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The Effects of the Different Woods on Hot-Smoking Vacuum Packed Atlantic Bonito (*Sarda sarda*) Stored at 4°C

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Abstract: The effect of 2 kinds of wood on quality of hot smoking vacuum-packed Atlantic bonito (*Sarda sarda*) was investigated during storage at 4°C. The quality changes of samples during storage were evaluated by pH, TVB-N, TMA, TBA and sensory analyses. The contamination of Benzo (a) Pyrene (B[a]P) in smoked fish samples were also analyzed. B[a]P was found 0.220±0.35 ppb in samples smoking with mixed woods (Group A) and 0.088±0.21 ppb in samples smoking with apple wood (Group B). Significant differences were observed between group A and B in terms of pH and TVB-N ($p<0.05$). The TMA and TBA values weren't significantly different at the end of the storage period. A significant decrease was found in sensory scores of the samples for Group A and Group B after 60 days of storage. There were significant difference between the groups for colour, odour and taste at the end of storage period ($p<0.05$).

Key words: *Sarda sarda*, smoking, vacuum-packed, quality indicators, B[a]P, self life

INTRODUCTION

Preservation of fish by exposure to wood smoke is one of the oldest methods. Originally the purpose was to inhibit growth of meat spoiling bacteria and improve the preservative qualities of products. However, smoking is now primarily used to impart the desirable colour and flavour of smoked food rather than as a preservative. The main chemical component found in wood smoke are phenols, organic acids, alcohols, carbonyls, hydrocarbons and some gaseous components such as carbon dioxide, carbon monoxide, oxygen, nitrogen and nitrous oxide. Phenolic compounds are responsible for imparting microbiological and oxidative stability and desirable organoleptic properties of the smoked fish products (Kjallstrand and Petersson, 2001). The nature of the wood used in the smoking process can effect the relative concentration of phenolic compounds in these products (Guillen and Ibargoitia, 1998; Guillen and Marzanos, 1997).

The most common traditional smoking process is hot smoking and still widely used in fish processing. One of the major problems with this traditional smoking is the occurrence of polycyclic aromatic hydrocarbons, which are a class of ubiquitous chemical compounds, known to be carcinogenic, originating from incomplete combustion processes which occur whenever wood, coal or oil are burnt (Vazquez Troche *et al*., 2000; Kishikawa *et al*., 2003; Lage Yusty and Cortizo Daviña, 2005; Okuda *et al*., 2006; Tfouni *et al*., 2007; Philips, 1999; Stolyhwo and Sikorski, 2005). According to The Scientific Committee on Food (2002), a number of heavy PAHs are carcinogens (genotoxic) and benzo[a]pyrene can be used as a marker for the occurrence and effect of these carcinogenic. As a marker of PAHs, its maximum permissible limit has been also set by other countries e.g. Germany, Austria, Czech Republic, Switzerland, Italy and the Slovak Republic to a level of 1 ppb (Simko, 2002; Stolyhwo and Skorski, 2005). European Commission suggests that the B[a]P value should be between 2 and 5 ppb for smoked fish. However, there is an argument between the member countries about its value since some member of European countries accepts the lower limit than 2 ppb. Thus there is no agreement for B[a]P value (Anonymous, 2004).

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Atlantic bonito (*Sarda sarda*) is an economically important commercial species in Turkey with 6000 ton production per year (DIE, 2003) and are generally sold fresh or frozen or are processed by curing or smoking.

The aim of the present study was to determine the effects of different woods on chemical and sensory quality of hot-smoking vacuum packed Atlantic bonito stored at 4°C.

MATERIALS AND METHODS

Sample preparation: Atlantic bonito (*Sarda sarda*) were caught from the Black Sea Region, Sinop, Turkey. After harvest, the fish were immediately placed into boxes and covered with ice. Having been transported to the laboratory, the bonitos were slaughtered, gutted and washed. The mean length and weight of fish sample were 29.06±0.77 cm and 248.50±5.26 g, respectively.

Smoking process: Cleaned bonitos were immersed in brine at a ratio of 1:1 (w/w) containing 20% NaCl for 1 h. Brined fish were then laid in trays to drain for 30 min. After draining, the fish were transferred to the smokehouse (AGK model, Germany). Samples were smoked at 40°C for 1 h and heated at 80°C for 1 h to an internal temperature of average 76°C at the end of the process. Temperatures were recorded before and after smoking using with digital thermometer (model WT-2). In order to compare the effects of the different woods, the production of smoke was performed by 2 kinds of wood sawdust; mixed woods (oak-beech-poplar) called as Group A and apple woods called as Group B. The smoked product, chilled 4 h at 15°C was vacuum-packed into bags consisting of 70% polystyrene and 30% polyamide. After packing, samples were immediately stored at 4°C. After 0, 1, 15, 30, 45, 50, 55 and 60 days of storage, analyses of chemical and sensory quality parameters were carry out. At each sampling, three fish was used for each group. All chemical analyses were performed in triplicate.

Chemical analysis: For pH measurement (Orion research Model pH meter), 10 g *bonito* were homogenised (Ultraturax T25 IKA, 7500 rpm) in 10 mL of distilled water solution (Curran *et al*., 1980). Total Volatile Bases (TVB-N), Trimethylamine (TMA) and Thiobarbituric Acid (TBA) were analyzed according to Botta *et al*. (1984), AOAC (1990) and Tarladgis *et al*. (1960), respectively. Dry matter, crude ash and crude protein were determined using the method of AOAC (1980). Lipid was performed according to the method of Bligh and Dyer (1959). B[a]P analysis was carried out according to method of Willett *et al*. (1995).

Sensory evaluation: Sensory quality of smoked bonito fillet was evaluated at each sampling time by using 8th semi-trained panellists members of the academic staff (Amerina *et al*., 1965). Before sensory assessment, samples were removed from the refrigerator and held for 30 min at room temperature. Fish samples were evaluated for overall acceptability with regarding to colour, texture, taste and odour. A hedonic scale was used with numerical scores from 1-10. A score of 7-10 indicated ''very good'' quality, a score of 4.0-6.9 showed ''good'' quality, a score of 1.0-3.9 denoted as spoiled.

Statistical methods: Data were analyzed by one-way Analysis of Variance (ANOVA), using the SPSS 10.0 for Windows. Significance of differences was defined at $p \leq 0.05$. Statistical comparison was based on 3 samples for each treatment for each specific storage time.

RESULTS AND DISCUSSION

Meat yield of Atlantic bonito used in the experiment was found to be higher than other species (average 86%, before smoking process. Table 1 shows meat yield of group A and B were 76.17±0.61 and 70.09±1.59%, respectively after smoking of the bonito. Group A meat yield higher than group B ($p<0.05$). Although, in this study the mean raw meat yield was found 86.32%, Samsun *et al*. (2004) reported that the mean raw meat yield was 74.4%. Erkoyuncu *et al*. (1994) also reported that Atlantic bonito was the highest meat yield within 15 commercial fish species which are caught in Black Sea.

The concentrations of benzo(a) pyrene in Atlantic bonito from traditional hot-smoking with different kind of woods are shown in Table 1.

In this study, B[a]P content was found to be 0.220±0.35 ppb for group A and 0.088±0.21 ppb for group B (Table 1). The B[a]P contents in examined fish samples were below the maximum limit levels of 1 ppb by Turkish Food Codex Regulations. Smoked fish normally contains less than 1 ppb of B[a]P (0.1- 0.5 ppb), but this value can be exceeded in heavily smoked products (Larsen and Poulsen, 1987). Steining and Meyer (1976) found benzo[a]pyrene's content of hot-smoked mackerel in range 0.5-2.4 ppb. Yurchenko and Mölder (2005) showed that the B[a]P concentration in 20 of 84 samples of commercially hot-smoked fish varied from

Table 1: The mean meat yields and B[a]P for group A and B

Groups	Fresh yield (%)	Processed yield (%)	Within temperatures (°C)	B[a]P (ppb)
A	86.48±0.38a	76.17±0.61a	76.17±0.40a	0.220±0.35a
B	86.17±0.34a	75.09±1.59a	77.0±0.71a	0.088±0.21b

a, b: Different letters in mean values are significantly different ($p<0.05$)

Table 2: The effect of different woods on chemical quality parameters of hot smoking vacuum packed atlantic bonito

	Crude ash (%)		Crude protein (%)		Crude lipid (%)		Dry matter (%)	
Days	A	B	A	B	A	B	A	B
0	1.17±0.00a	1.19±0.03a	20.35±0.02a	20.20±0.24a	8.76±0.04c	8.46±0.06c	30.42±0.20a	30.03±0.14a
1	0.99±0.18a*	2.18±0.10b	22.88±0.16b	23.30±0.33b	10.13±0.06d	10.61±0.20e	35.60±0.18b*	36.94±0.04b
15	2.00±0.10d	2.43±0.06ab	23.93±0.08c*	27.61±0.10c	10.26±0.02d*	6.50±0.02c	38.01±0.49d*	36.59±0.29b
30	2.37±0.09e	2.19±0.02ab	24.33±0.07c*	29.68±0.07e	10.30±0.03d*	5.21±0.05b	36.33±0.11bc	36.88±0.21b
45	1.97±0.08d	2.29±0.04cb	27.99±0.06d*	30.53±0.42f	6.07±0.03a*	4.48±0.03a	36.62±0.07c*	37.49±0.23c
50	1.63±0.06c*	2.39±0.09db	28.32±0.14de*	29.27±0.17e	7.31±0.08b*	6.31±0.02c	38.47±0.17d	37.98±0.12c
55	1.58±0.06bc*	2.47±0.016eb	28.52±0.12e*	29.75±0.02e	8.75±0.09c*	4.56±0.04a	39.13±0.28e*	36.86±0.23b
60	1.53±0.16b*	2.87±0.16fb	28.65±0.023e	28.05+0.04d	8.89±0.03c*	7.37±0.11d	39.18±0.05e*	36.88±0.27b

a,b,c, Different letters in mean values for each group are significantly different ($p<0.05$), *: Mean values between groups in rows are significantly different ($p<0.05$)

Table 3: The chemical analysis of hot-smoked vacuum-packed bonito

	pH		TVB-N (mg/100 g)		TMA (mg/100 g)		TBA (mg malonaldehit/kg)	
Days	A	B	A	B	A	B	A	B
0	5.60±0.01a	5.59±0.02a	11.67±0.47a*	11.96±0.56a	0.88±0.05a	0.85±0.02a	0.64±0.04a	0.70±0.03a
1	5.72±0.02a	5.70±0.01a	13.87±0.64a*	20.07±0.93b	1.89±0.37b	1.17±0.03b	1.66±0.07b*	3.59±0.29b
15	5.92±0.01c*	5.74±0.02b	20.53±2.03b*	27.07±0.47c	3.12±0.14c*	4.60±0.13c	2.39±0.14c*	6.32±0.23c
30	6.07±0.01b	5.69±0.04ab	21.13±0.93b*	29.60±0.64c	3.34±0.03c*	4.46±0.05c	3.90±0.16c*	6.71±0.11dc
45	6.17±0.02b*	5.70±0.02ab	26.74±0.89c*	32.67±0.47d	4.1±0.04d*	6.31±0.23d	4.02±0.06d*	7.10±0.12ce
50	6.46±0.04d*	5.71±0.06ab	29.34±0.27c	30.40±0.90c	6.79±0.11e	6.58±0.14d	5.72±0.07e*	7.38±0.05de
55	6.69±0.06e	6.70±0.03c	33.32±0.64d	34.0±0.27e	7.25±0.09f*	7.86±0.05e	7.20±0.09f*	7.79±0.04ge
60	6.84±0.02f	6.854±0.02d	39.67±0.93d	37.23±0.43f	8.22±0.07f	8.47±0.06f	8.32±0.21g	8.47±0.18g

a,b,c, Different letters in mean values for each group are significantly different ($p<0.05$), * :Mean values between groups in rows are significantly different ($p<0.05$)

0.33-1.09 μg kg^{-1}. Zabik *et al.* (1996) found that benzo[a] pyrene's content in hot smoked lean and fat trout fillet ranged from 5.12 and 8.43 ppb B[a]P, respectively. The concentration of PAHs can vary in considerable range, especially depending on several variables like the properties of the fish, method and parameters of smoking, degree of smoking, composition of the smoke and exposure of the edible parts to the smoke (Lawrence and Weber, 1984; Stolyhwo and Sikorski, 2005; Yurchenko and Mölder, 2005). Statistical analysis indicated that there was a significant difference in B[a]P content between Group A and B ($p<0.05$). Results obtained from this study proved that the kind of wood used can effect the B[a]P concentrations in hot-smoked fish. Similar statement were revealed by Stolyhwo and Sikorski (2005), in which most of the PAHs content in smoked foods come from the wood smoke.

Proximate composition: The chemical composition of fresh and smoked bonito for Group A was shown in (Table 2). Crude ash, crude protein, crude lipid and dry matter were 1.17, 20.35, 8.76 and 30.42% at beginning of the study and 1.53, 28.65, 8.89 and 39.18% at the end of study, respectively. At the beginning of the storage period, the proximate composition of Group B was found to be as 1.59% for crude ash, 20.20% for crude protein, 8.46% for lipid and 30.03% for dry matter, whereas these values were found to be as 2.87% for crude ash, 28.05% for crude protein, 7.37% for lipid and 36.88% for dry matter in Group B at the end of the storage. There were significant differences between the amount of crude ash in Group A ($p<0.05$), while there were no significant differences between the amount of crude ash in Group B during the storage periods ($p>0.05$). The amount of crude protein significantly increased in both groups for the duration of storage ($p<0.05$). The lipid content of Group A did not change significantly ($p<0.05$), while the lipid content of Group B decreased significantly at the end of storage period ($p>0.05$). Similarly, Gomez-Guillen *et al.* (2000) reported that the percentage of dry matter, crude protein and crude ash in brining and smoking fish significantly increased during storage period ($p<0.05$). This might be explain the present result in which an increase of the amount of dry matter, crude protein and ash content is accompanied by smoking.

Chemical analysis: The effect of different woods on TVB-N, TMA-N, TBA and pH values in fresh and hot smoking vacuum packed bonito stored at 4°C up to 60 days is given in Table 3.

The initial pH values significantly increased from 5.60±0.01 to 6.84±0.02 in Group A and from 5.59±0.02 to 6.854±0.02 in group B ($p<0.05$) at the end of the storage period (Table 2). This pH value is in agreement with that of Kolodziejska *et al.* (2002), who reported a pH values in hot smoked mackerel ranged from 6.13+0.05 to 6.22+0.07 during the 21 days of storage (Table 3).

Table 4: The sensory scores of smoked atlantic bonito in vacuum- packing at 4°C

	Colour		Texture		Odour		Taste-flavour	
Days	A	B	A	B	A	B	A	B
0	10.0±0.0e	10.0±0.0e	10.0±0.0g	10.0±0.0e	10.0±0.0f	10.0±0.0f	-	-
1	10.0±0.0e	10.0±0.0e	9.6±0.25f	10.0±0.0e	9.8±0.2f	9.2±0.2e	9.6±0.25e	9.8±0.20e
15	9.00±0.32d	9.2±0.2d	7.8±0.58e	8.6±0.4d	7.6±0.21e*	6.2±0.49d	7.2±0.58d	7.6±0.40d
30	9.00±0.32d*	7.8±0.2c	7.2±0.20de	7.6±0.6c	6.6±0.40d*	5.6±0.40cd	6.4±0.25c*	7.4±0.51d
45	8.4±0.25cd	7.4±0.25bc	6.6±0.25cd	7.2±0.2c	6.2±0.20cd	5.8±0.58c	6.4±0.40c	6.0±0.63c
50	7.6±0.25bc	6.8±0.37b	6.0±0.32bc	5.8±0.2b	5.8±0.20bc	5.6±0.40bc	6.0±0.0bc*	5.0±0.0b
55	7.2±0.20b*	5.0±0.32a	5.6±0.25b	5.8±0.37b	5.2±0.20b	5.0±0.32b	5.6±0.25b	5.2±0.2b
60	5.6±0.25a*	4.4±0.25a	4.6±0.25a	3.4±0.4a	4.0±032a*	3.0±0.45a	4.4±0.25a*	3.2±0.37a

a,b,c, Different letters in mean values are significantly different ($p<0.05$), *: Mean values in rows are significantly different ($p<0.05$)

The initial TVB-N values (11.67±0.47 mg/100 g for group A and 11.96±0.56 mg/100 g for group B) are indicative of freshness of raw fish material. Similar results were revealed in research conducted by Goulas and Kontominas (2005), in which the initial TVBN content in raw bonito was 10.93 mg N/100 g and by Kaya *et al.* (2006) in fresh bonito was 10.44 mg N/100 g. As results show, these values increased to 39.67±0.93 mg/100 for Group A and 37.23±0.43 mg/100 for Group B at end of storage period (60 days) (Table 2). Similarly, the increase in hot-smoked bonito with time of storage was also found by Kaya *et al.* (2006). The kind of wood used in this research did not affect the TVB-N values of smoked bonito at the end of the storage period. The TVB-N content of smoked samples (Group A and Group B) exceeded the above limit of 35 mg N/100 g after approximately 60 days of storage fish set by the EU (EEC, 1995). The increase in TVB-N might be caused by microbiological contamination and autolytic decomposition of amino acids and the complete microbial reduction of TMAO to TMA (Truelstrup *et al.*, 1996).

The TMA values significantly increased from 0.88±0.05 to 8.22 mg/100 g in the samples of Group A and from 0.85±0.02 to 8.47 mg/100 g in the samples of Group B for the duration of storage ($p<0.05$). The TMA values of all investigated bonito were lower than the legal limit set by the EU for fish (12 mg/100 g N) after 60 days of storage. Similar results were revealed in research conducted by Kaya *et al.* (2006). It has been shown that the TMA-N levels produced by bacterial action rather than the action of intrinsic enzymes can change depending on species, age, time of year, muscle type and diet of fish (Connell, 1990; Reddy *et al.*, 1994; Leroi *et al.*, 1998; Dondero *et al.*, 2004).

The TBA values as an indicator of the degree of lipid oxidation in fresh fish were 0.64±0.04 mg malonaldehyde/kg in group A and 0.70±0.03 mg malonaldehyde/kg in group B (Table 2). These values gradually increased up to 8.32±0.21 mg malonaldehid kg^{-1} in group A and 8.47±0.18 mg malonaldehyde kg^{-1} in Group B throughout the 60 days of storage ($p<0.05$) (Bhuiyan *et al.*, 1986; Goulas and Kontominas, 2005; Yanar *et al.*, 2006; Duman and Patır, 2007). Data show that the kind of wood did not affect retard of lipid oxidation during storage at 4°C.

Sensory evaluation: Results of the sensory assessment of hot-smoked Atlantic bonito stored at 4°C are shown in Table 4. The tendency decrease was obtained for the colour, texture, odour and taste of the fish. Autolytic enzymes can have a major impact on the loss of textural quality of hot-smoked bonito during the early stages of deterioration, but they did not produce the characteristic off-flavours and off-odours, which are typical of microbiological activity. There were significant difference between the groups for colour, odour and taste at the end of storage period ($p<0.05$). Shelf life was found to be 55 days for samples. According to panellist, sensory quality was higher in group A than in group B. Truelstrup *et al.* (1996) and Kolodziejska *et al.* (2002) reported that the shelf-life of processed seafood decrease during storage periods due to the microbial activity. In order to obtain high quality, storage temperature, packing methods and the kind of sawdust such as beech, oak and hornbeam are important factors for smoking preservation techniques.

CONCLUSION

Smoking of the bonito is one of the best processing techniques since the shelf-life of bonito was 55 days by using different kind of wood sawdust. According to Turkish Food Codex, the smoked fish was safe in terms of B[a]P value since its concentration was found low (1 ppb). Consequently the shelf-life of fish was increased by hot smoking and vacuum packing. In addition to this the smoked fish with mixed sawdust were more preferable than apple sawdust by panellists.

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