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Influence of processing methods on mycoflora changes during storage of raw and processed Atlantic horse mackerel (*Trachurus trachurus*)

Olaluwa T. Adeyemi[1], Odutola, Osilesi[1], O. O. Adebawo[1], F. D. Onajobi[1], Sunday O. Oyedemi[2] and Anthony J. Afolayan[2]*

[1]Department of Biochemistry, Bencarson Senior School of Medicine, Babcock University, Ilisan Remo, Ogun state, Nigeria.
[2]Botany Department, University of Fort Hare, Alice 5700, South Africa.

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Study assessed the influence of processing on mycoflora of kote fillet, skin, head and bones (SHB) during storage for 28 days at ambient temperature of (32 ± 2°C). Fish samples were prepared by smoking (wood and coal) and poaching using standard methods. Fungi associated with raw and processed fillets and SHBs included the species of *Absidia glaucus*. *Absidia, Aspergillus flavus, Aspergillus niger, Aureobasidium* sp., *Candida tropicalis, Candida krusei, Fusarium* spp., *Rhizopus* sp., and *Penicillium expansum*. The various fungi was isolated using the direct plating and dilution plate methods indicated that wood smoke processing method had the highest ($p<0.001$) amount of mycoflora, which was followed by the coal smoke and poaching method. Also the SHB samples (fillet and SHB) had markedly low ($p<0.05$) fungal count than in the fillet. Thus, the SHB showed great promise in having lower ($p<0.01$) mycoflora which could be gathered and utilized at little costs for human food and animal feed, invariably reducing costs of feeds due to highly priced amount of casein, soybean meal and groundnut cake.

Key words: *Trachurus trachurus*, temperature, smoked fish and mycoflora.

INTRODUCTION

Fish is highly perishable but very important food stuff, due to its high levels of protein and polyunsaturated fatty acids as well as its affordability by the masses compared with beef. One of such species is the Atlantic horse mackerel (*Trachurus trachurus*), a medium-fat fish species abundant in the North-east Atlantic (Zimmermann and Hammer, 1999; Adeyemi et al., 2013). Nonetheless, chemical breakdown of protein, fat and water contents contribute to quick spoilage of fish (Adeyemi et al., 2013). Therefore various fish processing methods are used, to discourage/reduce the growth of spoilage organisms (Fayemi, 1999; Adetunji et al., 2007; Fagbohun et al., 2010) and increase the shelf-life of the stored product. Fish processing methods, like

*Corresponding author. E-mail: aafolayan@ufh.ac.za. Fax: +27866282295.

salting/brining, poaching, boiling, drying and smoking have been used for decades, this is because they allow for better preservation and storage as well as increase fish availability to the consumers (Egbal et al., 2010). More importantly is the skin, head and bone (SHB) of these processed fish could be gathered and utilized as a form of protein concentrate at little costs thus reducing costs of animal feeds due to highly priced casein, fish meal, soybean meal and groundnut cake (GNC) (Adeyemi, 2013).

Fungi dominate the micro flora of stored products, due to their ability to grow at low water content (Deible and Swamson, 2001). Mycoflora utilizes the nutrient contents of dry edible products, thus decreasing the value of food materials. Based on relative humidity field fungi attack developing and matured seeds in the field, while storage fungi are predominantly species of *Aspergillus* and *Penicillum* which attack stored products (Christensen, 1957). It is therefore important to know the quality of mycoflora of poached, coal and wood smoked *kote* and the cut off point of dried *kote* stored at ambient temperature (32 ± 2°C). Therefore, the objective of this work was to determine the effect of processing on the mycoflora of *Trachurus trachurus* fillet, skin, head and bones (SHB) during storage for 28 days at ambient temperature (32 ± 2°C).

MATERIALS AND METHODS

Collection of samples

Sample preparation and processing

A total of 20 kg (approximately 100 fish) of horse mackerel was collected 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 packing of samples

A portion of the fish was poached in water at 60°C for 15 min and the remaining portion was 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, preheated 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 place in cane woven basket to cool off, after which portions of the processed fish were packaged in an insect free labelled transparent polythene bags and kept in the laboratory (32 ± 2°C).

Organoleptic test

A total of 10 member's panel evaluated the quality of the raw and processed products through sensory evaluation. Score sheet of sensory evaluation used in study was based on the method described by Standard National Indonesia, (1991). Sensory assessment was conducted via categorical ranking methods as described by DOCE (1989) and Eyo (2001). Four categories were ranked: highest quality, good quality, fair quality, and rejectable quality. The sensory assessment of the skin, eyes, gills, flesh odour, consistency, flavour, texture, colour and flesh appearance of the fish samples were also considered and scores among panelists were collected, statistically analyzed and expressed as the mean ± standard error (S.E.) (n=10), the significant differences between means were compared amongst the different processing methods using the least significant difference test after ANOVA for one-way classified data (Duncan, 1995). This was done to determine the taste, odour, texture and general appearance for the raw, poached and smoked *T. trachurus* samples. Products were scored on a scale of 10 - Excellent, 8 - Very Good, 6 - Good, 4 - Fairly good, 2 – Poor and 0 - extremely poor.

Analytical method

Cooking process was done without adding any ingredient. After poaching and smoke processes, a known portion of each fish species was oven dried to constant weight at 60°C, and the flesh of each fish was 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 stored on the shelf at ambient temperature for 28 days. After which the samples were examined for changes in the mycoflora periodically on day; 0, 3, 7, 14, 21 and 28, respectively.

Culture media

The media used in this study was nutrient agar. The media were prepared according to the manufacturer's specification. These media were sterilized in an autoclave at 121^0C for 15minutes.

Isolation of micro-organism

One gram of each sample (raw and processed kote fillet and SHB) was serially diluted, 1 ml of an appropriate dilution was inoculated on nutrient agar plates and the plates were incubated for 24 h at 30°C. After 24 h sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared sterile nutrient agar and MRS agar plates, then incubate for 24 h at 30°C in order to get pure cultures. The routine laboratory method of Cruickshank et al. (1975) as modified by Alexopoulous et al., (1996) was used to characterize different isolates. The isolates were identified using their macroscopic, cultural, physiological and biochemical characteristics.

Direct plating method

From the ambient stored processed *kote* samples, 10 g were examined randomly for external mouldness. They were surfaced sterilized with ethanol and later washed with sterile distilled water. Using a sterile dissecting forceps, the surface of the stored sun dried plantain chips were scrapped and were aseptically plated on potato dextrose agar (PDA) plate and incubated at room temperature for 5-7 days as described by Amusa (2001). The fungi cultures were further subcultured until pure colonies were obtained

Table 1. Fungi isolated from raw fillet and SHB stored at ambient temperature (32 ±2^{0}C) using different isolating methods.

Fungal		*A. niger*	*A. flaus*	*A. fumigatus*	*Mucor*	*Fusarium*	*Rizopus*	*Penicillum expansum*	*Aureobasidum*	*Candida tropicalis*	*C. krusei*	*Absidia*	*A. glaucus*
Dilution		**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**
FILLET	0	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- -
	3	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- -
	7	+ -	- -	- -	-	+ -	- -	- -	- -	+ -	- -	- -	- -
	14	+ -	- -	- -	+ -	+ -	- -	- +	- -	+ -	- -	- -	- -
	21	- -	- -	- -	+ -	+ +	+ -	- -	- -	-	- -	- -	- -
	28	- -	- -	- -	- -	+ -	- -	- -	- -	+ -	- -	- -	- -
		A B	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**	**A B**
SHB	0	- -	- -	- -	- -	- -	- -	- -	- -	- +	- -	- -	- -
	3	- -	- -	- -	+ +	- -	- -	- -	- -	- +	- -	- -	- -
	7	- -	- -	- -	+ -	- -	- -	- -	- -	+ -	- -	- -	- -
	14	- -	- -	- -	+ -	- -	- -	- -	- -	+ -	- -	+ -	- -
	21	- -	- -	- -	+ -	- -	+ -	- -	- -	- -	- -	- +	- -
	28	- -	- -	- -	+ -	- -	- -	- -	- -	+ -	- -	- -	- -

A = Direct plating method B = Dilution plate method + = present (isolated) - = absent (not isolated). SHB= Skin head and bone.

by successive hyphae tip transfer (Egbebi et al., 2007). The cultures were examined under the microscope for fruiting bodies, hyphae to determine the common fungi present.

Dilution plate methods

This method was used to determine the type of fungi present in the ambient stored processed *kote* samples. About one gram of the sample was sterilized with ethanol and grinded with 10 ml of sterile distilled water. This was shaken thoroughly and 1ml of suspension was pipetted into a sterile test tube containing 9ml of distilled water. This was thoroughly mixed together. The sample was serially diluted and 1 ml each of aliquots of 10^{-4} and 10^{-5} were added to molten PDA plates. The plates were swirled gently to obtain thorough mixing and were allowed to solidify and incubated at room temperature for 5 - 7 days. The fungal colonies were counted every 24 h. Successive hyphae tip were transferred until pure cultures of each of fungus was obtained.

Identification of mycoflora

The associated fungi were identified by their cultural and morphological features (Alexopoulous et al., 1996). The isolates were examined under bright daylight for the colour of the culture and further examination was carried out.

RESULTS AND DISCUSSION

The microorganisms isolated from the raw, coal and wood smoked as well as poached (fillet and SHB) samples using different methods are represented in Tables 1 to 4 respectively. The scores done according to the Standard National Indonesia (1991) scheme for each parameter in the raw and processed samples are presented in Tables 5 and 6, respectively. A total of fungus were isolated from ambient (32 ± 2°C) stored raw and processed fillet and SHB, based on their cultural and morphological characteristics. The fungi include**:** *A. niger, A. flavus, A. fumigatus, Mucor, Fusarium*, *Rizopus, Penicillum expansum, Aureobasidum* spp*, Candida tropicalis, C. krusei, Absidia* and *A. glaucus* sp. The results indicated that *A. niger*, *A. flavus, A. fumigatus, Mucor*, *Fusarium*, *Penicillum expansum, Rizopu and Absidia)* were found in the coal smoked (fillet and SHB) stored samples; *A. niger, A. flavus, A. fumigatus, Mucor*, *Fusarium*, *Rizopus*., *Penicillum expansum, Aureobasidum, Candida tropicalis, C. krusei, Absidia* and *A. glaucus* spp) were found in the wood smoked stored. *A. niger, A. fumigatus, Mucor,*, *Penicillum expansum, Aureobasidum, Candida tropicalis,* and *Absidia*) in the poached

Table 2. Fungi isolated from coal smoked fillet and SHB stored at ambient temperature (32 ± 2°C) using different isolating methods.

Fungal		*A. niger*	*A. flaus*	*A. fumigatus*	*Mucor*	*Fusarium*	*Rizopus*	*Penicillum expansum*	*Aureobasidum*	*Candida tropicalis*	*C. krusei*	*Absidia*	*A. glaucus*
Dilution		A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B
FILLET	0	- +	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- -
	3	- -	- -	- -	+ -	- -	- -	- -	- -	- +	- -	- -	- -
	7	+ -	+ -	+ -	+ -	- -	- -	+ -	- -	- -	- -	- -	- -
	14	- -	- -	- -	+ -	+ -	- -	- +	- -	+ -	-	+ -	- -
	21	- -	- -	- -	+ -	+ -	- -	- -	- -	- -	- -	- -	- +
	28	- +	- -	- -	+ -	- -	- -	- +	- -	- -	- -	- -	- -
		A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B
SHB	0	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	3	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	+ -	- -
	7	+ -	- -	- -	- -	+ -	- -	- -	- -	+ +	- -	+ -	- -
	14	+ -	- -	- -	- -	- -	- -	- -	- -	+ -	- -	- -	- -
	21	+ -	- -	- -	+ -	+ -	- -	+ -	- -	+ -	- -	- -	- -
	28	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- -

A = Direct plating method, B = Dilution plate method, + = present (isolated), - = absent (not isolated), SHB= Skin head and bone.

Table 3. Fungi isolated from wood smoked fillet and SHB stored at ambient temperature (32 ±2°C) using different.

Fungal spp		*A. niger*	*A. flaus*	*A. fumigatus*	*Mucor Spp*	*Fusarium Spp*	*Rizopus*	*Penicillum expansum*	*Aureobasidum spp*	*Candida tropicalis*	*C. krusei*	*Absidia*	*A. glaucus*	*A. terreus*	*P. chanlybeum*
Dilution		*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*
FILLET	0	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	3	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	7	- -	+ -	- -	-	+ -	- -	- -	- -	+ -	- -	- -	+ -	- +	-
	14	+ -	- -	- -	+ -	+ -	- -	- -	- -	+ -	- -	- -	- -	- -	- -
	21	- -	- -	- -	+ +	+ -	+ -	- -	- -	- -	- -	- -	- -	- -	- -
	28	+ -	- -	+ -	+ -	+ -	- -	- -	- -	- -	- -	+ -	- -	- -	+ -
		A B	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*	*AB*
HEAD	0	- -	- -	- -	+ -	+ -	- -	- -	- -	- +	- -	- -	- -	- -	- -
	3	- -	- -	- -	+ -	- -	- -	- -	- -	- +	- -	- -	- -	- -	- -
	7	- -	- -	- -	+ -	+ -	+ -	- -	- -	- -	- -	- -	- -	- -	- -
	14	- -	- -	- -	+ -	+ -	- -	- -	- -	+ +	- -	- -	- -	- -	- -
	21	- -	- -	- -	+ +	+ -	+ +	- -	- -	- -	- -	- -	- -	- -	- -
	28	- -	- -	- -	+ -	+ -	- -	- -	- -	+ -	+ -	+ -	- -	- -	- -

Table 4. Fungi isolated from poached smoked fillet and SHB stored at ambient temperature (32 ± 2°C) using different isolating methods

Fungal		*A. niger*	*A. flaus*	*A. fumigatus*	*Mucor*	*Fusarium .*	*Rizopus*	*Penicillum expansum*	*Aureobasidum*	*Candida tropicalis*	*C. krusei*	*Absidia*	*A. glaucus*	*Penicillum*
Dilution		A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B
FILLET	0	- -	- -	- -	+ -	- -	- -	- -	- +	- -	- -	- -	- -	- -
	3	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	7	+ -	- -	- -	+ -	+ -	- -	- -	- -	- -	- -	- -	- -	- -
	14	+ -	- -	- -	+ -	+ -	- -	- -	- -	+ -	- -	+ -	- -	- -
	21	- -	- -	- -	- -	- +	- -	- -	- -	- -	- -	- -	- -	- -
	28	- -	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- +
		A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B
HEAD	0	- -	- -	- -	- -	- -	- -	- -	- +	- -	- -	- -	- -	- -
	3	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
	7	- -	- -	- -	- -	+ -	- -	-	- -	- -	- -	+ -	- -	- -
	14	+ -	- -	- -	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -
	21	+ -	- -	- +	+ -	+ -	- -	- -	- +	- -	- -	- -	- -	- -
	28	- -	- -	- -	+ -	- -	- -	- -	- -	- -	- -	- -	- -	- +

A = Direct plating method, B = Dilution plate method, + = present (isolated), - = absent (not isolated). SHB= Skin head and bone.

Table 5. Showing Result of Sensory Evaluation of Processed *T. trachurus*.*

Parameter	CSK	WSK	PK
Odour	9.20 ± 0.32^{a}	8.80 ± 0.32^{a}	8.72 ± 0.55^{a}
Flavour/taste	9.20 ± 0.32^{a}	9.80 ± 0.20^{a}	8.00 ± 0.54^{b}
Texture	8.00 ± 0.79^{a}	8.20 ± 0.62^{a}	7.09 ± 0.62^{b}
Colour	9.60 ± 0.27^{a}	9.60 ± 0.40^{a}	7.27 ± 0.82^{b}

*Data= Mean ± SEM, n=10. Values with different superscripts along a row are significantly different ($P < 0.05$). CSK: Charcoal smoked *Kote*; WSK: Wood smoke *Kote*; PK: Poached *Kote*

samples compared to raw stored samples that had only fungi species (that is, *A. niger, Mucor*, *Fusarium, Rizopus, Penicillum expansum, Candida tropicalis,* and *Absidia*). Species of *Aspergillus, Mucor, Fusarium, Rhizopus,* and *Penicillum* fungi are known to be surface contaminant of most food products that induces decay. In this study the increase in quality of fungi in all isolates was similar to the report of Fagbohun et al. (2010), and Oladipo and Bankole (2013) but with the report of Ogundana et al. (1970) a decrease in fungi quantity in stored products was noticed. The fungi were likely to originate mainly from contamination from air and fish handling during processing, which was

Table 6. Showing Result of Sensory Evaluation of Raw *T. trachurus**

Parameter	Eye	Gill	Skin colour	Flesh texture
Rk	6.40 ±0.97	8.00±0.42	8.80 ±0.44	9.20±0.44
GRADE	2.60±0.85	1.70±0.26	1.70±0.26	1.70±0.26

*Data= Mean ± SEM, n=10. RK values were graded as described by Baremo de Classification de Frescura, (1989) & Eyo, (2001) (See Appendix I). Rk: Raw *kote*

detected from day 0 of storage. This fungus have been reported in immunosuppressed hosts such as in AIDS patients, non-AIDS patients with hematological malignancies and those receiving antifungal antibiotics that could alter the microbiota of human (Selik et al., 1997). *Penicillium* infections results in keratitis, endophtalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis, urinary tract infections, mucocutaneous, genitourinary, gastrointestinal, pulmonary and disseminated infections like the clinical features (Lueg et al., 1996; Mitchell et al., 1996; Kontogiorgi et al., 2007). Three species were isolated and identified from sundried plantain chips.

Aspergillus spp are common mould living in soil, hay etc. and the second most commonly recovered fungus in opportunistic mycoses. By transplantation, extensive use of immunosuppressive drugs which include corticosteroids predisposes human to *Aspergillus* infections (Douglas, 2007). The clinical features of *Fusarium* infections include keratitis, endophthalmitis, otitis media, onychomycosis, cutaneous pulmonary infections, endocarditis and fungemia (Lueg et al., 1996). Proper heating of food, elimination of infected and suspected food by *Fusarium* spp are the major preventive measures (Odds et al., 1998). *Rhizopus* spp and *C. krusei* were the least frequently encountered fungi in this study. The isolation was made at the 2nd week of storage.

Kontogiorgi et al. (2007) reported *Rhizopus* to causs rhinocerebral mucormycosis, mucocutaneous, genitourinary, gastrointestinal, pulmonary and disseminated infections. It is also responsible for the damage of blood vessels and nerves. Vascular invasion by *Rhizopus* causes necrosis of the infected tissue. Treatment of *Rhizopus* infections remains difficult due to its property to invade vascular tissues, infarction of the infected tissue is common and mortality rates are very high *Rhizopus* infections can be prevented by avoiding contact with contaminated object as well as maintaining a proper hygiene (Welsh and Kaplan, 1998).

Data obtained from sensory evaluation via categorical ranking method (Eyo, 2001), revealed that both the raw and processed fillet and SHB parts were in superior ($p < 0.05$) quality that warrant general acceptance (Tables 5 and 6). In addition, because of peroxidative damage to cellular membranes nutritional muscular dystrophy, fatty liver degeneration, anaemia, exudative diathesis, erythrocyte haemolysis, haemorrhages and depigmentation often observed in fish deficient of vitamin E (He and Lawrence, 1993; Mehrad et al., 2012); this was not the case in present study for both raw and processed (fillet and SHB) samples. Although PK was lowest in odour (8.72 ± 0.55), flavour (8.00 ± 0.54), texture (7.09 ± 0.62) and colour (7.27 ± 0.82), this values were still above the average score of 5 points for each fish product out of a maximum of 10 points, hence confirming that PK was also significantly ($p < 0.05$) acceptable for human food. Lastly, the average values of 6.40 ± 0.97 for eye, 8.00 ± 0.42 for gills, 8.80 ± 0.44 for skin colour and 9.20 ± 0.44 for flesh texture of indicated raw samples were of good quality and significantly high ($p < 0.05$) organoleptic acceptance. Nonetheless handling and processing of fish products, apart from good hygiene caution must be taken to reduce contamination by pathogens. This is because the isolated fungi can degrade both fillet and SHB as substrate, and pose a threat to the consumers by either infecting them or elaborating metabolites that can affect organs of the body.

Conclusion

Present study provided evidence for pathogenic fungi to enter, survive and grow within locally processed fish (kote fillet and SHB) samples. The samples subjected to the poaching method had markedly the least ($p<0.001$) presence followed by the charcoal and wood smoking method. The SHB samples also recorded significantly ($p<0.05$) low amounts of presence in all the (raw and processed) samples compared to the fillet (raw and processed). Since, the SHB showed great promise in longer ($p<0.01$) keeping quality at ambient temperature than the fillet. Results suggests that foods processed from the SHB could serve as healthy low cost food that would help increase the importance of wastes which if left uncared may cause pollution to the environment. Due to the high cost of protein concentrate and fish meal used in animal feeds, the SHB could be converted to nourishable feeds.

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