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Effect of chitosan coatings on proximate parameters and essential minerals composition of smoked catfish

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Abstract

This work had been carried out with the aim of evaluating the effect of chitosan-based edible coating on the proximate parameters (moisture; crude protein; fibre; fat; ash and carbohydrate contents) and essential minerals (Na; Fe; Mg; Zn; K; and Ca) of smoked and coated *clarians gariepinus*. Wholesome catfish was purchased at Yankura fish market in Kano, Nigeria, and transported immediately in water to FCAPT. Kano; Food Science Laboratory for processing and smoking using a newly designed and fabricated improved smoking kiln. Samples of the catfish were then coated with formulated chitosan-based edible polymer using a dip method of application and re-dried with a laboratory oven drier at 35oC for five hrs. Samples were then subjected to proximate parameters and essential elements analysis. The result of proximate parameters showed that there was an increase in moisture, ash, fiber, and carbohydrate contents from 9.73±0.8%, 4.04±0.6%, 3.50±0.5%, and 3.08±0.5 respectively before coating, to 10.27±1.2%, 6.48±1.3%, 4.33±0.6%, and 4.58±0.6% respectively after coating. There was no significant ($p<0$) difference in crude protein and fat content (67.45±11 and 7.42±0.3 respectively) before coating and (67.05±0.8% and 7.49±0.3% respectively) after coating. The result of the essential minerals component showed that there was no significant difference in the values of zinc, iron, potassium, magnesium, and sodium for both coated and uncoated smoke fish. Only Ca shows a significant increase in the value of coated compared with uncoated smoke catfish. It was concluded that chitosan coating of catfish preserves its nutritional qualities and it can also be a means of fortifying the fish with other minerals to improve its nutritional qualities.

Keywords: Chitosan; Polymer; Edible coating; Dip method; Proximate parameters; Essential elements

1. Introduction

Fish is a cold-blooded vertebrate animal living either in fresh or salt water. The various species available have adapted themselves to different aquatic habitats such as shallow waters, deep seas, rivers, dams, streams, and rocky shores (McHugh, D. J. 2003; FAO. 2000; FAO. 2012). It has the most economic value among aquatic organisms and is also very important to the human diet as it contains a high level of protein necessary for healthy growth and development (Eyo, 2001; Da-Silva, 2002). The presence of omega-3 oils and essential minerals necessary for healthy living increases the desire to consume fish, more so it is the cheapest source of protein in many developing countries of the world. The major constraint militating against the all-year availability of fish is its perishable nature. Different methods of preservation adopted to extend the shelf-life of fish include smoking, drying, frying brining, freezing, and cooking. Of these methods, smoking is the most widely used in Africa and other developing countries of the world. Packaging is another area where losses can be experienced in smoked fish production.

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Food packaging is the enclosure of food materials or products to protect and preserve their nutritional quality and increase their shelf-life for sustainable food value chains (Lindh *et. al.* 2016, United Nations, 2015). The problems of packaging have led to huge post-harvest losses of food materials the world over. The commonly used synthetic food packaging materials such as polyethylene, terephthalate, polyvinylchloride, polyethylene, polypropylene, polystyrene, and polyamide are petrochemically-based (Jacob *et. al.* (2020); Patricia and Manuel; 2019, Muncke *et. al.* 2016). This plastic is not biodegradable and thus contributes to environmental pollution (Lindani *et al*. 2020). Life in the ocean is affected by the toxicity released by these plastics, which can pose a hazard to human health as humans consume seafood through the food chain. Ozone layer depletion is another consequence of non-degradable waste from synthetic food packaging materials. As a result of these problems, food regulators in many countries are now encouraging their food industries to reduce if not eliminate the use of synthetic food packaging materials (Miguel *et al*. 2011). Through research, recent developments in the food packaging industries are replacing synthetic materials with materials that are biodegradable, non-toxic, environmentally friendly, and edible. According to Ashok *et al.*, (2016) and Lindani *et al*., (2020), the biodegradable food packaging material is a material that can be broken down from longer molecular chains into smaller molecular components when subjected to decomposition agents such as temperature, humidity, and biological enzymes. Biodegradable polymers for coatings can be divided into polysaccharides, proteins, and lipids-based, or may be composite (combinations of two or more of the above). Patricial and Manuel (2019) reported that the application of these materials or their combinations depend on the ability to prevent moisture loss, aroma loss, transport of dissolved substances, water absorption in the matrix's food, or oxygen permeation.

The aim of this present work is to determine the effect of chitosan coatings on the proximate and minerals composition of smoked catfish. Chitosan is a polysaccharide produced by the deacetylation of chitin. Chitin is obtained from crustacean exoskeletons, algae, and fungal cell walls (Inmaculada *et. al.* 2021). Of the biopolymers, chitosan is the most available coatings and additive material with film formulations to high reliability and it outperforms other products in extending the shelf life of food and agricultural products (Inmaculada *et a*l. 2021; Julie *et al*., 2021). Other properties that make chitosan a good choice for a coating material include its non-toxic properties and antibacterial and antifungal activities. Biodegradability and biocompatibility with other materials such as composite coatings and films (Utami *et al*., 2021; Jasor *et al.*, 2014; Zambrano-Zaragoza *et al.,* 2013). The antiseptic properties of chitosan have been widely exploited with promising results in agriculture, the food industry, biotechnology, and biomedicine (Zhang *et.al*. 2016).

Edible coating or film according to Samira et al. (2017); Pramod et. al. (2016); and Vijaykumar et, al. (2017); Azzadin et. al. (2020) is any food-grade thin material that is used for wrapping or enclosing food products to increase their shelf-life and can be consumed together with the product without causing any ill-heath to the consumer. Edible coatings act as moisture and gas barriers to reduce or minimize the interactions of the product with its immediate environment. It can provide adequate protection for the product against mechanical, physical, chemical, and microbiological attacks in the course of post-production handling and transporting from one place to the other. The edible coatings can also serve as carriers of food fortifying materials such as vitamins, minerals, antioxidants, antibacterial and antifungal (Vijaykumar *et, al*. 2017) There are different methods of coating applications on food products. These are dipping, brushing, spraying, casting, and electrostatic methods. The type of method to be adopted for a particular food product will depend on its nature and the polymer material to be used.

2. Material and methods

2.1 Source of Fish Samples for the Experiment

Samples of African Mud Catfish (*Clarias gariepinus*) for this research were purchased from Galadima fish market, Kano, and transported in water to the Food Science Technology Laboratory of Federal College of Agricultural Produce Technology, Hotoro, Kano. Catfish was chosen for this research work because it is the most common type of fish raised by many fish farmers through aquaculture in Nigeria.

2.2 Samples Preparation

Fish samples for the experiment were prepared according to the method Omodara *et. al.* (2016). Upon receiving, the catfish was butchered, completely degutted, and washed to remove mucilage and blood stains. It was then given osmotic treatment (soaking in a brine solution of 350g of salt to 50 liters of water for 30mins. This treatment was to reduce moisture from fish tissue before smoking and also to add taste. Thereafter they were arranged on drying trays under room temperature for the surface brine to drain.

2.2.1 Smoking and Drying of Samples

Catfish for this research work were smoked and dried using a developed smoking kiln. After drying to safe moisture content, trays were removed and fish were allowed to cool under room temperature before packing and kept for analysis.

2.2.2 Coating of Smoked Fish Samples

Application of edible coating solution on the smoked African Mud Catfish *(Clarias garipenius)* was done by dipping method. Wholesome smoked fish were sorted out among the lot, a clean tiny thread of 60cm in length, initially soaked inside alcohol solution and dried was used to tie each of the fish samples to be coated. This is to prevent direct touching of samples during and after coating. It also serves as a hanger for the samples on the line during setting and drying at room temperature. Edible solutions of different chitosan concentrations with and without plasticizers were then prepared inside a 1000ml beaker. The solution was cooled to 50^{o}c and wholesome smoked *Clarias garipenus* were dipped into the solution one after the other and allowed to be totally submerged so that every part of them was coated with the solution. They were then removed using the thread as the handle and tied on an already prepared line for an excess solution to drain and the coating to set overnight on the fish at room temperature of about 28^{o}C. Further drying is done after setting by arranging the samples inside oven set at 35^{o}c for 3-4h. Thereafter they are packaged and stored for analysis under ambient temperature.

2.3 Proximate Analysis on both Fresh and Coated Samples

Proximate analysis such as crude protein, moisture content, carbohydrate, crude fiber, fat content, and ash for the smoked fish was analyzed using the method of AOAC (2005).

2.3.1 Analysis of Crude Protein Content

Crude protein is also known as the total protein content in food substances. Kjeldahl method of protein analysis was used. Essentially, 0.2 grams of each sample was weighed, wrapped in filter paper, and placed in a Kjeldahl digestion flask. 10ml of concentrated H_2SO_4 and 0.5g of catalysts mixture (Na_2SO_4+ $CaSO_4$+SeO, 10:5:1w/w) was added to the materials in the flask. Four pieces of anti-bumping granules were added and the mixture was digested by heating on a Kjeldahl digesting apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in a standard volumetric flask. Aliquots (10ml of the diluted solution), and 10ml 45% NaOH solution were poured into the distillation apparatus and distilled into 100ml of 2% boric acid containing 4 drops of Bromo cresol green/methyl red indicator until 70ml of distillate was collected. The distillate was now titrated against standardized 0.01M HCL until the color changed to a grey endpoint. The percent nitrogen in the original sample was then calculated using the following expression:

$$\% \text{ Nitrogen} = \frac{(a-b)\times 0.001\times 14\times 100}{w\times c} \quad \text{---------- (1)}$$

Where:
a= Titre value of the digested sample
b= Titre value of the blank sample
v= Volume after dilution (100ml)
w= Weight of dried sample (mg)
c= Aliquot of the sample used (10ml)
% Protein = % Nitrogen x protein conversion factor (6.25).

2.3.2 Moisture Content

The oven dry method as described by Onwuka, (2005) was adopted to determine the moisture content of smoked fish. First, the dish was washed and dried in the oven for 30 mins, cooled inside a desiccator for another 30 mins, was weighed and recorded as W1, and 2g of sample was weighed and recorded as W_2. The oven was put on and set at 105^{o}C and dried for 3h. Thereafter removed and cooled inside a desiccator. Sample plus dish was weighed after cooling. Drying continued until the weight was constant and recorded as the final weight after cooling (W_3). The moisture content was calculated from the equation below given by Onwuka, (2005) as

$$\%\text{Moisture Content} = \frac{w_2 - w_3}{w_2 - w_1} \quad \text{---------- (2)}$$

Where:
W_1 = Weight of empty dish
W_2 = Weight of dish + Sample
W_3 = Weight of dish + oven dried sample.

2.3.3 Determination of Crude Fat

Soxhlet extraction method as described by Onwuka, (2005) was adopted to determine the crude fat. Labeled boiling flasks of 250ml was washed and oven dried at 103°C and transferred into a desiccator for cooling. Thereafter 2g of samples were accurately weighed on an electric scale into labeled extraction thimbles. The thimbles were plugged slightly with cotton wool and placed inside the assembled Soxhlet apparatus. The flasks were filled up to three quarters with petroleum ether of 45°C boiling point. The extraction was run for 6hr. The petroleum ether was removed by evaporation on a water bath while the extractives in the flask were dried in electric oven at 800°C for 30 mins followed by cooling and weighing. Percentage crude fat was calculated by the equation below:

$$\% \text{ Crude Fat} = \frac{C-B}{A} \times 100 \quad \text{---------- (3)}$$

Where:
A = Weight of sample
B = Weight of assembled Soxhlet apparatus
C = Weight of assembled Soxhlet apparatus + Extractives

2.3.4 Determination of Crude Fiber

Crude fiber is the residue left after samples have been subjected to light petroleum ether treatment. It was determined by weighing 2g of crushed samples and boiling for 30mins under reflux containing 200ml of 1.25g of H_2SO_4 per 100ml of solution and then filtered with linen on a fluted funnel. The insoluble material was washed with boiling water until it is acid-free. The residue was then washed back into a beaker and boiled for 30mins with a solution containing 1.25g of carbonate-free sodium hydroxide per 100ml. The final residue was filtered through already washed and ignited asbestos in a Gooch Crucible and dried at 100°C for 2hr followed by cooling in a desiccator and weighing (W_1). The dried, cooled and weighed residue was then transferred into a muffle furnace and incinerated at 600°C for 4hr followed by weighing (W_2). The percentage of crude fiber content was calculated from

$$\% \text{ Crude fiber} = \frac{W_1 - W_2}{W_1} \times 100 \quad \text{---------- (4)}$$

2.3.5 Determination of Ash Content

Ash content of food materials is the residue of minerals left after moisture and organic materials (proteins, fats, carbohydrates, vitamins, organic acids, etc.) have been burnt away at a temperature of about 500°C. Finely ground 5g of sample was weighed into a silica crucible that has already been heated to 600°C followed by cooling in desiccators. The weight of the empty crucible (W_1) after cooling was taken as well as the weight of the crucible and sample (W_2) and recorded. The sample was then transferred into a pre-heated muffle furnace at 550°C for 2hr or until light grey ash results indicating that all organic matter has been burnt. The sample was then removed from the furnace and cooled in a desiccator after which the final weight (W_3) was taken and recorded. Percentage ash content was calculated with the formula below:

$$\% \text{ Ash content} = \frac{w_2 - w_3}{w_2 - w_1} \times 100 \quad \text{---------- (5)}$$

Where:
W_3 – Final weight of crucible + sample
W_2 – Initial weight of crucible + sample
W_1 – Empty weight of crucible

2.3.6 Determination of Carbohydrate Content

The Difference method as described by Mičulis, (2011) was used to determine the percentage carbohydrate content. This is given as:

% Carbohydrate = 100 – (% crude protein + % ash + % crude fat + % moisture + % crude fiber). ---------- (6)

2.3.7 Determination of Essential Elements

Essential mineral contents of the smoked fish were determined using the standard AOAC official method Azzadin, (2020) as described by Ana, (2011) 2g of ground samples was burnt down to ashes in a porcelain crucible at 450^{o}C all-night and then processed with 5ml of 6M HCL, boiled until all liquid solution evaporated on a hot plate and then allowed to cooled inside a desiccator. 10ml of 0.1M nitric acid was added to make a solution of the ash left-over. The solution was later poured inside 50ml round bottom flask after 2h with the addition of distilled water up to the mark. The mixture was now used in the determination of the mineral contents of the smoked fish sample. The following minerals Ca, Mn, Zn, Fe, and Pb underwent Atomic Absorption Spectrometer for their determination while Flame Photometer was used for Na, and K determination. Concentration levels of each element was then calculated from the solution.

3. Results and discussion

3.1 Effect of chitosan coatings on proximate compositions of the coated cat fish

The results of proximate analysis for both coated and uncoated smoked catfish are shown in Table 1. The mean value of moisture content for coated and uncoated from table 1 were 10.27% and 9.73% respectively. The moisture content of coated African mud catfish samples increases after going through the process of coating. Both coated and uncoated moisture content were far less than 30% which was recommended by Nwaehujor *et. al*. (2021) for safe storage against microbial infection. Moisture content is of great importance to the keeping quality of the fish. High moisture content could account for a reduced shelf-life. The higher moisture in coated sample compared with uncoated could be as a result of difference in individual moisture content of the sample compositions (chitosan coatings + smoked fish). The values of Ash content of coated and uncoated smoked catfish from Table varied between 6.48±1.3% and 4.04±0.6 % respectively. An increase in the ash content of coated samples compared with uncoated samples indicates an increase in the mineral content of coated samples. This could be as a result of the mineral content (calcium) present in the chitosan material used in coating. As observed from Table 1, the crude protein of coated and uncoated samples was 67.05±08 and 67.45±11 respectively. There was no significant ($p<05$) difference in protein value between coated and uncoated samples of dried fish (Adeyeye *et. al.* 2015). It can also be observed from the Table that there was no significant difference in values of the crude fat for uncoated smoked fish (7.42±0.3) and coated smoked fish (7.49±0.3). Fibre and carbohydrate values for coated fish is significantly different and higher than those of the uncoated smoked fish. This finding could be as a result of the fact that chitosan being a polysaccharide increases the fibre and carbohydrate content in the coated fish.

Table 1 Proximate Analysis of Coated and Uncoated Catfish

Samples	Moisture Content (%)	Ash Contents (%)	Crude Protein (%)	Crude Fat (%)	Fiber Content (%)	Carbohydrate (%)
Uncoated smoked Fish	9.73±0.8b	4.04±0.6b	67.45±11b	7.42±0.3a	3.50±0.5b	3.08±0.5b
Coated smoked Fish	10.27±1.2a	6.48±1.3a	67.05±0.8a	7.49±0.2a	4.33±0.6a	4.58±0.6a

3.2 Effect of chitosan coatings on mineral compositions of the coated catfish

The results of the effects of coatings on mineral compositions of the catfish are presented in Figure 1. It can be observed from the figure that there is no significant difference in the values of zinc, iron, potassium, magnesium, and sodium for both coated and uncoated smoke fish. One can conclude that coating the smoke fish with chitosan-based bio-coating does not have an impact on the aforementioned minerals. But there was a significant ($p<0.05$) difference in calcium composition between coated and uncoated fish samples. The Ca composition in coated fish was higher than that of uncoated. This can be attributed to the presence of calcium in chitosan composition which was added to that present in

the fish sample. It can then be concluded that coating catfish with chitosan improves the minerals content of the coated fish. Calcium in human diet gives stronger bones and healthy teeth most especially in growing children.

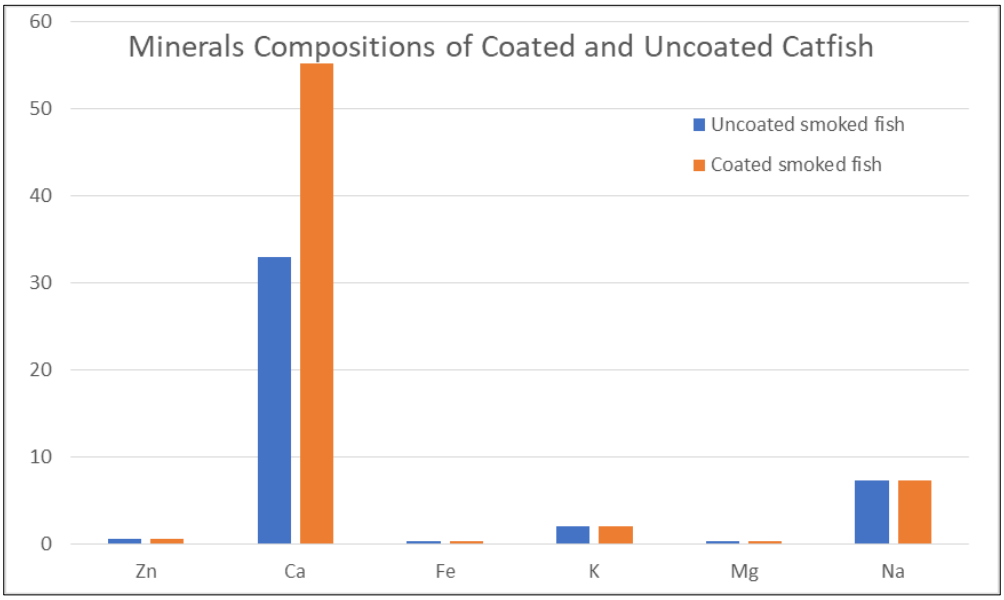

Figure 1 Graph of Mineral Composition for Coated and Uncoated Catfish

4. Conclusion

The result of this research work has established the effect of chitosan-based edible coating on smoked catfish. This has made vital information available concerning the proximate parameters (moisture content, crude protein, crude fiber, ash, crude fat, and carbohydrate content) and essential minerals (Na, K, Fe, Zn, Mg, and Ca) of smoked and coated *clarians gariepinus.* It can be concluded that the chitosan edible coating of catfish improves its nutritional qualities aside from its preservative qualities and it can also be a means of fortifying the fish with other minerals to improve its nutritional qualities. Smoked fish is a common source of protein in the diet of Nigerians and other developing countries of the world; therefore, it is recommended that regular analysis of its proximate parameters and essential minerals compositions should be carried out by food regulatory bodies to ascertain its nutritional qualities and to make sure that essential minerals are present up to the required levels so as to guide against nutritional deficiency of the consumers.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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