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Impact of Refrigerated Storage on Quality of Oil from Freshwater Jarko (*Wallago attu)* Fish.

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Abstract

The effect of refrigerated storage on the quality of freshwater fish oil Jarko (*Wallago attu)* was evaluated by measuring fatty acid profile, free fatty acids (FFA), peroxide value (PV), acid value (AV), sponification value (SV), iodine value (IV) and poylene index (PI) up to the time period of 120 days. After 120 days storage, mono unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) contents were decreased by 24.89% and 33.70%, respectively. While, saturated fatty acids (SFA) content was found to be increased by 26.82%, against the actual value. The change in polyunsaturated fatty acids during refrigerated storage was measured by the PI value. The PI decreased during storage due to lipid oxidation, but remained nearly constant after 90th day of storage. The results of PV, AV and FFA demonstrates that *Wallago attu* fish oil remained acceptable for consumption for 60 days but eventually exceeded the recommended values after 60 days of refrigerated storage.

Keywords: Wallago attu, Oil quality, Poylene value, Lipid oxidation, Refrigerated storage.

Introduction

Fish foods have recently received more attention from consumers due to their positive benefits on human health and nutrition [1]. Recent studies have clearly shown the importance of *n-3* and *n-6* fatty acids for human health and nutrition [2 - 4]. Fortunately, fish oil is one of the best sources of dietary supply of these fatty acids. Many studies have shown that fish oil has important roles in prevention of cardiovascular diseases and some types of cancer, including colon, breast and prostate [4-6]. In addition, fish oil also helps to prevent brain aging and Alzheimer's disease [7]. Many health experts suggest that two to three servings per week of fish food should be consumed in order to meet the recommended level of essential fatty acids for pregnant women, children and elderly people [8- 9]. Fish food is the best source of dietary supply of *n-3* fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It has been suggested that consuming EPA and DHA may reduce the risk of mortality from cardiovascular disease in people who have already experienced a cardiac event [4].

Fresh fish is susceptible to spoilage caused by both microbiological and chemical reactions. Lipid deterioration easily takes place and limits the shelf-life of oily fish during storage [10- 11]. Both hydrolytic and oxidative rancidities in fish muscle are associated with quality deterioration [12]. Due to its high content of polyunsaturated fatty acids, including EPA and DHA, fish oil is highly susceptible to oxidative spoilage [13] and the rate of fish oil oxidation is significantly different from that of other oils. Fish oils also include high concentrations of phospholipids, containing unsaturated fatty acids, which make them even more sensitive than other oils. Hydrolysis, induced by lipases and phospholipases, produce free fatty acids that undergo further oxidation to produce low-molecular weight compounds that are

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responsible for the rancid off-flavor and taste of fish and fish products [14]. Exposure of fish products to air (as is very common in the display of fish under retail conditions) results in oxidation of PUFAs. This causes rancidity of the product, which most often results in the deterioration of color, texture and flavor [15-16] and even the nutritive value of the food [17]. Therefore the main purpose of this study was to investigate the stability of fish oil during refrigerated storage with respect to its nutritional value and fitness for human consumption.

Materials and Methods

Fish samples

Ten *Wallago attu* (Jarko), samples with an average weight of 1500–1600 g, were captured from Indus river Jamshoro Sindh, Pakistan. After capture the fish, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the National Center of Excellence in Analytical Chemistry, University of Sindh Jamshoro, Pakistan. Whole fish were immediately washed, cleaned and kept in to the refrigerator. The flesh was chopped to uniformity and the total lipids were extracted from a sample of the minced fillets using chloroform: methanol (2:1 by vol) extraction solutions by Folch *et al*. 1957 method [18], extracted lipids were used for analyses during refrigerated storage.

Fatty acid profile

Fatty acid profile was determined as fatty acid methyl esters (FAMEs). The FAMEs were prepared according to the IUPAC method [19]. The prepared methyl ester was injected to the gas chromatography (Perkin Elmer) equipped with the flame ionization detector (FID). A polar capillary column (100 m x 0.25 mm**:** Supelco, Inc., Bellefonte, PA, USA) fitted with a non bonded *biscynopropyl siloxane* stationary-phase. The analytical conditions were: Injection port temperature of 260 ^{0}C and Detector temperature of 270 ^{0}C. The oven was programmed from 150 to 270 ^{0}C at a rate of 4 ^{0}C /min. Retention times of FAME standards (Sigma, St. Louis, MO, USA) were used to identify chromatographic peaks of the samples. Fatty acid content was calculated, based on the peak area ratio and expressed as g fatty acid/100 g oil.

Oxidative stability of fish oil

Determination of free fatty acids (FFA)

The free fatty acids content of the oil was determined volumetrically using aqueous sodium hydroxide (0.1 M) and phenolphthalein indicator (1% ethanol) according to AOCS (1998) method Ca 5a-40. A neutral mixture of diethyl ether: ethanol (1:1) (50 ml) was used as a solvent. FFA values were reported as % oleic acid by weight.

Determination of peroxide value (PV)

The peroxide value was determined and expressed as meq O_2/kg oil, according to AOCS (1998) method Cd 8b-90. Oil samples were dissolved in chloroform and mixed with glacial acetic acid (Sigma) and freshly prepared saturated potassium iodide solution. Liberated iodine was titrated with standard sodium thiosulphate (0.01 M) solution using starch indicator (1%).

Determination of the iodine value (IV)

The iodine value was determined according to the AOAC (1999), method 993.20, using carbon tetrachloride as solvent. Dissolved oil sample was mixed with Wijs' solution and freshly prepared potassium iodide (10%) solution. Liberated iodine was titrated with standard potassium thiosulphate (0.1 M) solution, using carbon tetrachloride as a blank and starch as an indicator.

Determination of acid value

Acid value was determined according to AOAC (1995). Acid value was analyzed by titration of approximately 0.5 g of lipid, dissolved in a mixture of 100 ml of ethanol and diethyl ether (1:1; v/v), with 0.01 N potassium hydroxide. Phenolphthalein was used as indicator. The results were expressed as mg KOH/g lipid.

Saponification value

Saponification value was determined according to (AOCS Cd 3-25) method. It is the number of KOH required to saponify 1 gram of oil. Saponification is the hydrolysis of ester under alkaline condition.

Polyene value

The polyene index (PI) is the ratio 20:5+ 22:6/16:0 of fatty acids and was calculated the method as described by Alicia *et al*, 2007 [25].

Statistical analysis

The results presented are means ± standard deviation obtained from the analysis of ten fish. The difference between the mean values of parameters examined were submitted to One-way analysis of variance (ANOVA) using SPSS 10.0 (SPSS 1990, SPSS Inc., Michigan Ave, Chicago, Illinois, USA), statistically significant differences were reported at ($P<0.05$). If the overall F-test was significant ($p<0.05$), then a Fishers T- test was performed to discern differences among the storage days.

Results and Discussions

Changes in fatty acid profile

Changes in saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were observed in oil of *Wallago attu* fish throughout the refrigerated storage are presented in Table1. The SFA level was found higher as compare to MUFA and PUFA. The PUFA level was observed to decrease during storage time in contrast to SFA similar study was shown by Nazemroaya, *et al* [20] during frozen storage of *Scomberomorus commersoni* and *Carcharhinus dussumieri* fish. The represented GC-FID chromatogram for *Wallago attu* fish were presented in (Fig. 1.)

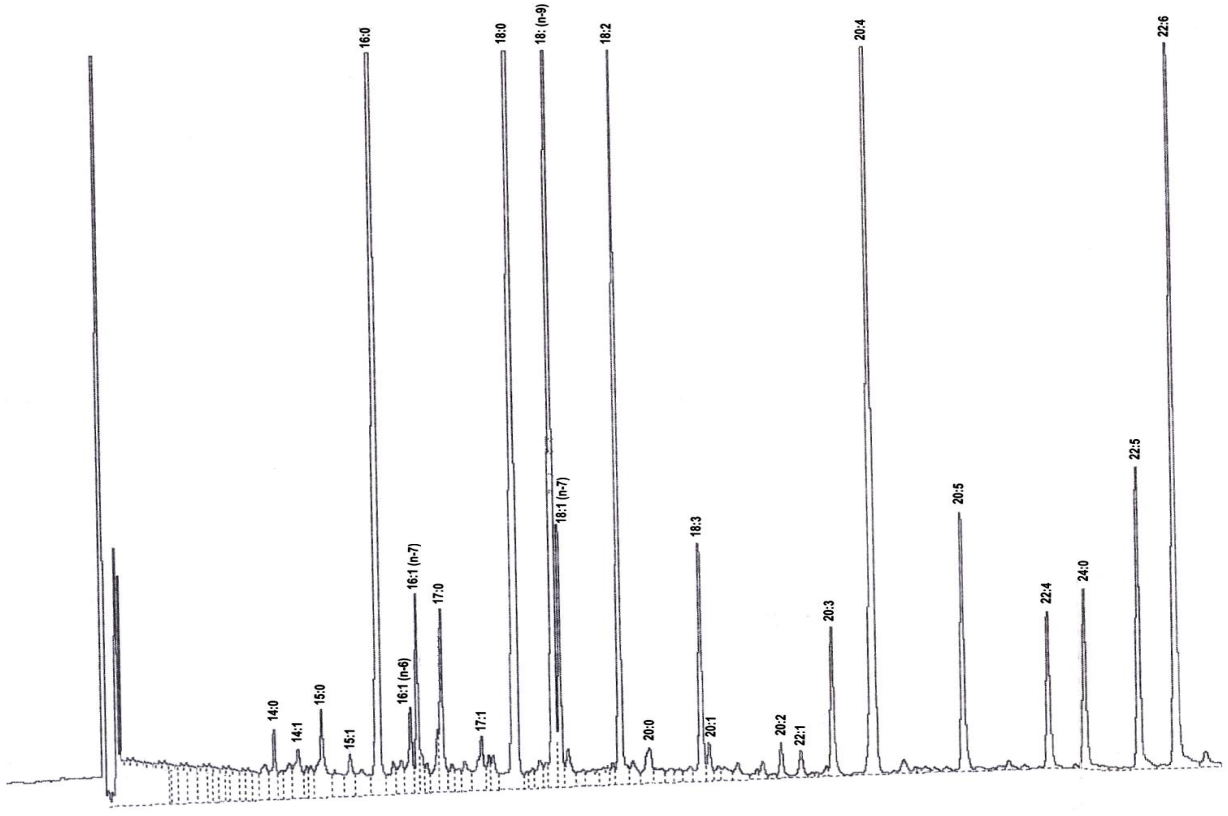

Figure 1. **Represented GC-FID Chromatogram of Fatty acids methyl esters for *Wallago attu* fish oil.**

Note: Elution order of fatty acids with respect to retention time of Fatty acids 14:0, 14:1, 15:0, 15:1, 16:0, 16:1(n-6), 16:1(n-7), 17:0, 17:1, 18:0, 18:1, 18:1, 18:2, 20:0, 18:3, 18:3, 20:1, 20:2, 22:0, 22:1, 20:3, 20:4, 24:1, 20:5, 22:4, 24:0, 22:5, 22:6, Retention time, 8.37, 9.11, 9.87, 11.12, 11.71, 12.64, 12.86, 13.63, 14.86, 15.97, 17.17, 17.35, 19.22, 20.14, 21.78, 22.1, 23.5, 24.2, 25.88, 17.18, 29.97, 32.62, 33.72, 35.41, 36.60, respectively.

Fresh *Wallago attu* muscle lipids comprised 37.32% SFA, 27.96% MUFA and 34.69% PUFA. Among PUFA, docosahexaenoic acid 22:6 (*n*-3) (DHA) was the most abundant followed by eicosapentaenoic acid 20:5 (*n*-3) (EPA). Similar findings with abundance level increase by 2-3 times are also reported earlier [20]. In the present study, DHA was 2.4 times greater than EPA. During refrigerated storage, decreases in PUFA, especially EPA were observed, particularly as the storage time increased. During refrigerated storage, EPA decreased by 8.49%, 20.57%, 30.10% and 43.37% at day 30, 60, 90 and 120, respectively. For DHA, it decreased by 4.18%, 8.07%, 11.83% and 16.43% at day 30, 60, 90 and 120, respectively. The marked decrease in EPA and DHA might be due to their susceptibility to oxidation. At day 120 of refrigerated storage, MUFA and PUFA contents decreased by 24.89% and 33.70% respectively, whereas SFA content increased by 26.82%, compared with that found at day 0.

Oxidative stability of fish oil

Fish oil containing high level of PUFAs, is very labile to hydrolytic spoilage and especially oxidative deterioration. In the present study the oxidative stability of *Wallago attu* fish oil were determined by using different analytical methods, such as FFA, PV, SV, IV, AV and PI index to assess the quality of fish oil under refrigerated storage conditions. The results are presented in (Table 2).

Table 1. **Changes in main fatty acid classes (%) of *Wallago attu* fish oil during refrigerated storage.**

Fatty acids	Storage days				
	0	30	60	90	120
∑SFA	$37.32^{a} \pm 0.01$	$40.35^{b} \pm 0.02$	$44.5^{a} \pm 0.01$	$47.11^{c} \pm 0.03$	$51.0^{a} \pm 0.01$
∑MUFA	$27.96^{b} \pm 0.02$	$25.46^{a} \pm 0.01$	$23.79^{c} \pm 0.03$	$21.93^{d} \pm 0.04$	$21.0^{b} \pm 0.02$
∑PUFA	$34.69^{c} \pm 0.11$	$30.95^{c} \pm 0.15$	$28.68^{b} \pm 0.02$	$26.27^{a} \pm 0.01$	$23.0^{a} \pm 0.01$
20:5	$6.71^{c} \pm 0.05$	$6.14^{d} \pm 0.15$	$5.33^{e} \pm 0.21$	$4.69^{b} \pm 0.02$	$3.80^{a} \pm 0.00$
22:6	$14.12^{a} \pm 0.01$	$13.53^{b} \pm 0.02$	$12.98^{c} \pm 0.03$	$12.45^{d} \pm 0.10$	$11.8^{a} \pm 0.01$

Note: Each value is an average of ten samples, with its standard deviations. Different superscripts in the same row indicate significantly different values are at ($p < 0.05$).

Table 2. **Change in chemical properties of *Wallago attu* fish oil during refrigerated storage.**

Storage Days	Free Fatty Acid (%)	Peroxide Value (meq O_2/Kg)	Sponification value (mgKOH/g)	Iodine value (mg I/g oil)	Acid Value (mg KOH/g)	Polyene index (20:5+22:6/16:0)
0	ND	ND	$172^{a} \pm 0.01$	$188^{c} \pm 0.05$	$3.15^{a} \pm 0.01$	$1.05^{e} \pm 0.03$
15	$0.60^{a} \pm 0.11$	$1.37^{a} \pm 0.12$	$175^{a} \pm 0.01$	$184^{c} \pm 0.01$	$4.85^{a} \pm 0.01$	$1.03^{e} \pm 0.01$
30	$1.07^{a} \pm 0.02$	$2.81^{b} \pm 0.03$	$177^{b} \pm 0.12$	$183^{c} \pm 0.03$	$5.93^{b} \pm 0.02$	$0.92^{d} \pm 0.15$
45	$2.15^{b} \pm 0.01$	$3.92^{b} \pm 0.01$	$179^{b} \pm 0.15$	$180^{c} \pm 0.01$	$7.68^{b} \pm 0.04$	$0.80^{c} \pm 0.12$
60	$4.38^{c} \pm 0.12$	$4.15^{b} \pm 0.01$	$183^{c} \pm 0.02$	$176^{b} \pm 0.11$	$8.50^{b} \pm 0.05$	$0.61^{b} \pm 0.01$
75	$5.82^{c} \pm 0.01$	$6.99^{c} \pm 0.13$	$184^{c} \pm 0.04$	$173^{b} \pm 0.12$	$10.32^{c} \pm 0.12$	$0.68^{b} \pm 0.05$
90	$7.91^{d} \pm 0.01$	$8.05^{d} \pm 0.14$	$185^{c} \pm 0.01$	$170^{b} \pm 0.31$	$13.95^{c} \pm 0.04$	$0.57^{a} \pm 0.21$
105	$10.43^{e} \pm 0.03$	$7.02^{d} \pm 0.02$	$192^{d} \pm 0.05$	$168^{a} \pm 0.04$	$15.25^{d} \pm 0.01$	$0.56^{a} \pm 0.01$
120	$11.65^{e} \pm 0.15$	$5.34^{c} \pm 0.01$	$206^{e} \pm 0.12$	$163^{a} \pm 0.15$	$16.78^{d} \pm 0.01$	$0.56^{a} \pm 0.03$

Note: Each value is an average of ten samples, with its standard deviations. Different superscripts in the same column indicate significantly different values at ($p < 0.05$). ND= not detected.

The free fatty acid formation due to the lipid hydrolysis has provided a suitable means for assessment of fish oil damage during storage and can be used as quality index for fish and for other food products [22]. In the present study increase in free fatty acid content was not detected during the 1st storage day however little changes were observed to start from the 15th day of storage and FFA values were gradually increase with increasing storage time period. Moreover, as per quality specifications for crude fish oil, maximum acceptable values of 5% FFA are proposed [23]. This study showed that the free fatty acid level of *Wallago attu* fish oil reached 5% limit during 60th day of refrigerated storage.

An important stage in the oxidation is the reaction of oxygen with the unsaturated fatty acid molecules to form hydroperoxides; the amount of these can be used as a measure of the extent of oxidation in the early stages. The peroxide test is a measure of the formation of hydroperoxides. An increase in the PV is most useful as in index of the earlier stages of oxidation; as oxidation proceeds and peroxides are degraded the PV can start to fall. The initial changes in PV of *Wallago attu* fish oil was 1.37 meq O_2/Kg detected during the 15th day of storage. The PV increased significantly until the 90th day of storage than decrease on day 105 of storage. The decrease of the PV at the end of storage may occur owing to decomposition of hydroperoxides in to secondary oxidation products. Although literature review mentioned the PV of crude fish oil was between 3 and 20 meq O_2/Kg [24-25], yet the present study indicated the PV of examined fish oil sample not exceeding 20 meq O_2/kg oil. Where as, the acceptability limit for PV of crude fish oil is 7–8 meq O_2/kg oil [13], examined fish oil samples during refrigerated storage reached this acceptability limit in 75 days.
SV is a measure of molecular weight and defined as the amount of alkali required to saponify fatty acid in a given weight of oil. Hydrolysis and oxidation bring about lipid breakdown, forming free fatty acids or aldehydes and ketones as the end product. The SV of analyzed fish oil increases with increasing storage period, it is possible that the end products of oxidation, such as aldehydes and ketones may contribute to increase in SV.

Conversely there was gradual decrease in IV for analyzed *Wallago attu* fish oil. General decreased in IV indicates the decrease in the degree of unsaturation during storage time period of refrigerated storage.

AV also increased with increasing time period; increase in AV is generally associated with lipase activity originating from microorganisms or biological tissue. It is important to note that the samples studied neither sterilized nor studied them under aseptic conditions. Thus, it is possible that some enzyme or microorganism contamination might have taken place during sample removal which is in contrast to earlier studies which reported that hydrolysis of fish was greatly reduced upon sterilization [26]. The acceptable limit for AV is reported to be 7-8mg KOH [23]. Our results showed that this limit was exceeded beyond 45 day of storage.

Damage to polyunsaturated fatty acids during refrigerated storage was measured by the PI value. PI (20:5+22:6/16:0) might provide a meaningful tool to measure oxidative stability of fishery products because it includes 2 major long chain essential polyunsaturated fatty acids that the level of which can be measured with reasonable accuracy as they are the essential FA. The PI decreased during storage due to lipid oxidation, but remained nearly stable after 90th day of storage. This study demonstrates that *Wallago attu* fish species was acceptable for consumption for 60 days of refrigerated storage and eventually becomes unacceptable after that.

(Fig. 2) shows the relationship between iodine value and polyene index of *Wallago attu* fish oil for different refrigerated storage days. The linear relationship was found in iodine value versus polyene index having regression value R^2= 0.8635 the following regression equation was generated Y = 0.00227 X - 3.2449.

In the present study inverse relation was observed between the iodine value and free fatty acids of *Wallago attu* fish oil as shown in (Fig. 3). The excellent regression value (R^2= 0.9929) was found with regression equation Y = -1.8875 X + 184.9. In similar fashion Figure 4 represents the inverse relationship between iodine value and acid

value in *Wallago attu* fish oil during refrigerated storage with regression value R^2=0.9748 as calculated with regression equation Y = -0.5728 X + 110.48.

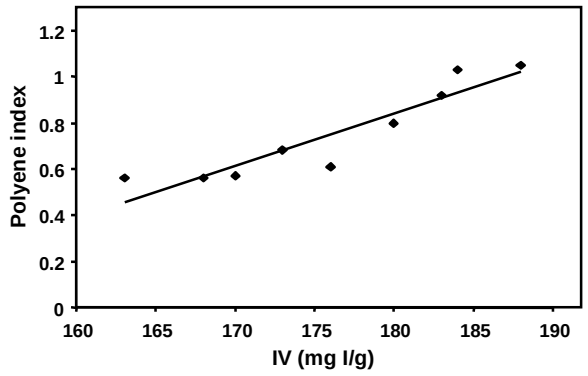

Figure 2. **Relationship between Iodine value and polyene index in *Wallago attu* fish oil during refrigerated storage.**

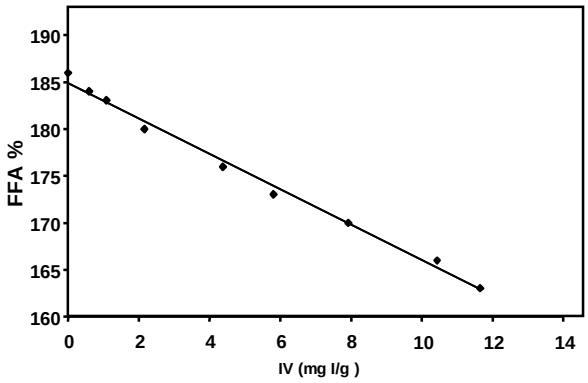

Figure 3. **Relationship between iodine values and free fatty acids in *Wallago attu* fish oil during refrigerated storage.**

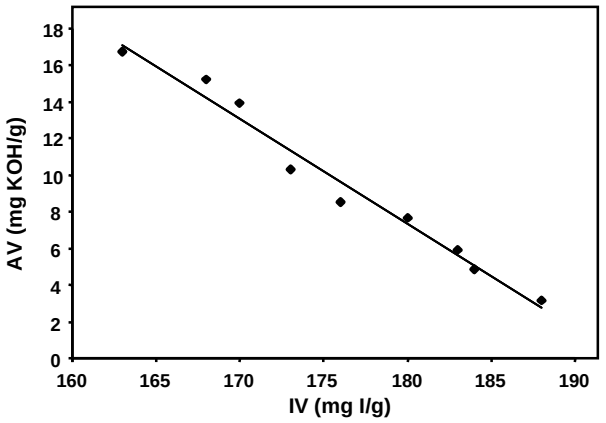

Figure 4. **Relationship between Iodine value and acid value in *Wallago attu* fish oil during refrigerated storage.**

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