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Physicochemical and Functional Properties of the Royal Spiny Lobster, *Panulirus Regius* (De Brito Capello, 1864)

[1]Lawal-Are, A.O., *[2]Moruf, R.O., [1]Oriaghemuoria, O.M., [1]Paimo, E.O and [1]Adewale, A. B.
[1]Department of Marine Sciences, University of Lagos, Akoka - Lagos, Nigeria
[2]Department of Fisheries and Aquaculture, Bayero University, Kano, Nigeria
****Corresponding Author: tunjimoruf@gmail.com***

Abstract
An investigative study was carried out to determine the colour attribute, oleic acid content and functional properties of raw and grilled meat of the Royal Spiny Lobster (*Panulirus regius*) using standard methods. The intensity of lightness was higher than that of yellowness and redness in both raw and grilled lobster meats. The highest values of lightness (79.63) and redness (7.10) were obtained in grilled meat. However, grilling significantly ($P < 0.05$) reduced yellowness from 27.67 to 20.37. The oleic acid content in grilled lobster meat (2.83 %) was higher than the percentage value in raw lobster meat (2.77 %), although this was not observed to be significantly different. Relatively, grilling increased the absorption and emulsion capacities, emulsion stability, swelling power, dispersibility, specific gravity with significantly ($P<0.05$) higher loose bulk density. However, foam capacity (10.64±6.72 %), foam stability (53.06±29.11 %), solubility (9.97±7.02 %) and packed bulk density (0.95±0.02 g/ml) were non-significantly higher in the raw lobster meat. The good functional properties exhibited by the raw and grilled meats are indications that the Royal Spiny Lobster will be useful as food ingredient.

Keywords: Cooking process, Food properties, Grilling, Lobster meat, Seafood.

Introduction
A protein is built up from a long polymer chain of amino acids, a polypeptide chain. The variable side chains give each protein chain its distinctive character. Proteins are important in food processing and food product development, because they are responsible for many functional properties that influence the consumer acceptance of food products (Tornberg, 2005). Proteins that are utilised in food processing are of various origins, and can be roughly grouped into meat proteins (e.g. gelatine), plant proteins (e.g. soya protein, peanut protein and wheat protein) and animal-derivatives protein (e.g. milk proteins) (Sanni *et al*., 2019). Many of the meat proteins require processing to provide a food material having acceptable functional properties, such as emulsification, fat and water absorption, texture modifications, colour control and whipping properties, which are attributed primarily to the protein characteristics (Garba and Kaur, 2014). The utilization of such proteins as food ingredients will however depend on its physicochemical and functional characteristics (Timilsena *et al*., 2016).
Lobsters are the focus of valuable fisheries worldwide; they are often regional icons, and one of the most researched aquatic crustaceans (Phillips *et al*., 2013).
The lobster species, *Panulirus regius* (De Brito Capello, 1864) is caught as by-catch by trawlers in Nigeria (Lawal-Are *et al*., 2018a). In present time, the best means to obtain the benefits of crustacean-consumption is to utilize its components as ingredients, such as protein isolates. The successful use of such protein ingredients depends upon their abilities to fulfill one or more functional requirements, e.g. good solubility, emulsion/foam stabilization, or gel formation. Enzymatic modification of proteins is a useful mechanism to improve the functionality compared with the native unhydrolyzed proteins (Garcés-Rimón *et al*., 2016; Hall *et al*., 2016).
Lobster meat in general can be used to prepare diet supplements, to obtain chitin, and as a source of astaxanthin for aquaculture (García-López *et al.,* 2016). Although, nutritional status of different species of lobsters has been dealt with in various part of the world (Ayanda *et al*., 2018; Haryono *et al*, 2018; Varisco *et al.*, 2020; Moruf *et al*., 2021a), details on the functional properties of lobster meat have not been evaluated. Therefore, the aim of this study was to report and compare the functional properties of raw and grilled meats of the Royal Spiny Lobster, *P. regius* collected along the eastern parts of the Lagos Harbour, Nigeria.

Materials and Methods
Sample Preparation
A total of 90 wet samples of lobster were obtained from commercial trawl catches at the landing site of the 2km wide

Lagos Harbour, between May and October, 2019. The sampling station lies along the eastern parts of the Lagos Harbour (6° 39' 16"N and 3° 40' 11" E), which is at the mouth to the Atlantic Ocean. The lobsters were kept in ice-chest before being taken to Marine Sciences Department, University of Lagos, for further analysis. The samples (average size of 153 g) were thoroughly washed, measured, de-shelled and carapace region was discarded. The samples were separated into two parts; one part was analyzed raw while the second part was cooked by grilling. Specimens in the grilled process were prepared in a flat top griddle (HG4, Hobart Corp., Troy, OH) with the thermostat set at 100°C. After the set temperature was attained, samples were grilled for 15-17 min. Head, claw and cephalothorax meats were picked by hand, and the muscle tissues from each treatment (grilled and raw meat) were separately blended to prepare homogenate samples. These samples were kept in polythene bags and frozen separately (−20°C) until further analysis.

Analytical Procedures

Colour attributes of raw and grilled lobster meats were measured using a colorimeter (Precise color reader HP-2000, calibrated with white and black glass standards) as described by Martínez-Maldonado *et al*. (2018). To determine Free Fatty Acid (FFA) as Oleic, One (1) g of the test sample was poured into a conical flask with 20 mL of solvent mixture (1:1 v/v ethanol: diethyl ether) and 0.2 mL of phenolphthalein indicator solution, titrated while shaking with 0.1 N KOH till pink colour appeared and persisted for 10 sec. Estimation of FFA was based on Equation 1.

$$\%FFA \text{ (as oleic acid)} = \frac{(\text{V} - \text{B}) \text{ x N x } 28.21}{W} \qquad (1)$$

Where V = Volume of titrant (KOH) consumed for sample

B = Volume of titrant consumed for blank

N = Normality of titrant (KOH)

W = Weight of sample

Water absorption capacity (WAC) and oil absorption capacity (OAC) were determined by following the method described by Brishti *et al.* (2017). The 0.25 g of lobster meat was mixed with 5 mL distilled water or oil in pre-weighed centrifuge tube for 30 secs using a vortex. Sample was allowed to stand at room temperature (20 - 25ºC) for 15 min and centrifuged at 3000 rpm for 15 min. After centrifugation, the supernatant was decanted, and the centrifuge tubes + precipitate were re-weighed. The WAC and OAC were expressed as seen in Equation 2.

$$WAC \text{ or } OAC\ (g/g) = \frac{\text{W2}}{W1} \qquad (2)$$

Where W1 = weight of the dry sample (g)

W2 = weight of precipitate + centrifuge tube (g)

The modified methods reported by Souissi *et al*. (2007) were used to determine the emulsion capacity and emulsion stability. Foam capacity as determined by the aeration method proposed by Pacheco-Aguilar (2008) with modifications. Briefly, 0.75 g of protein was dispersed in 25 mL of dd-water (final pH 6.8) and equilibrated by stirring with a stir bar for 10 min at room temperature. Protein mixture was aerated using a Sorvall Omni Mixer with macro-attachment assembly (Norwalk, CT, U.S.A). FC was expressed as percent increase after aeration. Volumes were recorded before and after aeration (Equation 3) as;

$$\text{Foam capacity (\%)} = \frac{Volume\ after\ aeration - Volume\ before\ aeration}{Volume\ before\ aeration} X100\% \qquad (3)$$

Foam stability (% FS) was calculated as the percentage of foam remaining after 10, 30, 60, and 90 min inactive periods.

Specific gravity was determined in the lobster meats according to weight under water method as described by Solaiman *et al*. (2015), while bulking density was analyzed following the method of Asoegwu *et al*. (2006) and calculated using Equation 4:

$$Bulking\ density = \frac{\text{Weight of sample (g)}}{Volume\ of\ sample\ (ml)} \qquad (4)$$

The dispersibility of the lobster meats was evaluated according to Lee and Lian (2002) as the ease of dispersing the thawed mince (300 g) during mixing for 1 min in a Kitchen-Aid bowl mixer at a setting of "5" (1 very difficult - 9 very easy to mix). Swelling power and solubility were determined by following the method described by Pranoto *et al*. (2014) with slight modification. A sample (0.2 g) was put in a pre-weighed centrifuge tube, added with 10 mL distilled water and mixed using vortex. The sample was allowed to stand at room temperature (20-25ºC) for 5 min and then put in a water bath at 95ºC for 30 min. After that, it was cooled at 20-25ºC for 10 min. The sample was centrifuged at 3000 rpm for 15 min to separate gel and supernatant. The gel after separating from supernatant was expressed as swelling power. While the supernatant is placed on a plate that has been known to weigh and then dried in an oven to a constant weight. Swelling power was calculated by using Equations 5.

$$Swelling\ power\ (g/g) = \frac{\text{W2} - \text{W1}}{W0} \qquad (5)$$

Where W0 = weight of the dry sample (g),

W1 = weight of the dry sample + centrifuge tube (g),

W2 = weight of gel + centrifuge tube (g)

In the determination of solubility, sample (200 mg) were dispersed in 20 mL buffers of pH 3.0. The buffer mixture was stirred with a magnetic stir bar at room temperature for 30 min and centrifuged at 7500 x g (4 °C) for 15 min. Protein content of the supernatant and total protein in samples were determined using the bicinchoninic acid protein assay (BCA) method with bovine serum albumin as a standard following manufacture's protocol.

Protein solubility expressed as seen in Equations 6:

$$Solubility\ (\%) = \frac{Protein\ content\ in\ supernatant}{Total\ protein\ in\ sample} X100\% \qquad (6)$$

Statistical Analysis

Results were expressed as mean and standard error. Student t-test was used to sort out the differences in two means at a significant level of P≤0.05. Data were analyzed using Microsoft Excel 2010.

Results

Colour Concentration

The results of the colour attributed to raw and grilled meats of the spiny lobster are shown in Figure 1. The intensity of lightness was higher than that of yellowness and redness. The highest values of lightness (79.63) and redness (7.1) were obtained in grilled lobster meat. However, grilling significantly (P < 0.05) reduced yellowness from 27.67 to 20.37 in the lobster meat.

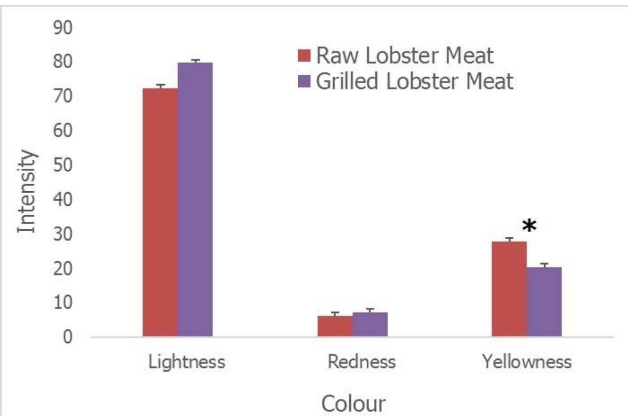

Figure 1: Colour intensity of the Royal Spiny Lobster (*Panulirus regius*) meat
(* significant difference)

Free Fatty Acid Content

The Free Fatty Acid (FFA) content as oleic acid in spiny lobster (*Panulirus regius*) is shown in Figure 2. The % FFA level in grilled lobster meat (2.83 %) was higher than the percentage value in raw lobster meat (2.77 %), although this was not observed to be significantly different.

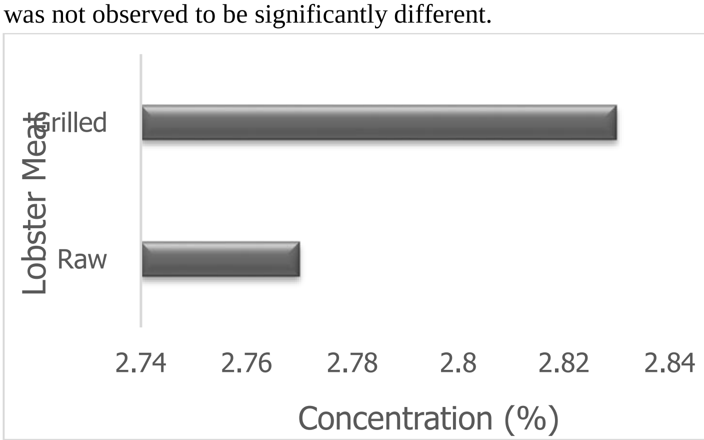

Figure 2: Free Fatty Acid (as Oleic) in the Royal Spiny Lobster (*Panulirus regius*) meat

Functional properties

The result of the functional properties of raw and grilled lobster meats is presented in Table 1. Relatively, higher range values of water absorption capacity (108.91±0.25 %), oil absorption capacity (50.32±0.53 %), emulsion stability (39.20±0.53 %), swelling power (106.71±0.25 %), dispersibility (27.00±0.50 %), specific gravity (0.42±0.00 g/ml), emulsion capacity (2.50±0.37 ml/g) with significant (P<0.05) higher loose bulk density (0.52±0.02 g/ml) were recorded in the grilled lobster meat. However, foam capacity (10.64±6.72 %), foam stability (53.06±29.11 %), solubility (9.97±7.02 %) and packed bulk density (0.95±0.02 g/ml) were non-significantly higher in the raw lobster meat.

Table 1: Functional Properties of the Royal Spiny Lobster (*Panulirus regius*) Meat

Parameters	Raw Meat	Grilled Meat	p-value
Water Absorption Capacity (%)	85.15±18.27	108.91±0.25	0.26
Oil Absorption Capacity (%)	46.21±2.11	50.32±0.53	0.13
Emulsion Stability (%)	27.33±11.73	39.20±0.53	0.37
Foam Capacity (%)	10.64±6.72	3.88±0.07	0.37
Foam Stability %	53.06±29.11	37.00±1.15	0.61
Swelling Power %	75.06±35.89	106.71±0.25	0.43
Solubility %	9.97±7.02	3.08±0.25	0.38
Dispersibility %	16.72±6.99	27.00±0.50	0.22
Packed Bulk Density (g/ml)	0.95±0.02	0.88±0.02	0.89
Loose Bulk Density (g/ml)	0.41±0.01	0.52±0.02	0.01*
Specific Gravity (g/ml)	0.34±0.05	0.42±0.00	0.15
Emulsion Capacity (ml/g)	2.40±0.06	2.50±0.37	0.80

*Significant difference

Discussion

Seafood consumers consider colour as a quality indicator, hence colour attribute is an important quality parameter in fish restructured products. In the present study, lightness in lobster meat was higher than yellowness and redness. Possibly because the lobster muscle metabolized energy with less oxygen, so it has less myoglobin and thereby lighter in color. Lawal-Are *et al.* (2020) reported similar colour attribute for mince and whole jumbo lump of *Portunus validus*. Higher percentage concentration of oleic acid was recorded in the grilled lobster meat, similar to the report of Lawal- Are *et al.* (2018b) for processed cuttlefish (4.47±0.10 %). It has been observed that processing method influences the level of oleic acid in shellfish.

The absorption capacities (water and oil) in this study were lower than that of grilled *Cardiosoma armatum* meat (339.96 %) (Moruf *et al.*, 2021b) and cooked mollusc meat (292.59 %) (Moruf, 2021). WAC reflects the extent of denaturation of the protein while OAC acts as a flavour retainer and improves the mouth feel of foods (Butt and Batool, 2010; Lawal-Are *et al*., 2018b). Accordingly, WAC is affected by pH and ionic strength while OAC depend on the amount of non-polar amino acids in the side chain and structure of the proteins (Butt and Batool, 2010; Lone *et al*., 2015).

The difference observed in the emulsion capacities of the raw and grilled lobster meats was not significant. There are few reports in the literature on emulsifying properties of invertebrates that can serve as a comparison. Adebowale *et al.* (2005) reported an adequate emulsification but poor stability in whole giant African cricket (*Gryllidae sp*) powder. In contrast, Omotoso (2015) reported both high emulsion formation and stability in moth (*Cirina forda*) larva and silkworm *(Bombyx Mori*) powders. An increase in emulsion capacity compared with unhydrolyzed controls, was also observed in chickpea (Ghribi, *et al.*, 2015) and Pacific whiting (*Merluccius productus*) (Pacheco-Aguilar *et al.,* 2008).

Foamability is an important food property by which proteins form a flexible cohesive film to entrap air bubbles (Lone *et al.*, 2015). Foams are two-phase colloidal systems with a continuous aqueous phase and dispersed gas phase. Good foam expansion requires rapid migration, unfolding and rearranging at the air/water interface to reduce surface tension (Khaled *et al*., 2014). In this study, the foaming capacities and stabilities are in close agreement with the foaming capacity of *Cirina forda* larva (7%) (Omotosho, 2006) and foaming stability of raw cuttlefish (46%) (Lawal-Are *et al*., 2018b).

In the present study, grilling increased the swelling power, dispersibility, specific gravity with significant ($P<0.05$) higher loose bulk density. However, foam stability, solubility and packed bulk density were non-significantly higher in the raw lobster meat. The low bulk raw lobster density would be an advantage in the formulation of complementary foods. On the other hand, high density indicates that the product can function as a good thickener in food products as well as their suitability for use in processed foods (Appiah *et al*., 2011). Specific gravity is one of the most reliable predictor of carcass lean content, which increases with the maturity of animal. The dispersibility of fish mince is an important quality requirement for the ease of mixing with other ingredients during formulation (Lee and Lian, 2002).

Higher swelling power in the grilled lobster meat indicates that lobster can be applied to improve the characteristics of baked products. This phenomenon can be attributed to additional interactions between nutrient and other components in relation to heating temperature (Zuluaga *et al*., 2007). Solubility is regarded as a critical functional property and the first property examined when developing a new protein ingredient (McCarthy *et al*., 2013). Generally, the variation in solubility recorded in the present study according to the view of Ratnawati *et al*. (2019), is a function of the ionogenic groups present in protein molecules in the lyophilic colloidal systems of protein solutions.

Conclusion

The meat of *Panulirus regius* is a good source of free fatty acid (oleic acid) with attractive colour attributes. Grilling process facilitates muscle detachment from shell, while increasing important functional properties such as swelling power, dispersibility, specific gravity and loose bulk density of the lobster meat, making it potentially useful in many food formulations.

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