

## Proximate analysis and antinutritional factors of water-melon seeds (*Citrullus lanatus*).

Formatted: Font: Italic

### ABSTRACT

Watermelon (*Citrullus lanatus*) is a flowering plant species of the Cucurbitaceae family. It is rich in an amino acid called citrulline and may help move blood through the body and also widely used as flavouring agent and also contributes substantially towards obtaining a balanced diet. This research was aimed to evaluate the proximate and anti nutritional factors of the plant. The proximate composition was done by Soxhlet extraction, AOAC, Kjeldhal and other standard procedures while anti nutrients were analysed using standard procedures. The results of the research revealed the proximate composition of the water-melon seeds such as moisture content, crude protein, lipids, ash content, crude fibre and carbohydrate. Low moisture content ( $5.20 \pm 0.003$ ), high proteins ( $17.844 \pm 0.012$ ) and lipids ( $18.208 \pm 0.004$ ) with others detected in moderate amount was obtained. Anti nutritional factors such as oxalate, phytate, cyanide, nitrate, and tannin were also analyzed which revealed low oxalate content ( $0.005 \pm 0.003$ ) with moderate amount of phytate ( $4.366 \pm 0.141$ ), nitrate ( $47.309 \pm 0.539$ ) and cyanide ( $41.510 \pm 0.138$ ). Therefore, water-melon seeds might be vital in maintaining an optimum nutritional status to avoid malnutrition, morbidity or mortality.

Formatted: Font: Italic

Comment [Im1]: Unit?

Comment [Im2]: Unit?

KEY; Water-melon, proximate, anti-nutritional.

### INTRODUCTION

Comment [Im3]: Introduction should highlight the importance of watermelon, why this fruit used as study matter, the composition of watermelon in term of nutrition etc .... Significance of the study

**Watermelon** (*Citrullus lanatus*) is a flowering plant species of the Cucurbitaceae family. A scrambling and trailing vine-like plant, it was originally domesticated in Africa (Bahari et al., 2012). It is a highly cultivated fruit worldwide, with more than 1,000 varieties (Boualem et al., 2016). Wild watermelon seeds have been found in the prehistoric Libyan site of Uan Muhuggiag. There is also evidence from seeds in Pharaoh Tombs of watermelon cultivation in Ancient Egypt. Watermelon is grown in favorable climates from tropical to temperate regions worldwide for its large edible fruit, which is a berry with a hard rind and no internal divisions, and is botanically called a *pepo*. The sweet, juicy flesh is usually deep red to pink, with many black seeds, although seedless varieties exist. The fruit can be eaten raw or pickled, and the rind is edible after cooking. It may also be consumed as a juice or as an ingredient in mixed beverages.

Other crops of importance in this family include cucumber (*Cucumis sativus* L.), melons (*C. melo* L.) and squash (*Cucurbita moschata* Duch.). The crop is a warm, long-season, trailing, prostrate, annual which has monoecious and/or andro-monoecious sexuality (Boualem et al., 2016). Watermelon fruit has a thick rind (exocarp) that has variable pigmentation with a solid or striped appearance, a fleshy mesocarp, and an endocarp which varies in color from white to yellow or red (Bahari et al., 2012; Munisse et al., 2013). Three types of watermelon are grown in drier regions of Africa: the dessert type (*C. lanatus* var. *lanatus* L.), the cooking type [*C. lanatus* (Thunb.) Matsum. & Nakai var. *citroides*] and the seed type (*C. colocynthis* L. Schrad.). Dessert watermelon is grown worldwide, has a characteristic sweet taste, a low-calorie fruit used mostly in salads and juices (Bahari et al., 2012). The cooking type, also called cow watermelon, is normally used as animal feed, for cooking thick porridge, or mixed in dry maize (*Zea mays* L.) grain (Mujaju et al., 2011). The seed type watermelon is mostly grown in Central to West Africa and is used to extract oil, make egusi soup, snacks, and flour (Jensen, 2012).

Watermelon flesh is a source of carotenoids, Vitamins A, B6, C, lycopene, and antioxidants. Watermelon rinds are edible containing many nutrients (Jensen et al., 2011). Pickled watermelon rind is consumed in the Southern US. In China, rinds are stir-fried, stewed, or pickled. Watermelon juice can be made into wine, or other traditional brews. In Sudan and

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Comment [Im4]: Scientific names

Formatted: Font: Italic

Egypt, watermelon seeds are roasted, salted, and eaten as snacks. Watermelon fruit is used as a source of drinking water during drought seasons in parts of Sudan and Nigeria (Ayodele and Shittu, 2013; Goda, 2007).

## MATERIALS METHODS

All chemicals and reagents used were of analytical grade.

### SAMPLE PROCESSING

Water-melon seeds used in this research was purchased from the Sokoto main market. It was identified in the Department of Biological Sciences, Sokoto State University, Sokoto.

The samples were allowed to dry at room temperature of 22°C for 5 days then the samples were ~~granded~~grinded using ~~morte~~mortar and ~~pistol~~, then the samples were ready for analysis.

Comment [Im5]: ? pestle

### PROXIMATE ANALYSIS

Comment [Im6]: Methods should write in paragraph, past tense, use proper English

#### -DETERMINATION OF MOISTURE CONTENT (AOAC, 1990)

##### Principle

The principle is based on heating the sample to eliminate all the water content in the sample. This is achieved by placing the sample in an oven at 105°C for 24 hours. High temperature is needed to avoid decomposition of some organic compound.

##### Procedure

An empty dish was cleaned and dried in an oven at 80°C for about 30 minutes and was weighed ( $W_1$ ). 5g of the sample was placed in the dish and weighed ( $W_2$ ), it was then placed in hot air oven and dried at 105°C for 24 hours. It was cooled in a desiccator for 20 minutes and weighed ( $W_3$ ). The procedure was repeated, drying for about 3 hours for each subsequent drying until a constant value was obtained out of which was calculated.

Comment [Im7]: Brand?

##### Calculation

$$\% \text{ Moisture} = \frac{\text{loss in weight due to drying}}{\text{Weight of fresh sample}} \times 100$$

[W1, W2, W3?](#)

#### DETERMINATION OF ASH CONTENT (BAKARE, 1985)

##### Principle

When a food material is ashed in a muffle furnace at high temperature of 600°C for 5 hours, all the organic matter is burnt off leaving the inorganic substance in the form of ash.

##### Procedure

A clean crucible was ignited in a hot furnace for one minute, the crucible was then removed and cooled in a desiccator and weighed ( $W_0$ ). 5g of the sample was placed in the empty crucible and weighed ( $W_1$ ). The crucible containing the sample was then heated in muffle furnace at 600°C for 5 hours to burn off all the organic matter after which the crucible was cooled in a desiccator and weighed ( $W_2$ ).

Comment [Im8]: Brand?

##### Calculation

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of fresh sample}} \times 100$$

[W0, W1, W2?](#)

#### DETERMINATION OF CRUDE PROTEIN (MICRO KJELDAHL'S METHOD)

##### Principle

The kjeldahl's digestion involves oxidation of organic matter with conc.  $H_2SO_4$  and a tablet of kjeldahl's catalyst. The sulphuric acid converts all form of nitrogen to ammonium sulphate. Subsequent addition of excess amount of NaOH neutralizes the acid and releases ammonia which is distilled into boric acid solution and titrated against 0.01N HCl end point so that the amount of HCl consumed can be calculated.

Comment [Im9]: ?

##### Procedure

The analysis involves 3 stages or steps

**Comment [Im10]:** Write in paragraph.  
Past tense

- 1. Digestion:** 2g of sample will be collected and put in a clean dry 100ml kjeldahl flask. One tablet of the mixed catalyst and 20ml concentrated  $H_2SO_4$  will be added. Little amount of distilled water will also be added to the mixture to digest the organic matter present. The flask will then be heated in a fume cupboard until a clear solution is obtained, heating will be continued until the ammonium sulphate and the organic matter are converted to carbon (iv) oxide in the presence of oxygen. The content will be cooled and transferred into a volumetric flask.
- 2. Distillation:** 10mls of aliquot will be put into kjeldahl flask, 20ml of 40 % NaOH and 50ml distilled water will be added to make up the solution to extract out the amount of ammonia present in the sample which will be evaporated into boric acid indicator. 20ml of boric acid indicator will be used as the receiver of the nitrogen extracted. The ammonia will be liberated into the boric acid until the volume is made up to 40ml in the conical flask. Color change from green to pink will be observed.

$$\% \text{ Nitrogen} = \frac{TV \times NA \times 0.014 \times 50}{\text{Weight of sample (g)} \times \text{mls of aliquot}} \times 100$$

- 3. Titration:** The collected sample with ammonia will then be titrated against 0.01N HCl to end point, which will give the actual amount of protein in the sample. The colour change from pink to green, the end point and the titre value will be recorded.

#### Calculation

$$\% \text{ Crude protein} = \%N \times 6.25$$

Where

TV= Titre value

NA= Normality of acid

50 = Dilution factor i.e. volume of acid

6.25 = Conversion factor

## DETERMINATION OF LIPID CONTENT

**Method:** semi-continuous method (soxhlet)

### Principle

It is based on continuous extraction of lipid content from the sample using petroleum ether in a soxhlet extractor. Non-polar component of the sample was easily extracted into organic solvent.

### Procedure

Soxhlet extractor with reflux condenser and a small round bottom flask were fixed and 2g ( $W_0$ ) of the sample was placed in the thimble which has been dried and weighed ( $W_1$ ), the mouth of the porous thimble was covered with clean white cotton in order to distribute the draping petroleum ether. The thimble was placed in the extractor and petroleum ether was added until it was half in the flask. The flask was then heated for five hours. The thimble was removed and the petroleum ether in the top container was collected. The extract was removed from the soxhlet extractor when it was almost free of petroleum ether. Finally, the extraction flask containing the oil was weighed ( $W_2$ ) to know the content of the crude lipid.

### Calculation

$$\% \text{ Crude lipid} = \frac{\text{weight of lipid extracted}}{\text{Weight of sample}} \times 100$$

$$\% \text{ Crude lipid} = \frac{W_2 - W_1}{W_0} \times 100$$

Where  $W_1$  = weight of flask + sample

$W_2$  = weight of extracted lipid

$W_0$  = weight of sample

## **-DETERMINATION OF CRUDE FIBER**

**Method:** AOAC (1990) Method was used

### **Principle**

Boiling the sample with sulphuric acid (acid hydrolysis) removes free sugars and starch. Alkaline hydrolysis or treatment with NaOH removes proteins and some carbohydrates, after which the sample will be transferred into a crucible for ashing. Percentage fibre can then be calculated by weight difference.

### **Procedure**

The residue (2g) obtained from crude lipid extraction was placed in a conical flask; 200ml of distilled water and 20ml of  $H_2SO_4$  was added and fixed on a heater and boiled for 30 minutes to maintain a constant volume. The sample was filtered in a muslin cloth, rinsed with warm water and spatula was used to scrape the sample into the flask, 20ml of  $H_2SO_4$  and 10% of NaOH was added to the contents. The content was placed 30minutes then filtered with muslin cloth and the sample was rinsed with petroleum ether. It was then allowed to drain and the residue was scraped into a crucible and placed in an oven and was dried for one hour at  $105^{\circ}C$  and allowed to cooled in a desiccator and weighed ( $W_1$ ). It was then placed in a muffle furnace to ash for two hours at  $600^{\circ}C$  and allowed to cool in a desiccator and weighed ( $W_2$ ). Percentage fibre was then calculated.

### **Calculation**

$$\% \text{ Crude fibre} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100$$

Where  $W_1$  = weight after drying

$W_2$  = weight after ashing

## **-DETERMINATION OF CARBOHYDRATE CONTENT (DIFFERENCE METHOD OYELEKE, 1984)**

### **Principle**

The total proportion of carbohydrate in a sample can be obtained by difference method which is by deducing other nutritional component from 100%.

### **Procedure**

The sum of % ash, % moisture, % crude protein, % crude lipid and % crude fibre subtracted from 100%-. The remainder account for the total percentage of carbohydrate in the sample

### **Calculation**

*Percentage of carbohydrate*

$$= 100\% - (\% \text{ moisture} + \% \text{ ash} + \% \text{ lipid} + \% \text{ protein} + \% \text{ fibre})$$

## **DETERMINATION OF ANTINUTRITIONAL FACTORS**

### **-DETERMINATION OF NITRATE**

Nitrate was determined using method of ILTA (1988)

**Procedure:** 0.1g of powder sample was added into 100ml conical flask, 10ml of distilled water was added and boil for 30 minutes, filter using filter paper

Chart 1:

REAGENT	TEST	STANDARD	BLANK
Sample	0.2ml		
Standard sodium nitrate		0.2ml	
DH <sub>2</sub> O			0.2ml
5% salicylic acid	0.8ml	0.8ml	0.8ml
Mix and incubate for 20min.			
2n NaOH	19ml	19ml	19ml
Mix and allow for cooling and measuring			
Absorbance at 410nm			

Comment [Im11]: Concentration?

Comment [Im12]: w/v?

Comment [Im13]: ?

[Write in paragraph, use past tense](#)

Calculation: (% nitrate mg %)

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

## **DETERMINATION OF TANNINS (TREASE AND EVANS, 1978).**

### **Principle**

The method is based on quantitative consumption of tannins and pseudo tannins to iodine in alkaline medium, a character which is attributed to their phenolic nature. True tannins, in contrast to pseudo tannins can be removed from the extract by precipitation with gelatin, this can permit the determination of each group of constituents alone. Excess iodine is determined by titration rendering acidic with sodium thiosulphate standard solution.

### **Procedure**

Sample (100g) of was placed in a 100ml conical flask and 50ml of distilled water was added and boiled for 30 minutes in a boiling water bath, it was then filtered using filter paper, the filtrate was used for the test. 3 test tubes were arranged and labeled as test, standard and blank, 5ml of the sample (filtrate) was added to the test tube labelled test, 5ml of standard reagent was added to the standard test tube and 5ml of distilled water was added to the blank test tube. 5ml of 17% sodium carbonate was added to each sample and then 2.5ml of folindenins reagent was also added to each test tube, the contents were mixed and distilled water was used to make the volume up to 50ml, the contents were incubated for 20 minutes at room temperature and the absorbance was taken at 760nm.

Comment [Im14]: ?

Comment [Im15]: w/v?

#### Calculation

$$\% \text{ Tannic acid} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

#### DETERMINATION OF PHYTATE (LUCAS AND MARAKAKA, 1975).

#### Principle

4g of each sample was weighed. Hundred ml of 2% concentrated hydrochloric acid was used to soak each sample into conical flask for 3h and filtered through a filter paper. 25ml of each filtrate was placed in 250ml beaker and 107ml of distilled water was added in each case to give proper acidity. 5ml of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron (III) chloride solution which contained 0.00195g iron per ml. The end point was slightly brownish yellow which persisted for 5min.

#### Calculation

$$\% \text{ Pytate} = \text{phytin phosphorus} \times 3.55$$

*Titre value* × 1.19 = *phytin phosphorus*

#### DETERMINATION OF CYANIDE (RAILS, 1992):

**3.0** Sample (0.5g) was measured and placed in a 100ml conical flask and 50ml of distilled water was added, the contents were boiled for 30 minutes in a boiling water bath, then it was filtered using filter paper, the filtrate is used for the test. 3 test tubes were arranged and labelled test, standard and blank, 1ml of sample (filtrate) was added to the test tube labelled test, 1ml of standard KCN was added to the standard test tube and 1ml of distilled water was added to the blank test tube. 4ml of alkaline picrate was added to each test tube; the contents were mixed.

**Formatted:** Indent: Left: 0.63 cm, No bullets or numbering

**Comment [Im16]:** concentration?

**Comment [Im17]:** Concentration?

#### How to determine the cyanide content?

#### RESULTS

Table 1. Result of the Proximate Analysis of Water Melon Seeds

Parameter	values
Moisture content	5.20±0.003
Protein	17.844±0.012
Fibre content	4.805±0.003
Ash content	5.165±0.003
Lipid content	18.208± 0.004
Carbohydrate	11.604±3.708

**Comment [Im18]:** Unit for each parameter?

#### Statistical analysis?

Table 2. Result of the Anti-Nutritional Parameters of Water Melon Seeds

Parameters	values
Nitrate	47.309±0.539
Tannins	92.614±0.637
Cyanides	41.510±0.138
Oxalate	0.005±0.003
Phytates	4.366±0.141

**Comment [Im19]:** Unit for each parameter?

**Comment [Im20]:** In Methods, not mention on how to determine the oxalate content?

### Statistical analysis?

### Discussion

The proximate composition of water-melon seeds constituent is presented in [Table 3.1](#) shows, the moisture content of the seeds ( $5.20 \pm 0.003$ ) which is within the [acceptable range for a good keeping period](#). The results show low moisture content ([Table 3.1](#)) and this is consistent with 6.39% reported for water-melon seeds ([Olorode \*et al.\*, 2014](#)). The relatively low moisture content is an indication that this seeds will have high shelf life especially when properly packaged against external conditions. The crude protein content ( $17.844 \pm 0.012$ ) obtained for the seed was high. And this is in line with the study of ([Pugalenthin \*et al.\*, 2014](#)) which shows that the [proteins](#) are essential component of the diet need for the survival of animals and humans, which function basically in nutrition by supplying adequate amount of required amino acids. The crude fibre content was ( $4.805 \pm 0.003$ ). It is believed that fibre reduces the level of cholesterol in human blood and decreases the likelihood of different cancers. The ash content ( $5.165 \pm 0.003$ ) which is close to that reported for melon seeds varieties. ([Eelinge \*et al.\*, 2012](#)). [The high fat content](#)

**Comment [Im21]:** Rewrite the Discussion, compare the results with previous study, explain the similarity or differences, the mechanism involved nutrient synthesis in seeds etc...

**Comment [Im22]:** References?

(lipid)  $18.208 \pm 0.004$  obtained in the water-melon seeds are comparable to commonly known oil seeds likely groundnut may reach up to 50% (Pancholly *et al.*, 1978). The carbohydrate obtained from water-melon seeds ( $11.604 \pm 3.708$ ). The carbohydrate was in the range of 10.68% to 53.58% in agreement with the findings of (Penuel *et al.*, 2013). Many health's issues are attributed to carbohydrate component of legume. Seeds constituent with low carbohydrate content might be ideal for diabetic and hypertensive patient requiring low sugar diet (Olorode *et al.*, 2014).

The anti-nutritional composition of *Citrullus lanatus* seed is presented in Table 3.2. The concentrations of oxalate, phytate, nitrate, tannins and cyanide in the water-melon seeds are  $0.005 \pm 0.003$ ,  $4.366 \pm 0.141$ ,  $47.309 \pm 0.539$ ,  $92.614 \pm 0.637$  and  $41.510 \pm 0.138$  respectively. The high value of tannins in human diet can cause side effect such as irritation, nausea, vomiting and liver damaged. Regular consumption of herbs or seeds with high tannins concentration seems to be associated with an increased chance of developing nose or throat cancer (Cehung *et al.*, 1998).

### Conclusion

This study has showed that *Citrullus lanatus* seeds are a good source of fat (lipid) and protein. It also contains moderate amount of some of the anti nutritional factors indicating that the *Citrullus lanatus* has anti nutritional benefit in humans and animals. The high amount of tannins indicates that the *Citrullus lanatus* has high content of tannins which might be harmful to human beings. Therefore the plant seed is beneficial as it rich in amino acids, proteins and vitamins. Thus, these nutrients together helps boost the body metabolism.

### REFERENCES

Ayodele, O.J (*et al.*,2013). Cost-benefit analysis of melon (egusi) seed and seed- oil yield responses to phosphorus fertilizer application. Int. Res. J. Agric. Sci. Soil Sci. 3:152–155.

Formatted: Font: Italic

Comment [Im23]: No Discussion on the parameters tested

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Comment [Im24]: In complete

Bahari, M. Remison, H.E. Ahamefule, K.O. Azeez, and P.O. Fesobi 2012. Combining ability analysis incomplete diallel cross of Watermelon. *Sci. World J.* 2012:1–6. doi: 10.1100/2012/543158.

Bakare, R., S. Vinyard, E.R. Wiley, E.D. Brown, J.K. Collins, P. and Perkin-Veazie . 1985. A comprehensive review on watermelon seed oil – An underutilized product. *J. Pharm.* 71:2250–3013.

Boualem, A., A. Lemhemdi, M.A. Sari. 2016. The andromonoecious sex determination gene predates the separation of *Cucumis* and *Citrullus* genera. *PLoS One* 11(5):1–13. doi: 10.1371/journal.pone.0155444.

Elinge, E.K., S.U. Remison, H.E. Ahamefule, K.O. Azeez, and P.O. Fesobi. 2012. Performance of watermelon (*Citrullus lanatus* L.) in response to organic and NPK fertilizers. *Agric. Environ.* 9:5–17. doi: 10.1515/ausae-2017-0001.

Jensen, B.D. 2012. African watermelons and their uses. Proceedings Xth EUCARPIA meeting on Genetics and Breeding of Cucurbitaceae, Antalya, Turkey, 15–19 Oct. doi: 10.1094/PDIS-11-11-0999-PDN.

Jensen, B.D., F.M. Touré, and M. Ag. 2011. Watermelons in the sand of Sahara: Cultivation and use of indigenous landraces in the Tombouctou Region of Mali. *Ethnobotany Res. Appl.* 9:151–162. doi: 10.17348/era.9.0.151-162.

UNDER PEER REVIEW

UNDER PEER REVIEW

