Aquatic Science and Fish Resources

http://asfr.journals.ekb.eg

Print ISSN: 2682-4086 Online ISSN: 2682-4108

Effect of Hot Smoking Conditions on Nutritional Quality of Indian Mackerel and Pangasius Fish Fillets

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ARTICLE INFO

Article history:
Received Dec. 20, 2022
Received in revised form JAN. 18, 2023
Accepted JAN. 18, 2023
Available online JAN. 21, 2023

Keywords:
Indian mackerel
Pangasius
hot smokin,
quality properties
lipid quality

ABSTRACT

The main aim of this work was performed to investigate of lightly hot smoking on the nutritional quality of Indian Mackerel (*Rastrelliger kangurata*) and Pangasius (*Pangasius hypophthalmus*) fillets. The results obtained showed that hot smoking conditions caused an increase in protein content while the values of both moisture and lipid decreased. Nutritional value was higher of hot-smoked Pangasius fillets than other one. Values of salt, TBA and TVB-N decreased by <3%, <3 mg MDA/kg and <20 mg/100g in both hot smoked samples, respectively. The SFAs content was dominant followed by MUFAs and PUFAs and the PUFAs/SFAs ratio of both hot smoked samples was within the recommended limit of WHO. Some lipid quality indices; n-3/n-6, TI, FLQ and ΣEPA + DHA of smoked Indian mackerel were better than in pangasius under the same conditions. Total viable bacterial counts (TVC) and yeast and mold were lower than the limit of Egyptian standard. Moreover, hot smoked fish fillets had distinct organoleptic qualities especially Indian mackerel. In conclusion, the effect of hot smoking on nutritional quality was closely attributed with species, source, age, sex, size, and total quality of fish studied.

INTRODUCTION

The fish processing industry in developing countries needs to marine and/or aquaculture fish which had a low economic value. The process of adding value to fish is associated with increase of sensory attributes, shelf life, and techniques used (**Priyadarshini et al., 2021**).

Smoking is one of the oldest techniques for preserving fish. Smoking depends on method used, smoke source and temperature used (**Dore, 1993; Wheaton and Lawson, 1985**). According to **Alcicek and Atar (2010)**, hot smoking fish is performed at temperatures (70 to 80°C) for long enough to cause the protein coagulate.` Hot smoked fish can be consumed without additional preparation (**Gates, 2015**). Concerning the effect of smoking on the nutritional quality of fish, this process affects the nutritional value and biological availability of proteins and fats and shelf life too. Furthermore, it is crucial for the fish industries to determine the lipid profile of native fish as reviewed by several researchers (**Hall, 1997**; **Pertiwi, et al., 2020; Silva et al., 1993 and Sokamte et al., 2020).**

Many vegetable oils, including those from corn, soybeans, and olives, are abundant sources of n-6 series monounsaturated (MUFAs) and polyunsaturated (PUFAs), whereas fish oils are

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doi: 10.21608/ASFR.2023.182521.1033

the sources of n-3 PUFAs (**Cavali et al., 2022; Rodrigues et al., 2017**).

According to the literature, the effect of hot smoking on the percentage and content of various nutrients in fish varies depending on the species of fish, lipid content, and the smoking parameters (**Bienkiewicz et al., 2019; Rasmussen and Morrissey, 2007; Stolyhwo et al., 2006; Tokarczyk et al., 2011**). According to **Hosseini et al. (2014)** and **Sioriki et al. (2015)**, heat treatments may cause a decrease in the amount of fatty acids in fish (**Domiszewski, 2013; Golgolipour, et al., 2019; Leung, et al., 2018**). Since informaion related to the effects of smoking techniques on the quality and lipid profile of Indian mackerel and Pangasius fish are scarce in the literature. Actually, there have been scarce in researches especially on hot smoked Indian mackerel and Pangasius. Furthermore, very few findings were found in the literature about the fatty acid composition as a result of this hot smoking method. In Egypt, both Indian mackerel and Pangasius fish are an inexpensive, and they are raised in the wild and aquaculture. Therefore, the purpose of this study was to investigate the effect of hot smoking technique on the nutritional value and lipid quality parameters of hot smoked marine Indian Mackerel (*Rastrelliger kangurata*) and farmed Pangasius (*Pangasius hypophthalmus*) fillets.

2. Materials and methods:

2.1. Materials

2.1.1. Fish samples:

Twenty five kilograms of both fish Indian mackerel and pangasius were obtained as follows: fresh marine Indian mackerel (*Rastrelliger kangurata*) known locally as Cascombry with an average length of 26 cm and weight of 150g were purchased from El-Ansary fish local market, Suez, Egypt during November 2021. And, freshwater pangasius (*Pangasius hypophthalmus*) with an average length of 30 cm and weight of 0.950-1.200 kg cultivated in raceway unit of the Faculty of Fish Resources, Suez University were purchased at the same time. All samples were delivered in polystyrene boxes with crushed ice to the laboratory of fish processing unit, Faculty of Fish Resources, Suez Univ., Egypt.

2.1.2. Others:

Refined salt made by El-Nasr Saline's company, sawdust of white hardwood and vacuum polyethylene bags were purchased from Suez commercial market, Egypt.

2.2. Technological process:

2.2.1. Hot smoking process: As illustrated in Fig.1, the fresh Indian mackerel and pangasius fish were manually filleted with a sharp knife. The fillets were brined in 15% brine solution for 10 min then drained partially for 10 min over the racks of the local electric smoking kiln. The conducted smoking regime included three stages; the first was drying stage at 30°C for 60 min with a slight intensity smoke followed by cooking stage at 55°C for 60 min with a moderate intensity smoke and the final stage which conducted at 80°C for 60 min with a heavy smoke. The hot smoked fillets were then cooled at room temperature for 20 min before being packed in polyethylene packs and subjected to further analysis.

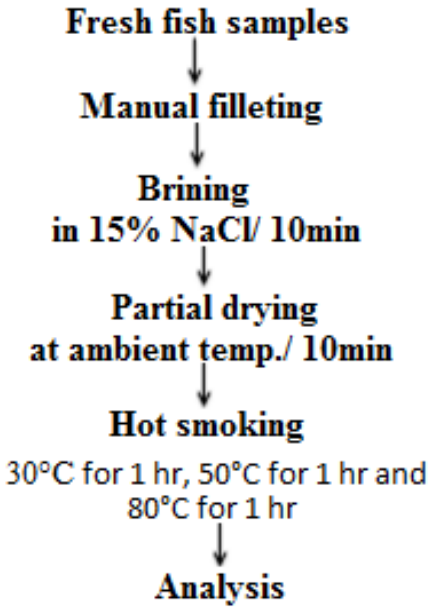

Fig.1. Layout of fish hot-smoking process

2.3. Analytical methods:

2.3.1. Proximate composition:

Chemical composition; moisture, salt and ash contents (**AOAC, 2000**), crude protein content

(**ISO 5983-2: 2009**) and total lipids (**Bligh and Dyer**, **1959**) were determined. Carbohydrate was calculated by difference.

The caloric values (kcal/100g) of smoked fish fillets of both Pangasius and Indian mackerel were calculated using the following formula **(Falch et al*., 2010).***

Nutritional value (kcal/100g) = (%protein× 4) + (%carbohydrate× 4) + (%lipid× 9)

2.3.2. Physicochemical indices:

2.3.2.1. pH value:

The pH value was determined after homogenizing 5 g of macerated sample with 45 ml of distilled water using a calibrated pH meter (OHAUS STARTER 2100 Bench pH meter, OHAUS Instruments, USA).

2.3.2.2. Thiobarbituric acid (TBA) value:

It was determined as pointed out by **Pearson (1976)**. The procedure entailed boiling of the samples and blank with TBA reagent for 35 min in a simmering water bath. After cooling, the samples′ optical densities (O.D.) were measured at 538 nm using T60 UV-Visible Spectrophotometer and the TBA value was calculated as follows:

TBA value (as *mg* Malonaldehyde/ *kg* sample) = 7.8 × O.D. (at 538 nm)

2.3.2.3. Total volatile basic nitrogen (TVB-N):

It was carried out according to the **Antonacopoulos** modification technique's distillation process **(1989)**. The generated distillate was titrated with 0.1 N HCl in the present of Tashiro indicator until the neutral point was obtained. The TVBN content of fish sample was calculated as follows:

TVBN (*mg*/100 *g*) = ml 0.1 N HCl × 14 × 100 / *weight of sample*

2.3.2.4. Fatty acid profile determination:

Total lipids were extracted in triplicate using the Trans esterification method by acid catalysis (**Folch, et al., 1957**). The fatty acid composition of raw and hot smoked fillets of both Indian mackerel and Pangasius fish were analyzed (**Park et al., 2001**). According to the approach suggested by **Hartman and Lago (1973)**, each sample (100 mg) was methylated, shaken, heated (60°C for 5 h), and dissolved in 5 ml of sulfuric acid/methanol. Samples were agitated until phase separation occurred after chilling, adding 1 ml of Milli-Q distilled water and 2 ml of n-heptane pro-analysis.

Gas chromatography (GC) (Scion 456- GC) with a capillary column (100 m × 0.25 mm i.d., 0.20 µm film thickness) and flame ionization detector were used to measure the fatty acid methyl esters (FAMEs). The temperature was raised to 175°C at a rate of 13 °C/min and maintained for 27 min, and then raised to 215°C at a rate of 7°C/min and maintained at 230°C for 5 min. The GC-oven programme temperature was as follows; the first temperature was set at 70°C for 4 minutes. The injector and detector had respective temperatures of 250°C and 300°C. 1.8 ml/min of hydrogen was employed as the carrier gas. An automated injector was used to administer samples (1 µl). By contrasting the samples' methyl esters' retention periods with those of fatty acid standards, fatty acids were determined (Supelco TM Component FAME Mix, cat 18,919 Supelco, EUA). The areas of the methyl esters were normalized to determine the amount of fatty acids, and the data were represented as a percentage area of total fatty acids (% wet weight)].

2.3.2.5. Indices of lipid quality

According to the recommendations of the WHO, the ratio of ∑PUFAs/∑SFAs and the ratio of ∑PUFAs (n-6/n-3) were calculated using a pooled set of fatty acid profile data. The additional data discovered by lipid quality indices were contrasted with indices suggested by **Ulbricht and Southgate (1991)**; **HernándezMartínez et al. (2016)**; **Passos et al. (2016)**; **Rodrigues et al. (2017)**; and **Souza et al. (2017)**.

The atherogenicity index (AI), thrombogenicity index (TI), and the ratio of hypocholesterolemic to hypercholesterolemic fatty acids (h/H) were used to determine the nutritional quality of the lipid fraction from the fatty acid profile (**Santos-Silva et al., 2002; Ulbricht and Southgate, 1991**).

These indices of lipid quality estimated according the following equations:

AI = [C12:0 + 4×C14:0 + C16:0] / [ΣMUFAs + Σn-6 PUFA + Σn-3 PUFA] (1)

TI = [14:0 + 16:0 + 18:0] / [(0.5×ΣMUFAs) + (0.5×Σn-6 PUFA) + (3×Σn-3 PUFA) + (Σn-3/n-6)] (2)

h/H = [18:1n-9 + 18:2n-6 + 20:4n-6 + 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3] / [14:0 + 16:0] (3)

Flesh-lipid quality *(FLQ)*: The FLQ displays the percentage relationship between the important n-3PUFA (EPA + DHA) and total lipids. The quality of the dietary lipid source is indicated by a higher index value (**Senso et al., 2007**).

FLQ = 100 × (EPA + DHA) / (% total fatty acids) (4)

Nutritional contribution of linoleic acid (LA) and **Contribution of both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA):**

Nutritional contribution of

LA %=[(C × M)/ DRI]×100 (5)

Nutritional contribution of

ΣEPA + DHA % = [(C × M) / DRI] × 100 (6)

Where; C represents fatty acid concentration (LA or ΣEPA + DHA) as *mg*/100 *g* fish sample, M represents the meal portion in *g* (the meal size was set at an average of 227 *g* for an adult consumer with a 70 *kg* body weight, as recommended by **US-EPA, 1995**), and DRI represents dietary reference intake in *mg* (an average of 1300 *mg*/*day* was used to estimate the nutritional contribution of LA in human consumption for both men and women (**NHMRC, 2006**). A level of 500 *mg*/*day* was used to calculate the nutritional contribution of EPA + DHA, using the method proposed by **Saavedra et al. (2017)**.

2.3.3. Microbiological examination:

2.3.3.1. Samples preparation:

Fresh and hot-smoked fillets samples were aseptically ground with a sterile electronic mixer. A representative sample of each product (5 g) was mixed with 45 ml peptone water (0.1%) and used to prepare serial dilutions.

2.3.3.2. Total viable bacterial count (TVC):

According to the spread plate method of **Buck and Cleverdon (1960);** plate count agar media (MPN, UK) was used to enumerate the mesophilic bacteria. The incubation time and temperature of plates were 35±2°C for 24 hours for TVC (**APHA, 2001).** Plates containing between 30 and 300 colonies were counted as CFU/g.

2.3.3.3. Yeasts and molds:

Yeasts and molds were enumerated on Potato Dextrose Agar medium (lab M, UK). The incubation period was achieved at 25±2°C for 3-5 days and plates containing 10-150 colonies were examined and expressed as CFU/g **(APHA, 2001)**.

2.3.4. Sensory attributes:

Smoked fish fillets of both Indian mackerel and Pangasius were evaluated their appearance, color, odor, flavor, taste, texture and overall acceptability by fifteen trained panelists from Fish Processing and Technology Department of Faculty of Fish Resources at Suez University, Egypt. The evaluation was performed using a 9-point hedonic scale, whereas excellent equal 9 and poor/ unacceptable equal 1 **(Muzaddadi, 2013).** The score of each sensory attribute was expressed as the mean value.

2.4. Statistics Analysis:

All data were analyzed statistically using IBM SPSS Statistics version 20. The Standard deviation and t- test analysis were conducted to define the variation between values.

3. Results and discussion

3.1. Proximate composition and nutritional value:

The chemical composition and nutritional value of raw and hot smoked fillets of Indian mackerel and pangasius fish were presented in Table (1). The moisture, protein, lipid, ash, carbohydrate and nutritional values were 74.4±0.38%, 17.8±0.29%, 3.0±0.23, 1.26±0.41%, 2.54±0.25%, and 108.36 Kcal/100g for fresh Indian mackerel and 69.4±0.25%%, 15.3±0.23%, 12.9±0.19%,

1.2±0.37%, 1.2±0.43%, and 182.1 Kcal/100g for pangasius fish, respectively.

There were significant lower values in moisture, protein and carbohydrate while a significant higher value was found in the fat for fresh pangasius which led to an increase in its nutritional value compared with Indian mackerel. According to **Thammapat et al. (2010)** and **Visentainer et al. (2007),** the variety, origin, species, sex, age, nutritional status, and the reproductive cycle of *Pangasius sp.* could affect the protein and lipids content in fish, which is usually in the range (ww) of 11–24% and 1- 6%, respectively. The lipid content in pangasius in this study was higher than the previous range, may be related to the type of feed used during feeding season. Our findings for *R. kanagurta* was congruent with **Nisa and Asadullah (2011)** and **Shamsan, et al. (2019)** who found that the ranges for moisture, protein, lipid, and ash were 70.11-74.41%, 16.02- 20.09%, 3.0- 12%, and 0.89-1.35%, respectively.

As appeared in the same Table 1, the proximal composition and nutritional values of hot smoked fillets of both Indian mackerel and pangasius fish were as follows: moisture 63.2±0.27% and 57.2±0.19%, protein 25.1±0.42% and 22.1±0.25%, lipids 2.5±0.22% and 12.1±0.32%, ash 3.9±0.32% and 4.1±0.43%, and carbohydrate 5.3±0.23% and 4.5±0.52%, respectively. The salt content of fresh Indian mackerel and pangasius fish were 0.28±0.14% and 0.19±0.14%, respectively while its content increased to 2.45±0.25% and 2.01±0.23% of smoked Indian mackerel and pangasius fish fillets, respectively. This is due to brining step before smoking process, reduction in fat and moisture content after hot smoking process. The results of salt content in raw and hot smoked pangasius were close with **Sokamte et al. (2020);** they found that the results of salt content of fresh and smoked fish were 0.32 ± 0.02% and 3.09 ± 0.17%, respectively.

The nutritional value of hot smoked pangasius was higher (215.3 kcal/100g) than Indian mackerel (144.1 kcal/100g), due to higher lipid content of pangasius. The results observed a decrease in moisture and lipid content and increase in protein, carbohydrate, and ash content as the temperature was raised to 80°C during the hot smoking process. Our results were in agreement with those of **Sokamte et al. (2020)** on smoked *Pangasius hypophthalmus* fillets. On the contrary, the smoked Indian mackerel results in this study were different compared with the results by **Hanumanthappa and Chandrasekhar (1987)** where moisture, protein, fat and ash were 44.88%, 35.28%, 13.72% and 7.0%, respectively.

Table 1. Proximate composition, salt content (%, ww) and nutritional value of raw and hot smoked fillets of Indian mackerel and Pangasius fish.

Item	Indian Mackerel		Pangasius	
	Raw	Smoked fillets	Raw	Smoked fillets
Moisture	74.4±0.38^{a}	63.2±0.27^{b}	69.4±0.25^{a}	57.2±0.19^{b}
Protein	17.8±0.29^{b}	25.1±0.42^{a}	15.3±0.23^{b}	22.1±0.25^{a}
Fat	3.0±0.23	2.5±0.22	12.9±0.19^{a}	12.1±0.32^{b}
Ash	1.26±0.41^{b}	3.9±0.32^{a}	1.2±0.37^{b}	4.1±0.43^{a}
Salt	0.28±0.14^{b}	2.45±0.25^{a}	0.19±0.14^{b}	2.01±0.23^{a}
Carbohydrate	2.54±0.25^{b}	5.3±0.23^{a}	1.2±0.43^{b}	4.5±0.52^{a}
Nutritional value (Kcal)	108.36	144.1	182.1	215.3

a, b values in each raw of each product with different super- scripts are significantly different ($p < 0.05$); Data are Mean ± SD.

This variation is due to the method and conditions used of processing (using short time of brining and hot smoking compared with dry salting for 3h and smoking at 70 ± 5° C for 5 h in the previous study).

3.2. Physicochemical parameters

Some physicochemical parameters including pH, TVB-N and TBA were investigated before and after hot smoking process to define the quality of both Indian mackerel and Pangasius fish (Table 2).

3.2.1. pH value

The pH of food has a significant impact on microbial development and food degradation. The pH values of fresh and hot smoked mackerel and pangasius fillets recorded (6.47±0.17and 6.53±0.07) and (6.59±0.17and 6.82±0.07), respectively (Table 2). **Leksono et al. (2014)** reported that fresh *Pangasius hypophthalmus* had a pH close to 7 and decreased to range of 6.62 - 6.66 after hot smoking.

3.2.2. TVB-N content

Total volatile basic nitrogen (TVB-N) in fish and fish products serves as a gauge for the level of putrefaction, decomposition, and proteinase constituent breakdown. As shown in Table 2, the raw Indian mackerel fish had the lowest TVB-N content (2.71±0.43 mg/100g) compared with raw pangasius fish (12.97±0.26 mg/100g). The TVB-N values in hot smoked Indian mackerel fillets increased significantly more than seven times (19.61±0.25 mg/100g) after smoking, due to effect of high temperature however, this content was within the limits of good quality according to **EOS (2005)**. These results were agreement with **Amitha et al. (2019)** for fresh Indian mackerel (4.57 mg/100g) and **Hanumanthappa and Chandrasekhar (1987)** for hot smoked Indian mackerel (19.6 mg/100g). Also, the same trend was found in hot smoked pangasius fillets where TVB-N content increased slightly to 16.42±0.31 mg/100g which was within the **EOS's guidelines for good quality (2005)** (35 mg/100 g).

3.2.3. TBA value

To assess the level of fat oxidation in food products, the TBA test is frequently employed. **Pegg (2004)** stated that TBA was used to calculate the accumulated secondary oxidation products, which are expressed as mg malonaldehyde/kg of flesh. As illustrated in Table 2, TBA values of raw Indian mackerel (lean fish) and pangasius (fatty fish) recorded 0.13±0.25 and 0.23±0.25 mg MDA/kg, respectively. After hot smoking, TBA values increased to 1.43±0.25 and 2.01±0.11 mg MDA/kg in hot smoked Indian mackerel and pangasius fillets. Hot smoking conditions may be increased the oxidation of fish lipids. The known permissible threshold for TBA is 4.5 mg MDA/kg (**EOS, 2005**). Researchers claim that the TBA number in a good material shouldn't be ≥5 and should be ≤3 in extremely good materials (**Sinnhuber and Yu, 1958; Varlik et al. 1993**). The TBA values found in our investigation fell into the category of extremely good products.

Table 2. Physicochemical properties of raw and hot smoked fillets of Indian mackerel and Pangasius fish

Parameters	Indian mackerel		Pangasius	
	Raw	Smoked fillets	Raw	Smoked fillets
pH	6.47±0.17	6.53±0.07	6.59±0.17	6.82±0.07
TVB-N (mg/100g)	2.71±0.43^{b}	19.61±0.25^{a}	12.97±0.26	16.42±0.31
TBA (mg/kg)	0.13±0.25^{b}	1.43±0.25^{a}	0.23±0.25^{b}	2.01±0.11^{a}

a, b values in each raw of each product with different super- scripts are significantly different ($p < 0.05$); Data are Mean ± SD.

3.3. Fatty acids composition

As shown in Table 3, the fatty acid profile of both fresh and smoked Indian mackerel and pangasius are identified. In fresh Indian mackerel and pangasius contained 48.68 and 45.35% saturated fatty acids (SFAs), 18.27and 39.64%

monounsaturated fatty acids (MUFAs) and 33.05 and 15.01% polyunsaturated fatty acids (PUFAs), respectively. These results were higher than those findings by **Alkuraieef et al. (2021)**; they found that Indian mackerel contained SFAs 27.01%, PUFAs 22.01% and MUFA 4.01%. Our results agreed in PUFAs (32.61%) while differed in both SFAs (17%) and MUFAs (50%) of Indian mackerel fish as recorded by **Sonavane et al. (2017**). With regard to the FAs composition of pangasius, our results are lower than the results reported by **Artar et al. (2022)**; they found the highest concentration was SFAs (57.09%) followed by PUFAs (29.06%) and MUFAs (22.16%) while **Sokamte et al. (2020)** found that the highest proportion was PUFAs (38.02%) followed by SFAs (31.14%) and MUFAs (23.89%). Fatty acids levels and availability differed amongst fish species, depending on sex, age, feeding behavior, etc. (**Parthasarathy and Joseph, 2011**; **Petenuci et al., 2019**).

In both raw Indian mackerel and pangasius, the dominant fatty acids were palmitic acid (28.01% and 32.75%, respectively) was followed by stearic acid and myristic acid, further oleic acid (7.23% and 37.13%, respectively) and DHA (24.30%) in Indian mackerel while linoleic acid (12.89%) in pangasius as shown in Table (3). It is well recognized that palmitic acid is one of the key factors leading to an increase in total blood cholesterol, particularly LDL cholesterol, which is connected to the risk increased of dying from coronary heart disease and its occurrence (**Mensink and WHO, 2016**). Consuming MUFAs-rich meals, especially those high in oleic acid, are associated with reduced LDL cholesterol and, thus, lower risk of coronary heart disease death (**Cintra et al., 2006**). The PUFAs n-3 (as functional lipids) and n-6 (as deposited as store lipid) work together to play a critical role in the growth and normal development of nerve cells as well as in brain function. They are also crucial for lowering LDL cholesterol and heart disease risk (**Thammapat et al., 2010**).

From Table 3, it could be noticed that Indian mackerel after hot smoking, SFAs increased slightly to 49.87% and followed by MUFAs which increased by more than 49.9% (27.20%) while PUFAs decreased by 30.65% and reached to 22.92%. These results indicated that PUFAs more vulnerable to oxidation and alteration to SFAs and MUFAs by cooking (**Domínguez et al., 2019**). Among the SFA, myristic, palmitic, pentadecyclic and arachidic acids increased while stearic, tricosylic and lignoceric acids decreased. Oleic (n-3), palmitoleic (n-7) and erucic (n-9) acids increased as the most abundant MUFA. Among PUFA, DHA (n-3) and arachidonic (n-6) acids decreased to 11.81% and 0.14, respectively while Docosadienoic (n-6), Dihomo-γ-linolenic (n-6) and Linolenic (n-3) acids increased. In contrast to these results, **Popelka et al. (2021)** found that raw mackerel contained more SFAs than smoked mackerel while PUFAs lowered in raw mackerel compared to smoked fish. They stated that DHA was the major differentiating FA owing to its increased presence in smoked mackerel while oleic, myristic and stearic acids were main differentiating variables because of their lower quantities in smoked mackerel.

Concerning of hot smoked pangasius fillets, the SFA and MUFA were reduced slightly to 43.04% and 39.17%, respectively while PUFA increased slightly to 17.79%. These results in pangasius revealed that PUFAs are structural lipids in the muscle, so they are less vulnerable to cooking or dehydration (**Bouzgarrou et al., 2020**). Palmetic and stearic acids were slightly decreased while Tricosylic acid appeared as SFAs. Also among of MUFA, oleic acid was decreased and Erucic acid was increased. Among PUFAs, Dihomo-γ-linolenic acid became dominant followed by Linolenic acid. **Sokamte et al. (2020)** reported that fatty acids profile of smoked *Pangasius hypophthalmus* fillets were palmitic (22.28 ± 0.67) and stearic (9.80 ± 0.10) as dominant in SFA, Oleic (19.35 ± 0.10) as dominant in MUFA and linoleic (16.78 ± 0.21), arachidonic (12.72 ± 0.17), 4,7,10,13,16-docosapentaenoic acid C22:5n-6 (3.69 ± 0.20) and DHA (3.15 ± 0.10) as dominant in PUFA.

Table 3. Effects of hot smoking process on fatty acids composition (%) of Indian mackerel and Pangasius fillets.

Fatty acids	Indian mackerel		Pangasius	
	Raw	Smoked	Raw	Smoked
Caproic Acid (C6:0)	**0.10 ± 0.01^{a}**	**UD^{b}**	**0.03± 0.01**	**0.05± 0.01**
Capric Acid (C10:0)	**0.17± 0.02**	**0.12± 0.01**	**UD**	**0.01± 0.01**
Undecylic acid (C11:0)	**0.65± 0.05^{a}**	**0.13± 0.01^{b}**	**0.05± 0.02**	**0.06± 0.02**
Lauric acid (C12:0)	**0.53± 0.04^{a}**	**0.35± 0.03^{b}**	**0.22± 0.02**	**0.26± 0.03**
Tridecyclic acid (C13:0)	**0.13± 0.01**	**0.13± 0.02**	**0.02± 0.01^{b}**	**0.05± 0.01^{a}**
Myristic acid (C14:0)	**3.71± 0.06^{b}**	**7.01± 0.11^{a}**	**4.86± 0.09^{a}**	**4.50± 0.07^{b}**
Pentadecyclic acid (C15:0)	**0.94± 0.05^{b}**	**1.78± 0.09^{a}**	**0.21± 0.02^{b}**	**0.31± 0.03^{a}**
Palmitic acid (C16:0)	**28.01± 0.12^{b}**	**30.23± 0.14^{a}**	**32.75± 0.14^{a}**	**29.99± 0.13^{b}**
Margaric acid (C17:0)	**1.20± 0.09**	**1.57± 0.08**	**0.12± 0.02**	**0.13± 0.01**
Stearic acid (C18:0)	**11.47± 0.10^{a}**	**7.55± 0.08^{b}**	**6.50± 0.07^{a}**	**5.83± 0.06^{b}**
Arachidic acid (C18:0)	**0.23± 0.01^{b}**	**0.49± 0.07^{a}**	**UD**	**UD**
Behanic acid (C22:0)	**UD**	**UD**	**0.59± 0.05**	**0.56± 0.04**
Tricosylic acid (C23:0)	**0.76± 0.06^{a}**	**0.12± 0.02^{b}**	**UD**	**1.30**
Lignoceric acid (C24:0)	**0.78± 0.08^{a}**	**0.39± 0.05^{b}**	**UD**	**UD**
Σ Saturated Fatty Acids (SFA)	**48.68± 0.17^{b}**	**49.87± 0.19^{a}**	**45.35± 0.13^{a}**	**43.04± 0.14^{b}**
Myristoleic acid (C14:1 n-5 (c9))	**0.47± 0.07^{a}**	**0.13± 0.02^{b}**	**0.06± 0.02**	**0.10± 0.03**
Pentadecenoic acid (C15:1 n-5 (c10))	**1.67± 0.05**	**1.57± 0.07**	**0.17± 0.02^{b}**	**0.25± 0.02^{a}**
Palmitoleic acid (C16:1 n-7 (c9))	**2.77± 0.07^{b}**	**6.62± 0.09^{a}**	**1.54± 0.05**	**1.59± 0.06**
heptadecenoic acid (C17:1 n-7 (c10))	**0.27± 0.02^{b}**	**0.41± 0.05^{a}**	**0.07± 0.01^{b}**	**0.10± 0.01^{a}**
Elaidic acid (C18:1 n-9 (t9))	**UD**	**UD**	**0.14± 0.02**	**0.14± 0.03**
Oleic acid (C18:1 n-9 (c9))	**7.23± 0.10^{b}**	**11.05± 0.09^{a}**	**37.13± 0.15^{a}**	**34.18± 0.17^{b}**
Erucic acid (C22:1 n-9 (c13))	**5.60± 0.11^{b}**	**7.09± 0.13^{a}**	**0.55± 0.04^{b}**	**2.81± 0.08^{a}**
Nervonic acid (C24:1 n-9 (C15))	**0.27± 0.02**	**0.33± 0.04**	**UD**	**UD**
Σ Monounsaturated Fatty Acids (MUFA)	**18.27± 0.11^{b}**	**27.20± 0.18^{a}**	**39.64± 0.15^{a}**	**39.17± 0.13^{b}**
Linoleic acid (C18:2 n-6 (c9,c12))	**1.90± 0.07**	**1.87± 0.05**	**12.89± 0.13^{a}**	**12.19± 0.14^{b}**
γ-linolenic acid (C18:3 n-6 (c6,c9,c12))	**UD ^{b}**	**0.13± 0.02^{a}**	**0.27± 0.04**	**0.35± 0.06**
Linolenic acid (C18:3 n-6 (c9,c12,c15))	**1.16± 0.07^{b}**	**1.91± 0.04^{a}**	**1.69± 0.09**	**1.67± 0.07**
Eicosadienoic acid (C20:2 n-6 (c11,c14))	**UD ^{b}**	**0.27± 0.01^{a}**	**UD**	**UD**
Dihomo-γ-linolenic acid (C20:3 ω_6 (c8,c11,c14))	**0.93± 0.05^{b}**	**1.17± 0.09^{a}**	**0.05± 0.01^{b}**	**2.38± 0.10^{a}**
Arachidonic acid (C20:4 n-6 (c5,c8,c11,c14))	**0.91± 0.07^{a}**	**0.14± 0.01^{b}**	**UD ^{b}**	**0.42± 0.05^{a}**
Docosadienoic acid (C22:2 n-6 (c13,c16))	**3.85± 0.11^{b}**	**5.64± 0.17^{a}**	**0.10± 0.02^{b}**	**0.33± 0.03^{a}**
EPA (20:5) n-3	**UD**	**UD**	**UD**	**UD**
Docosahexaenoic acid (DHA) (C22:6 n-3 (c4,c7,c10,c13,c16,c19))	**24.30± 0.18^{a}**	**11.81± 0.12^{b}**	**0.01± 0.01^{b}**	**0.44± 0.05^{a}**
Σ Polyunsaturated Fatty Acids (PUFA)	**33.05± 0.17^{a}**	**22.92± 0.13^{b}**	**15.01± 0.13^{b}**	**17.79± 0.12^{a}**

a, b values in each raw of each product with different super- scripts are significantly different ($p < 0.05$); Data are Mean ± SD. UD: undetectable.

3.4. Indices of lipid quality:

The ratio between PUFAs and SFAs decreased in Indian mackerel from 0.68 to 0.46 while it increased in pangasius from 0.33 to 0.41 after hot smoking as presented in Table (4). The PUFAs/SFAs ratio is a measure recommended by WHO for assessing lipid quality; with levels less than 0.45 deemed harmful (**WHO, 2005; Wood and Enser, 1997**). **Stołyhwo et al. (2006)** and **Sokamte et al. (2020)** revealed that smoked Indian mackerel and pangasius had PUFAs/SAFs ratio equal 2.77 and 1.36, respectively which is more than in the present study. According to the previous studies, losses in FA vary greatly based on the circumstances of smoking and the type of fish smoked (**Akintola et al., 2013; Kaya et al., 2008; Zakipour Rahimabadi et al., 2016**). **Kołakowska et al. (2002)** noticed that fish protein absorbs salt during brining beside some impurities cations which play a role in prooxidative activity. This peroxidation rate of FA may increase during initial stages of drying and heating and before the concentrations of phenolic smoke antioxidants increased.

Although, Σ(n-3)PUFAs in smoked Indian mackerel was decreased dramatically from 25.46% before smoking to 13.71% but still more than that of smoked pangasius (2.11%) (Table 4). Σ(n-6)PUFAs in both smoked Indian mackerel and pangasius increased to 9.21% and 15.68%, respectively and it was more pronounced in pangasius. Fish lived in warm water which distinct as low soluble oxygen may have high level of polyunsaturated fatty acid, especially n-6 due to lower oxygen solubility (**Smith and Miller, 1980**). The n-3/n-6 PUFA ratio is also a helpful indicator for assessing the nutritional content of fish oils (**Piggott and Tucker, 1990).** In the present study, this ratio was decreased after hot smoking of raw Indian mackerel fillets from 3.36 to 1.49 while this ratio did not change after smoking of pangasius fillets (0.14). The present n-3/n-6 PUFA ratio was the same as **Sokamte et al. (2020)** found with smoked fillets of *Pangasius hypophthalmus* (0.14). Chronic inflammation-related disorders are suppressed by an increase in omega-3 PUFA (a high n-3/n-6 ratio). Indian mackerel as lean fish may absorb fewer phenols than pangasius which is fatty fish in this study. Phenols play more antioxidant role in lipid of pangasius while oxidation affected on lipid of Indian mackerel. The WHO advises maintaining the consumption of PUFAs at a ratio of n-3/n-6 >0.1, which is congruent with the ratio discovered in this study (n-3/n-6 = 0.14). The n3/n6 ratio that has been recognized as ideal for nutritional purposes is 1:1 (**Simopoulos, 2002**). Due to a combination of declining consumption of fish and other n-3 PUFA-rich foods and a constant increase in dietary vegetable oils rich in n-6 PUFAs, the n-3/n-6 ratio in modern human diets has dramatically fallen to 1:4-1:6 in the Eastern diet and 1:15- 1:20 or higher in the Western diet to achieve human health benefits (**Gebauer et al., 2006; Simopoulos, 2008; Strobel et al., 2012**).

The n-7 fatty acid is a nutrient that improves insulin sensitivity and wards off type 2 diabetes. It lowers inflammatory processes and LDL cholesterol levels while also enhancing artery flexibility. In a brief, it aids in the treatment of metabolic disorders. (**Blanco and Soengas, 2021; Cavali et al., 2022; Frigolet and Gutiérrez-Aguilar, 2017 and Passos et al., 2016**). Also, n-9 Palmitoleic acid represents the dominant n-7 in both raw Indian mackerel and pangasius (2.77% and 1.54%, respectively). Hot smoking process caused more than double increasing in Indian mackerel (6.62%) while did not in pangasius (1.59%) (Table 3). Oleic acid was the dominant n-9 in both hot smoked Indian mackerel (11.05%) and in pangasius (34.18%). **Albuquerque et al. (2016)** found that oleic acid can assist with sepsis by lowering metabolic dysfunction. So, the ΣPUFAs (n-7) and ΣPUFAs (n-9) of hot smoked Indian mackerel (7.03% and 18.47%, respectively) and hot smoked Pangasius (1.69% and 36.99%, respectively) have beneficial role in diets (Table 4).

According to Table 4, the atherogenicity index (AI) and thrombogenicity index (TI) comped in raw Indian mackerel (0.85 and 0.47) was lower

and favorable than in raw pangasius (0.96 and 1.39). After hot smoking process, AI and TI values increased in Indian mackerel (1.17 and 0.74) due to oxidation of PUFA while decreased somewhat in pangasius (0.85 and 1.19). The AI value of hot smoked pangasius fillets is more favorable than hot smoked Indian mackerel while for the TI value is the vice versa. Human health is harmed by AI and TI values that are more than 1.0 (**Siddik et al., 2019**). Lower AI values of fatty acids are preferable for preventing cardiovascular problems, but lower TI values are preferable for preventing blood clots in veins (**Rodrigues et al., 2017; Ulbricht and Southgate, 1991**). According to **Zaborskienė et al. (2021),** raw Atlantic salmon fillets had the highest AI (0.13) and TI (0.17) indices for human health, while cold-smoked mackerel samples had slightly lower indices (AI - 0.23, TI - 0.25).

Table 4. Hot smoking effects on omegas and indices of lipid quality of Indian mackerel and Pangasius fillets.

Nutritional Parameter	Indian mackerel		Pangasius	
	Raw	smoked	Raw	smoked
Omegas				
Σn −3 PUFAs	25.46± 0.17^{a}	13.72± 0.12^{b}	1.70± 0.07^{b}	2.11± 0.10^{a}
Σn −6 PUFAs	7.59± 0.19^{b}	9.22± 0.15^{a}	13.31± 0.13^{b}	15.67± 0.12^{a}
Σn −7 PUFAs	3.04± 0.11^{b}	7.03± 0.16^{a}	1.61± 0.05	1.69± 0.07
Σn −9 PUFAs	13.10± 0.08^{b}	18.47± 0.12^{a}	37.68± 0.16^{a}	36.99± 0.19^{b}
Σn −5 PUFAs	2.14± 0.06^{a}	1.70± 0.04^{b}	0.23± 0.03^{b}	0.35± 0.03^{a}
Indices of lipid quality				
PUFAs/SAFs ratio	0.68	0.46	0.33	0.41
Σn −3 PUFAs / Σn −6 PUFAs	3. 35	1.49	0.13	0.14
AI	0.85	1. 17	0.96	0.85
TI	0.47	0.74	1.39	1.19
h/H	1.12	0.72	1.38	1.42
FLQ	24.3	11.8	0.01	0.44
Nutritional Contribution of LA	331.77	326.53	2250.79	2128.56
Nutritional Contribution of ΣEPA + DHA	11032.20	5361.74	4.54	199.76

a, b values in each raw of each product with different super- scripts are significantly different ($p < 0.05$); Data are Mean ± SD.

AI: atherogenicity index; TI: thrombogenicity index; h/H: ratios between hypocholesterolemic and hypercholesterolemic fatty acids; FLQ: Flesh lipid quality

According to Table 4, the value of h/H was higher before and after hot smoking in pangasius (1.38 and 1.42) while, it decreased in Indian mackerel after hot smoking (1.12 and 0.72). The health of people is benefited by greater h/H values (>1.0 ± 0.2) (**Turan et al., 2007**). These results indicated that lipid of pangasius was not affected by the hot smoking temperature compared to Indian mackerel lipid. The loss of lipids and consequent loss of PUFA and MUFA that happened during hot smoking treatment was the predominant factor in the reduction of the h/H index after hot smoking of Indian mackerel. The observed variations in that index were probably caused by

the fact that SFA was more difficult to elute with cooking loss than PUFA and MUFA. Because this indicator is associated with the specific effects of fatty acids on cholesterol metabolism, higher h/H ratios are regarded to be beneficial to human health (**Hernández-Martnez et al., 2016**).

The flesh lipid quality (FLQ) displays the percentage relationship between the important n-3 PUFA (EPA + DHA) and total lipids (Table 4). The quality of the dietary lipid source is indicated by a higher index value (**Senso et al., 2007**). FLQ decreased sharply in Indian mackerel from 24.3 to 11.8 while it increased in pangasius from 0.01 to 0.44 after hot smoking (Table 3). Although Indian mackerel lipid was affected by hot smoking treatment, the quality of mackerel fat remains higher than pangasius lipid. Our study's findings of an increase in FLQ related to EPA and DHA content in hot-smoked pangasius fillets could be explained by both natural concentrations brought on by the sample's dehydration and more effective lipid extraction from muscle tissue. **Castro-González et al. (2015)** and **Pietrzak-Fiećko et al. (2017)** noted a rise in EPA and DHA levels in a variety of fish species following heat treatment. On the other hand, cooking loss more than oxidation processes was likely to be responsible for the observed drop in EPA and DHA levels after hot smoking of Indian mackerel. In addition to water and proteins, lipids are also lost during cooking (**Kong et al., 2007**). The British Nutrition Foundation recommends eating fish as a balanced and nutritious meal, with everyone consuming 1.5 g or 0.2 g of EPA + DHA per week (**Özyurt et al., 2005**).

The raw and hot smoked pangasius differed considerably in the quantity of LA (%) that it contributed to the diet. However, compared to raw and hot smoked pangasius, the nutritional contribution of EPA + DHA (%) in Indian mackerel was greater (Table 3). Regarding the dietary contribution of EPA + DHA reported in this study, 568 g of hot-smoked pangasius fillets or 22 g of hot-smoked Indian mackerel fillets seem to be closer to the requirement and virtually fulfill the quantity of 500 mg advised by the European Food Safety Authority (**EFSA, 2017**). Similar to how this study described the dietary contribution of LA, 53.5 g of hot-smoked pangasius fillets or 87.5 g of hot-smoked Indian mackerel fillets appear to be closer to the requirement and practically fulfill the amount of 1300 mg recommended by **EFSA (2017)**.

3.5. Microbial load:

As presented in Table 5, the microbial loads in both types of raw Indian mackerel and pangasius fish were close despite the fact that the catch environments for both fish were different. The raw Indian mackerel and pangasius fish had TVC counts

3.8×10^4 and 6.5×10^4 CFU/g, respectively while yeast and mould counts were 6.2×10^2 and 5.2×10^3 CFU/g, respectively. These outcomes may be because of the differences in aquatic environment for both species and to many activities in which the fish handler is involved. In addition to that, both fresh fish have highest values of moisture and pH of which could support the growth of most microorganisms. **Tong Thi et al. (2016)** examined the microbiological quality of frozen Pangasius sold in Belgium and found that total mesophilic aerobic counts were between 3.8 and 4.8 log CFU/g, whereas total psychrotrophic aerobic counts varied from 3.8 to 5.2 log CFU/g.

Table 5. Total viable bacterial counts (TVC) and yeast and mold in raw and hot smoked fillets of Indian mackerel and Pangasius fillets.

Criterion	Indian mackerel		Pangasius	
	Raw	Smoked fillets	Raw	Smoked fillets
TVC (CFU/g)	$3.8 \pm 0.3 \times10^{4a}$	$2.5 \pm 0.3 \times10^{3b}$	$6.5 \pm 0.2 \times10^{4a}$	$5.3 \pm 0.4 \times10^{3b}$
Yeast and mold (CFU/g)	$6.2 \pm 0.4 \times10^{2a}$	$0.97 \pm 0.2 \times10^{2b}$	$5.2 \pm 0.3 \times10^{3a}$	$1.3 \pm 0.2 \times10^{2b}$

a, b values in each raw of each product with different super- scripts are significantly different ($p < 0.05$); Data are Mean ± SD.

Hanumanthappa and Chandrasekhar (1987) found that the total plate count and yeast and mould of frozen raw Indian mackerel were 3.5 $\times 10^3$ CFU/g and 0.4 $\times 10^2$ CFU/g, respectively.

After hot smoking a great decline in TVC and yeast and mold counts were observed in both Fillets of smoked Indian mackerel (2.5×10^3 and 0.97×10^2 CFU/g) and pangasius (5.3×10^3 and 1.3×10^2 CFU/g), respectively (Table 5). This good microbial quality resulted from the antimicrobial smoke compounds along to procedures of hot smoking process (**Cyprian et al., 2015**) which caused reduction in moisture contents and increased the salt contents. These results of aerobic bacterial counts were less than the Egyptian standard (**ESO, 2005**) acceptable limit of smoked fish, 10^5 CFU/g. **Hanumanthappa and Chandrasekhar (1987)** found that the total plate count and yeast and mold of smoked Indian mackerel were 1.4 $\times 10^2$ and 0.3 $\times 10^2$ CFU/g, respectively.

3.6. Organoleptic acceptability:

The mean scores of sensory properties of hot smoked fillets of Indian mackerel and pangasius were given in Table 6. The panelists evaluated the smoked fillets in terms of appearance, colour, taste, texture, odour and overall acceptability where the scores were ranged between excellent and very good for Indian mackerel while they ranged from very good to good score for pangasius. The panelists preferred hot smoked Indian mackerel fillets than Pangasius one. Nowadays, modern smoked goods stand out for their distinct organoleptic qualities, higher moisture content, and reduced salt concentrations in the flesh.

Table 6. Sensory properties of hot smoked fillets of Indian mackerel and Pangasius fish.

Sensory characters	Smoked fillets	
	Indian mackerel	Pangasius
Appearance	8±0.5	8±0.5
Color	8±0.5	7±0.7
Taste	8±0.4	8±0.3
Odour	8±0.5	7±0.5
Texture	8±0.4	8±0.3
Overall acceptability	8±0.5	7±0.6

4. Conclusion:

Hot smoked Indian mackerel and pangasius in short time process had distinct organoleptic properties with low salt content and less loss in moisture content. Hot smoked Indian mackerel fillets had less nutritional, TBA values, TVC and yeast and mold counts but had

more salt, moisture, TVB-N values and overall acceptability scores compared with smoked pangasius fillets. Hot smoked Indian mackerel fillets had omegas n-9> n-3> n-6>n-7 while hot smoked pangasius fillets had n-9> n-6>n-3. The PUFAs/SFAs ratio of both hot smoked fish was in the recommended limit of WHO. Fish lipid indices were indicated that these hot smoked products had good contribution in the human healthy diet.

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