Eurasian Journal of Food Science and Technology 2022; Vol:6, Issue:1, pp:1-7

Effect of Freezing and Smoking on The Proximate and Mineral Composition of Flat Sardinella (*Sardinella Eba)*

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Abstract

A study was conducted to determine and compare the effects of freezing and smoking on the proximate and mineral composition of *Sardinella eba.* The results of the proximate composition were observed to be higher in the smoked fish samples compared to the frozen samples, with all parameters significantly higher ($P<0.05$) in the smoked samples than in the frozen samples apart from moisture content. Eight minerals were quantified in *S. eba*, including five macro minerals (Ca, K, P, Na, and Mg) and three trace minerals (Fe, Zn, and Mn). The mineral content values of the smoked fish samples in all the parameters studied were statistically significantly higher than in the frozen fish samples, particularly for the trace minerals such as Zn and Mn, than in the smoked samples. In conclusion, the information obtained in this study could be useful to fish consumers, processors, and nutritionists in the efficient post-harvest management of fish resources.

Keywords: Proximate composition; mineral elements, freezing, smoking, *Sardinella eba.*

Research article
Received date: 13 March 2022
Accepted date: 19 June 2022

INTRODUCTION

In the modern world, fish provides 16% of total animal protein consumed globally (FAO, 2016). Its nutritional properties have been highlighted in several studies showing that fish contains important micro-nutrients (riboflavin, iron and calcium) and fatty acids, such as omega-3, all of which are important for human health, particularly during childhood (FAO, 2016). In Nigeria, the demand for fishery products have grown more and more even with the acute shortage of fresh fish there is wide acceptance of frozen fish (Olopade, 2015). Frozen fish dominates other fish forms in total "wet equivalent" of fish consumption in Nigeria (Liverpool-Tasie et al., 2018).

Freezing preserves fish for extended periods because it prevents the growth of microorganisms that cause both food spoilage and foodborne illness. In Nigeria, the complexity of the marketing and distribution of frozen fish, couple with erratic power supply for cold storage warehouses to maintain constant freezing temperature on the fish and with higher ambient temperature make fish quality deteriorates very rapidly (Olopade, 2015). As a result of poor handling of frozen fish outside the low temperature storage space fish are being warmed and even thawed and need refreezing (Olopade 2015). Pourshamasian et al. (2012) reported that during frozen storage some of the deterioration still occurs in the stored food, during which the freezing rate and temperature fluctuation are affecting the extent of quality loss. The quality of raw material before processing is an important factor in determining the product quality.

Lack of cold storage facilities usually forces people to rely on the traditional processing methods of smoking to retain the quality and extend the shelf life of the imported frozen fish. However, the conditions of raw material are a very important factor for the quality and shelf life of the final product (Arason et al. 2014). If the fish is not fresh or is of low quality before processing, the final product quality is compromised. Fish smoking/processing will not improve the quality of the final product if the raw material was not good before processing (Oehlenschläger, 2014). Biochemical composition of the whole body indicates the fish quality. The assessment of the fish's proximate composition is important to know its nutritive value, and its better processing and preservation (Mridha et al. 2005). The principal components of fish muscle include water, protein and fat while the minor components include carbohydrates, minerals and vitamins, and extractives, such as, sugars, free amino acids and nitrogenous bases (FAO, 2014).

The measurement of some proximate profiles such as protein contents, carbohydrates, lipids, moisture contents and ash percentage is often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Watermann, 2000). Minerals components such as potassium magnesium, calcium, iodine, phosphorus are important for human nutrition (Erkan and Ozden, 2007).

Sardines are widely distributed throughout the tropical and subtropical seas including the Mediterranean and Black seas (Froese and Pauly, 2017). The genus *Sardinella* species is an essential source of food for the human population and thus contribute to food security. The demand and consumption of sardines are on the rise due to availability and low cost (Kirema, 2012). *Sardinella eba* is the more commonly consumed frozen fish in Nigeria. The aim of this study was to analyze and document the proximate composition and mineral elements content of frozen (stored at an abused temperature) and later smoked *Sardinella eba.*

MATERIAL AND METHODS

Samples of *Sardinella eba* were purchased from a frozen fish market in Port Harcourt, Nigeria. The fish samples were immediately transported in ice cooled boxes to the Department of Fisheries at the University of Port Harcourt for laboratory analysis. The fish samples were washed and prepared to remove all traces of blood staining and spilled enzymes before being immediately refrigerated to keep them fresh and free of pest and bacterial infestation. The *Sardinella eba* samples were divided into two parts; one was used to determine the proximate compositions of the frozen fish, and the other was dried using a traditional locally constructed smoking kiln, which was made using a drum with the base completely opened to allow free flow of smoke to enhance the smoking of the fish

Proximate composition of fish

Proximate analysis of the muscles was carried out according to standard methods of the Association of Official Analytical Chemists (AOAC, 2000) Moisture determination Moisture was determined gravimetrically by ° desiccation at 105 C and 77 mmHg for 5 h. % Moisture = initial weight (g) - final weight (g) x 100 % initial weight (g) Ash content Ash content of the sample was determined by incinerating in a muffle furnace at 600°C for 3 h. % Ash = weight of ash after heating x 100 % weight of fresh sample Protein content The total nitrogen was determined by the Kjedahl method (Vlieg, 1984). Total protein content was obtained by multiplying protein content by 6.25.

Crude fibre content Crude fibre was determined as loss in weight on ash after acid and base digestion of the sample. Lipid Extraction Lipid was extracted from the muscles of the fish, in triplicates, according to methods as described by Bligh and Dyer (1959) with slight modifications, as described by Widjaja et al. (2009). Five grams (5 g) of the muscle sample (1 g of liver) was homogenized with 80 ml methanol, 40 ml chloroform and 28 ml of distilled water for 2 minutes. Chloroform (40 ml) and distilled water (40 ml) was added and homogenization continued for about 2 minutes. After homogenization, it was filtered in a glass funnel, using a Whatmann No. 1 filter paper. The residue was put back in a fresh beaker and was re-homogenized with 40 ml chloroform: methanol (1:1 v/v) for about 30 seconds, then filtered. Filtrates were then combined and transferred to a separating funnel to allow for phase separation. The bottom chloroform layer was then collected after being passed through a 2.5 cm thick layer of anhydrous sodium sulphate (Na_2SO_4). The remaining aqueous layer was washed with 20 ml chloroform. The collected organic chloroform layer, containing the extracted lipids was then evaporated under vacuum at 40°C to remove the solvent and the obtained lipid kept in the refrigerator. The weight of the extracted lipid was recorded. Lipid Content (%) Lipid content (%) = amount of lipid extracted (g) x 100 % weight of original sample (g).

Mineral Determinations

For minerals analysis (Na, Mg, Fe, Mn, K, Ca, P, Zn) the ash samples were digested with 2.5 ml HNO_3 and 60% perchloric acids according to AOAC method (2005). The digested samples were used for selected minerals analysis, using atomic absorption spectrophotometer (Model A A-6200, Shimadzu, Corp., Kyoto, Japan). Two grams from the ash sample were placed in a digestion tube and pre-digested using10ml of HNO_3 and 1ml of HClO3 acids were added and temperature maintained at 135oC until the liquor was colourless. The digested liquors were then filtered through a whatman 1 filter paper and diluted to 25ml with distilled water. Suitable standard solutions were prepared and their absorbance measured to prepare a standard curve. The standard curve was used to calculate the concentration of mineral.

Data Analysis

The analyses were performed in triplicate and all data were expressed as mean ± SD and compared by student's t- test.

RESULTS AND DISCUSSION

Proximate composition of frozen and smoked samples of *sardinella eba*

The proximate compositions of smoked and frozen samples of *Sardinella eba* are presented in Table 1. The results of this study revealed variations in the values of proximate composition with all parameters were significant higher ($P<0.05$) in the smoked samples than in frozen samples apart from moisture content. Eyo (2001) reported low protein, crude fibre, ash, and high moisture, carbohydrate, and lipid content in frozen fish. It has been observed that the gradual denaturation of protein leads to a decrease in water holding capacity, thus when frozen fish is thawed, drip is produced and nutritional substances are drained away with the drip (Ciarlo et al. 1985).

The apparent increases in some proximate component contents after smoking were mainly due to water loss by evaporation during the process. In agreement with these results, similar changes in the content of essential nutrients in thermally treated fish were reported by Bastías et al. (2017).This finding is in agreement with the observations of Puwastien et al. (1999).

The percentage moisture content value ranged from 35.11±0.02 recorded for the smoked samples to 79.33±0.02 for frozen samples. Pereira et al. (2005) found 71.02% moisture in defrosted fresh sardines. Clucas and Ward (1996) reported that flesh from healthy fish contained 70–80 % water. The decrease in moisture content occurring during smoking is the consequence of water loss during this process. According to Sigurgisladottir et al. (2000), the weight loss is due to dehydration during smoking.

Akinwumi (2014), which found that freezing and smoking were more efficient preservation methods in terms of the retention of the protein value and reduction of moisture content. The percentage value of crude protein recorded for the frozen sample was 16.83±0.05 significantly lower than the smoked sample value of 48.84±0.05. Clucas and Ward (1996) reported that flesh from healthy fish contained (15–24%) protein. This result was similar to values reported in the muscles of other fish species, such as sardines and mackerel (Pourshamsian, 2012). It was observed that the crude fat content was significantly higher in the smoked samples (5.25±0.02) than in the frozen samples (0.33±0.01). Clucas and Ward (1996) reported that the flesh of healthy fish contained 1–22% fat. After the smoking process, the increase in lipid content observed could be the result of moisture evaporation (Eyo, 2001). Based on this result, the fish species can be classified as fatty fish. Fish are often classified according to fat content. Lean fish have <0.5% fat, semi-fat fish contain 0.5–2% and fatty fish have more than 2% (Clucas and Ward, 1996). The ash content in any fish sample is an indication of its mineral content (Huda et al., 2010). The ash contents were found to be in the range of 1.13±0.02 to 4.24±0.02 for frozen and smoked samples, respectively. Szenttamásy et al. (1993) found ash contents ranging from 0.08 to 1% for fresh fish meat. An increase in the ash content in smoked fish can be due to the significant reduction in the moisture content occurring during the smoking process. Fish smoking results in concentration of nutrients such as proteins and ash (Doe and Olley, 1983).

Further results revealed that the percentage of dry matter ranged from 20.47±0.02 for frozen samples to 64.90±0.02 recorded for smoked samples. The values of the nitrogen free extract of smoked samples (6.58±0.00) were significantly higher than the frozen samples (2.18±0.02).

Table 1. Percentage mean proximate values of frozen and smoked samples of *sardinella eba*

Parameters	***Frozen***	***Smoked***
%Crude Protein	16.83±0.05 [a]	48.84±0.05 [b]
%Crude Fat	0.33±0.01 [a]	5.25±0.02 [b]
%Crude Fiber	0.00±0.00	0.00±0.00
%Ash	1.13±0.02 [a]	4.24±0.02 [b]
%Moisture	79.33±0.02 [a]	35.11±0.02 [b]
%Dry Matter	20.47±0.02 [a]	64.90±0.02 [b]
%Nitrogen free extract	3.18±0.02 [a]	6.58±0.00 [b]

[ab]*Means with different superscript along same row are significantly different (p<0.05)*

Mineral compositions of frozen and smoked *sardinella eba*

Table 2 shows the mineral contents of the frozen and smoked *Sardinella eba.* Eight minerals were quantified in *S. eba*, including five macro minerals (Ca, K, P, Na, and Mg) and three trace minerals (Fe, Zn, and Mn), suggesting that these fish could be used as good sources of minerals. The smoking process contributed to multidirectional changes in the content of mineral elements in the fish. The mineral content values of the smoked fish samples in all the parameters studied were statistically significantly higher than those in the frozen fish samples, apart from the Fe element, which was very high in the frozen sample. Magnesium and Zinc were particularly abundant in the fish analysed. Fish meat is a rich source of minerals and the most abundant microelements are Zinc (Zn), Iron (Fe) and Copper (Cu) (Saadettin et al, 1999). Sofoulaki et al. (2018) reported this health benefit.

Fresh fish contains a significant amount of minerals in general, but processed fish, such as dried fish, have higher values (Kinsella 1986). Marimuthu et al. (2012) indicated that mineral contents in snakehead fish increased depending on the cooking methods. The results of mineral content in this study are in line with the findings of Adewoye et al. (2003) who reported that variations exist in the mineral composition of fish. The concentrations of Zn, Mn and Fe in the frozen and smoked samples are lower than the toxic levels described by (FAO/WHO 2001). The macro minerals (Ca, K, P, Na, and Mg) and three trace minerals (Fe, Zn and Mn) reported in this study were within the limits of FAO (2010) values for fish muscles.

Table 2. Mineral compositions of frozen and smoked *sardinella eba*

Minerals	Frozen	Smoked
%Ca	0.12±0.00 [a]	0.30±0.00 [b]
%P	0.08±0.00 [a]	0.25±0.00 [b]
%Mg	0.07±0.00 [a]	0.17±0.00 [b]
%K	0.09±0.00 [a]	0.21±0.00 [b]
%Na	0.07±0.00 [a]	0.15±0.00 [b]
Fe(mg/kg)	104.16±0.01 [a]	0.12±0.00 [b]
Zn(mg/kg)	10.58±0.02 [a]	18.56±0.02 [b]
Mn(mg/kg)	88.07±0.02 [a]	118.74±0.02 [b]

[ab]*Means with different superscript along same row are significantly different ($p<0.05$)*

CONCLUSION

The effects of freezing and smoking on the proximate composition and mineral contents of *Sardinella eba* were examined. Results reveal that freezing conserves the chemical composition of the fish species and the smoking process reduces the content of water, which contributed to the relative increase in the concentration of nutrients, including crude ash and crude protein, content of mineral elements and reduced the fat content. The overall results obtained from the present study indicated that both freezing and smoking processes are important preservation methods that could enhance the nutritive values of fish and possibly reduce post-harvest losses. It is concluded that *Sardinella eba* subjected to frozen abuse and smoking can still provide healthy fish in terms of minerals and nutrients.

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