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Study on chemical composition and physical properties of the Hamri (*Barbus luteus*) and Balaout (*Chondrostoma regium*) fish meat, oil and impact of its oils on cholesterol, triglyceride, HDL and blood sugar of laboratory rats

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ABSTRACT

The study aimed at investigating the meat chemical composition and physical properties of oil of the Hamri (*Barbus luteus*) and Balaout (*Chondrostoma regium*) fish and its oil content or fatty acids, and also to know the impact of its oils on the level of cholesterol, triglyceride, high density lipoprotein (HDL) and blood sugar levels of laboratory rats. The study area extended from the province of Shirqat and Balad district to the province of Salah al-Din. The approximate percentages of meat from Hamri were 72.13, 19.74, 5.07 and 1.60 % for the moisture, protein, fat and ash respectively, and 71.63, 19.98, 4.96 and 2.04% respectively from Balaout. The extract oil from 2 types of fish differed significantly in Iodine value, Peroxide value, and Acid value and in saponification number. The fatty acids profiles results showed that oils from Hamri and Balaout fish meat consisted of 44.31 and 55.76% of Saturated fatty acid, 36.10 and 25.41% of poly unsaturated fatty acid, and 18.17 and 25.41% poly unsaturated fatty acids respectively. The experiment laboratory rats showed decreases in cholesterol, triglyceride and blood sugar level, and increases in high density lipoprotein (HDL). In conclusion, it is recommended that this oil can be used in human diet for health benefits.

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1. Introduction

Fish is one of the natural sources of nutrients that necessary for the human body to build tissues and for its activities. Some species of fatty fish contain 25% fat as well as meat, considered as a rich source of minerals, calcium, phosphorus and iodine; especially marine fish. Feeding fish regularly helps to decrease malnutrition

and diseases associated with lack of protein since fish meat contains a high percentage of protein and different essential amino acids. Also, its fat is rich in saturated fatty acids (Omega 3 fatty acids) which is heart-friendly and hence, helps reduce cholesterol level in human blood, as well as a moderate source of energy in the human body (Pang et al., 2017). Fish should therefore be eaten at least, two to three times a week, (Jiménez-Colmenero et al., 2001).

Approximately, 1,000 fish and shellfish species of varying nutritional and sensorial characteristics are consumed worldwide on a daily basis (Fraser and Sumar, 1998). Medical and nutritional researches show that marine fish oils are of high health and therapeutic importance because they contain unsaturated fatty acids, omega-3, EPA and DHA, which have a positive effect on public health and reduced blood sugar (Mozaffarian and Wu, 2011). There are many different types of fish in the Tigris and Euphrates rivers.

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Most of these species are used as food for people in these areas. There is a lack of information about the chemical composition of these fishes, especially their unsaturated fatty acids contents and their effect on human health.

Therefore, the aim of this study was to investigate the oil or fatty acids content as well as the chemical composition and physical properties of the oil of the fishes Hamri (*Barbus luteus*) and Balaout (*Chondrostoma regium*). The work further aims to investigate the impact of this oil on the level of cholesterol, triglyceride, and high density lipoprotein (HDL) and blood sugar levels of laboratory rats upon consumption of the fishes.

2. Material and methods

2.1. Source of fish

The study involved the meat of two types of Iraqi freshwater fish *Barbus luteus* and *Chondrostoma regium* locally known as Hamri and Balaout respectively (Al-Daham, 1982) (Fig. 1). Both species of fish are most common in the Tigris River in all seasons of the year. The study area extended from the province of Shirqat and Balad district to the province of Salah al-Din. This study was not a part of fauna survey, fish were caught by using gill net without making stress to other types of aquatic animal, and handling and catching of fish were done according to the animal ethic and laws (Directive, 1986). After fish catch fish were anaesthetized by using mustard before killing. Surgical process was done at laboratory that belonged to the University of Tikrit. As Fish samples were refrigerated at 8 °C until lipid extractions were performed. Most of the measurements and tests were conducted in the Graduate Laboratory in the Department of Food Science at the College of Agriculture, University of Tikrit. The head, the viscera, the skin and the bones were removed manually to obtain the meat, and the meat pieces of one type were completely mixed and crushed in a mortar to get well-crushed meat.

2.2. Proximate composition

2.2.1. Moisture content

Moisture content was determined when samples were dried in the convection oven at 105 °C until the weight was stabilized (Chemists, 1990).

2.2.2. Protein content

Protein content was determined according to the method of Chemists (1990) by using micro Kjeldahl and was calculated as follows:

$$\text{Protein \%} = \text{nitrogen} \times 6.25$$

2.2.3. Fat contents

The percentage of fat in fish meat samples was estimated by taking a known weight of dried samples and extracted with diethyl ether using the Soxhlet apparatus. The amount of fat was calculated based on the method described in Chemists (1990).

2.2.4. Ash content

Ash content was determined according to the method of Chemists (1990) by taking a known weight of flesh and placing it in a muffle furnace at 550 °C for 16 hrs. The ash percent was determined as follows:

$$\text{Ash \%} = \frac{W_1}{W_2} \times 100$$

where W_1 = weight of ash, and W_2 = initial weight

2.3. Oil extraction

Cold oil extraction was performed using a mixture of hexane, chloroform, ether and petroleum ether in equal proportions according to the method reported by Chemists (1990) to avoid exposure to any heat treatment. The extraction was repeated four times at room temperature (25.6 °C). The extract was left in a separate funnel to allow the oil to be separated by gravity. Two layers were obtained. The upper layer was separated from the pure oil and the bottom layer represented a mixture of the pure oil and some emulsifying materials. The oil was placed in plastic containers (250 ml vials) and stored in a freezer for testing directly.

2.4. Oil properties

2.4.1. Iodine value

The number of iodized fish oil was calculated according to the method of Chemists (1990). 0.25 g of oil was dissolved in 10 ml of chloroform in a beaker. 30 ml of Hans solution was then added and the beaker was well covered and kept in the dark for 30 min. 10 ml of 15% potassium iodide was then added and the contents well mixed. After that 100 ml of distilled water was added and the mixture calibrated with sodium sulfate (0.1 M), using a



Fig. 1. left; Hamri (*Barbus luteus*) and Balaout (*Chondrostoma regium*) in the right side.

starch guide. The steps were then repeated without adding the oil, to serve as blank.

2.4.2. Peroxide value

This was estimated by dissolving 5 ml of oil in a soluble mixture (ice acetic acid + chloroform (60 + 40 volume/volume)) and then adding 0.5 ml saturated potassium iodide solution. The beaker was then closed and the mixture stirred in a circular motion for 15 min. 100 ml of water was added. The solution was then kept in a flask and the iodine calibrated with sodium thiosulfate (0.1 M) with the evidence of starch and an extreme rejuvenation. The steps were repeated without adding oil, so as to get the blank and then according to the size of thiosulfate necessary for calibration (Chemists, 1990).

2.4.3. Acid value

The acid value was estimated by taking 5 g of oil and adding 50 ml of 95% neutral ethanol to it. The contents was then heated to dissolve the fat, after which 0.1 ml samples of sodium hydroxide solution was added with phenolphthalein as an indicator, according to the method reported by Chemists (1990).

2.4.4. Saponification number

This was also calculated according to the method of Chemists (1990) by adding 5 ml of potassium hydroxide (0.5 M) to the sample (5 g of oil) with a stirrer installed on the nozzle of the flask. The sample was then heated in a water bath for half an hour, and then cooled by running water from the outside and calibrating with HCl (0.5 M) in the presence of phenolphthalein. The process was repeated without adding oil to be used as blank.

2.5. Fatty acid profile

2.5.1. Preparation of fatty acids methyl ester

Fatty acids were acquired by transmethylating off into fatty acid methyl ester as described by Simionato et al. (2010). Lipid weight of approximately 150 mg was added to 5.0 ml of 0.25 molL⁻¹ of sodium methoxide in methanol-diethyl ether (1:1) and it was stirred vigorously for 3 min. After that 3 ml of isooctane and 15 ml of saturated sodium chloride were added. The content was stirred vigorously again and allowed for the phase separation. The upper layer was transferred to GC Vial for analysis. Fatty acids were identified by comparing standard retention time with fatty acid retention time of samples. Fatty acids standard was purchased from Supelco (16823–0048), USA and contains 37 fatty acids.

2.5.2. GC-FID analysis of fatty acids

GC analysis was performed using GC-FID equipment with SGE-BPX 70 column. The condition of GC during the analysis was set as follows: rates used were 1.4 ml min⁻¹ carrier gas (H₂), 30 ml min⁻¹ make-up gas (N₂) and 30 and 300 ml min⁻¹ flame gases, (H₂) and flame synthetic air, respectively. The injection sample took rate 1/100 with detector temperatures 235 °C. The column temperature was 65 °C for 4 min, followed by a ramp of 16 °C min⁻¹ up to 185 °C, kept for 12 min. A second ramp of 20° C min⁻¹ was run up to 235 °C for 14 min. The total analysis time was 40 min.

2.6. Biological experiment

Male laboratory rats (weighed 145–210 g) that aged between 45 and 50 days, on a well-known diet containing two type of fish oil were used. T1 control (normal feed), T2 [6% of Hamri (*Barbus luteu*) oil] and T3 [6% of Balaout (*Chondrostoma regium*) oil]. After day- 28 controlled experiment, cholesterol, high density lipoprotein, triglyceride and blood sugar level of laboratory rats were esti-

ated by an enzymatic colorimetric method by using commercial kit (Biolabo, Maizy, France).

2.7. Statistical analysis

The results of the experiment were statistically analyzed, using the complete randomized design (CRD) statistical program (Institute, 1999). Differences between the means were tested by Duncan's multiple range tests. The level of significance was chosen at P < 0.05.

3. Results

The percentage of proximate analysis of 2 types of fish is shown in Table 1. The percentages in Hamri meat were 72.13, 19.74, 5.07 and 1.60%, while those of Balaout meat were 71.63, 19.98, 4.96 and 2.04% for moisture, protein, fat and ash respectively. The iodine value was 77.20 in the Hamri fish meat oil, and 86.00 in the Balaout fish meat oil. The results (Table 2) show that oil from Hamri and Balaout fish meat consisted of 44.31 and 55.76% of saturated fatty acid, 36.10 and 25.41% of poly unsaturated fatty acid, and 18.17 and 25.41% mono unsaturated fatty acids respectively. The results (Table 3) also show that Stearic acid (6.18%) in Hamri meat oil and palmitic fatty acid (3.95%) in Balaout meat oil were the predominant fatty acids in the saturated fatty acid group. The Palmitoleic acid (C16:1) recorded high percentages of 24.83 and 27.56% in Hamri and Balaout fish respectively. The percentages of oleic acid (C18:1) were 23.26 and 22.63% in Hamri and Balaout meat oil respectively. The Stearic acid, Palmitoleic acid (C16:1) and the percentages of oleic acid (C18:1) for common carp were 2.97%, 10.40% and 36.54%, respectively (Stancheva and Merdzhanova, 2011). Furthermore, A significantly decrease (P < 0.05) in serum cholesterol level was fed on a diet containing 6% oil of Hamri and Balaout fish with a total of 88.13, 97.30 Mg/Deciliters respectively, while in the serum of controlled treatment, rats fed on a diet containing animal fat concentration of 6%(175.76 Mg/Deciliters). Results of triglycerides levels showed significant decrease (P < 0.05) (101.54, 151.43 Mg/Deciliters) in the serum of T2 and T3 rats respectively compared to the controlled sample (222.70 Mg/Deciliters).

4. Discussion

Results of Table 1 agreed with results of other researches on 11 fresh water fishes where the fat percentage was between 0.6 and 7.6 % (Grela and Dudek, 2007); (Łuczyńska et al., 2008); (Khidhir, 2011). The difference in proximate composition may be due to the nature of nutrition, season and the surrounding environment (Pirestani et al., 2009); (Abii et al., 2007); (Tulgar and Berik,

Table 1

Proximate analysis and Some Properties of (mean values ± sd) Hamri (*Barbus luteus*) and Balaout (*Chondrostoma regium*) meat oil. (n = 10).

Parameters	Hamri (<i>Barbus luteus</i>)	Balaout (<i>Chondrostomaregium</i>)
Moisture %	72.13 ± 1.23a	71.63 ± 1.99a
Ash %	1.60 ± 0.35a	2.04 ± 0.15a
Fat %	5.07 ± 1.12a	4.96 ± 0.30a
Protein %	19.74 ± 1.20a	19.98 ± 0.85a
Iodine value%	77.20 ± 3.65a	86.00 ± 2.85a
Peroxide value (meq oxygen/kg fat)	15.56 ± 1.62a	10.85 ± 1.17a
Acid value (mg KOH/g oil)	1.74 ± 0.14a	1.46 ± 0.04a
Saponification number (mg/g oil)	19.74 ± 1.20b	138.43 ± 5.42a

Different letter in same column mean significant differences (P < 0.05).

Table 2
Fatty acid profile (mean values \pm sd) of Hamri (*Barbus luteus*) and Balaout (*Chondrostoma regium*) meat oil. (n = 10).

Fatty acid%	Hamri (<i>Barbus luteus</i>)	Balaout (<i>Chondrostoma regium</i>)
SFA	44.31 \pm 14.61	55.76 \pm 6.39
Poly USFA	36.10 \pm 1.67	25.41 \pm 2.10
MonoUSFA	18.17 \pm 0.53	16.57 \pm 0.58
Total fatty acid%	98.59 \pm 36.61	97.733 \pm 5.53
C12:0	2.48 \pm 0.12	4.05 \pm 0.10
C14:0	5.08 \pm 0.09	3.92 \pm 0.25
C16:0	4.42 \pm 0.167	4.6700 \pm 0.46
C18:0	6.18 \pm 0.59	3.95 \pm 0.11
C14:1	3.06 \pm 0.06	2.28 \pm 0.54
C16:1	24.83 \pm 1.83	27.56 \pm 4.10
C18:1	23.26 \pm 2.47	22.63 \pm 2.09
C20:1	5.15 \pm 1.13	3.29 \pm 0.34
C18:3	7.03 \pm 0.08	8.93 \pm 0.52
C20:2	4.11 \pm 0.17	3.89 \pm 0.91
C20:4	1.14 \pm 0.39	3.91 \pm 1.42
C22:5	2.02 \pm 1.12	6.91 \pm 1.01
C22:6	3.51 \pm 0.42	4.44 \pm 0.73

Table 3
Some Rats blood traits (mean values \pm sd) feeding on 6% Hamri (*Barbus luteus*) and Balaout (*Chondrostoma regium*) meat oil. (n = 5).

Treatment	Cholesterol (Mg/Deciliter)	Triglyceride (Mg/Deciliter)	High density lipoprotein (HDL) (Mg/Deciliter)	Blood sugar level (Mg/Deciliter)
T1 (Control)	175.76 \pm 5.59a	222.70 \pm 22.26 a	21.07 \pm 0.94b	216.80 \pm 9.07a
T2 (6%) of Hamri (<i>Barbus luteu</i>) oil	88.13 \pm 5.51b	101.54 \pm 8.93b	25.09 \pm 1.38a	127.06 \pm 8.21b
T3 (6%) of Balaout (<i>Chondrostoma regium</i>) oil	97.30 \pm 6.94b	151.43 \pm 8.37b	26.06 \pm 1.42a	154.60 \pm 16.02b

Different letter in same column mean significant differences ($P < 0.05$).

2012). Chemical composition is unique for each species or in other words different from one type to another (Shearer, 1994). However, according to Huss (1995) all values within the natural ranges were approximate (i.e., water 66–84%, protein 15–25 %, fat 0.1–24 %). Lipid of fish is one of the most important natural sources of polyunsaturated fatty acids (Patrick Saoud et al., 2008). The proteins, fats and water of fish meat are also of essential importance to human health; hence, scientists and manufacturers take this into consideration when processing fish, so as to satisfy consumers (Murray and Burt, 2001).

The increase in iodine may also be due to the high percentage of unsaturated fatty acids in the studied fish meat because the iodine is a standard for unsaturation. The peroxide values were 15.57 and 10.85 meq oxygen/kg fat in Hamri and Balaout meat oil respectively. It is consistent with Khidhir et al.(2013) whose study revealed Peroxide values (PV) for Bizz, Grass carp, Shabbout, Common carp and Silver carp to be 3.40, 4.47, 5.29, 7.25 and 8.86 meq oxygen/kg fat respectively. From the results the Saponification number of the fish meat oils for Hamri and Balaout were 112.56 and 138.43 mg/g respectively (Table 1).

The difference of results of oil from Hamri and Balaout fish meat and saturated fatty acid, poly unsaturated fatty acid, and mono unsaturated fatty acids may be due to the variation on nutrition and presence of some feed on aquatic environment (Taşbozan et al., 2016). The composition of fat and hence the composition of fatty acids in fish vary, depending on different factors; usually, their aquatic environment (marine water, fresh water, cold or warm water) and the biological, physical and chemical properties of that environment. Also, seasonal changes, migration, sexual maturity, spawning period, species, food habits, and whether they are bred in aquaculture or are left in their natural habitats affect the composition of fat/fatty acids (Aras et al., 2002). The benefits of n-3 PUFA (polyunsaturated fatty acids) are associated with the synthesis of eicosanoids such as prostaglandins, thromboxanes and leukotrienes (Abbas et al., 2009).

Moreover, Palmitoleic acid is one of the important pharmaceutical applications of fatty acids. It is supposed to have anti-clotting effects, which can help prevent stroke (Abraham et al., 1989). It was clear that the palmitic acid percentage was lower than Hamri and Balaout fish. The benefits of oleic acid include the following: modification in the plasma concentrations of fat and fatty proteins, inhibition of blood clotting, improved or balanced glucose level, and reduction in inflammation and oxidation states in fasting conditions (López et al., 2010). The oil from Balaout fish meat recorded of Arachidonic fatty acid (C4:20) is 6.91%, which forms the basis for prostaglandin formation and also in biological creation of thrombin that pervades in the clotting of blood (Memon et al., 2011).

Table 3 shows the effect of dietary fish oil on the level of cholesterol, triglyceride, high density lipoprotein (HDL) and serum glucose level in the laboratory rats after feeding on a diet containing 6% concentration of oil, compared with the controlled sample. Moreover, feeding on the diet containing 6% Hamri and Balaout oil raised the level of high-density lipoprotein (HDL), (25.09 and 26.06 Mg/Deciliters) respectively for Hamri and Balaout. The results showed a high sugar level of about 216.80 Mg/Deciliters in the rats of the control group, while the level of blood sugar in groups fed on a diet containing fish oil were 127.06 and 154.60 Mg/Deciliters) respectively for Hamri and Balaout.

Furthermore, fish consumption reduces the risk of heart and cardiovascular diseases, including heart failure and atrial fibrillation, stroke and myocardial infarction, lethal and sudden cardiac death in several monitoring studies (Mozaffarian and Wu, 2011); (Roth and Harris, 2010).

5. Conclusion

Hamri (*Barbus luteus*) and Balaout (*Chondrostoma regium*) are important and common types of fish in Iraq. Approximate compositions of both types of fish were approximately same. Its oils had

significant effect on cholesterol, triglyceride, high density lipoprotein (HDL) and blood sugar levels of laboratory rats. It can be concluded that the fats in the studied fishes contained good levels of unsaturated fatty acids and this may have positive effects on human health.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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