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Influence of Medicinal Plant Powders on Fungal Degradation of the Proximate Contents of Egusi Melon Seeds

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ABSTRACT

Egusi melon (Colocynthis citrullus L.) seed samples were obtained from farmers, in each of the three local government areas of Ohafia, Isiala Ngwa South and Ikwuano in Abia State; Aguata, Aniocha and Orumba in Anambra State; Ishiagu, Ezza and Afikpo South in Ebonyi State; Nkanu, Nsukka and Udenu in Enugu State; Orlu, Ideato and Mbaitoli in Imo State. The seeds were subjected to seed health testing using blotter method and Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus granulosis, Botryploidia theobromae, Fusarium solani and Penicillum citrinum were isolated. The proximate analysis of inoculated seeds with the above isolates showed a significant reduction in protein, dry matter, crude fiber, ash and oil contents while carbohydrate and moisture content increased significantly. However the inclusion of plant powder in seed inoculated with isolates improved their fat and carbohydrate contents significantly.

Keywords: Egusi melon, plant powders, proximate contents, fungal isolates.

INTRODUCTION

Egusi melon (*Colocynthis citrullus*(L.) belongs to the family Cucurbitaceae (Ogbonna and Obi, 2007; Ntui, 2010). It has also been referred to in some texts as *Citrullus lanatus (*Oguremi, 1978; Okoli, 1984), *Citrullus vulgaris* (Philip, 1977, Umechuruba, 1997, Makinde *et al*., 2007) and *Cucumeropsis manii* (Nyananyo, 2009). Oyolo (1977) proposed that the vernacular name 'egusi' be attached to *Colocynthis citrullus* to prevent confusion due to several names given to the crop and its relatives.

Egusi melon is an important oil seed crop which contains 53% oil, 36% protein and rich in fat and Vitamin A, B_1, B_2 and C (Bankole *et al*., 2005b; Ntui, 2010). It also contains a fairly high amount of unsaturated fatty acids with percentage composition by weight of oil: Lauric, 0.21%, Palmitic, 13.45%, Myristic, 0.78%; Stearic, 13.71; Oleic, 14.50%, Linoleic, 56.94; Linoleic, 0.46% (Oluba et. al., 2008).

In Abia State, Nigeria, the communities use ground egusi melon with edible mushroom (*Pleurotus tuberregium*) to make egusi balls, a delicacy which is a substitute for protein in their daily diet (Nwokolo and Sim, 1987). Inaddition, egusi melon can be used as condiments known as 'ogiri' made from fermented the seed in a process which improves the nutritional quality and digestibility of the seed. Egusi seed produces high quality of oil which is in high demand in Sudan and Ethiopia (Giwa*et al*, 2010). In the cropping system egusi melon provides effective weed suppression in traditional intercropping with yam, maize, cassava and plantain (Yusuf et. al., 2008).

One of the major constraints on egusi melon production in South-Eastern Nigeria is fungal infection of egusi while growing in the field and during storage. Some of the fungal pathogens of egusi include *Sclerotium rolfsii, Botryodiplodia theobromae, Cercospora citrulina, Alternaria cucumerina, Collectotrichum lagenarium, Fusarium oxysporium* and *Aspergillus spp (*Umechuruba, 1997; Chiejina, 2006; Hegazy, 2011).

However, proper executions of disease management strategies such as the use of botanicals possess antifungal properties and act as protectants during the storage period. Local farmers preserve their

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agricultural produce under storage by using some of these botanicals. In Nigeria, reasonable quantity of dry pepper is used to protect and preserve cowpea and other seeds against insect pest during storage. Also, in Burkina Faso, unbroken leaves of *Cassaia nigricans* are used as layers to store pulses. Bankole and Joda (2004) have demonstrated that lemon grass powder and essential oil could have preservative effect against mold and aflatoxin contamination of melon seeds. Adekunle and Uma (2005) also reported that storing egusi melon seeds in powdered leaves of *Ocimum gratissimum* and *A. indica* protected them against some fungal infection during storage. This paper examines the impact of medicinal plant powders in preservation of egusi melon from some fungal infections during storage.

MATERIALS & METHODS

The spore suspensions of some contaminating fungi (*A. flavus, A. fumigatus, A.granulosis, A. niger, B. theobromae, F. solani and P. citrinum*) isolated from egwusi seed in Umudike farms were used in this trial at Michael Okpara University laboratory of College of Crop and Soil Sciences. Each fungal isolate containing 20 ml of suspension was introduced into each of the 21 glass jars with 35g shelled egusi melon seeds (35g seed/ jar) mixed with 35g of each of the plant powders (**ginger, lemon grass and neem**). A second batch of 7 glass jars, each containing 35g of egusi melon seeds was mixed with the samt amount of spore suspensions while a third batch glass jar containing 35g of healthy uncontaminated seeds (without any spore suspensions and plant powders) served as the control. The experiment was replicated three times. The jars were incubated under room temperature (30^{o}C) with manual shaking twice daily. After 14 days of incubation the treated seeds were subjected to the following analysis shown below:

Dry matter and moisture content

A laboratory crucible was washed and dried in an oven at 105±2^{o}C for 1 hour. It was then weighed after cooling in a desiccator. 10g each of the pulverized treated egusi melon seeds was then added to the crucible and the total weight determined. The crucible, together with its contents was transferred into an oven at 105±2^{o}C and dried for 3 hours, after which it was weighed. This process was repeated until difference in weight between two successive dryings was less than 0.1g. The difference in weight between the original sample and the dried sample was calculated using the formula according to AOAC and Ibitoye (2005) and modified by Moses et. al. (2012):

$$\% \text{ weight loss} = \% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where

W_1 = initial weight of the empty dish

W_2 = weight of dish + undried sample

W_3 = weight of dish + dried sample

% Dry matter = 100% - % Moisture content

Crude fibre content

5g each of the pulverized treated egusi melon seed was put into 200ml of 1.25% sulphuric acid and boiled gently for 30 minutes and filtered through a muslin cloth into a Buchner funnel. The residue obtained was washed with hot sterile distilled water to remove acids. The acid-free residue was put into 200ml 1.25% sodium hydroxide and boiled for 30 minutes, after which it was filtered and then washed thrice with petroleum ether. The resulting residue was then put in a crucible and dried to constant weight after which it was cooled in a dessicator and weighed. Then the crucible containing the residue was then subjected to ashing in a muffle furnace at 300±10^{o}C for about 30 minutes and cooled in a desiccator after which it was reweighed. (AOAC, 2005; Ibitoye, 2005; Moses et. al., 2012).

The % crude fibre was calculated using the formula:

$$\frac{W_2 - W_3}{W_1} \times 100$$

Where,

W_3 = weight of crucible + ashed residue

W_2 = weight of crucible + sample before ashing

W_1 = weight of sample used.

Ash content determination

10g each of the pulverized treated egusi melon seeds was placed into a pre-weighed and dried crucible and heated for 3 hours at 100°C. Afterwards, the crucible was transferred into a muffle furnace and the temperature slowly increased from 200-450°C, until ashing was complete, as indicated by white colour of the sample. The sample was then carefully removed and cooled in a dessicator to room temperature and reweighed immediately. (AOAC, 2005; Ibitoye, 2005).

% Ash content was calculated using the formula:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where,

W_3 = weight of dish plus ashed sample

W_2 = weight of dish plus sample

W_1 = weight of dish only

Determination of percentage fat content

5g each of the pulverized treated egusi melon seed was loosely wrapped in a filter paper and put into a thimble which is fitted to a dried round-bottom flask of known weight, containing 120ml of Petroleum ether. The sample was heated via a heating mantle and allowed to reflux for 6 hours after which the thimbles were removed. The solvent was then recovered by evaporation and the extracting flask with its oil content was dried in an oven at 60°C for 30 minutes to remove any residual solvent. After cooling in a dessicator, the flask was reweighed.

The difference in weight of the flask was received as mass of fat.

% Fat content was calculated using the formula (AOAC, 2005; Moses et. al., 2012).

$$\% \text{ Fat} = \frac{W_2 - W_1}{W_3} \times 100$$

Where,

W_1 = weight of initial empty extraction flask

W_2 = weight of flask + extracted oil

W_3 = weight of sample

Determination of protein content

Percentage crude protein was determined by Kjedhal method. The total nitrogen content was determined and multiplied by 6.25 to obtain the percentage crude protein.

0.5g each of the pulverized treated egusi seed sample was mixed with 10ml of concentrated sulphuric acid in a Kjedhal digestion flask. One tablet of Kjedhal catalyst was added to it and the mixture heated under a fume cupboard until a clear solution was obtained in a separate flask. The acid and other reagents were digested but without sample to form the blank control.

All the digests were carefully transferred to a 100ml volumetric flask using distilled water and made up to the mark in the flask. A 100ml portion of each digest was mixed was distilled and the distillate collected into 10ml of 4% boric acid solution containing three drops of mixed indicators (bromocresol

green and methyl red). A total of 50ml distillate was obtained and titrated against 0.02M sulphuric acid solution. Titration was done from the initial green colour to a deep red end point.

The nitrogen content was calculated as shown below (AOAC, 2005; Moses et. al., 2012):

$$\%N_2 = \frac{100 \times N \times 14 \times V_f \times T}{W \times 1000 \times V_a}$$

Where,

W = weight of sample analyzed

N = Concentration of sulphuric acid titrant

V_f = Total volume of digest

V_a = Volume of digest distilled

T = Titre value - Blank

Determination of carbohydrate content

The total carbohydrate was determined by subtracting the sum of percentage crude fat, percentage crude protein, percentage fiber and percentage ash from one hundred, thus:

% crude oil + % crude protein + % fiber + % ash – 100 = % carbohydrate content.

RESULTS & DISCUSSION

Moisture Content

The result in Table 1 shows that the uninoculated egusi melon seeds which served as a control gave moisture content of 4.30% while the seeds inoculated with each of the following fungi, *F. solani, P. citrinum, A. granulosis, A. fumigatus, A. niger, A. flavus,* and *B. Theobromae* gave moisture contents of 5.08%, 6.07%, 6.71%, 6.73%, 6.74%, 6.75% and 6.75% respectively. There was a significant difference between the moisture contents of each of the inoculated seeds and the control. It was also observed that egusi melon seeds inoculated with *F. Solani* and treated with each of the plant powders of ginger, lemon grass and neem, recorded moisture contents of 4.81%, 4.72% and 4.62% respectively. These moisture contents differed significantly (P=0.05) when compared with *F. solani* inoculated seeds without the plant powder treatments. Similar results of decrease in moisture contents were obtained in egusi seeds inoculated with *P. citrinum, A. granulosis* and *B. theobromae,* treated with each of the plant powders tested in this experiment.

Dry matter

The dry matter ranged from 95.73% of the control to 93.26% of egusi seeds inoculated with *A. Flavus* and the difference was significant.

Crude Fiber

Uninoculated egusi melon seeds (control) gave the highest crude fibre content of 13.43%. The seeds inoculated with F. solani had the lowest content of 10.52% which differed significantly (P=0.05) with the control. The result also shows significant differences between the fibre content of both the control and the other inoculated seeds used in this experiment. Similar results were obtained with inoculated seeds treated with each of the plant powders from ginger, lemon grass and neem.

Ash content

The result of the analysis of variance showed that except the ash contents of egusi melon seeds inoculated with *B. theobromae, F. solani,* and *P. citrinum,* which were higher than the control, the seeds inoculated with *A. fumigatus, A. granulosis, A. flavus,* and *A. niger,* had ash content of 3.35%, 3.35%, 3.34%, and 3.33% respectively which were lower than 3.87% of the control. The seeds inoculated with *A. fumigatus,* treated with each of the plant powders from lemon grass, ginger and neem, tested in this experiment, gave 3.31%, 3.41%, and 4.18%, respectively. The above treatments differed significantly from each other.

Fat content

The fat contents of egusi seeds inoculated with *A. fumigatus, A. niger, A. flavus, A. granulosis, B. theobromae, F. solani, and P. citrinum,* were 22.99%, 36.26%, 36.27%, 36.24%, 29.57%, 35.75%, and 24.87%, respectively. Each of their fat contents differed significantly (P=0.05) when compared with 42.20% of the control. Moreso, the fat contents of the seeds inoculated with *A. fumigatus, B. theobromae, F. solani,* and *P. citrinum* significantly (P=0.05) differed with each other.

Protein content

High protein content of 25.21% was recorded in the control compared with 16.21% of the seed inoculated with *B. theobromae.* The protein contents of the two treatments differed significantly at P=0.05. The protein contents of egusi seeds inoculated with *A. niger*and *P. citrinum,* varied significantly. The variation ranges from 16.25% to 20.13%. Each of these protein contents differed significantly when compared with the control.

Carbohydrate content

The lowest carbohydrate content of 11.07% was recorded in the control while the highest 31.68% recorded in *F. solani,* inoculated egusi melon seeds and they differed significantly (P=0.05) from each other.

Table1. *Effects of plant extracts on the fungal degradation of the proximate contents of egusi melon seeds incubated at room temperature (25±2°C) for 7 days*

	% Parameters							
Treatments	Moisture	Ash	Protein	Fat	CHO	Dry Matter	Crude Fiber	Mean
Control	4.3	3.83	25.21	42.2	11.07	95.73	13.43	27.97
A. fumigates	6.74	3.35	16.26	22.99	16.35	93.28	11.9	24.41
A. fumigatus + ginger	6.67	3.41	16.18	36.27	26.76	93.32	10.66	27.61
A. fumigatus + lemon grass	6.66	3.31	16.21	36.21	36.98	93.27	10.63	29.04
A. fumigatus + neem	4.71	4.18	15.23	35.67	29.61	95.46	10.6	27.92
A. niger	6.73	3.33	16.26	36.26	26.37	93.28	11.06	27.61
A. niger + ginger	6.67	3.43	16.18	36.28	26.64	93.29	10.67	27.59
A. niger + lemon grass	6.64	3.31	16.21	36.23	26.97	93.29	10.64	27.61
A. niger + neem	6.7	3.41	16.21	14.18	26.89	93.28	10.61	24.47
A. flavus	6.73	3.34	16.24	36.27	26.37	93.26	11.06	27.61
A. flavus + ginger	6.67	3.41	16.19	25.3	26.78	93.31	10.67	26.05
A. flavus + neem	6.71	3.41	16.2	36.18	26.88	93.28	10.61	27.61
A. granulosis	6.71	3.35	16.23	36.24	26.37	93.28	11.05	27.60
A. granulosis + ginger	6.67	3.41	16.21	36.18	26.89	93.28	10.64	27.61
A. granulosis + lemon grass	6.65	3.3	16.22	36.18	26.89	93.28	10.64	27.59
A. granulosis + neem	6.7	3.41	16.2	36.18	26.89	93.28	10.62	27.61
B. theobromae	6.75	4.26	20.19	29.57	27.09	93.94	12.15	27.71
B. theobromae + ginger	6.72	4.2	20.2	29.55	27.09	93.95	12.19	27.7
B. theobromae + lemon grass	6.73	4.21	20.19	29.56	27.11	93.96	12.18	27.71
B. theobromae + neem	6.8	4.13	20.19	29.65	27.11	93.95	12.17	27.71
F. solani	5.08	4.28	15.22	35.75	39.68	94.92	10.61	29.36
F. solani + ginger	4.81	4.23	15.24	35.67	39.42	95.17	10.55	29.30
F. solani + lemon grass	4.73	4.15	15.16	35.62	29.82	95.28	10.52	27.90
F. solani + neem	4.63	4.18	15.23	35.67	29.48	95.4	10.6	27.88
P. citrinum	6.07	5.32	20.16	24.87	29.4	93.93	12.16	27.42
P. citrinum + ginger	6.02	5.22	20.13	27.12	29.4	93.89	12.14	27.70
P. citrinum + lemon grass	6.1	5.04	20.11	27.1	29.4	93.9	12.25	27.7
P. citrinum + neem	6.05	5.06	20.11	27.12	29.53	93.98	12.12	27.71
Mean	6.183	3.91	17.48	32.36	27.83	93.91	11.25	
$LSD_{0.05}$	0.05*	0.02*	0.03*	9.13*	0.1*	0.05*	0.46*	

Fungi are the major cause of post-harvest deterioration of cereals, legumes and oil seed (Reddy et. al., 2010; Aboloma and Ogunbusola, 2012). The changes that occur when seeds deteriorate in storage include discolouration, caking and abnormal odours (Bankole and Joda, 2004). Nutrients are lost as a result of these changes which occur in the carbohydrate, protein, lipid and vitamin contents. Abolomaet *al* (2012) has shown that deterioration caused by seed-borne microorganisms especially fungi can lead to deterioration in the proximate composition of cucurbits, including consequent changes in organoleptic properties such as taste, flavour and texture.

Carbohydrates decompose to carbon dioxide, water and the sugar content is used by fungi during respiration. This leads to a decrease in dry weight as the protein content increases.

Other results of proximate analysis also show that fungal activities cause the conversion of fat and oil content of infected seeds to free fatty acids. This result is in agreement with Bankole and Jodas report (2004) of 10.6% free fatty acid with *A. flavus*, 10.3% with *A. tamari* against 0.4% in uninnoculated seeds. Except under very rare conditions, the mineral contents of seeds and their products do not change during storage. The ash content of infected seeds increases. This is as a result of losses of other seed constituents such as carbohydrates. This agrees with findings by Abolomaet *al* (2012).

Some of the fungi reported in this work produce mycotoxins, espcially Aflatoxins, which predispose to liver cancer, a food-borne carcinogenic agents (Hegazy, 2011; Bankoleet *al*, 2005a). Bankoleet *al* (2005b) has shown that aflatoxins occur in detectable quantities in egusi melon found in Nigeria. Reddy *et al* (2010), reported that aflatoxin B1, fumomosin B1 and ochratoxin A are toxic to mammals causing hepatotocity, teratogenicity and mutagenicity which in diseases such as toxic hepatitis, haemorrage, oedema, immune suppression, hepatic carcinoma, equine leukoen cephatomalacia, osophageal cancer and kidney failure. Several outbreaks of mycotoxicosen disease in human and animals caused by various mycotoxins have been reported after the consumption of mycotoxin contaminated food and feed (Reddy and Laghavender, 2007). The above mentioned health problems can be averted if seeds are subjected to certain treatments before they are stored.

Storage fungi also reduce seed quality and, hence, market value. Fungal deterioration of stored seeds constitutes a great challenge to the storage system in Nigeria, leading to huge losses to the farmers, consumers and traders. The evaluation of various methods of controlling these fungi using plant extracts show that the extracts were effective against seed-borne fungi of egusi melon. These plants are not toxic to both animals and humans. They are very cheap and readily available as they are abundantly distributed in all parts of Nigeria. Seed treatment with plant powders, disinfectants, heat or vegetable oils is also necessary before they are stored, especially if they have evidence of fungal infection. Seeds that are intended for food should be treated with powders of ginger, lemon grass.

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