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Changes in the nutritional quality of *Citrillus Colocynthis* (Melon) induced by processing

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Abstract
Citrullus colocynthis seed is used as food condiment in Eastern Nigeria. The study was aimed at isolating and comparing the incidence of fungal infection in self processed and commercially processed seed flour sold in the open market. Investigate the effect of fungal infection on the nutrient and anti-nutrient composition of self and commercially processed *Citrillus colocynthis* seeds. Shelled *C. colocynthis* seeds and grounded seed (flour) samples were purchased from a market at Woji, in Port Harcourt, Rivers State, Nigeria. The seeds were dehusked manually and the whitish and visually healthy seeds were then grinded using a sterile electric blender and immediately stored in air tight container. A suspension of each sample (self and commercially processed) was made and dispensed in Petri dishes containing Sabroud Dextrose Agar (SDA) incubated for five days. The fungi were later identified on the basis of spore characterization. The nutritional analysis (proximate, mineral and photochemical) was done according to the procedure recommended by the association of official analytical chemists (AOAC 2005)**.** Commercially processed samples recorded higher fungal incidence relative to self processed samples. *Aspergillus Niger* and *Aspergillus flavus* were dominant species among the fungi isolated. The values of some essential nutrients (Moisture, lipid, Carbohydrate and protein) decreased in commercially processed sample relative to self processed sample. All phytochemical contents were higher in commercially processed samples. The information on fungal infection available from this study has shown that processed *C. colocynthis* seed flour sold in the open market is a good substrate for fungal culture.

Keywords: fungi, *Citrillus colocynthis*, Nutrients, anti-nutrient, processed

Introduction
Citrullus colocynthis (melon is a creeping annual belonging to the Cucurbitacea family (Obute and Ndukwu 2005 [23]; Odiaka and Schippers, 2004) [24]. The melon plant has smooth spherical fruits of the size of cucumber sometimes or as big as a small ball. The fruit is green when young and somewhat yellow when ripe and contains a soft spongy pulp in which is embedded numerous oval-shaped, compressed, white or brown seeds (Ude *et al*., 2002) [30]. *C. colocynthis* seeds are high in protein (34.86%), oil (42. 29 %') minerals such as sodium [162.76ppm), potassium (8.28%), Calcium (1.49%), Copper (3.37ppm) and Zinc (13.46ppm) (Abiodun and Adeleke, 2010) [2]. Thus *C. colocynthis* is a major source of protein in the tropical regions of Africa and Asia where staples are very low in protein. Due to its high protein content, it is used as food condiment and soup thickener. The seeds are roasted and ground into a coarse, nutritional meal which is a local staple in Kalahari regions of Africa. Also in Sudan and Egypt C. *colocynthis* seeds are roasted and the pulp is eaten as a dish called 'tasali' (Van Dersvossen *et al.*, 2004). In Nigeria, *C. colocynthis* seeds are fermented and eaten as "Ogiri" (Abiodun and Adeleke, 2010) [2]. The seeds can also be roasted, fried and then boiled to prepare a sweeter known as 'Igbalo' (Van dervossen *et al*., 2004) [32]. *C. colocynthis* seeds, however have a challenge of field and storage fungal contamination.
Experience shows that commercially processed seeds sold in the open market is laden with micro-organisms. Bankole *et al.*, (2005) [7] among other researchers reported the presence of aflatoxins in *C. colocynthis* grown in Nigeria. This study will add to the body of knowledge on fungi associated with *C. colocynthis* seeds in storage. However, there is dearth of information on microbial and nutritional comparison of self processed and commercially processed *C. colocynthis* seed flour sold in the market. This study was therefore aimed at isolating and identifying fungi implicated in the commercially and self processed *C. colocynthis* seeds and evaluate the nutritional status of both samples.

Materials and Methods
Collection of Samples
Shelled *C. colocynthis* seeds and grounded (flour) samples were purchased from a market at Woji, in Port Harcourt, Rivers State, Nigeria. The seeds were dehusked manually and those seeds with evident fungal infections and coloured seeds were discarded. The whitish and visually healthy seeds were then grinded using a sterile electric blender and immediately stored in air tight container. The samples were taken to the plant pathology laboratory of the Department of plant science and Biotechnology, Rivers State University for further studies.

Media Preparation
The medium used for fungal isolation was the Sabraud Dextrose Agar (SDA). This was prepared by weighing 32.8g of Sabraud Dextrose Agar (SDA) into a 500ml conical flask, distilled water (500ml) was added into the flask with a measuring cylinder integrated with chloramphenicol and stirred to homogenize. The mouth of the conical flask was plugged with sterile cotton wool and wrapped with foil. The conical flask with its contents was autoclaved for 15 minutes at 121ºC at 15 PSI pressure. Sterile Petri dishes were prepared and the mixture dispensed into them while still hot and allowed to solidify.

Isolation of Fungi from Commercially and Self Processed Samples

One gram of commercially processed sample of *C. colocynthis* was added to 25ml sterile distilled water. The suspension was thoroughly shaken and serially diluted three (3) times. Each dilution was kept in sterile Petri-dish. SDA previously prepared was poured onto suspension and mixed thoroughly and allowed to set. This procedure was repeated for self processed sample in triplicate. They were left on the laboratory bench at 28±1°C and left for 5 days. The fungi were identified on the basis of spore characteristics and the nature of mycelium. Alexopoulous *et al.,* (2002) [30] viable counts were also made and recorded as colony forming unit. Pure cultures of the isolates were made after series of isolation.

Nutritional Analysis

Nutrient analysis of various proximate compositions; Moisture, Lipid, Protein, Fiber, Ash and carbohydrate as well as mineral; calcium, potassium, phosphorus, sodium, iron and magnesium composition were determined. Also, analysis of the anti-nutrient content.

(Tannin, Saponin, Total oxalate and Cynogenic glucoside) of the seed was carried out following procedures recommended by the Association of Official Analytical Chemists (AOAC, 2000).

The results of each component were subjected to statistical analysis using the software statistical package SAS at 5% level of significance and mean separation was done using Tukey's range test.

Results

Results on the percentage incidence of fungi Isolated from self processed and commercially processed seed flour are presented in Table 1, proximate, mineral and photochemical constituents are presented in Table 2, 3 and 4 respectively.

Table 1: Percentage Incidence of Fungi Isolated from Self Processed and Commercially Processed *C. colocynthis* Seed Flour.

	Fungal Isolates					
Parameter	***Aspergillus niger***	***Aspergillus flavus***	***Penicillium italicum***	***Botryodiplodia theobromae***	***Fusarium oxysporium***	***Rhizopus stolonifer***
Self-Processed Sample	15.00±1.41^{b}	10.00±1.42^{b}	5.00±1.42^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Commercially Processed Sample	55.00±0.00^{a}	50.00±2.83^{a}	20.00±1.42^{a}	30.00±1.42^{a}	25.00±2.83^{a}	40.00±1.42^{a}

Means that do not share a letter are significantly different

Significant differences occurred among fungal species isolated from self and commercially processed samples. *Aspergillus Niger*, *Aspergillus flavus* and *penicillium italicum* were dominant in both samples while *Botryodiplodia theobromae*, *Fusarium oxysporium* and *Rhizopus stolonifer* were dominant in commercially processed sample.

On a general note, the percentage incidence of fungi was higher in commercially processed sample.

Table 2: Proximate Composition of Self and Commercially Processed *C. colocynthis* Seed Flour

	Proximate Composition (% ww)/100g					
Parameter	**Moisture**	**Ash**	**Fiber**	**Lipid**	**Carbohydrate**	**Protein**
Self-Processed Sample	13.40±0.28^{a}	5.70±0.23^{a}	2.50±0.14^{a}	41.60±2.82^{a}	11.25±1.42^{a}	25.40±1.42^{a}
Commercially Processed Sample	13.20±0.14^{a}	5.70±0.00^{a}	2.50±0.00^{a}	40.02±1.42^{b}	11.08±1.42^{a}	24.30±1.42^{a}

Means that do not share a letter are significantly different

Moisture, lipid, carbohydrate and protein depreciated in commercially, processed seed flour relative to self processed sample while ash and fiber had the same value in both samples.

Table 3: Mineral Composition of Self and Commercially Processed *C. Colocynthis* Seed Flour

	Mineral Composition (%ww)/100g					
Parameter	**Calcium**	**Phosphorus**	**Sodium**	**Potassium**	**Iron**	**Magnesium**
Self-Processed Sample	1.17±0.00^{a}	3.10±0.14^{a}	0.04±0.14^{a}	0.68±0.00^{a}	0.75±0.00^{a}	4.40±0.14^{a}
Commercially Processed Sample	1.37±1.42^{a}	3.10±0.00^{a}	0.02±0.14^{a}	0.68±0.04^{a}	0.75±0.00^{a}	3.50±0.14^{b}

Means that do not share a letter are significantly different

The values of sodium and magnesium decreased, while calcium increased in commercially processed sample. However, phosphorus, potassium and iron maintained the same value in both samples.

Table 4: Photochemical Content of Processed *C. colocynthis* Seed

Parameter	**Tannin**	**Total Oxalate**	**Saponin**	**Hydrogen Cyanide**
Self-Processed Sample	0.81± 0.01^{b}	0.15±0.01^{b}	0.10±0.01^{b}	0.00±0.00^{a}
Commercially Processed Sample	1.30±0.04^{a}	0.65±0.03^{a}	0.50±0.01^{a}	0.00±0.00^{a}

Means that do not share a letter are significantly different

Photochemical content analysis revealed the presence of Tannin, Total oxalate and Saponin. However, hydrogen cyanide was not present.

Discussion

Fungal Contamination

Results from percentage incidence of fungi in *C. colocynthis*

Seed flour revealed higher incidence and fungal loads in commercially processed samples significant differences existed among fungal contamination. *Aspergillus Niger* and *Aspergillus flavus* were dominant species among the fungi isolated as seen in Table 1. Self processed sample had very minimal fungal contamination relative to commercially processed sample. This result was not unexpected, commercially processed seed flour sold in the open market encourage fungal to thrive. This is on account of the fact that the environments in which the seeds are processed are unaseptic.

The method of processing, handling, and preservation affect the level of contamination and influence microbial load. The climatic conditions prevalent in an open markets also favour the survival of these fungi. This confirms the findings of Chuku *et al.*, (2009) and Ebimieowei and Emiri (2016) [13]. Generally, six fungal species were isolated, viz *Aspergillus niger, A. flavus, P. italicum, Botryodiplodia theobromae*, *Fusarium Oxysporium and Rhizopus stolonifer* from commercially, processed sample. It thus suggests that commercially processed sample is heavily contaminated.

A lot of studies abound that shows that fungi have the potential to contaminate a variety of food condiments. Various genera of fungi that have been isolated from Nigeria food stuffs and food ingredients include *Aspergillus species, Fusarium, Rhizopus*, *penicillium* and *Botryodiplodia.* Bankole *et al.,* (2005) [7] Jomoh and Kolapo (2008) [26]. Akinyele and Oloruntoba (2013); Ebana *et al.*, (2014) [12] Bankole *et al.,* (2005) [7] Isolated similar fungal species from *Citrullus colocynthis*. The findings from this study confirms their assertion.

Proximate Composition

Results on proximate composition revealed that the values of moisture, protein, carbohydrate and lipid depreciated in commercially processed *C. colocynthis* seed flour relative to self processed sample. However, the values of Ash and fiber remained the same in both samples. The reduction of moisture content could be attributed to the fact that the seed sold in the market is usually exposed to air which encouraged drying. Generally, the moisture content of 13.20% is relatively high and would encourage fungi to thrive. The moisture content from this work is relatively higher than 7.10% reported by Jacob *et al.*, (2015) [18], but lower than 87.15% reported by Ebana *et al.*, (2014) [12] on *C. colocynthis* seed flour. The decrease in moisture from this study agrees with Sanyaolu (2014) [27] who reported a decrease in moisture content of fungal infected seeds of *irvingia gabonensis*.

Generally, the changes in the nutritional value of *C. colocynthis* as observed in this study agrees with the findings of Oladimeji and Kolapo (2008) [26] who asserted that different micro-organisms that were inclusive of fungi were seen to cause a reduction in the nutritional worth of some oil seeds in Nigeria. The high content of lipid in *C. colocynthis* (41.60%) in this work lends the seed its credence as an oily seed. The decrease in lipid seems to suggest that these fungi have high lipase activities. Fat provides energy for the body. The lipid content (41.6%) from this work is lower than 49% reported by Jacob et al., (2015) [18] and Ebana *et al.*, (2014) [12].

The protein content (25.40%) is comparable to those reported for *C. colocynthis* seeds 28.63% (Bankole *et al.*, 2005) [7]. The low carbohydrate value (11.25%) is an indication that *C. colocynthis* seed is poor in carbohydrate. The value is significantly lower than 20.17% reported for the same seed (Igwenyi *et al.*, 2011).

The decrease and carbohydrate conforms the findings of Sanyolu (2014) on *Irvingia gabonensis* which has similar composition as *C. colocynthis*, also used as soup thickener. This observation however negates the assertion of Falaye and Fagbotiun (2012) who reported an increase in carbohydrate of groundnut (*Arachis hypogea*) infected with *R. stolonifer* and moulds.

Reduction in protein content may be caused by the degradation and dissolution of the seed tissue by the fungi. The protein might have been broken down by the fungi into smaller molecules that they absorbed Nweke and Ibiam (2012). Earlier researchers had reported depletion of protein in fungal infected crops and vegetables. Ndife *et al.*, (2013) reported protein content of cocoa beans were significantly depleted when infected with some fungal species. Shehu and Aliero (2010) have also reported that infected onion leaf showed a significant decrease in the quantity of protein content.

Fiber content neither increased nor decreased, which suggest that the fungi infection had no impact on these nutrient contents. The ash and fiber contents were higher than those reported by Igwenyi (2011) on *C. colocynthis* seeds but comparable to those reported by Jacob *et al.*, (2015) [18] on same seed. Fiber supplements or fibre-rich foods may function as normal dietary agents by modulating the digestive and absorptive process Okaka *et al.*, (2006). It is also believed that fiber reduces the level of cholesterol in human blood and decreases the likelihood of cancer.

Mineral Analysis

The result on mineral analysis as shown in Table 3 revealed that the most abundant mineral found was magnesium followed by phosphorus, while sodium was least. The values of sodium, (0.04%) potassium (0.68%), phosphorus (0.68%) iron (0.75%) and magnesium (4.40%) observed from this result is significantly lower than the values reported by Jacob *et al.*, (2015) [18], as follows; sodium (0.21%), potassium (4.94%), iron (14.4%) and magnesium (20.46%), phosphorus (5.77%) for the same seed. However the value of calcium (1.17%) from this work was higher than that reported for calcium (0.10%) by Jacob et al., (2015) [18]. The values of these mineral contents from this study are however comparable to the values reported by Abiodun and Adeleke (2010) [2].

The values of magnesium and sodium reduced in commercially processed sample relative to self processed sample, while calcium increased. However, the values of phosphorus, potassium and iron remained the same in both self processed and commercially processed seed flour. The increase in calcium agrees with the findings of Emiri and Enaregha (2020) [14] on Biochemical changes of *Mucuna sloanei* seeds induced by some pathogenic fungi. Similarly, Nwaukwu and Ikechi-Nwogu (2012) [22] reported a decrease in Magnesium contents of *Dialium guineense* infected with similar fungal isolated in this research.

Mineral are essential elements that exist in non-organic form and are normally required in small amounts hence they, like vitamins are tagged micro-nutrients. They are essential to life and an element is said to be essential when a deficiency in intake produces an impairment of function and physiological amounts of only that element can prevent or

alleviate the impairment (Boukari *et al.*, 2001) [10]. Magnesium, which is most abundant in this study is beneficial to blood pressure and helps to prevent sudden heart attack, cardiac arrest and stroke. Calcium, found in *C. colocynthis* is an important component of bone and contribute to its structural development and it's dietary deficiency together with phosphorus and vitamin D causes rickets in children, osteoporosis and osteomalacia in adults. (Voet and Voet, 2004) [33].

Anti-nutritional (Photochemical) Composition

The anti-nutritional composition of *C. colocynthis* is presented in table 4. Result revealed the presence of Tannin, (0.81), Oxalate (0.15) and Saponin (0.10) which are low, while Hydrogen cyanide was not detected. These values were significant lower than the values reported by Jacob et al., (2015) [18] for same seed. It thus suggest that *C. colocynthis* is a healthy diet, as Hydrogen cyanide have been reported to cause risk for human health. Higher value of oxalate in human diet can increase the risk of renal calcium absorption and has been implicated in kidney stores, Chai and Liebman (2004) [11]. Higher values of oxalate in human diet can increase the risk of renal calicium absorption and has been implicated in kidney stores, (Chai and Liebman 2004) [11]. Higher values of tannin in foods interferes with protein absorption and digestive enzymes. A neurological disease known as Tropical Ataxis Neuropathy (TAN) is linked to consumption of high level cyanide in cassava based diet. (Hassan and Umar, 2004) [16]. The concentrations of oxalate, tannin and Saponin in *C. colocynthis* seed flour obtained from the result of this study are low to cause any health risk. According to Betancur-Ancona (2008) [9], only plants with more than 200mg of hydrocyanic equivalent acid per 100mg fresh weight are considered dangerous.

Conclusion and Recommendations

The information on fungal contamination available from this study has shown that processed *C. colocynthis* seed flour sold in the open market is a good substrate for fungal culture which in turn utilized the nutritional constituent of their substrate (*C. colocynthis)* for their growth, hence the depletion of some of the macro and micronutrients. The low concentrations of anti-nutritional (photochemical) constituents suggest that the seed flour is a good source of food for human and animals. Self processed seed flour is recommended. However, there is need to create awareness on good sanitary and aseptic measures to food condiment vendors and the general public in method of processing and handling of food condiments.

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