International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 8 No. 4 Oct. 2014, pp. 1612-1617

http://www.ijias.issr-journals.org/

Nutritional and Microbial Analysis of Melon (*Citrullus colocynthis Linn*) Cake and its Components - A Traditional Snack in South - South Nigeria

Rose U. Ebana[1], Comfort Aloysius Etok[2], and Uwem Okon Edet[1]

[1]Microbiology Department, Obong University, Obong Ntak, Akwa Ibom State, Nigeria

[2]Microbiology Department, University of Uyo, Uyo, Akwa Ibom State, Nigeria

ABSTRACT: The nutritive value of mushroom melon cake was investigated. Our study indicates that the raw melon possessed high moisture, protein and fat content of 87.15±0.03, 45.20±0.01, and 48.83±0.01g/100 dry weight, respectively. However, it was low in fibre and carbohydrate. Another major component of the melon cake, *Pleurotus tuber regium* proximate composition gave 48.20±0.02, 15.50±0.02, 0.84±0.02, 3.31±0.02 and 1.63±0.03 for moisture, protein, fat, fibre and carbohydrate, respectively. Following 96 hours of fermentation, the protein content increased from 46.47±0.02 to 47.78±0.01 in the group aided by Baker's yeast and was higher than the natural fermentation group whose protein went from 45.55±0.05 to 47.50±0.02. Phytochemical screening showed the presence of cardiac glycosides in *Combretum micranthum* but not in *Pleurotus tuber regium*. *C. miranthum* also showed good antimicrobial activity against the isolated microorganisms from the contaminated the melon. Further work should be carried out on *C. micranthum* antimicrobial properties.

KEYWORDS: Melon Cake, *C. micranthum*, Proximate, Cardiac glycoside and Mushroom

1 INTRODUCTION

Nigeria aims to be among the top 20 economies in the world by 2020. To attain such noble objective would mean achieving food security, and also meeting the Millennium Development Goals (MDGs) of eradicating hunger and poverty in the country, among other things. As a ray of hope, the country is blessed with a variety of food crops and trees that are very rich in nutrients and other properties [1], [2], [3]. *Citrullus colocynthis*, melon is an important food crop locally and abroad and is usually grown for its seeds and not the bitter pulp. It belongs to the family cucurbitaceae [3], [4]. It is a creeping annual plant that grows well on rich light soils in the hot climates of Africa [2], [5]. The seeds are used in all part of Nigeria as a condiment in a delicacy called Egusi soup, a very popular soup in Nigeria [6], [7], [4]. Studies have shown that the seed contains oil with possible commercial importance. In addition to the oil, it has also been found to contain carbohydrate, protein, fat, vitamins and minerals. These make the seeds a complete source of balanced diet [9], [10], [8], [3], [11]. It is a popular soup thickener and flavouring agent in some parts of the country especially in the south-east and south-south [4], [7]. The seeds are also used to prepare mushroom melon cakes, a special cake and delicacy in Nigeria. This delicacy is commercially available on the streets, markets and motor parks of Nigeria but native to Akwa Ibom and Cross River State. It is an important snack served in traditional occasions. The Cake is usually prepared with grounded melon seed, sclerotium of pleurotus tuber regium, salt, fresh pepper, and a local leave called Landaga or Asakka locally(*C. micranthum*) but usually used for colouring the melon cake. The need for richer sources of foods was the impetus to evaluate and highlight the nutritive and medicinal values of mushroom melon cake and its components .The study aimed to firstly evaluate the chemical and the nutritive value of mushroom melon cake, sclerotium of the mushroom, melon seeds, the fermented melon, and the commercial melon. Secondly, to isolate potential spoilage microorganisms from the fermented melons. Thirdly, to carry out phytochemical screening of *C. micranthum* and sclerotium of the mushroom. Lastly, to test their antimicrobial activity against the isolated spoilage microbes.

Corresponding Author: Uwem Okon Edet

2 MATERIALS AND METHODS

Material

The leaves of *C. micranthum* were obtained from Ikot Ekpene in Akwa Ibom State. Test organisms used for the study were isolated from the melon employed in the study. Raw unshelled melon was purchased from Watt market in Calabar and was deshelled manually by hand. The commercial melon cake and Baker's yeast *Saccharomyces cerevisiae* were purchased from Watt market in Calabar. Reagents for phytochemical analysis were obtained from a reputable distributor and included Dragendroff reagent, ferric chloride, Mayer Reagent, Fehling Solution and Molisch reagent.

Preparation of Extracts and Phytochemical Screening

The aqueous and ethanolic extracts of the leaf were prepared as briefly described below. For the aqueous extract, about 8g of the properly grinded leaf were dissolved in 20ml of sterile distilled water and allowed to stand for about 24 hours, filtered, dispensed into sterile bottles, heated in a water bath to concentrate it, and kept at 4^0C. Another 8g was weighed, dissolved in 20ml of 97% ethanol and allowed to stand for 24hours. After 24 hours, it was filtered and allowed to evaporate leaving behind an oily substance.

The *C. micranthum* leaf and the mushroom extract pleurotus tuber-regium extract were tested for alkaloids, saponins, tannins, phlobatinin and cardiac glycosides using the method described by Sofowora [12]. Equally, the extracts were also tested for anthranoid and polyphenols, reducing compounds, hydromethyl anthraquinones, flavonoids and anthroquinones were also tested via a method described by Gundidza [13].

Preparation of Media and Dyes

Phenol red, lacto phenol in cotton blue, NNNN-Tetramethyl-p-phenylene diamine hydrochloride, normal saline, Gram iodine, Gram crystal violet and Gram safranin were all prepared according to manufacturers' instructions. Muller- Hinton, Malt agar, Nutrient Agar, Methyl Red Voges- Proskauer, and fermentation broth were all prepared following manufacturer's instruction.

Preparation, Treatment of Cake Components and Fermentation of Samples

Dried melon seeds of *Citrullus colocynthis* were deshelled manually and those seeds with evident fungal infections and coloured seeds were discarded to eliminate any toxin contamination risk. The whitish and healthy seeds were then grinded using a sterile electric blender and immediately placed in an air tight container to avoid rancidity of the oil and stored at 4^oC for usage. The red pepper were washed in clean water, and blended to obtain a paste. The *C. micranthum* leaves were washed thoroughly, oven dried and grinded into a fine powder. For the purpose of the study, two methods of fermentations were employed namely natural and aided fermentation. To the aided fermentation, about 5g of Baker's yeast was added to 10g of prepared melon but the natural add no yeast was added to the melon preparation. All manipulations were done aseptically to avoid contamination. The melon in each process were cut and wrapped in small clean bags and stored at room temperature for 24, 48, 72 and 96 hours.

Disk Diffusion Method

The antimicrobial effect was tested by disk diffusion method as summarised below. Filter papers of size 5mm were cut using a cork borer, wrapped with foil paper and sterilized in hot air oven. A colony of the test organisms were sub cultured on nutrient broth, incubated at 37^oC for 6 hours. After which the organisms were transferred onto solidified Muller-Hinton Agar plates. Filter papers were then soaked in the extracts and placed on the agar plates. Plates were then incubated at 37^oC for 24hours. Zones of inhibitions were then measured and recorded in triplicates. Mean values for each taken and the standard deviation calculated.

Proximate Composition

The methods for the chemical analysis of the samples were those of the Association of Official Analytical Chemist (AOAC, 1980) [14] and same was used to estimate the moisture content, ash content, crude protein, petroleum ether(lipid content), crude fibre, and carbohydrate content of samples.

Isolation of Microorganisms from samples

A gram each of both aided and natural yeast were dissolved differently in 9ml of sterile distilled water , thoroughly shaken and serial dilutions up to 10^8 were made for each samples. Dilutions 10^3, 10^4, 10^5 were then poured into separate sterile petri dishes , freshly prepared nutrient agar poured into them and allowed to solidify. The plates were then incubated at 28^oC for 24 hours and then examined for growth. Isolated colonies were sub cultured to obtain pure stock cultures. The

same method of isolation used for bacteria was also used for the fungi except that for the fungi, malt extract agar was poured into the plates and upon solidification plates were incubated at 28^{o}C for 3days and then examined for growth. The isolates were characterised by microscopic and macroscopic examinations. The macroscopic examinations revealed the colour of the aerial and substrate mycelia for the fungi and also the type shape, and solubility of pigment produced for both bacteria and fungi. A number of biochemical test were done to properly identify the isolates. They included Catalase, oxidase, coagulase, urease, utilization of carbon, methyl Red and Voges- Proskauer.

3 RESULTS

The results obtained from the study are as presented in the tables below. All values presented in the tables are mean of triplicates and standard deviations from the mean.

Table 1: Phytochemical Screening of C. micranthum and Pleurotus tuber regium

Chemical Constituents	*C. micranthum*	*Pleurotus tuber regium*
Alkaloids	+	+
Cardiac Glycoside	+	-
Saponin	+	+
Tannin	-	-
Flavonoid	+	+
Polyphenol	+	+
Hydroxymethyl-Anthraquinone	+	+
Phelobatinin	+	-
Anthraquinone	-	-

N/B: + means present while – means absent. The table above shows the results of the photochemical screening that was carried out. The C. micranthum only lacked anthraquinones and tannins while the pleurotus tuber regium lacked phlobatinin and cardiac glycosides in addition.

Table 2: Proximate composition of raw melon, Ikot Ekpene processed self processed melon cake and pleurotus tuber regium (g/100 dry weight).

Samples	Moisture	Ash	Crude Protein	Crude fat	Crude fibre	Carbohydrate
Raw melon	*87.15±0.03	3.41±0.01	45.20±0.01	48.83±0.01	3.16±0.01	0.61±0.04
Ikot Ekpene Processed	54.30±0.03	2.45±0.01	44.80±0.01	46.61±0.01	4.50±0.02	1.15±0.04
Self Processed Melon Cake	49.40±0.01	2.20±0.02	46.09±0.01	47.30±0.02	3.43±0.02	0.96±0.01
Pleurotus tuber regium	48.20±0.02	2.19±0.01	15.50±0.02	0.84±0.02	3.31±0.02	1.63±0.03

**Values presented here are expressed as Mean±SD. Raw melon was the richest in moisture, ash, crude fat. It had lowest amount of carbohydrate and fibre. Its protein content and fibre was almost same with other samples examined. The melon cake had the highest protein content while the Ikot Ekpene processed had the highest carbohydrate.*

Table 3: Composition of raw natural and aided fermented melon after 24, 48, 72 and 96 hours

Samples	Moisture	Ash	Crude Protein	Crude Fat	Crude Fibre	Carbohydrate
24 Hours						
Natural	46.46±0.01	2.44±0.04	45.55±0.05	46.47±0.02	2.71±0.03	5.09±0.20*
Aided	71.74±0.01	1.83±0.01	46.47±0.02	47.14±0.02	1.47±0.02	3.09±0.06
48 Hours						
Natural	74.43±0.03	2.54±0.04	46.20±0.02	47.65±0.03	2.43±0.03	1.29±0.26
Aided	76.32±0.02	2.14±0.03	46.91±0.03	47.80±0.02	1.10±0.02	1.41±0.17
72 Hours						
Natural	85.35±0.02	3.10±0.02	47.32±0.02	48.42±0.40	0.52±0.02	0.45±0.03
Aided	87.49±0.09	2.20±0.02	47.70±0.02	49.30±0.02	0.48±0.02	0.47±0.03
96 Hours						
Natural	88.49±0.02	3.21±0.03	47.50±0.03	48.90±0.01	0.22±0.02	0.11±0.03
Aided	93.34±0.03	2.21±0.01	47.78±0.01	50.07±0.02	0.04±0.01	0.02±0.02

**Values also expressed as Mean±SD. The melon with enhanced fermentation was richer that the natural one in moisture, protein and fat. However, the natural fermentation had more fibre and ash than the aided fermentation. For carbohydrate, aided fermentation was only slightly higher than natural after 48 hours and 72 but not at 24 and 96hours.*

Table 4: Proximate Composition of cooked and fermented melon after 24, 48, 72 and 96 hours

Samples	Moisture	Ash	Crude Protein	Crude Fat	Crude Fibre	Carbohydrate
24 Hours						
Natural	69.74±0.04	2.45±0.02	46.26±0.17	48.25±0.02	2.90±0.02	0.67±0.32*
Aided	70.35±0.01	2.24±0.02	47.30±0.01	48.38±0.01	1.67±0.06	0.43±0.05
48 Hours						
Natural	80.38±0.02	2.64±0.04	46.83±0.003	47.87±0.03	1.23±0.06	1.42±0.13
Aided	76.71±0.02	2.30±0.01	47.12±0.02	48.22±0.02	1.34±0.04	1.10±0.02
72 Hours						
Natural	80.38±0.02	2.73±0.02	47.02±0.02	48.62±0.01	1.28±0.06	0.34±0.03
Aided	79.13±0.02	2.39±0.01	47.45±0.01	49.10±0.01	0.79±0.01	0.29±0.01
96 Hours						
Natural	82.75±0.01	3.00±0.01	47.12±0.02	49.32±0.02	0.25±0.01	0.22±0.01
Aided	84.33±0.03	2.62±0.02	47.75±0.04	49.48±0.01	0.09±0.02	0.05±0.02

**Values also expressed as Mean±SD. The cooked melon with enhanced fermentation was richer that the natural one in protein and fat. However, moisture was only higher in the aided group after 24 and 96 hours. Fibre was the highest in the natural group just like with the raw melon above in table 3. For carbohydrate, natural fermentation had more than aided.*

Table 5: Inhibitory Activity of the Aqueous Extract and Ethanolic Extract of C. micranthum on Bacteria Isolates (mm).

Bacteria	Aqueous Extract	Ethanolic Extract
Staphylococcus aureus	*10.60±0.020	15.20±0.112
Escherichia coli	11.80±0.211	11.60±0.125
Staphylococcus epidermis	11.70±0.401	11.20±0.011
Klebsiella Sp	11.00±0.001	11.00±0.201
Micrococcus	11.00±0.010	11.40±0.111
Bacillus Sp	11.50±0.012	11.30±0.091
Psuedomonas Sp	11.00±0.201	11.70±0.010

Values are expressed Mean±SD. The highest zone of inhibition was seen with the ethanolic extract on S. aureus and the least zone was seen with the aqueous extract. Other organisms had fairly similar zones of inhibition as shown above.

4 DISCUSSION

A lot of studies abound on the nutritive and medicinal value of plants in Nigeria [5], [6], [7], [9], [10], [11], [15], [16]. Our study has not only evaluated the food composition of raw melon but also that of fermented and cooked melon in addition to the phytochemical screening of the melon cakes components. When compared to the raw melon, cooked melon appeared to have much lowered protein content see tables 2 and 3. Fermentation is already been established as a way of adding flavour to food, increasing its palatability and increasing its protein value [6]. Among the fermented samples, the effect of fermentation was very obvious and the samples that went through natural fermentation were slightly lower in most nutrients than the aided samples. After 24 hours, of fermentation, the protein content was 45.55±0.05 and 46.47±0.02g/100 dry weight for natural and enhanced fermentation, respectively. The use of yeast a single cell protein did increase the overall protein content of the samples. This has been also observed previously [9], [15]. The protein content of the fermented foods rose steadily with time to 47.50±0.02 after 96hours of fermentation. Even though the protein content did increase over time following fermentation, it was observed that the cakes lost their ability to be moulded into cakes after 24 hours and also it lost its organoleptic properties after 72 hours as they did not appear palatable any more. The moisture content in the raw fermented samples had more moisture and carbohydrate than the cooked samples. Crude fibre and carbohydrate also dropped in both cooked and fermented samples over time. We proposed here that the *Pleurotus tuber regium* used in processing the melon cake contributes to both the protein and probably more of the carbohydrate which were generally low in the raw melon proximate composition.

A lot of studies abound that shows that fungi and bacteria have potential to contaminate a variety of foods [6], [17], [18]. Fungi isolated from our study included *Aspergillus niger*, *Aspergillus* species, and *Penicillium* species. Bacteria isolate included *Staphylococcus aureus*, *Esherichia coli, Bacillus* species, *Staphylococcus epidermidis*, *Klebsiella* species, *Micrococcus* and *Pseudomonas* species. In an earlier study, *Penicillium, Fusarium, Rhizopus and Aspergillus* were isolated from unfermented and fermented melon [6]. In the same study, Bacillus, Lactobacillus and Leuconostoc species dominated the bacteria isolates and varied with time of fermentation. The highest contamination of the melon was seen in those samples sought from the market and this could be as a result of aseptic handling of the melon by dealer in the market. The zone of inhibition shown by both the ethanolic and aqueous extracts of the *C. micranthum* leaves indicate that its addition to the melon cakes together with the *Pleurotus tuber regium* may have had some antimicrobial effect on the microorganisms that could cause contamination and spoilage of the melon cake. The extracts of *C. micranthum* inhibited all the isolates from melon. A lot of plants are known to contain phytochemical and show good antimicrobial properties [12], [13], [19], [20]. Both *C. micranthum* and the *Pleurotus tuber regiu*m on analysis contain alkaloid, polyphenol, flavonoid reducing compounds and other phytochemical bases that could be used as potential precursors of various drugs and other important properties

5 CONCLUSION

Our findings show that melon cakes both fermented and raw melon contains basic diets such as protein, carbohydrate, oil, fibre and moisture and if properly processed, could help the country in its drive for attainment of food security and vision 2020.

ACKNOWLEDGEMENT

This work is dedicated to the memory of **ANDY ENO** who started the preliminary studies of this work. May her gentle soul rest in peace.

REFERENCES

[1] J. C. Okaka, E. N. T. Akobundu and A. N. C. Okaka, Food and Human Nutrition An Integrated Approach, OCJ. Academic publishers, Enugu. Nigeria, pp. 135 – 368, 2006.

[2] S. H. Cobley, An Introduction to the Botany of Tropical Crops Longmans, Green & Co., pp. 293 –301, 1957.

[3] M. O. Adeniran and G. F. Wilson, "Seed type classification of egusi melon in Nigeria" Paper presented at the 6th African Horticultural Symposium, University of Ibadan, 9th – 25th, July, 1981.

[4] E. C. Oriaku, C. N. Agulnna, and H. U. Nwannewuihe, Comparative Performance Analysis of Melon (*Colocynthis Citrullus L.)* De-Husking and Separation Machines by Principles of Impact and Attrition, *Intl Journal of Multidisciplinary Sci and Eng*., (7):53- 59, 2013.

[5] V. O.E. Akpambang, I. A. Amoo and Izuagie, Comaprative compositional anlaysis on two varieties of melon(colocynthis citrullus and Cucumeropsis) and a variety of almond (Prunus amygdalus, *Research Journal of Agric and Biological Sciences*, 4: 639-642, 2008.

[6] B. J. Akinyele and O. S. Oloruntoba, Comparative Studies on *Citrullus vulgaris*, *Citrullus colocynthis* and *Cucumeropsis mannii* for Ogiri Production, *British Microbiology Research Journal*, 3(1):1-18, 2013.

[7] I. O. Igwenyi, C. A. Eze, , B. N. Azoro, C. E Offor, and C. P. Nwuke, Proximate, Mineral and Amino Acid Compositions of *Irvigna gabonesis* and *Citrullus colocynthis* Used as Soup Thickener in South Easter Nigeria, *Intl Journal of Biotechnology and Biochemistry.* 7(4): 493-499, 2011.

[8] E. A. Ogunremi, "Effect of nitrogen on melon (Citrullus lanatus)" at Ibadan Nigeria, *Exploratory Agriculture*, 14: 357 – 365, 1978.

[9] O. U. Eka, and J. K. Edijala, Chemical Composition of some traditionally prepared Nigerian food. . *Nigerian Journal of Science*. 6: 177-178, 1972.

[10] N. A. Obasi, , J. Ukadilonu, E. Eberechukwu, E. I. Akubugwo, and U. C Okorie, Proximate Composition, Extraction Characterisation and Comparative Assessment of coconut (cocos nucifera) and Melon (colocynthis Citrullus) seeds and seeds oils, *Pakistan Journal of Biological Sciences,* 15(1):1-9, 2012.

[11] V.A. Oyenuga and B. L. Fetuga, "Some aspects of biochemical and nutritive value of the water melon seeds (Citrullus colocynthis)" *Journal of Sciences and Food Agriculture*, 26: 843–854, 1975.

[12] E. A. Sofowora , Medicinal Plants and Traditional medicine in Africa, Wiley and Sons New York, pp. 256-259, 1984.

[13] M. Gundidza, Phytochemical screening of Zimbabween medicinal plants, Central African Journal of Medicine. 31(21): 238-270, 1985.

[14] A. O. A. C. (1980). Official methods of Analysis. 13th edition Association of Official Analytical Chemists, Washington, D.C.

[15] O. U. Eka, and C. Hobbs, Effect of cooking on nutritive value of Danwake, *Nigerian Journal of Sciences.* 12(6):317-325, 1978.

[16] Oke, O. L., Nutritive value of Nigeria water melon, *Nature*, 207:192, 1965.

[17] M. J. Pelazar, E. C. S. Chan and N. R. Kreig, Microbiology of food in Microbiology Concepts and Applications. Mc Graw-Hill, Inc. USA. 840-850, 1993

[18] J. M. Jay, Microorganisms In: Modern Food Microbiology, Aspen Publisher Inc. Maryland USA, pp 53-171.

[19] E. E. Madunagu, R. U. B Ebana, and E. D. Ekpe, Antibacteria and Antifungal Activity of some medicinal Plants of Akwa Ibom State, West African *Journal of Biological and Applied Chemistry*. 35(1-4): 25-30, 1990.

[20] R. U. B. Ebana, E. E. Madunagu, E. D. Ekpe, and I. N. Otung , Microbiological Exploitation of Cardiac Glycosides and Alkaloids from *Garcinia kola, Borreria ocymoides, Kola nitida and citrus aurantifolia*, *Journal of Applied Bacteriology.* 71:398- 410, 1991.