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Microbial and proximate analysis of sea food exposed to different preservation techniques

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Abstract

The study examined the microbial load of bacteria and fungi of samples stored from 0- 10 days. The study samples were subjected to proximate analysis. The study showed at day zero that fish was the highest having moisture content of 71.43%, while periwinkle had ash content of 4.62%. Shrimps was the least having 0.74% of ash. Periwinkle had crude protein of 53.1% and carbohydrate of 24.09. The result for minerals shows that all the samples were high in mineral content. Fish was highest in Calcium (Ca) 52.67, Magnesium (Mg) 63.18, Potassium (K) 64.72 while Periwinkle was lowest in Zinc (Zn) 0.48, Iron (Fe) 2.47 and Calcium (Ca) 36.42. Finally Shrimps was lowest in Magnesium with 31.78% respectively, however there is a reduction in all the proximate analysis carried out from day 0 to day 10 in carbohydrate, crude protein and crude lipid. Thus, reduction in water activity as a result of smoking fish slow down spoilage and extend shelf life of the sample. In the samples treated, smoking have been shown to be more effective in the preservation than Refrigeration.

Keywords: Microbial; Proximate; Preservation; Sea Food; Techniques

1. Introduction

Foods undergo spoilage due to microbial, chemical, or physical actions. Therefore, foods are required to be preserved to retain their quality for longer period of time. Food preservation is defined as the processes or techniques undertaken in order to maintain internal and external factors which may cause food spoilage. The principal objective of food preservation is to increase its shelf life retaining original nutritional values, color, texture, and flavour. The history of 'Food Preservation' dates back to ancient civilization when the primitive troupe first felt the necessity for preserving food after hunting a big animal, which could not be able to eat at a time. Knowing the techniques of preserving foods was the first and most important step toward establishing civilization. Conventional food preservation techniques like drying, freezing, chilling, pasteurization, and chemical preservation are being used comprehensively throughout the world. Scientific advancements and progresses are contributing to the evolution of existing technologies and innovation of the new ones, such as irradiation, high pressure technology, and hurdle technology (Freedman, 2011). The processing of food preservation has become highly interdisciplinary since it includes stages related to growing. The processing of food preservation has become highly interdisciplinary since it includes stages related to growing, harvesting, processing, packaging, and distribution of foods. The Aim is to determine the effect of Microbial and Proximate Analysis of Sea Food Exposed to Different Preservation Techniques. Therefore, an integrated approach would be useful to preserve food items during food production and processing stages. Several groups of pathogenic bacterial have been found associated with seafood such include, *Salmonella, Listeria, Escherichia coli* and *Vibroparahaemolyticus*. The prevalence of these pathogenic bacterial on Ready-to-eat (RTF) seafood may be due to handling during and after

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processing or contamination in the inherent habitat of the fish, when this contaminated seafood are consumed by human it brings negative impact which may include illness or death (Kim *et al*., 2014), they further allude that pathogenic bacteria is the causative agents in human if infective dose are consumed, to them 100 viable cells are infective to human when host-parasite relationship are considered. Food spoilage due to physical changes or instability is defined as physical spoilage. Moisture loss or gain, moisture migration between different components, and physical separation of components or ingredients are the examples of physical spoilage (Rahman, 2007; Balasubramanian& Viswanathan, 2010). The key factors affecting physical spoilage are moisture content, temperature, glass transient temperature, crystal growth, and crystallization. Food serves general purpose of keeping the body healthy and strong, it can also serve as transmission medium to pathogenic microorganisms. Several groups of pathogenic microorganisms have been involved in foodborne disease but the most implicated foods are vegetables, fruits, frozen food and many more, the associated organisms include, *Salmonella, Listeria monocytogenes*and *Listeria ivanovii, Vibroparahaemolyticus, Pseudmonas aeruginosa* and *Staphylococcus aureus*. The central for Diseases Control and Prevention in 2020) estimated that 48 million illness, 128.000 hospitalized and. 3.000 death occurred in 2017 - 2020 due to foodborne disease. In any food processing sector microbiological analysis such as specification, harzard analysis and critical control point should be set and monitored in order to reduce or totally eliminate the spread of pathogenic microorganisms also vendors and manufacturer' scan follow microbiology specified guidelines during preparation, storage, packing and distribution to totally bring the growth and proliferation rate of the organisms to a hault.

2. Material and methods

The study was carried out in vendors within and around Choba and Rivers state University campus, in Port Harcourt, Rivers State. The choice of both location is associated with population of people including students and staff of host tertiary institutions located around these study areas.

2.1. Sample Collection

A total of 36samples samples made up of 12 Fishes, 12 Prawns, and 12 Periwinkles were purchases at random from Choba market, Port Harcourt. The samples were purchased and divided into 2 equal halves and wrapped in a sterile nylon bag. The samples were transported to the Microbiology laboratory within minimum time.

2.2. Sample Preparation.

Each half of the samples were kept inside the freezer while the other half was smoked and samples analyzed within 2days intervals (i.e day 2,4,6,8 and 10).

The Periwinkle sample was washed with clean water to remove debris or sand. The periwinkles were then roasted in a pot at minimal temperature and time with constant steering on the pot. The periwinkle was then brought out of the shell and divided into portions.

2.3. Isolation and Characterization

Depending on the level of contamination, serial dilutions was made for Total Heterotrophic Count (THC), and Total Fecal ColiformCounts (TFCC) (Temesgen*et al.,* 2016) using nutrient agar plates, MacConkey agar and Eosin Methyl Blue (EMB) plates and incubated at 37^{o}C for 24-48 hours. The plate count were compared with the standard plate count level set for microbiological quality of pea nut butter.

2.4. Enumeration of Bacteria

A homogenate was prepared by measuring 10g of each of the each of the samples purchased from the fish, prawn and Periwinkle vendors and was transferred into 9ml of normal saline and homogenized for 30 seconds aseptically. A 6 10fold dilution tubes containing 9ml of sterile saline was used and 1ml was transferred from the residue homogenate aseptically using a sterile syringe, to the first dilution tube, the same procedure was used. 1ml was aseptically withdrawn from the first dilution bottle to the second dilution bottle. This was repeated until the dilution was completed. Using a new 1ml pipette 0.1ml was aseptically transferred from the dilution tubes labelled 10^{-4}, 10^{-5}, 10^{-6}, aseptically to a freshly prepared and dried Nutrient agar plates (for Total Heterotrophic Count) and Mac Conkey agar plates (for Total Fecal Coliform Count) and spread with a sterile bent rod aseptically. The plates were then be incubated at 37^{o}C for 24hrs. This was done in duplicate.

The number of colonies was counted and the average taken, the colony forming unit of each average was calculated using average divided by the dilution factor, multiplied by the volume plated. Total population were expressed as Colony Forming Units per gram (Cfu/gm). This was repeated for all samples from day o-day10.

2.5. Enumeration of Fungi

A homogenate was prepared by measuring 10g of each of the each of the samples purchased from fish, prawn and Periwinkle vendors the and was transferred into 90ml of normal saline and homogenized for 30 seconds aseptically. A 6 10fold dilution tubes containing 9ml of sterile saline was used and 1ml was transferred from the residue homogenate aseptically using a sterile syringe, to the first dilution tube, the same procedure was used. 1ml was aseptically withdrawn from the first dilution bottle to the second dilution bottle. This was repeated until the dilution was completed. Using a new 1ml pipette 0.1ml was asceptically transferred from the dilution tubes labelled 10^{-4}, 10^{-5}, 10^{-6}, aseptically to a freshly prepared and dried Potato Dextrose agar (for Total Heterotrophic Fungi) and spread with a sterile bent rod aseptically. The plates shall then be incubated at ambient temperature of 25^{0}C. This was done in duplicate.

The number of colonies was counted and the average taken, the colony forming unit of each average was calculated using average divided by the dilution factor, multiplied by the volume plated. Total population shall be expressed as Colony Forming Units per gram (Cfu/gm).

2.6. Isolation of Microorganisms

Pure culture of isolates obtained were repeated subculture on freshly prepared nutrient agar, MaConkey agar, Centrimide agar, Eosine Methyl Blue agar, Manitol Salt agar, Salmonell Shigella agar and Saboraud Dextrose agar .The isolates further was stored on slants at 4°C refrigeration temperature for identification. Identification of characteristic bacteria isolates was based on colonial morphology, microscopy and biochemical tests.

2.7. Standardization of Bacterial Inoculum

A loop full of test organism shall be inoculated on nutrient broth and incubated for 24 hours. Exactly 0.2 ml from the 24 hours culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 3-5 hours to standardize the culture to 0.5 McFarland standards (106 cfu/ml)

2.8. Proximate Analysis Methods

The moisture, crude protein, crude fat, crude fiber, Carbohydrate and ash contents as well as the mineral contents of the fishes, Prowns, and Periwinkle were determined.

2.9. Moisture Determination

2g of each of the samples was accurately weighed in a clean, dried petri dish of a known weight (W1). It was quickly placed in a conventional oven at 105 °C for 6 h. The petri dish was then placed in a desiccator for 30 min to cool. After cooling, it was weighed again (W2). The percent moisture was then calculated.

2.10. Crude Protein Determination

2g of each of the samples was weighed into a digestion tube, 5 grams of catalyst and 1 glass bead together with 10 mL concentrated sulfuric acid was also added. Digestion tubes were placed in the digester. Digestion commenced initially at low temperature to prevent frothing and boiling at a temperature of 320°C until the solution was clear which an indicator of complete oxidation is. Erlenmeyer flask (250mL) containing 50 mL of 4% boric acid with indicator was placed in a distillation unit and distillation commenced for 10 min whiles the tip of the condenser extended below the surface of the acid solution. 100 mL of water and 70 mL of 50% sodium hydroxide (excess) was added to the digests during the distillation process to ensure complete release of ammonia. 150 mL of distillate was obtained when condenser with ice cold water was used to effectively capture all distilled ammonia and the receiver flask was lowered to ensure that the delivery tube was properly placed for continues distillation. The delivery tube was rinsed with water and the washing was made to drain into the flask. Titration of the distillate was done with a standardized 0.1 N hydrochloric acid until 16 the first appearance of the pink colour was obtained. Result was recorded to the nearest 0.05 mL volume and calculated.

2.11. Crude Fiber

5g of each of the samples was taken from the zip lock bags and defatted before subjecting to analysis. 2g of peanut samples were weighted into a flat bottom flask and 200 mL of boiling sulphuric acid (1.25%) was added for 30 min. The resulting solution was filtered through cheesecloth using a funnel and then washed with hot water until it was free from the acid. The residue on the cloth was transferred into a flask and 200 mL of boiling sodium hydroxide solution (1.25%) was added. The flask was immediately connected to the digestion apparatus and boiled for 30 min. The flask was then

removed and immediately the solution was filtered and washed thoroughly with boiling distilled water until washing was no longer basic. The residue was rinsed with 15 ml of alcohol. It was transferred into porcelain crucibles and dried at 105°C in an oven for 24 h. It was cooled to room temperature in a desiccator and weighed. The crucible and its weight was incinerated in a muffle furnace at 550 °C. It was cooled to room temperature in a desiccator and weighed. The difference between the two weights was recorded and the percentage crude fiber calculated.

2.12. Crude Fat Determination

Approximately 1 g of dried samples from the moisture analysis was weighed and wrapped in a filter paper and placed in a fat free thimble and then placed into the extraction tube. Clean and dried receiving beaker was weighed and filled to about three quarter with petroleum ether and then fitted into the Soxhlet apparatus. The tap was turned on to allow the flow of water, and heater was also turned on to start the 17 extraction process. After 6h, siphoning allowed ether to evaporate and condense back into the receiving beaker and the process was repeated until extraction was complete. The beaker was disconnected before the last siphoning. Extract was placed in a water bath for the remaining ether to evaporate; the dish was then placed in an oven at 105oC for 2 hours and cooled in a desiccator. The percent crude fat was determined.

2.13. Ashing

Clean empty crucible was placed in a muffle furnace at 600 °C for an hour, it was cooled in a desiccator and then weighed and weight noted W. 1g of sample was weighed and transferred into the crucible and noted W2. The sample was ignited over a burner with the help of blowpipe, until charred. Then the crucible was placed in a muffle furnace at 600°C for 6 h for complete oxidation of all organic matter in the sample. After the process, Crucible was cooled in the desiccator and the weight was noted W3. Percent crude ash was calculated and recorded.

2.14. Total Carbohydrate

Total carbohydrate was determined by difference between 100% and the sum total of determined proximate component.

3. Results and discussion

The result of the proximate analysis at day zero shows that fish was the highest having moisture content of 71.43%, while periwinkle had ash content of 4.62%. Shrimps was the least having 0.74% of ash. Periwinkle had crude protein of 53.1% and carbohydrate of 24.09. The result for minerals shows that all the samples were high in mineral content. Fish was highest in Calcium (Ca) 52.67, Magnesium (Mg) 63.18, Potassium (K) 64.72 while Periwinkle was lowest in Zinc (Zn) 0.48, Iron (Fe) 2.47 and Calcium (Ca) 36.42. Finally Shrimps was lowest in Magnesium with 31.78%.

The result shows that there was a decrease in the entire result obtained from proximate analysis. Each of the Parameters experiences a decline. Fish still had the highest moisture content although decreased to 62.82%. Periwinkle still had the highest percentage of Ash although it reduced to 3.58%. Shrimp still maintained the highest crude fiber with 3.21%. Although Periwinkle was richest in carbohydrate though reduced. For elements, Potassium (K) was highest and found in fish after refrigeration. As shown in table1

Thus, reduction in water activity as a result of smoking fish slow down spoilage and extend shelf life of the sample.

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Table 1 The Proximate analysis of Fish, Shrimps and Periwinkle from Day Zero (0) and Day Ten (10)

Sample	Periwinkle (Day 0)	Periwinkle (Day10)	Shrimp (Day 0)	Shrimp (Day 10)	Fish (Day 0)	Fish (Day 10)
Moisture content (%)	16.33	14.74	67.29	54.28	71.43	62.82
Ash (%)	4.62	3.58	0.74	0.63	1.58	1.24
Crude fiber (%)	0.58	0.51	4.64	3.21	1.46	1.08
Crude protein (%)	53.1	48.6	18.94	15.73	21.45	17.50
Crude lipid (%)	1.28	1.01	1.63	1.48	1.37	1.26
Carbohydrate (%)	24.09	31.56	7.04	24.67	2.71	16.1
Ca (mg/100g)	36.42	24.59	47.91	31.62	52.67	44.83
Mg (mg/100g)	58.24	48.13	31.78	22.47	63.18	51.05
Fe (mg/100g)	2.47	2.08	4.82	4.31	3.91	3.25
K(mg/100g)	38.19	33.84	42.69	36.76	64.72	57.18
Zn (mg/100g)	0.48	0.29	1.77	0.84	1.58	1.13

4. Conclusion

The microbial evaluation of Fish, Periwinkles and Prawns sold within Choba preserved by smoking and freezing was carried and the result showed that ten bacteria and two fungi genera were isolated from the entire samples and they was a considerable reduction in number of viable cell count after some days of the storage methods used (Smoking and Freezing) although there was a considerable degrease in physicochemical and nutritional components of each of the stored samples. One of the major revolutionary inventions of human civilization was acquiring the knowledge to preserve foods as it was the precondition to man to settle down in one place and to develop a society. However, increasing shelf lives of food items without compromising original food properties is still critical and challenging. Food is an organic perishable substance, which is susceptible to spoilage due to microbial, chemical, or physical activities. Different traditional techniques, such as drying, chilling, freezing, and fermentation, had been evolved in the past to preserve foods and to maintain their nutrition value and texture. With time and growing demands, preservation techniques have been improved and modernized. To ensure food safety and long shelf life of foods, it is important to understand food spoilage mechanisms and food preservation techniques.

Compliance with ethical standards

Acknowledgment

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Disclosure of conflict of interest

No conflict of interest to disclose.

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