUFA and PUFA. The quantity of PUFA derived from the physical extraction was higher compared to the acid fermentation method which agrees with the results of some other studies on the extraction of fish waste oil, on contrary to the MUFA rate, which was closely similar between the two extracted oils, a result consistent with previous studies (Al-Hussainy, 2007; Nazir et al., 2017).

Fatty acid profile, peroxide, iodine and free fatty acids (FFA) values are among the most important indicators for the quality of oils (Khoddami et al., 2012). The current study showed that there were significant differences ($p<0.05$) between chemical and physical characteristics of the extracted oils. Iodine value indicates the degree of unsaturation of fatty acids in oils where higher values reflect the higher oil content of unsaturated fatty acids, which in turn is a good indicator of the source or type of oil (Yi et al., 2014). Free fatty acids FAA are formed as a consequence of the hydrolysis process that occurs due to the exposure of oil to heat and water. Values recorded in the current study were close to Al-Hussainy (2007) in the fish oil wastes of *Ilisha compressa* (0.99%) and lower than the value of 3% in Abd El-Rahman et al. (2018). The differences in the ratios of free fatty acids reflect moisture and iron content of these oils which are important factors in hydrolysis acceleration and free fatty acid release (Aidos, 2002).

Iodine value in wet extraction could increase as a result of oxidation of the double bonds in fatty acid representing the temperature profile of extraction mixture. The iodine value of 110.51g/100g oil was recorded for physically extracted oil from mackerel which is close to the finding of the current study as well as to Bako et al. (2017). The saponification value is correlated with the molecular weight of the fatty acids in the oil, and is inversely proportional to the molecular weight of the fatty acids present in the chlorides. The difference in saponification value reflects the type of fatty acids and the length of the carbon chain associated with soap crystals (Bonilla-Méndez & Hoyos-Concha, 2018). TBA is an important measure of oxidative rancidity. The values recorded in the current study are similar to that of Al-Hussainy (2007) who recorded a value of 2.43 for oil extracted from fish viscera.

The peroxide test is used to determine the onset of rancidity in oil but it does not indicate the progression of lipid oxidation. It does not reflect the extent or amount of oil breakdown caused by various oxidative factors and should not exceed 10 mg/kg which is comparable to the present results as well as to those of Nazir et al. (2017). The values are also consistent with those obtained by Jayasinghe et al. (2013) where the value of peroxide was 2.9 meq/kg for the oil extracted from the fish viscera. Saponification value is correlated with the molecular weight of the fatty acids in the oil, which is inversely proportional to the molecular weight of the fatty acids present in the chlorides. The difference in saponification value reflects

the type of fatty acids and the length of the carbonate chain associated with soap crystals (Bonilla-Méndez & Hoyos-Concha, 2018). TBA is an important measure of oxidative rancidity. The values recorded in the current study are similar to that of Al-Hussainy (2007) who recorded a value of 2.43 for oil extracted from of fish viscera.

The refractive index is used to determine the purity of oils where values increase with the percentage of unsaturated fatty acids in the oil. The values of the refractive index were close in both extracted methods which subsequently were in line with that of Al-Hussainy (2007) in carp waste oil. The present study also concurred with Bako et al. (2017) for *Scomber scombrus* oil with the refractive index of 1.415.

The specific weight was 0.878 g in physical oil and 0.801 g in chemical oil, The density of the oils and the specific weight vary depending on the composition of the unsaturated fatty acids and their molecular weights due to the higher density of some oils than others due to the high content of unsaturated fatty acids with double bonds (Inguglia et al., 2020). The specific gravity was 0.891 g/ml in physical oil and 0.914 g/ml in chemical oil. The results are consistent with Sathivel et al. (2002). The specific gravity was 0.854 g/ml for oil extracted from catfish *Clarias gariepenus* and 0.853 g/ml for oil extracted from mackerel (*S. scrombrus*). The values were similar to those of Al-Hussainy (2007) in carp viscera oil where the specific gravity was 0.994 g/ml. Viscosity is defined as gravity-resistant fluid and increases with increasing saturation. The presence of impurities is adversely affected by temperature. Any increase in temperature by 1 °C leads to a 2% decrease in viscosity (Razon, 2009). Viscosity was 34.14 cm^2/sec in physical oil and 39.21 cm^2/sec in chemical oil, and such values are consistent with Abd El-Rahman et al. (2018) for oil extracted from the fish viscera who reported the viscosity as 33.37 cm^2/sec.

Fog and spill points are also important physical properties in the assessment of oil quality. The focal point is based on the presence of both polyunsaturated and unsaturated fatty acids.

Oil color is one of the factors that determine consumer acceptance and are very important in determining its subsequent use. The darker the colour of the oil, the longer it is stored. Colour is influenced by several factors including processing temperature and the amount of oxygen available in addition to the existence of pigments such as carotenoids. On the other hand, oil flavour expresses volatile compounds found in extracted oil which could be detected as odours (Warm et al., 2000).

Studied oils possessed a distinctive odour which is very similar to its fishy origin. The physically extracted oil has a clear fish odour while the chemical oil has a fishy odour with little acid note. Physical oil was light amber in contrast to chemical oil which was yellowish-brown. This result is in line with Al-Hussainy (2007).

In conclusion, fish wastes could be considered as a suitable and sustainable source of fish oil production with acceptable chemical, physical and sensory properties. The physical (boiling) and chemical (acid fermentation) recovery

methods were easy-to-use and low-cost techniques for extracting fish oil with suitable specifications although they could be improved further by modifying some processing parameters.

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