Effects of brine concentration on lipid oxidation and fatty acids profile of hot smoked tuna (*Thunnus albacares*) stored at refrigerated temperature

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Abstract This work evaluated the lipid oxidation and the changes in fatty acids in hot-smoked tuna (*Thunnus albacares*) as a function of brine concentration. Fresh, commercially harvested tuna fish samples were purchased from a local supermarket. The fish were first immersed for 30 min in a brine solution at 5, 10, or 15% sodium chloride concentration and were then smoked at 50 °C for 3 h followed by 1 h at 60 °C and 3 h at 105 °C. The fish were then dried for 17 h, cooled and stored at 4 °C. Oxidative rancidity was measured by the peroxide value (PV), and thiobarbituric acid number (TBA) and fatty acids profile by GC-MS. Oxidative rancidity increased with storage time. The PV and TBARS values were more pronounced for samples immersed in 10% brine solution during the first 27 days of storage, whereas the lowest increase was observed for samples treated with 15% salt. Fatty acid concentration exhibited changes after smoking, and this was varied with salt concentration. The palmitic acid and stearic acid, the two main saturated fatty acids in tuna, increased after smoking at all brine concentration, whereas the contents of oleic acid, eicosapentaenoic acid and docosahexaenoic acid decreased. In conclusion, 15% NaCl-treated tuna gave smoked product with less lipid oxidation and a fatty acid profile comparable to that for 5 and 10% NaCl-treated samples.

Keywords Tuna · Brine · Smoking · Lipid oxidation · Fatty acids

Introduction

There is an ever-increasing demand for fresh and processed fish in the developed and developing countries due to its nutritional value. Fish, in particular fatty fish, is attracting a great attention because it is good source of the long chain n-3 polyunsaturated fatty acids (PUFAs), mainly the docosahexaenoic acid (C22:6n-3) and the eicosapentaenoic acid (C20:5n-3). The PUFAs have been claimed for their positive role on human health. They play an important role in the prevention of cardiovascular disease (Sinclair 1993; Stone 1997) and may decrease the development of cancer (Collett et al. 2001; Terry et al. 2001). The lack of PUFA in human diet might lead to physiological disorders like insufficient growth, skin alterations, and a greater susceptibility to infections (Pawar et al. 2011). Hence, preserving these fatty acids during processing became a priority to the food industry.

Fish and shellfish are perishable protein sources for human consumption. Scientists have been constantly searching for improved methods to preserve or extend the shelf life and safety of various aquatic food products (Chang et al. 1998). Smoking is one of the oldest conservation methods, especially in the fishing industry. It works by action in the fish of several of the smoke individual component namely: aldehydes, ketones, alcohols, acids, hydrocarbons, ester, phenols, ether, etc. (Doe 1980; Leroi and Joffraud 2000; Guillen and Errecalde

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2002). It imparts characteristic flavor and color to the fish in addition to increasing its shelf life by the combined action of dehydration as well as the antimicrobial and the antioxidant activities of the smoked constituents (Goulas and Kontominas 2005). There are three different stages of the total smoking process: salting, heating and smoking (Aminullah Bhuiyan et al. 1986). Salt may be added in the first step prior to smoking by injection, dry salting or brining; the two last processes being most widely adopted by industry (Espe et al. 2002; Mørkøre et al. 2001). Salt is used to preserve and add flavor to smoked fish. The functions of salt in meat products for curing are to inhibit microorganism growth, to add flavor, and to extract salt soluble proteins (Cheng et al. 2007). However, the contact of salt with fish has been reported to enhance lipid oxidation of the highly unsaturated lipids, directly related to the production of off-flavors, protein denaturation and textural change (Harris and Tall 1994). Lipid oxidation is the primary reason for quality changes of meat during storage (Gecgel 2011). Lipid oxidation primarily affects fatty acids in general and PUFA in particular (Haile et al. 2011).

Fish and fish oil are highly susceptible to oxidation due to their high content of polyunsaturated fatty acids mainly eicosapentaenoic acid (EPA, C-20:5) and docosahexaenoic acid (DHA, C-22:6) (Kulås and Ackman 2001). Cooking, thermal treatment and higher storage temperatures are known to increase the susceptibility of n-polyunsaturated fatty acids (PUFA) toward lipid oxidation in general (Regulska-Ilow and Ilow 2002; Atayeter and Ercoşkun 2011), and the losses of unsaturated fatty acids have often been related to lipid oxidation (Bhuiyan et al. 1993; Atayeter and Ercoşkun 2011). Since these fatty acids occur in high amounts only in seafood, it is necessary to study the effect of processing on their stability. This type of work will ensure the quality and safety of smoked fish.

Yanar et al. (2006) reported that although the shelf life of smoked tilapia immersed in 15% salt concentration was longer to 5 and 10% brine but it was less effective lipid oxidation during storage at 4 °C. Optimum storage stability was obtained with the use of 5% brine. Similarly, Augbourg and Ugliano (2002) reported that increasing the salt content in fish accelerates the rate of oxidative rancidity in salted horse mackerel during frozen storage. The growth of microorganisms was also found to be affected by salt concentration and storage conditions (Augbourg and Ugliano 2002).

Tuna and tuna-like species are important fish species due to their high global economic value and their prevalence in international trade for canning and sashimi (Guizani et al. 2005). Yellowfin tuna (*Thunnus albacares*) are large pelagic fish that prevail in the tropics and

subtropics; with landings accounting for about 22% of the world's tuna catch (Guizani et al. 2005). They are commercially important in many countries, including Oman, and there is high demand from international markets.

For the fishing industry to be sustainably developed, the resources have to be as widely used as possible and not only traded as captured. Processing, such as smoking will certainly provide a higher production and cause less waste since it does not require specialized labor and requires a low production cost (Andrade and Oliveira 2000).

The objective of this study was to use smoking technique as a way of adding value to tuna and to assess the effect of brine concentration prior to smoking on some quality parameters (lipid oxidation and fatty acid profiles) of the final product.

Material and methods

Sample preparation and smoking process

Whole fresh tuna (*Thunnus tongol*) (local name, Sahwa) was purchased from local fish market (at Al-Seeb, Sultanate of Oman) in the Month of November. The fish were placed into plastic boxes, covered with ice and transported to the Food Science Laboratory, Sultan Qaboos University. The average mass and length of the whole fish were about 4 kg and 0.65 m, respectively. The fish were washed in tap water and then filleted. The fillets were immersed in brine solution in different brine concentration 5, 10 or 15% at a ratio of 1:10 (w/w) for 30 min.

Brined fish were laid in trays and transferred to the smoking kiln (MK2, AFOS) for smoking using Mendi (local name) wood. Samples were smoked at 50 °C for 3 h followed by 1 h at 60 °C and 3 h at 105 °C. The smoked fish were then dried for 14 h by turning the heater off for an additional 14 h with the fan motor on, cooled at room temperature and stored at 4 °C. Experiments were performed in triplicates.

Lipid oxidation

Lipid oxidation was assessed by peroxide value (PV) and the thiobarbituric acid reactive substances (TBARS) value. The PVs were measured using the procedure developed by Egan et al. (1981). Smoked tuna was ground into a powder with a grinder and 0.5 g of the powder was mixed with 25 ml solution of acetic acid and chloroform (ratio 3:2) and 1 ml of saturated potassium iodide. The mixture was stored in the dark for about

10 min prior to the addition of 30 ml of distilled water and 1 ml of freshly prepared 1% starch (w/v) solution. After shaking, the sample was titrated with 0.01 N sodium thiosulfate until the blue color disappeared. The PV were expressed as milliequivalents of peroxide oxygen per kg of sample (mEq/kg).

Thiobarbituric acid reactive substances (TBARS) in fresh and smoked tuna were determined by the extraction method of Sørensen and Jørgensen (1996). Absorbance was measured at 532 nm by an Ultraspec 30009 against a blank containing 5 mL of distilled water and 5 mL of thiobarbituric acid reagent. Thiobarbituric acid reactive substances, expressed as milligrams of malondialdehyde per kilogram of meat, were calculated from the standard curve of TEP (1,1,3,3-tetraethoxypropane).

Fatty acids analysis

The fatty acid profile of smoked tuna was determined according to the procedure described in AOAC (1990). The GC-MS analyses of the methyl esters were performed on a Hewlett Packard 5890 GC equipped with an 30m_0.32 mm OmegaWax1 (Supelco, Inc. USA) fused-capillary column and a HP5989B mass detector. Individual FA were identified by comparing their retention time with commercially available mixtures of saturated fatty acids, and polyunsaturated fatty acids from a marine source (Supelco Inc., USA) and confirmed by NIST98 mass spectral computer library. The FA were quantified (mg g^{-1} fresh weight) using heneicosanoic acid (C19) as internal standard.

Statistical analysis

All data were expressed as means $\pm$ standard deviation of three experiments. Duncan's test was used to determine the difference of means and level of $P < 0.05$ was considered to be statistically significant (SAS 2001).

Results and discussion

Lipid oxidation

Lipid deterioration is the main cause of low shelf life of fatty fish due to progress in oxidation and enzymatic hydrolysis of unsaturated fatty acids in fish. The stability of lipids in smoked tuna during storage at 4 °C was evaluated by following the development of lipid oxidation by assessing peroxide value and the TBARS values. Figure 1a shows that there is a trend towards an increase in PV up to a certain point during the storage period regardless of salt concentration; followed

by either, a decrease in these values or remained nearly same. Results showed also a general increase with storage time up to day 27 days of storage followed by a decrease thereafter for all samples except for the control. Similar lipid oxidation trend was observed by Rahman et al. (2009) for freeze-dried grouper during storage at different temperatures and moisture contents, and by Atayeter and Ercoşkun (2011) who reported that PV and TBARS values increased with storage time and higher storage temperatures for European squid. The PV showed an inverse relationship with salt concentration. Samples treated with 10% salt had significantly higher peroxide value than those treated with 5 and 15% salt. It seems that salt at level of 10% accelerates lipid oxidation and has a protective effect at higher concentrations. Rhee and Ziprin (2001) claimed that the addition of NaCl at 0.5–2.5% to be prooxidative and therefore to promote lipid oxidation. Salt has an effect on the water activity of foods, it generally lowers it. The autoxidation of acyl lipids is also influenced by water activity (aw) of food. The reaction rate is high for both dehydrated and water-

Fig. 1 PV and TBARS of smoked tuna brined in different salt concentrations. Results are the mean $\pm$ SD, $n=3$

containing food, but is minimal as the water activity is lowered towards the monolayer moisture. Salt has a strong effect on the adsorption of water by protein molecules. One of the factors determining the behavior of the fish fillets in different salting media is the NaCl interaction with the protein matrix (Offer and Trinick 1983). The state of the proteins in the fish muscle has been found to be mainly related to the salt concentration of its water phase (Barat et al. 2002). At low salt concentrations, maximum muscle swelling occurs as a result of high water-protein interaction (maximum water-holding capacity). At higher salt concentrations, the proteins may have strong protein-protein bonds, resulting in dehydration (Gallart-Jornet et al. 2007). It is worth noting that the PV in all smoked tuna samples treated with 15% NaCl were kept below 20–40 meq/kg during the whole storage time, which is normally considered for a noticeable rancid taste in oil (Egan et al. 1981).

The formation of TBARS followed a similar trend as PV. Figure 1b shows that TBARS increased after 27 days storage time regardless of salt concentration. However there was a significant ($p < 0.05$) differences between samples treated with different salt concentrations. Highest TBARS values were obtained for samples treated with 10% salt concentration followed by 5% salt. The TBARS values reached 1.012 and 1.953 malonaldehyde/kg samples after 27 days of storage for the 5 and 10% salt-treated samples respectively. In contrast the TBARS value of the untreated samples and of the 15% samples reached maximum value of 0.952 and 0.397 mg malonaldehyde/kg sample, respectively which are below the values of 1–2 mg DMA/kg usually regarded as the limit for the development of an objectionable odour/taste (Connell 1990). These results confirm those obtained for the PV. Lower salt concentrations promote lipid autoxidation. The increase in TBA value during storage may be attributed to the partial dehydration of fish and to the increase of oxidation of unsaturated fatty acids as a result of smoking at relatively high temperature. This observation is in agreement with results by Goktepe and Moody (1998a, b) who observed two fold increase in TBA value of raw catfish after smoking (smoke temperature up to 82 °C) and by Goulas and Kontominas (2005) for chub mackerel (smoked temperature up to 70 °C).

TBARS values decreased for all samples after the 27 days refrigerated storage. This observation is in agreement with results reported by other authors (Curzio and Quaranta 1982; Fernandez et al. 1997; Gomes et al. 2003; Goulas and Kontominas 2005). The decrease of TBA value after 27 days of storage could be attributed to the interaction between MDA (the end product of lipid oxidation) and proteins, amino acids, glycogen, etc. (Fernandez et al. 1997; Gomes et al. 2003).

Fatty acids profile

The fatty acids profile was analyzed in the fresh tuna samples and smoked tuna at different brine concentrations. Results are presented in Table 1. The concentration of total saturated, monounsaturated and polyunsaturated fatty acids are in the range of those reported by Siriamornpun et al. (2008) for canned tuna. From the GC-MS results for fatty acids, it is obvious that the fatty acid profile exhibited changes after smoking irrespective of the salt concentration. The total saturated fatty acids content of raw tuna was 23.47% with palmitic (C16:0) and stearic acid (C18:0) being the main fatty acids comprising 53% and 32% of the total SFA respectively. The total SFA increased after smoking in particular the palmitic and stearic acids, with the greatest increase ($p > 0.05$) being in the 15% NaCl-treated samples. The total monounsaturated fatty acids content was 13.99% with oleic acid (C18:1 n-9) being the main monounsaturated fatty acid in raw tuna, contributing 68% of “MUFA”. The MUFA increased significantly ($p > 0.05$) in smoked brined in 5% salt but did not change for smoked brined in 10 and 15% salt. Oleic acid content increased in samples brined in 5 and 15% salt but did not change in samples brined in 10% salt. The total PUFA content of raw tuna fish was 13.77% of the total fatty acids, with DHA (C22:6n3) and EPA (C20:5n3) contributing 72% and 9% of the total PUFA respectively. The content of PUFA, EPA and DHA decreased after smoking for all samples regardless of the brine concentration. The loss of health beneficial ω -3 fatty acids, EPA and DHA was reported in skipjack tuna subjected to different types of heat processing (Stephen et al. 2010). The losses were higher with canning followed by frying, while cooking and microwaving heating was found to induce minimum losses.

Conclusions

The salt concentration had effect on the increase of lipid oxidation (PV- values and TBARS). The effect was inversely proportional to salt concentration; the highest PV and TBARS values were obtained in the 10% NaCl-treated samples during the 27 days of storage. Smoking but not salt had an effect on the fatty acids of tuna samples. Saturated fatty acids significantly increased whereas the unsaturated fatty acids significantly decreased after smoking regardless the salt concentration. The mono unsaturated fatty acids showed slight or no changes after smoking. Only the 5% NaCl-treated samples exhibited a significant increase in their total MUFA.

Table 1 Fatty acids concentration (mg/100 g dry matter) of smoked tuna brined in different salt concentrations

Fatty acids	Fresh tuna	Smoked tuna		
		5% brine	10% brine	15% brine
C8:0	ND	0.08±0.02	0.07±0.03	0.05±0.04
C10:0	ND	0.03±0.03	0.06±0.04	0.05±0.05
C12:0	0.17±0.04	0.11±0.02	0.18±0.05	0.14±0.03
C13:0	0.05±0.02	0.13±0.02	0.12±0.08	0.06±0.09
C14:0	0.14±0.15	0.46±0.05	0.49±0.04	0.46±0.07
C15:0	0.20±0.05	0.36±0.04	0.34±0.04	0.30±0.02
C16:0	12.50±1.58	16.28±1.94	15.62±1.96	14.75±0.96
C17:0	1.33±0.17	1.30±0.08	1.46±0.18	1.25±0.13
C18:0	7.53±0.58	9.77±1.12	10.10±1.15	9.30±0.48
C20:0	0.64±0.21	0.09±0.04	0.10±0.18	0.11±0.16
C21:0	0.19±0.06	0.22±0.02	0.31±0.06	ND
C22:0	0.31±0.01	0.04±0.01	0.08±0.09	0.03±0.03
C23:0	0.23±0.02	0.06±0.07	0.01±0.00	0.04±0.01
C24:0	0.18±0.01	0.03±0.01	0.08±0.07	0.02±0.01
SSFA	23.47±1.71 ^a	28.98±0.59 ^c	29.02±1.81 ^c	26.57±1.56 ^b
C14:1	0.04±0.036	0.04±0.00	0.04±0.02	0.02±0.02
C16:1	1.74±.23	1.57±0.17	1.48±0.16	1.27±0.13
C17:1	0.34±0.05	0.60±0.07	0.65±0.06	0.60±0.04
C18:1 n-9t	0.38±0.017	0.12±0.02	0.23±0.12	0.10±0.02
C18:1 n-9c	9.51±0.78	11.78±0.87	9.58±0.73	10.44±0.91
C20:1	0.19±0.06	0.22±0.02	0.31±0.06	0.22±0.01
C22:1	0.05±0.01	0.03±0.01	0.05±0.01	0.01±0.02
C24:1	0.76±0.20	1.28±0.05	1.35±0.13	1.25±0.10
SMUFA	13.99±1.05 ^a	15.64±0.88 ^b	13.68±1.03 ^a	13.91±1.13 ^a
C18:2n-6t	0.24±0.04	0.34±0.03	0.38±0.06	0.34±0.01
C18:2n-6c	2.19±0.08	1.45±0.07	1.30±0.14	1.11±0.08
C18:3n6	0.03±0.02	0.03±0.00	0.04±0.00	0.06±0.06
C18:3n3	0.78±0.03	0.24±0.02	0.27±0.01	0.19±0.01
C20:3n6	0.60±0.02	0.19±0.01	0.34±0.08	0.24±0.03
C20:3n3	6.09±0.29	5.30±0.37	4.67±0.55	5.13±0.39
C20:5n3	4.91±0.38	4.15±0.30	4.04±0.64	4.60±0.42
C22:6n3	38.83±2.94	36.79±2.09	36.26±7.15	35.55±3.33
SPUFA	53.77±1.55 ^a	48.47±1.38 ^b	47.28±1.79 ^b	47.22±1.51 ^b

Means in the same row with the same superscript are not significantly ($p<0.05$) different, $n=3$

SSFA Sum of saturated fatty Acids; *SMUFA* Sum of monounsaturated fatty acids; *SPUFA* Sum of polyunsaturated fatty acids; *ND* not detected; C8:0, caprylic acid; C10:0, capric acid; C12:0, lauric acid; C13:0, tridecyl acid; C14:0, myristic acid; C15:0, pentadecyl acid; C16:0, palmitic acid; C17:0, margaric acid; C18:0, stearic acid; C20:0, arachidic acid; C21:0, heneicosyl acid; C22:0, behenic acid; C23:0, tricosyl acid; C24:0, lignoceric acid; C14:1, myristoleic acid; C16:1, palmitoleic acid; C18:1n-9c, oleic acid; C18:1n-9t, elaidic acid; C20:1, Eicosenoic Acid; C22:1, Erucic acid; C24:1 nervonic acid; C18:2n-6c, linoleic acid; C18:2n-6t, linoelaidic acid; C18:2n6c Linoleic acid; C18:3n6 γ -Linolenic acid; C18:3n3, α -Linolenic acid; C20:3n3, eicosatrienoic acid; 20:3 n6, dihomo- γ -linolenic acid; C20:5n3, eicosapentaenoic acid; C22:6 n-3, Docosahexaenoic acid

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