Research Journal of Chemical Sciences _____ ISSN 2231-606X
Vol. **4(4),** 28-32, April **(2014)** *Res. J. Chem. Sci.*

Effect of Heat Treatment on Nutrient and Anti-nutrient Components of Melon (*Citrullus colocynthis*) Husks

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Available online at: www.isca.in, www.isca.me
Received 28th January 2014, revised 25th February 2014, accepted 20th March 2014

Abstract

The effects of heat treatment (boiling and autoclaving) on the nutrient and anti-nutrient components of melon husk were investigated. The proximate compositions (crude protein, lipid, fibre, ash and carbohydrate), mineral constituents (Calcium(Ca), Potassium(K), Sodium(Na), Iron(Fe), Copper(Cu), Zinc(Zn), Magnesium(Mg) and Phosphorous(P)) and antinutrients compositions (Tannin, Oxalate, Phytate, Hydrogen cyanide (HCN), Tripsin inhibitor (TIA), Amylase inhibitor (AIA) and Lectin) were determined using standard methods in three portions of melon husk: Raw, Boiled and Autoclaved. The results of the proximate analysis revealed that variations in the proximate composition of the differently treated husks were significant except for the lipid content. However, there was no significant difference between the proximate compositions of raw and autoclaved melon husks except for ash and carbohydrate contents. The results of mineral analysis showed that boiling caused significant reductions in all the mineral elements analyzed. All the detected antinutrients were significantly reduced by heat treatments (boiling and autoclaving) and autoclaving was found to be more effective in reducing the levels of antinutrients than boiling. The results therefore showed that autoclaving is more effective in reducing the levels of antinutrients and had lesser effect on the nutrient composition of melon husk than boiling. It is concluded that melon husk, if heat treated, could be an alternative source of feed for live stock.

Keywords: Melon husk, nutrient, antinutrient, autoclaving, boiling.

Introduction

Melon (*Citrullus colocynthis*), commonly known as bitter apple, colosynth, vine-of-Sodom, is a cucurbitaceous plant[1]. It is a crawling crop that is widely grown in tropical Africa for its seeds which are deshelled, dried and ground into a paste that is added to vegetable soup to confer aroma, improve taste and to thicken the soup[2]. Known as egusi in West Africa[3], this plant family is known for its great genetic diversity and widespread adaptation which includes tropical and subtropical regions, arid deserts and temperate locations[4]. It has a large, fleshy perennial root, which sends out slender, tough, angular, vine-like stems. The stems are angular and rough; the leaves are rough, 5–10 cm in length, deeply 3–7 lobed; solitary pale yellow blooms. Each plant produces 15–30 round fruits, about 7–10 cm in diameter, green with undulate yellow stripes, becoming yellow all over when dry. Seeds are small (6 mm in length), smooth and brownish when ripe[5]. The seeds are removed from the pulp, washed and air-dried.

The most important use of the seeds is as a thickener in soup. The popular egusi soup is a kind of soup thickened with the ground seeds with considerable local variation. The seeds can also be fermented and used as condiments[6]. According to Ziyada and Elhussien[7], melon oil has good potential to be used for edible purposes and bio-diesel production. Un-extracted melon seeds are rich sources of energy (oil content of 51% to 55%) and crude protein (32.5% to 38.7%). These give melon seeds the potential of supplying both energy and protein in poultry diets, and may reduce feed cost. Although melon seed is low in lysine, an essential amino acid, when it is used as the main source of protein in a balanced ration, it is readily digestible, and its biological value and efficiency of its utilization were observed to be inferior only to those of animal protein[8]. In a study on the seed, Oloyede *et al* showed that processing of melon seeds by cooking and fermentation made the protein in melon seed more available to the broiler chicks, and also removed some or all of the antinutritional factors in raw melon seed[9].

It has been reported that the seeds are also used as domestic remedy for urinary tract infection, hepatic congestion, intestinal worms and abnormal blood pressure[10].

Melon seeds are covered by husks which are shelled in the process of processing. These husks are not only thrown away as a waste but they also constitute potential environmental pollutant.

With Corn starch and soybeans which are the main sources of energy for livestock also being important staples in human diet particularly in developing nations, the competition between the

animals and humans for the not adequately available sources of energy results in inadequate production and high cost of livestock which in turn makes it difficult for the humans to meet their protein need. To this effect, nutritional researches are turning to agricultural by-products in search of alternative sources of energy for livestock. Agricultural produces and residues attract widespread attention for renewable energy generation and also for their large-scale combustion[11] and melon husks could be one. However, there is a dearth of information on the nutritional and antinutritional composition of the husks as well as on the effect processing could have on the nutrient and antinutrient components of the husks.

Antinutrients are secondary metabolites synthesized by plants as a defense mechanism against predators, pathogens and adverse environment. Also called natural toxicants, the antinutrients are broadly divided into two categories: proteins (such as lectins and protease inhibitors) and others such as phytic acid, tannins, oligosaccharides, saponins and alkaloids[12]. The negative impacts of the ingestion of antinutritional factors have extensively been reported. For instance, some factors, like trypsin inhibitor, affect protein utilization and digestion, others, like phytic acid and tannins, affect mineral utilization. Also, lectin causes disruption of the small intestinal metabolism and morphological damage to the villi[13]. In order to inactivate or reduce anti-nutrients, various conventional, simple processing methods such as heat treatment, have been used. Such treatments increase the bioavailability of the nutrients in food stuffs.

This research work was therefore, carried out to determine the nutritional and antinutritional components of melon (*Citrullus colocynthis*) husk, and to evaluate the effect of heat treatment on these components.

Material and Methods

Sample preparation: Melon seeds were bought from En kwura market in Kano state of Nigeria and screened to remove bad ones. The husks were then manually removed from the seeds. The husks were washed with tap water to remove any dirt, sun-dried and then divided into portions A, B, and C. Portion A was left raw, while portions B and C were subjected to heat treatments. Portion B was boiled by immersing the husks in boiling water and allowing boiling for 90 minutes and then sun-dried. Portion C was autoclaved for 30 minutes at 120^{o}C. The three portions were separately ground to flour and separately stored in clean polyethylene bags.

Proximate analysis: The carbohydrate, ash, crude fat, crude fiber and crude protein contents of the raw and treated husks were determined using the recommended methods of the Association of Official Analytical Chemists[14].

Mineral analysis: Ca, K and Na were determined using the method described by AOAC[14]. In this method, raw and treated husk samples were turned to ash after being burnt in a muffle furnace for 5hours at a working temperature of 550^{o}C. The ash of each sample obtained was digested by adding 5ml of 2MHCl to the ash in the crucible and heating to dryness on a heating mantle. 5ml of 2MHCl was added again, heated to boil, and filtered through a Whatman No.1 filter paper into a 100ml volumetric flask. The filtrate was made up to mark with distilled water, stoppered and made ready for reading of concentration of Calcium, Potassium and Sodium on the Jenway Digital Flame Photometer (PFP7 Model).

Fe, Cu, Zn, and Mg were estimated using an atomic absorption spectrophotometer as described by AOAC[15]. Here, the samples were turned to ash and digested as were done for calcium and potassium determination. The digest was washed into 100ml volumetric flask with distilled water and made up to mark. This solution was aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

Phosphorus was determined by spectrophotometric method. Here ash of each sample obtained was treated with 2MHCl solution as described for calcium determination above. 10ml of the filtrate solution was pipetted into 50ml standard flask and 10ml of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development.

Determination of the anti-nutritional factors: Phytate: Phytate contents were determined using the method of Wheeler and Ferrel[15]. This involved soaking the sample in 100 ml of 2% concentrated HCI for 3 hours. 50 ml of the filtrate was placed in 250 ml beaker and 100 ml of distilled water added to each sample. 10 ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard Iron (III) chloride solution which contained 0.00195 g iron per ml.

Tannin: Tannin was determined by measuring 0.2 g of finely ground samples into a 50 ml beaker. 20 ml of 50% methanol was added and covered, and placed in a water bath at 79^{o}C for 1hour and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 ml volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. 1 ml of sample extract was pipetted into 50 ml volumetric flask, and 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2 $C0_3$ were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 minutes when a bluish-green colouration developed. Standard Tannic Acid solutions of range 0-10 ppm were treated similarly as 1 ml of sample above[16]. The absorbances of the Tannic Acid Standard solutions as well as samples were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm.

Oxalate: The oxalate content of the differently processed melon husk was determined according to the method of Dye[17]. This involved extraction by boiling 2 g of each of the samples in 40 ml of water for 30 minutes in a reflux condenser. 10 ml of 20% Na_2C0_3 was added and boiled for another 30 min. The liquid extract was filtered and washed with hot water till wash water showed no alkaline reaction. The combined wash water and filtrate was concentrated to a small volume and cooled. HCI (1:1) was added drop wise with constant stirring until the final acid concentration after neutralization was about 1% at which stage a heavy precipitate appeared, which was allowed to flocculate. Extract was carefully filtered into 250 ml flask, made up to mark and kept overnight. Supernatant liquid was filtered through a dry filter paper in a dry beaker. An aliquot of the filtrate in a 400 ml beaker was diluted with water to 200 ml and reacidified with acetic acid. 10 ml of a 10% Calcium Chloride solution was added to the medium and stirred very well to induce calcium oxalate precipitate to appear and left to settle overnight. The clear supernatant liquid was carefully decanted off through Whatman No. 42 filter paper. The precipitate was dissolved in HCI (1:1). Oxalic acid was precipitated by adjusting the pH with ammonium hydroxide solution. The content was boiled and allowed to settle overnight. Oxalic acid was determined by titrating against 0.05 N $KMnO_4$ solution.

The cyanide contents of raw and treated samples of melon husk was determined using the method of Bradbury *et al.*[18], while the Trypsin Inhibitor, Amylase Inhibitor and Lectin were determined using the methods of Kakede *et al.*, Jeffe and Kortt respectively[19-21].

Statistical Analysis: Data generated from the experiment were analysed using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

Results and Discussion

Results of the proximate analysis of the raw and treated melon husk are presented in table 1. Variations in the proximate composition of the differently treated husks were significant ($p<0.05$) except for the lipid content. However, there was no significant difference ($p<0.05$) between the proximate compositions of raw and autoclaved melon husks except for ash and carbohydrate contents. The boiled melon husk had the least crude protein (14.77%), while there was no significant difference ($p<0.05$) between the protein contents of the raw (15.5%) and autoclaved (15.29%) melon husk. The same trend was observed for fibre contents. Boiling caused significant ($p<0.05$) reductions in all the mineral elements analyzed (table 2). The significant reduction in the fibre, protein and mineral contents of boiled melon husk could have been due to degradation and leaching of the nutrients into the cooking medium. Previously, it had been observed that nutrients may be lost during cooking in two ways. First, by degradation, which can occur by destruction or by other chemical changes such as oxidation, and secondly by leaching into the cooking medium[22]. Although heating results in the loss of some nutrients, it produces the desired texture, flavour and palatability needed in food and improves digestion.

Table-1
Proximate composition of raw and heat-treated melon husk

	Raw Melon Husk	Boiled Melon Husk	Autoclaved Melon Husk
Protein (%)	15.500 ± 0.126^a	14.77 ± 0.120^b	15.29 ± 0.173^a
Fibre (%)	6.07 ± 0.107^a	4.55 ± 0.035^b	5.98 ± 0.058^a
Lipid (%)	2.05± 0.023^a	1.99 ± 0.010^a	2.01 ± 0.118^a
Ash (%)	6.79 ± 0.105^a	5.93 ± 0.088^a	5.85 ± 0.104^b
Carbohydrate (%)	69.59 ± 0.215^a	70.89 ± 0.087^b	72.75 ± 0.250^c

Results are means of 3 determinations ± S. E.M.,
Values along the same row with the same superscript are NOT significantly different (P>0.05), and are significantly different if the superscripts are different

Table-2
Mineral components of raw and heat-treated melon husk

	Raw Melon Husk	Boiled Melon Husk	Autoclaved Melon Husk
Na (%)	0.41 ± 0.002^a	0.30 ± 0.002^b	0.39 ± 0.001^a
K (%)	0.71 ± 0.004^a	0.52 ± 0.002^b	0.69± 0.002^a
P (%)	0.22 ± 0.002^a	0.20b± 0.003^b	0.21 ± 0.002^a
Ca (%)	0.1 ± 0.003 ^{a}	0.07± 0.002^b	0.09 ± 0.001^a
Mg (%)	0.39 ± 0.004^a	0.21± 0.006^b	0.38± 0.025^a
Fe (mg/kg)	22.17 ± 0.296^a	16.97± 0.136^b	21.77± 0.285^a
Zn (mg/kg)	18.68 ± 0.214^a	14.87± 0.203^b	18.18± 0.163^a
Cu (mg/kg)	7.13 ± 0.203^a	5.07 ± 0.146^b	7.63 ± 0.203^a

Results are means of 3 determinations ± S. E. M, Values along the same row with the same superscript are NOT significantly different (P>0.05), and are significantly different if the superscripts are different

The results of the analysis of antinutritional components of raw and treated melon husk are presented in table 3. The levels of Tannin (0.034%), Oxalate (0.137%), and Phytate (0.290%) in raw melon husk were low as compared to 15.15%, 0.71% and 2.05% respectively reported by Ogbe and George[23]. Although, the levels of Cyanide (16.28mg/kg) and Trypsin inhibitor

(23.51TIU/kg) were high when compared to 0.06 mg/kg and 2.01TIU/kg respectively reported by Ogbe and George[23], they are in reasonable agreement with values reported for some other commonly consumed foods. The lectin content was found to be higher than the values reported by Udensi *et al*., for *Mucuna cochinchinensis* (42.67 Hu/g) and raw *Mucuna utilis* (15.27 HU/g)[24] but lower than 126.48 Hu/g reported for *Treculia africana* by Ugwu and Oranye[25]. Furthermore, significant ($p<0.05$) reductions in the levels of these antinutrients were observed in the heat treated samples, with 30-minute autoclaving being more effective than 90-minute boiling in most but not all cases. Similarly, autoclaving treatment has been reported to be more effective than boiling in reduction of various antinutritional compounds in *Bauhinia purpurea*[26] and *Entada scandens*[27].

Table-3
Antinutrient components of raw and heat-treated melon husk

	Raw melon Husk	Boiled melon Husk	Autoclaved melon Husk
Tannin (%)	0.034 ± 0.002^{a}	0.016 ± 0.002^{b}	0.013 ± 0.003^{b}
Oxalate (%)	0.137 ± 0.003^{a}	0.105 ± 0.0024^{b}	0.083 ± 0.003^{c}
Phytate (%)	0.290 ± 0.005^{a}	0.215 ± 0.002^{b}	0.232 ± 0.006^{b}
HCN (mg/kg)	16.28 ± 0.015^{a}	11.34 ± 0.012^{b}	11.20 ± 0.113^{b}
TIA (TIU/g)	23.51 ± 0.020^{a}	17.89 ± 0.017^{b}	15.45 ± 0.147^{c}
AIA (AIU/g)	19.32 ± 0.020^{a}	13.95 ± 0.049^{b}	12.01 ± 0.018^{c}
Lectin(HU/g)	55.66 ± 0.010^{a}	31.61 ± 0.210^{b}	23.90 ± 0.570^{c}

Results are means of 3 determinations ± S. E. M., Values along the same row with the same superscript are NOT significantly different ($P>0.05$), and are significantly different if the superscripts are different.

High levels of antinutrients have been implicated in a number of malnutrition conditions. Phytic acid has a strong binding affinity to minerals such as calcium, magnesium, iron and zinc. This results in precipitation, making the minerals unavailable for absorption in the intestines. Phytic acids are common in the hulls of nuts, seeds and grains[28]. Amylase Inhibitors prevent the action of enzymes that break the glycosidic bonds of starches and other complex carbohydrates, preventing the release of simple sugars and absorption by the body[29]. Like phytic acid, oxalate is also a culprit in mineral mal-absorption. In the body, oxalic acid combines with divalent metallic cations such as calcium (Ca^{2+}) and iron (II) (Fe^{2+}). These oxalates can form larger kidney stones that can obstruct the kidney tubules. An estimated 80% of kidney stones form crystals of the corresponding oxalates which are then excreted in urine as minute crystals[30]. Tannin and trypsin inhibitor adversely affect protein utilization. While condensed tannins inhibit herbivore digestion by binding to consumed plant proteins and making them more difficult for animals to digest, and by interfering with protein absorption and digestive enzymes, trypsin inhibitor obstructs the action of trypsin, a pancreatic enzyme that digests protein. It interferes the physiological process of digestion where the normal functioning of pancreatic proteolytic enzymes is prohibited in non-ruminants, leading to severe growth depression[31]. Like amylase inhibitor, lectin negatively interferes with carbohydrate utilization as it has great ability to bind specific sugars or glycoproteins.

Conclusion

The low levels of antinutrients factors, particularly, when heat-treated suggests that melon husk could be an important source of energy as the nutrients have the potential of being properly utilized in the biological system. However, the mere presence of nutrient(s) in a foodstuff does not actually tell how efficiently such nutrient(s) could be utilized by the body. It is therefore our recommendation that further researches be conducted on the husks using experimental animals to ascertain the possibility of incorporating it into livestock feeds.

References

1. Alkofahi A., Batshoun R., Owis W. and Najib N., Biological activity of some Jordanian plants extracts, *Fitoterapia,* **5,** 435–42 **(1996)**

2. Achu, M.B., Fokou, E., Tchiegang, C., Fotso, M., and Tchouanguep, F.M.: Nutritive value of some curbitaceae oilseeds from different regions in Cameroon, *Afr. J. Biotechnol*., (**11**), 1329–1334 (**2005**)

3. Egusi, Wikipedia, the free encyclopedia, Retrieved June 08 2012 **(2012)**

4. Oluba M.O., Ogunlowo Y.R., Ojieh G.C., Adebisi K.E., Eidangbe G.O. and Isiosio I.O., Physicochemical properties and fatty acid composition of citrullus lanatus (Egusi melon) seed oil, *J. Biol. Sci*., (**8**), 814–817 **(2008)**

5. Schafferman D., Beharav A., Shabelschy E. and Yaniv Z., Citrullus colocynthis, *J. Arid of Environs.,* **40**, 431-39 (**1998**)

6. Yusuf A.A., Adewuyi S. and Lasisi A.A., Physicochemical composition of leaves, meals and oils of fluted pumpkin (*Telfairia occidentalis*) and molon (*Citrullus vulgaris*), *Agric. J*., (**1**), 32-35 (**2006**)

7. Ziyada A.K. and Elhussien S.A., Physical and Chemical Characteristics of *Citrullus lanatus* Var. Colocynthoide Seed Oil, *J. of Physical Sci*., **19(2),** 69–75 (**2008**)

8. Oyenuga V.A., Nigerian Foods and Feeding Stuffs, Their Chemistry and Nutritive Values (Pp. 8 - 16; 86-89), Ibadan: Ibadan University Press (**1978**)

9. Oloyede O.B., Otunola G.A. and Apata D.F., Assessment of protein quality of processed melon seed as a component of poultry feed, *Biokemistri*, **16**(**2**) 80-87 (**2004**)

10. Moerman D., Native American Ethnobotany, Timber press, Oregon, 453- 459 (**1998**)

11. Isak R.S. and Alamgir A.S., Utilization of Wheat Husk Ash as Silica Source for the Synthesis of MCM-41 Type Mesoporous Silicates: A Sustainable Approach towards Valorization of the Agricultural Waste Stream, *Res. J. Chem. Sci.,* **3**(**11**), 66 (**2013**)

12. Martín-Cabrejas M.A., Aguilera Y., Pedrosa M.M., Cuadrado C., Hernandez T., Diaz S. and Esteban R.M., The impact of dehydration process on antinutrients and protein digestibility of some legume flours, *Food Chem.*, **114**, 1063-1068 (**2009**)

13. Grant G., Lectins In: "Toxic Substances in Crop Plants" ed. by F.J.P.D'Mello, C.M. Duffus and J.H.Z. Duffus. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge CB4 4WF, Cambridge, 49-67 **(1991)**

14. Association of Official Analytical Chemists (A.O.A.C.). Official Methods of Analysis, 14th Edition, Washington D.C. (**1995**)

15. Wheeler E.L. and Ferrel R.E., A method for Phytic acid Determination in Wheat Fractions, *Cereal Chem.,* **48,** 312-320 (**1971**)

16. Swain T., Tannins and Lignins In: Herbivores: Their Interactions with Plant Metabolites, Rosenthal, G.A. and D.H. Janzen (Eds.), Academic Press, NewYork (**1979**)

17. Dye W.B., Chemical studies on halogton glomeruius, *Weeds*, **4**, 55-66 (**1956**)

18. Bradbury M.G., S.V. Egen and J.H., Bradbury. Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits, *J. Sci., Food Agric.*,**79**, 593-601 (**1999**)

19. Kakade M.L., Hoff D.E. and Liener I.E., Contribution of trypsin inhibitor of the deleterious effects of unheated soyabeans fed to rats, *J. Nutr*., **103,** 1772-1779 (**1974**)

20. Jaffe, W. G. Moreno, R. and Wallis, V. Amylase inhibitor in legume seeds, *Nutr. Rep. Int*. **7**:169 (**1973**)

21. Kortt, A.A. Purification and properties of the basic lectins from winged bean seed [Phosphocarpus tetragonolobus (L) D.C.], *European J. Biochem*., **138**, 519 (**1984**).

22. FAO Food Composition tables for the Near East. Food and Agricultural Organization (FAO/UN), Rome, Food and Nutrition Paper, 26 (**1982**)

23. Ogbe A.O. and George G.A.L., Nutritional and Anti-nutrient Composition of Melon Husks: Potential as Feed Ingredient in Poultry Diet, *Research Journal of Chemical Sciences,* **2(2)**, 35-39 (**2012**)

24. Udensi E.A., Onwuka G.I. and Okoli E.C., Effect of processing on the levels of some Antinutritional factors in Mucuna utilis, *J. Plant Prod. Res*., (**1**), 1-7 (**2003**)

25. Ugwu F.M. and Oranye N.A., Effects of some processing methods on the toxic components of African breadfruit (Treculia africana), *African J. of Biotech.,* **5**(**22**), 2329-2333 (**2006**)

26. Vijayakumari K., Pugalenthi M. and Vadivel V., Effect of soaking and hydrothermal processing methods on the levels of antinutrients and in vitro protein digestibility of Bauhinia purpurea L. seeds, *Food Chemistry,* **103**, 968 – 975 (**2007**)

27. Vadivel V., Pugalenthi M. and Megha M., Biological evaluation of protein quality of raw and processed seeds of gila bean (Entada scendens Benth), *Tropical and Subtropical Agro ecosystem,* **8,** 125-133 (**2008**)

28. Phytic acid, Wikipedia, the free encyclopedia, Retrieved Jan 08 **(2012)**

29. Amylase inhibitor, Wikipedia, the free encyclopedia, Retrieved Jan 08 **(2012)**

30. Evan A. and Worcester E., Kidney stone disease, *J. Clin Invest*., **115**(**10**), 598–608 (**2005**)

31. Gogoi R., Niyogi, U.K. and Tyagi, A.K. Reduction in Trypsin Inhibitor Activity in Jatropha cake by Chemical, Thermal and Radiation Treatment, *Res. J. Chem. Sci*., **4**(**1**), 17-19, January **(2014)**