Assessment of Food Quality and the Associated Mycoflora of Okpa, a Local Recipe from Bambara Groundnut

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Abstract
Okpa is a local cake produced from Voandzeia subterranean (Bambara groundnut). Studies on the food quality of okpa and its associated fungal flora were carried out in the Department of Plant Science and Biotechnology and the Food Science and Technology Laboratory respectively in the Rivers State University. The proximate composition of the okpa revealed the presence of moisture, ash, fibre, lipid, carbohydrate and protein, although moisture, lipid, protein and fibre had higher values for the spoilt sample, while ash and carbohydrate were highest in healthy samples. The mineral content found were iron, phosphorus, potassium, sodium and magnesium and all the parameters accessed recorded higher values in healthy samples. Four fungal organisms were isolated from spoilt samples and include: Cryptococcus neoformans, Rhizopus stolonifer, Aspergillus niger and Fusarium oxysporium. The highest percentage incidence was observed for R. stolonifer (70%) while 10% was the least recorded for C. neoformans.

Key word: *Okpa, Food quality, Fungal flora and Incidence*

Introduction

Voandzeia subterranean L. (synonym: *Vigna subterranean*) commonly known as Bambara groundnut is a legume indigenous to Africa and belongs to the family Fabaceae. It goes by the names Okpa (Igbo), Epa-roro (Yoruba) and Kwaruru (Hausa) in Nigeria and it is regarded as an important legume following its socioeconomic and utilization values (Akpalu *et al*., 2013). The plant is annual and herbaceous although small with a height ranging from 0.30 to 0.35m and is characterized by three leaflets of compound leaves. The seed of Bambara groundnut possesses a similar shape as peanut *(Arachis hypogea*) but reveals a length of half inch with wrinkled and round pods (Bamshaiye *et al*., 2011). Having the ability to grow in areas with high and low rainfall, *V. subterranean* is regarded as a drought tolerant and has been cultivated in semi-arid areas like Ghana, South Africa and Nigeria. However, its cultivation has also been reported in South East Asia (Indonesia, Thailand and Malaysia), Europe (Spain, Italy and Portugal) and places within the Mediterranean climate such as Isreal and Lebanon (Azam- Ali *et al*., 2001).

The seeds contain 19% of protein, 63% of carbohydrate and 6.5% of oil; and can be processed into flour (Okonkwo and Opara, 2010). The flour is used to prepare different porridges, soups and cakes. Okpa is a processed cake from bambara groundnut and is cherished mostly by the South Eastern Nigerians. Its production process involves weighing of desired quantity of flour, mixing with necessary ingredients and small water, wrapping in nylon or put in small plates and boiled before being consumed. It is most preferably served with cooled soft drink (Alobo, 1999).
Following the unhygienic practices in the production of okpa, several fungal organisms have

been implicated to cause contamination and spoilage of the cake. Organisms such as *Aspergillus niger, Penicillium spp, Trichophyton spp, Chrysosporium spp, Mucor spp, Syncephalastrum spp, Geotrichum spp and Rhizopus spp.* have been reported to be associated with the spoilage of the prepared recipe. The presence of these organisms do not only reduce the nutritional values of the cake but also have severe health implications as these organisms have been noted by early researchers to cause several harmful diseases (Nnaji and Rao, 2017).

Materials and Methods

Sample Collection

Samples of ready to eat Okpa (*Voandzeia subterranean)* were bought from Mile 3 market Diobu Port Harcourt and brought to the Department of Plant Science and Biotechnology Laboratory where it was observed for spoilage.

Mycological studies

Preparation of mycological medium

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120^{o}C for an hour after washing with soap, while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Agrios, 2005). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminium foil. The conical flask containing the mycological medium was autoclaved at 121^{o} C and pressure of 1.1kg cm-3 for 15 minutes. The molten agar was allowed to cool to about 40 ^{o} C and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation of fungi from *Voandzeia subterranean*

One gram of *Voandzeia subterranean* sample showing visible signs of spoilage by moulds was inoculated onto Sabouraud Dextrose Agar in Petri dishes onto which ampicillin was added to hinder the growth of bacteria. The inoculation was done in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25^{o} C ± 3^{o} C (Baudoni, 1988, Chuku, 2009, Samson *et al*, 1981). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates was obtained after a series of isolations**.**

Identification of fungal organisms from *Voandzeia subterranean*

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Samson *et al*, 1981 and Olds, 1983).

Pathogenicity studies

Pathogenicity studies were carried out on *Voandzeia subterranean* to check if the fungi isolated from *V. subterranean* were capable of causing spoilage of the fresh samples. The methods of (Agrios, 2005, and Trigiano, 2004) were basically followed. The fungal isolates were introduced into *V. subterranean* and observed for seven days. The set up was monitored regularly for growth.

Determination of nutrient components of *Voandzeia subterranean*

The samples of *Voandzeia subterranean* were sent to the Food Science and Technology Laboratory for the determination of nutrient compositions. The methods of AOAC (2005) were used for the analysis.

Results and Discussion

Table 1: Proximate composition of fresh and spoilt okpa

Parameters	**Percentage Composition (%)**	
	Fresh	Spoilt
Moisture	46.50	62.85
Ash	4.50	3.20
Lipid	30.45	32.10
Fibre	1.35	2.75
Carbohydrate	52.35	25.25
Protein	13.65	15.60

Table 2: Mineral composition of fresh and spoilt okpa

Parameters	**Percentage Composition (%)**	
	Fresh	Spoilt
Calcium	1.10	0.95
Iron	0.31	0.30
Magnesium	0.52	0.40
Sodium	6.50	6.10
Potassium	0.40	0.30
Phosphorus	0.25	0.20

Table 3: Fungal isolates and their percentage incidence

Fungal isolates	**Percentage incidence (%)**
Rhizopus stolonifer	70
Cryptococcus neoformans	30
Aspergillus niger	10
Fusarium oxysporium	15

Result of proximate composition for Okpa is presented in Table. Moisture values increased from 46.5 in fresh okpa samples to 62.85 in spoilt samples. A higher value of ash (4.50) was observed for the fresh sample compared to 3.20 recorded for the spoilt. The values recorded for lipid were 30.45 and 32.10 for fresh and spoilt samples respectively. The highest value for fibre (2.75) was recorded for the spoilt sample with a lower value of 1.35 for the fresh samples. Carbohydrate value (52.35) recorded for the fresh samples were higher compared to 25.25 found in the spoilt okpa samples. However, a reduced value for protein (13.65) for the fresh samples and an increased value of 15.60 for the spoilt samples were also observed. It is important to state that moisture, lipid, protein and fibre recorded higher values in spoilt samples, while ash and carbohydrate had the highest values in fresh samples. The results for the proximate composition of healthy okpa in this study is in line with Mazahib *et al* (2013) analysis on bambara groundnut, although 18% of protein was reported which was relatively higher than what was got in this study. More so, values of carbohydrate and protein were slightly lower than that reported by Okonkwo and Opara (2010).

Table 2 revealed that calcium, iron, magnesium, sodium, potassium and phosphorus recorded 1.10, 0.31, 0.52, 6.50, 0.40 and 0.25 values respectively for the fresh samples. However, values of 0.95, 0.30, 0.40, 6.10, 0.30 and 0.20 were also reported for calcium, iron, magnesium, sodium, potassium and phosphorus respectively in the spoilt samples. Generally, it was observed that all the parameters accessed were highest in healthy samples and as such a higher food quality as against the spoilt samples where some food nutrients reduced.

Four fungal organisms were isolated in this study from the spoilt okpa as shown in Table 3 and they are as follows; *Cryptococcus neoformans, Rhizopus stolonifer, Aspergillus niger and Fusarium oxysporium.* The highest percentage incidence was observed for *R. stolonifer* (70%) and this was followed by 15% and 10% for *F. oxysporium and A. niger* respectively. Furthermore, the least incidence of 5% was observed for *C. neoformans.* The above organisms were subjected to pathogenicity test and were observed to cause spoilage in the fresh samples. The current study agrees with Nnaji and Rao (2017) who also isolated similar fungal organisms found to be associated with the grain and flour of Bambara groundnut. They also reported that, *Rhizopus and Aspergillus* were the highest contaminants of *V. subterranean*. However, the deteriorative effect of fungi on the nutrient composition of food including stored grains has been reported by early researchers (Amadi and Adeneyi, 2009).

Conclusion

The current study has revealed the presence of fungal organisms in the ready to eat okpa sold in the market which could due to the poor hygienic method adopted. This stands as a great threat to the human health as these organisms can cause diseases. The isolation of Aspergillus species from this important delicacy is frightening bearing in mind the fact that they harbour mycotoxins capable of causing cancer in man. But proper handling during the production process like preparation in sterile environment, use of clean water, clean nylon or plate and adoption of proper hygiene will not only reduce the incidence of occurrence of these organisms but will also make the cherished okpa safe for eating.

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