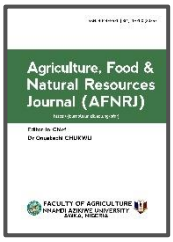
*Agr. Foo. Nat. Res. J. Volume 4, Issue 1, Page 79 - 85*

**e-ISSN: 1597-7153 | p-ISSN: 3043-5420**

**Agriculture, Food and Natural Resources Journal**

***The Official Journal of the Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Nigeria***

Journal homepage: https://journals.unizik.edu.ng/afnrj




Original Article

# Nutritional safety assessment of giant tiger shrimp (*Penaeus monodon*): Impact of smoking

OPEN ACCESS

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**DOI:** https://www.doi.org/10.5281/zenodo.15109501

***Editor:*** *Dr Onyekachi Chukwu, Nnamdi Azikiwe University, NIGERIA*

***Received:*** *January 5, 2025*
***Accepted:*** *February 13, 2025*
***Available online:*** *March 31, 2025*

***Peer-review:*** *Externally peer-reviewed*

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**Conflict of Interest:** *The authors have no conflicts of interest to declare*

***Financial Disclosure:*** *The authors declared that this study has received no financial support*

**ABSTRACT**

*This study examined the impact of smoking on the proximate composition, mineral content, mineral ratios, and mineral safety index (MSI) of the shell and flesh of the Giant Tiger Shrimp (Penaeus monodon). Fresh and smoked samples were analyzed using standard of the* Association *of Official Analytical Chemists' (AOAC) methods for proximate composition, while mineral content was determined via atomic absorption spectrophotometry. Mineral ratios and MSI were calculated to assess dietary safety. Results showed that smoking significantly reduced moisture content, leading to a concentration of nutrients. Protein, crude fat, total ash, and nitrogen-free extract (NFE) increased notably in smoked samples compared to raw ones, with protein levels rising by 63.82% in the shell and 73.29% in the flesh. Mineral concentrations, including calcium, sodium, and potassium, also increased significantly post-smoking. While mineral ratios such as Ca/Mg, Ca/P, and Na/K mostly remained within recommended dietary limits, the Na/Mg ratio showed deviations. MSI analysis indicated that calcium and sodium levels exceeded recommended thresholds, suggesting potential health risks if consumed in excess. Given the high sodium content, moderate consumption of smoked P. monodon is advised, particularly for individuals with hypertension or cardiovascular concerns. Additionally, pairing smoked shrimp with potassium-rich foods may help balance sodium intake and mitigate potential health risks.*

K E Y W O R D S : *Biochemical profile, Mineral ratio, Mineral safety index, Shellfish, Shrimp*

## INTRODUCTION

Important crustaceans such as shrimp, prawns, crayfish, lobster, and crab are significant sources of nutritious food for humans, providing substantial amounts of dietary protein and lipids across many countries (Lawal-Are *et al*., 2021). As a key commodity in international fishery trade, there has been a notable increase in the global consumption of crustaceans (Manan and Ikhwanuddin, 2020). Shrimps, in particular, have become a highly traded seafood commodity, serving as a crucial source of foreign exchange earnings for numerous developing nations, with catches surpassing 336,000 tons in 2018 (FAO, 2020), while marine shrimps dominate the production of farmed crustaceans in coastal aquaculture systems and hold significant importance for both local economies and global markets.

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Penaeid shrimps are recognized as one of the most valuable resources in the global crustacean fishery and aquaculture sectors (Manan and Ikhwanuddin, 2020; Moruf *et al*., 2021a). Giant Tiger shrimp (*Penaeus monodon*) is among the most economically and nutritionally significant seafood globally. It serves as a vital source of high-quality protein, omega-3 fatty acids, vitamins, and minerals, making it an essential component of balanced diets (Abuzar *et al.*, 2023). Beyond its nutritional value, *P. monodon* supports livelihoods in aquaculture and fisheries, particularly in developing countries. However, its nutritional benefits and safety are influenced by post-harvest processing methods, which are critical in ensuring consumer health and meeting market standards.

Seafood is highly perishable due to its high-water activity, neutral pH, and nutrient-rich composition, which provide favorable conditions for microbial growth (Aberoumand *et al.*, 2024). Consequently, effective processing methods are required to extend its shelf life, enhance microbial safety, and preserve its nutritional quality. Among the commonly employed methods, hot smoking stands out for its wide usage and contrasting mechanisms.

Processing methods can broadly be categorized into heat treatments, such as hot-smoking, boiling, steaming, frying, and grilling, and non-heat treatments, including freezing, irradiation, high-pressure processing and marination. Heat treatments are widely adopted due to their efficacy in eliminating microbial pathogens and extending shelf life (Moruf *et al*., 2021b; Dash *et al*., 2022). Despite these advantages, heat treatments often degrade heat-sensitive nutrients, such as polyunsaturated fatty acids (PUFAs), vitamins, and antioxidants, thereby diminishing the nutritional quality of the shrimp (Tian *et al*., 2023). Non-heat treatments, on the other hand, have emerged as innovative alternatives aimed at preserving the nutritional and sensory qualities of seafood.

Hot smoking, a heat-based processing method, involves cooking seafood at elevated temperatures (typically 65–85°C) while exposing it to smoke. This process achieves dual objectives: microbial inactivation and flavor enhancement. However, it has been associated with certain drawbacks. The high temperatures used in smoking can degrade heat-sensitive nutrients such as polyunsaturated fatty acids and vitamins (Vishwakarma *et al*., 2024).

Despite the widespread use of this method, there is a limited understanding of it comparative effect with untreated form on the nutritional safety of shrimp. While studies have individually examined the impacts of hot smoking, there is insufficient evidence regarding how this method differs in preserving nutrients and maintaining overall quality. Addressing this issue is critical for informing public health guidelines, optimizing shrimp processing techniques, and meeting consumer expectations for safe and nutrient-rich seafood. This study seeks to fill this gap by conducting a comparative analysis of heat-treated and untreated Giant Tiger Shrimp, with a focus on their nutritional safety and implications for dietary recommendations.

## MATERIALS AND METHODS

### Sample Collection and Preparation

Samples of harvested *P. monodon* from Lagos Lagoon, were purchased from the Lagos Lagoon Makoko jetty in Lagos State, Nigeria, between November 2023 and April 2024. The harvested shrimps were immediately stored in an ice chest for transport and subsequently preserved in a laboratory deep freezer at –20°C for further chemical analysis. A total of 180 samples (30 individuals per catch) of uniform sizes were randomly selected for analysis. The samples were then separated into two parts; one part was analyzed raw, while the second part was subjected to oven-smoking. The process involves marinating the shrimp and arranging them on a wire rack or tray. The smoking operation utilizes charcoal as the smoke source, placed in a Lodge L8SK3 Cast Iron Skillet (Model: L8SK3), a heat-resistant pan. The shrimp are then placed in a preheated oven at a temperature range of 80–100°C and smoked for 15 minutes. This slow-cooking process allows the smoke to infuse the shrimp with a rich, smoky flavor while also enhancing their shelf life.

### Laboratory Analysis

Before analysis, the samples were separated into fillet extract and exoskeleton components. These components were dried and homogenized at 105°C. The crude protein content was determined using the Micro-Kjeldahl Method, while crude fat was extracted with the Soxhlet apparatus using a chloroform/methanol mixture (2:1 v/v). Moisture, ash, and crude fiber contents were measured following AOAC (2006) protocols, and carbohydrate content was calculated by difference. Mineral determination involved digestion of the samples in $HNO_3$/HCl, followed by analysis of elements such as Ca, P, Mg, Na, and K using the Buck Scientific 210 GVP model of the Varian Spectra Atomic Absorption Spectrophotometer, as per the procedure of Santoso *et al*. (2020). Mineral ratios and the Mineral Safety Index (MSI) were calculated using methods outlined by Watts (2010) and Hatcock (1985), respectively.

### Data Analysis

The data were subjected to descriptive statistics using Microsoft Excel (2010). The significance of the results (LSD test) at $p < 0.05$ was tested by means of a student T-Test.

## RESULTS AND DISCUSSION

### Proximate Composition

The proximate composition of the shell of Giant Tiger Shrimp reveals significant nutritional variations between raw and smoked samples (Table 1). Smoking reduced moisture content drastically from 79.07 g/100 g to 38.11 g/100 g, with a mean of 58.59 g/100 g, indicating a 51.8% reduction due to dehydration during smoking. Protein content increased notably from 12.77 g/100 g in raw shells to 20.92 g/100 g in smoked shells, a

63.82% enhancement attributed to moisture loss concentrating the protein content. Similarly, crude fat and total ash levels were significantly elevated in smoked samples, increasing by 324.76% and 189.04%, respectively, reflecting the concentration of lipids and minerals during the smoking process. Nitrogen-Free Extract (NFE) also rose markedly, with a 754.96% increase, indicating the effect of heat treatment on residual carbohydrate-like components. These findings align with study by Abankwa and Acquaye (2023), which reported that smoking reduces moisture content while concentrating proteins, lipids, and ash in crustaceans, thereby enhancing their nutritional density and potential use in value-added products. The high variability (CV%) across parameters highlights the significant impact of processing on the shrimp shell composition.

**Table 1: Proximate composition of Giant Tiger Shrimp (Shell)**

| Parameters | Raw (g/100 g) | Smoked (g/100 g) | Mean | SD | CV % | % D |
|---|---|---|---|---|---|---|
| **Moisture** | 79.07 | 38.11 | 58.59 | 28.96 | 49.43 | 51.80 |
| **Protein** | 12.77 | 20.92 | 16.85 | 5.76 | 34.21 | -63.82 |
| **Crude Fat** | 1.05 | 4.46 | 2.76 | 2.41 | 87.52 | -324.76 |
| **Total Ash** | 4.29 | 12.40 | 8.35 | 5.73 | 68.72 | -189.04 |
| **NFE** | 2.82 | 24.11 | 13.47 | 15.05 | 111.80 | -754.96 |

*Keys: Nitrogen Free Extract (Carbohydrate CHO), SD = standard deviation (Raw: Smoked), CV % = coefficient of variation percentage, % D = percentage difference between exoskeleton and flesh.*

Table 2 presents the proximate composition in the flesh of Giant Tiger Shrimp, highlighting the effects of smoking on nutrient concentration. Moisture content decreased substantially from 78.84 g/100 g in raw samples to 43.61 g/100 g in smoked samples, resulting in a mean value of 61.23 g/100 g and a 44.69 % reduction due to dehydration during smoking. Protein content increased significantly from 18.38 g/100 g to 31.85 g/100 g, a 73.29 % increase, as water loss concentrated the protein fraction. Similarly, crude fat and total ash increased markedly, with changes of 426.67 % and 167.46 %, respectively, reflecting the enhanced nutritional density after smoking. NFE exhibited the most dramatic increase, rising from 1.37 g/100 g to 20.38 g/100 g (1,387.59 %), indicating significant changes in carbohydrate-like substances or residual energy components during heat treatment. These findings align with studies by Akintola (2015), which demonstrated that smoking reduces moisture while increasing protein, fat, and ash contents in crustaceans, enhancing their nutritional value and shelf life. The high coefficients of variation (CV %) observed, particularly for crude fat and NFE, suggest variability in these parameters due to processing conditions.

**Table 2: Proximate composition of Giant Tiger Shrimp (Flesh)**

| Parameters | Raw (g/100 g) | Smoked (g/100 g) | Mean | SD | CV % | % D |
|---|---|---|---|---|---|---|
| Moisture | 78.84 | 43.61 | 61.23 | 24.91 | 40.69 | 44.69 |
| Protein | 18.38 | 31.85 | 25.12 | 9.52 | 37.92 | -73.29 |
| Crude Fat | 0.15 | 0.79 | 0.47 | 0.45 | 96.29 | -426.67 |
| Total Ash | 1.26 | 3.37 | 2.32 | 1.49 | 64.45 | -167.46 |
| NFE | 1.37 | 20.38 | 10.88 | 13.44 | 123.61 | -1387.59 |

*Keys: Nitrogen Free Extract (Carbohydrate CHO), SD = standard deviation (Raw:Smoked), CV % = coefficient of variation percentage, % D = percentage difference between exoskeleton and flesh.*

**Mineral Composition**

The mineral composition of Giant Tiger Shrimp shell shows notable differences between raw and smoked samples, reflecting the effects of smoking on mineral concentration (Figure 1). Calcium (Ca) increased from 1905.34 mg/100 g in raw samples to 2100.55 mg/100 g in smoked samples, though the change was not statistically significant (P = 0.15). Sodium (Na) and potassium (K) content were significantly higher in smoked shells, increasing from 1114 mg/100 g to 1276.76 mg/100 g (P = 0.02) and from 1532.56 mg/100 g to 1885.55 mg/100 g (P = 0.04), respectively, likely due to dehydration concentrating these minerals. Magnesium (Mg) and phosphorus (P) also increased slightly, from 18.54 mg/100 g to 25.3 mg/100 g and from 953.55 mg/100 g to 1011.24 mg/100 g, respectively, though these changes were not statistically significant (P>0.05). These findings are consistent with studies such as Abankwa and Acquaye (2023), which reported enhanced mineral content in shrimp shells post-smoking, highlighting their potential for use as mineral-rich by-products in food and feed applications. The observed increases demonstrate the value of processing techniques like smoking in enhancing the nutritional and functional quality of shrimp shell waste.

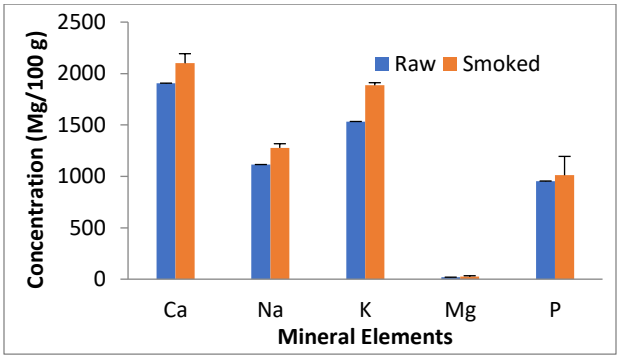



**Figure 1: Mineral concentrations in the shell of Giant Tiger Shrimp**

The mineral composition of the flesh of Giant Tiger Shrimp shows that smoking significantly enhances certain mineral concentrations, while others remain relatively unchanged (Figure 2). Calcium (Ca) increased from 1461.29 mg/100 g in raw samples to 1743.34 mg/100 g in smoked samples, although the difference was not statistically significant (P = 0.17). Sodium (Na) rose significantly from 1599.26 mg/100 g to 1890.07 mg/100 g (P = 0.05), and phosphorus (P) also showed a significant increase, from 1210.86 mg/100 g to 1450.85 mg/100 g (P = 0.01), reflecting the concentration effects of moisture loss during smoking. Potassium (K) and magnesium (Mg) exhibited slight increases from 1092.37 mg/100 g to 1185.31 mg/100 g and 10.43 mg/100 g to 14.41 mg/100 g, respectively, but these changes were not statistically significant (P>0.05). Similar trends have been reported in studies by Abu and Eli (2018), where smoking increased mineral concentrations in shrimp flesh due to reduced water content. These findings emphasize the nutritional benefits of smoking, particularly its ability to enhance essential minerals, making shrimp flesh a more nutrient-dense food product post-processing.

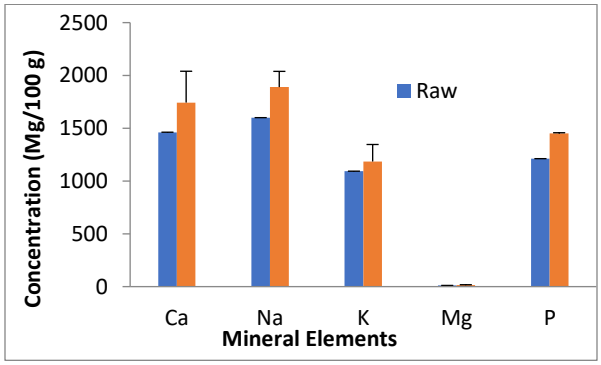



**Figure 2: Mineral concentrations in the flesh of Giant Tiger Shrimp**

**Mineral Ratio**

The mineral ratios in the shell of the Giant Tiger Shrimp exhibit variations between raw and smoked samples, reflecting changes in mineral balance due to processing (Table 3). The Ca/Mg ratio was higher than the acceptable ideal range (3 – 11), with values of 102.77 for raw shells and 83.03 for smoked shells, indicating a significant excess of calcium relative to magnesium, which may limit bioavailability (da Paixão Teixeira *et al*., 2022). The Ca/K ratio, within the ideal range (2.2 – 6.2), decreased slightly from 1.24 in raw shells to 1.11 in smoked shells, while the Ca/P ratio remained consistent across treatments at approximately 2.04, aligning well with the acceptable range (1.5 – 3.6) and supporting its suitability for dietary calcium-phosphorus balance. The Na/K ratio, crucial for electrolyte balance, was 0.73 in raw and 0.68 in smoked shells, below the ideal range (1.4 – 3.4), suggesting a relatively lower sodium content compared to potassium. The Na/Mg ratio was substantially higher than the ideal range (2 – 6), at 60.09 for raw and 50.46 for smoked shells, emphasizing a sodium-dominated profile. The [K/(Ca + Mg)] ratio remained stable between 1.06 and 1.15, slightly below reference balance (2.2), highlighting processing-induced shifts in potassium relative to calcium and magnesium. These findings align with Truong *et al.* (2023), who reported that processing alters mineral ratios in crustacean shells, influencing their potential use in functional feed formulations.

**Table 3: Mineral ratios in the shell of the Giant Tiger Shrimp**

| Parameter | Reference balance | Acceptable ideal range | Raw | Smoked | Mean | SD | CV % |
|---|---|---|---|---|---|---|---|
| Ca/Mg | 7 | 3 to 11 | 102.77 | 83.03 | 92.90 | 13.96 | 15.03 |
| Ca/K | 4.2 | 2.2 to 6.2 | 1.24 | 1.11 | 1.18 | 0.09 | 7.75 |
| Ca/P | 2.6 | 1.5 to 3.6 | 2.00 | 2.08 | 2.04 | 0.06 | 2.74 |
| Na/K | 2.4 | 1.4 to 3.4 | 0.73 | 0.68 | 0.70 | 0.04 | 5.01 |
| Na/Mg | 4.0 | 2 to 6 | 60.09 | 50.46 | 55.28 | 6.80 | 12.31 |
| [K/(Ca + Mg)] | 2.2 | | 1.06 | 1.15 | 1.11 | 0.06 | 5.77 |

*Keys: Standard deviation (SD), Coefficient of variation per cent (CV%)*

The mineral ratios in the Giant Tiger Shrimp flesh (Table 4) reveal significant deviations from reference balances and acceptable ideal ranges, particularly in the Ca/Mg and Na/Mg ratios, likely due to the effects of smoking on mineral concentration. The Ca/Mg ratio was markedly higher than the ideal range (3 – 11), with values of 140.10 in raw flesh and 120.98 in smoked flesh, reflecting a strong dominance of calcium over magnesium, which could limit the bioavailability of magnesium (da Paixão Teixeira *et al*., 2022). The Ca/K ratio remained within the acceptable range (2.2 – 6.2), increasing slightly from 1.34 to 1.47, while the Ca/P ratio, consistently at 1.20, fell below the ideal range (1.5 – 3.6), indicating a relative phosphorus dominance, which is critical for skeletal health. The Na/K ratio, crucial for maintaining electrolyte balance, stayed within the ideal range (1.4 – 3.4), rising from 1.46 to 1.59 post-smoking. However, the Na/Mg ratio was significantly higher than the recommended range (2 – 6), at 153.33 in raw flesh and 131.16 in smoked flesh, suggesting sodium dominance that may affect nutritional balance. The [K/(Ca + Mg)] ratio, slightly below the reference balance of 2.2, decreased from 1.01 to 0.94,

indicating a processing-induced reduction in potassium relative to calcium and magnesium. These trends align with findings by Tenyang *et al.* (2022), who reported that processing methods like smoking significantly alter mineral ratios, enhancing certain mineral concentrations while disrupting the balance of others.

**Table 4: Mineral ratios in the flesh of the Giant Tiger Shrimp,**

| Parameter | Reference Balance | Acceptable ideal range | Raw | Smoked | Mean | SD | CV % |
|---|---|---|---|---|---|---|---|
| Ca/Mg | 7 | 3 to 11 | 140.10 | 120.98 | 130.54 | 13.52 | 10.36 |
| Ca/K | 4.2 | 2.2 to 6.2 | 1.34 | 1.47 | 1.40 | 0.09 | 6.70 |
| Ca/P | 2.6 | 1.5 to 3.6 | 1.21 | 1.20 | 1.20 | 0.00 | 0.31 |
| Na/K | 2.4 | 1.4 to 3.4 | 1.46 | 1.59 | 1.53 | 0.09 | 6.04 |
| Na/Mg | 4 | 2 to 6 | 153.33 | 131.16 | 142.25 | 15.68 | 11.02 |
| [K/(Ca + Mg)] | 2.2 | | 1.01 | 0.94 | 0.97 | 0.05 | 4.93 |

*Keys: Standard deviation (SD), Coefficient of variation per cent (CV%)*

**Mineral Safety Index**

The mineral safety index (MSI) values for the shell of *P. monodon* highlight variations in mineral concentrations between raw and smoked samples (Table 5), with implications for dietary safety and bioavailability. Calcium (Ca) exhibited MSI values of 15.88 for raw and 17.5 for smoked shells, both exceeding the recommended acceptable index (MSItv = 10), with percentage differences (% D) of -58.78 % and -75.05 %, respectively, indicating a potential risk of excessive calcium intake if consumed in large quantities. Magnesium (Mg) showed MSI values well within the safe threshold (MSItv = 15), at 0.7 for raw and 0.95 for smoked shells, with % D values of 95.37 % and 93.68 %, suggesting safe levels. Phosphorus (P) had MSI values below the acceptable limit (MSItv = 10), at 7.95 for raw and 8.43 for smoked, with modest % D values (20.54 % and 15.73 %), confirming it remains within safe consumption levels. Sodium (Na) presented a concern, with MSI values significantly exceeding the recommended limit (MSItv = 4.8), at 10.69 for raw and 12.26 for smoked shells, with high negative % D values (-122.8 % and -155.35 %), aligning with findings by Vandecasteele *et al.* (2021), who reported elevated sodium levels in processed shrimp shells. The higher sodium and calcium MSI values emphasize the need for caution in dietary formulations incorporating smoked shrimp shells.

The MSI values for the flesh of *P. monodon* indicate notable differences between raw and smoked samples (Table 6), reflecting changes in mineral concentration due to processing. Calcium (Ca) exceeded the recommended MSI threshold (MSItv = 10) in both raw (12.18) and smoked (14.53) flesh, with negative percentage differences (% D) of -21.77 % and -45.28 %, respectively, suggesting potential overconsumption risks if intake is excessive. Magnesium (Mg) remained significantly below the MSI threshold (MSItv = 15), with values of 0.39 in raw and 0.54 in smoked flesh and high % D values (97.39 % and 96.4 %), consistent with safe consumption. Phosphorus (P) values approached the threshold (MSItv = 10) with 10.09 for raw and 12.09 for smoked flesh, showing minimal % D (-0.9 % and -20.9 %), aligning with reports by Truong *et al*. (2023) that phosphorus in shrimp flesh is nutritionally adequate yet safe. Sodium (Na) levels, however, significantly surpassed the MSI threshold (MSItv = 4.8), with raw and smoked values at 15.35 and 18.14, and large negative % D (-219.85 % and -278.01 %), corroborating AlFaris *et al.* (2022), who identified elevated sodium levels in processed shrimp products. These findings emphasize the importance of moderating shrimp consumption, particularly in smoked forms, to avoid exceeding sodium and calcium safety levels.

**Table 5: Mineral safety index of shell of the Giant Tiger Shrimp**

| Mineral | RAI (mg) | $MSI_{tv}$ | Raw | | Smoked | | Mean | SD | CV % |
|---|---|---|---|---|---|---|---|---|---|
| | | | $MSI_{cv}$ | % D | $MSI_{cv}$ | % D | | | |
| Ca | 1200 | 10 | 15.88 | -58.78 | 17.5 | -75.05 | 16.69 | 1.15 | 6.89 |
| Mg | 400 | 15 | 0.7 | 95.37 | 0.95 | 93.68 | 0.82 | 0.18 | 21.81 |
| P | 1200 | 10 | 7.95 | 20.54 | 8.43 | 15.73 | 8.19 | 0.34 | 4.15 |
| Na | 500 | 4.8 | 10.69 | -122.8 | 12.26 | -155.35 | 11.48 | 1.1 | 9.63 |

*RAI – recommended adult intake, $MSI_{tv}$ – MSI table value, $MSI_{cv}$ – MSI calculated value, % D – percentage difference between $MSI_{tv}$ and $MSI_{cv}$, SD – standard deviation, CV % – coefficient of variation percentage.*

**Table 6: Mineral safety index of flesh of the Giant Tiger Shrimp, *Penaeus monodon***

| Mineral | RAI (mg) | $MSI_{tv}$ | Raw | | Smoked | | Mean | SD | CV % |
|---|---|---|---|---|---|---|---|---|---|
| | | | $MSI_{cv}$ | % D | $MSI_{cv}$ | % D | | | |
| Ca | 1200 | 10 | 12.18 | -21.77 | 14.53 | -45.28 | 13.35 | 1.66 | 12.45 |
| Mg | 400 | 15 | 0.39 | 97.39 | 0.54 | 96.4 | 0.47 | 0.11 | 22.66 |
| P | 1200 | 10 | 10.09 | -0.9 | 12.09 | -20.9 | 11.09 | 1.41 | 12.75 |
| Na | 500 | 4.8 | 15.35 | -219.85 | 18.14 | -278.01 | 16.75 | 1.97 | 11.79 |

*RAI – recommended adult intake, $MSI_{tv}$ – MSI table value, $MSI_{cv}$ – MSI calculated value, % D – percentage difference between $MSI_{tv}$ and $MSI_{cv}$, SD – standard deviation, CV % – coefficient of variation percentage.*

## CONCLUSION AND RECOMMENDATION

Smoking significantly modifies the nutritional composition of Giant Tiger Shrimp, leading to the concentration of essential nutrients and minerals while decreasing moisture content. This process enhances the levels of protein, crude fat, total ash, and nitrogen-free extract (NFE) in both the shell and flesh. Additionally, minerals such as calcium, sodium, and potassium show notable increases. However, deviations in certain mineral ratios, particularly Na/Mg, raise concerns about dietary balance. Furthermore, the Mineral Safety Index (MSI) analysis indicates potential health risks due to elevated calcium and sodium levels.

The study recommended the following:

i. Consumers should limit the intake of smoked shrimp to avoid excessive calcium and sodium levels.

ii. Complementary food sources should be considered to correct mineral imbalances, particularly Na/Mg ratios.

iii. Further research should explore improved smoking techniques that enhance nutrient retention while minimizing health risks.

iv. Health professionals should educate consumers on the benefits and risks of smoked shrimp consumption.

v. Food safety authorities should establish limits for mineral concentrations in smoked seafood products to ensure dietary safety.

**Acknowledgement**

Authors appreciate their institutions for providing a conducive research environment.

**Authors' Contributions**

ROM conceived the research topic and managed the manuscript, KAA performed the research, MUA wrote and contributed to the manuscript and AOL supervised the research. All authors reviewed and approved the final version of the manuscript.

**Ethical Statement**

Not applicable

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