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Influence of Pepper Additive and Packaging Styles on Nutrient, Fungi and Aflatoxin Compositions of Stored 'Robo' (Deffatted Melon Snack)

Gbolagade S. Jonathan[1], Ganiyat B. Abdulrauf[1], Oluwatosin B. Ogunsanwo[1*] and Michael D. Asemoloye[2*]

[1]Mycology & Applied Microbiology Group, Department of Botany, University of Ibadan, Ibadan, Nigeria.

[2]School of Pharmaceutical Science and Technology, Tianjin University Nankai District, 92 Weijin Road, Tianjin, 300072, China.

***Corresponding authors**

Oluwatosin Babatunde Ogunsanwo,
Mycology & Applied Microbiology Group,
Department of Botany,
University of Ibadan, Ibadan,
Nigeria.

Michael Dare Asemoloye
School of Pharmaceutical Science and Technology,
Tianjin University Nankai District,
92 Weijin Road, Tianjin, 300072,
China.

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Abstract

Robo, an indigenous defatted melon snack commonly consumed in Nigeria generates a percentage of rural cash earnings for its producers. However, studies on the possible aflatoxin contamination of this product due to associated fungi and their control using cost effective measures are scarce. This study demonstrates the production of Robo, reports its nutrient composition, effects of pepper additive, packaging styles, and its possible fungi/aflatoxin contamination. Powdered chilli pepper was supplemented to paste of melon seed at different concentrations; 50g (T_1), 100g (T_2), 150g (T_3) and 200g (T_4) during Robo production while those with no pepper additive were treated as control. Thereafter, they were packaged in sterile polyethylene bag, plastic container and white paper and stored at 28±2°C for 12 weeks. The nutrient, fungi and aflatoxin compositions of fresh and stored Robo samples were determined. The result showed that paper-stored Robo samples had the highest carbohydrate (20.53±4.91), crude protein (40.96±3.79), ash (7.47±0.89), fibre (8.90±1.47) and pH (6.81±0.55) and least moisture (8.14±0.58) contents. Most dominantly associated fungi with this food are Aspergillus flavus, A. parasiticus, Fusarium oxysporium, Penicillium sp, Candida albicans, A. ustus, A. niger, Trichoderma sp, Rhizopus stolonifers and A. oryzae. The polyethylene bag-stored samples record the highest fungal incidence (88) followed by plastic-stored samples (81) while the paper stored samples had the least (76). Plastic-stored samples had the highest aflatoxin content AFB_1 of 1.02±0.19 while the polythene stored samples had the highest AFB_2 (0.45±0.31), AFG_1 (0.02±0.41) and AFG_2 (0.02±0.02) respectively. However, paper-stored samples had the least AFB_1 (0.52±0.29), AFB_2 (0.34±0.09), AFG_1 (0.00±0.10) and AFG_2 (0.01±0.04). Interestingly, it was observed that increased concentrations of chilli pepper from T_1-T_4 resulted in the corresponding decrease in their aflatoxin compositions. In addition pepper addition improved the organoleptic properties of the Robo samples as T_2 was generally accepted after twelve weeks of storage. The implications of these findings are discussed.

Keywords : Robo, packaging materials, pepper additive, nutrient, fungi, aflatoxin contamination.

Introduction

Robo is a snack processed by frying melon seeds into paste. It is one of the most prominent snacks from melon in Nigeria (1). Robo is the fried residue obtained during oil extraction from melon seeds and it is rich in protein and crude fat (2). Although this food is popularly consumed across Southwestern part of Nigeria, yet very few data are currently available on this food product as regards its nutritional qualities and safety. Spoilage of food materials by fungi is a major concern challenging the food security and it is mandatory to develop an improved control measures for the prevention of food spoilage (3,4). Due to the carcinogenicity, residual toxicity, teratogenicity and long-term degradation of synthetic chemicals they are not been used for the control of food spoilage moulds (5). Therefore, there is a dire need for natural products having antimicrobial activities without any side effect on the body.

Aflatoxins are secondary metabolites which are synthesized in stored food products by *Aspergillus flavus* and *A. parasiticus* (6). They are known for food hazards. Aflatoxins are associated with grains and seeds during storage (6). Incidences of aflatoxins have been widely reported in various Nigerian foods. From these reports concerns about the health hazards posed by aflatoxins were raised and the need for a biological control measures that can reduce their contaminations in various food products were also suggested (7, 4).

Chili pepper (*Capsicum annuum*) is a well-known cooking spice that is rich in vitamins, proteins, carbohydrates and phytochemicals (8). In addition, extracts from chilli pepper have been demonstrated for inhibition of some food bacteria and fungi (9, 10). Also, packaging styles and the quality of packaging materials are some of the factors that determine the safety of stored food product. Depending on the prevalent environmental conditions, the shelf life of a product is often times determined by the quality of various materials used in packaging and storing them (11,12). This study therefore undertakes to investigate the influence of packaging materials and chili pepper additive on the nutrient, fungi and aflatoxin compositions of 'Robo'.

Materials and Methods

Collection of Melon Seed

Healthy samples of shelled melon seed and chilli pepper were procured from two agro-seed shops; one at Bodija market and the other at Oja Oba market both in Ibadan, Oyo state. The samples were packaged in clean polythene bags. Samples were thereafter conveyed to the Mycology/Plant Pathology Laboratory, Botany department, University of Ibadan, where the research was conducted. The preparation of 'Robo', its fortification with pepper storage and fungal analysis were carried out in the laboratory.

Production and fortification of Robo with chilli pepper

Traditional method of producing Robo was employed in this study but it was done under sterile condition to avoid contamination by microorganisms. In order to obtain clean melon seeds, broken and spoilt and seeds other unwanted materials were sifted from the melon seeds. Thereafter fine healthy melon seeds were roasted in aluminium pot for 20 – 25 minutes. The melon seeds were stirred at interval in order to ensure proper roasting. The roasted melon seeds were milled using attrition mill after allowing them to cool down. In order to extract oil from the milled melon seeds, it was made into paste and pressed vigorously with wooden stirrer such that oil began to flow out of the paste.

Fresh chilli pepper was dried using a dehydrator at 55°c for 24h and milled into fine powder using Excella grinder. The fortification of the melon paste with pepper was carried out as follows:

Melon seeds (1000g) + pepper (50g) = 5 % (treatment T_1)
Melon seeds (1000g) + pepper (100g) = 10 % (treatment T_2)
Melon seeds (1000g) + pepper (150g) = 15 % (treatment T_3)
Melon seeds (1000g) + pepper (200g) = 20 % (treatment T_4)
Melon seeds (1000g) = 0 % (Control)

The treatment was done in three replicates. After the fortification, the deffated paste was moulded into small balls after which the extracted oil was used to fry them. The whole process follows the pattern presented in Fig. 1.

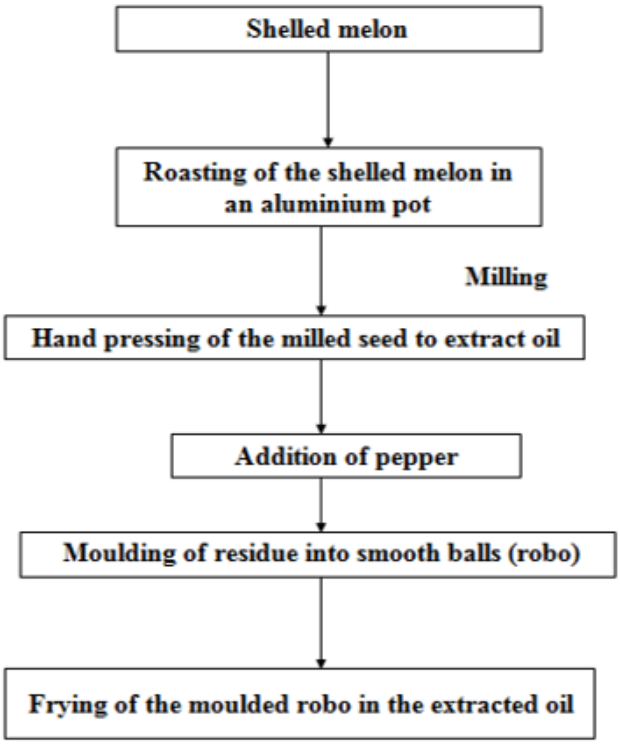

Figure 1: flowchart showing the steps in preparing Robo

Packaging styles and storage conditions

Three commonly used packaging materials namely; polythene bags, plastic containers and paper wrap were adopted for packing the Robo samples. This was done in order to determine the effect of these packaging styles on the nutrient and aflatoxin compositions of Robo samples. The fortified and unfortified samples of Robo were all packaged using these materials and stored inside a woody cupboard under the ambient condition (28+2°C). The storage lasted for 12weeks.

Nutrient analysis of the Robo samples

The method described by Association of Official Analytical Chemist (13) was used to analyse the proximate compositions of the samples after the 12th weeks of storage. The crude ash, crude fat, crude fibre, and moisture were all determined. Percentage carbohydrate was calculated by adding the percentages of other fractions (crude fibre, crude fat, crude protein, ash and moisture content) and subtracting them from 100. All the analyses were carried out in three replicates.

Isolation and characterization of bio deteriorating fungi associated with the Robo samples

This was done following the method of Fasidi and Jonathan (14). Fungi were isolated from the Robo samples before and after storage. The isolation was done at 4 weeks interval (i.e. 0, 4th, 8th and 12th week) (Fig 2-4). Using a sterile scalpel, small portion was excised from every replicates of Robo samples in each treatment and aseptically plated on sterile potato dextrose agar (PDA) media. After these, the PDA plates were incubated for 5 days at 28±2°C. Each colony was separated on new plates to observe a pure culture and the percentage incidence of the fungal isolates was thereafter carried out based on the number of times they occurred in all the samples. Percentage incidence was calculated according to the equation below (15).

$$\text{Percentage incidence} = \frac{\textit{Total number of a fungus isolated}}{\textit{Total fungi isolated}} \text{ x } 100$$

Of all the fungi, those ones with percentage incidence above 50 % were considered to be the most dominant. The morphological and microscopic characteristics of the dominant fungi were further studied. Fungi isolates were identified by comparing the examined macro and microscopic characters recorded with fungi identification reported by (16). The microscopic features of the fungal isolates were determined using Olympus photomicrograph (BX51). The features that were studied include: the shape of conidia head, the conidiophore structures and shape of the vesicle.

Analysis of the aflatoxin content

Extraction: The samples were analysed for aflatoxin contents after the 12th weeks of storage. Thin layer Chromatography (TLC) was used for the determination of the aflatoxin contents of the samples using the method of Thomas et al. (17). Concentrations of Aflatoxins (AFB_1, AFB_2 AFG_1 and AFG_2) were determined in the samples.

To do this, 2 and 5 g of the samples were pulverized. The pulverised samples were weighed into 500 ml Erlenmeyer flasks after which 250 ml of methanol and distilled water (60:40v) was added. The flask was shaken in a shaker for approximately 30 minutes. The solution was thereafter filtered using Whatsman filter paper no. 1 and the filtrate was collected. For further extractions, the filtrate was dissolved in 30 ml of the sodium chloride and 50ml of hexane, 50 ml of chloroform and 5 g of cupric carbonate respectively. Whattman paper No. 42 was used to filter the final solution over a bed of anhydrous sodium sulphate. For cleaning, 25ml of chloroform was used to wash the culpric carbonate after which it was filtered through the sodium sulphate bed. The extract was later dried in a water bath. For the detection of aflatoxins, the cuvettes of the spectrophotometer were developed in 1ml of chloroform and read at 250nm wavelength. The virtual intensity of the fluorescence was compared with that of standards. For the confirmation of AFB_1, the cuvettes were sprayed with aqeous sulphuric acid (50:50v). AFB_2 was derived from AFB_1 as dihydro derivative. The plate fluoresced deep yellow colour after changing from the pale blue colour which indicated the presence of AFB_2. While AFG_1 and AFG_2 flouresced yellow green and pale yellow green colours respectively when they were exposed to UV light. Aflatoxins concentration was quantified and calculated according to the method of Association of Official Analytical Chemists (18).

Sensory evaluation of Robo samples

This was done according to the method of Jonathan et al. (4). The effect of pepper additive on the taste and other organoleptic properties of Robo samples at different concentrations (5-20 %) was studied. For the measurement of the sensory values of the samples, Respondents were provided with Robo samples while questionnaire was also given to them. The organoleptic characteristics that were measured include: taste, aroma, flavour, colour and texture as well as their overall acceptability.

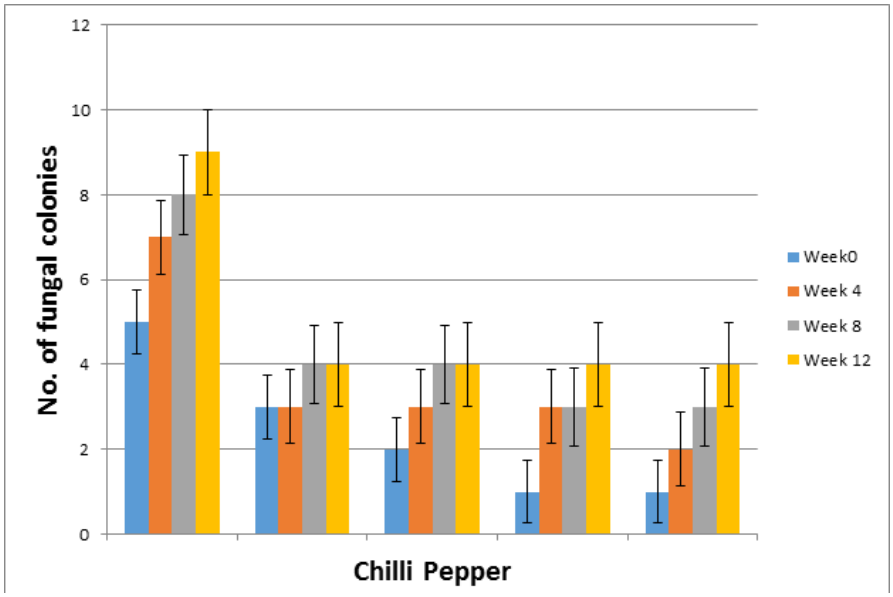

Figure 2: Effect of Chilli Pepper Variation on the Number of Colonies in Samples Stored in Paper

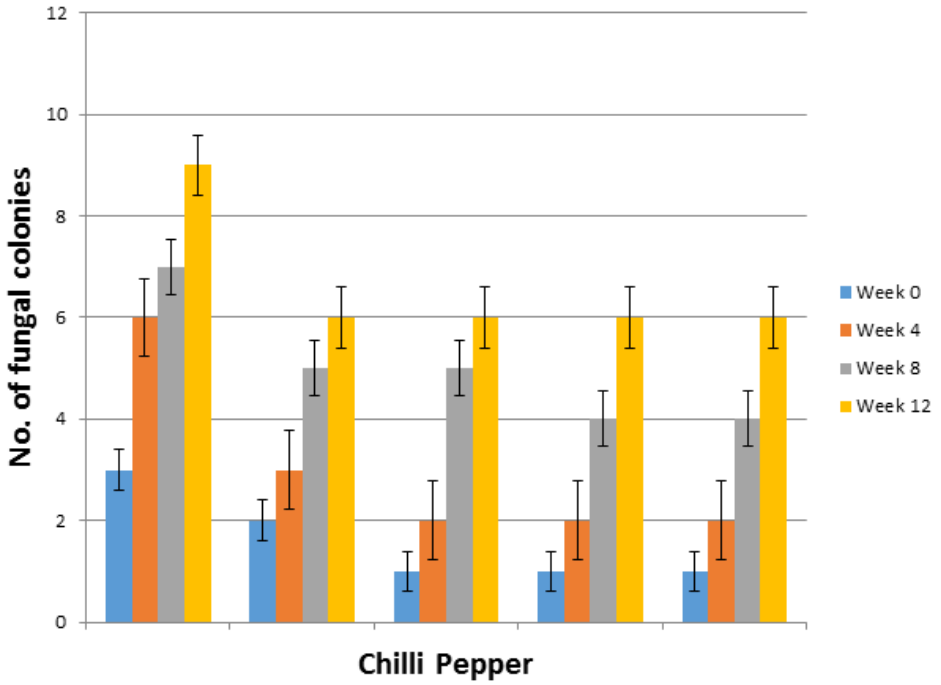

Figure 3: Effect of Chilli Pepper Variation on the Number of Colonies in Samples Stored in Plastic

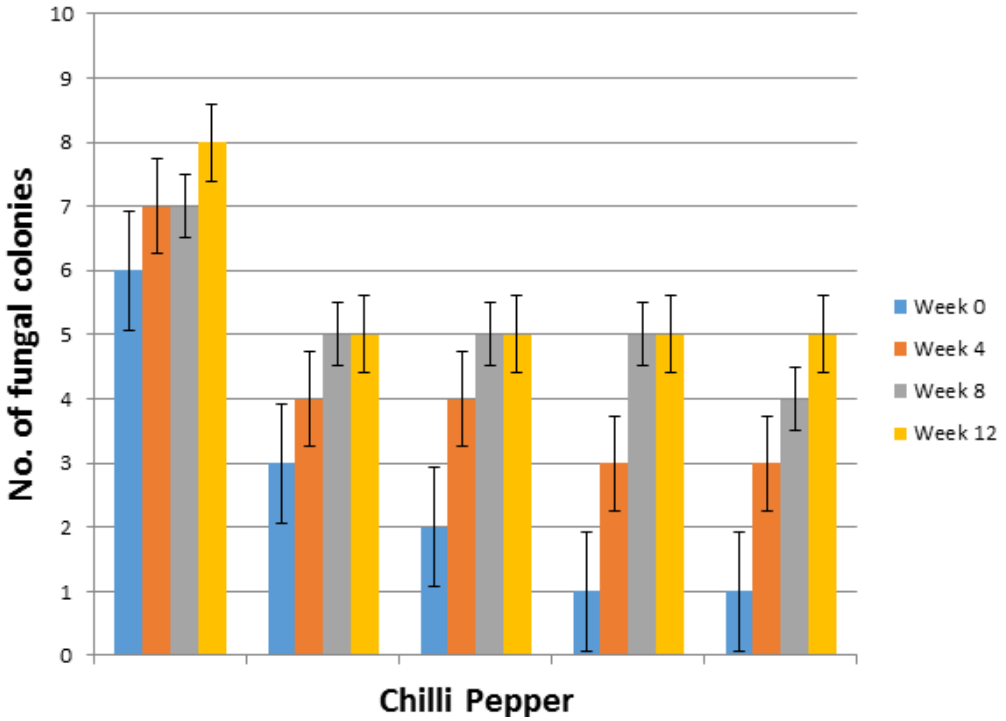

Figure 4: Effect of Chilli Pepper Variation on the Number of Colonies in Samples Stored in Polyethylene Bag

Statistical data analysis

The means of data generated were separated using the Duncan's multiple range test (DMRT). The generated data were also subjected to analysis of variance (ANOVA) using SAS 9.3 version. The significance level was tested at probability levels of $p<0.05$.

Result

Effect of the pepper additive and packaged materials on the proximate composition of Robo

The results obtained from the proximate analysis of Robo samples stored under different packaging style (paper, polyethylene bags and plastic containers) show significantly varying nutrient values. Generally, higher protein and carbohydrate contents with lower fat and moisture contents were observed in all the samples under study (Table 1-3). Interestingly, it was observed that

the pepper additive at different concentrations had positive effects on the samples which were packaged with various materials. Fresh unfortified samples had higher nutrient compositions than the stored samples after twelve (12) weeks of storage while the nutritional compositions of fortified samples increased with increased pepper additives. However, samples with pepper additives had higher nutrient values compared to the unfortified samples.

Treatments	Carbohydrate	Protein	Fat	Fibre	Ash	Moisture	pH
Control(fresh)	19.15±0.14^{a}	37.78±0.49^{b}	18.23±0.01^{c}	7.97±0.08^{d}	7.15±0.06^{f}	9.99±0.01^{a}	6.66±0.01^{e}
Control(stored)	11.21±0.39^{b}	36.77±0.29^{b}	12.11±0.01^{c}	7.14±0.04^{e}	6.22±0.01^{e}	7.67±0.06^{b}	5.60±0.01^{d}
T_1	13.81±0.90^{b}	37.59±1.97^{b}	13.37±11.58^{ab}	7.87±0.78^{d}	6.67±0.11^{d}	8.44±0.01^{e}	5.93±0.06^{c}
T_2	14.78±0.34^{b}	43.82±0.30^{a}	17.35±0.43^{c}	9.14±0.13^{c}	7.96±0.06^{c}	8.67±0.21^{d}	6.83±0.01^{a}
T_3	20.15±0.17^{a}	44.85±0.45^{a}	20.17±0.16^{b}	10.11±0.11^{b}	8.20±0.26^{b}	8.77±0.10^{d}	6.87±0.01^{b}
T_4	22.08±0.08^{a}	44.93±0.67^{a}	23.46±0.47^{a}	11.18±0.17^{a}	8.62±0.06^{a}	9.33±0.01^{c}	6.88±0.01^{a}
Grand mean	20.53±4.91	40.96±3.79	18.28±5.08	8.90±1.47	7.47±0.89	8.14±0.58	6.81±0.55

Values are means of three replicates±Standard Deviation, values with the same letter in a column are not significantly different from each other at p>0.05.

Table1: Effect of storage on the proximate composition of paper-packed Robo samples

Treatments	Carbohydrate	Protein	Fat	Fibre	Ash	Moisture	pH
Control(fresh)	19.15±0.14^{c}	37.78±0.49^{cd}	18.23±0.01^{d}	7.97±0.08^{c}	7.15±0.06^{c}	9.40±0.72^{a}	6.66±0.01^{bc}
Control(stored)	16.47±0.45^{c}	37.17±0.10^{d}	18.23±0.89^{d}	7.94±0.10^{c}	6.67±0.01^{b}	7.72±0.03^{a}	6.60±0.00^{c}
T_1	16.85±0.26^{c}	37.59±0.36^{c}	18.48±0.51^{cd}	8.23±1.66^{c}	7.17±0.38^{b}	8.27±0.74^{c}	6.63±0.02^{c}
T_2	17.00±0.01^{b}	38.34±0.01^{bc}	19.38±0.25^{c}	9.22±0.02^{bc}	7.24±0.01^{b}	8.56±0.01^{c}	6.77±0.01^{ab}
T_3	22.33±2.08^{a}	38.96±0.49^{b}	20.63±0.65^{b}	9.57±0.15^{a}	7.43±0.29^{b}	8.77±0.01^{bc}	6.77±0.02^{ab}
T_4	23.06±0.65^{a}	44.72±0.31^{a}	21.88±0.10^{a}	9.99±0.01^{a}	7.88±0.02^{a}	8.77±0.10^{bc}	6.80±0.00^{a}
Grand mean	19.14±2.85	39.09±2.69	19.47±0.47	8.82±0.10	7.26±0.41	8.91±0.62	6.71±0.01

Values are means of three replicates±Standard Deviation, values with the same letter in a column are not significantly different from each other at p>0.05.

Table 2: Effect of storage on the proximate composition of plastic-packed Robo samples

Treatments	Carbohydrate	Protein	Fat	Fibre	Ash	Moisture	pH
Control(fresh)	19.15±0.14^{ab}	37.78±0.49bc	18.23±3.30^{cd}	7.97±0.08b^{c}	7.15±0.06^{f}	9.95±0.01^{a}	6.66±0.01^{d}
Control(stored)	17.22±0.02^{b}	35.88±1.20^{bc}	17.30±0.34^{cd}	7.82±0.16^{c}	6.70±0.05^{e}	8.97±0.03^{ab}	6.45±0.01^{c}
T_1	17.23±0.01^{b}	36.78±0.58^{d}	19.33±0.11^{c}	7.89±0.22^{bc}	7.34±0.11^{d}	7.92±0.02^{c}	6.59±0.06^{b}
T_2	18.05±0.10^{ab}	38.83±0.49^{b}	19.97±0.02^{b}	8.47±0.46^{b}	7.48±0.06^{b}	8.55±0.01^{b}	6.84±0.04^{a}
T_3	18.46±0.47^{ab}	39.20±0.71^{bc}	20.44±0.59^{b}	9.89±0.62^{a}	7.64±0.04^{c}	8.67±0.06^{ab}	6.87±0.01^{a}
T_4	19.49±2.62^{a}	42.15±0.13^{a}	22.88±0.20^{a}	10.12±0.01^{a}	7.88±0.01^{a}	8.77±0.12^{ab}	6.89±0.01^{a}
Grand mean	18.27±1.28	38.77±1.84	19.86±1.64	8.69±1.02	7.37±0.39	8.81±1.04	6.72±0.17

Values are means of three replicates±Standard Deviation, values with the same letter in a column are not significantly different from each other at p>0.05.

Table 3: Effect of storage on the proximate composition of polythene bag-packed Robo samples

Furthermore, it was observed that packaging materials also influenced the variations in nutrient compositions of the samples before and after storage. When fresh samples, 5% and 20% fortified stored samples packaged in various materials were compared, Robo samples which were packed with paper had the highest carbohydrate (20.53±4.91) crude protein (40.96±3.79), ash (7.47±0.89), crude fibre (8.90±1.47) and pH (6.81±0.55) and least moisture (8.14±0.58) contents. However, samples packed with polythene had the least carbohydrate (18.27±1.28), crude protein (38.77±1.84) crude fibre (8.69±1.02) and highest fat (19.86±1.64) contents while samples packed with plastic had the lowest pH (6.71±0.01), ash (7.26±0.41) and highest moisture (8.91±0.62) contents.

Effect of pepper additive on fungi associated with Robo samples

Results obtained from this study showed that some fungi belonging to 7 fungal genera namely: *Aspergillus, Penicillium, Trichoderma, Rhizopus, Candida*, and *Fusarium* were isolated from the samples during storage. The isolated fungal strains were identified as *Aspergillus flavus, A. parasiticus, Fusarium oxysporium, Penicillium sp, Candida albicans, A. ustus, A. niger,*

Trichoderma sp, Rhizopus stolonifers and *A. oryzae* (Fig 5).

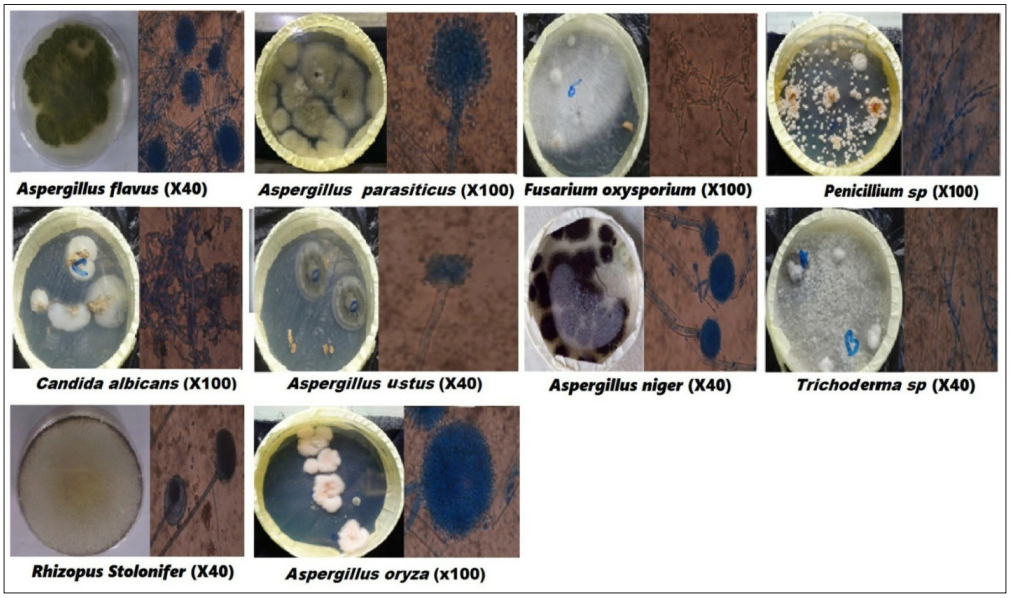

Figure 5: Plate and Photomicrographic structure of Fungal Strains Isolated from Melon Cake (Robo) Samples.

After twelve weeks of storage, it was generally observed that pepper additive had effects on the associated fungal incidence in samples of Robo packed with various materials. Robo samples without pepper had the highest fungal incidence compared to other samples (Fig 2-4). Also, the fungal strains decreased with the increased pepper concentrations in the samples. However, Robo samples packed in paper had the least fungal incidence while the Robo samples packaged in the polythene bag had the highest fungal incidence (table 4).

Fungi	Paper-packed samples		Plastic-packed samples		Polythene bag-packed samples	
	Occurrence	% incidence	Occurrence	% incidence	Occurrence	% incidence
Aspergillus flavus	12	15.78	16	19.75	18	20.45
A. parasiticus	13	17.1	15	18.51	15	17.05
Fusarium oxysporium	4	5.26	5	6.17	4	4.55
Penicilium sp.	6	7.89	4	4.94	8	9.09
Candida albicans	7	9.21	5	6.17	8	9.09
A. ustus	7	9.21	7	8.64	4	4.55
A. niger	7	9.21	9	11.11	6	6.82
Trichoderma sp.	9	11.84	10	12.35	10	11.36
Rhizopus stolonifer	5	6.58	5	6.17	9	10.23
A. oryzae	5	7.89	5	6.17	6	6.82
Total	76	100	81	100	88	100

Table 4: The dominant fungi associated with 'Robo' samples stored in different materials

Effect of pepper treatments and packaging materials on the aflatoxin composition of Robo

The results obtained from the aflatoxin analysis of Robo samples which were packed in different materials (paper, polyethylene bags and plastic containers) and stored under the same environment/conditions showed significantly varying aflatoxin compositions. Generally, higher aflatoxins B1 and B2 contents with lower aflatoxins G1 and G2 were observed in all the samples (table 5-7).

Treatments	Aflatoxin (µg/kg)				
	AFB_1	AFB_2	AFG_1	AFG_2	Total Aflatoxins
Control(fresh)	0.05 ± 0.11^{c}	0.07 ± 0.11^{b}	0.00 ± 0.02^{d}	0.02 ± 0.03^{a}	0.14^{b}
Control(stored)	1.59 ± 0.14^{a}	1.25 ± 0.07^{a}	0.01 ± 0.06^{a}	0.01 ± 0.06^{ab}	2.86^{a}
T_1	1.43 ± 0.09^{b}	0.69 ± 0.12^{b}	0.04 ± 0.04^{b}	0.01 ± 0.07^{ab}	2.17^{a}
T_2	0.02 ± 0.02^{d}	0.01 ± 0.32^{c}	0.01 ± 0.03^{d}	0.01 ± 0.04^{b}	0.05^{c}
T_3	0.02 ± 0.05^{d}	0.01 ± 0.04^{c}	0.00 ± 0.02^{d}	0.00 ± 0.03^{c}	0.03^{c}
T_4	0.01 ± 0.01^{e}	0.00 ± 0.04^{d}	0.00 ± 0.02^{d}	0.00 ± 0.01^{c}	0.01^{c}
Grand mean	0.52±0.29	0.34±0.09	0.00±0.10	0.01±0.04	

Values are means of three replicates±Standard Deviation, values with the same letter in a column are not significantly different from each other at p>0.05

Table 5: Effect of storage on the aflatoxin content of paper-packed Robo samples

Treatments	Aflatoxin (µg/kg)				
	AFB_1	AFB_2	AFG_1	AFG_2	Total Aflatoxins
Control(fresh)	0.77 ± 0.08^{c}	0.01 ± 0.06^{c}	0.01 ± 0.71^{a}	0.02 ± 0.05^{a}	0.81^{a}
Control(stored)	2.14 ± 0.01^{b}	0.03 ± 0.13^{a}	0.01 ± 0.00^{c}	0.01 ± 0.17^{c}	2.19^{b}
T_1	3.03 ± 0.12^{a}	0.03 ± 0.05^{a}	$0.01\pm0.06b^{c}$	0.00 ± 0.81^{b}	3.07^{a}
T_2	2.07 ± 0.17^{b}	0.03 ± 0.05^{a}	0.01 ± 0.11^{b}	0.00 ± 0.31^{c}	$2,11^{b}$
T_3	1.04 ± 0.03^{c}	0.03 ± 0.08^{a}	0.01 ± 0.03^{c}	0.00 ± 0.01^{c}	1.08^{c}
T_4	1.03 ± 0.01^{c}	0.02 ± 0.01^{c}	0.01 ± 0.01^{c}	0.00 ± 0.01^{c}	1.06^{c}
Grand mean	1.02±0.19	0.02±0.20	0.01±0.15	0.01±0.41	

Values are means of three replicates±Standard Deviation, values with the same letter in a column are not significantly different from each other at p>0.05.

Table 6: Effect of storage on the aflatoxin content of Robo samples packed in plastic bags

Treatments	Aflatoxin (µg/kg)				
	AFB_1	AFB_2	AFG_1	AFG_2	Total Aflatoxins
Control(fresh)	0.07 ± 0.08^{c}	2.02 ± 0.10^{b}	0.01 ± 0.01^{b}	0.00 ± 0.01^{c}	2.1^{a}
Control(stored)	2.34 ± 0.01^{bc}	2.78 ± 0.11^{a}	1.89 ± 0.04^{a}	1.00 ± 0.07^{ab}	6.01^{b}
T_1	1.00 ± 0.73^{a}	2.72 ± 0.32^{a}	1.88 ± 0.22^{a}	1.00 ± 0.12^{a}	5.6^{b}
T_2	1.78 ± 0.41^{b}	$2.72\pm0.\ 17^{a}$	1.88 ± 0.06^{a}	1.00 ± 0.02^{ab}	6.38^{b}
T_3	0.40 ± 0.11^{c}	1.74 ± 0.06^{c}	1.88 ± 0.21^{a}	1.00 ± 0.04^{b}	3.02^{c}
T_4	0.63 ± 0.05^{d}	0.56 ± 0.02^{d}	0.02 ± 0.01^{b}	0.02 ± 0.01^{a}	1.11^{a}
Grand mean	0.60±0.31	0.45±0.31	0.02±0.41	0.02±0.02	

Values are means of three replicates±Standard Deviation, values with the same letter in a column are not significantly different from each other at p>0.05.

Table 7: Effect of storage on the aflatoxin content of Robo samples packed in polyethylene bags

Also, it was observed that the pepper additive at different concentrations had positive effects in reducing aflatoxin contents of the samples. The untreated stored sample (Stored 0% pepper) in paper had higher aflatoxins B1 and B2 compared to the fresh samples. The aflatoxins compositions of the stored Robo samples treated with pepper additives were however lower than the concentrations of aflatoxins in both fresh samples and untreated stored (0% pepper) samples.

This study revealed that apart from the pepper additives and storage time, packaging materials that were used for the storage of the Robo samples had a significant influence on their aflatoxin contents. The aflatoxin result shows that the samples packed using different materials (paper, polyethylene bags and plastic containers) and stored under the same storage environment/condition had varying aflatoxin contents after 12 weeks of storage. It was observed that the samples packed in paper had the least AFB1, AFB2, AFG1 and AFG2 contents (0.52±0.29, 0.34±0.09, 0.00±0.10 and 0.01±0.04 µg/kg) followed by the samples packed in plastic containers (1.02±0.19, 0.02±0.20, 0.01±0.15 and 0.01±0.41 respectively) while the samples packed in polyethylene bags had the highest (0.60±0.31, 0.45±0.31, 0.02±0.41 and 0.02±0.02).

Effect of pepper additive on the sensory value of Robo

Most of the respondents preferred the aroma, colour, taste, flavour, texture of the Robo with 10% pepper additive over other treatments and control. The samples with 10 % pepper additive had the highest overall acceptability while the lesser number of the respondents preferred the control (0% pepper). In addition, it was observed that the pepper additive showed statistically significant effects ($p \leq 0.05$) on the aroma, colour, taste, texture and the overall acceptability as presented in table 8. However, the pepper additive did not significantly influence the aroma and the flavour.

Treatment (%)	Aroma	Colour	Taste	Flavour	Texture	Overall Acceptability
Control	5.50 ± 0.50^{d}	5.07 ± 1.01^{c}	4.50 ± 0.62^{c}	6.00 ± 2.65^{b}	4.33 ± 1.53^{c}	3.33 ± 1.53^{c}
T_1	6.93 ± 1.10^{c}	6.00 ± 0.60^{b}	5.80 ± 0.53^{b}	6.67 ± 1.15^{b}	5.87 ± 0.42^{b}	5.63 ± 0.47^{b}
T_2	8.33 ± 3.21^{ab}	8.93 ± 3.00^{a}	9.53 ± 2.14^{a}	9.27 ± 2.53^{a}	9.84 ± 3.37^{a}	9.03 ± 3.21^{a}
T_3	7.87 ± 1.86^{ab}	5.93 ± 0.31^{b}	5.67 ± 0.41^{b}	5.97 ± 0.25^{b}	5.60 ± 0.36^{b}	5.90 ± 0.26^{b}
T_4	7.67 ± 2.08^{ab}	5.33 ± 0.58^{c}	5.77 ± 0.21^{b}	8.67 ± 3.06^{a}	5.94 ± 0.21^{b}	5.93 ± 0.70^{b}

Values are means of three replicates±Standard Deviation, values with the same letter in a column are not significantly different from each other at $p>0.05$

Table 8: Sensory evaluation of 'Robo' samples with pepper additive supplemented at different concentrations

Discussion

Robo is a cake that is produced after the oil extraction of melon and sold as ready to eat snacks in various parts of Southwestern Nigeria (2). The nutrient analysis of the Robo samples revealed the presence of higher protein and carbohydrate content with the corresponding lower fat and moisture content in all the samples. This could be a reflection of the processing style for Robo which involves the extraction of fat from the melon. The oil is removed and dried completely through frying to give it the desired taste and choice (preferably eaten dried and crunchy). This study revealed that the increased concentrations of pepper additives influenced the increase in the nutritional composition of the Robo samples. It can therefore be deduced that the nutritional compositions of chilli pepper lead to the increase in the nutritional compositions of the Robo samples (18). Nutrient analysis of Chilli pepper revealed that it contains carbohydrate, fats, fibre, protein, ash, minerals and vitamins (8, 20). The highest carbohydrate, protein and ash content recorded in samples of Robo packed with paper could be due to the fact that the paper used allowed aeration thereby preventing the samples from having high moisture content which might have enhanced the nutrient compositions of the samples. However, the lower nutrient compositions recorded in samples packed in polythene bags and plastic containers could be due to lack of aeration in the packaging materials. Moisture increases in a packaging material that does not support aeration thereby causing the moisture content of food samples in such material to increase which affects its nutrient composition and shelf life (11).

In this study, the fungal content of Robo samples stored in paper was observed to be lower than those stored in polyethylene bags and plastic containers. This could be because paper absorbed oil from the samples which further reduced the oil contents and by this, their quality deteriorated less quickly as compared to the samples stored in plastic and polyethylene bags. It is also suspected that absorption and loss of moisture by the paper from the samples to the surrounding environment further enhanced the dried condition of the sample (Marsh & Bugusu, 2007). Conversely, high moisture content in polyethylene and plastic bags observed during the study could have resulted from lack of aeration. In a similar report, Marin *et al.* (21) reported that the moisture content of stored peanut packaged in polypropylene and jute bags lined with polypropylene was significantly higher than the one packaged in paper. Samples stored in facilities where there is poor aeration in their environments are often prone to deterioration (22).

The storage fungi isolated from Robo samples packaged in different materials while they were under storage belong to genera *Fusarium*, *Aspergillus* and *Trichoderma*. Storage fungi often have the capacity to make use of hydrolytic enzymes when present in oil and seed products to produce free fatty acids and glycerol thereby changing their fat quality (23, 24), which leads to lower quality or rejection of foodstuffs (25). In a similar study, it was reported that increase in the storage period of peanuts significantly increased the level of its fatty acids content (25). Makun *et al*. (26) stated that drying generally inhibits moulds' growth, aflatoxin production and off-flavors formation due to fungal lipase action and oxidative rancidity which have been reported to increase the moisture of dried foodstuffs. Whereas studies on the antifungal and antiaflatoxigenic effect of some spices have been carried out, there is little or no report on the effect of Chilli pepper on fungal and aflatoxin control in Robo (Defatted melon snack). Fungal composition of the Robo samples in this study showed that samples fortified with pepper (especially T_2 and T_4 concentrations in Robo) were less affected by storage fungi compared to the unfortified samples (both fresh and stored). This shows that deteriorative effect of fungi in the Robo samples was inhibited by pepper additives. Fungi often cause the deterioration of food samples under storage (4, 27). Storage fungi are often thermotolerant hence they can survive in dry conditions (28). Some of the storage fungi isolated from Robo samples include *Aspergillus flavus*, *A. parasiticus*, *Fusarium oxysporium*, *Penicillium sp*, *A. ustus*, *A. niger*, *Trichoderma sp* and *A. oryzae*. However, their incidences were lower in Robo samples fortified with pepper but higher in unfortified samples.

Due to fungal contamination, there have been concerns about the safety of some snacks that are commonly sold in Nigeria (29). Storage moulds cause food deteriorations which usually

result in contamination of mycotoxin, nutritional and chemical changes in food stuffs (30). Aflatoxin contamination of foodstuff is a potent threat and a huge challenge to developing countries due to the poorly designed agricultural and food processing systems in these countries (7). Moreover, aflatoxin contamination in food crops within developing countries have been linked to various postharvest conditions such as handling, storage and processing (31).

The extraction of aflatoxins from the Robo samples in this study shows that some fungi were responsible for the incidence of the mycotoxins in the Robo sample. The two major fungi that produce aflatoxins; *Aspergillus flavus* and *A. parasititcus* were isolated from the Robo samples. However, it was observed that the chilli pepper additive in Robo at different concentration (especially T_2 and T_4 concentrations in Robo) reduced the aflatoxin contents in the Robo samples. This supports the report of Kiin-Kabari et al. (32) who asserted that pepper additives preserved perishable foods like fish. In an in vitro study, aqeous extract of pepper has been reported to have reduced biosynthesis of aflatoxin in aflatoxigenic species by 99% (33). Interestingly, fortification of Robo samples with different concentrations pepper had some level of inhibitory effect on aflatoxigenic fungi in Robo after storage compared to the control/untreated samples and as such there was significant decrease in aflatoxin accumulation in the pepper treated stored samples compared to the control. This result is an eye opener to the potency of pepper for the control of aflatoxin in Robo samples under storage. Various plants materials such as whole plants, aqeous extracts and volatile compounds have been shown to be very effective for the control of aflatoxigenic fungi and aflatoxins concentrations in feeds and various food products (33). Sanchez *et al.* (35) reported that extracts of *Agave asperrima* and *A. striata* inhibited the growth of A. flavus and A. parasiticus which resulted to the reduction of aflatoxin production in the infected crops (35). In a separate study by Atanda *et al*. (36), the leaves of *Laurus nobilis* and oils of *Cinnamomum cassia* were reported to have drastically lowered the biosynthesis of aflatoxin in *A. parasiticus* CFR 223 without affecting its growth. Various concentrations of ginger have been proven to be effective against the aflatoxins composition of stored ogi samples (18). In addition, the aflatoxin contents of the Robo sample was below the tolerable limit hence they were safe for consumption. However, there has been concerns about the possible increase in aflatoxins compositions of food products under storage for a long duration (37,4). The higher concentrations of AFB_1 and AFB_2 compared to AFG_1 and AFG_2 extracted from the Robo samples could be a reflective of the fact that aflatoxins B_1 and B_2 are the first metabolites produced during the aflatoxin biosynthesis (7).

The bioactive compounds in the chilli pepper used in this study may be responsible for the inhibition and higher percentage reduction of fungi and aflatoxins in the pepper treated Robo samples. Gas Chromatography Mass Spectrometry analysis of distilled oil of pepper revealed the presence of various compounds such as myristicin, dillapiol, safrole caryophylene and limonene are some of the bioactive compounds presence in the sample (38,39,40). Essential oil of members of Piperaceae family are known to contain high contents various bioactive compounds (41,38,39,40). There have been reports on the inhibitory effects of these bioactive compounds on aflatoxigenic fungi and aflatoxin production (42,43,44,45). Hence, the presence of these bioactive compounds in the chilli pepper could be responsible for low fungal and aflatoxin content observed in the pepper treated samples. In addition, the presence of phenols, glycosides, flavonoids, isoflavonoids, alkaloids, tannins, terpenes which have been reported to be present in aqeous extract of pepper could be responsible for the antiaflatoxigenic effect of the chilli pepper against some aflatoxin producing species (34). This must have happened when aqeous extract of different concentrations of Chilli pepper were used to fortify the Robo samples. Generally, bioactive compounds along with some other compounds are more effective in their vapour phase which makes them a potent fumigants for the protection of food products under storage (35).

The popularity of paper packaging among Nigerian Robo consumers and traders could be ascribed to the fact that it is cost effective and readily available. When compared to plastic materials, paper is preferred based on the earlier stated attributes. Unlike polyethylene bags and plastics, paper absorb moisture easily but allows good aeration while plastic and polyethylene containers are non-absorptive and don't allow good air flow rather they retain heat (46), therefore promoting fungal growth and aflatoxin contamination (47). This may be worse if the samples are not dried properly before storing them (48). Packaging methods of food products play vital roles in their quality after storage as their deterioration depend on conditions under which they were stored. The recommended safe moisture levels for defatted oil seeds in general is $\leq 10\%$ (49). However, in many tropical countries their environments are characterized by high humidity hence, this has made it difficult to keep the tolerable moisture level of dried foodstuffs after storage thus increasing the risk of aflatoxin contamination (7). Robo are commonly stored for about 6-12 weeks within households in Nigeria during which they are consumed or sold out. In both commercial and household practices, Robo is commonly packed in paper, polyethylene bags or plastic containers in which they are kept until completely consumed or sold out. However, Robo are preferably consumed dried and crunchy but the problem is how to maintain this requirement after storage. This supports the report of Barro *et al.* (50) who noted that the length of storage usually has significant effects on physical damage and quality of foodstuffs. To maintain quality, it is necessary to facilitate aeration during storage (51).

Samples stored in polyethylene and plastic containers, respectively had higher incidence of aflatoxin contamination than samples stored in paper. This could ascribed to the fact that paper packed samples showed least fungal incidence and hence least aflatoxin contents while the polyethylene packed samples showed the highest fungal incidence hence higher aflatoxin contents. Higher incidences of fungi in a stored food sample increases the chances of the accumulation of aflatoxin concentrations in such sample (4). Furthermore, occurrence of higher aflatoxin concentrations in polyethylene

and plastic stored samples compared to paper stored samples can also be ascribed to the trapping of heat and moisture in both polyethylene and plastic which encouraged the growth of fungi as well as production and accumulation of aflatoxin in the stored samples compared to paper. In addition, storage duration of Robo samples significantly influenced the aflatoxin contamination of the sample as stored Robo samples contained more aflatoxin than the fresh ones. This supports the reports of Hell *et al*. (47) who observed that there was correlation in the increase in the levels of aflatoxin in maize samples and the increase in the storage period.

The acceptability of a food product depends on the overall perception of the consumers about the food product. In this study, the respondents preferred Robo samples fortified with 10% concentration of chilli pepper to other treated samples. Spices generally increase the organoleptic properties of food samples and their acceptability (18). In various parts of the world, spices are been used to improve the flavour and aroma of various food products (52). Also, consumers have preferences when it comes to taste and values of a food product (18).

Conclusion

From the foregoing, this study has shown that the nutrient compositions of the unfortified Robo samples such as carbohydrate, crude protein, fats were enhanced when they were fortified with different concentrations of chilli pepper. Chilli pepper is therefore a good spice for the enrichment of Robo snacks.

Although both fresh and stored samples of Robo contained aflatoxigenic fungi and aflatoxins during and after storage but Chilli pepper in treated Robo samples reduced the fungal incidence and aflatoxins concentration of the snack. The major aflatoxigenic fungi belonging to the genera *Aspergillus* isolated from the samples are *A. flavus* and *A. parasiticus*. Aflatoxins concentration detected in treated, untreated, stored and fresh ones were below the tolerable limit. In addition, the packaging styles that were used to pack the Robo samples had effect on their nutrient, fungal and aflatoxin compositions. As observed in this study, Robo samples packed with polythene and plastic had higher fungal and aflatoxins composition compared to samples packed with paper which had the least fungal and aflatoxin content with relatively higher nutrient compositions. The respondent preferred T2 (10% chilli pepper) as the sample with the best orgonoleptic properties.

Therefore, Chilli pepper at 10% (w/w) and the use of paper are recommended for the fortification and packing of both fresh and stored samples of Robo. Further work can be carried out on the effect of Chilli pepper and the packaging styles used in this study on other mycotoxins in Robo samples.

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