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CHEMICAL BIO-COMPOUNDS AND FUNCTIONAL PROPERTIES OF RAW AND PROCESSED CUTTLEFISH, *Sepia officinalis* (MOLLUSCA: CEPHALOPODA)

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Abstract

Cuttlefish is one of the most economically important cephalopods with rich taste and few inedible parts. The study was aimed at evaluating the nutrient, non-nutrients, free fatty acid and cholesterol compositions and functional properties of raw and processed Sepia officinalis using standard methods. Moisture and raw protein in raw sample of the cuttlefish were significantly higher ($P < 0.05$) than in processed samples. Raw fat showed significant differences with fried sample having highest value (22.39±0.54 %) to raw (16.24±0.10 %) and boiled samples (4.09±1.42 %). Raw sample contained more ash with highest mg/100g levels of calcium, phosphorus, magnesium, iron and manganese. Processed cuttlefish registered significant reduction in levels of trypsin inhibitor, phytate and oxalate compared to the raw. Raw protein of fried cuttlefish exhibited positive relationships with all the non-nutritional factors while total ash only showed positive relationships with phytate ($r = 0.998$) and trypsin inhibitor ($r = 0.999$). Cholesterol content of raw cuttlefish was of 0.923±0.02 mg/100g being reduced by boiling (0.711±0.02 mg/100g) and increased by frying (1.037±0.02 mg/100g). A significantly high percentage of oleic was observed in fried cuttlefish (4.47±0.10) while boiled sample had the lowest value (1.4±0.05). The results showed significantly high percentage of water and oil absorbing capacities for raw cuttlefish while foam and emulsion stabilities were higher in the boiled sample. By frying, the highest foam and emulsion capacities of 8.8 and 4.4% respectively were obtained. The study suggests that Sepia officinalis will be highly desirable for preparing comminuted sausage products due to its good functional and nutritional properties.

Keywords: *nutritional, mollusc, shellfish, Nigeria*

1. Introduction

Sepia officinalis (Cephalopoda) is one of the most important demersal marine invertebrates inhabiting the continental shelf. Global annual catch of cuttlefish ranged from 11,000 to 15,000 tons [1] where Mediterranean countries are the main exporters of frozen cuttlefish in the world. The connective tissue of cephalopods is highly developed compared to fish in general [2-3]. Cuttlefish is rich in taste and has few inedible parts. As fat content of fishes varies with species, age, size and season, cuttlefish contains low level of lipid; however, omega-3 polyunsaturated fatty acid presented the majority of the total lipid [4, 2]. The fat content of raw fishes can also influence fat exchanges and interactions between the culinary fat and that of the fish during processing [5]. According to Okpanachi *et al.*[5], various processing methods (boiling, frying, smoking, roasting etc) used in the preparation of raw fish for consumption could have varying effects on the fish nutrient, texture and flavor. Nutrient chemical bio-compounds are traditionally used as nutritional indicators of fish. The Proximate composition and mineral profiles of fish are determined largely by several factors e.g species, sex, size, maturity, feeding regimes and season [6-7]. Information on daily dietary intake of nutrients, especially cholesterol, is quite important for especially those with cardiovascular problems [8]. The nutritional composition of cuttlefish has been reported by some researchers [9, 10, 2]. However, this composition could vary among species due to geographical differences of fishing grounds [11]. In addition, compositions can also vary with processing methods. Data on the macronutrient content of cuttlefish is only available for raw *Sepia spp* and there seems to be a scarcity of information on the processed ones. Cuttlefish was chosen because they are readily available, cheap, affordable and within the reach of an average Nigerians. This work is thus a preliminary investigation on the proximate composition, mineral, anti-nutrient, cholesterol contents and functional properties (absorbing, foaming and emulsion capacities) of raw, boiled and fried Cuttlefish, *Sepia officinalis* commonly retailed in Nigeria with a view to showcase the nutritive significance for human health benefit.

2. Materials and methods

2.1 Sample preparation

The cuttlefish samples (Fig. 1) used in this study were obtained from Ajeloro Fish Market in Apapa, Lagos State-Nigeria. The fishes were thoroughly washed, cut into pieces (50 g) and washed again with distilled water. The head region was discarded. The samples were then separated into three parts, one part was analyzed raw; a second part was boiled in water while the third part was deep-fried with vegetable oil in a frying pan. Boiling was done in distilled water, kept boiling for about 20 minutes until the pieces were cooked and tender. Frying was achieved within 15 minutes and the temperature was about 240°C. All processing methods followed the usual procedures used to prepare fish for table consumption in Nigeria but without the addition of any ingredient. All samples were homogenized prior to analysis.

Fig. 1: Dorsal view of the Cuttlefish, *Sepia officinalis* (Mollusca: Cephalopoda)

2.2 Analytical Procedures

Proximate analysis was carried out according to standard procedure [12]. For the moisture content, fish muscle duplicate samples were kept in an oven, at 102-105°C for 24 h. The ash content was determined by incineration of 5 g of the sample at 600°C for 8 hours. The determination of crude fat content was conducted by soxhlet extraction method using n-hexane as solvent. The N-content was multiplied by 6.25 to estimate the protein of the samples.

The content of total carbohydrates was determined by difference according to the formula below:

100 – [% water + % proteins + % lipids + % ashes].

The proximate compositions were measured in dry matter basis.

Mineral components were evaluated from solution obtained by first wet-ashing the samples and dissolving the ash with de-ionized water and concentrated hydrochloride acid in standard flask. The solution was analyzed for minerals content using Atomic Absorption Spectrophotometer. Phosphorus was analyzed for by employing the method reported by Vanado Molybate and read on colorimeter [12].

The estimation of anti-nutritional contents was by the colorimetric procedure as modified by [13]. All determinations were done in triplicate. Cholesterol content was estimated using Liebermann-Burchard reagent. Standard cholesterol solution used was 0.4 mg/mL. Liebermann-Burchard reagent was prepared with 0.2 mL concentrated sulfuric acid and 2 mL glacial acetic acid and was covered with aluminum foil. Fatty acid of fat extracted from samples was determined by gas chromatography (GC) of methyl esters. Methyl esters were prepared by transmethylation using 2 M KOH in methanol and n-heptane according to the method described by AOAC with minor modifications [12].

The modified methods reported by Souissi *et al*. [14] were used to determine the oil absorbing, water absorbing, emulsion and emulsion stability. The foam formation and the foam stability were determined by optical measurements. The foams were produced with a homogenizer for 2 min at 17 500 rpm, in 3 mL of solution (50 mMTris–HCl – 0.5 M NaCl, pH 7.5), which contained 1.5% protein. The initial height of the solution and the foam height were recorded at intervals of 0, 2, 10, 20 and 30 min, using a caliper. The foaming capacity17 was expressed as the proportion of foam height at 0 min to solution height. The foaming stability (FS) was conveyed by the percentage of foam height at some time to 0 min. The measurement of the height was rapid and accurate to three digits after the decimal point.

2.3 Statistical Analysis

Data obtained was subjected to analysis of variance (ANOVA), Pearson Correlation Coefficient and where there was significant difference at $P \leq 0.05$, Duncan Multiple Range Test (DMRT) was used to sort out the differences in the means.

3. Results

3.1 Proximate Composition

The moisture and crude protein contents for raw sample of cuttlefish were significantly higher ($P < 0.05$) than the processed samples (Table 1). Also, the raw fat showed significant differences between the samples with the fried sample (22.39±0.54%) having significantly higher values than the raw (16.24±0.10%) and boiled samples (4.09±1.42%). Raw sample contained more ash compared to the processed form. Raw fiber was not detected in the cuttlefish; raw or processed.

3.2 Mineral Content

Raw sample of cuttlefish has the highest mg/100g levels of calcium, phosphorus, magnesium, iron and manganese as follows: 342.16±3.99, 5.75±0.04, 368.81±4.21, 1.22±0.03 and 4.07±0.02 respectively. With the exception of calcium, frying had a significant increase in mineral level as compared to boiling (Table 1).

Table 1

Proximate and mineral compositions of raw and processed cuttlefish (*Sepia officinalis*)

Composition	Raw	Boiled	Fried
% Moisture	73.33±0.68 [a]	68.00±0.42 [b]	60.19±0.67 [c]
% Raw Protein	78.55±0.76 [a]	75.96±0.62 [b]	73.7±0.94 [c]
% Raw Fat	16.24±0.10 [b]	4.09±1.42 [c]	22.39±0.54 [a]
% Raw Fibre	ND	ND	ND
% Total Ash	3.35±0.09 [a]	3.18±0.04 [b]	2.12±0.01 [c]
% Carbohydrate	1.86±0.03 [b]	16.77±0.04 [a]	1.79±0.20 [c]
Calcium (mg/100g)	342.16±3.99 [a]	321.05±2.85 [b]	319.76±1.09
Phosphorus (mg/100g)	5.75±0.04 [a]	5.11±0.01 [c]	5.35±0.08 [b]
Magnesium (mg/100g)	368.81±4.21 [a]	306.74±1.92 [c]	325.67±3.31 [b]
Iron (mg/100g)	1.22±0.03 [a]	0.94±0.02 [c]	0.97±0.02 [b]
Manganese (mg/100g)	4.07±0.02 [a]	3.12±0.21 [c]	3.95±0.02 [b]

3.3 Non-nutrients

The non-nutrient content (oxalate, phytate and trypsin inhibitor) was significantly ($P< 0.05$) higher in raw sample compared to the processed sample (Fig.2). In this work, frying was found to have greater efficiency in the elimination of the levels of oxylate and phytate available in the cuttlefish than boiling which seemed to eliminate trypsin inhibitor more efficiently when compared with frying.

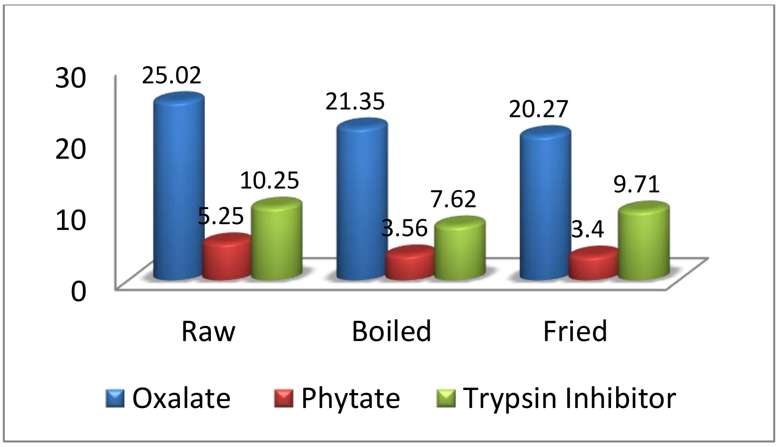

Fig.2: Non-nutrient levels in raw and processed Cuttlefish (*Sepia officinalis*) samples

3.4 Correlation Analyses

The correlation matrix showed some important relationships between the chemical bio-components analyzed. Table 2 showed the correlation matrix for raw cuttlefish in which case there were approximately perfect positive relationships between crude protein and phytate (r = 0.967) and between crude protein and trypsin inhibitor (r = 0.992). Similar relationship was exhibited by total ash with phytate (r = 0.995) and trypsin inhibitor (r = 0.974) with significance at the 0.05 level. Oxalate showed negative relationship with all the proximate composition except with carbohydrate (r =

0.942). For boiled cuttlefish sample, only crude fat showed negative relationship with the non-nutritional factors; oxalate (r = -0.607), phytate (r = -0.006) and trypsin inhibitor (r = -0.356) (Table 3). The crude protein of fried cuttlefish exhibited positive relationships with all the non-nutritional factors while total ash only showed positive relationships with phytate (r = 0.998) and trypsin inhibitor (r = 0.999) at significance of 0.05 levels (Table 4).

Table 2

Correlations for raw cuttlefish (*Sepia officinalis*) sample

	Moisture	*Raw Protein*	*Raw Fat*	*Total Ash*	*Carbohydrate*	*Oxalate*	*Phytate*	*Trypsin Inhibitor*
Moisture	1							
Raw Protein	0.999	1						
Raw Fat	0.919	0.923	1					
Total Ash	0.937	0.939	0.999	1				
Carbohydrate	-0.064	-0.058	0.335	0.289	1			
Oxalate	-0.395	-0.389	-0.001	-0.048	0.943	1		
Phytate	0.966	0.967	0.989	0.995	0.197	-0.148	1	
Trypsin inhibitor	0.991	0.992	0.963	0.975	0.067	-0.272	0.992	1

Table 3

Correlations for boiled Cuttlefish (*Sepia officinalis*) sample

	Moisture	*Raw Protein*	*Raw Fat*	*Total Ash*	*Carbohydrate*	*Oxalate*	*Phytate*	*Trypsin Inhibitor*
Moisture	1							
Raw Protein	0.196	1						
Raw Fat	-0.975	-0.409	1					
Total Ash	0.521	0.939	-0.697	1				
Carbohydrate	0.583	0.911	-0.749	0.997	1			
Oxalate	0.415	0.974	-0.607	0.993	0.982	1		
Phytate	0.217	0.914	-0.006	0.721	0.667	0.799	1	
Trypsin inhibitor	0.140	0.998	-0.356	0.918	0.886	0.959	0.936	1

Table 4

Correlations for fried Cuttlefish (*Sepia officinalis*) sample

	Moisture	*Raw Protein*	*Raw Fat*	*Total Ash*	*Carbohydrate*	*Oxalate*	*Phytate*	*Trypsin Inhibitor*
Moisture	1							
Raw Protein	0.867	1						
Raw Fat	0.801	0.396	1					
Total Ash	0.124	0.602	-0.495	1				
Carbohydrate	0.806	0.403	0.999	-0.488	1			
Oxalate	0.500	0.001	0.919	-0.798	0.916	1		
Phytate	0.182	0.649	-0.443	0.998	-0.435	-0.760	1	
Trypsin inhibitor	0.169	0.638	-0.455	0.999	-0.447	-0.770	0.999	1

3.5 Cholesterol and Oleic contents

The cholesterol content of raw cuttlefish was 0.923±0.02mg/100g, being reduced by boiling (0.711±0.02 mg/100g), but increased by frying (1.037±0.02 mg/100g).

A significantly high percentage of oleic was observed in fried cuttlefish sample (4.47±0.10 %), while the boiled sample had the lowest value (1.4±0.05 %)(Table 5).

Table 5

Cholesterol and free fatty acid contents of raw and processed cuttlefish (*Sepia officinalis*)

	Raw	Boiled	Fried	*P-value*
Cholesterol (mg/100g)	0.923±0.02	0.711±0.02	1.037±0.02	0.00*
FFA as Oleic (%)	3.95±0.10	1.4±0.05	4.47±0.10	0.00*

3.5 Functional Properties

The result as shown in the Fig.3 revealed significantly high percentage of water and oil absorbing capacities of raw cuttlefish sample, while foam and emulsion stabilities were the highest in the boiled sample. By frying, the highest foam and emulsion capacities with 8.8 and 4.4% respectively were obtained.

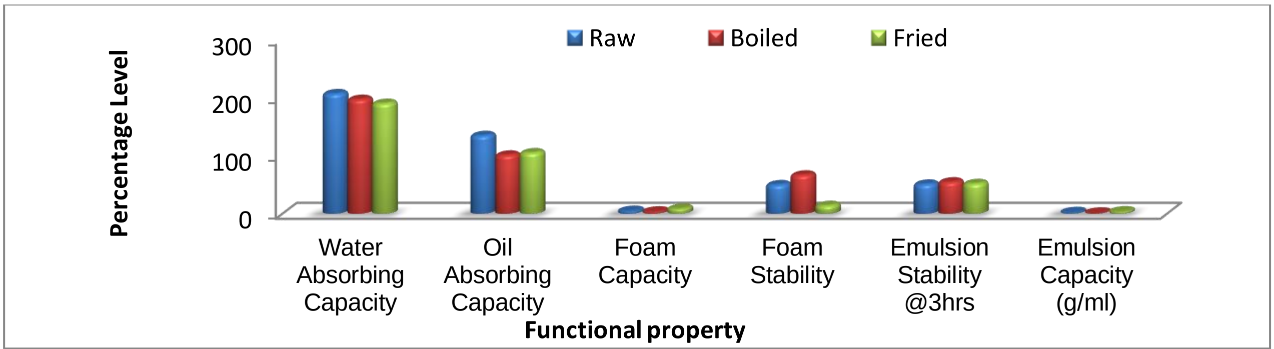

Fig. 3: Functional properties of raw and processed cuttlefish (*Sepia officinalis*)

4. Discussion

4.1 Proximate and Mineral contents

The nutritional elements showed variable values in the cuttlefish analyzed, raw protein recording the highest values both in raw and processed forms. The significant decrease in raw protein levels ($P < 0.05$) in boiled and fried, when compared with the raw sample, is in agreement with the report of Okpanachi *et al*.[5] and this suggested that the protein was leached and denaturized in the boiling and frying respectively. As observed from the results, frying decreases the moisture content that result in desirable non-enzymatic browning reactions, but increases the raw fat content of the cuttlefish. Fat contents are vital in the structural and biological functioning of cells and they help in the transportation of nutritionally essential fat soluble vitamin [15]. Ash, a measure of the mineral content of food item indicates that the cuttlefish is a good source of minerals. The amount of carbohydrate obtained in this study was higher than the amount observed in the report of [2] on *Sepia recurvirostra* which might be due to genetic factors as well as extrinsic factors such as feeding regimes and/or exercise significant change in some structural and flesh quality parameters of the two cuttlefish species. According to [16], cephalopods living in hypo-osmotic environment absorb minerals using digestive gland as they swallow massive quantities of sea water during and after feeding. Raw sample of the cuttlefish recorded the highest values for all the measured minerals while boiled sample recorded the lowest with the exception of calcium which lowest value was observed in fried sample. In general, there were significant influences of boiling and frying on the proximate and mineral compositions of the cuttlefish.

4.2 Non-nutritional content

Non-nutritional factors are generally toxic and may negatively affect the nutrient value of fish by impairing protein digestibility and mineral availability. However, they are heat labile and hence may be inactivated by processing methods involving heat generation [17]. The lower values of phytate and oxalate suggest that the nutritive value of cuttlefish would be impaired to a comparatively lesser extent. Nwosu[18]indicated a significant reduction in phytate and trypsin inhibitor contents following cooking which is in agreement with this research. Generally, processing reduced the level of all the non-nutrients analyzed to their permissible levels.

4.3 Correlation

The correlation analyses for raw and processed samples suggest that phytate and trypsin inhibitor will negatively affect the raw protein and mineral level (ash), making these nutrients unavailable. In the fried cuttlefish sample, oxalate has no significant effect on mineral level (ash) which is contrary to the report of [19] that suggests that oxalate forms complexes with minerals. According to Ndidi *et al*. [17], although processing reduces non-nutrient level to permissible limits, however some quantity of the nutrients could still be affected. This may offer some explanations why processing does not increase, in some cases, the level of nutrients.

4.4 Oleic acid and Cholesterol contents

Significant differences in oleic acid and cholesterol content were observed among raw and processed samples ($p < 0.05$). Similar observation was reported by Ozogul *et al*.[20] on frying process significantly reducing the sterol content of some selected mollusc except for common cuttlefish. Several studies have shown that cephalopod lipid profiles display high concentration of phospholipids, cholesterol and Polyunsaturated Fatty Acids. [21] reported a content of cholesterol in several cephalopods such as 123 mg/100 g in cuttlefish, 180 mg/100 g in squid and 139 mg/100g in octopus.

4.5 Functional properties

The cuttlefish (*Sepia officinalis*) has a low value of water absorbing capacity in both the raw and processed samples as compared to that reported by [22] on *Clarias lazera* while oil absorbing capacity is similar to the report of [23] on *Gymnarcus niloticus*. Water absorbing capacity reflects the extent of denaturation of the protein while oil absorbing capacity acts as flavor retainer and improves the mouth feel of food. Foam formation and stability are a function of the type of protein, PH, processing method, viscosity and surface tension [15]. [24] reported that foams are used to improve the texture, consistency and appearance of foods. Fried sample of the cuttlefish has the highest values of foam and emulsion capacities while boiling received the highest values for foam and emulsion stabilities. The cuttlefish sample in the present study has low values of emulsion capacity and emulsion stability in comparison with *Clarias lazera*[22] but higher than the values reported for some selected sea foods [25]. This suggests that cuttlefish would be highly desirable for preparing comminuted sausage products.

5. Conclusion

Different nutritional components of cuttlefish undergo different changes at elevated temperatures. Processing drastically reduced the level of non-nutrients in cuttlefish with minimal effect on the nutritional quality. Therefore, in view of the nutrient availability, low non-nutritional content, and the quality of functional properties after processing, the consumption of *Sepia officinalis* could help to combat malnutrition in developing countries. Further work should be geared toward essential and non-essential amino acid analyses and organoleptic property of this species of cuttlefish for knowledge of its complete nutritional benefit.

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