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NUTRITIONAL VALUE AND FATTY ACID COMPOSITION OF HOUSEHOLD COOKING ON FISH FATTY ACIDS PROFILE USING ATHEROGENICITY AND THROMBOGENICITY INDICES

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A B S T R A C T

Different cooking methods were effects on the fatty acids and food nutritional qualities. Fish is the most nourished food containing a higher ratio of essential fatty acids. This study is contacted to determine the effect of different cooking methods (microwaving, boiling and grilling) for preparing salmon, mackerel, sardine and tuna had on the fatty acid profiles, in particular the ω-3 fatty acid. Depending on estimated polyunsaturated/saturated ratio and ω-6/ ω-3 ratio as a measure of the propensity of the fish and cooked fish to influence the incidence of coronary heart disease indices of atherogenicity and thrombogenicity which were identified nutritional benefit from fish lipid. Also determine the level of minerals and toxic contaminant in fish meat according different cooking methods. Study was carried on salmon, sardine, mackerel and tuna fishes. The samples were cooking by different methods. These were microwaving, grilling and boiling. By lipid extraction, fatty acids profile was determined in all fresh and processed fish. From the fatty acid profile, the atherogenicity index (AI) and thrombogenicity (TI) indices were calculated. Mineral and toxic hazardous mineral were also identified in the cooked fish. It could be concluded that the use of heating process of fish especially microwave is advantageous over conventional cooking like grilled and blanched fish especially with salmon and mackerel. It also, the ω3 fatty acids retarded the decline in cognition over time. So, then ω-3 also produces a potent anti-thrombotic effect by decreasing production of thromboxane. There is evidence that the type of fat is more important than the total amount of fat in the quantification of cardiovascular disease risk; therefore, the Atherogenicity Index (AI) and the thrombogenicity Index (TI) were also evaluated. Using grilling and boiling process in the most of the fishes were able to decrease the level of cadmium in all studied fish.

Keywords: fatty acids, ω6/ ω3, fish oils, boiling, microwave, grille, atherogenicity, thrombogenicity.

INTRODUCTION

Lipids in marine foods consist mainly of long-chain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Kinsella *et al.*, 1990) which belongs to the physiologically important group of n-3 fatty acids. Several epidemiological studies have demonstrated the protective role of fish and fish oil consumption against coronary heart diseases (Kris-Etherton *et al.,* 2003). The increase of unsaturated fatty acids, along with the reduction of saturated fats, supports the lowering of blood cholesterol in humans (Kinsella 2005) and causes a positive impact on human nutrition. Specifically, the increase of unsaturated fatty acids contributes to the prevention of chronic diseases. Polyunsaturated fatty acids ω-3 (PUSFA ω-3) plays a role in preventing heart disease and has anti-inflammatory and anti-thrombosis affects (Connor 2000). Also, ω-3 and ω-6 polyunsaturated fatty acids are considered essential cannot be synthesized in the human body; they must be obtained through diet (Mahan *et al.,* 2005). These nutritional benefits of fish consumption were mainly attributed to the effect of ω-3 polyunsaturated fatty acids, which are thought to have several potential cardio protective actions (Din *et al.,* 2004). Long chain polyunsaturated fatty acids (LCPUFAS) play an important role in maintaining health

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conditions (Rizzo *et al.,*2000). The main effects of n-3 fatty acids on human health can be divided into three main categories viz., 1. Their essentiality in specific organs; 2. Their significant role in lowering blood lipids; 3. Their role as precursors for mediating biochemical and physiological responses. Diets with a high content of these fatty acids may reduce not only the risk of cardiovascular diseases but also the risk of hypertension and arthritis (Lee *et al.,* 1985). Activation of platelets and thrombosis are crucial events in atheromatic plaque formation and they have become a common therapeutic target in coronary syndromes (Lee *et al.,* 2013) lowering cholesterol levels and blood pressure (Horrocks and Yoe 1999). Bouzan (2005) stated that, the health benefit of polyunsaturated of long chain fatty acids has been linked to the inhibiting of formation of blood clots and reduction of stroke risk .The effect of ω-3 fatty acid on platelet function and thrombosis are controversial, suggesting that other substances, apart from ω-3 fatty acids, may be responsible for the antithrombotic properties of marine fish (Kristensen 2001).

Evidence from cellular and molecular research studies indicates that the cardioprotective effects of n-3 PUFA result from a synergism between multiple, intricate mechanisms that involve anti-inflammation, pro resolving lipid mediators, modulation of cardiac ion channels, reduction of triglycerides, influence on membrane micro domains and downstream cell signaling pathways and antithrombotic and antiarrhythmic effects. n-3 PUFAs inhibit inflammatory signaling pathways (nuclear factor-κ B activity) and down-regulate fatty acid (FA) synthesis gene expression (sterol regulatory element binding protein-1c) and up-regulate gene expression involved in FA oxidation (peroxi some proliferate activated receptor α). Moreover, DHA, long chain polyunsaturated ω-3fatty acids are essential for infant brain development and eye function (Birch *et al.,* 1998). This includes potential therapeutic benefits of ω-3fatty acids for rheumatoid arthritis, as there is a mechanism that involves immune system modulation to reduce the action of inflammatory compounds (Darlington and Stone. 2001). Simopoulos (2004) indicated that, the high ratio of ω-6 to ω-3fatty acids in Western diets may contribute to higher risk of chronic disease. The international society for the study of Fatty Acids and Lipids also recommends at least 0.5 g per day of EPA plus DHA for cardio protective benefits in healthy adults .Increased ratio of ω-6/ω-3 is attributed to increase of the high stability of salmon lipid against frying temperature (Candela *et al.,* 1998). Feeding fish oil to the transgenic mice expressing pig's PPAR (peroxi some proliferator activated receptor) decreased lipogenesis (down-regulated SREBP-1c (sterolresponse element-binding protein), lipoprotein lipase and adipose tissue FABP), increased lipolysis(up-regulated hormone-sensitive lipase and uncoupling protein-2) and increased b-oxidation (up-regulated acyl-CoA oxidase, carnitine palmitoyl transferase-1 and long-chain acyl CoA dehydrogenase) with final consequence of the total body fat reduction (Yu *et al.,* 2010) Meanwhile, the cooking of fish improves their digestibility, enhances palatability, and provides a safe eating by killing harmful bacteria, other microorganisms, and parasites (Gokoglu *et al.,* 2004), enhance its flavor and taste (Unusan 2007). Traditionally, there are numerous fish cooking methods varying among different countries and even within the same country, depending on the species of the fish (Gokoglu *et al.,* 2004). During cooking, chemical and physical reactions occurred therefore digestibility is increased due to protein denaturation but the content of thermo labile compounds and polyunsaturated fatty acids is often reduced (Finot 1997). Modifications in the fatty acid profiles by different culinary technologies, such as frying, boiling, roasting, microwave cooking, grilling or cooking with steam, have been studied in some fish species (Gladyshev *et al.,* 2005). Since most fish species are consumed cooked, the nutritive value of the final cooked product is of major importance for human health.

Unfortunately, polyunsaturated fatty acids (PUFAs) are susceptible to oxidation and to thermal damage due to excessive heat. Modifications of fatty acids during cooking could be related to three mechanisms: oxidation, loss of fatty acids by diffusion (in roasting) or fatty acid exchange between fish and oil (in frying). The nutritive value of fish can be affected by processing or cooking methods. The effects of different processing and cooking methods on nutritive values of different fish species have been previously studied (Garcia-Arias *et al.,* 2003). Polyunsaturated fatty acids are known to be highly susceptible to oxidative breakdown (Sant'ana and Mancini-Filho 2000) and heat catalysts strongly for the initiation of lipid peroxidation (Kingston *et al.,* 1998).The oxidation and changes in lipid profile of the fish lipid resulting during cooking can lead to certain medical disorders such as higher risk of atherosclerosis

(Modugo *et al.,* 2011), oxidative stress, and exacerbate atherogensis by incorporating into lipoproteins (Penumetcha 2000). It has been recognized that lipid oxidation product exert toxic carcinogenic and mutagenic effects (Yang *et al.,* 1998) and causing a decrease of fatty acid digestibility and adsorption as a result of cross-linking reactions of secondary lipid oxidation with protein (Kirk 1984). Several studies have shown that cooking methods effect on fatty acid compositions and the lipid class composition of fish (Garcia-Arias *et al.,* 2003). The effect of cooking methods on the fatty acid profile has been studied however, there were moisture and lipid losses during cooking amongst the different methods. The fatty acid profile showed only minor differences between the methods apart from an increase in PUFAs in the deep fried salmon due to linoleic acid uptake from the frying oil (Danae *et al.,* 2010).

Most of cooking methods such as poach, steam, microwave and oven baked showed good preservation of ω-3 fatty acids, and this is attributed to internal protection of ω-3 fatty acids in king salmon. It also was the effect of heating on fish lipid sprat, herring and bream. Furthermore, the increasing of peroxide value was proportional to heating temperature. DHA increased by 20% after 1 h heating at 100°C ; a 45% decrease after 15 min heating at 160°C and a 70% loss after 1 hr at the same temperature. EPA under the same conditions reported losses of less than 20% (Kolakowska *et al.,* 2010).

The effects of essential heavy metals were observed in fast-growing young children; this effect might be extrapolated to toxic metals. These theoretical considerations are compared with epidemiological evidence. Clinical manifestations of heavy metal intoxications are chronically, toxic Cd intake causes a microcytic hypochromic anemia in young rats. Anemia and reduced intelligence scores were recently observed in children after exposure to very low levels of Pb (Ernahrungswiss 1990) Cadmium and lead are non-essential and toxic metals which are distributed and release into the aquatic environment by industrial sources such as mining, refining of ores (Rashed 2001).

The aim of this study was to determine the effect of different cooking methods (microwaving, boiling and grilling) for preparing salmon, mackerel, sardine and tuna had on the fatty acid profiles, in particular the ω-3 fatty acid. This research also aimed to investigate, the polyunsaturated/saturated ratio and ω-6/ ω-3 ratio as a measure of the propensity of the fish and cooked fish to influence the incidence of coronary heart disease depending on indices of atherogenicity and thrombogenicity. However, these indices have an important role to identify nutritional benefit from fish lipid. Moreover, effect of these cooking methods on level of minerals and toxic contaminant in fish meat.

MATERIALS AND METHODS

Samples: The salmon, sardine, mackerel and tuna fish approximately 5kg with size about 250-350 g for each, which obtained from in 2011 from grocery stores and local market in Giza, Egypt. Sample preparation and different cooking methods were conducted by Remedios *et al.,* (2011) with some modification. They placed into a laboratory in an ice tank; samples were cleaned and washed with tap water. Each group of fish divided into four groups. One of them was studied as fresh fish, while the three others from each group were employed for each of the cooking methods under study. Inside each group, fish specimens were distributed into three batches (n=3 five specimens per batch). Fish were prepared using a handling process, such as gutting, deboning, filleting and washing.

Chemicals: All chemical reagents and standard fatty acids (Spulco) were purchased from Sigma Aldrich, Chemical Company. P.O. 145508, St. Lous, USA.

Methods: When choosing the cooking methods were taken into consideration. Different cooking treatments were selected as common cooking procedures used by consumers. These were microwaving, grilling and boiling. The fish was grilled over hot coals using black bean for about 10 min per side, and then for barbecuing, on commercial charcoal were used. The fish were blanched into bleaching and put in brine solution 5%. The boil was carried out in stainless steel pan of boiling 100°C water about 1litre and cooked for 5 min. After this cooking method, samples were catching up and drained on punched pan. Microwaving, the fish fillets were performed in a microwave oven (Panasonic, NE-S262, Japan), samples wrapped in the aluminum foil, placed on a clear Pyrex microwave plate and using frequency of 2450 MHz, 650W for 5 min. With no additional ingredient added. After cooking the samples were placed on absorbent paper. Raw fillets were used as the reference.

Lipid extraction: About 100 gram of fish muscle was homogenized immediately after cooking, separately

using a Warring blender. The lipids were extracted following by (Bligh and Dyer 1959). After separation of the solvent layer using a separating funnel, the chloroform phase was kept, dried and filtered with sodium sulfate anhydrous, and evaporated at 40 °C in a rotary evaporator. The remaining oil was used for the fatty acid determination. Nitrogen flushed into fish lipid samples with tightly closed and stored at -20°C until analysis.

Fatty acid analysis: Preparation of fatty acid methyl esters was performed by esterified fatty acid by boron trifluoride in methanol, according to (AOAC 2012). Fatty acid methyl esters (FAMEs) were separated on a Spulco column of DB-Wax (30×0.25mm id., × 0.25 µm film thicknesses, J&W, USA) and quantified using a Simadzu model 2010 gas chromatography fitted with auto sampler. A system equipped with a flame ionization detector (FID) and helium (99.999%) as carrier gas flow 30ml/min, and split ratio of 1:100. Operating condition were as follows, the inlet and detector temperatures of 250°C and oven temperature maintained at 200°C. Retention times and peaks of fatty acid methyl esters (FAMEs, from Sigma) were identified quantified and quantified by comparison with the retention times of known FAMEs of Sigma fatty acid standards. The obtained results of fatty acids were expressed in GC area % of total fatty acid.

Calculated Indices: From the fatty acid profile, the atherogenicity index (AI) and thrombogenicity (TI) indices were calculated, as proposed by Ulbricht and Southgate (1991) to relate the profile of fatty acids with the risk of cardiovascular disorders, through the equation:

IA = [C12:0 + (C14:0 × 4) + C16:0] / (Total unsaturated fatty acids)

Where: C12 = the percentage of lauric acid in relation to TFA; C14 = the percentage of myristic acid in relation to TFA; and C16 = the percentage of palmitic acid in relation to TFA.

TI = ∑ (C14:0 +C16:0 +C18:0) / (0.5×cis C18:1 + 0.5 × ∑ MSFA + 0.5 × ∑ (ω-6) + 0.5× ∑ (ω-3) + (ω-3/ω-6)

Where: **ω-6** is fatty acids containing omega-6 and **ω-3** is fatty acids containing omega-3.

Also the saturated/unsaturated fatty acids (Usat / Sat) , PUFAs/ Sat and ω-3/ω-6 ratios and ∑ of EPA+DHA were calculated using the fatty acid profile in extracting lipid from different cooked fish species under different cooking processes.

Estimation of mineral profile: The mineral contents Fe, Mn, Cu, Zn, Ca, and Mg in each composite fish and processed according to the method described in AOAC (2012). Fe, Mn, Cu, Zn, Ca and Mg were determined by digesting 0.5 g sample in concentrated HNO_3 at a temperature of 85 C˚ and then in $HClO_4$ at temperature of 180C ˚ until 1-2 ml of digested samples were left. The digested sample was then filtered and volume was made up to 25 mL. These samples were then run through an Atomic Absorption Spectrophotometer (Varian, AA240, and Victoria, Australia) using air acetylene flame to determine the mineral content.

Statistical analysis: All the analyses in this study were carried out in triplicate and the results were reported as mean values ± standard deviation. Correlation was analysis by Microsoft Excel statistical software (Microsoft Office Excel 2007, Microsoft Corp., and Redmond, WA, USA).

RESULTS AND DISCUSSIONS

The influence of the microwaving, boiling and grilling cooking methods on the fatty acids and their nutritional qualities are shown in Table 1 and Table 2. The aim of this study was to investigate the changes which occur under the influence of different heating methods in the composition of fatty acids. It also helps to know their nutritional quality indicators in fillets of four fish species. These fishes were a widely consumed throughout the world, study to investigate for atherogenicity and thrombogenicity properties of their lipid fractions. Fatty acids of cooked fishes were compared to their raw fishes. The extractions of lipids were carried out by the (Bligh and Dyer 1959). After different cooking treatments of various types of fish in this current study, lipid profile of fishes appeared to change and significant differences were found in comparison with uncooked fish samples (Table 1). The extracted lipids were used to identify their fatty acid composition. Culinary processes like boiling and, grilling whether done conventionally or using a microwave oven, lead to a reduction in the n-3 PUFAs fraction of the total fatty acid content. From Table I, the total of saturated fatty acids (SFA) of salmon lipid was increased from 19.2%, 20.8%, 24.9% and 24.5% in raw, microwaving, boiling and grilling, respectively. Boiling and grilling, resulting an apparent increasing in the total SFA in salmon fish, especially at level of C16:0 and$C_{18:0}$ than raw and microwaving method, probably due to its turnover of instability of unsaturated fatty acids by a

destructive heating process. Generally, C16:0, C18:0 and C20:4 of fatty acids of these three methods were found at the highest content among saturated fatty acids of salmon lipid. Microwaving and grilling of lipid of mackerel fish appears more effective on the saturated fatty acid content, SFA was determined as 56.7% and 66.4%, respectively. Meanwhile, blanch method was shown a fairly effect difference as 49.8% than these two methods. Most of these changes in SFA were concerned firstly with increasing of C16:0 and C18:0 follows by C20:4 fatty acids. The lipid and their fatty acid composition in the cooked fish fillets was increased higher in microwaving and grilling methods than raw and blanched mackerel fish. This might due to the lower water content of the cooked fillets (Regulska-Ilow and Ilow. 2002).

A culinary cooking method like microwaving was involved higher percentage of the total SFA (78.0%) in the lipid of sardine fish. Most of this increasing was occurred in palmitic acid and stearic acid when compared to raw lipid of sardine. Meanwhile boiling method occurred decrease of total SFA of sardine compared to the raw lipid of sardine. The grilling method was shown a slightly lower in the total SFA compared to raw lipid of sardine. A positive influence was occurred in two cooking methods of microwave and, grilling in total SFA in tuna lipid. Particularly increasing was occurring among levels of palmitic and stearic acids. Observations showed that boiling tuna fish lipid causes the most of SFA to be depress (6.82%) in this method compared to raw lipid of tuna fish and different cooking method under this study. The SFA was dominated by palmitic acid (C16:0) and stearic acid (C18:0). The SFA was changed between cooking methods, although there was a deeply decrease occurred in blanched, tuna fish. This decrease was also apparent in the levels of total SFA of boiling tuna compared to raw lipid of tuna fish. This negatively decrease occurred by consequences absorption of the culinary sunflower oil which added into the boiling tuna (Gokoglu *et al.,* 2004).

The culinary processes can alter significantly of the content; the consumption and the biological activity of the fish lipids, several reactions involved are often related cooking time and temperature treatment. Microwave ovens change regular electricity into high – frequency microwaves that water, lipid and sugar can absorb causing food particle vibration, and therefore results in heating of food (Garcia-Arias *et al.,* 2003). The composition of the fatty acids in different fish species in Table 1, presented and evidence of the fact that fishes are a very good source of ω-3 PUFAs. Of note is the fact that both of raw mackerel and sardine containing a higher proportion of total EPA (C20:5) and DHA (C22:6) as 28.3 % and 13.4% respectively, follows by raw salmon and raw tuna (10.1 and 5.8 % respectively).

The total SFA of sardine was highly increased in microwave process 78.0%, this is concerning in C16:0 (56.0%) compared with raw sardine fish (28.0%). Meanwhile, the total PUFAs were deeply decreases from 23.7 % in raw sardine fish into 15.7 % in the microwave process. Also, the grilled on sardine fish had a drastically effected on total PUFAs 8.3% compared with raw of sardine was 23.7%. While the total monounsaturated MUSFA and polyunsaturated PUFAs for grilled sardine were generally 51.6 %. The blanched sardine had a higher content of polyunsaturated fatty acids (54.7%). From this obtained result, the boiling method of sardine fish improves the quality and quantity of sardine PUFAs comparing to raw sardine fish.

Boiling process effect of increased monounsaturated fatty acids C18:1ω 9 (24.0%) and C18:2 ω 6 (51.7%) while were 8.5 % and 1.5% in raw sardine fish. The worst treatment of grill was corresponding to decrease PUSFs in grilled sardine into 8.3% while; the raw sardine monounsaturated was 23.7%. The main changes in fatty acid profile of PUFAs were occurred in C20:5 ω3 (2.7 %), while in raw was 13.4%. Loss of total ω-3 content in microwave and grill cooking sardine fish were higher than those of fish cooked by other methods. This is also in agreement by (Turkkan *et al.,* 2008). Fish lipids reduce thanks to their high content of ω-3 PUFAs reduces the amounts of cholesterol and triglycerides and the level of low density lipoprotein cholesterol in blood serum, and reduce the blood pressure and tendency the blood to form clots, thus reducing the risk of atherosclerosis, cardiovascular disease and arterial hypertension (Horrocks and Yeo 1999). Their recommendation for a week eating the amount of fish more than 300 g can be imparted on the significant role to prevent coronary heart disease (Horrocks and Yeo 1999). Unfortunately, the cooking method can be eliminated the polyunsaturated by oxidation into saturated fatty acids. This reason has a significant reason to decrease the nutritional value of used fish.

The qualitative of the lipid fraction of raw and cooked salmon, mackerel, and tuna with their atherogenicity

Table 1. Fatty acid content* (% of total fatty acids) in raw and cooked salmon, mackerel, sardine and tuna, results % ± S.D (Standard Deviation).

Fatty acids	Salmon				Mackerel				Sardine				Tuna			
	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling
Saturated fatty acids																
C6:0	-	-	-	-	-	-	-	-	0.5 ±0.2	-	-	0.7 ±0.3	-	-	-	-
C8:0	-	-	-	-	-	-	-	0.5 ±0.06	-	-	-	0.5 ±0.12	-	-	-	-
C10:0	0.7 ±0.2	-	-	-	0.2 ±0.3	0.3 ±0.33	-	-	-	-	0.1 ±0.01	-	-	-	-	-
C12:0	0.1 ±0.3	-	-	-	0.7 ±0.01	1.1 ±0.3	-	-	0.1 ±0.2	-	-	-	-	-	-	-
C14:0	3.3 ±0.2	3.8 ±0.4	3.9 ±0.30	4.0 ±0.35	6.6 ±0.14	3.8 ±0.22	7.4 ±0.02	9.7 ±0.21	12.1 ±0.22	3.2 ±0.42	0.8 ±0.0	2.6 ±0.25	1.5 ±0.24	5.6 ±0.24	0.1± 0.16	3.8 ±00.1
C15:0	0.5 ±0.32	-	-	0.3 ±0.1	1.3 ±0.33	2.8 ±0.22	0.6 ±0.23	3.4 ±0.33	1.0 ±0.27	1.3 ±0.14	0.2 ±0.14	1.2 ±0.01	0.8 ±0.22	1.7 ±0.15	0.1 ±0.24	1.6 ±0.04
C16:0	10.9 ±0.25	12.4 ±0.11	14.6 ±0.02	16.7 ±0.12	15.4 ±0.21	29.0 ±0.33	27.4 ±0.01	28.9 ±0.26	28.7 ±0.28	56.0 ±0.18	9.6 ±042	26.5 ±0.74	26.8 ±0.14	33.2 ±0.29	6.82 ±0.30	37.2 ±0.24
C17:0	0.3 ±0.30	0.4 ±0.04	0.3 ±0.14	-	0.4 ±0.22	6.7 ±0.23	5.3 ±0.04	8.6 ±0.18	1.5 ±0.32	2.2 ±0.21	0.5 ±0.15	1.5 ±0.10	2.8 ±0.11	-	0.1 ±0.01	2.3 ±0.03
C18:0	2.7 ±0.1	2.8 ±0.26	3.8 ±0.1	3.5 ±0.12	2.8 ±0.30	8.9 ±0.26	7.8 ±0.15	10.2 ±0.24	5.5 ±0.02	15.0 ±0.22	4.5 ±0.15	11.5 ±0.16	21.9 ±0.15	21.7 ±0.01	3.8 ±0.22	18.3 ±0.14
C20:0	0.6 ±0.9	0.2 ±0.65	0.7 ±0.3	-	0.2 ±001	2.1 ±0.18	1.0 ±0.12	3.3 ±0.01	1.2 ±0.39	0.3 ±0.25	0.3 ±0.18	-	-	1.0 ±0.12	0.3 ±0.14	-
C22:0	0.1 ±0.2	1.2 ±0.22	1.6 ±0.74	-	-	2.0 ±0.01	0.3 ±0.05	1.8 ±0.07	0.2 ±0.37	-	0.5 ±0.15	-	-	0.7 ±0.04	0.6 ±0.02	-
Unsaturated fatty acids																
C16:1ω7	4.2 ±0.11	4.7 ±0.08	-	4.9 ±0.25	4.4 ±0.11	4.8 ±0.12	15.7 ±0.25	4.0 ±0.24	10.6 ±0.29	2.4 ±1.3	0.8 ±0.2	1.9 ±0.3	3.0 ±0.21	6.6 ±0.14	0.3 ±0.18	3.6 ±0.04
C16:1ω9	0.3 ±0.6	-	4.6 ±0.1	0.3 ±01	0.4 ±0.46	0.8 ±0.22	1.6 ±0.14	1.1 ±0.12	0.2 ±0.18	-	-	-	-	5.4 ±0.01	-	-
C16:2ω4	0.6 ±0.33	0.6 ±0.3	0.7 ±0.12	0.7 ±0.12	0.8 ±0.11	0.9 ±0.33	1.2 ±0.01	0.8 ±0.42	1.3 ±0.62	1.1 ±0.26	-	1.3 ±0.18	1.2 ±0.05	2.3 ±0.01	-	1.7 ±0.01
C16:3ω4	0.6 ±0.12	0.5 ±0.01	0.4 ±0.35	0.6 ±0.14	0.6 ±0.12	0.5 ±0.14	-	0.1 ±0.21	1.0 ±0.23	0.2 ±0.01	-	-	0.6 ±0.01	0.8 ±0.14	-	-
C16:4ω3	-	-	-	-	-	-	-	-	0.2 ±0.02	2.3 ±0.05	-	-	0.6 ±0.16	-	-	-
C16:4ω1	-	-	-	-	-	-	-	-	-	0.3 ±0.14	-	-	-	7.0 ±0.1	0.3 ±0.12	-

C18:4ω1	0.3 ±0.33	-	-	-	0.2 ±0.2	-	-	0.2 ±0.11	0.1 ±0.3	0.4 ±0.14	0.1 ±0.0	-	-	7.0 ±0.20	-	-
C18:1ω9	31.5 ±0.32	34.2 ±0.28	30.1 ±0.08	26.0 ±0.01	8.1 ±0.3	2.7 ±0.16	6.2 ±0.25	5.5 ±065	8.5 ±0.21	5.3 ±0.15	24.0 ±0.54	6.2 ±0.61	15.1 ±0.27	3.2 ±0.29	25.0 ±0.01	15.1 ±0.14
C18:1ω7	3.3 ±0.01	3.6 ±0.05	2.4 ±0.1	4.3 ±0.0	1.8 ±0.12	3.6 ±0.25	2.7 ±0.24	3.8 ±0.14	6.0 ±0.33	4.3 ±0.62	1.0 ±0.19	3.2 ±0.23	2.5 ±0.01	1.7 ±0.22	0.6 ±0.0	4.2 ±0.19
C18:1ω5	0.2 ±0.12	-	0.1 ±0.01	-	0.6 ±0.30	0.2 ±0.32	-	-	0.1 ±0.14	-	-	-	-	-	-	-
C18:2ω6	11.1 ±0.13	9.0 ±0.12	5.9 ±0.15	9.1 ±0.33	-	0.1 ±0.17	1.0 ±0.19	0.8 ±0.14	1.5 ±0.25	2.3 ±0.54	51.7 ±0.25	2.0 ±0.22	8.8 ±0.18	-	57.8 ±0.14	6.3 ±0.15
C18:3ω6	-	-	-	-	-	-	0.2 ±0.12	-	0.1 ±0.1	0.5 ±0.24	-	-	0.7 ±0.21	-	-	-
C18:3ω3	3.9 ±020	3.3 ±0.31	2.1 ±0.24	2.03 ±0.60	1.3 ±0.1	1.0 ±0.01	1.0 ±014	1.3 ±0.22	0.1 ±0.14	1.1 ±0.12	0.5 ±0.0	0.8 ±0.1	1.0 ±0.31	-	0.6 ±0.4	1.8 ±0.25
C18:4ω3	0.9 ±0.15	0.8 ±0.03	0.9 ±0.11	1.1 ±0.02	2.2 ±0.0	0.8±0.0 1	-	0.9 ±0.45	1.6 ±0.0	0.5 ±0.22	1.0 ±0.22		0.4 ±0.14	-	-	-
C20:1ω9	5.7 ±0.91	5.6 ±0.14	5.4 ±0.02	7.4 ±0.52	12.9 ±0.33	-	0.1 ±0.12	0.1 ±0.31	0.6 ±0.88	-	0.2 ±0.2	32.0 ±0.45	-	-	0.2 ±0.1	-
C20:1ω7	0.9 ±0.02	0.3 ±0.01	0.2 ±0.01	-	0.4 ±0.21	-	0.2 ±0.2	0.1 ±0.15	0.2 ±0.4	-	-	-	-	-	-	-
C20:2ω6	0.9 ±0.22	0.7 ±0.3	0.7 ±0.02	0.5 ±0.3	0.3 ±0.24	0.3 ±0.2	0.1 ±0.13	0.2 ±0.2	0.2 ±0.05	0.3 ±0.01	-	-	-	-	-	-
C20:3ω6	0.4 ±0.12	-	0.3 ±0.3	-	0.4 ±0.16	0.2 ±0.1	0.4 ±0.0	0.4 ±0.3	0.2 ±0.22	-	-	-	0.6 ±0.60	-	-	-
C20:4ω3	1.1 ±0.31	3.2 ±0.4	2.0 ±0.22	1.0 ±0.01	0.8 ±0.01	0.7 ±0.2	-	-	0.5 ±0.22	-	-	-	-	-	-	-
C20:5ω3 (EPA)	5.0 ±0.48	4.5 ±0.5	-	5.9 ±0.23	4.4 ±0.03	3.8 ±0.33	-	2.4 ±0.14	13.4 ±0.27	5.2 ±0.36	-	2.7 ±0.25	5.8 ±0.33	2.2 ±0.27	-	3.6 ±0.35
C20:4ω6	-	0.3 ±0.03	4.8 ±0.21	0.5 ±0.12	0.5 ±0.31	0.3 ±0.12	-	0.1 ±0.01	1.5 ±0.12	3.3 ±0.12	-	1.5 ±0.16	4.7 ±0.56	1.6 ±0.26	-	-
C22:6ω3 (DHA)	5.1 ±0.33	-	3.9 ±0.3	3.5 ±0.06	23.9 ±0.25	13.1 ±0.42	0.3 ±0.3	2.5 ±0.1	-	-	-	-	-	-	-	-
C24:1ω9	0.3 ±021	-	0.5 ±0.01	-	-	0.2 ±0.01	-	1.0 ±0.02	-	-	-	-	-	-	-	-
C22:1ω9	-	-	-	-	1.2 ±0.12	0.8 ±0.12	-	0.2 ±0.03	0.3 ±0.11	-	-	-	-	-	-	-
C22:5ω3	-	-	-	3.4 ±0.33	-	-	-	-	1.5 ±0.36	-	1.4 ±0.16	-	-	-	-	-
Σ SFA	19.2	20.8	24.9	24.5	27.6	56.7	49.8	66.4	50.8	78.0	16.5	44.5	53.8	63.9	11.82	63.2
ΣMUFA	46.4	48.4	43.3	42.9	29.8	13.1	26.5	16.0	26.6	12.0	26.0	43.3	20.6	16.9	26.1	22.9
ΣPUFA	29.9	22.9	21.7	28.33	35.9	21.7	4.2	9.7	23.7	15.7	54.7	8.3	24.4	20.9	58.7	13.4

Table 2. Lipid fractions* of raw and cooked salmon, mackerel, sardine and tuna, and their atherogenicity and thrombogenicity indices.

Fatty acids	Salmon				Mackerel				Sardine				Tuna			
	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling
EPA+DHA	10.1	4.5	3.9	9.4	28.3	16.9	0.3	4.9	13.4	5.2	0	2.7	5.8	2.2	0	3.6
ω6/ ω3	1.29	1.18	0.76	1.67	27.17	21.55	0.76	4.73	4.94	1.42	0.056	1.00	0.53	1.37	0.01	0.88
ω3 PUFAs	16.0	11.8	8.9	16.9	32.6	19.4	1.3	7.1	17.3	9.1	2.9	3.5	7.8	2.2	0.6	5.4
ω6 PUFAs	12.4	10.0	11.7	10.1	1.2	0.09	1.7	1.5	3.5	6.4	5.17	3.5	14.8	1.6	5.78	6.3
Thermal oxidation ratio	0.468	--	0.267	0.209	1.552	0.452	0.011	0.087	--	--	--	--	--	--	--	--
Usat/Sat	3.97	3.43	2.61	2.91	2.34	0.62	0.62	0.38	0.95	0.36	5.09	1.14	0.78	0.64	7.31	0.57
PUFAs/Sat	1.56	1.1	0.87	1.16	1.26	0.39	0.09	0.14	0.43	0.2	3.52	0.14	0.39	0.38	5.1	0.21
Atherogenic index	0.543	0.648	0.600	0.628	1.197	3.392	3.634	4.554	0.8509	7.423	0.4939	1.949	1.862	6.078	2.69	3.043
thrombogenicityindex	0.209	0.223	0.211	0.499	0.170	0.434	1.839	1.056	0.589	2.820	0.247	1.025	0.739	3.075	0.189	1.436

* Values are mean of three measurements.

and thrombogenicity indices are shown in Table 2. The highest level of PUFAs ratio in the processed spices of fish under this study is attributed mainly to the highest level of essential fatty acids. In fact, the oleic acid, linoleic acid EPA and DHA were the most abundant fatty acid in the unsaturated fatty acids in all of both raw and cooked of different species fish as obtained in table 2. The results revealed that the EPA + DHA content significantly decreased during the boiling process in all fish lipid samples as demonstrated in Table (2). However, the EPA and DHA value in raw fish samples was varied from 5.8 % to 28.3 % according to the species of fish. The ratio of ω-6/ ω-3 is a good index for comparing the relative nutritional value of fish oils. This also in agreement with Mara Cristina Romero (2013) reported that , the dietary balance between n-3 and n-6 PUFAs has been evenly balanced with a n-3/n-6 ratio of approximately 1 ; the modern Western diet is now dominated by n-6 PUFA, with a n-3/n-6 ratio of approximately 0.06 . In addition to the above, it‘s possible correlation between total n-6/n-3 ratio and arachidonic acid / EPA ratio in RBC phospholipids and in whole blood total lipids. 1.432 healthy volunteers and patients with various pathologies were recruited and categorized according to their age, sex and any existing pathologies (Rizzo 2000). It has been suggested that a complex view on lipid interactions, which defines the resulting “lipidome” – the individual lipid profile, is necessary. Rather than individual fatty acids, a complex indicator combining the increased content of monounsaturated fatty

acids and the low n-6/n-3 ratio could be associated with protection against certain types of cancer (Kang 2005).

Continuously, the data in Table 2 illustrated that, then ω-6/ ω-3 ratio was 1.180%, 1.42 % and 1.375 % of salmon, mackerel and tuna, respectively with heating process by microwave comparing the other heating processes. However, lower thermal oxidation and lower ω-6/ ω-3 ratio was observed during cooking methods used (Wood *et al.,* 2003). The microwave process was tending to increase the ratio of ω-6/ ω-3in mackerel and tuna as 24. 25 % in mackerel and 1.375 % compared with raw mackerel 27.17% and raw tuna 0.527% and tuna are in agreement by (Hearn *et al.,* 1987) reported that microwave cooking did not alter significantly the lipid total content and percentage of fatty acids in sea water fish. Meanwhile, (Dasilva *et al.,*1993) detected that conventional oven baking verified on saltwater fish fatty acid did not significantly alter , whereas microwave oven cooking reduced PUFAs concentration.

These changes in ω-6/ ω-3 corresponding to increase the ratio of omega 3 polyunsaturated fatty acids and protective role of microwave process against the destruction of ω-3fatty acids. Wood *et al.,* (2003) recommended that the ratio of unsaturated/saturated fat in a human diet be above 0.4. Thus, even with reductions in the concentration of acids, the ratios of unsaturated/ saturated fat at all levels were above the recommended rate. The boiling process has a higher effect on the PUFAs/ Sat and ω-6/ ω-3 compared with their raw group of fish. Conversely, the ratio of thermal oxidation could be shown that blanched mackerel have a lower ratio of oxidation 0.011 % compared with raw and microwave of mackerel (1.552%).These results also indicated by Candela *et al.,* (1998) the effect of deep frying on the lipid fraction of three high fat fish; salmon, mackerel and sardines.

The salmon, which had the lowest amount of DHA and EPA to start with, had the highest level after cooking. Candela *et al.,* (1998) proposed that salmon lipids may have a relatively higher stability during frying. The n-6/n-3 ratio increased 8.92-fold in the salmon after frying and presented the best ratio in this study due to its greater DHA and EPA stability and lower intake of linoleic acid during frying.

Thermal oxidation produced in the cooked fish samples: The oxidation ratio of C22:6 ω 3 / C16:0 was used as an oxidation index during thermal processing. The ratio decreased for all samples for all treatment of canning, grilling and microwaving process (figure 1). The elevation of this ratio is corresponding to oxidation decomposition on essential fatty acids which taken place in those fish cooked samples of salmon and mackerel. While, in this ratio was completely none identified because of C22:6 ω 3 not found in the profile fatty acids of sardine and tuna. These obtained results in agreement by (Moradi *et al.,* 2009).

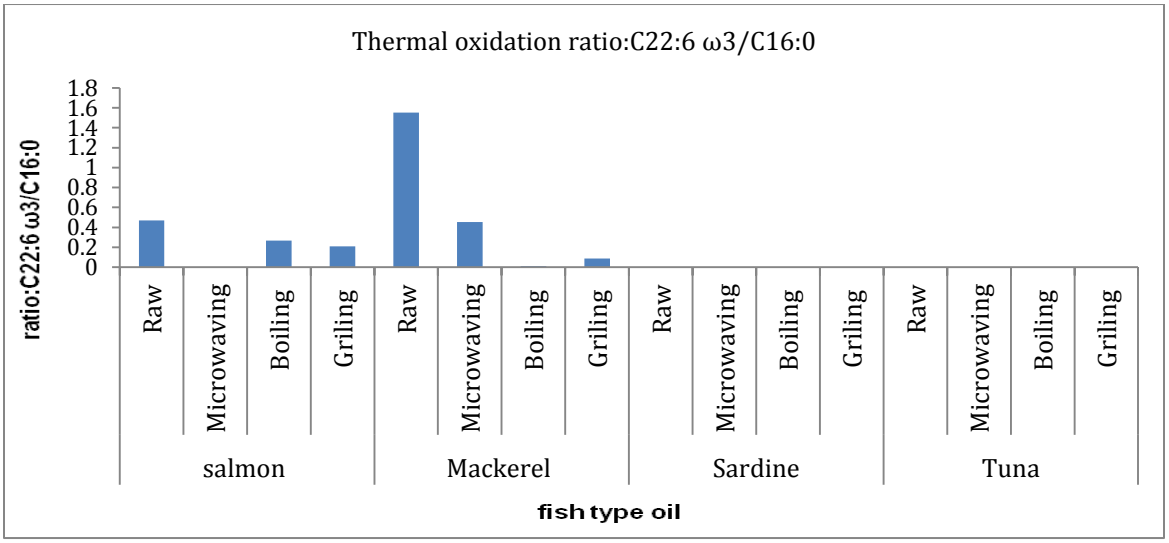

Figure 1. Thermal oxidation ratio for lipid of cooked fish

Among the ω-3 PUFA was the dominant values varied from 0.6 % to 19.4% during the heating process, however these change values differed according to the using heating process type and the species of fish. As a reported in table (2) the ω-6 PUFA, the content was at a minimum (0.9 %) and maximum (11.7%) at the

conditions of the heating process. However a good significant cooking type in PUFA concentration or lipid class distribution due to heat processing in grilled and blanched were demonstrated. For thermal oxidation ratio C22:6 ω3/ C16:0 values were 0.209 %, 0.267 % for grilled and blanched salmon, respectively. Meanwhile, this value was 0.011%, 0.087 % and 0.452 % for blanched, grilled and microwave mackerel, respectively. On the other hand, the ratio of Usat/Sat for lipid classes was 3.43% and 0.62 % for salmon and mackerel, respectively.

Additionally, these results reported that the main problem of fish lipid classes is the oxidative deterioration under the heat processing. However, it causes more nutritional problems of use fish lipids. Oxidation of unsaturated fatty acids on lipids produces offensive colors and off-flavor. However, its limits their use and decreases the nutritional values through the formation more of secondary reaction products. Similarly, table II illustrated the following values of the ratio of PUFAs /Sat: 0.87% blanched salmon, 0.09 % blanched mackerel, respectively. For the grilled sardine and tuna were 0.14% and 0.21%, respectively. The fatty acid DHA (C22:6) has a high number of double bonds, thus being highly susceptible to oxidation which may lead to its reduced percentage. Lipids can undergo several reactions during the cooking process, such as hydrolysis and oxidation, which may affect flavor, scent, color and texture, and its nutritional value. Alterations in lipid level after steaming and roasting are related to the lipid content of each species, temperature, species size and exposed surface (Dasilva *et al.,* 1993).

In general, even when a significant statistical variation was found between the cooking methods, the difference in the percentages of fatty acids was small, which demonstrates that baking and steaming had little influence on fatty acid composition of the species analyzed. Such differences may be rather attributed to the water and lipids leached out during thawing and cooking than to lipid reactions (such as oxidation) during heat treatment.

The incidence of coronary heart disease is reviewed in the light of evidence with regard to their functional role, either in protection or in a promotion. Detailed analysis of the evidence shows that the relations are more complex than the current lipid hypothesis suggests. It is proposed that, in particular, the polyunsaturated/saturated ratio as a measure of the propensity of the diet to influence the incidence of coronary heart disease should be replaced by indices of atherogenicity and thrombogenicity.It is known that, the atherogenic low density lipoprotein (LDL) cholesterol must be modified by oxidation, and that small, dense sub- fractions of it are attractive to macrophages. These macrophages become foam cells that injure the endothelium, causing adherence and activation of platelets. The platelets, in turn, release platelet- derived growth factor. This factor, along with other growth factors, stimulates proliferation of smooth muscle cells, fibroblasts, and collagen. The latter forms the occlusive plaque prone to rupture, fissure, sub initial hemorrhaging, and thrombosis (Austin *et al.,* 1988). Rizzo *et al.,*(2000) indicated that fish oil has a significantly effective on the lower rate of LDL synthesis in the normal subject. Fish oil has inhibitor roles to synthesis of VLDL. Thus the most likely mechanism for the hypolipidemic action dietary fish oil may be depression of VLDL and LDL synthesis. Fish oil markedly decreases the elevated chylomicron levels that usually occurred after fatty meals. The fish lipid is diminished absorption chylomicrons and slower entry of chylomicron into the absorption circulation and rapidly removed from absorption circulation According to the relative contents of particular cooked group of fish fatty acids, the lipid profile of these groups shows a fluctuated number of atherogenicity and thrombogenicity indices in comparison to raw lipid of salmon, mackerel, sardine and tuna. There was a little effect on the atherogenic index for fatty acid of salmon particular to the cooking process. Studies also indicate that high intakes of LCn3PUFAs would produce a lower platelet count, less platelet aggregation, a longer bleeding time, higher urinary prostacyclin (PGI2) metabolites, and lower concentrations of thromboxane metabolites (Scheurlen *et al.,* 1993).

The atherogenicity index was recorded the 0.543% in raw salmon, microwave: 0.648 %, blanched: 0.600%, and grilled: 0.628%. For the mackerel the story is totally different, grilled mackerel has a higher level of the atherogenicity index (4.554), and blanched mackerel (3.634) follows by microwave fish lipid (3.392), while the raw mackerel AI was 1.197. From these obtained results can be assumed that mackerel polyunsaturated is lower activity against lipid peroxidation and PUFAs in mackerel also was higher (about 36%). Corresponding to the destructive cooking process on mackerel fish also

increase the total of saturated fatty acids, this also increase atherogenicity index and nutritional are affected by the suffering of CHD. This also in agreement with Ulbricht and Southgate (1991) reported that, PUFA is very liable to peroxidation and we therefore have the paradox that diets high in PUFA may actually be dangerous in initiating CHD or cancer. Furthermore , Until recently the dietary balance between n-3 and n-6 PUFAs has been evenly balanced with a n-3/n-6 ratio of approximately 1 ; the modern Western diet is now dominated by n-6 PUFA, with a n-3/n-6 ratio of approximately 0.06 (Simopoulos 2002).

Rizzo *et al.,*(2000) indicated that, the arachidonic acid /EPA ratio is a more sensitive and reliable index for determining changes in total blood fatty acids. Moreover, Micallef *et al.,* (2009)reported that negative significant correlation between plasma concentrations of the high sensitivity CRP (hs-CRP; a marker of low-grade sustained inflammation), and EPA and DPA, respectively.

In Table (2) is illustrated that, the microwave process on sardine and tuna have a higher effect for AI in sardine: 7.423 and tuna: 6.078. However the AI for raw sardine was 0.850 and 1.862 for raw tuna. However, in this current study found highly trends for effect of ω6/ ω3 ratio of the atherogenicity indices apparent in all of cooked fish and related to the type of fish also. Atherogenicity index appearing that sardine, mackerel and tuna were defiantly affected after using cooking method, especially boiling and microwave have a legal effect on ratio ω6/ ω3 and AI. Otherwise, there were a little bed relation between ω6/ ω3 and AI of sardine. In addition, the table 2 shows also that atherogenic index value raised under heating process in all fish lipid samples. This also agreed with Beare-Rogers (1988) in the thrombogenicity index, the results clearly show that the different effects on values under a different heating process. The values varied from 0.209 % to 3.075 % during the heating process for a different lipid fraction of the studied samples.

The correlation between type of fatty acids of fish and effects cooking methods on AI and TI: According to the relative contents of the particular fatty acid profile of cooked fish groups (sardine , mackerel, salmon and tuna), showed fluctuation in AI and TI when compared with their raw fish group. The sensitivity of their fatty acids especially omega-3 towards oxidation and destruction, which occurred during cooking process were originally different (Table 2). The principle ratio of ω-6/ ω-3used for identifying the variation between type of fish and effects of cooking method of predicting the nutritional status of consuming these cooked fish. Herein, from the previous researches, there was large evidence that omega fatty acids particular ω-3 decreased serum triacylglycerol concentration primarily by inhibiting synthesis of VLDL from the liver. Also the ratio of ω-6/ ω-3 appeared a significant correlation with AI and TI. Adkins and Kelley (2010) regarded as a high ω -3 PUFA oil has a ratio of 0.5 whilst probably the most common vegetable oil in the modern human diet. Figures 2, 3, 4 and 5 illustrated the correlation between the ratio of ω-6/ ω-3 and with AI and also with TI among different cooked fish groups and cooking methods. In the Fig 2 could be found that the curve is moving simply towards upwards with TI, with salmon fish especially under different cooking method ranked as microwave , boiling and grueling process. This positive correlation between ω-6/ ω-3 and with TI is accounted for R^2 = 0.637 from the linear equation was y = 0.303x - 0.086. However, there is no evidence correlation between ω-6 and ω-3 and with TI. There is a lower tendency to correlate with ω-6/ ω-3 and with TI, which calculate R^2=0.016 from the linear equation y = 0.015x + 0.585.

In contrast, cooked mackerel showing, in Figure (3) the correlation curve is sifted down up with a higher correlation between ω-6/ ω-3 and either TI or AI. The calculation of correlating ω-6/ ω-3 and with TI equal R^2= 0.901, and R^2= 0.627 for AI for cooking mackerel fish at different cooking methods. Cooked sardine appears that, there were a lower correlation between ω-6 and ω-3 and with AI and follows by TI, these were calculated R^2=0.023 and R^2=0.009, respectively. Despite, cooked tuna had shifted curve upward, which was showing strongly and positively correlated between ω-6/ ω-3 and both of AI and TI calculated R^2= 0.627, and R^2= 0.937 , that observed after cooking method especially microwaving , boiling and grilling .

The mineral content: The mineral content in cooked fishes is given in Table (3). There was variation in the iron content in all the varieties of raw fish lowest level in salmon (7.41 mg/Kg) and Tuna was the highest level of iron (25.79 mg/Kg). The microwave process generally decreases the level of iron in all varieties of fish. There was not much of a difference in the magnesium content in most species of fish and the highest levels were in the raw salmon (17.54 mg/kg) in grilling is decreased into 9.07 mg/kg.

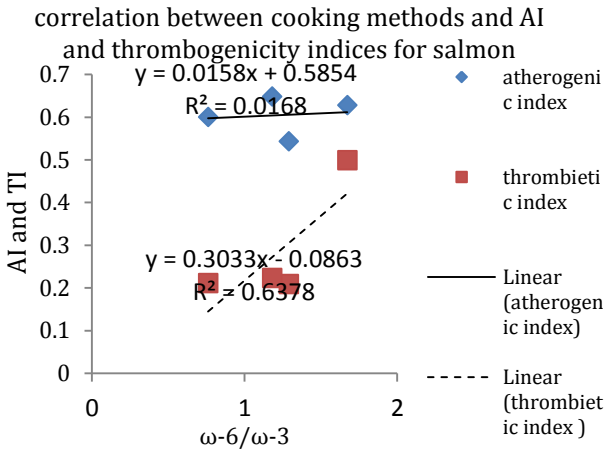

Figure 2. Correlation between cooking methods and Atherogenic (AI) and thromboieticityindices (TI)for salmon

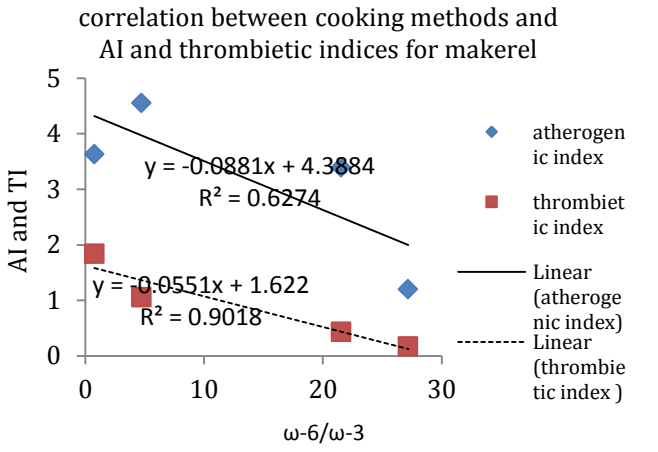

Figure 3. Correlation between cooking methods and Atherogenic (AI) and thromboieticityindices (TI)for mackerel

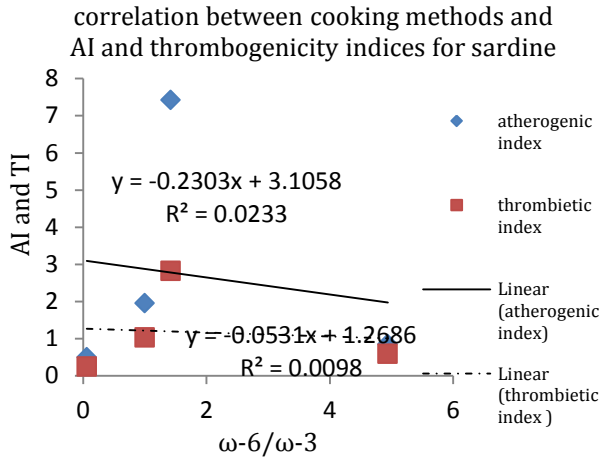

Figure 4. Correlation between cooking methods and Atherogenic (AI) and thromboieticity indices (TI) for sardine

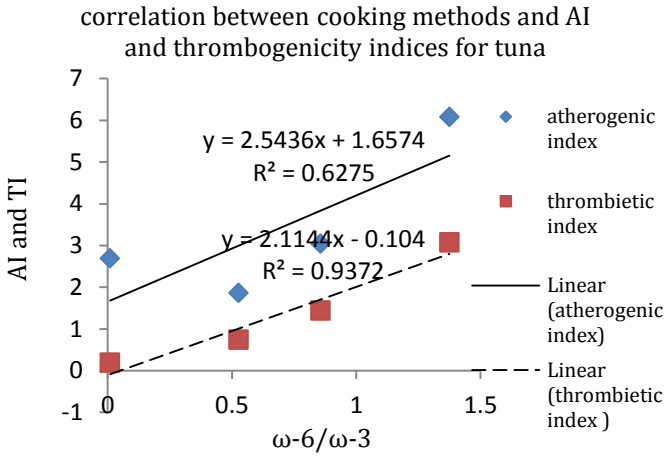

Figure 5. Correlation between cooking methods and Atherogenic (AI) and thromboieticity indices (TI) for tuna

Table 3. Mineral content and level of environmental pollutant ratio of heavy metal in cooked fishes

Mineral	Salmon				Mackerel				Sardine				Tuna			
	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling	Raw	Microwaving	Boiling	Grilling
Mg	276.8 ±0.92	271.4 ±1.02	250.2 ±0.52	282.2 ±0.17	365.1 ±0.99	356.8 ±0.87	241.2 ±0.74	298.8 ±0.78	33.9 ±0.24	30.4 ±0.89	23.18 ±0.25	28.4 ±0.91	521 ±0.28	466.0 ±0.84	401.0 ±0.76	322.6 ±0.71
Zn	6.16 ±0.25	5.99 ±0.87	5.18 ±0.45	5.89 ±0.14	10.25 ±0.24	10.11 ±0.51	12.44 ±0.12	12.88 ±1.33	11.54 ±0.45	11.16 ±0.16	8.33 ±0.25	11.44 ±0.18	22.69 ±0.95	18.25 ±0.98	12.48 ±0.02	18.48 ±0.95
Fe	7.41 ±0.33	6.48 ±0.63	8.14 ±0.12	9.03 ±0.85	18.99 ±0.84	17.20 ±0.02	12.6 ±0.35	17.51 ±0.52	16.55 ±0.12	14.33 ±0.01	8.77 ±0.45	15.24 ±0.12	25.79 ±0.65	24.13 ±0.32	18.44 ±0.42	24.25 ±0.94
Cu	1.07 ±0.12	0.99 ±0.25	2.12 ±0.65	2.45 ±0.03	5.411 ±0.65	4.77 ±0.94	4.12 ±0.99	3.34 ±0.18	-	1.44 ±0.25	0.88 ±0.47	0.67 ±0.97	8.547 ±0.02	8.44 ±0.12	7.15 ±0.09	6.45 ±0.01
Mn	17.54 ±0.35	16.14 ±0.16	10.24 ±0.25	9.07 ±0.12	7.904 ±0.2	8.04 ±0.03	9.48 ±0.08	9.09 ±0.07	-	7.33 ±0.88	6.75 ±0.24	6.16 ±0.25	13.33 ±0.65	12.44 ±0.04	7.45 ±0.19	7.66 ±0.02
Cd	< 0.007	0.005	0.003	0.002	< 0.008	0.007	0.002	0.003	< 0.008	0.002	0.001	-	< 0.010	0.004	0.002	0.002
Pb	0.003	0.003	0.010	0.008	0.006	0.007	0.009	0.010	0.007	0.005	0.004	0.004	0.005	0.005	0.008	0.009

There is no detectable level in raw sardine, while microwave at sardine showing a level 7.33 (mg/kg). Of the fishes the boiling process had the decreased levels of magnesium between 9.61, 33.9, and 10.32 % in raw salmon, mackerel and sardine respectively. Only tuna has a highest decrease was found in grilling process about 38%.

Lead content was generally low (<0.007 μg/g) in most of the species of fish. There was an increase occurred in lead content between boiling and grilling in all fishes , except for sardine was decreased than raw sardine. Vice reverse was occurring in the level of cadmium after grilling and boiling process, there were an appreciable decrease from 0.007 in sardine and mackerel less than 0.002 μg/g.

CONCLUSIONS

From these results, it could be concluded that the microwave is advantageous over conventional cooking like grilled and blanched fish especially with salmon and mackerel. Furthermore, the ω3 fatty acids retarded the decline in cognition overtime. So, ω-3 also produces a potential anti-thrombotic effect by decreasing production of thromboxane. There is evidence that the type of fat is more important than the total amount of fat in the quantification of cardiovascular diseases risk; therefore, the AtherogenicityIndex (AI) and the thrombogenicity Index (TI) were also evaluated.

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