



## **Comparative Analysis of the Nutritive Value of Smoked, Dried *Procambarus clarkii* (Freshwater Crayfish) from Three Nigerian States**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author OAO designed the study, Authors OBA and OTM sourced for the samples while authors OJO and AAB managed the analyses. Authors OAO and AAB wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The nutritive value of smoked, dried *Procambarus clarkii* sourced from Akwa Ibom, Rivers and Lagos states of Nigeria were evaluated and compared. The proximate composition of the crayfish samples were determined using official methods of analysis, mineral composition were determined using Atomic Absorption Spectrophotometer and the amino acid composition were analysed using Amino Acid Analyser. The proximate composition of the crayfish samples from three different locations (Akwa Ibom, Lagos and Rivers states) such as moisture, crude protein, crude fat and ash contents showed significant difference ( $p < 0.05$ ) across the selected locations while crude fibre and carbohydrate content showed no significant difference ( $p < 0.05$ ) across the selected locations. The amino acids composition gave the crayfish sample from Akwa Ibom state as the richest source of essential amino acids. The mineral contents of the crayfish samples such as potassium, phosphorus, magnesium, manganese and calcium showed significant difference ( $p < 0.05$ ) across

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the selected locations while calcium, zinc and sodium contents showed no significant difference ( $p < 0.05$ ) across the selected locations. Comparing their nutrient component, smoked, dried *P. Clarkia* from Awka Ibom state possess the richest nutritive value.

**Keywords:** Comparative; smoked; dried; *Procambarus clarkii*; Akwa Ibom; Rivers; Lagos.

## 1. INTRODUCTION

Crayfish is a freshwater crustacean categorized as animal polypeptide consisting of large amount of protein [1], it was reported to be a relatively cheaper source of protein than other animal proteins and possess high nutritive value [2]. It is reported to have no accumulation of heavy metals and chemicals because it is sensitive to polluted waters, hence, not toxic for consumption [1]. Crayfish is sold and consumed all over the world in various dishes. Crayfish is very low in carbohydrates, fat and easily digested than any type of meat due to its short muscle fibre which can prevent unnecessary weight gain when consumed [3,1].

Crayfish also known as crawfish are regarded as members of the super family Asteroidean and Parastacoidea [4]. *P. clarkii* is found in lentic and lotic freshwater habitats such as sluggish streams, swamps, ditches, sloughs and ponds, it avoids streams and ditches with strong flow where it is replaced by other species [5]. Fresh water Crayfish (*Procambarus clarkii*) are highly regarded and have had much impact on the economy of Louisiana state, USA both in aquaculture and wild capture which contribute about USD 150 million annually [6]. Supply of wild harvest of *P. clarkii* varies from year to year due to seasonal fluctuations, this fact made entrepreneurs to begin experimenting the farming of crayfish in order to fill the gap between consumption and production, this made pond reared crayfish constitutes the principal of the annual harvest of Louisiana which accounts for 90-95% of the total production in USA [6].

It is estimated that 12,000 metric tonnes of crayfish is usually produced annually in Nigeria which is low compared to the consumption, whereas, the demand for crayfish has been increasing [7]. Crayfish is usually prepared for consumption by smoking, and occasionally sundried to form an indispensable food in the diet of Nigerians [8]. Just like fish, diet, habitat, species and many factors may affect the chemical composition of crayfish. This research work is aimed at comparing the nutritive value of

smoked dried *Procambarus clarkii* from Akwa Ibom, Rivers and Lagos states of Nigeria.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Samples

Smoked, dried fresh water crayfish (*Procambarus clarkii*) was bought from three states in Nigeria (Agbalata market, Badagry Local Government, Badagry, Lagos State, Ikot Ambang market, Ikot Ekpene road, Ibiono Ibom Local Government, Uyo, Akwa Ibom State and Okrika market, Okrika Local Government, Port Harcourt, Rivers State. The smoked, dried crayfish samples were kept in clean, dried air tight containers for the period they were been analysed.

### 2.2 Determination of Proximate Components of the Crayfish Samples

The moisture content and crude fat were determined according to the methods of [9] while crude fibre and ash content were determined according to the methods of [10]. The crude protein was determined according to the method [11] with some modifications. The carbohydrate content was determined by the method of [12].

#### 2.2.1 Moisture content

The crayfish samples (2 g) each was placed in a crucible and dried in an oven at 105°C, cooled in a desiccator and dried again till constant weights were achieved. The percentage moisture content was calculated as:

$$\% \text{Moisture} = \frac{(\text{Initial weight} - \text{Final weight}) \times 100}{(\text{Weight of sample})}$$

#### 2.2.2 Ash content

The sample (5 g) each was weighed into a previously dried, cooled and weighed silica crucible. The crucible containing the sample was transferred into a muffle furnace and ignited at 550°C until a white ash was obtained. The ash was moistened with distilled water, dried on steam bath and then on hot-plate and re ashed

at 550°C to constant weight. The percentage ash content was calculated as follows:

$$\text{Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

### 2.2.3 Crude protein

The crude protein content was determined using microkjeldahl method. The digested samples were diluted, made alkaline with NaOH and distilled water. Liberated ammonia gas was trapped in a conical flask containing boric acid solution. The conical flask was positioned such that the steam of the condenser dipped into the boric acid solution. After collecting about 50cm<sup>3</sup> of the distillate, the receiver was lowered and the tip of the condenser was washed with distilled water, the ammonia solution in the distillate was titrated against 0.1M HCl. A blank determination was carried out using the same amount of the reagents in the absence of the sample.

$$\% \text{Nitrogen Content} = \frac{(\text{Titre value} \times M \times 0.0014 \times Df \times Cf)}{(\text{Weight of sample})}$$

Where:

M = Molarity of HCl = 0.01M

Df = Dilution factor = 50

Cf = Correction factor = 10

%Crude protein = %Nitrogen x 6.25

%Nitrogen was converted to percent crude protein by multiplying with 6.25, the conversion factor. Most proteins contain 16% Nitrogen, hence, the conversion factor is 6.25 (100/16 = 6.25).

### 2.2.4 Crude fat

Accurately weighed (2 g) of each sample was placed in extraction apparatus. Extraction was completed in 8 hrs and the solvent used (petroleum ether) was recovered using rotary evaporator. The extracted lipid was evaporated to dryness to completely remove all the solvent residues and then placed in a desiccator to cool. The percentage of lipid was calculated using the equation below:

$$\text{Weight of lipid} = \text{Weight of flask and content after extraction} - \text{Weight of flask before extraction}$$

### 2.2.5 Crude Fibre

100 ml of 0.25M H<sub>2</sub>SO<sub>4</sub> was added to 2 g each of the crayfish samples and brought to boil for 30 mins after which the hot mixture was filtered. The residue was washed free of acid with plenty of warm water. Each residue was transferred into

round bottom flasks to which 100 ml of 0.25M of NaOH was added and boiled again for 30 minutes. The mixture was then filtered and the residue washed free of alkali with warm water. The residue was then transferred into a dried, weighed silica dish and dried to a constant weight at 105°C for 90 mins, cooled in a desiccator and weighed. The weighed samples were burnt off and reweighed. The percentage of crude fibre was determined as follows:

$$\text{Initial weight of residue} - \text{Final weight of residue} \times 100$$

### 2.2.6 Carbohydrate

The carbohydrate content was determined by difference i.e.

$$\% \text{Carbohydrates} = 100 - (\% \text{Mo} + \% \text{As} + \% \text{Cf} + \% \text{Cp})$$

Where; %Mo= Percentage moisture content

%As= Percentage ash content

%Cf= Percentage crude fat

%Cp= Percentage crude protein

## 2.3 Determination of Mineral Composition of the Crayfish Samples

The mineral composition of the crayfish samples were determined according to the method adopted by [10].

The ash obtained by dry ashing the samples at 550°C in a muffle furnace during the determination of the ash content was dissolved in 5ml HNO<sub>3</sub>/HCl/H<sub>2</sub>O (1:2:3) and heated gently using heating mantle until the brown fumes disappeared. Distilled water (5mL) was added to the remaining content of the crucible and heated. The solution was filtered using whatman No 4 filter paper and made up to 100mL. Working standard solutions were prepared by diluting the stock solution. P, Mg, Fe, Ca, Zn and Mn in the fish samples were analyzed using atomic absorption spectrophotometer, (Model 215 VGP BUCK Scientific) equipped with flame and graphite furnace. Na and K was determined using flame photometer.

## 2.4 Determination of Amino Acid Composition of the Crayfish Samples

The amino acid profile of the crayfish samples were determined using amino acid analyser, technicon TSM-1 (model: DNA 0209) and methods described by [13]. The crayfish samples

were dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-sample Amino Acid Analyser (TSM). Approximately 200 mg each of the crayfish samples was dissolved in 0.7 mL distilled H<sub>2</sub>O and 0.5 mL 20 mM norleucine (internal standard). 500mL each of the extract was mixed with 50 mL 20 mM norleucine. Concentrated hydrochloric acid (HCl, 12 M) was added, to a final concentration of 6 M. The sample mixture was flushed with nitrogen gas for 15 s in order to minimize oxidation, before hydrolysis at 110°C for 24 h. Following hydrolysis, 100 mL aliquots of the hydrolysates were evaporated under nitrogen gas until complete dryness and re-dissolved to a suitable concentration in lithium citrate buffer at pH 2.2. All amino acids were analysed chromatographically using an ion exchange column followed by ninhydrin post column derivatization on a Biochrom 30 amino acid analyser (Biochrom Co., Cambridge, UK). Amino acid residues were identified using the A9906 physiological amino acids standard (Sigma Chemical Co., St. Louis, MO, USA).

### 3. RESULTS AND DISCUSSION

#### 3.1 Proximate Composition of the Crayfish Samples

Table 1 shows the proximate composition of the crayfish samples from three different locations (Akwa Ibom, Lagos and Rivers States). Parameters such as moisture content, crude protein, crude fat and ash content showed significant difference ( $p < 0.05$ ) across the selected locations. However, crude fibre and carbohydrate content showed no significant difference ( $p < 0.05$ ) across the selected locations. The crayfish sample from Akwa Ibom state had the lowest moisture content (3.27%). The results obtained for the moisture content was comparable to 3.10% obtained by [2] for freshly purchased smoked, dried *Penaeus monodon*.

Moisture content determines the water activity in a product. [14] deduced that both smoking and drying processes are responsible for low moisture contents in shrimps as the nutritional compositions of fresh, smoked and sundried *Penaeus monodon* were compared by this author. This indicates that crayfish purchased from Akwa Ibom state can be stored for a longer period compared to those from Rivers and Lagos states as it is widely known that low moisture content is an indication of shelf life extension. Crayfish sample bought from Akwa Ibom state also had the highest crude protein content (69.51%), this was close to 66.36% reported by [2] for freshly purchased smoked dried *Penaeus monodon* with 64.74% and 64.27% reported by [14] for smoked and sundried *Penaeus monodon* respectively. Crayfish sample purchased from Lagos State had the highest crude fat content (11.23%) but still lower than 14.95% reported by [2] for freshly purchased smoked, dried *Penaeus monodon*. This product may not be able to withstand long storage as it may be easily susceptible to oxidation. The smoking and drying processes the crayfish samples went through may be responsible for the high ash content. All the crayfish samples had high ash contents. Ash content in food gives the number of mineral elements present in a sample [15]. The crayfish samples studied all had low crude fibre and carbohydrate content. Glycogen which is not stored in marine animal's body is usually responsible for the low carbohydrate content [14].

#### 3.2 Amino Acids Composition of the Crayfish Samples

Table 2 shows the amino acids composition of the crayfish samples from three different locations (Akwa Ibom, Lagos and Rivers States). Parameters such as isoleucine, glutamate, methionine, phenylalanine, serine, glycine, alanine, tryptophan, arginine, proline and

**Table 1. Proximate composition of the crayfish (*Procambarus clarkii*) samples**

| Parameters (%)   | Akwa Ibom crayfish       | Lagos crayfish           | Rivers crayfish          |
|------------------|--------------------------|--------------------------|--------------------------|
| Moisture content | 3.27±0.015 <sup>c</sup>  | 3.76±0.023 <sup>a</sup>  | 3.41±0.12 <sup>b</sup>   |
| Crude protein    | 69.51±0.024 <sup>a</sup> | 68.79±0.05 <sup>c</sup>  | 69.22±0.01 <sup>b</sup>  |
| Crude fat        | 10.73±0.013 <sup>c</sup> | 11.23±0.018 <sup>a</sup> | 11.01±0.016 <sup>b</sup> |
| Crude fibre      | 1.17±0.015               | 1.03±0.03                | 1.12±0.023               |
| Ash content      | 13.14±0.12 <sup>a</sup>  | 12.78±0.025 <sup>c</sup> | 12.97±0.05 <sup>b</sup>  |
| Carbohydrate     | 2.18±0.01                | 2.41±0.014               | 2.27±0.05                |

Values are mean ± standard deviation of triplicate determinations

<sup>abc</sup>: Means within each row with different superscripts are significantly different ( $p < 0.05$ )

tyrosine showed significant difference ( $p < 0.05$ ) across the selected locations. However, histidine, leucine, threonine, valine, aspartic acid, lysine and cysteine showed no significant difference ( $p < 0.05$ ) across the selected locations. Glutamate composition was ranked highest among the amino acid in all the crayfish samples. This is in agreement with the reports of [16] for fresh *P. clarkii* and [14] for smoked and sundried *Penaeus monodon*. The high level of glutamate in dried products accounted for their high flavour and taste [14]. Leucine is the most abundant essential amino acid in all the crayfish samples. Leucine is usually one of the most abundant amino acids in high protein foods [17]. Leucine is important in preventing protein breakdown and muscle loss but should be consumed as part of a protein based diet rather than taken as supplement. Studies on rats that consumed 40% protein diet containing leucine recorded beneficial effects but different results occurred when leucine was simply dropped into the diet as a supplement [18]. Leucine is highly concentrated in animal sources but much less concentrated in plant sources [18]. Lysine is the next abundant essential amino acids in all the crayfish samples, lysine is important during fish spoilage due to its ability to produce biogenic amines by decarboxylation [19]. Serine which helps in fatty acid metabolism is the least amino

acid present in all the samples [20]. *P. clarkii* from Akwa Ibom state had the highest value of essential amino acids (56.16%) which indicates crayfish sample from this state is a rich source of essential amino acids compared to those from Rivers and Lagos states.

### 3.3 Mineral Contents of Composition of the Crayfish Samples

Table 3 shows the mineral contents composition of the crayfish samples from three different locations (Akwa Ibom, Lagos and Rivers States). Parameters such as zinc, potassium, phosphorus, magnesium, manganese and calcium showed significant difference ( $p < 0.05$ ) across the selected locations. Meanwhile, calcium, zinc and sodium contents showed no significant difference ( $p < 0.05$ ) across the selected locations. Crayfish sample from Akwa Ibom state had the highest mineral contents while the *P. clarkii* sample from Lagos state recorded lowest mineral contents. The result of the mineral composition showed phosphorus, sodium, potassium and magnesium having high concentration in all the crayfish samples. Phosphorus had the highest concentration of (381.40 mg/100 g, 315.10 mg/100 g and 338.25 mg/100 g) for crayfish samples from Awka Ibom,

**Table 2. Amino acid composition of the crayfish (*Procambarus clarkii*) samples**

| Amino acids (g/100 g) | Akwa ibom crayfish       | Lagos crayfish           | Rivers crayfish         |
|-----------------------|--------------------------|--------------------------|-------------------------|
| Histidine             | 5.38±0.012               | 4.61±0.025               | 5.29±0.03               |
| Isoleucine            | 3.99±0.06 <sup>c</sup>   | 4.89±0.018 <sup>a</sup>  | 4.76±0.023 <sup>b</sup> |
| Leucine               | 10.21±0.028              | 9.87±0.024               | 10.15±0.026             |
| Lysine                | 9.45±0.012               | 9.34±0.015               | 8.63±0.08               |
| Methionine            | 7.12±0.03 <sup>a</sup>   | 7.03±0.032 <sup>b</sup>  | 6.95±0.025 <sup>c</sup> |
| Phenylalanine         | 6.62±0.014 <sup>c</sup>  | 8.47±0.024 <sup>a</sup>  | 7.83±0.03 <sup>b</sup>  |
| Threonine             | 5.64±0.034               | 4.11±0.05                | 4.37±0.023              |
| Valine                | 6.77±0.013               | 4.92±0.08                | 5.54±0.015              |
| Tryptophan            | 0.98±0.03 <sup>a</sup>   | 0.0 <sup>c</sup>         | 0.69±0.12 <sup>c</sup>  |
| ΣEAA                  | 56.16                    | 53.24                    | 54.21                   |
| Glutamate             | 12.01±0.034 <sup>a</sup> | 11.65±0.014 <sup>c</sup> | 11.77±0.06 <sup>b</sup> |
| Serine                | 1.67±0.02 <sup>b</sup>   | 1.98±0.013 <sup>a</sup>  | 1.57±0.06 <sup>c</sup>  |
| Aspartic acid         | 9.56±0.015               | 8.79±0.025               | 9.23±0.04               |
| Glycine               | 3.69±0.026 <sup>c</sup>  | 3.13±0.018 <sup>a</sup>  | 3.56±0.013 <sup>b</sup> |
| Alanine               | 3.24±0.02 <sup>c</sup>   | 6.86±0.043 <sup>b</sup>  | 8.18±0.024 <sup>a</sup> |
| Cysteine              | 1.08±0.08                | 1.12±0.016               | 1.14±0.028              |
| Arginine              | 4.50±0.015 <sup>b</sup>  | 3.92±0.008 <sup>c</sup>  | 4.67±0.012 <sup>a</sup> |
| Proline               | 1.75±0.024 <sup>a</sup>  | 1.35±0.034 <sup>c</sup>  | 1.69±0.015 <sup>b</sup> |
| Tyrosine              | 2.04±0.05 <sup>c</sup>   | 3.87±0.024 <sup>a</sup>  | 3.43±0.014 <sup>b</sup> |
| ΣNEAA                 | 39.54                    | 42.67                    | 45.24                   |
| TAA                   | 95.70                    | 95.91                    | 99.45                   |

Values are mean ± standard deviation of triplicate determinations

<sup>abc</sup>: Means within each row with different superscripts are significantly different ( $p < 0.05$ )

**Table 3. Mineral composition of the crayfish (*Procambarus clarkii*) samples**

| Minerals (mg/100 g) | Akwa Ibom crayfish        | Lagos crayfish            | Rivers crayfish           |
|---------------------|---------------------------|---------------------------|---------------------------|
| Iron                | 37.50±0.02 <sup>a</sup>   | 17.25±0.016 <sup>c</sup>  | 28.90±0.023 <sup>b</sup>  |
| Zinc                | 15.12±0.05                | 9.19±0.018                | 11.12±0.026               |
| Sodium              | 180.00±0.023              | 118.20±0.03               | 132.71±0.02               |
| Potassium           | 165.10±0.015 <sup>a</sup> | 106.32±0.035 <sup>c</sup> | 124.10±0.018 <sup>b</sup> |
| Phosphorus          | 381.40±0.03 <sup>a</sup>  | 315.10±0.013 <sup>c</sup> | 338.25±0.05 <sup>b</sup>  |
| Magnesium           | 126.70±0.04 <sup>a</sup>  | 99.26±0.016 <sup>c</sup>  | 115.40±0.034 <sup>b</sup> |
| Manganese           | 4.12±0.023 <sup>a</sup>   | 2.32±0.012 <sup>c</sup>   | 3.96±0.02 <sup>b</sup>    |
| Calcium             | 145.43±0.035              | 136.25±0.013              | 129.40±0.05               |

Values are mean ± standard deviation of triplicate determinations

<sup>abc</sup>: Means within each row with different superscripts are significantly different ( $p < 0.05$ )

Lagos and Rivers states respectively which is lower than the recommended standard value of 700 mg [2]. This is slightly lower than 485mg/100g reported by [2]. Phosphorus is required for DNA and RNA synthesis but this study shows the crayfish samples are not good source of phosphorus. Calcium helps in muscle contraction and relaxation, blood clotting and absorption of vitamin B<sub>12</sub> [21]. The calcium concentration in this study is lower than the recommended value for calcium which is between 600-1400 g [22]. Hence, the crayfish samples are not good source of calcium. Iron has long been recognized as an essential constituent of haemoglobin, the red colouring matter of blood. Deficiency of iron results in nutritional anaemia, a condition in which the blood is deficient in red colouring matter [23]. The iron content of the crayfish samples (37.50 mg/100 g, 17.25 mg/100 g, and 28.90 mg/100 g) from Akwa Ibom, Lagos and Rivers states respectively were in close agreement to the result of [16] for fresh *P. clarkii* but higher than 12.33 mg/100 g reported by [2] for freshly purchased smoked, dried *Penaeus monodon*. However, in all the samples, the crayfish sample from Akwa Ibom state had the highest concentration of essential minerals.

#### 4. CONCLUSION

Comparing the proximate, mineral and amino acid composition of the crayfish samples, smoked, dried *P. clarkii* sourced from Akwa Ibom state had the highest nutritional value and rated the best of all the crayfish samples. However, this does not mean that smoked, dried *P. clarkii* sourced from Rivers and Lagos state are of low quality as they are also good source of nutritional diet to consumers.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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