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Biochemical factors involved in the resistance of some smoked tropical fish samples to insect attack

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Abstract: The role of mineral elements (ppm), protein and oil content (%), and saponification and free fatty acid values in contributing to resistance against insect infestation was investigated in 4 fish species: *Clarias gariepinus, Parachuma obscura, Hydrocynus* sp., and *Malapterus electricus.* Smoked fish samples were purchased from a local market in Ilorin, Kwara State, Nigeria, packaged, and maintained at 4 °C for 7 days. They were later brought out to acclimatise, and then observed for 4 months for possible natural insect infestation. Next, crushed samples of each species were analysed using conventional chemical analysis in order to assess the role of some elements and biochemical compounds in inducing resistance. The results showed that insect infestation did not occur after the period of storage (4 months) and chemical analysis showed that 3 samples lacked lead (Pb), a heavy metal not required in trace amounts, but contained other heavy metals required in trace amounts, such as Fe, Mn, Co, and Cu. Protein content in the 4 samples was average (50%), but oil content was low. *Hydrocynus* sp. and *Malapterus electricus* had an acid value below 50 mg KOH/g. Additionally, all the samples had high a saponification value (above 500 mL/g). The presence of iron (≥1.5), high protein (%) and saponification values, low oil content, and low acid values of between 35 and 90 were the elements and biochemical constituents that prolonged the shelf life of the fish species studied.

Key words: Acid value, fish, infestation, mineral content, resistance, shelf life

Böcek istilasına karşı bazı füme (smoked) tropikal balık örneklerinin direncini içeren biyokimyasal faktörler

Özet: Böcek istilasına karşı dirence katkıda bulunan mineral elementlerin (ppm), protein ve yağ (%)'ın, sabunlaşma ve serbest yağ asidi değerinin ilgisi isimleri verilen dört balık türü (*Clarias gariepinus, Parachuma obscura, Hydrocynus* sp. ve *Malapterus electricus*)'nde araştırıldı. Füme (Smoked) balık örnekleri Ilorin, Kwara, Nijerya'daki yerel bir marketten satın alındı ve yedi gün boyunca 4 °C' de paketli olarak saklandı. Örnekler daha sonra iklime alıştırılmak üzere dışarı çıkarıldı ve olası doğal böcek istilası için dört ay boyunca gözlemlendi. Bundan sonra, her türün ezilmiş örnekleri, dirence sebep olan bazı elementler ve biyokimyasal bileşiklerin rolünü değerlendirmek için, kimyasal analizlerin geleneksel yöntemleri kullanılarak, kimyasal analizleri yapıldı. Sonuçlar, 4 aylık depolama süresi sonunda örneklerin hiçbirinde böcek istilasının oluşmadığını göstermiştir ve kimyasal analizler, üç örnekte izlemede gerekli olmayan ağır bir metal olan kurşunun (Pb) eksik olduğunu, fakat Fe, Mn, Co ve Cu gibi izlemede gerekli olan diğer ağır metallerin bulunduğunu ortaya çıkarmıştır. Dört örnekteki protein içeriği ortama % 50 idi, fakat düşük yağ içeriği mevcuttu. *Hydrocynus* sp. ve *Malapterus electricus*'da KOH/g 50 mg'ın altında asit değerine sahipti. Ayrıca, tüm örneklerde 500 mL/g 'ın üstünde sabunlaşma değeri vardı. Demirin varlığı (≥ 1,5), yüksek protein (%) ve sabunlaşma değeri, düşük yağ içeriği ve 35-90 arasındaki asit değeri bu çalışmada balık türlerinin raf ömrünün uzun olmasına yardım eden elementler ve biyokimyasal bileşenlerdir.

Anahtar sözcükler: Asit değeri, balık, istila, mineral içerik, direnç, raf ömrü

Introduction

Fish are a major living aquatic resource and contain a high quantity of protein, vitamins, and minerals. Over 25,000 t (metric tons) of fish are used for human consumption and animal feed (1). According to the FAO (1), poor handling, storage, and distribution are responsible for 100% (by weight) of the loss of fish that are caught. Stored fish is often prone to spoilage because of its low acid value, which supports the growth of pathogens (2).

Oyeleye (2) posited further that improved handling and storage could improve the quality of stored fish in Africa. Fish can be preserved traditionally or by modern techniques, which retard spoilage and extend the shelf life (3). Eyo (4) advocated the adoption of fish smoking as a preservation method to protect fish against pathogenic micro-organisms and insect deterioration, while Buckel et al. (5) cited poor distribution and handling as sources of contamination. Other investigators (3) reported that immediately after fish are caught a complicated series of chemical and bacteriological changes take place, and that infestation by such insects as blow flies and beetles can cause spoilage; hence, there is a need for durable and reliable storage methods. In response to the above reports many methods of preservation have been suggested for fish, including salting, fermentation and boiling, frying, and smoking (6). Among these methods of preservation, smoking seems to be the most favoured, and a scientific basis that establish its usefulness and improvement of the method are necessary in order to meet the FAO standard.

Thus, we were prompted to investigate the timing and type of insect infestation after cold treatment, and the involvement and concentrations of mineral content in the tissue of some selected fish species. We also determined the types and concentrations of biochemical compounds in fish that are possible factors in resistance to insect infestation, thereby increasing the shelf life of the selected fish samples.

Materials and methods

This study was carried out at the department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Four common species of smoked fish were purchased from a market in Ilorin Kwara State, Nigeria, and were identified by fishery experts as *Clarias gariepinus, Parachnam obscura, Hydrocynus* sp., and *Malapterus electricus.* They were stored at 4 °C in polyethylene bags for 7 days to ensure the samples were free of insect infestation, and then they were brought out to equilibrate for 5 days. All the samples were again stored in polyethylene bags at room temperature for 4 months and checked once a month for insect infestation.

After the fourth month each sample was carefully broken and ground with a porcelain mortar and pestle. The head, trunk, and tail were crushed separately, packaged into clean screw cap bottles, and stored at 4 °C until chemical analysis.

Chemical analysis

Acid digestion (wet oxidation)

Two grams of each sample was weighed into a Kjeldahl flask and mixed with 20 mL of concentrated HNO_3; 10 mL of 50% hydrogen peroxide and 10% saturated ammonium oxalate were also added until dense fumes evolved. Mg, Fe, Co, and Pb were determined with an atomic absorption spectrophotometer (AAS).

Nitrogen digestion

Samples (0.5 g) were weighed and a digestion catalyst was added after which 20 mL of concentrated H_2SO_4 was added and placed on a digester for 2 h at 80 °C. Nitrogen content (%) was analysed with a nitrogen analyser and the results were multiplied by a dilution factor, i.e. 6.25 (a constant to obtain the percentage of protein content).

Oil extraction

Oil was extracted with a soxhlet apparatus and the percentage was determined using the following formula:

% oil in ground sample = weight of oil (g) / weight of sample (g) × 100

Saponification value

Samples (2 g) were refluxed with 50 mL of alcoholic KOH (2), boiled for 1 h, and cooled. Phenolphthalein indicator (1 mL) was used and titrated against 0.5 N HCl until the pink colour disappeared. The amount was calculated as follows:

Table 1. Mean value of some heavy metals present in 4 species of smoked fish (ppm).

Fish species	Heavy metals (ppm)				
	Fe	Mn	Co	Cu	Pb
C. gariepinus	1.70 b	0.00 b	0.00 b	0.00 b	0.00 a
P.obscura	0.00 c	0.07 b	0.03 b	11.37 a	0.00 a
Hydrocynus sp.	4.30 a	0.00 b	0.22 a	0.00 b	0.00 a
M. electricus	3.00 ab	0.62 a	0.18 a	0.00 b	0.00 a

NB: Values with the same letter are not significantly different at $P \leq 0.05$

Table 2. Biochemical compositions and acid value present in the fish samples (%).

Fish species	protein	oil	saponification (mL/g)	acid value (mg KOH/g)
C. gariepinus	69.54ab	16.93a	778.39a	52.88b
P. obscura	86.05a	13.20a	551.19b	86.06a
Hydrocynus sp.	82.83a	19.05a	242.63c	36.57b
M. electricus	73.90a	17.60a	712.48a	46.89b

NB: Values with the same letter are not significantly different at $P \leq 0.05$

28.05 × titre value of blank – titre value of saponification / weight of sample (g)

Free fatty acid

In 50 mL of neutral solvent 1 g of oil was dissolved. A drop of phenolphthalein was added and titrated against 0.1 N KOH to give a pink colour.

Acid value (mg KOH/g) = titre value × normality KOH × 56.1 / weight of sample (g)

Free fatty acid value was evaluated by dividing the acid value by 2:

FFA = acid value / 2

Results and discussion

Physical condition

None of the 4 fish samples showed any signs of microbial or insect infestation 4 months after storage. Additionally, the colour, texture, and flavour of the fish did not change. The minerals present were Fe, Mn, Co, Cu, and Pb, and their content (ppm), in increasing order, was as follows: Fe > Mn > Co > Cu (Table 1). The protein and oil values (%), and saponification (mL/g), and acid value (mg KOH) for the 4 species are as shown in Table 2.

Protein content was highest in *P. obscura* (86%) and lowest in *C. gariepinus* (69.54%), while oil content was relatively low in all the samples (between 13.20% and 19.05%). The lowest saponification value was recorded in *Hydrocynus* sp. (242.63%), whereas the highest was recorded in *C. gariepinus* (778.39 mL/g) (Table 2). Acid values (mg KOH/g) in increasing order were as follows: *Hydrocynus* sp. 36.57 → *M. electricus* 46.89 → *C. gariepinus* 52.88 → *P. obscura* 86.06 (Table 2).

During the storage period insect infestation did not occur. The samples were subjected to low temperature treatment (4 °C) before the commencement of the experiment in order to ensure they were free of infestation. Our findings are not consistent with those of Ashamo and Ajayi (7), in which *Dermestes* infestation occurred in smoked fish that was purchased from a local market after 10 days (after acclimatisation from cold treatment) in storage. The method of smoking and storage conditions was suspected to be responsible for the spoilage they observed. Again, poor handling during experimental procedures can cause rapid spoilage, especially if humidity is allowed to accumulate around the stored samples; 12% relative humidity is recommended for products stored at room temperature.

Living organisms require trace amounts of some heavy metals to support life; however, toxic levels of these essential nutrients can be detrimental. The heavy metals observed in the fish (fresh and smoked) in the present study were absorbed through the gills from water and were introduced by the smoking process. No defined order of occurrence of these metals was noted in any of the samples, except in *M. electricus* in which Fe > Mn > Cu was observed in increasing order (Table 1). Khan et al. (8) also reported a similar trend in fish samples that they analysed. Supporting the toxicity of these metals Windom et al. (9) concluded that although anthropogenic input might elevate the concentration of heavy metals in the surrounding water their accumulation in fish muscle and tissue might be biochemically regulated to exclude toxic concentrations; this then accounted for the quantities of the metals noted in the muscle of the samples, which were not hazardous to the health of humans, but protected the fish against microbial and insect spoilage.

Burgess (10) reported that the nutritional composition of fish muscle varies from species to species, within species, and seasonally, while Kirchgessner and Schwarz (11) suggested that within fish species mineral retention depends mainly on the feed and feeding rate used; however, in the present study the amount of nutritional composition varied from one sample of fish to the other (feeding was not monitored in this experiment). High acid values observed in some of the samples (≤50 mg KOH/g) prevented the oil from becoming rancid during storage; acid values of at least 36.57 mg KOH/g (Table 2) aided long-term storage and prevented biological deterioration.

According to the FAO (1), one of the causes of fish spoilage is bacterial enzyme and chemical reactions involving the oxidation of fat in fish tissue, and it has been postulated that smoking (as a means of preservation) increases the hydrocarbon content of the product; therefore, the skin prevented the hydrocarbon content from incorporating a greater proportion of this aliphatic hydrocarbon into the flesh (10). In the present study the absence of aliphatic hydrocarbon in the studied species resulted in an extended shelf life.

High protein (≤73.9%) and oil (≤17.60%) content, saponification values ≤715.48%, and a minimum acid value of 46.89 mg KOH/g were responsible, among other factors, for the long shelf life of the selected fish species that were smoked. The manipulation of these elements (and others contained in feed components) at the correct level is recommended for feed formulation and feeding regimes in pond treatment and fish management programmes.

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