Effects Of Associated Mycoflora On The Shelf Life Of Varieties of Groundnut (*Arachis hypogea* L.) Paste Preserved With Occium *gratissimum* (Scent Leaf)

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Abstract
This research explores the effects of associated mycoflora on shelf life of different varieties of Arachis hypogaea (groundnut) proceeded into paste and treated with scentleaf. The research was conducted in the Plant Science and Biotechnology laboratory, Rivers State University, Port-Harcourt, Nigeria. Three varieties of groundnut were used; Samnut 21, Samnut 23 and Samnut 24, identification was done at School to Land Farms, Rumuodumaya, Rivers State. 20kg of each of the groundnut varieties were bought from Rumuokoro market in Port-Harcourt. After identification, the seperated groundnut varieties were processed by salting with a sprinkle of water and fried locally (using firewood and a metallic frying pan) until light brown, thereafter ground under aseptic condition into paste, and samples properly labeled and set aside. Ocimum gratissimum (scent leaf) is the plant material used as treatment was sundried and ground to powder thereafter, it was applied to 10g of each variety of the groundnut paste. The effects of powdered scent leaf powder on the groundnut paste varieties resulted in significant reduction in lipids, calcium, Iron, potassium, while all other parameters such as moisture, fibre, carbohydrate, Sodium etc. significantly increased at ($p<0.05$) across the three species. The application of powdered scent leaf to the three varieties of groundnut paste, improved moisture content, reduced associated fungi and as well as improved shelf life and quality of the varieties of groundnut paste.

Key words: *Groundnut, microflora, shelf life and better leaf.*

Introduction

Groundnut is also known as peanut, goober pea, or monkey nut. The plant is a legume grown in both tropics and subtropics. Groundnut belongs to the family Fabaceae which are mostly legumes, they have the ability to fix nitrogen in the soil, this makes them valuable crops especially in crop rotation. The plant is native to; South America, Bolivia, Argentina and Brazil (Seijo *et al*, 2007) now domesticated all around the world. It is believed that groundnut was introduced into Nigeria

in the 16th century. In Nigeria, groundnut is consumed in various ways; either boiled, fried, roasted, etc. and can also be processed into various delicacies by different tribes in the country. The commercial production of groundnut has been hampered due to spoilage caused by *Aspergillus flavus* which can gain entry through openings on the seed kernel or naked seeds. There are several products and delicacies prepared from groundnut such as; Dankwa (a local delicacy prepared from groundnut, native pepper, sugar, salt, dried cereal, made into balls common amongst the Hausas), another local delicacy prepared from groundnut is Okwuse (prepared by frying and grinding groundnut seeds, common in Niger Delta. Groundnut can also be processed into a paste, and eaten with kolanuts or garden eggs during traditional occasions. Groundnut paste and/or products can be contaminated as a result of improper handling, poor production hygiene methods, and improper storage conditions, especially when the product is not consumed early within the first four weeks of production. There are several fungal pathogens associated with this deteroriation in groundnut paste and similar groundnut products, such fungi include; Rhizopus stolonifer, *Sclerocium rolfsii*, *Aspergellus flavus*, as well as Aspergellus species (Okogbule et al., 2023).

Scent leaf is also known as African basil, bush tea and fever plant is a shrub that can be grown in tropical and sub-tropical regions. Scent leaf is native to Africa, Madagaster, and southern Asia. The plant has aromatic properties and it is a popular flavouring agent added to soups (especially pepper soup) in southern part of Nigeria. In traditional medicine, scent leaf is used for the treatment of common cold, fever, convulsion in children, etc. The plant due to its pharmacological properties can be used as an anti-convulsant and sedative due to the anxiolytic properties of the leaves essential oil . The essential oil of scent leaf shows some evidence of antibacterial activity (Oboh *et al*, 2009). Scent leaf acts as insect repellent and it also has potential use as a food preservative (Nguefack *et al*., 2009).

MATERIALS AND METHODS

GROUND NUT PASTE PREPARATION

Twenty kilograms of 3 varieties of freshly harvested and shelled groundnut was purchased from Rumuokoro market in Port Harcourt metropolis and transported to School to Land Farm at Rumuodamaya for identification. The reported groundnut varieties were Samnut 21, Samnut 22 and Samnut 23. The groundnut was taken to Ozuoba for further preparation in Obio/Akpor Local Government Area, in Rivers State. Filter the plants, and separate the battered ones from the unblemished ones. Sprinkling with water and a slight amount of salt, the seeds were wetted and combined vigorously and sundried for an hour. Using a low heating local oven operated by firewood, the dry seeds were then fried in a metallic frying pan. Care was taken to ensure the seeds were fried well without causing burning to them. Frying the groundnut seeds in garri accomplished smooth frying. Before peeling they allowed the fried seeds to cool down. The seeds were ground in manual blender after peeling, and saved for further research (Chuku, 2011).

PREPARATION AND COLLECTION OF PLANT MATERIALS.

Preparation of Preservation Plant Material

The leaf sample was purchased from Rumuokoro market in Port Harcourt metropolis, washed and sun dried for about 5 days to remove moisture. Thereafter, it was blended into fine powder and stored in an airtight well labelled container.

DETERMINATION OF PROXIMATE AND MINERAL COMPOSITION OF GROUNDNUT PASTE AND THE PLANT MATERIALS.

The different samples prepared were taken to the Rivers State University Food Science and Technology Laboratory for study. This method of analysis used was the AOAC (2005).

BIOLOGICAL PROCEDURE

Pure culture of each isolate was inoculated on groundnut paste after the plant was sterilized with 70-80% ethyl alcohol on the surface and washed in sterile distilled water. Sterile scapula was used, where some portion of each isolate mycelium was added aseptically. Vaseline was used for securing the wound to the Inoculums. This will prevent contact with other micro-organisms. They were kept in a clean dish on the laboratory desk until rotted. Thereafter the biochemical parameter of the rotten plant was also analyzed.

CHEMICAL PROCEDURE

Proximate Composition was determined for protein, carbohydrate, Ash, fiber, lipid, and moisture. This procedure was carried out in accordance with the Association of Official Analytical Chemists (2005).

Mineral Content of determine the concentration of calcium, potassium, sodium, iron, nitrogen, phosphorus, on the plant material was done in accordance with the methods of Osborne (1978) and (AOAC 2005) procedures.

Vitamins Evaluation.

The analysis of certain vitamins in the groundnut paste was carried out. The composition of vitamin A, C, and E was determined in accordance with the AOAC (2005) procedures.

MYCOLOGICAL STUDIES

STERILIZATION OF GLASSWARE

Glassware such as pipettes, conical flask, test tubes, measuring cylinder, beaker etc were washed and sterilized in a hot air oven for 20 to 30 minutes. Also, distilled water and normal saline were autoclaved for 15minutes at 121^0C. All workbench surfaces were sterilized with sodium hypochlorite (Okogbule,2022).

PREPARATION OF NORMAL SALINE

This was done by dissolving 8.5g of salt (sodium chloride) in 1000ml of distilled water. After which 9ml of it was dispensed into different test tubes and autoclaved. This was later used for serial dilution.

PREPARATION OF SABOURAUD DEXTROSE AGAR (SDA)

The drug developed by the supplier was used to treat SDA. Measured and dissolved 65 g of SDA in 1000ml of distilled water, fully combined and autoclaved at 1210C for 15minutes. Tetracycline and ampicillin were applied to prevent development of the bacteria during autoclaving. 20ml of the prepared SDA was dispensed into sterile petri dishes after which cooling and solidification was allowed.

ISOLATION OF SPOILAGE FUNGI FROM GROUNDNUT PASTE SAMPLES.

A triple dilution was used in which 1 g of the samples was measured and moved to the first (original) test tube comprising 9ml of sterile normal saline and agitated. The 1ml from the first dilution was transferred into the first test tube and 1ml from the second dilution transferred into the third test tube. (Okogbule,2024)Different sterile pipette was used to conduct this process. An aliquot from the first and third dilution was inoculated into separate petri dishes containing SDA. This was immediately incubated for 7 days at 250C.

CHARACTERIZATION AND IDENTIFICATION OF FUNGAL ISOLATES

The fungal isolates were characterized and identified by Barnett and Hunter (1998) using macroscopic and microscopic examination. In the macroscopic test, the color of the colony was examined and noticed while the cotton blue throughout lacto phenol was stained in the microscopic center of a sterile glass slide and a small portion of the champignon was mounted on the slide using a sterile inoculation

PATHOGENICITY TEST

On freshly prepared groundnut paste samples, the pathogenic potential of the isolated fungi was checked in sterilized petri dishes and the fungal isolates were inoculated individually in the groundnut paste and incubated at room temperature (28OC ±3OC) for 7 days and examined for adjustments. The test samples were measured in grams at the end of the test and before inoculation. The weight loss of the checked samples in grams was used to calculate the degree of spoilage for the actual isolated species (Nmom *et al*, 2004).

Results and Discussion

Results

Treatment with *Occium gratissimum* (Scent Leaf)

The results of the effects of treatment of different variaties of groundnut paste with *Occium gratissimum* is presented in Table below. The proximate composition and Mineral Content were analyzed. The Treatment of groundnut paste with different concentrations of powdered plant of scent leaf (*Occium gratissimum*) shoes that there was significant reduction observed in Lipids, Calcium, Magnesium, Phosphorous, Potassium concentration of the different species of groundnut when compared with their respective control samples, while all other parameters such as Moisture Content and Sodium significantly increased at ($p<0.05$).

Table1.1 : Effects of treatment of *Occium gratissimum* (Scent Leaf) on the proximate composition of different species of groundnut paste.

Scent Leaf	Moisture%	Ash %	Lipid %	Fibre %	CHO %	Protein%
SAM NUT 21_4g	13.5 ± 0^{bc}	2.7 ± 0.24^{b}	32.43 ± 0.16^{ef}	2.5 ± 0^{a}	23.42 ± 0.2^{a}	25.45 ± 0.61^{abc}
SAM NUT 21_Ctrl	10.67 ± 0.41^{g}	3.22 ± 0.04^{b}	45.04 ± 0.51^{a}	1.52 ± 0.04^{d}	16.29 ± 0.35^{cd}	23.4 ± 0^{cd}
SAM NUT 23_2g	12.03 ± 0.59^{def}	3.8 ± 0.77^{ab}	41.45 ± 0.92^{bc}	1.73 ± 0.32^{d}	19.88 ± 1.83^{bc}	20.95 ± 2.64^{de}

SAMNUT 23_3g	12.35±0.51^{de}	3.77±0.71^{ab}	41.18±1.13^{abc}	1.4±0.24^{d}	18.28±0.66^{bcd}	23.52±1.7^{bcd}
SAMNUT 23_4g	12.47±0.83^{cd}	3.63±0.84^{ab}	38.75±4.85^{cd}	2.73±0.57^{a}	20.25±2.52^{abc}	23.42±1.39^{cd}
SAMNUT 23_Ctrl	11.33±0.75^{efg}	3.28±0.08^{ab}	43.45±1.41^{ab}	1.57±0.05^{d}	16.1±0.89^{cd}	23.77±0.75^{cd}
SAMNUT 24_2g	12.33±0.41^{de}	3.48±0.79^{ab}	41.68±0.97^{bc}	1.93±0.33^{cd}	21.02±1.95^{ab}	19.41±2.57^{e}
SAMNUT 24_3g	12.55±0.57^{cd}	3.47±0.72^{ab}	40.63±1.24^{c}	1.41±0.42^{d}	18.53±1.63^{cd}	23.74±2.29^{cd}
SAMNUT 24_4g	12.92±0.87^{bcd}	4.38±0.97^{a}	36.43±4.67^{e}	2.23±0.41^{bc}	21.5±2.45^{ab}	24±1.33^{bc}
SAMNUT 24_Ctrl	11±0.55^{fg}	3.25±0.05^{b}	44.04±1.33^{ab}	1.53±0.05^{d}	14.11±6.36^{d}	23.68±0.57^{cd}
Pr > F(Model)	< 0.0001	0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes

CHO=Carbohydrate

Table1.2 : Effects of treatment of *Occium gratissimum* (Scent Leaf Seed)on the Mineral Content (mg100g) composition of different species of groundnut paste.

Scent Leaf	Ca	Fe	Mg	P+	k	Na
SAMNUT 21_2g	25±0^{e}	3.7±0^{b}	22±0 ^{c}	305±0^{ab}	318±0^{c}	41.1±0^{b}
SAMNUT 21_3g	25.2±0de	3.8±0^{b}	24.1±0 ^{c}	306±0 ^{ab}	319±0 ^{c}	42±0 ^{b}
SAMNUT 21_4g	26±0^{cde}	3.9±0^{ab}	25±0 ^{c}	308±0 ^{ab}	476.67±258.2^{b}	44±0^{a}
SAMNUT 21_Ctrl	91.77±0.82^{a}	4.6±0^{a}	165±4.9^{a}	372.5±6.12^{a}	704.33±1.63 ^{a}	18.43±0.16^{e}
SAMNUT 23_2g	45.4±4.03^{bc}	4.3±0.62^{a} ^{b}	105.33±1.03 ^{b}	177.33±101.21^{b}	387.33±14.46^{bc}	25.77±0.52^{c}
SAMNUT 23_3g	45.83±3.36^{b}	4.33±0.57^{ab}	105.33±0.52 ^{b}	179±100.7 ^{b}	389.33±14.46 ^{bc}	26.17±0.52^{c}
SAMNUT 23_4g	46.83±3.36^{b}	4.27±0.36^{ab}	106.67±1.03 ^{b}	182.67±100.18 ^{b}	397±10.84 ^{bc}	24.57±1.61^{d}
SAMNUT 23_Ctrl	81.18±20.92 ^{a}	4.45±0.27^{ab}	167.17±0.98^{a}	353.33±7.53^{a}	669.17±49.74^{a}	18.9±0.46^{e}
SAMNUT 24_2g	42.8±4.03^{bcd} ^{e}	3.9±0.62^{a} ^{b}	104.67±1.03 ^{b}	242.67±101.21^{ab}	396.67±14.46 ^{bc}	25.43±0.52^{cd}
SAMNUT 24_3g	36.47±15.73 ^{bcde}	3.97±0.57^{ab}	105.67±0.52 ^{b}	244±100.7 ^{ab}	398.67±14.46 ^{bc}	25.83±0.52^{c}

SAMNUT 24_4g	44.67±3.36^{bc} ^{d}	4.03±0.36^{ab}	107.33±1.03 ^{b}	247.33±100.18 ^{ab}	404±10.84 ^{bc}	26.03±0.05^{c}
SAMNUT 24_Ctrl	80.62±20.89 ^{a}	4.33±0.29^{ab}	166.67±1.03^{a}	350.17±7.63^{a}	638.33±47.92^{a}	19.03±0.51^{e}
Pr > F(Model)	< 0.0001	00019.	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes

Ca=Calcium, Fe= Iron, Mg=Magnesium, P= Phosphorous, K=Potassium, Na= Sodium

The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months depicted low lipid content at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months recorded high Fibre content at the various concentration levels compared to the control irrespective of the concentration levels varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months recorded high content of carbohydrates at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months depicted little or no difference in the protein content at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months showed low Calcium content at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months showed little or no difference in the Iron content at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months showed low Magnesium content at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months showed low Phosphorous content at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for

Six months recorded low content of Potassium at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.The result of the treatment effect on the shelf life of groundnut paste with ground Scent leaf stored for Six months depicted high sodium content at the various concentration levels compared to the control irrespective of the concentration levels and the different varieties.

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