Mycoflora, Proximate Composition and Mineral Analysis during the Storage of Smoked Dried Crayfish (*Penaeus natialis* - Shrimps)

Abstract

This study was carried out to assess the changes in proximate composition, mineral content and mycoflora associated with smoked dried crayfish *Penaeus natialis* ~~(shrimps)~~ stored for twenty weeks. Smoked dried crayfish *Penaeus natialis* ~~(shrimps)~~ were purchased at Igbokoda, Ilaje Local Government Market, Ondo State, Nigeria. They were studied under storage for twenty weeks (6 months) and the proximate, mineral and mycofloral analyses were carried out at four weeks interval. The mycoflora were isolated using direct plating and dilution methods on Potato Dextrose Agar (PDA), Saboraud Dextrose Agar (SDA) and Malt Extract Agar (MEA) and identified using their cultural and morphological features with reference to standard procedures accordingly. The fungi isolated using direct plating methods and dilution methods were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Rhizopus* sp., *Phytophthora siskiyouensis*, *Penicillum* sp. and *Mucor* sp. The proximate analysis result showed a decrease in Ash, fat, and crude fibre content while moisture, crude protein and carbohydrate content increased ~~respectively~~ during the twenty four weeks storage. The mineral analysis result of the smoked dried crayfish *Penaeus natialis* ~~(shrimps)~~ showed a decrease in Sodium, Potassium, Calcium, Magnesium, Zinc, Iron, Copper, Manganese, Cadmium and Phosphorous respectively. This study showed that the smoked dried crayfish *Penaeus natialis* ~~(shrimps)~~ were contaminated by fungi; which is an indication that the market places where these products were displayed for sale were not hygienic coupled with leaving the products in open air without coverage which could allow products contamination with fungal spores leading to fungal spores germination, deterioration and spoilage of products during storage. Good hygiene, constant product checking and sensitization of the products processors, handlers and sellers will minimize exposure to fungal spores' contamination while mitigating deterioration and spoilage of the products during storage.

Keywords

Storage, Mycoflora, Proximate, Minerals, Shrimps

1. Introduction

Crayfish *Penaeus natialis* ~~(shrimps)~~ is an important flavour ingredient in many Nigerian local preparations. Crayfish are eaten worldwide like other edible crustaceans, only a small portion of the body of a crayfish is eaten in most prepared dishes, such as soups, bisques, only the tail portion is served [1]. Crayfish processing has

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become a large part of the crawfish industry. Crawfish processing is a modern industry that produces a high quality product available for consumption world-wide [2,3].

Preservation of crayfish *Penaeus natialis* ~~(shrimps)~~ is very important because it is easily susceptible to deterioration immediately after harvest and to prevent economic losses. The development of machinery that could be employed for effective handling, harvesting, processing and storage of sea foods such as fish and crayfish cannot be over-emphasized especially when aquaculture is growing fast in Nigeria [4]. The use of smoke in local fish preservation was reported by Eyo [5] and the implication of poor postharvest handling of crayfish has also been reported Kumolu-Johnson *et al* [6]. Smoke drying is done to partially cook, remove water, obtain brown colour, improve organoleptic flavor and control microbial and enzymatic actions that may cause spoilage. Preservation effects of smoke derived from the antioxidant and antimicrobial properties of its phenolic compound have been reported by Shehu *et al* [7] and Abou-zaid and Mohammed [8]. In local markets, crayfish is retailed open as small heaps on tables to attract consumers and information on duration of effectiveness of smoke drying on crayfish quality is scarce. The essence of processing is to preserve and stop microbial deterioration action on food and to retain the quality of the food [9]. However, there is little or no adequate information on the effectiveness of smoke drying on crayfish quality; hence this study is aimed at studying the changes in proximate composition, mineral content and mycoflora associated with smoked dried crayfish *Penaeus natialis* ~~(shrimps)~~ during twenty four weeks storage.

2. Materials and Methods

2.1. Collection of Samples

Samples of Crayfish namely *P. ~~enaeus~~ natialis* ~~(shrimps)~~ were randomly purchased at Igbokoda, Ilaje Local Government Market, Ondo State, Nigeria. The dried crayfish *P. ~~enaeus~~ natialis* ~~(shrimps)~~ samples were clearly-labeled, stored at room temperature in a sterile airtight container, and kept in a well-ventilated laboratory for a period of twenty four weeks (6 months) under investigation.

2.2. Mycoflora Isolation from the Stored Smoked Dried Crayfish *P. ~~enaeus~~ natialis* ~~(Shrimps)~~

The mycoflora associated with smoked dried crayfish *P. ~~enaeus~~ natialis* (~~shrimps~~) during storage were isolated using the methods described below:

2.2.1. Direct plating method

Visible mouldy sundried crayfish *P. ~~enaeus~~ natialis* ~~(shrimps)~~ were examined and randomly selected from the stored samples for mycofloral isolation using the method described by Amusa [10]. The sample surfaces were sterilized with ethanol and washed in sterile distilled water. The sterilized samples were aseptically placed on Potato Dextrose Agar plates with sterilized spatula and incubated at 28°C for 5 days. The hyphae tips of each fungal growth were successively sub-cultured on freshly prepared Potato Dextrose Agar plates until pure colonies were obtained [11]. The cultures were examined microscopically to assess the ~~fungi~~ presence of fungi.

2.2.2. Dilution plate method

The dilution plate method was done by placing 1g of smoked dried crayfish *P. ~~enaeus~~ natialis* ~~(shrimps)~~ in sterile distilled water and shaken thoroughly. One ml each of the standardized sample was pipette into 9 ml of sterile distilled water in test tube; and serially diluted in series of test tubes containing sterile distilled water. One

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ml each of aliquots of 10^{-2} and 10^{-3} was introduced into molten Potato Dextrose Agar (PDA) plates in duplicates for each isolate and incubated at 28°C for 5 days. The fungal growths were observed every 24 hours for the fruiting bodies and hyphae tips of each fungus were sub-cultured successively until pure cultures were obtained [11]. The cultures were examined microscopically to assess the fungi present.

2.3. Identification of Mycoflora

The mycoflora isolated from the stored smoked dried crayfish *P. ~~enaeus~~ natialis ~~(shrimps)~~* were identified by their gross cultural and morphological features. The mycoflora were examined under bright day light for colour of the culture and further examination were carried out using Needle mount preparation method as described by Tuite [12], Crowley *et. al*. [13] and Egbebi *et al.* [11] and Slide culture technique method as described by Fagbohun *et al*. [14].

2.4. Nutrient Analysis

2.4.1. Proximate Analysis

Samples of the stored smoked dried crayfish *P. ~~enaeus~~ natialis ~~(shrimps)~~* were analysed for the for ash, crude fiber, moisture and fat contents according to the methods described by Pearson [15] and A.O.A.C. [16]. The nitrogen was determined by Micro-Kjeldahl method as described by Pearson [15] and the percentage nitrogen was converted to crude protein by multiplying 6.25. The carbohydrate content was estimated by the difference in value obtained when all the chemical composition values were subtracted from 100%. All determinations were in triplicates and values of each constituent were expressed in percentage.

2.4.2. Mineral Analysis

The stored smoked dried crayfish *P. ~~enaeus~~ natialis ~~(shrimps)~~* were analysed for the minerals using the solution obtained by dry ashing the sample at 550°C and dissolving it in 10% HCL (25ml) and 5% lanthanum chloride (2ml), boiling, filtering and making up to standard volume with deionized water. Mn, Cu, Co, Zn, Fe, Mg, Na, and Ca were determined with a Buck Atomic Absorption Spectrometer (Buck Scientific, Model 200A/200, Inc. East Norwalk, Connecticut, U.S.A). Sodium was measured with a Corning 405 flame photometer (Corning Halstead, Essex, UK, Model 405) (AOAC) [16]. The detection limits had precisely been determined using the methods of Varian Techtron [17] as Mn 0.01, Cu 0.005, Co 0.05, Zn 0.005, Fe 0.02, Mg 0.002, Ca 0.04, Na 0.001, ppm (all for aqueous solutions). The optimum analytical range was 0.5 to 10 absorbance units with coefficient of variation of 0.05-0.40%. Phosphovanadomolybdate method using a spectronic 20 colorimeter (Galenkamp, London, UK) (AOAC) [16]. All chemicals were BDH analytical grade

3.0. Results and Discussion

The proximate content of smoked dried crayfish *P. ~~enaeus~~ natialis ~~(shrimps)~~* during twenty four weeks storage is shown in Table 1 below

Table 1: Results of proximate analysis of smoked dried crayfish *P. ~~enaeus~~ natialis ~~(shrimps)~~* during 24 weeks storage (g/100g)

Weeks of Storage	Ash	MC	CP	FAT	CF	CHO
Fresh	13.45 ± 0.07^{E}	6.40 ± 0.14^{C}	68.46 ± 0.79^{A}	5.10 ± 0.28^{C}	ND	6.40 ± 0.57^{A}
4	13.38 ± 0.04^{E}	6.39 ± 0.02^{BC}	68.19 ± 0.03^{A}	5.40 ± 0.02^{C}	ND	6.66 ± 0.04^{A}
8	13.42 ± 0.01^{E}	6.36 ± 0.01^{B}	68.26 ± 0.01^{A}	5.36 ± 0.01^{C}	ND	6.62 ± 0.02^{A}

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12		13.24±0.02^{D}	6.42±0.02^{BC}	68.36±0.04^{A}	5.32±0.01^{C}	ND	6.63±0.05^{A}
16		12.25± 0.01^{C}	6.52±0.02^{C}	68.13±0.04^{A}	4.26±0.02^{B}	ND	8.86±0.01^{B}
20		11.68±0.01^{B}	6.36±0.01^{B}	68.21±0.01^{A}	3.99±0.03^{AB}	ND	9.79±0.00^{C}
24		10.29±0.01^{A}	6.21±0.01^{A}	68.07±0.01^{A}	3.89±0.16^{AB}	ND	11.53±0.16^{D}

MC: Moisture content, CP: Crude protein, CF: Crude Fiber, CHO: Carbohydrate, ND: Not Detected. Means for each treatment with the same alphabet in each row are not significantly different at 5% level of significance (p< 0.05), while different alphabets in each row are significantly different at 5% level.

The mineral content of smoked dried crayfish *P. ~~enaeus~~ natialis* ~~(shrimps)~~ during twenty four weeks storage is shown in Table 2.

Table 2: Results of mineral analysis of smoked dried crayfish *P. ~~enaeus~~ natialis* ~~(shrimps)~~ during twenty four weeks storage (mg/100g)

ks ge	Na	K	Ca	Mg	Zn	Fe	CU	Mn	CD	P
	58.90±0.14^{F}	66.30±0.14^{A}	86.65±0.01^{A}	49.90±0.14^{E}	0.63±0.01^{D}	6..33±0.11^{C}	0.28±0.04^{E}	0.99±0.01^{E}	0.23±0.04^{EF}	
	57.89±0.03^{D}	65.40±0.04^{A}	76.90±14.15^{A}	49.29±0.08^{D}	0.62±0.01^{C}	6.62±0.01^{E}	0.24±0.01^{DE}	0.91±0.01^{D}	0.24±0.02^{F}	
	58.12±0.02^{E}	65.49±0.02^{A}	87.03±0.03^{A}	49.43±0.04^{D}	0.68±0.02^{D}	6.55±0.01^{b}	0.19±0.01^{C}	1.12±0.02^{F}	0.19±0.02^{DE}	
	57.97±0.05^{D}	60.46±0.09^{A}	85.67±0.03^{A}	49.35±0.02^{D}	0.61±0.01^{C}	6.49±0.01^{D}	0.15±0.02^{C}	0.99±0.02^{E}	0.16±0.01^{CD}	
	56.88±0.04^{B}	64.81±0.38^{A}	83.63±0.01^{A}	48.78±0.33^{C}	0.54±0.01^{B}	5.68±0.01^{B}	0.11±0.01^{A}	0.59±0.01^{C}	0.12±0.01^{BC}	
	55.65± 0.21^{A}	60.39±0.02^{A}	81.52±0.02^{A}	47.39±0.01^{B}	0.48±0.04^{A}	5.52±0.01^{A}	0.07±0.01^{A}	0.50±0.00^{A}	0.08±0.01^{AB}	100.08±0.15^{A}
	56.56±0.02^{B}	60.39±0.01^{A}	81.29±0.03^{A}	45.80±0.01^{A}	0.48±0.04^{A}	5.55±0.01^{A}	0.09±0.08^{A}	0.54±0.01^{B}	0.06±0.01^{A}	

Na: Sodium, K: Potassium, Ca: Calcium, Mg: Magnesium, Zn: Zinc, Fe: Iron, Cu: Copper, Mn: Manganese, CD: Cadmium, P: Phosphorus. Means for each treatment with the same alphabet in each row are not significantly different at 5% level of significance (p< 0.05), while different alphabets in each row are significantly different at 5% level.

The mycoflora isolated from smoked dried crayfish *P. ~~enaeus~~ natialis* ~~(shrimps)~~ during twenty four weeks storage is shown in Table 3.

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Table 3: Mycoflora isolated from smoked dried crayfish *P. ~~enaeus~~ natialis* ~~(shrimps)~~ during twenty four weeks storage (mg/100g)

Mycoflora	Week of storage													
	0	**0**	**4**	**4**	**8**	**8**	**12**	**12**	**16**	**16**	**20**	**20**	**24**	**24**
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Aspergillus niger	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Aspergillus fumigatus	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Rhizopus sp.	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Phytophthora siskiyouensis	-	-	-	-	-	-	+	+	+	+	+	+	-	-
*Penicillium*sp.	-	-	-	-	-	-	-	-	+	+	+	-	-	-
Mucor sp.	-	-	-	-	-	-	-	-	-	-	-	-	+	+

1: Dilution method, 2: Direct plating method, (+): isolated, (-): not isolated

3.1. Proximate Analysis

There was a significant decrease in ash content (13.45 - 10.29 g/100g), fat (5.10 -3.89 g/100g), moisture content (6.40 - 6.21 g/100g)~~,~~ and crude protein (68.46 - 68.07 g/100g) ~~a~~but the carbohydrate content ~~nd there was an~~ was increased ~~in carbohydrate~~ (6.40-11.53 g/100g). ~~while~~ The crude fibre was not detected during the twenty ~~h~~ four weeks storage of smoked dried crayfish *P. ~~enaeus~~ natialis* (~~shrimps)as shown in~~ (Table 1). This result is in agreement with the findings of Girard [18] who reported a significant reduction in ash content of cattle hide from (1.67-0.83) mg/100g after storage for months. Decrease in ash content indicates loss of nutrients as the storage progressed. Ash content in food contributes to the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid etc.) have been incinerated at a temperature of about 500^{o}C. Ash content is generally taken to be a measure of the mineral content of the original food [19]. However, this result contradicts that of Oladejo and Adebayo-Tayo [20] who reported an increase in crude protein (21.68-54.16) mg/100g of "Banda" dried meat during storage and Rodolfo *et al*. [21] who found out that fungi increase the protein content of the samples on which they grow. This result is also different from the findings of Lawal *et al.* [22] who reported a decrease in the proximate content such as carbohydrate content of sundried coco yam chips during storage. Crude fiber was not detected in stored smoked dried crayfish ~~(shrimps)~~ which is similar to that of Eleazu [23] who reported that crude fiber was not found in the 10~~%~~, 30~~%~~, or 40% NRCRI cassava bread samples or in the 100% wheat bread. There was a reduction in the moisture content from 6.40 – 6.21 (g/100g). This result is in agreement with the work of Ajai *et al* [24] who reported a decrease in the moisture contents of milk samples after storage ~~from~~ (8.32 - 7.51) g/100g. It is known that products that have low fat values normally have high moisture contents. Decrease in water content in this study could be attributed to the fact that infecting fungus utilizes the moisture content for its survival and growth. The shelf life of any product is influenced by the amount of water present in it [25]. Moisture content is a widely used parameter in the processing and testing of food. It is an index of water activity of many foods and determines the shelf life or keeping quality of the food. The observed value in this study implies that smoked dried crayfish ~~(shrimp)~~ will have a long shelf life because of the low moisture content.

3.2. Mineral Analysis

The summary of the mineral composition of smoked dried crayfish *Ppenaeus natialis* (shrimps) during twenty four weeks storage showed a decrease in Sodium (58.90 - 56.56 mg/100g), Potassium (66.30 - 60.39 mg/100g), Calcium (86.65 - 81.29 mg/100g), Magnesium (49.90 - 45.80 mg/100g), Zinc (0.63 - 0.48 mg/100g), Iron (6.33 - 5.55 mg/100g), Copper (0.28 - 0.09 mg/100g), Manganese (0.99 - 0.54 mg/100g), cadmium (0.23 - 0.06 mg/100g) and Phosphorous (106.10 - 100.89 mg/100g) (as shown in Table 2). This result supports the findings of Oladejo and Adebayo-Tayo [20] who reported a reduction in Sodium (0.35 - 1.55) mg/100g in "Banda" dried meat during storage. This result is in contrast to the work of Hassan *et al.*(2005), who reported an increase in sodium content in of *Vernonia amygdalina* leaf protein concentrates of (57.5±0.34 mg/100g). High sodium content in food is of great concern for health because of its implication in high blood pressure [26]. The result of this study indicated that eating of smoke dried crayfish (shrimp) could not lead to high in blood pressure. Low sodium content is beneficial in the treatment of hypertension and renal diseases [27]. The manganese content of stored smoked dried crayfish (shrimp) observed in this study significantly decreased from 0.99 - 0.54 mg/100g. The result of this work is different from that of Mensah, [28], who reported a significant increase in Mn from (2.7 - 20.1) mg/kg for meat hides. Thus, certain trace elements such as copper, iron and manganese constitute essential part of any balanced diet. The RDA for manganese varies between 2.7mg/kg to 3.1mg/kg (RDA, 2001). However, the manganese content observed in this study was low when compared to the RDA value for manganese.

3.3. Mycoflora of smoked dried crayfish *P. enaeus natialis* (Shrimps)

The mycofloral associated with smoked dried crayfish *P. enaeus monodon* (shrimps) during twenty four weeks storage were *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Rhizopus* sp., *Phytophthora siskiyouensis*, *Penicillum* sp.,and *Mucor* sp. This result supports that of Adebayo-Tayo *et al.* [29] who reported the isolation of *Aspergillus flavus*, *Aspergillus tereus*, *Aspergillus fumigatus*, *Abisidia sp.*, *Rhizopus sp.*, *Aspergillus niger*, *Mucor* sp., *Cladosporum* sp., *Penicillium viridatus*, *Candida tropicalis* and *Fusarium moniliformis* from selected smoked fish from different markets sites in Uyo, Akwa Ibom state. The implication of mycofloral in these products could be attributed to the ever increasing demand for smoked dried crayfish *P. enaeus monodon* (shrimps) and in the quest of the retailers to meet this need the fish are overloaded on the smoking kiln during processing; as a result they are exposed to a reduced intensity of heat for short period of time. This leads to improper processing and vulnerability of the fish to fungal contamination [30]. The market place where the smoked dried crayfish products are displayed for sale most times are not clean or hygienic, such as in open trays without coverage Hassan *et al* [26] Fungi found in stored food are divided into two groups namely the field fungi and the storage fungi. Most at times it is difficult to distinguish between the two as fungal growth may start both in the field and during storage. Species of *Aspergillus*, *Rhizopus* and *Penicillum* have been reported as storage fungi which infect crops on the field and may persist and proliferate in storage resulting in increased fungal and mycotoxin contamination with increased duration of storage [31].

4.0. Conclusion

This current study indicated that the stored smoke dried crayfish (shrimps) were contaminated with fungal species with significant loss of nutrients during the twenty four weeks storage. Therefore, special attention should be paid to the microbial investigation to minimize the threats posed to public health. The crayfish

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~~(shrimps)~~ must be properly dried to reduce the moisture content before packaging to prevent fungal invasion and enhance the good keeping and storage quality. Good sanitary practices including good storage practices must be followed and microbiological standards must be adhered to by checking production procedures and handling until the stored smoke dried crayfish ~~(shrimps)~~ reach the consumer's table. Stored smoked dried crayfish ~~(shrimps)~~ sellers should be sensitized on the importance of good hygienic practices, good housekeeping and proper storage conditions to prevent deterioration of their product

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