Effects of Different Type of Processing Methods on Biochemical Changes of Skipjack Tuna

Katsuwonus pelamis

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Abstract: Nutritional value of Skipjack tuna (Katsuwonus pelamis) which was processed with curry in coconut milk and fried in coconut oil respectively were analysed. The n-3 polyunsaturated fatty acids (PUFAs) are significantly higher in the fresh flesh (33.91%) of skipjack tuna than fish cooked in curry (15.65%) and fried fish (7.61%). n-6 PUFAs showed an increase in fish cooked with coconut milk (19%) and a decrease in fried fish (10%) compared to fresh fish (12%). Particularly, lipids considered to have health benefits, EPA and DHA, showed decreased amounts in both fish cooked in curry (2.26%, 11.38%) and fried fish (1.18%, 5.56%) compared to fresh fish (4.06%, 27.77%), respectively. Tocopherol content was higher in fresh fish (1048.88 µg/100 g) than in the fish cooked in curry (661µg/100 g) and fried fish (931.16 µg/100 g). Thrombogenic (TI) and atherogenic (AI) indices increased upon cooking, which makes the fish less healthy for consumption. The processed fish indicated lesser nutritive values.

Keywords: skipjack tuna, fatty acid composition, tocopherol, proximate composition, atherogenicity, thrombogenicity, health benefits

Practical Applications
Processed tuna fish with coconut oil and coconut milk causes low nutritive value of tuna consumption due to loss of health beneficial fatty acids (EPA and DHA) and the tocopherol content in muscle. The lipid indices increase in processed tuna fish consumption (lower) less effects on anti-inflammatory action in atherosclerosis. This type of tuna processing method is most popular in Asian fish food industries. The raw tuna fish consumption gives better nutritive content than the processed fish.

1. Introduction

Many people eat fish for the beneficial impacts on human health. In fact, the wide range of healthy fatty acids (FAs) in fish, including their high content of polyunsaturated fatty acids (PUFAs) (Yang et al. 2013) [1], is raising the commercial appeal of fish. The health benefits of fish consumption, believed to be due to the n-3 fatty acids in fish, include decreasing the risk of cardiovascular diseases [2] (Stone et al. 1996), reducing the serum triacylglycerol level [3] (Harris 1997), reducing blood pressure [4] (Howe 1997), and impeding the development of insulin resistance, which is related to modulating glucose metabolism [5] (Samane et al. 2009). The cooking process can stimulate lipid oxidation and the loss of long chain n-3 PUFAs, which reduces the nutritive value of fish [6] (Aouburg et al. 2010). Among the PUFAs, linoleic acid (LA) (18:2n-6), belonging to the n-6 PUFAs with anti-atherogenic action, and the more important n-3 PUFA components such as linoleic acid (LNA) (18:3n-3), EPA (20:5n-3) and DHA (22:6n3) which have anti-thrombogenic effects are considered beneficial [7] [8] (Thies et al. 2003; von Schacky et al. 1999). However, the saturated fatty acids (SFAs), lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), are recognized as carrying health risks [9] (Mensink et al. 1994). The monounsaturated fatty acids (MUFA)s present in fish, particularly DHA, have been shown very efficient in reducing the risk of coronary disease. Indeed, MUFA have been recognized as being as beneficial as the n-3 PUFAs because of their effectiveness in lowering blood cholesterol [10] (Ander et al. 2003). The FA composition of fish is affected by many factors such as species, capture, area, temperature and fishing season; however, it is believed that the dietary fatty acids such as EPA and DHA, in fish have a direct influence on its FA composition [11] (Lei et al. 2013). To assess the nutritional quality of lipids in fish, the indices of atherogenicity (AI), showing the inhibition of the aggregation of plaque and diminishing the levels of esterified FAs, cholesterol, and phospholipids, thereby preventing the appearance of micro-and macro-coronary diseases) and thrombogenicity (TI, showing the tendency to form clots in the blood vessels) have been proposed by [12] Ulbrich and Southgate (1991). The tocopherol contents of seafood items have been reported by [13] Gotoh et al. 2009. The effects of various storage temperatures, storage times and cooking styles on vitamin E levels of fish muscle were also observed [14] (Arslan et al. 1997).

In this study, we focused on the skipjack tuna Katsuwonus pelamis (Linnaeus 1758) which is commonly consumed by Asian people. Cooked skipjack tuna as curry with coconut milk and fish fried with palm oil, which are both considered food delicacies and within tradition for Sri Lanka and worldwide [15] (Collette and Nauen 1983). The skipjack tuna, K. pelamis (Linnaeus, 1758), is harvested more than any other tuna species in the marine fisheries of Sri Lanka [16] (Acharige et al. 2012).

The aim of this study was to characterize the fatty acid components, health-related lipid indices (AI & TI) and tocopherol content of the muscle tissue of skipjack tuna uncooked, cooked in curry with coconut milk and fried with coconut oil. Our data provides useful information for food scientists, nutritionists and consumers on the nutritional values of these delicacies.
2. Materialia and Methods

2.1. Fish Fillet Samples

Wild skipjack tuna (K. pelamis) (n= 05), with a meanweight of 1.63±0.73 kg and length of 52.24±2.45 cm, were randomly selected from the Eravur market, Eastern Province, Sri Lanka during the month of March 2016 and brought to the laboratory on ice within a polyethylene bag. Then it was gutted and well cleaned skin-less fish (flesh) packed in polyethylene bag and stored in flake ice until delivered to the Industrial Technology Laboratory (ITT), Colombo, and were in transit for 5 hours. Upon arrival at the laboratory, flesh samples were kept at -80°C until FA analysis.

2.2 Preparation of Traditional Fish Curry

Fish curry was prepared in a traditional Asian style with coconut milk. The fish were cleaned thoroughly and muscle tissue (trunk and abdominal parts) were cut into pieces and weighed. The fish pieces were transferred into a bowl and sprinkled with two teaspoons of salt (5.25 g) and one teaspoon of turmeric (2.5 g) and were set aside for 10 min. A medium size aluminium pan was used to cook fish in this study and was kept over medium heat. First, coconut oil (50 ml) was added, then mustard seeds, fenugreek, fennel seeds, curry leaves and cinnamon sticks were added and left for 30 seconds. The garlic, green chillies and onion (chopped) were added and cooked for 4-5 min until the onion became translucent. After that, sliced tomatoes were added and left for 2 min, followed by chilli powder, coriander powder/ cumin powder and salt, which were added and stirred well. Next, two litres of diluted coconut milk were poured in, followed immediately by tamarind pulp. Cooking continued for 4 min as the liquid heated to boiling. The gravy was allowed to boil for 5 min and the pieces of fish were gently added into the gravy and left for another 5-10 min cooking in low flame with the pan closed. Finally, two cups of concentrated coconut milk (first time taken) (50 ml) was added into the boiling gravy, which was left for another 5 min in low flame. Before the fish was removed from the flame, fresh coriander leaves were sprinkled over the top.

2.3 Preparation of Fried Fish

The fish were cleaned thoroughly and the scales, head and tail were removed; only the trunk was weighed. The fish were transferred into the bowl and sprinkled with two teaspoons of salt (5.25 g) and one teaspoon of turmeric (2.25 g) and were set aside for 10 min to marinate.

Coconut oil (Vimal, Cooking oil, made in Sri Lanka) was used as the frying oil. One litre of coconut oil was added into the frying pan and left boiling for 5 min. The temperature of the oil was noted and was about 92±2°C when the marinated fish were gently added into the oil for frying. The contents of the pot were stirred gently for 30 min until it turned a golden colour.

2.4 Proximate Analysis

All the proximate components were analysed in triplicate according to the respective standard methods available in the Association of Official Analytical Chemists (AOAC) Official Methods of Analysis (1995) [17]. All the reagents used for analysis were analytical reagent grade. In order to determine the recovery percentage in crude protein analysis, samples spiked with (NH₄)₂SO₄ were used; recovery values were within the acceptable range of 90-110%.

2.5 Lipid Extraction

The raw fish flesh, cooked fish in curry and fried fillet with palm oil from skipjack tuna each fish was homogenized using a mixed grinder (Sumeet, domestic DXE plus, India). Samples of approximately 3.0 g of homogenized muscle from each fish were weighed in triplicate using an analytical balance (AG204, Mettler, Toledo) and placed in dried conical flasks. Muscle tissue samples were hydrolysed by adding 8 mL of distilled water and 10 mL of concentrated HCl and incubated at 95°C in a boiling water bath for 45 min. The samples were cooled and transferred to Mojonnier flasks. Fat was serially extracted three times with 25 mL volumes of petroleum ether: diethyl ether (1:1 v/v). The upper phase containing the lipids was evaporated to dryness and weighed for further analysis.

2.6 Fatty Acid Methyl Esters (FAMEs)

Fatty acid methyl esters (FAMEs) were prepared from muscle sample. Sample were extracted with petroleum ether/diethyl ether solvent mixture according to method from the AOAC Official Methods of Analysis (948.16). The FAs, part of the total lipid extracted, were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and trans-esterified with 14% BF₃ (v/v) in methanol [18] (Paquot 1979).

2.7 Fatty Acids Analysis

The FAMEs were analysed on a Shimadzu-14A model gas chromatograph (GC) (Shimadzu, Japan), equipped with a flame ionization detector (FID) and fitted with a capillary column (Superlcowax-10 polyethylene glycol, length = 100 m, I.D. = 0.25 μm) (Sigma-Aldrich Co LLC, St. Louis, MO). Injector and detector temperatures were 200°C and 220°C. The oven program was initially held at 60°C for 10 min, then increased at a rate of 1°C/min to 200°C over 10 min and then held at 200°C for 55 min. Total run time was 75 min. The flow rate of the N₂ carrier gas was 1°C min⁻¹. GC analysis of FAMEs was repeated three times for each sample. FAMEs were identified by comparison of peak retention times to those of standards (NU prep check- SD 461, USA). Samples were run in split mode (50:1). Results were expressed as FID response area relative percentages of peak areas obtained from the GC-FID chromatogram. The results are given as mean ± SD in Table 1.

2.8 Indices of Lipid Quality

The atherogenicity (AI) and thrombogenicity (TI) indices, which were calculated from the fatty acid profile, as

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proposed by [19] Ulbricht and Southgate (1991), relate the profile of FAs with the risk of cardiovascular disorders, using the following equations:

\[ A1 = (C12:0 + (C14:0 × 4) + C16:0) / (\text{total unsaturated FAs}) \]  

Where C12 is the percentage of lauric acid in relation to total fatty acid (TFA); C14 is the percentage of myristic acid in relation to TFA; and C16 is the percentage of palmitic acid in relation to TFA.

\[ TI = \Sigma(C14:0 + C16:0 + C18:0) / (0.5 × \text{cis C18:1} + 0.5 × \Sigma \text{MSFA} + 0.5 × (n-6) + 0.5 × (n-3) + (n-3n-6)) \]  

Where: MFSA is monounsaturated fatty acid, n-6 is FAs containing omega-6 and n-3 is FAs containing omega-3. In addition, the unsaturated/saturated (USAT/SAT), n-3/n-6 ratio and Σ (EPA+DHA) were calculated using the fatty acid profile after extracting lipid from muscle (Table 1).

**Table 1: Proximate composition (%) of the flesh of fresh skipjack tuna, fish cooked with curry in coconut milk and fish fried in palm oil**

<table>
<thead>
<tr>
<th>Component</th>
<th>Fresh fish</th>
<th>Fresh fish</th>
<th>Curry fish</th>
<th>Fried fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>28.34±2.34</td>
<td>24.33±2.01</td>
<td>30.21±1.22</td>
<td>29.32±1.12</td>
</tr>
<tr>
<td>Total Lipid</td>
<td>16.00±0.32</td>
<td>0.41±0.56</td>
<td>17.62±0.23</td>
<td>21.04±1.92</td>
</tr>
<tr>
<td>Moisture</td>
<td>73.23±0.93</td>
<td>73.28±0.89</td>
<td>81.13±2.16</td>
<td>73.43±2.14</td>
</tr>
<tr>
<td>Crude Ash</td>
<td>2.14±0.25</td>
<td>1.43±0.22</td>
<td>1.89±0.45</td>
<td>1.12±0.67</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± SD on a flesh weight basis

**Table 2: Fatty acid composition of fresh flesh, fish cooked with curry in coconut milk and fish fried with coconut oil**

### 2.9 Tocopherol Analysis

The basic extraction procedure and fast HPLC method were used as described by [20] Nirungsan and Thongnopua (2006) to determine the level of tocopherol in freshwater and marine fish. HPLC analyses were conducted using a Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with an RF-551 fluorescence detector and pump (Shimadzu, Jasco, UK), and was packed with reversed-phase C18, Developsil 5 μm RP AQUEOUS (150 × 4.6 mm). Tocopherol standard, α-tocopherol (Sigma Chemicals, UK), was used for the determination of tocopherol content from the lipid extract of each fish. The mobile phase consisted of acetonitrile and methanol. Chromatographic separation was carried out using continuous isocratic elution with HPLC-grade acetonitrile (eluent A) and methanol (eluent B). The HPLC isocratic profile was 50% acetonitrile and 50% methanol, and the flow rate was 1.0 mL/min throughout the whole separation. The total separation time was 12 min and the overall elution was run for 15 min to ensure full separation. The injection volume was 20 μL and detection was monitored with a UV fluorescence detector (RF-551, Shimadzu Fluorescence detector, Kyoto, Japan) at wavelengths of 298 and 330 nm for excitation and emission, respectively. The signals of fluorescence intensity were integrated with a C-R6A Chromatopak (Shimadzu, Kyoto, Japan). The detected signals were quantified using a standard calibration curve prepared using authentic standards (Sigma Chemicals UK). The regression coefficient of the four replicate standard injections was 0.998 for each tocopherol at 0, 2, 4, 6, 8, 10 and 12 μg/mL.

### 2.10 Statistical Analysis

Statistical analysis was performed using the STATISTICA package. A non-parametric multivariate analysis was applied on data through a Mann-Whitney test and with a Principal Component Analysis (PCA). Data were analysed statistically using one-way analysis of variance (ANOVA), p < .05, using Statistical Analysis System (SAS 9.3). Means were compared using the Least Significant Difference (LSD) multiple comparison test.

### 3. Results and Discussion

Fatty acid components, tocopherol and health-related lipid indices in skipjack tuna fish samples cooked with curry in coconut milk, fried in coconut oil and uncooked were quantified. Gas chromatography (GC) was used to detect several FA components and their concentrations are expressed as area percentage (٪) in Table 2. Our statistical analysis emphasized the PUFAs, MUFAAs, n-3 /n-6, PUFA/SFA ratio and the health-related lipid indices, the atherogenic index (AI) and thrombogenic index (TI). The tocopherol content in the muscle was determined using high pressure liquid chromatography (HPLC) and expressed as μg/100 g (Table 2).
compared in the season, 

found by Turkkan (2011) that the degradation could be decreased. 

Nurjanah (2009 and 2015) also reported that the content of fresh fish was 52.24±2.45 cm and the mean total weight was 1.63±0.73 kg. 

Table 1. Parameters of proximate composition of fresh, fried and curry fish.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>C22:4n6</th>
<th>C22:5n6</th>
<th>C22:6n6</th>
<th>C22:5n6</th>
<th>C22:6n6</th>
<th>N-6PUFA</th>
<th>N-4PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0.35±0.04*</td>
<td>0.44±0.47*</td>
<td>0.07±0.01*</td>
<td>0.39±0.02*</td>
<td>0.44±0.02*</td>
<td>0.07±0.01*</td>
<td>0.39±0.02*</td>
</tr>
<tr>
<td>Fried</td>
<td>11.22±0.70</td>
<td>17.26±1.59</td>
<td>9.61±0.15</td>
<td>11.22±0.70</td>
<td>17.26±1.59</td>
<td>9.61±0.15</td>
<td>11.22±0.70</td>
</tr>
<tr>
<td>Curry</td>
<td>0.11±0.01*</td>
<td>0.08±0.01*</td>
<td>0.16±0.02*</td>
<td>0.11±0.01*</td>
<td>0.08±0.01*</td>
<td>0.16±0.02*</td>
<td>0.11±0.01*</td>
</tr>
</tbody>
</table>

* Data are represented as mean ± SD. a, b, c- same letters are not significantly different and different letters are significantly different

3.1 Biological Parameters

The mean length of the analysed fish flesh samples obtained from the skipjack tuna fish was 52.24±2.45 cm, and the mean total weight was 1.63±0.73 kg. Five of the analysed samples were female and four were male fish.

3.2 Proximate Composition

The results of proximate components analysis of the skipjack tuna samples are given in Table 1.

Moisture content of cooked fish is higher than fresh fish and fried fish due to the fish cook in liquid medium. In fried fish, moisture replaced by the fat during frying, there by moisture content get decreased. Protein content of fresh, cooked and fried skipjack tuna samples were more or less same. Due to the ingredients used to make curry and the oil for frying, the proximate components were different from the fresh flesh (Table 1) in lesser amounts. Total lipid in fresh fish is significantly higher compared to the fresh and cooked fish. Similar results reported by Gokoglu et al. (2004), who observed a rise of fat by 9.26% in rainbow trout (Oncorhynhus mykiss). The increase has been attributed to oil absorption (Zahra et al. 2013). Further, our analysis found a much higher amount of total lipids in fresh skipjack tuna compared to the study of Mahaliyana et al. (2015); this can be explained as described by Bligh et al. 1988 and the fat content in fish can be influenced by habitat, season, food source, activity level, growth phase, spawning and maturity, as well as muscle type.

The n-3 PUFAs are significantly higher in fresh flesh (33.91%) of skipjack tuna than curry (15.65%) and fried fish (7.61%) and this result agrees with the work done by Nurjanah et al. (2015). It may be due to the use of a higher temperature during frying speeds up the processes of degradation and oxidation of the frying oil as supported by Sartika (2009). EPA (4.06%) and DHA (27.77%) showed decreased levels in both curry (2.26%, 11.38%) and fried fish (1.18%, 5.56%) compared to fresh fish (Table 2). Turkkan et al. (2008) stated that frying had the effect of reducing EPA and DHA in sea bass (Dicentrachuslabrax) by 30% and 28%, respectively and Domiszewskiet al. (2011) reported that the process of frying at 180°C for 6 min could increase fat content 2.23% to 9.65 % in catfish fillets. Weberet al. 2008 explained that because the oils used in the frying process had no DHA or EPA, oil absorption would always reduce the fractional content of these FAs. The content of the n-6PUFAs had increased in fish cooked in curry (17.26%) and decreased in fried fish (9.61%) compared to fresh fish (11.22%).

Figure 1: Changes of fatty acid composition (%) in fresh flesh, fish cooked with curry in coconut milk and fish fried with palm oil. a-fresh, b-curry, c-fried

As shown in Fig 1, the overall fatty acid composition showed variation after cooking with curry or frying. The percentages of total fat that were SFAs had increased from the 30% value of fresh flesh to 40% after cooking with curry and 41% after frying due to the ingredients of coconut milk and oil, respectively. N3-PUFAs decreased from their high values in fresh flesh (37%) to much lower values after cooking in curry (17%) and frying (8%). N6-PUFAs showed that C22:6n3 (DHA), a PUFA, was dramatically reduced by frying (27.77% vs 5.56%). C18:1n9 (Oleic acid), a MUFA, was increased in fried fish (32.22%) compared to fresh fish (11.12%), whereas C16:0 (Palmitic acid), an SFA, showed an increase in fried fish (32.04%) compared to fresh fish (18.46%) (Table 2). The FAs present in palm oil, which is used to make fried fish, were reported by Ebong et al. (1999).
increased after cooking in curry (19%), but decreased after frying (10%), compared to fresh flesh (12%). As stated by [27], [31] Little et al. 2000 and [32] Sioen et al. 2006, the FA composition of the frying oil could influence the changes in FAs and total lipid contents. The ratio of n-6/n-3 lipids in fried fillets increased by a factor of 1.26 and in fish cooked in curry by a factor of 1.10, presumably due to the quantity of C18:2n6 in the frying oils, as explained by [33] Candela et al. 1998. Those authors further claimed that the increased n-6/n-3 ratio would limit the positive effects of the high n-3 PUFA level of raw fish. The PUFA:SFA ratio showed a decreasing trend from fresh fish (1.60) to curry (0.88) to fried fish (0.44) (Table 2), which reveals that frying fish with coconut oil may have caused it to lose its nutritive value. Consuming fried fish seemed to increase the amount of low density lipoprotein cholesterol (LDL-C) in serum in research conducted by[34] Kinsella et al. 1990.

3.4 Tocopherol Content

The high tocopherol content of fresh flesh, was reduced upon cooking with curry in coconut milk, but did not decrease as significantly when the fish was fried with palm oil (Fig 2). It may be due to the availability of plant tocopherol in coconut oil. Tocopherol is a vital nutrient, but it is unstable and can be lost during processing, heating and cooking. An interrelated series of reactions, including hydrolysis, oxidation of the oil and polymerization of fat molecules can occur [35] (Saguy and Dana 2003). [36] Gotoh et al. 2011 found that heating processes, such as boiling, grilling and frying significantly decreased the α-tocopherol contents of fish meat.

Both atherogenic and thrombogenic indices in skipjack tuna were low in fresh fish, whereas fried fish had higher TI values (>1), due to the absorbance of coconut oil. This is in agreement with other literature reports [19] (Ulbritich and Southgate 1991). [37] Ghaeni et al. 2013 studied the AI and TI in wild species and found seasonal variation. The health beneficial FAs (EPA, DHA) are shown significantly decreased by traditional cooking methods in Asian tuna fish dishes. By using coconut oil for frying and coconut milk for processing, the nutritional quality of fish is decreased. In raw fish, the health beneficial fatty acids and tocopherol were significantly higher than processed fish.

4. Conclusions

The lipids in fish fillets play an important role in giving taste, flavour, smell and texture to the fish. Fatty acid compositions changed significantly during preparation by either cooking in curry or frying in coconut oil and showed lower nutritive values than raw fish. Particularly, the healthy lipids, EPA and DHA were lost. Likewise, tocopherol, being heat sensitive, showed significant losses after both cooking methods. The thrombogenic index was significantly increased in fried fish, adding to the evidence that consuming fried fish is less beneficial to human health than eating fish cooked with curry in coconut milk.

5. Acknowledgements

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References


