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Effect of cooking on physical, biochemical, bacteriological characteristics and fatty acid profile of Tilapia (*Oreochromis mossambicus*) fish steaks

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ABSTRACT

Fish makes a vital contribution to the survival and health of a significant portion of the world's population. Fish is especially important in the developing world. In some of Asia's poorest countries people derive as much as 75% of their daily protein from fish. In West Africa, fish accounts for 30% of animal protein intake, and this number would be larger if the poor could afford to buy more. In the present study, the effect of cooking on the changes in physical, biochemical, and bacteriological characteristics and fatty acid profile of fresh tilapia (Oreochromis mossambicus) meat were studied. The physical parameters like color, textural and pH was analyzed, while the chemical parameters like Total Volatile Basic Nitrogen (TVB-N), Thiobarbutric Acid (TBA) value, fatty acid profile and bacteriological analysis like Total plate count (TPC) were also analyzed. Along with the physical, biochemical, and bacteriological parameters, proximate analyses like moisture, protein, fat and ash content were also analyzed to assess the nutritional quality of tilapia fish. A significant ($p<0.05$) reduction in moisture and fat content was observed in cooked tilapia meat with increased protein and ash content. The parameters of color viz. lightness (L), redness (a), yellowness (b), hue angle (arcton, b/a) and saturation index ($a^{*2}+b^{*2})^{0.5}$ significantly changed ($p<0.05$) in the cooked fish steaks. Textural profile analysis of tilapia fish steaks revealed significant decrease ($p<0.05$) in hardness, adhesiveness, gumminess, resilience, cohesiveness and chewiness, with a slight increase in the springiness and stringiness. Cooking of tilapia fish steaks had a significant influence on the TBA content and microbial load. The levels of TBA, salt, TVBN and TPC of raw/fresh meat of tilapia, which were 0.77±0.01mg%, 0.58±0.01 %, 3.57±0.01mg% and 4.9 $x10^4$ cfu/g, was changed to 1.61±0.06mg%, 0.41±0.01%, 3. 59±0.01mg% and <1 $x10^2$ cfu/g respectively, after cooking. The fatty acid composition of the cooked tilapia fish steaks significantly decreased in the levels of monounsaturated fatty acids and increased the n-3/n-6 Poly-Unsaturated Fatty Acid (PUFA) ratio. While cooking significantly increased the contents of eicosa-pentaenoic acid (EPA) and decosa-hexaenoic acid (DHA) in tilapia meat, no significant ($p>0.05$) impact was observed on the contents of decosa-pentaenoic acid (DPA). Therefore tilapia meat appears to be a valuable source of n-3 PUFAs.

Key words: Tilapia, fatty acids, color, texture.

INTRODUCTION

Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as a source of high quality protein and other elements for the maintenance of healthy body [1]. Tilapia fish are widely cultured in tropical and sub tropical regions of the world and constitute the third largest group of farmed finfish, with annual production growth rate of about 12% [2]. Among the freshwater fish, tilapia has become popular in the United States and this trend is expected to continue as consumption is projected to increase from 1.5 million tons in 2003 to 2.5 million tons in 2010 [3], probably because of its availability to consumers as live or in a very fresh form i.e from pond to table [1].

In India, fresh tilapia is mainly marketed in the whole or fillet forms and has a storage life of 10 -14 days in chilled condition [4]. Among the various methods of processing fish, application of heat is one of the most important methods available for fish preservation, quality improvement and consumer convenience and/or to increase their market value. Cooking has the additional benefits of inactivating endogenous enzymes and stopping microbial growth. Although fish in India are commonly consumed as pan-fried, the consumer has minimum or no knowledge about the nutritive values of raw and cooked fish. In the canning industry, cooking is used mainly to reduce excess moisture, so that the total exudates released in thermal canned / retort products are minimum, thereby improving the sensory, physical and chemical qualities of the product and increasing the shelf life [5].

Regular consumption of food with appropriate content of eicosa-pentaenoic acid (EPA) and docosa-hexaenoic acid (DHA) are believed to prevent and treat coronary heart disease (CHD), Brain development and mental health, Hypertension, Diabetes, obesity, Cancer, thrombosis, lung disease, and some other diseases [6]. However, there is no clear report on the effect of cooking on color, texture, fatty acid profile and quality of fish. Since tilapia (*Oreochromis mossambicus)* is one of the commercially important fish varieties that is chilled and marketed from the natural waters and the knowledge fatty acid composition, color, texture & quality parameters is still limited, the present study was undertaken to investigate the effect of cooking on the color, texture, biochemical, bacteriological and fatty acid profile of fresh tilapia (*O. mossambicus*) steaks, which contains appreciable amount of omega-3 fatty acids.

MATERIALS AND METHODS

SAMPLES

Fresh tilapia (*O. mossambicus)* caught by gill net off the natural water body (reservoir) of Muthukur, Andhra Pradesh, India was iced in the ratio of 1:1 and transported in insulated containers to laboratory in chilled condition. The time gap between capture of fish and analysis was less than 6 h. The average length and weight of the randomly selected fishes were 20 to 24 cm and 240 to 270 g, respectively. The fish were descaled by using a descaler (Koneteollisuus OY, SF-01801 Klaukkala, Finland), eviscerated and beheaded (to remove scales, gut & head) and washed thoroughly in portable chilled water and kept in flake ice (Sanyo Electric Co., Ltd., Japan) until further processing. The dressed fish were later cut into steaks of 2.0 cm size and divided into 2 groups. Fish steaks from the 1st group (raw) were randomly selected for measuring the color and texture of the meat. For the measurement of pH, fatty acid profile, proximate composition and other chemical characteristics the homogenized meat of fish steaks (without bones) were used. Aseptically collected fish meat samples were used for enumeration of total plate count of bacteria.

Fish steaks were steam cooked for 5 min. [7] to form the 2nd group (cooked). Randomly selected samples were measured for the changes in the physical, biochemical and bacteriological characteristics and fatty acid profiles. All the measurements, except color were carried out in triplicates.

PHYSICAL ANALYSES

Color measurements

Instrumental color was measured using a Hunter - Lab scan XE – spectrocolorimeter (Hunter Associates Laboratory, Reston, USA.). The samples were evaluated for Commission Internationale de L 'Eclairage's lightness (L*). redness (a*), yellowness (b*) and hue angle (arcton, b*/a*), which describes hue or color of the ground meat and saturation index $(a^{*2}+b^{*2})^{0.5}$ which describes the brightness or vividness of color [8]. All values were determined from the mean of eight measurements of each fish steak at 28 ± 2^{0}C using the A/10^o observer unless otherwise specified. The spectrocolorimeter was standardized using white (L*=100), and black (L*=0) standard tiles

and working standards, before being used. The results of the color profile analysis were tabulated using Easymatch software (EasyMatchQC, Version 4).

Texture measurements

A compression test was carried out by placing the sample on the base plate and compressed twice and the texture profile analysis (TPA) was measured with a TA-XT2i Texture Analyzer (Stable Microsystems, UK) with a cylindrical stainless steel probe of 5mm diameter for fresh and cooked fish steaks. The load cell used was of 50 kg capacity with the following test conditions - pre test speed: 1.0mm/s, test speed: 0.5mm/s, post-test speed: 10.0mm/s, distance: 3mm/s for fish steaks, trigger force: 5g, return distance:15mm and contact force: 5g. Force by time data from each test was used to calculate mean values for the TPA parameters. The values for hardness, cohesiveness, springiness, stringiness, adhesiveness, resilience, gumminess and chewiness were determined at 28 ± 2^0C as described by Bourne [9]. The results of TPA were tabulated using Texture Expert Exceed software.

pH measurements

Ten grams of fish muscle was blended with 90 ml distilled water in a homogenizer (LAB-MED LTD, Laboratory Blender CE, England) for 30 sec. and the pH of the fish homogenate was measured using a digital pH meter (Eutech Instruments, Singapore) standardized at pH 4 and 7 [10].

CHEMICAL ANALYSES

Total Volatile Basic Nitrogen (TVB-N)

Ten grams of fish muscle was homogenized with 20ml 20% trichloroacetic acid (TCA) in a blender. The homogenate was filtered through Whatman no.1 filter paper into a 100ml standard flask. The residue was diluted with 1% TCA and made up to 100 ml. The filtrate was used for determining the TVBN content by the micro-diffusion method of Conway [11] and expressed as mg/100g of muscle.

Thiobarbutric Acid (TBA) value

Thio-barbutric acid (TBA) value was determined using the method of Tarladgis *et al*. [12]. The TBA number was expressed as mg malonaldehyde equivalents per kg sample. The absorbance was determined using a spectrophotometer (Thermo Scientific, U.K. Model UV 4.1) at 532 nm against a blank containing distilled water and TBA solution.

Fatty Acid Profile

Total lipids of the fish muscle were extracted using the method described by Folch *et al*. [13]. Tissue samples weighing 3g were used for the extraction of total lipid and the fatty acids in the total lipids were converted into fatty acid methyl esters (FAME) by transmethylation using methanolic sodium hydroxide, BF_3 methanol and n-heptane as described by AOAC [14]. Fatty acid methyl esters were analyzed using gas chromatography – mass spectrometry (Shimadzu QP2010quadrupole, Kyoto, Japan) equipped with ionization energy of 70 eV operating in positive electronic impact set to 100 µA, connected to a GC 8060 gas chromatograph (Shimadzu) equipped with a Carbowax (25 m × 0.25 mm; 0.25-µm film thickness) column (Cromlab S.A, J&W Scientific CA, USA), with helium as the carrier gas. Injector and detector temperatures were set at 250°C. Injection was performed in split mode (1:15). The column temperature was programmed to initially be at 50°C for 2 min and then to increase at a rate of 10°C per min to a final temperature of 230°C. FAME was separated at constant pressure (23.1 kPa). The mass spectrometer was tuned to get the relative abundances of m/z from 40 to 550. The identification of the methyl esters of fatty acids was done by matching with retention time and fatty acid and reported as percentage of total fatty acids.

PROXIMATE ANALYSES

The moisture content of raw and cooked meat of tilapia was determined by using an automatic moisture analyzer (Denver Moisture Analyzer, model IR 120, Colorado, Germany). The sample was heated at initial temperature of 100˚C and final temperature of 170˚C. Moisture content corresponds to the weight loss of the sample. Crude protein content was calculated by multiplying the nitrogen content determined by the Kjeldahl's method by 6.25 [14]. Fat was determined by the method described by the AOAC [14] using the Soxhlet extraction system. Ash content was determined by ashing in a Phoenix microwave furnace (CEM Corporation, North Carolina, U.S.A) at 550°C ± 10°C for 5 hr and sodium chloride content determined by using AOAC method [14].

BACTERIOLOGICAL ANALYSES (TOTAL PLATE COUNT)

The total plate counts of bacteria were estimated using the spread plate technique. Ten grams of the sample was weighed aseptically, transferred into a sterile polythene pouch and soaked in 90 ml normal saline for 15 min. after which it was blended in a Stomacher (Seward, West Sussex, England) for 60 sec. at normal speed. Using a sterile pipette, 1 ml of the supernatant was aseptically transferred into a 9 ml saline, Vortexed and a 10 fold set of serial dilutions prepared. Aliquots (0.1 ml) of the appropriate dilutions were spread plated on to nutrient agar. The plates were incubated at 37°C for 48 hr and the individual colonies counted. The counts were expressed as cfu/g of meat as described by Hitching *et al*. [15].

STATISTICAL ANALYSES

The significance of difference among the means was determined by one-way ANOVA using SPSS 10.0 for windows (SPSS Inc., Chicago, IL). The Pearson correlation coefficients between the parameters were carried out using the same software.

RESULTS AND DISCUSSION

RESULTS

The proximate composition of raw and cooked tilapia fish steaks is shown in Table 1. The decrease ($p<0.05$) in the levels of moisture and fat was mainly due to the loss of tissue liquid during the process of cooking. The levels of thio-barbituric acid (TBA), salt, total volatile base nitrogen (TVBN) and total plate count (TPC) of raw/fresh meat of tilapia, which were 0.77±0.01mg%, 0.58±0.01%, 3.57±0.01mg% and 4.9 $x10^4$ cfu/g, changed to 1.61±0.06mg%, 0.41±0.01%, 3.59±0.01mg% and <1 $x10^2$ cfu/g respectively, after cooking. While the TBA and TVBN values increased on cooking the tilapia meat, the content of salt decreased slightly. Cooking reduced the TPC of tilapia meat to <1 $x10^2$ cfu/g.

The pH value of steam cooked tilapia meat increased from 6.8±0.02 (raw) to 6.83±0.04. The increase in pH ($p>0.05$) of the samples may be due to breakage of hydrogen bond and electrostatic interactions. The L*, a*, b*, hue angle and saturation index values of raw and cooked fish steaks are given in Fig.1. The color measured in raw meat was found to be significantly different ($p<0.05$) compared to cooked meat. The color values of raw meat for a*, b* and saturation index resulted in a highly significant positive correlation with all the corresponding cooked meat color values; a*=r=0.413 and b*=r=0.178; whereas, L* value index resulted in a highly significant negative correlation with the corresponding cooked meat color value (L*=r=-0.315).

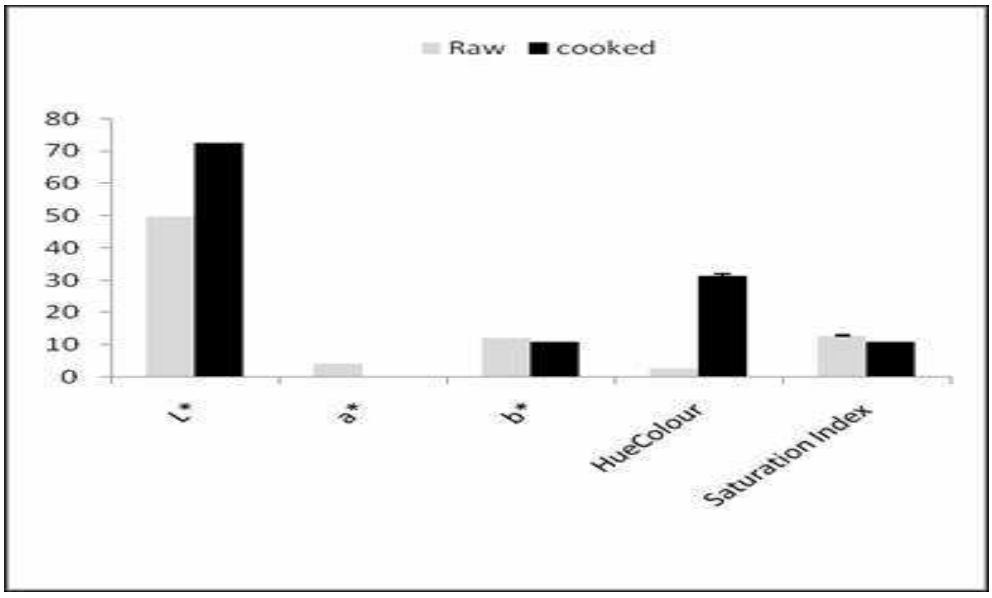

FIG. 1: changes in the color values of tilapia meat on cooking
(L: 'Eclairage's lightness, a: Redness, b: Yellowness)*

The fatty acid composition of raw and cooked tilapia steaks is given in Table 3. The total saturated fatty acids of cooked tilapia meat were slightly higher than fresh meat and the increase in the fatty acids were mainly contributed by palmitic acid (16:0). The level of monounsaturated fatty acids (MUFA) of fresh tilapia meat decreased after cooking and this may be due to the hydrogenation of MUFA and leaching during cooking (Fig. 2).

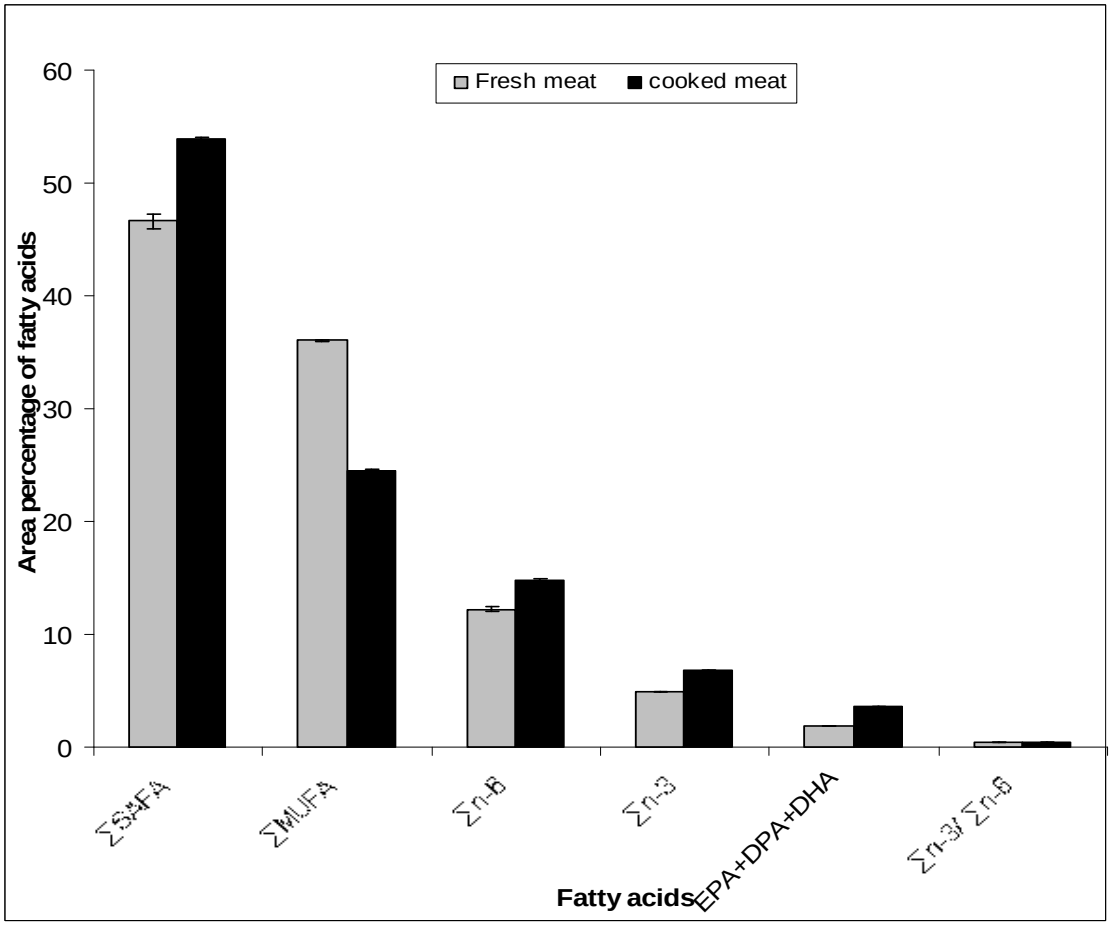

FIG. 2: Changes in the fatty acids content of tilapia fish meat

(SAFA: Saturated Fatty Acids; MUFA: Mono-unsaturated Fatty Acids; n-3: Omega 3 Fatty Acids; n-6: Omega 6 Fatty Acids; EPA: Eicosa-pentaenoic Acid; DPA: Decosa-pentaenoic Acid; DHA: Decosa-hexaenoic Acid)

DISCUSSION

The levels of protein, fat, and ash in fish usually change according to nutrition, living area, fish size, catching season, seasonal and sexual variations as well as environmental conditions [16, 17]. The increase in TBA value of raw and cooked meat in this study was mainly due to oxidation and the results are in general agreement with those of beef and fish meat [18].

Table 1: Changes in the proximate composition* of tilapia steaks

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Raw	79.85±0.50	15.50±0.21	1.37±0.06	1.07±0.04
Cooked	78.20±1.19	17.38±0.37	1.21±0.04	1.12±0.06

** Data are expressed as mean± standard deviation, n =3*

Table 2: Changes in the textural characteristics of tilapia steaks on steam cooking

Texture characteristics*	Raw muscle	Cooked muscle
Hardness (gf)	112.80±1.78	57.95±5.15
Adhesiveness (gs)	1.55±0.37	0.05±1.62
Stringiness (mm)	1.13±0.05	1.39±0.06
Cohesiveness	0.60±0.01	0.52±0.02
Resilience (gs)	0.43±0.02	0.34±0.01
Springiness	1.02±0.01	1.18±0.02
Gumminess	68.08±1.09	30.36±3.92
Chewiness	69.37±1.45	35.66±4.51

**Each value is represented by the mean ± standard deviation of 8 observations*

Table 3: Changes in the fatty acid profile of fresh and cooked tilapia steaks (% of total fatty acids*)

Fatty acids	Fresh meat	Cooked meat
C12:0	0.43±0.01	0.15±0.01
C13:0	0.05±0.01	0.05±0.01
C14:0	6.09±0.34	5.29±0.04
C15:0	1.15±0.01	3.33±0.04
C16:0	28.26±0.27	37.13±0.14
C17:0	2.08±0.16	0.00±0.00
C18:0	8.41±0.12	8.00±0.01
C20:0	0.16±0.01	0.00±0.01
∑SAFA	**46.62±0.36**	**53.94±0.14**
C16:1n-9	0.49±0.01	0.00±0
C16:1n-7	9.38±0.02	6.73±0.03
C18:1n-9	20.65±0.01	15.18±0.16
C18:1n-7	4.86±0.02	2.61±0.02
C18:1n-5	0.34±0.01	0.00±0
C20:1n-9	0.31±0.01	0.00±0
∑MUFA	**36.03±0.04**	**24.52±0.16**
C18:2n-6	9.23±0.01	11.02±0.26
C20:2n-6	0.24±0.01	0.00±0.00
C20:3n-6	1.17±0.01	0.82±0.01
C20:4n-6	2.05±0.09	2.90±0.03
∑n-6	**12.19±0.21**	**14.73±0.27**
C18:3n-3	2.41±0.02	2.66±0.02
C20:3n-3	0.25±0.01	0.60±0.01
C20:4n-3	0.30±0.02	0.00±0
C20:5n-3	0.31±0.02	0.67±0.02
C22:5n-3	0.77±0.01	0.82±0.02
C22:6n-3	0.89±0.02	2.07±0.03
∑n-3	**4.92±0.02**	**6.82±0.03**
EPA+DPA+DHA	**1.88±0.03**	**3.56±0.05**
∑n-3/ ∑n-6	**0.40±0.01**	**0.46±0.01**

**Data are expressed as mean± standard deviation, n= 3*

Heating of muscle or isolated myofibrils usually results in an increase of pH [19, 20, 21]. The color and appearance are important in the selection and purchase of fish fillets and steaks in the market. It is found to be reported that brown discoloration in white-fleshed fish upon heating [22]. Changes in the salmon color pigments upon heating have been studied [23]. It is found to be stated that free ribose accounts for much of the Maillard's type of reaction when fish is heated in presence of carbohydrates [24]. The textural characteristics of tilapia meat like hardness,

adhesiveness, gumminess, resilience, chewiness and cohesiveness decreased drastically after cooking (Table 2). This may be due to the denaturation of proteins induced by cooking and also due to uncoiling of polypeptide chains [25]. The texture of cooked fish usually tends to become soft compared to raw fish probably due to the heat induced conversion of collagen to gelatin. On the other hand, cooking had a significant impact on the stringiness and springiness of fish muscle.

While studying the effect of pre-cooking on the lipid classes at different loci of albacore recorded an increase in polyunsaturated fatty acids (PUFA) and a decrease in MUFA [26]. A wide range of experiments showed no differences in the content of PUFA, even at different cooking conditions [26, 27, 28, 29]. A similar study on sardines showed a significant loss of fat during pre-cooking, without affecting the fatty acids - with an increase in the saturated fatty acid and n-3 PUFA and decrease in MUFA and n-6 PUFA [30]. n-3/n-6 ratio as the most important indicator of fish lipid quality, which ultimately reflects the quality of fish as food [31]. In our study, the ratio of n-3/n-6 in raw fish (0.4) increased to 0.46 on cooking. Scientific evidence also showed that n-3 fatty acids are important in the amelioration of cardiovascular disorders, suggesting that the n-3/n-6 ratio could be used as a biomedical index [32]. The levels of EPA, DPA and DHA almost doubled on steam cooking, thus making the cooked tilapia steaks a valuable source of n-3 fatty acid. On the contrary, no significant difference ($p>0.05$) were detected in the fatty acid composition of raw, microwave, baked ad grilled seabass fillets [33].

CONCLUSION

Cooking of tilapia fish steaks significantly ($p<0.05$) altered the pH, color and texture of meat and drastically reduced the bacterial load. The TVBN and TBA contents were well below the acceptable limit. Heat treatment in general did not decrease the levels of EPA and DHA in tilapia. Tilapia meat appears to be a valuable source of n-3 PUFAs, viz., EPA, DPA and DHA. Significant decrease in the values of MUFA and increase in the levels of n-3/n-6 PUFA ratio indicates positive effects of cooking tilapia fish meat, due to increase in the level of n-3 PUFA level. Although, the ratio of n-3/n-6 PUFA in tilapia muscle is better, it is very low compared with the other freshwater fishes and therefore requires fortification in the development of products from these fishes.

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