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Effect of Different Processing Methods on the Nutrient Composition and Sensory Properties of *Ethmalosa fimbriata*

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Abstract

The proximate, mineral, microbial and sensory properties of *Ethmalosa fimbriata* processed using four different processing methods was evaluated. The methods include frying, smoking, oven-drying and boiling. The moisture content of the smoked sample was significantly different from the oven-dried. Protein, lipid, ash, fibre, carbohydrate, calcium, magnesium, potassium and phosphorus contents of the samples were significantly different in all the samples. Total heterotrophic bacteria, coliform and fungal counts were highest in the fried sample. The fried and oven-dried samples were most accepted. Frying and oven-drying can be employed for large scale processing of nutritionally high quality *E. fimbriata* to meet the protein needs of the populace in Nigeria.

1. Introduction

Sea foods are important source of food worldwide. They rank second as staple as well as cheap animal protein food after meat and poultry [1]. There is a growing interest in consumption of fish as a healthy alternative to red meat in recent times [2]. Demand for fish has been reported to exceed supply worldwide and even in Nigeria [3]. Available data on domestic fish supply showed an average supply of over 600 thousand tonnes and importation of more than 700 thousand tonnes [4]. Fish supplies more than thirty percent of the protein requirement of the average Nigerian's diet. Consumption of poorly processed fish leads to disease as fish is usually prone to bacterial contamination [5, 6].

To avoid fish spoilage, processing is inevitable. Fish processing not only increases its shelf life but also improves it nutritional quality. Fish processing industries have contributed greatly to the economy of Nigeria and created employment opportunities for her teaming population. In the past, fishing and fish processing was abandoned for only the less privileged in the society. Recently, available modern techniques for fishing has aroused interest of people from all works of life into fish farming. Fish being a product that is quick to perish, needs to be processed to increase its shelf life and availability [7].



Lack of adequate processing method has remained a constraint to the availability of fresh fish in most rural communities [8]. The differences in location, distance and time of fish capture could often led to decomposition and spoilage, hence the need for adequate preservation and processing method. Such a method must have the potential to retain significantly, the nutritional quality of the fish.

E. fimbriata is a popular fish consumed in Nigeria both as a source of protein and for taste. It is commonly known as bonga fish in the southern part of Nigeria. Jay [9] reported that most people in Nigeria prefer to use it fresh, but this desire has poorly been achieved due to its poor shelf life. This research therefore aimed at improving post-harvest loss of *E. fimbriata* by the use of improved processing techniques.



Figure 1. Ethmalosa fimbriata.

2. Materials and Methods

2.1. Material Procurement

Fresh *E. frimbriata* were obtained from Oron beach market in Oron Local Government area of Akwa Ibom state and kept in sterile containers. They were transported to the laboratory in polystyrene boxes which were perforated at the upper side so as to allow for entrance of air. Identification of the sample was carried out in the Department of Fisheries and Aquaculture, University of Uyo. All the chemicals and reagents used were of analytical grade and obtained from Departments of Food Science and Technology and Microbiology respectively.

2.2. Sample Preparation

The procured fish samples were carefully cleaned to remove slime, blood and harmful bacteria. They were eviscerated followed by gills removal, leaving the skin on the fish. The fish were cut into uniform pieces (fillet). After evisceration, they were dipped into 80% brine solution and were allowed to stay for 5 minutes and thereafter salted using "Kent salting" and kept in sterile container. The fish samples were then divided into five parts and labeled A, B, C, and D. Sample A was fried, sample B smoked dried, sample C oven dried and sample D cooked. All the samples were subsequently subjected to the same analyses.

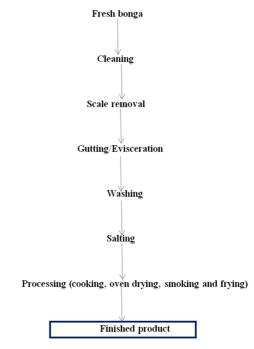


Figure 2. Flow chart for the production of processed fish sample (Source: Essuman, [10]).

2.3. Proximate Composition

2.3.1. Protein Content Determination

The protein content was determined using a micro-kjedahl method as described by AOAC [11], (2005). The sample (1g) was accurately weighed into a standard 250ml kjedahl flask containing 1.5g CuSO₄ and 1.5g of Na₂SO₄ as catalyst and 5ml concentrated H₂SO₄. The kjedahl flask was placed on a heating mantle and was heated gently to prevent frothing until a clear bluish solution was obtained. The digested solution was allowed to cool, quantitatively transferred to 100ml standard flask and made up to the mark with distilled water. Twenty (20) ml proportion of the digest was pipetted into a semi micro kjedahl distillation apparatus and treated with equal volume of 40% NaOH solution. The ammonia evolved was steam distilled into a 100ml conical flask containing 10ml solution of saturated boric acid to which 2 drops Tashirus indicator (double indicator) was added. The tip of the condenser was rinsed with a few millimeters of distilled water in the distillate which was then titrated with 0.1M HCl until a purple- pink endpoint was observed. The crude protein was obtained by multiplying the % Nitrogen content by a factor (6.25).

Calculation

% Nitrogen =
$$\frac{(\text{Sample titre - Blank titre})}{\text{Weight of sample}} \times \frac{0.1}{10} \times 0.014 \times 20 \times 100 \times 6.25$$
(1)

Crude Protein = % Nitrogen \times Kjedhal factor

(2)

2.3.2. Fat Content Determination

The method described by AOAC [11] was employed. The sample (2g), wrapped in a filter paper was placed in a clean extractor thimble. One hundred and fifty (150) ml of petroleum ether (boiling point: $60^{\circ}C-80^{\circ}C$) was poured into 250ml capacity round bottom flask. The soxhlet extractor was fitted into the round bottom flask which was settled on a heating mantle. The soxhlet apparatus was assembled and allowed to reflux for about 4 hours. The extract was poured into a dried pre-weighed beaker (W₁) and the thimble was raised with a little quantity of the ether back to the beaker. The beaker was heated on a steam bath to evaporate the excess solvent. The beaker was then cooled in desiccator and weighed (W₂).

Calculation:

% Total fat content =
$$\frac{W_2 - W_1}{Weight of sample} \times \frac{100}{1}$$
 (3)

Where:

 W_2 = weight of beaker + fat

 W_1 = weight of empty beaker only

2.3.3. Ash Content Determination

Ash content was determined using the method described by AOAC [11]. The sample (5g) was put into pre-weighed crucible. The weight of the crucible and its content (sample) was taken (W₂). The crucible and its content was then transferred to a muffle furnace and heated at 500°C- 600°C to burn off all the organic matter. It was left at this temperature for 4 hour. The crucible was allowed to cool in a desiccator and weighed (W₃).

% Ash =
$$\frac{\text{Weight of ash}}{\text{Weight of sample}} \times \frac{100}{1}$$
 (4)

$$= \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1} \tag{5}$$

Where;

 W_1 = weight of empty crucible

 W_2 = weight of crucible + sample before ashing

 W_3 = weight of crucible + ash

2.3.4. Crude Fibre Determination

Crude fibre was determined using the method described by AOAC [11]. The sample (2g) was defatted with petroleum ether for 2hours. It was boiled under reflux for 30 minutes with 200ml of 1.25% of H_2SO_4 . It was then filtered and washed with boiling water. The residue was transferred into a beaker and boiled for another 30 minutes with 200ml of a solution containing 1.25g of NaOH per 100ml. The final residue was filtered and washed with boiling water. The residue was filtered into pre-weighed crucible and oven dried at $105^{\circ}C$. It was then incinerated in a furnace at $550^{\circ}C$ for 4 hours. It was cooled in a desiccator and reweighed.

Calculation:

% Crude fiber =
$$\frac{1_a - 1_o}{\text{Weight of original sample taken}} \times \frac{100}{1}$$
 (6)

Where;

 I_0 = weight of sample crucible

 I_a = weight of crucible and its content after incineration (Ash fiber)

2.3.5. Total Carbohydrate Determination

Carbohydrate content was determined by difference using the method of Ihekoronye and Ngoddy [12]. This was done by subtracting the total sum of the percentage fat, ash, crude fibre and protein content from 100.

Calculation:

% Total carbohydrate = 100 - (protein + fat + moisture + ash + crude fibre) (7)

2.3.6. Total Energy Determination

The calorie value was calculated using Atwater factor as described by Osborne and Voogt, [13] (1978). The formula is as follows:

Caloric value = 4 Protein \times 9 fat + 4 carbohydrate (Kcal/g) (8)

2.4. Determination of Mineral Content

The samples were ashed at 550°C in a muffle furnace for 4 hours. The ash was dissolved in 10% HCl and made up with distilled water to 100ml in a standard flask. Calcium was analyzed by alpha Atomic Absorption Spectrophotometer. Sodium and potassium were estimated by corning 405 flame photometer [14]. Phosphorus was analyzed by employing vanadomolybdate methods and absorption was read on colorimeter AOAC [14]. Three determinations were made for each treatment.

2.5. Determination of Microbial Flora of the Fish Samples

2.5.1. Plating Technique

A portion of 1g of macerated fish sample was dissolved in 1ml of distilled water. One ml of each was aseptically pipetted and dissolved in 9ml of sterile distilled water in test tubes. A quantity of 1ml of the aliquot was pipetted and transferred into a second 9ml of sterilized water in other test tubes (10^{-2}) . The serial dilution procedure was conducted in 10^{-1} to seven (10^{-7}) test tubes designated diluents. One ml of the diluents was plated out on each sterile petri-dish. Using aseptic techniques, the medium was poured and gently swirled to obtain a uniform distribution of organisms. Thereafter, it was allowed to set and incubated at 37° C for 24 to 48 hours. After the incubation of the inoculated culture plates at appropriate condition (temperature and time), the plates were examined for their cultural characteristics.

2.5.2. Determination of Total Coliform Count

Molten MacConkey Agar 5.3g was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was sterilized in an autoclave at 121°C for 15 minutes at 15psi. The sterile molten medium was allowed to cool to approximately 45°C before it was poured into plates

containing 1ml of the diluents, gently rotated and allowed to set on a bench top, packed and incubated at 37°C for 24 to 48 hours. Colonies that developed after incubation with milky colour were counted and reported as cfu/ml [15].

2.5.3. Determination of Total Bacterial Count

A portion of 2.8g of Nutrient Agar was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was sterilized in an autoclave at 121°C for 15 minutes at 15psi. The sterile molten medium was allowed to cool to about 45°C before it was poured into Petri-dishes each containing 1ml of the diluents and gently rotated. The plates were allowed to set, packed and incubated at 37°C for 24 to 48 hours. Colonies that developed after incubation were counted and reported in cfu/ml [16].

2.5.4. Determination of Total Mycological Count

A portion of 6.3g of Sabourad Dextrose Agar (SDA) was weighed and mixed in 100ml of distilled water in a 25ml conical flask. The mixture was sterilized in the autoclave at 121°C for 15 minutes at 15 pound per square inch (15psi). On cooling to about 45°C the sterile medium was poured into petri-dishes containing diluents, and gently rotated to obtain homogenous distribution of organisms. It was allowed to set on a bench top and incubated at ambient temperature for 5 to 7 days. Colonies of moulds and yeast were observed after incubation, enumerated and reported in cfu/ml of sample [15].

2.5.5. Determination of total *Escherichia coli* Count

A quantity of 4.3g of Eosin Methylene Blue Agar was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was sterilized as usual in an autoclave at 121°C for 15 minutes at 15psi. The sterile molten medium of about 45°C was poured into plates containing 1ml of the inoculums and gently rotated to obtain a uniform distribution of organism. The plates were allowed to set and incubated at 37°C for 24 hours. Colonies that developed after incubation with greenish colour were counted and reported as cfu/ml [15].

2.5.6. Determination of Total Salmonella and Shigella Count

A quantity of 3.8g of Salmonella/Shigella Agar (SSA) was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was sterilized in an autoclave at 121°C for 15 minutes at 15psi. The lukewarm molten medium was poured into plates containing 1ml of diluents and gently rotated to obtain a uniform distribution of organisms. It was allowed to set and incubated at 37°C for 24 to 48 hours. Colonies that developed were counted and reported as cfu/ml [15].

2.6. Sensory Evaluation

The sensory evaluation was carried out according to the method described by Iwe [17]. A 20 member semi-trained panelist selected from the students of the Department of Food Science and Technology, University of Uyo were used for the sensory evaluation of the product. A 9-point hedonic scale (with a score of 1- indicating dislike extremely, 2-dislike very much, 3- dislike moderately, 4- dislike slightly, 5- neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, and 9-like extremely). The panelists were presented with coded samples labeled 546, 624, 426, and 428 that have been well packaged. The panelists were allowed to evaluate such attributes as appearance, aroma, taste, texture and general acceptability. Spoons and water were provided for the panelists to rinse their mouths between each successive evaluation.

2.7. Statistical Analysis

The data obtained were expressed as mean \pm standard deviation. One way analysis of variance (ANOVA) was used to determine whether there were any significant difference (P< 0.05) between the mean of samples using the Statistical Package for Social Statistics (SPSS Version 20). Means were separated using the Duncan's Multiple Range Test (DMRT).

3. Results

3.1. Proximate Composition of Processed Fish Samples

The result of proximate composition of the processed fish samples is as shown in Table 1. Result showed that the moisture content of samples ranged between 15.30 - 18.68%. The moisture content of samples B and C were significantly different. The protein, lipid, ash, fibre and carbohydrate content of all the samples were significantly different from each other.

Table 1. Proximate composition of processed fish samples.

Parameters	Α	В	С	D
Moisture (%)	15.30 ^b ±0.05	$18.68^{a}\pm0.02$	16.33°±0.03	15.30 ^b ±0.05
Protein (%)	41.04°±0.02	42.34 ^b ±0.01	50.33ª±0.03	$40.28^{d}\pm0.02$
Lipid (%)	20.33 ^b ±0.02	$16.46^{\circ} \pm 0.04$	23.60 ^a ±0.05	$15.32^{d} \pm 0.02$
Ash (%)	1.70°±0.02	1.94 ^b ±0.02	$0.42^{d}\pm 0.02$	2.63ª±0.02
Fibre (%)	10.34 ^b ±0.04	9.06 ^d ±0.03	9.18°±0.02	16.14 ^a ±0.02
Carbohydrate (%)	26.59 ^b ±0.01	30.20 ^a ±0.02	$16.47^{d}\pm0.03$	25.63 ^c ±0.03

Values are means of triplicate determinations \pm standard deviation.

Means with different superscript are significantly different (P < 0.05) along rows and column.

Key: A = Fried; B = Smoked; C = Oven dried; D = Boiled

3.2. Mineral Composition of the Processed Fish Samples

The mineral composition of the processed fish samples is as shown in Table 2. Calcium, magnesium, potassium and phosphorus content were all significantly different for all the samples.

Table 2. Mineral composition of processed fish samples.	
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Parameters	Α	В	С	D
Calcium (mg/g)	80.33 ^d ±0.02	94.20 ^b ±0.05	150.78 ^a ±0.02	89.66°±0.03
Magnesium (mg/g)	109.33 ^d ±0.03	120.42 ^b ±0.02	143.74 ^a ±0.01	122.30°±0.05
Potassium (mg/g)	$161.80^{d} \pm 0.02$	230.33 ^b ±0.02	264.66 ^a ±0.03	201.52°±0.01
Phosphorus (mg/g)	110.22 ^b ±0.01	108.31°±0.01	112.80 ^a ±0.05	$104.30^{d}\pm0.05$

Values in this Table are means of triplicate determinations \pm standard deviation.

Means with different superscript are significantly different (P < 0.05) *along rows and column.*

Key: A = Fried; B = Smoked; C = Oven dried; D = Boiled

3.3. Result of the Sensory Evaluation of the Processed Fish Samples

The result of the sensory evaluation is as presented in Table 3. It shows that appearance, colour, taste, texture, and acceptability were not significantly different in all the samples.

Parameters	A	В	С	D
Appearance	3.20 ^a ±1.60	2.95 ^a ±1.31	2.65 ^a ±1.42	2.65 ^a ±1.42
Colour	3.05 ^a ±1.35	3.10 ^a ±0.96	2.80 ^a ±1.54	3.40 ^a ±1.50
Aroma	2.90 ^b ±1.51	$3.20^{ab} \pm 1.60$	$3.20^{ab} \pm 1.36$	4.05 ^a ±2.06
Taste	2.60 ^a ±1.53	3.05 ^a ±1.31	3.05 ^a ±1.53	3.20 ^a ±1.23
Texture	3.00 ^a ±1.45	3.45 ^a ±1.66	3.15 ^a ±1.22	3.70 ^a ±1.59
Acceptability	2.80 ^a ±1.43	3.30 ^a ±1.78	$2.80^{a}\pm1.28$	3.50 ^a ±1.39

Values are means of triplicate determinations \pm standard deviation.

Means with different superscript are significantly different (P < 0.05) along rows and column.

Key: A = Fried; B = Smoked; C = Oven dried; D = Boiled

3.4. Result of Microbial Analysis from the Processed Fish Samples

The total heterotrophic bacterial count, total coliform count and total fungal count ranged from 1.3×10^3 to 0.7×10^3 , 1.0×10^3 to 4.0×10^3 and 1.0×10^3 to 3.0×10^3 respectively.

Table 4. Total microbial counts from processed fish samples.

TEST	Α	В	С	D
THBC (Cfu/g)	6.0×10^3	4.0×10^3	1.3×10^{3}	0.7×10^3
TCC (Cfu/g)	4.0×10^3	3.0×10^{3}	1.0×10^{3}	3.0×10^3
TFC (Cfu/g)	3.0×10^3	2.0×10^3	1.0×10^{3}	3.0×10^3

KEY: A = Fried; B = Smoked; C = Oven dried; D = Boiled

THBC = Total Heterotrophic Bacterial Count

TCC = Total Coliform Count

TFC = Total Fungal Count

4. Discussion

The moisture content is a measure of how well a product can store. The range observed in the samples compared reasonably well with that reported by Akinneye *et al.* [18]. Protein content of samples ranged from 40.28-50.33%. Although all the samples showed reasonable protein mean values which is in-line with that reported by Opara [19]. Oven dried sample had the highest protein content (50.33%). This means that oven drying is the best method in reducing protein loss during processing. Fat content was found to be highest in sample C (Oven dried sample). Oven dried sample retained more fat and is liable to become rancid due to fat oxidation if not properly stored or packaged. Sample D (Boiled sample) has the least fat content. The reduced fat content of the boiled sample may have been due to the loss of fat components during the boiling process. Ash content is an indication of the mineral content of a product [20]. Values close to 0.5% ash contents are good representation of the mineral content [20]. The result obtained in this study reveal that all the samples were of good ash content except sample C. The low ash content of sample C may have been due to the loss of volatile mineral components of the sample during the oven drying process. Foods rich in crude fibre, have been found to aid peristalsis movement of food through the digestive tract [21]. Crude fibre values obtained in the study

were in-line with that reported by Omojowo and Raji [22]. The carbohydrate content showed that the product is a poor source of carbohydrate as indicated in table 1 [23]. Nutritionally, the processed fish samples had reasonable proximate parameters.

All the four samples had reasonable mineral values (calcium, magnesium, potassium and phosphorus) (Table 2). This is attributed to the high ash content of all the samples except sample C. The high mineral mean values of sample C for all the minerals despite the low ash content may have been due to low level of oxalic and phytic acids being the main chelators of essential minerals thereby releasing them for biological activities [24].

The sensory scores for the processed fish sample indicate that all the samples were generally accepted. Sample C was most preferred in terms of appearance and colour. Sample A was most preferred in terms of aroma and texture whereas sample A and C shared the same preference for general acceptability. This indicates that these processing techniques will be widely acceptable if adopted in commercial production.

The microbial count of the processed fish samples indicates that sample A (fried) had the highest Total Heterotrophic Bacterial and coliform count. Coliform count is a measure of the sanitary condition of that product. The coliform count obtained in this study is within acceptable permissible limit as reported by Jay [9] (2011). Total fungal count ranged from $1.0x10^{3}$ cfu/g to 3. $0x10^{3}$ cfu/g with sample A and D having the highest fungal count. The scanty count witnessed on Total Heterotrophic Bacterial Count plate may have been due to the effectiveness of the processing methods used which greatly inhibited the growth of enteric bacteria on the samples. Total fungal count was highest in sample A and least in sample C. All the samples had microbial load less than 10^{5} cfu/g recommended for consumption by Jay [9] (2000). This means that all the samples were safe for consumption.

5. Conclusion

E. fimbriata has not received proper utilization due to its perishability and the presence of sharp, pin-like bones. However, the findings of this experiment have shown that it could be well-utilized as a processed fish product using processing methods like cooking, frying, oven drying and smoking which not only softened the pin-like bones but also preserved the product from spoilage organisms. This product which serve as a complementary food delicacy with very little patronage could be used as a veritable source of nutrient especially protein, fat and minerals for all classes of people, if proper processing technique is used.

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