

Full Length Research Paper

Stability study of smoked fish, horse mackerel (*Trachurus trachurus*) by different methods and storage at room temperature

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The present study assessed the effect of hot smoke using wood and charcoal dry heat on keeping quality of *Trachurus trachurus* fish fillet, skin, head and bones (SHB). *T. trachurus* was smoked using the altona kiln at 80 to 105°C for eight hours, placed in cain woven baskets to cool off, packaged in sealed transparent polythene bags and left on shelf for 7 days at ambient temperature (32 ± 2°C). Biochemical indexes assayed were: pH, total volatile nitrogen (TVBN), peroxide value (PV) and total viable count (TVC). pH of raw *T. trachurus* fillet was 6.85 ± 0.00 but significantly reduced with wood smoke at day 0 to 6.70 ± 0.02, while TVBN in raw *T. trachurus* fillet was 27.17 ± 1.61 mgN/100 g. After smoking, TVBN of *T. trachurus* fillet at day 0 was 21.83 ± 0.38 mgN/100 g (wood) and 23.05 ± 1.36 mgN/100 g (charcoal). pH of raw *T. Trachurus* SHB was 5.87 ± 0.03 and increased (p<0.05) with smoking, that is, 6.43 ± 0.21 (wood) and 6.43 ± 0.00 (charcoal). TVBN in the raw *T. Trachurus* SHB was 28.12 ± 2.17 mgN/100 g, and after smoking on day 0, it was 31.90 ± 0.3 mgN/100 g (wood smoked) and 24.44 ± 1.90 mgN/100 g (coal smoked). The PV was highest in charcoal smoked SHB (CSHB) of *T. trachurus* at day 5 (108.40±36.80) and TVC was highest in the CSHB on day 5 (3.48 logcfu/g). The overall study showed that *T. trachurus* fish samples (fillet and SHB) prepared via charcoal smoking method had the lowest (p<0.05) storage time amongst the smoked fish samples, when compared with those that were processed via wood smoke.

Key words: *Trachurus trachurus*, ambient temperature, smoked fish and seafood quality.

INTRODUCTION

Fish is greatly perishable but very important food stuff, especially in third world countries, due to its high protein content and nutritional value of unsaturated fatty matter and affordability by the masses when compared with beef. One of such species is horse mackerel (*Trachurus trachurus*), a medium-fat species abundant in the north-east Atlantic (FAO, 1996; Olatunde, 1998; FAO, 1998?; Zimmermann and Hammer, 1999).

The rate of fish spoilage depends on handling during processing, acidity level, species of fish, weather, mode

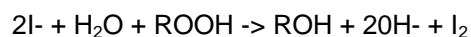
of storage and temperature during transportation (Oladusun et al., 1996). Chemical breakdown of protein, fat and water contents contribute to quick spoilage of fish. Quality losses might occur very rapidly after catch, especially in hot climates and tropical areas where cold preservation techniques are often missing. Saliu (2008) reported that fish spoilage in Nigeria is influenced to a large extent by high ambient temperatures, considerable distances of landing ports to points of utilization and poor as well as inadequate infrastructure for post-harvest

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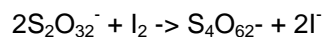
processing and landing (Saliu, 2008). Thus, traditional fish processing, such as salting/brining, drying and smoking, allow better preservation and storage and increase fish availability to the consumers (Egbal et al., 2010).

The processes of smoking and drying are the affordable and most widely used method for fish preservation in Nigeria (Food Inc, 2009) that dates back to civilization (Oladosun et al., 1996), it also aimed at preventing or reducing post-harvest losses (Govindan, 1985; Shepherd, 2000). Smoking involves the application of wood smoke to impart a smoky or smoked flavour and to partially dry a fish, or part of fish such as fillets, to produce a smoked fish product and also to extend the shelf life of the product under some conditions. In many parts of the world, preservation is still the main purpose of smoking. Components in the fuel (wood/charcoal) via pyrolysis are broken down in the process of burning to form smoke, which imparts on the fish a unique aroma, improves taste and color of fish (Afolabi, 1984; Olley et al, 1988), largely due to the presence of a range of phenolic compounds, nitrites and formaldehyde present in the smoke (IMPPFA, 2010).

Keeping quality of raw and processed fish is done via measuring various parameters in the fish samples like, pH, total viable count (TVC) and peroxide value (PV). Peroxide value is a major indicator of fat oxidation (rancidity), which is brought about by the action of air (oxidative rancidity) or by microorganisms (ketonic rancidity) in oil. In oxidative rancidity, oxygen is taken up by the fat with the formation of peroxides. The degree of peroxide formation and the time taken for the development of rancidity differ among oils (Cox and Pearson, 1962). The peroxide value measures the amount of iodine formed by the reaction of peroxides (formed in fat or oil) with iodide ion.



The base produced in the above reaction was taken up by the excess of acetic acid present. The iodine liberated was titrated with sodium thiosulphate.



The acidic conditions (excess acetic acid) prevents formation of hypiodite (analogous to hypochlorite), which would interfere with the reaction. The indicator used in this reaction is a starch solution where amylose forms a blue to black solution with iodine and is colorless where iodine is titrated. A precaution that was observed was adding the starch indicator solution only near the end point (the end point is close to when fading of the yellowish iodine color occurs) because at high iodine concentration, starch is decomposed to products whose indicator properties are not entirely reversible.

The aim of this work therefore, was to determine the effect of wood (*Acacia seyal* and *Citrus lemon*) and charcoal smoke on the keeping quality of *Trachurus trachurus*;

also bearing in mind that the skin, head and bones (SHB) of this fish which are most often thrown away during processing in developed countries, may contain vital nutritive values that are considered insignificant by majority of the people.

MATERIALS AND METHODS

Collection of samples

Sample preparation and processing

A total of 20 kg (approximately 100 fish) of horse mackerel was purchased from two popular major cold fish distributors (Asake and Heritage fisheries) in Ipata market, Ilorin, Nigeria. The mean length and weight of the fish was 30.52 ± 0.22 cm and 197.66 ± 3.67 g, respectively. *T. trachurus* was prepared using handling process that is, thoroughly washed, eviscerated and cooked by poaching and smoking using firewood (*A. seyal* and *C. lemon*) and charcoal. The processing methods were grouped into four (WSK: wood smoked kote; CSK: charcoal smoked kote; SK: poached kote; RK: raw kote).

Processing and packaging of samples

A portion of the fish was poached in water at 60°C for 15 min and the remaining portion was hot smoked using either charcoal or firewood in a conventional smoke kiln as described by FAO/WHO/UN (2007). The fish smoking kiln was operated by first loading firewood into the heat chamber, preheating for 20 min and closed for 30 min to allow the smoking to take place after which fish samples were loaded into the central chamber. Fish was smoked at 80°C for 4 h; temperature was later increased to 105°C for 2 h and then returned to 80°C until the fish was properly smoked. The smoking time, temperature and ambient conditions were monitored using a thermometer during the smoking operation. Smoking was terminated when fish was properly dried after 8 h. The smoked fish were placed in cane woven baskets to cool off, after which portions of the processed fish were packaged in transparent polythene bags and kept in perforated plastic containers for further analysis. Fresh and smoked samples were analyzed for fat, protein, moisture content, ash and crude fiber contents.

Analytical method

All cooking processes were done without addition of any ingredient. After the poaching and smoke processes, a known portion of each fish species and was oven dried to constant weight at 60°C, and the flesh of each fish was then separated from its bones, skin and head. The skin, head and bones were collectively homogenized while the fillet alone was homogenized using a kitchen blender and analyzed to determine the proximate composition in each of the fish samples on dry matter basis. The crude fiber was analyzed according to the method of Antia et al. (2006) whereas moisture, fat, ash and protein of the fish samples were determined following the method described by AOAC (2002). Five grams of dry ground sample was digested in 100 ml of 1.25% H_2SO_4 for 30 min. The digested sample was cooled and filtered and the residue was collected into a beaker and further digested with 100 ml of 1.25% NaOH. The residue was collected after filtration and oven dried at 100°C to constant weight. Dried residue was incinerated in the muffle furnace at 550°C for 5 h. Crude fiber value was obtained from loss in weight of the fish samples on ignition of dried residue remaining after digestion of free fat samples. Percentage crude

fiber = (loss of weight on ignition/weight of sample used) × 100%. Moisture content was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 105°C. Crude fat was obtained by exhaustively extracting 5.0 g of each sample in a Soxhlet apparatus using petroleum ether (40 to 60°C) as the extractant. Crude protein (percentage total nitrogen × 6.25) was determined by the Micro-Kjeldahl method of AOAC (1984) modified by Okalebo et al. (2002). Percentage nitrogen was calculated using the equation obtained from the calibration curve using various concentrations of the standards. Ash content was carried out by incinerating 1.0 g of the fish sample in a muffle furnace maintained at 550°C for 5 h. While known portions each of the raw, wood and charcoal smoked fish samples were stored at ambient temperature (32 ± 2°C) for 7 days and examined for changes in the mycoflora.

Keeping quality assessment of smoked fish samples

pH

Ten grams of *T. trachurus* raw and processed samples were homogenized in 50 ml of distilled water and the mixture was filtered using Whatman filter paper No.1. The pH of the filtrate was measured using a CRISON pH 25 at ambient temperature after calibration using standard buffers of pH 7 and 4 at 25°C. The pH meter was used to read measurements accurately to ±0.01 pH units (Goulas and Kontominas, 2005).

Total volatile basic nitrogen (TVBN)

Approximately ten grams sample of fish fillet and SHB were homogenized with 50 ml of distilled water. The mixture was centrifuged in Lab-line CF-622 centrifuge (Vadodara, Gujrat, India) at 400 rpm for 5 min and the supernatant was filtered through a Büchner funnel using Whatman No.1 filter paper. Two grams of MgO was added followed by one drop of antifoaming agent. A 250 ml Erlenmeyer flask containing 25 ml of 3% aqueous solution of boric acid, 0.04 ml of a mixture of methyl red and methylene blue indicators was used as the indicator. Distillation was continued until a final distillate volume of 125 ml was obtained. The distilled TVBN was titrated with an aqueous 0.1 N HCl solution. TVBN content was expressed as mgN/100 g of fish flesh (Goulas and Kontominas, 2005). Methyl red was prepared as the indicator by dissolving 0.1 g of methyl red in 300 ml of 95% ethyl alcohol, and made to 500 ml with distilled water.

Calculation:

$$\text{TVBN} = (V \times C \times 14 \times 100) / 10$$

Where V is the volume of hydrochloric acid added and its concentration (C); 10 represent the weight of the sample, while 14 = molecular weight of nitrogen.

Peroxide value

Lipid extract obtained from the crude fat was used for the determination of peroxide value using method of AOAC, (2000) as modified by Lee et al. (2002). A known weight of oil sample (1 g) was accurately weighed into 250 ml of chloroform (containing 0.01% butylated hydroxytoluene (BHT), and shaken for 30 s to dissolve the lipid. 50 ml of solvent mixture (glacial/acetic acid: chloroform 3:2 v/v) was then added and the flask was gently rotated to facilitate mixing. After which 1 ml of saturated potassium iodide was added, mixed and kept in a dark cupboard for 3 min. 100 cm³

of distilled water was added to the mixture and the liberated iodine was titrated with 0.05 M sodium thiosulphate solution using 2% freshly prepared starch as an indicator.

Calculation:

$$\text{Peroxide value } (\mu\text{eq peroxide/kg sample}) = \frac{S \times N \times 1000}{\text{Weight of sample (g)}}$$

Where S = ml Na₂S₂O₃ (test-blank) and N = normality of Na₂S₂O₃.

Total viable count

Total viable count was determined according to the method of Fawole and Oso (1995) as modified by Caglak et al. (2008) and Amusa (2001). Smoked fish products were packed in low-density polyethylene (LDPE) bags and stored at ambient room temperature of 30 ± 2°C, until microbial growths were observed on the fish products. 0.1 ml samples of serial dilutions (1:10, diluents water) of fish fillet and skin, head and bone (SHB) homogenates were aseptically plated on the surface of Potato Dextrose Agar (PDA) using pour plate technique described by Harrigan and MacCance (1976) in nutrient agar and incubated at 37°C for 24 h. Colonies were counted by making the colony on the opposite side of the plate on its position sides in the colonies counter apparatus. Then converted to log colony forming unit /g (log cfu / g).

Calculation:

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2) \times (d)]}$$

Where: N = number of colonies per ml or g of product; $\sum C$ = sum of all colonies on all plates counted; n₁ = number of plates in first dilution counted; n₂ = number of plates in second dilution counted; d = dilution from which the first counts were obtained

Statistical analysis

The data from all the analyses were collected and statistically analyzed and expressed as the mean ± SD (n = 3), the significant differences between means were compared for each group of rats using the least significant difference test after ANOVA for one-way classified data. SPSS 14.0 (2002) statistical tool was used to analyze data obtained.

Results were considered statistically significant at a level of p < 0.05, chosen as the minimum for significance with Duncan's multiple range test (Duncan, 1955). Values were expressed as mean ± standard error (s.e).

RESULT

Proximate analysis

The results of proximate analysis of processed fillet are presented in Table 1. The percentage moisture content of poached *kote* fillet (PKF) had the highest value followed by raw *kote* fillet (RKF) and the least was wood smoked *kote* fillet (WSKF). Among the processing methods, coal

Table 1. Proximate composition (%) of processed *T. trachurus* fillet.

Group (%)	RKF	PKF	CSKF	WSKF
Protein	65.65±1.01 ^d	66.68±1.19 ^b	66.80±0.54 ^c	66.24±2.70 ^a
Fat	3.32±0.01 ^c	3.46±0.01 ^c	4.06±0.00 ^b	8.87±0.01 ^a
Ash	8.60±0.20 ^a	4.93±0.68 ^c	4.40±0.81 ^c	6.50±0.06 ^b
Crude fibre	2.13±1.09 ^a	2.06±0.24 ^b	1.40±0.12 ^c	2.26±0.67 ^a
Moisture	73.56±0.01 ^b	74.02±0.02 ^a	69.78±0.01 ^c	52.76±0.02 ^d

Data = Mean ± SD, n=3. Values with different superscripts along a row are significantly different ($p < 0.05$). CSKF: charcoal smoked *kote* fillet; WSKF: wood smoked *kote* fillet; PKF: poached *kote* fillet; RKF: raw *kote* fillet.

Table 2. Proximate composition (%) of processed skin head bone (SHB)..

Group (%)	RKSHB	PKSHB	CKSHB	WKSCHB
Protein	58.15±3.77 ^b	54.48±7.05 ^b	55.78±6.71 ^a	55.80±3.50 ^c
Fat	5.67±0.01 ^b	7.28±0.02 ^a	6.08±0.01 ^b	7.45±0.02 ^a
Ash	22.00±1.02 ^b	26.20±1.80 ^a	17.73±0.24 ^d	19.40±1.80 ^c
Crude fibre	15.73±0.57 ^b	17.33±0.48 ^a	14.46±0.66 ^c	13.26±1.75 ^d
Moisture	73.56±0.01 ^b	74.03±0.10 ^a	69.77±0.00 ^c	52.76±0.04 ^d

Data = Mean ± SD, n = 3. Values with different superscripts along a row are significantly different ($p < 0.05$). CSSHB: charcoal smoked *kote* SHB; WSSHB: wood smoked *kote* SHB; PSHB: poached *kote* SHB; RSHB: raw *kote* SHB.

smoked *kote* fillet (CSKF) had the least crude fiber content, whereas others had comparable amount in comparison with the raw fish. The protein composition of raw fish fillet, in this study is similar to that observed by Simeonidou et al. (1998), for horse mackerel, Atlantic bonito, striped mullet, Nile Tilapia, Mediterranean hake and sardine. With respect to percentage crude protein content, WSKF had the highest value (6.80 ± 0.54), followed by PKF (6.68 ± 1.19) and CSKF (66.24 ± 2.70) as compared to RCF (65.65 ± 1.01). Highest fat concentration was recorded in WSKF (8.87 ± 0.01), while the least value was found in PKF (3.46 ± 0.01) when compared with the RCF (3.32 ± 0.01). Previous reports of McGill et al. (1974), Josephson and Lindsay (1989), Arannilewa et al. (2005), Saliu (2008) and Egbal et al. (2010) indicated that reduction in fat is associated with the oxidation of fat. This however was not the case in the present study, because all processing methods increased ($p < 0.05$) levels of fat in both the fillet and SHB samples, respectively.

The effect of processing on the proximate composition of *T. trachurus* (SHB) is depicted in Table 2. Data shows that raw SHB had the highest value of protein ($58.15 \pm 3.77\%$), and reduced ($p < 0.05$) with all processing methods when compared with raw SHB. All the processed fish SHB had significant amount of fat content but the highest value was obtained in the wood smoked ($7.45 \pm 0.02\%$) and poached SHB ($7.28 \pm 0.02\%$), while the least concentration were recorded in charcoal smoked SHB ($6.08 \pm 0.01\%$). The processing methods significantly ($p < 0.05$)

altered the moisture content of the fish with wood smoked SHB having the lowest ($p < 0.05$) value. Poaching of SHB recorded highest ash and crude fiber content, respectively. These findings emphasized that both SHB and fillet of *T. trachurus* had significant equal amount of protein content. Meanwhile, SHB from *T. trachurus* was found to have significantly higher ($p < 0.05$) values of fat, ash and crude fibre contents in SHB than the processed fillets.

Shelf life studies

pH

Changes in the pH of raw and smoked *T. trachurus* stored at ambient temperature ($32 \pm 2^\circ\text{C}$) for 7 days are shown in Tables 3 and 4, respectively. The pH of the raw *kote* fillet was similar to that reported by Erkan and Ozden (2008) who indicated that the normal pH of live fish muscle is close to the value 7. However, post mortem pH varies from 6.0 to 7.1 (Pacheco-Aguilar et al., 2000). Also pH reduced ($p < 0.05$) with processing and storage time in all the fillet samples, except in WSKF where no ($p > 0.05$) difference was observed when compared with the raw sample. In the SHB variety, pH significantly ($p < 0.05$) increased with both processing and storage time ($32 \pm 2^\circ\text{C}$) when compared with the raw fish sample on Day 0: RSHB (5.87), CSHB (6.29), WSHB (6.32); Day 5: CSHB (6.40), WSHB (6.28) and Day 7: CSHB (6.34).

Table 3. Changes in pH, TVBN and PV of raw and processed *T. trachurus* fillet at Day 0, 5 and 7.

Group	RKF (Day 0)	PKF (Day 0)	CSKF (Day 0)	WSKF (Day 0)	CSKF (Day 5)	WSKF (Day 5)	CSKF (Day 7)
PH	6.85±0.00 ^a	6.64±0.08 ^c	6.85±0.12 ^a	6.70±0.02 ^b	6.70±0.01 ^b	6.81±0.03 ^a	6.46±0.01 ^c
TVBN (mg/100g)	27.17±1.61 ^d	21.73±1.38 ^f	23.05±1.36 ^e	21.83±0.38 ^f	36.25±8.09 ^a	32.34±3.14 ^c	34.20±3.08 ^b
PV (meq/Kg)	23.76±1.16 ^e	11.32±4.98 ^f	28.38±1.35 ^d	11.32±4.98 ^f	96.03±5.11 ^a	72.93±0.40 ^b	52.40±6.80 ^c

*Values with different superscripts along a row are significantly different ($P < 0.05$). WSF: wood smoked *kote* fillet; CSKF: charcoal smoked *kote* fillet; PKF: poached *kote* fillet; RKF: raw *kote* fillet.

Table 4. Changes in pH, TVBN and PV of raw and processed *T. trachurus* SHB at day 0, 5 and 7.

Groups	RSHB (Day 0)	PSHB (Day 0)	CSHB (Day 0)	WSHB (Day 0)	CSHB (Day 5)	WSHB (Day 5)	CSHB (Day 7)
pH	5.87±0.03 ^d	5.79±0.08 ^d	6.43±0.00 ^a	6.43±0.21 ^a	6.41±0.00 ^a	6.28±0.01 ^b	6.34±0.01 ^b
TVBN (mg/100g)	28.12±2.17 ^e	25.22±1.43 ^f	24.44±1.90 ^f	31.90±0.37 ^d	34.50±9.92 ^c	52.37±04.73 ^a	42.37±5.68 ^b
PV (meq/Kg)	22.37±2.31 ^e	23.19±0.55 ^e	26.71±1.87 ^d	27.47±0.20 ^d	108.40±36.80 ^a	73.82±6.14 ^b	70.49±4.68 ^c

Values with different superscripts along a row are significantly different ($P < 0.05$). WSSHb: wood smoked *kote* SHB; CSSHB: charcoal smoked *kote* SHB; RSHB: poached *kote* SHB; PSHB: raw *kote* SHB.

Total volatile nitrogen (TVBN)

The results for total volatile basic nitrogen are represented in Tables 3 and 4, respectively. The TVBN values increased ($p < 0.05$) with storage time in both the fillet and SHB when compared with the raw fish samples on day 0. Results of current findings showed that on day 0, both fillet and SHB samples of raw and smoked fish were within the accepted TVBN limits for raw fish samples, because both fish varieties (fillet and SHB) had values less than 30 mgN/100 g (Daramola et al., 2007). Pearson (1982) and Connell (1995) reported and also recommended that the limit of acceptability of fish is 20 to 30 mgN/100 g, while Huss (1988) and Kirk and Sawyer (1991) suggested a value of 30 to 40 mgN/100 g as the upper limit. Although, the TVBN value for RKF on day 0 in this study was 27.17 mgN/100 g, which is within the accepted limit, value in the present investigation was higher ($P < 0.05$) than the report of Trinidad and Estrada (1986); who reported that TVBN value of *Heterotis niloticus* was 21.03 mgN/100 g in fillet in the 8th week. This could be attributed to the difference in the fish species and moisture content of the smoked fish relative to the surroundings (Daramola et al., 2007). The decreasing order of magnitude for TVBN in stored *T. trachurus* fillet was CSKF (day 5; 36.25 mgN/100 g) > CSKF (day 7; 34.20 mgN/100 g) > WSKF (day 5; 2.34 mgN/100 g) in the fillet, and CSHB (day 7; 42.37 mgN/100 g) > WSHB (day 5; 52.37 mgN/100 g) > CSHB (day 7; 42.37 mgN/100 g) in the SHB, respectively.

Peroxide value (PV)

The PV results are presented in Tables 3 and 4, respectively. Hydro-peroxide quantified by peroxide value

(PV) emerges as a result of the oxidation of fat and oil (Daramola et al., 2007). The slightly oxidized fat and oil having PV at levels of only 100 meq/kg are reported to be neurotoxic (Gotoh et al., 2006; Gotoh and Wada, 2006). Results in the present study, indicated that the initial PV value was less than 20 meq/kg on day 0 for WSKF (11.32 ± 4.98), but within 20 to 40 meq/kg range on day 0 for RKF (23.76±1.16) and CSKF (28.38 ± 1.35). PV value for stored smoked fish samples was highest ($p < 0.05$) in the charcoal products, that is, CSHB (108.4 meq/kg) and CSKF (96.03 meq/kg) on day 5, the same significantly ($P < 0.05$) decreased in WSKF (73.82 meq/kg) and CSKF (52.40 ± 6.80 meq/kg) on day 7 ($P < 0.05$) (Table 4). Overall, the PV values of all the stored smoked samples were higher ($P < 0.05$) in the SHB when compared with the fillet, values ranged from 23.76 to 96.03 meq/kg in fillet and 26.27 to 108.40 meq/kg in SHB within the 7 day storage period.

Total viable count

The TVC result is shown in Figure 1 as the log of colony forming unit/g of fish (log cfu/g). The appearance of moulds on the surface of the smoke fish samples was used as a measure of spoilage in the smoked fish stored at room temperature (32 ± 0.02) for seven days. Fish spoilage starts when trimethylamine oxide present in fish tissues is broken down to trimethylamine in the presence of lactic acid derived from break down of fish glycogen by microorganisms (Rehman, 1980). Whitish growth was observed on the surface of the smoked fish samples on different storage days. Microbial growths were observed on day 5 or all the wood smoked fish samples. However, this was not similar to the coal smoked fish samples, because microbial growth was observed on the surfaces

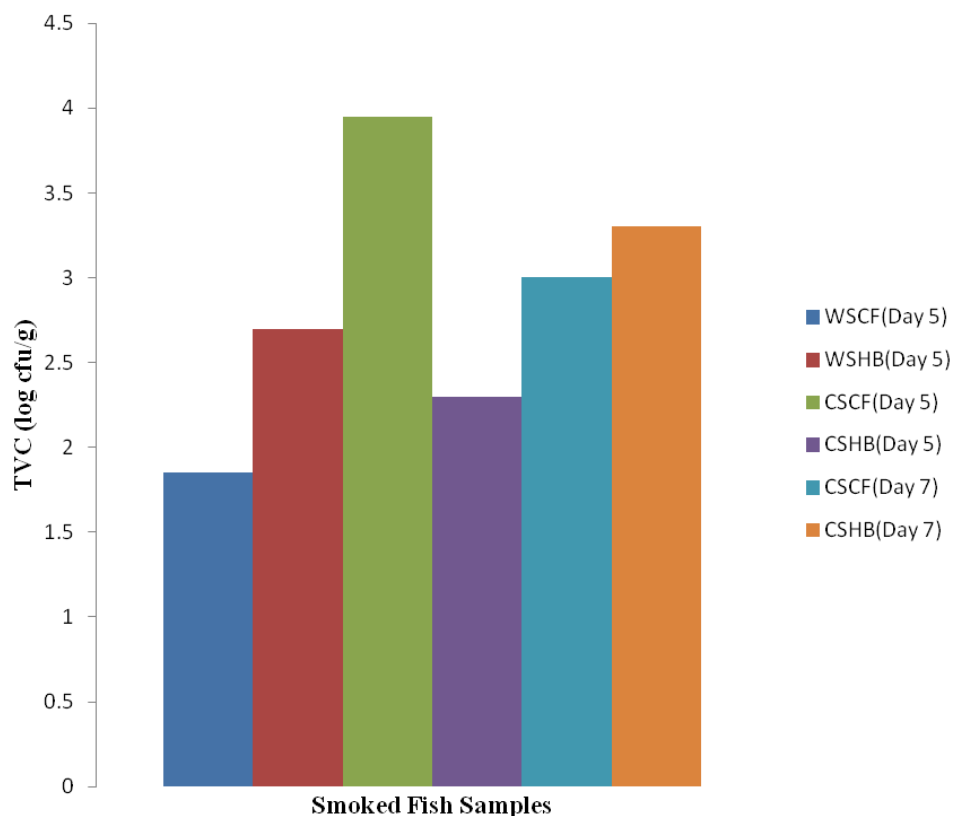


Figure 1. Total viable counts of charcoal and wood smoked *T. trachurus* fillet and SHB stored at ambient temperature ($32 \pm 2^\circ\text{C}$) for seven days. WSKF: wood smoked *kote* fillet; CSKF: coal smoked *kote* fillet; WSSHB: wood smoked *kote* SHB; CSSHB: charcoal smoked *kote* SHB.

of some of the coal smoked *T. trachurus* samples on day 5 and on the remaining fish samples on day 7 (Figure 1). In addition, the TVC was found to have the least ($p < 0.05$) value in WSKF ($1.85 \log \text{cfu/g}$), while the highest ($p < 0.05$) value was seen in the CSKF ($3.95 \log \text{cfu/g}$) on day 5. The essential ingredients like phenols and other aromatic compounds necessary to kill microorganisms including pathogens in smoked fish is smoke and/or heat attained during smoking (Clifford et al., 1980). Earlier works done by Pettet and Lane (1940) analysed the content of smoke and found that smoke contains more than 170 substances of whose functional group classes include aldehydes, ketones, acids, lactones, phenols and other aromatic compounds. These compounds are collectively known as pyrolyginous acids; the amount, type and nature of each compound are dependent on the type of wood used (Eyo 1979). Hardwood provides less smoke, but gives more intense heat than charcoal depending on the degree of aeration of the fire (Lea, 1933; Eyo, 1979). Therefore, the high TVC value observed in the CSKF ($3.95 \log \text{cfu/g}$) was as a result of less intense heat transfer efficiency (HTE) of the charcoal smoke, which invariably resulted in the production of lesser phenolic compounds for the charcoal smoked *T. trachurus* samples.

DISCUSSION

The pH value is a reliable indicator of the degree of freshness or spoilage, because it is used as an indicator of the extent of microbial spoilage in fish (Eyo, 1993). In present study therefore, the earlier observed decrease ($p < 0.05$) in the pH of coal smoked fillet with storage time at $32 \pm 2^\circ\text{C}$ may be as a result of the acids produced by the proteolytic microbes after decomposition of carbohydrate, thereby increasing the acid level of the medium, this concurs with the report of Eyo (1993) indicating that a decrease in pH is due to the fact that carbohydrate of the fish was fermented to acids. And the observed increase ($p < 0.05$) in the smoked SHB samples agrees with the findings of Erkan and Ozden (2008) that an increase pH is due to the increase in volatile bases from decomposition of nitrogenous compounds by endogenous or microbial enzymes. The pH of fish flesh has an important influence on its freshness because of its influence on bacterial growth; the lower the pH of a fish muscle, the slower the bacterial growth, and vice versa (Pacheco-Aguilar et al., 2000). Similar result has been indicated by Vasiliadou et al. (2005) that during storage, low pH confirms increased putrefaction (spoilage), besides Okoye et al. (2009) reported increase in pH during storage which is

similar to the former report that the pH of the Nile perch fish stored in ice over a period of 22 days increased with storage time

The TVBN decreased with processing in the fillet and SHB (with the exception of WSSHB), but increased during storage period. According to EEC (1995), the TVB-N value of both fillet and SHB of the raw and processed fish (day 0) fell within the acceptable upper limits of 25 to 35 mg/100 g for some fish species. As expected, a significant increase ($P < 0.05$) in WSSHB TVBN value was observed in wood smoked SHB. The increased TVB-N in the hot wood smoked samples was less than that obtained by Goulas and Kontominas (2005). They observed that TVBN values in mackerel (*Scomber japonicus*) almost doubled after hot smoking (Bilgin et al., 2008). In another study, a significant increase ($p < 0.05$) in TVB-N was observed after hot smoking of sea-bream (Vasiliadou et al., 2005). Whereas, result in the present study indicates that the TVB-N level increased gradually with the duration of storage (Table 4) in both the coal and wood smoked fish fillet and SHB. The TVB-N limit of 35 mg/100 g of muscle was exceeded by CSKF on the 5th day, whereas in the SHB, TVB-N limit was significantly exceeded by both the CSSHB and WSSHB on 5th and 7th days, respectively (Table 4). The present study showed that the effects of the 3 processing methods were significant ($p < 0.05$) for TVBN.

The peroxide value which is a primary indicator of oxidation of fat (rancidity) increased with storage time; ranging from 23.70 to 96.03 meq/kg on the 5th day in both raw and smoked horse mackerel samples. The peroxide values corresponding to incipient spoilage are usually in the order of 20 to 40 meq/kg of oxygen in wild turbot (Ozogul et al., 2005). Fat oxidation is a self catalyzing reaction, which is affected by the age of the raw material as well as oxidation of fats during processing and storage (AOAC, 1993). The oxidation of fats, which is usually at the site of unsaturated bonds in the fatty acid chains results in a variety of compounds being formed including free radicals and hydro-peroxides. Hence at the onset of oxidation, peroxide levels increase but are eventually oxidized to aldehydes and ketones, invariably resulting in lesser levels of peroxide in the later stages of oxidation (AOAC, 1993) This explains the reason why the peroxide values were higher ($p < 0.05$) on day 5 in both the fillet and SHB of the coal smoked *T. trachurus* fish. In addition, PV values have been reported for a number of species: 0.8 to 1.2 meq/kg for herring (Smith et al., 1980?), 5.60 meq/kg for wild turbot (Ozogul et al., 2005) and 27.6 meq/kg for fresh sardine (Cho et al., 1989). The PV values for RKF in this study was lower ($P < 0.05$) than that reported by Cho et al. (1989). Furthermore, the peroxide values corresponding to incipient spoilage are usually in the order of 20 to 40 milli-equivalents of oxygen per kg of sample (meq/kg). Also, Connell (1988)? Indicated that when peroxide value is above 10 to 20, fish develop rancid taste and smell. Thus, the observed PV

values for RKF, CSKF and WSKF on day 0 might be indicative of the beginning of spoilage. Raj and Liston (1963) indicated that anaerobic bacteria counts are very important in product commercialization, which is because the preservative effect of smoke itself is largely due to the presence of a range of phenolic compounds, nitrites and formaldehyde (Ahmed et al., 2010). Besides, Karra (1978) reported that smoking caused a decrease in TVC by an average of 94.7% of the original number in dogfish fillets; this was similar for the wood smoked horse mackerel fillet variety of the present investigation. Furthermore, the TVC values for coal and wood smoked horse mackerel indicated that spoilage ($p < 0.05$) was observed on the CSCF and CSHB samples on days 5 and 7, respectively (Figure 1). A higher ($p < 0.05$) degree of spoilage was recorded in the coal smoked horse mackerel samples, which may be as a result of the less intense heat attained from the charcoal smoke (Eyo, 1979; Clifford et al., 1980). Various workers have reported the effect of heat intensity on the shelf life of smoked product. CO₂ is reported to have an important effect on microbial growth, exerting a selective inhibitory action (Huss, 1992; Molin et al., 1983). Also, phenolic compounds produced by the more intense heat of hard wood (Nzeako, 1981) act as electron acceptor which terminates the rancidity chain reactions (Emmanuel, 1955; Dann, 1969; Lea, 1933; Olsen, 1976). The total phenol levels on the surface layers of hot smoked fish may reach 40 to 50 mg per kg weight of fish (Eyo, 1979). Also, Kleickman and Schellece (1979) evaluated the microbial numbers of smoked fish and discovered that the average plate count amounted to 10⁶/g of fish. Thatcher and Clark (1968) stated that when fish has a count of more than 10⁶ organism/g (6.0 log cfu/g), it is considered to have a very short potential shelf life or may be an early sign of incipient spoilage, this was however not the case in the present study because the highest ($p < 0.05$) TVC level of 3.95 and 3.30 log cfu/g were recorded in the CSCF (day 5) and CSSHB (day 7), respectively. Chemical methods are reliable measures of freshness or state of deterioration of product, because the concentrations of chemicals are dependent on storage time and temperature. Nonetheless, total viable or aerobic counts do not indicate the presence of some harmful anaerobic psychotropic bacteria, which results in detrimental consequences to the consumer's health (Caglak et al., 2008). Thus, findings in the present study indicated that horse mackerel processed via charcoal had the lowest ($p < 0.05$) storage time amongst the smoked fish samples (fillet and SHB), this might be as a result of less intense heat produced by the coal smoke that contained low levels of aromatic compounds like phenols responsible for killing microorganisms present in the fish.

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