Chapter- 1: introduction

Food processing produces a huge amount of wastage or by products like as bark, seed, pulp or other stingy materials. It is very expensive and complex for industry to dispose of these materials. By-products are vital source of minerals, fiber, organic compound, sugar, phytochemical compounds etc. (Djilas et al., 2009). Nutrients are added to food products during food preparation by utilization of this spare part of foods are normally discarded by industries. In this way, new countenance on the use of these spare parts as by-products, for reusing in the preparation of various types of food supplements or additives with high nutritional value have incited great attention as they are high value product and their restoration may be economically profitable for food industries (Claudia et al., 2014). Disposal of the by-products which are produced during food processing is one of the major problems in food industries (Hussein et al., 2011). The waste material causes environmental problems due to the growth of insects and rodents, so appropriate techniques are needed for transforming these unused materials into usable form for financial benefits. The edible portions of fruits are processed into food products like as juice, puree, ketchup, sauce, jam, jellies and canned slices, but the seeds are rejected as waste materials because these are not used for commercial motive, though the seeds are important source of nutritional components (Ajila et al., 2007).

Bangladesh has favorable environment to cultivate different types of crops such as paddy, wheat, pea, soybean, sunflower, mustard etc. and also several kinds of fruits such as olive, jackfruit, mango, watermelon etc. (Hoque et al., 2015). Watermelon (*Citrullus lanatus*) is a very common tasty and juicy fruit in Bangladesh which belong to the family of cucurbitaceae. It is an important crop during hot season to meet the demand of water as it contains 90 percent of water. It contains vitamins (A, B, C) and minerals. Watermelon is produced in different areas of Bangladesh. There are cultivated different indigenous varieties of watermelon such as sugar baby, Charleston gray, Crimson red etc. in our country (Rabbany et al., 2013). The seed of watermelon is considered as highly nutritious. It is an excellent source of fat, protein, minerals such as Mg, K, P, Fe, Zn, Na, Ca, Cu, vitamins (A, B) and others phytochemical compounds (Braide et al., 2012). The watermelon seed has economic advantage. The seeds are grinded into flour and used in preparation of sauces, snacks. The oil obtains from

Watermelon seed is used in cooking food and manufacture of cosmetics product (Jensen et al., 2011). Watermelon seed is rich in protein; it constitutes of nine essential amino acid along with glutamic acid, lysine and tryptophan. The seed of watermelon contains low amount of carbohydrate but large amount of calories. For athlete, roasted watermelon seed can be a best choice as a supplement. Watermelon seed contains different types of B vitamins such as thiamin, riboflavin, niacin, pantothenic acid, pyrodoxin which are essential in trace amount for altering the foods into energy and maintenance of other functions of our bodies. The watermelon seed contains the micronutrients such as magnesium, phosphorous, iron, sodium, calcium, zinc and manganese. Magnesium assists in regulation of blood pressure. Iron is an essential element needs for our body to bear proper oxygen throughout our whole bodies and promote the growth of cell in the body. The seed is also a good source of different types of fats like as monounsaturated fat, polyunsaturated fat and omega-6 fatty acid which assist in retrenchment of high blood pressure. Polyunsaturated fat help in reducing blood cholesterol level and cut down the risk of heart disease and type 2 diabetes. The seeds are very useful to cure from illness, restoration of health and improve our memory (Gopalan et al., 2007). Protein energy malnutrition is one of the leading nutritional health problems among the children and women with lower socio- economic status with having the deficiency of calcium, iron and magnesium. Particularly women are more suffering from calcium deficiency after menstrual period. So for removing malnutrition and mineral deficiency, it is best option that increases the use of unconventional seeds and cereals like grains as a good source of protein and minerals (Virginia et al., 2014). Functional properties such as water absorption capacity, oil absorption capacity, emulsion activity, emulsion stability, foam stability, foam capacity, bulk density are the idiosyncratic physiochemical properties which affect the protein content of food products during manufacturing and storage of food (Onimawo and Akubor, 2005). Fruit and fruit seed are excellent natural source of nutritional elements. These natural elements are plant originated and play a vital role in maintaining human health, especially in prevention of disease, growth and development of our bodies. In the modern era, the demand of phyto-nutrients which are found in the seed of fruits and vegetables are increasing due to public awareness regarding their health (Naz et al., 2013).

Considering the health and economic benefits of unutilized watermelon seed, present research work was planned to evaluate the chemical composition and functional properties of two varieties of locally grown watermelon seed in Bangladesh.

1.1 Aims and Objectives of the Study

- To estimate the nutritional composition of two varieties of watermelon seed (Crimson red and Charleston gray).
- To determine the phyto-chemical compounds present in watermelon seed.
- To observe the functional properties of the watermelon seed.

Chapter-2: Review of Literature

A conceptual framework for the study based on the ideas and concepts gathered from review work of existing literature will facilitate planning the study in a comprehensive manner.

2.1 Watermelon

Watermelon is one of the familiar crops of Cucurbitaceae family and its scientific name is *Citrullus lanatus*. It has an exterior rind which is light or dark green in color with or without stripes. The red color pulp in the interior part of fruit contain the seed which is adorned middle third of the pulp (Maynard, 2001). Watermelon is a fruit which different part such as flesh, seed, rind are rich in different types of vitamin, minerals, protein and fat (Wani et al, 2011). The indigenous name of watermelon is Tormuj in Bengali, Tarbooz in Hindi and Urdu. It is also called Tarbuj in Manipuri, Indrak in Gujarati (Verma and Tomar, 2017). Watermelon is cultivated in Bangladesh as an ancillary crop but it possess a significant rank among the fruit crops which are cultivated in Bangladesh due to its large amount of yield and farmers select the fruit for commercial cultivation as profitable crop for economic benefit (Rabbany et al., 2013). The flesh of the fruit is good source of carotene and lycopene compound which are necessary for resisting free radicals in human bodies (Oseni and Okoye, 2013).

2.2 Watermelon Seed

The watermelon seed is a good source of fat, protein, fatty acid, vitamins and minerals such as manganese, potassium, zinc, magnesium, copper etc. Due to the important health beneficial components of watermelon seed, day by day the potentiality of financial benefit of watermelon seed is increased (Mariod et al., 2009). The seed of watermelon is used in the production of different types of protein based products due to rich source of proteins (El- Adway and Taha, 2011). The seed is incorporated in baby food for the development of child nutrition because seed contains high amount of protein, fat and phytochemical compound (Maynard et al., 2001). The seed is very useful in baking due to its high protein content and good functional properties. The seed of watermelon is utilized as food additive such as soup thickener and flavoring agent (Fokou et al., 2004). Though seed is small in size however it contains essential nutritional elements such as protein, fiber, vitamins, minerals, omega-3 fatty acid and phytochemical compound which enhancing our life by helping our body in prevention of disease and flourish a healthy sound living (Omorayi and Dilworth, 2007).

2.3 Proximate Composition of Watermelon Seed

Mehra et al. (2015) conducted an analysis on chemical composition of watermelon seed where found the percentage of moisture, ash, protein, fat, fiber and energy were 3.575 %, 3.636%, 34.22%, 31.999%, 0.1% and 531.151 Kcal/100g respectively. The study revealed that watermelon seed were rich in protein, fat and energy. In another study reported by Mogotlane et al. (2018) two types of indigenous watermelon seed were analysed in Limpopo of South-Africa for comparing the proximate composition between the two types of watermelon seed. Results showed that the fat content of the seed of Capricorn district was 34.4% which is higher than the fat content of the seed of Sekhukhune district was about 31.6% and other proximate components found were ash (3.99%), protein (14.9 to 16.5%), fat (31.6 to 34.6%), and carbohydrate (3.16 to 5.26%) respectively**.** Tabiri et al. (2016) evaluated the nutritional composition of three types of watermelon seed such as Charleston gray, Crimson sweet and Black diamond. In the analysis, they found that the proximate content of three types of watermelon seed were a range of moisture (7.4 to 8.0%), ash (2 to 3%), protein (16.33 to 17.75%), fat (26.50 to 27.83%) and energy (354.05 to 369.11 Kcal/ 100g) respectively. In another study reported that the moisture, ash, protein, fat of watermelon seed flour were 9.59%, 3.63%, 25.33%, 45.66%, 11.86 to 15.31% respectively (Akusu and Kinn-Kabiri, 2015). An analytical study reported by Egbuonu (2015) estimated the nutritional component of watermelon seed and rind where revealed that the watermelon seed contained 2.48% of ash, 3.81% of moisture, 28.05% of carbohydrate, 2.37% of fiber, 21.46% of protein and 531.15Kcal/ 100g of energy respectively. Jacob et al. (2015) investigated the proximate composition of melon where found the moisture, ash, fiber content were 7.10%, 2.7%. 6.4% respectively. Hannah and Krishnakumari (2015) documented that the watermelon seed extract contained about 1.60% of ash, 7.03% of moisture, 26.4% of fat and 465.68 Kcal /100g of energy respectively.

2.4 Minerals Content of Watermelon Seed

Watermelon seed was evaluated for minerals content by Mehra et al. (2015**).** In the analysis, ICP- OES was used for determination of mineral content in watermelon seeds. In the study, it was revealed that the watermelon seed contained high amount of K, Mg, Ca, P and less amount of Fe, Na, and Zn. The amount of K, Mg, Ca, P, Fe, Na and Zn were 7599ppm, 4496 ppm, 2477ppm, 9249 ppm, 164 ppm, 74 ppm and 75 ppm respectively. Hannah and Krishnakumari (2015) estimated the minerals content of seed extract and ash of watermelon seed. In the study, they reported that the seed extract sample was high in Fe and low in Ca and P than ash sample of watermelon seed. The percentage of Fe, P and Ca in watermelon seed extract were 2.29mg/g, 0.83mg/g and 29.63mg/g respectively and in ash sample 2.25mg/g for Fe, 1.07mg/g for P and 45.14mg/g for Ca. From the analysis it was found that both of the seed extract and ash sample of watermelon seed were rich in calcium. An analytical study was conducted to compare the nutritional component of two types of watermelon seed collected from two district of Limpopo in South Africa. The two district were Capricorn landrace and Sekhukhune district. The study showed that, the minerals content such as zinc, copper, Iron and calcium were higher in the seed of landrace than the seed of Sekhukhune (Mogotlane et al., 2018).

Tabiri et al. (2016) conducted an investigation to estimate the chemical properties of three types of watermelon seed (Charleston gray, Crimson sweet and Black diamond). They revealed that calcium, magnesium, potassium, phosphorus and copper content were almost same in three types of watermelon seed. The iron and Zinc content were different in three types of seed. The Iron content of the three types of seed were 3.71mg/ 100g, 2.72mg and 4.60mg per 100g and the zinc content of the three types of seed were 3.71mg, 0.81mg and 0.66mg per 100g respectively. In the study it was reported that the iron content was higher in Charleston gray than others two types of seed and the zinc content was higher in Black diamond than other two types of watermelon seed. Jecob et al. (2015) analysed the minerals content of watermelon seed. They showed that the Iron content was higher about 144.70 mg/100g than others minerals in the watermelon seed. The seed also contained a good amount of manganese, zinc and magnesium about 22.73mg, 21.05mg and 20.46mg per 100g respectively.

2.5 Phytochemical Compound of Watermelon Seed

Mehra et al. (2015) analysed the phytochemical compound of watermelon seed. They reported that the seed of watermelon contained different phytochemical compounds like as alkaloid, flavonoids, crude phenol and saponin. The study revealed that the phytochemical content in watermelon seed such as flavonoid, saponin and crude alkaloid were 4.22%, 0.041%, and 15.688% respectively. It showed that crude alkaloid was higher than other phytochemical compounds in watermelon seed. Mogotlane et al. (2018) conducted an analytical study at Limpopo in South Africa on phytochemical components of two varieties of watermelon seed where found that the range value of flavonoid content in two varieties of watermelon seed was 0.015 to 0.347%.

2.6 Functional Properties of Watermelon seed

Egbuonu (2015) evaluated the chemical compositions and functional properties of watermelon seed and rind. In this study, the functional properties such as Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), Foam Capacity (FC), Foam Stability (FS) and Emulsion Stability (ES) were estimated. The result found for the functional properties of watermelon seed in this study were 116.30% for WAC, 123.50% for OAC, 21.5% for FC, 60.5% for FS, .28% for ES respectively. It reported that the values of functional properties of watermelon seeds were higher than the values of rind functional properties such as 7.13% for WAC, 1.65% for OAC, 5.65% for FC, 20.75% for FS respectively. A research study was conducted by Marie et al. (2015) to estimate the functional properties of four varieties of watermelon seed flour. They reported that the range of the functional properties of the four types of seeds flour were 130.23 to 254.42% for water absorption capacity, 107.50 to 140.11% for oil absorption capacity, 3.96 to 5.77% for foam capacity, 39.56 to 52.94% for foam stability, 23.48 to 29.99% for emulsion activity and 11.53 to 14.67% for emulsion stability respectively.

2.7 Health Benefits of Watermelon Seed

Watermelon seed is highly nutritious and is considered as good source of protein. The seed is used as protein rich foods like others different types of protein rich seed such as soybean, mustard, nut (Mustafa and Alamin, 2012). The seeds is contained about 25 – 40% of protein (Younis et al., 2000). The protein of watermelon seed constitute of different types of amino acids like as leucine, glutamic acid, arginine (Mello et al., 2001). As the seed contain high amount of protein, these can be used as ingredient for preparation of protein rich products and also used as nutrient supplement and additives like thickener (El-Adawy and Taha, 2011).

The pulp of watermelon contains the seed which is rich in fat or oil**.** Due to high fat content, the seed of watermelon is used in oil industry (Nyam et al., 2009). The seed of watermelon has a great demand in the Africa due to high nutritional value of watermelon seeds oil. The seeds are removed from the flesh of rind and sun drying. After drying, oil is extracted from seed by squeezing (Sui et al., 2011).

The oil of watermelon seed contains high amount of fatty acid which promote the function of different organs of the human body like as liver, eye, kidney, brain. Some fatty acid such as oleic acid and linoleic acids which decrease the low density lipoprotein level is known as bad fat and enhance the high density lipoprotein in the blood which decreases the risk of different types of heart-disease and stroke and high density lipoprotein is called good cholesterol which is helpful to our body (Njuguna et al., 2014).

2.8 Conclusion

The chemical component such as proximate composition, minerals content, phytochemical compound and functional properties of watermelon seed and the health benefits of watermelon seed have been reviewed and evaluated for the justification to carry out the present research work.

Chapter-3: Materials and Methods

The present study on "Comparative Evaluation of Nutritional Composition and Functional Properties of Two Varieties of Watermelon Seed in Bangladesh" was conducted in the year 2019. It was performed with the preparation of watermelon seed powder, determination of proximate composition, mineral content, phyto-chemical compound and evaluation of functional properties of watermelon seed powder. The materials and methods were applied for the study works are described in this part.

3.1 Study Location and Period

The study was conducted in Chattogram Veterinary and Animal Sciences University, Khulshi-4225, Chattogram, Bangladesh. The research work was carried for a period of six months from 1st July, 2019 to 30th December, 2019.

3.2 Sample Collection

Two varieties of watermelon seed such as Crimson red (Black color seed) and Charleston gray (Brown color seed) were collected from local seed supplier of Chattogram, Bangladesh.

Figure 3.2: Watermelon Seed Collection Location of Chattogram

3.3 Processing of Watermelon Seed

The collected watermelon seed was taken in dry and clean steel tray. The dirt, foreign materials, immature seed were removed from fresh mature seed and thoroughly cleaned. The watermelon seed was placed on cabinet dryer for drying at 60 ℃ for 24 hr. Then the dried seed was blended by electrical blender to convert the seeds into powder form. During blending, the husk was separated from seed powder. The seed powder was packed in polyethylene bag and stored in plastic container.

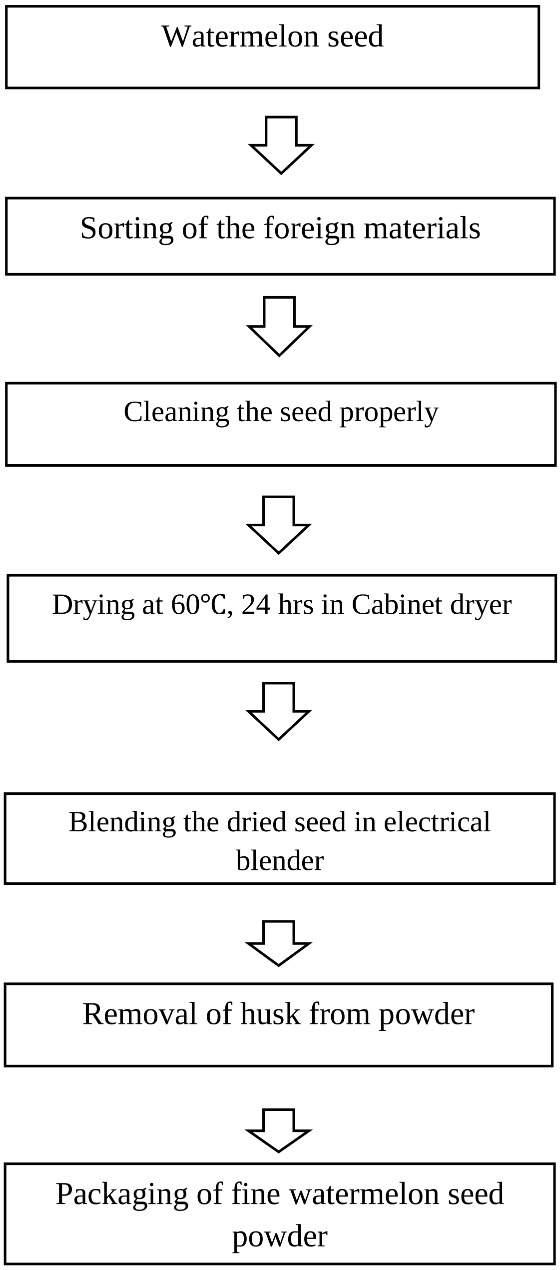

Figure 3.3: Processing of Watermelon Seed

3.4 Determination of Proximate Composition of Watermelon Seed

The proximate composition of watermelon seed powder such as moisture, ash, crude protein, crude fat, crude fiber were determined in triplicate by flowing AOAC method (AOAC, 2005). The total carbohydrate was estimated by using subtracting method (Pearson, 1976). The energy value of seed was calculated according to the formula used by Nile and Khobragade (2009). All proximate contents were expressed in percentage.

3.4.1 Moisture Content

An empty crucible was weighed and dried. A total of 10 g of sample (watermelon seed powder) was taken in the crucible and again weighed the crucible with sample. Then the crucible was kept in hot air oven and dried at 105°C for 48 – 72 hours. After finishing the drying, the crucible was removed from the air oven, covering the crucible and then it was placed in desiccators for cooling. Finally, the crucible was removed from desiccators and weighed. Re-drying the sample was repeated for obtaining constant weigh. The moisture content was calculated by using following formula:

$$\% \text{ Moisture } = \frac{\text{Loss of weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.4.2 Ash Content

A clean empty crucible was dried in oven, then it was placed in desiccators for cooling and taken weigh. 5gm of watermelon seed powder was taken in the crucible and burned the seed powder. Then the sample with crucible was cooled and placed in muffle furnace for inflaming at 550-600°Cfor 6-8 hours. The crucible was transferred into desiccators for cooling and weighed. The ash content was calculated by using following formula:

$$\% \text{ Ash content} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}} \times 100$$

3.4.3 Protein Content

Digestion

At first 1 g of sample (watermelon seed powder) was weighed and taken into digestion flask. Then 5 gram of digestion mixture and 20 ml of concentrated sulfuric acid (H_2SO_4) were added to the digestion flask. The digestion flask was put on the Kjeldahl digestion chamber. It was heated for digestion until obtain clear residual.

Distillation

After digestion, 20 ml distilled water was added to the content and it was transferred to the distillation flask. Then 100ml of 40% sodium hydroxide (NaOH) solution was added and the condenser was set. About 20ml of 2% boric acid and mixed indicator were added in conical flask. Then the distillation flask was heated gradually until obtain 100 ml of distillate.

Titration

The distillate was titrated with N/10 HCl solution and recorded the titration volume of the solution and the titer volume was calculated. The percentage of nitrogen in the sample was estimated by using following formula:

$$\%\ \text{Nitrogen} = \frac{(T_S - T_B) \times \text{Normality of acid} \times \text{meq.}\, N_2}{\text{Weight of Sample (gm)}} \times 100$$

Where,

T_S = Titer value of Sample (ml)

T_B = Titer value of Blank (ml)

Meq. Of N_2 = 0.014

The percentage of crude protein was calculated by using the following formula, where protein factor is 6.25.

% Crude Protein = % Nitrogen × 6.25

3.4.4 Fat Content

About 2g of watermelon seed powder was weighed and placed into the thimble. Then the thimble was transferred into the extractor and the top covered with cotton. The extractor was fitted and ether was run up to siphoning. Once again ether was effused haft of the previous volume. Then it was allow to boiling constantly at 40℃ to 60℃ for 6–8 hrs. The extraction flask was removed after ending the extraction period and put on the water bath for drying. Then the flask was put on the hot air oven for heating at 100℃ until obtain precise weight. At the last, the flask was placed in desiccators for cooling and the weight of ether extract was determined.

The percentage of fat (ether extract) was estimated by using following formula:

$$\% \text{ Fat (Ether extract)} = \frac{\text{Loss of ether soluble materials}}{\text{Weight of sample}} \times 100$$

3.4.5 Crude Fiber Content

About 2g of sample (watermelon seed powder) was weighted and taken into beaker. Then 125ml 1.25% H_2SO_4 solution and 3-5 drops of n–octanol were added to the beaker. The solution was allowed to boil for 30 minutes. Then it was rinsed with distilled water for 3 times to remove acid from it. At the end of rinsing, 125 ml 1.25% NaOH and 3-5 drops of antifoam were mixed. Again it was allowed to boil for 30 minutes. The residual substance was filtered and rinsed out. Again the residues were rinsed with 1% HCl solution to free from acid. Then residual matter was allowed to dry at 105℃ in hot air oven. The residual matter was placed in desiccators for cooling and weighed. The residual matter was incinerated. The residue was illuminated in muffle furnace at 550 – 600 ℃ for 4 – 6 hrs till obtaining white ash and the ash was weighed.

The percentage of crude fiber was estimated by using formula

$$\% \text{ Crude fiber} = \frac{\text{Weight of residue (crucible)} - \text{Weight of ash (crucible)}}{\text{Initial weight of sample}} \times 100$$

3.4.6 Carbohydrate Content

Carbohydrate was estimated by subtracting the percentage amount of moisture, ash, crude protein, crude fat and crude fiber from 100 according to subtracting method.

Carbohydrate was calculated by following formula:

% Carbohydrate = 100 – (% Moisture + % Ash + % Crude Protein + % Crude Fat + % Crude Fiber)

3.4.7 Energy Value

Energy value was calculated by following formula:

Energy value (Kcal/ 100g) = 4× percentage of carbohydrate + 9 × percentage of fat + 4× percentage of protein

3.5 Determination of Minerals Content of Watermelon Seed

Biochemical analyzer (Humalyzer 3000) was used for estimation of minerals content in watermelon seed by following colorimetric method. Commercial Biochemical kit was used for the experiment.

3.5.1 Sample Preparation

About 2 g samples were weighed and kept in conical flask. Then 5 ml HNO_3 and 2 ml 30% H_2O_2 were added and then the flask placed in microwave oven for digestion for 30 min. Then removed the digestion flask from microwave oven and cooled at room temperature. The solution was filtered on a filter paper & taken the filtrate to a volumetric flask (100ml) and made up to the mark which was used for mineral contents determination.

3.5.2 Calcium (Ca) Content

Principle

O-CresolPhthalein complexone form a violet complex by reaction with Calcium ions in an alkaline medium.

Colorimetric method: O-Cresolphthalein complexone, without deproteinization.

Assay

Wavelength /filter :	Hg 578nm (550 – 590 nm)
Spectrophotometer:	570nm
Temperature :	20 - 25℃/ 37℃
Light path :	1 cm

Calculation formula

$$\text{Calcium conc.}\left(\frac{\text{mg}}{\text{dl}}\right) = \frac{\text{(A)Sample}}{\text{(A)Standard}} \times \text{Standard conc.}\left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.3 Potassium (K) Content

Principle

Potassium reacts with sodium tetraphenyl boron and produce a fine turbidity of potassium tetraphenyl boron. The intensity of turbidity is directly proportional to the concentration of potassium in the sample.

Assay

Wavelength /filter :	630 nm (Hg 623) / Green
Temperature :	Room Temperature
Light path :	1 cm

Calculation formula

$$\text{Potassium conc.}\left(\frac{\text{mmol}}{\text{L}}\right) = \frac{\text{(A)Sample}}{\text{(A)Standard}} \times \text{Standard conc.}\left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.4 Iron (Fe) Content

Principle

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with ferrozine a colored complex. The intensity of the color formed is proportional to the Iron concentration in the sample.

Assay

Wavelength /filter	:	562 nm
Temperature	:	37 ℃ / 15 - 25℃
Light path	:	1 cm

Calculation formula

$$\text{Iron conc.}\ (\mu g/dl) = \frac{(A)\text{Sample} - (A)\text{Blank sample}}{(A)\text{Standard}} \times \text{Standard conc.}\ (\frac{mg}{dl})$$

3.5.5 Magnesium (Mg) Content

Principle

Magnesium ion reacts with xylidyl blue in alkaline solution and forms purple colored complex and complexes with calcium ion in presence of GEDTA. The Intensity of the purple color is proportional to the magnesium concentration.

Assay

Wavelength /filter : 520nm, Hg 546nm, 500 – 550 nm		
Temperature	:	20 - 25℃ / 37℃
Light path	:	1 cm
Measurement	:	Against reagent blank

Calculation Formula

$$\text{Magnesium conc.}\left(\frac{\text{mg}}{\text{dl}}\right) = \frac{(\text{A})\text{Sample}}{(\text{A})\text{Standard}} \times \text{Standard conc.}\left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.6 Phosphorus (P) Content

Assay

Wavelength /filter :		340 nm, Hg 334 nm
Temperature	:	20 - 25℃
Light path	:	1 cm
Measurement	:	Against reagent blank

Calculation Formula

$$\text{Phosphorus conc.}\left(\frac{\text{mg}}{\text{dl}}\right) = \frac{(\text{A})\text{Sample}}{(\text{A})\text{Standard}} \times \text{Standard conc.}\left(\frac{\text{mg}}{\text{dl}}\right)$$

3.5.7 Copper (Cu) Content

Principle

In an acidic medium ceruloplasmin releases copper which reacts with Di-Br – PAESA and formed a colored complex. Intensity of the complex formed is directly proportional to the amount of copper present in the sample.

Assay

Wavelength /filter	:	580 nm, Hg 578 nm / yellow
Temperature	:	room temperature (25 ℃)
Light path	:	1 cm
Measurement	:	Against reagent blank

Calculation Formula

$$\text{Copper conc.} \left(\frac{\mu g}{dl}\right) = \frac{(A)\text{Sample}}{(A)\text{Standard}} \times \text{Standard conc.} \left(\frac{mg}{dl}\right)$$

3.5.8 Zinc (Zn) Content

Principle: In an alkaline medium, Zinc reacts with Nitro – PAPS to form a purple colored complex. Intensity of the complex formed is directly proportional to the amount of zinc present in the sample.

Assay

Wavelength /filter	:	570 nm, Hg 578 nm / yellow
Temperature	:	room temperature (25 ℃)
Light path	:	1 cm

Calculation Formula

$$\text{Zinc conc.} \left(\frac{\mu g}{dl}\right) = \frac{\text{(A)Sample}}{\text{(A)Standard}} \times \text{Standard conc.} \left(\frac{mg}{dl}\right)$$

3.6 Determination of Phytochemical Compound of Watermelon seed

Crude alkaloid was determined according to the method described by Herborne (1973). Saponin content was evaluated by following the method reported by Obadoni and Ochuko (2001**).**

3.6.1 Crude Alkaloids Content

2.5g of watermelon seed powder was weighed and mixed with 100 ml of 10% acetic acid in ethanol. The mixture was allowed to incubation at 25℃ for 4 hr and then percolated the solution. The filtrate solution was placed on a hot water bath to thicken it up to one -fourth of actual amount. Then the concentrated NH_4OH was added drop by drop for precipitation. The precipitates were collected by allowing the mixture to settle down and rinsed with NH_4OH. Then it was allowed to filtration. Finally the weight of dried alkaloid was taken and estimated by following formula

$$\%\ \text{Crude Alkaloid} = \frac{\text{Weight of Crude Alkaloid}}{\text{Weight of sample}} \times 100$$

3.6.2 Saponin Content

5 g of watermelon seed powder was taken and 50 ml of 20% aqueous ethanol was added to it. The mixture was placed on hot water bath for heating at 55℃ for 4 hours and filtrate the solution. The residual were turning out with 50 ml 20% ethanol solution. The concentration of extracts was decreased to about 10 ml by heating on hot water bath at 90℃ . Then it takes into a separating funnel and mixed 20 ml of diethyl ether and agitated the solution. The hydrous was restored when the ether layer substituted. The clarification system was resolved by adding 15 ml of n- butanol and washed the n- butanol extracts were rinsed two times with 10 ml 5% NaCl. The residual mixture was placed on water bath and allowed to dry to obtain a constant weight. Then, the saponin content was estimated as a percentage by using the following formula

$$\% \text{ Saponin} = \frac{\text{Weight of Saponin}}{\text{Weight of sample}} \times 100$$

3.7 Determination of Functional Properties of Watermelon Seed

The water absorption capacity of the watermelon seed flour was determined by following the method of Phillips et al. (1988). The oil absorption capacity was evaluated according to the method of Sosulski (1962) and Njintang et al. (2001). The emulsion activity and stability were estimated by the method of Naczk et al. (1985). The foam capacity and foam stability were determined by the method of Coffmann and Garciaj (1977).

3.7.1 Water Absorption Capacity (WAC)

2g of seed powder ($M_{0)}$ was taken in centrifuge tube and added 20 ml distilled water and agitated for 30 min, then the solution was placed on a hot water- bath at 37℃ for 30 minute and it was centrifuged at 5000 rpm at 10 minutes. After finishing centrifuge, the obtaining sediment (M_2) was weighed and it was allowed to dry at 105℃ to constant weight ($M_{1).}$ The water absorption capacity was estimated by following formula:

$$\% \text{ WAC} = \frac{M2-M1}{M1} \times 100$$

3.7.2 Oil Absorption Capacity (OAC)

1g of sample (M_0) were mixed with 10 ml of soybean oil and agitated the mixture for 30 min., then centrifuged the mixture for 10 min at 4500 rpm and taken the weight of obtaining sediment (M_1).The oil absorption capacity was determined by following formula:

$$\% \text{ OAC} = \frac{M1-M0}{M0} \times 100$$

3.7.3 Emulsion Activity (EA)

3 g of seed powder was mixed 25 ml distilled water and blended for 30s. 50 ml oil was added to it and allowed to homogenization. Then centrifuged the emulsion at 1500 rpm for 5 min and determined the emulsion layer. The emulsion activity was determined by following formula:

$$\%\ \text{EA} = \frac{\text{Height of emulsified layer}}{\text{Height of whole layer in centrifuge tube}} \times 100$$

3.7.4 Emulsion stability (ES)

The emulsion stability was determined after heating the emulsion contained in calibrated centrifuged tube at 85 °C for 15 min in a water- bath, allowed to cool for few minutes and again centrifuged at 1500 rpm for 5 minutes. Emulsion activity was determined by the following formula:

$$\%\ \text{ES} = \frac{\text{Height of remaining emulsified layer}}{\text{Original emulsion height}} \times 100$$

3.7.5 Foam Capacity and Stability (FC and FS)

5g of seed powder sample was taken in a 250 ml cylinder. The volume of seed powder was recorded and 100 ml of distilled water was mixed with the sample. Then, the mixture was shaken few minutes to obtain foam and recorded the foam volume and foam stability was estimated by recording the foam capacity at 10, 30 and 60 minutes interval.

The foam capacity and stability were evaluated by following formula:

$$\%\ \text{FC} = \frac{\text{Volume after homogenization} - \text{Volume before homogenization}}{\text{Volume before homogenization}} \times 100$$

$$\%\text{FS} = \frac{\text{Volume after time (t)}}{\text{Initial foam volume}} \times 100$$

3.8 Statistical Analysis

All data obtained from estimation of proximate composition, minerals content, phytochemical compound and functional properties of watermelon seed powder were imported in Microsoft Excel 2007 spread sheet for statistical analysis. Statistical data analysis was carried out by using IBM SPSS STATISTICS 25. Data were estimated for statistical significance by using independent sample ‘t’ test for the comparison of mean values, where n=3 obtained from triplicate test for each sample. The statistical data analysis was conducted at 5% level of significant, where $P < 0.05$ indicated the mean value difference is significant and $P > 0.05$ indicated mean value difference is not significant. All result values were expressed as Mean ± SD (Standard deviation).

Chapter-4: Results

The results of the analytical research study on two varieties of watermelon seed in terms of proximate composition, energy value, minerals content, phytochemical compound and functional properties of two varieties of seed are presented in this section.

4.1 Proximate Composition of Watermelon Seed

In Table 4.1, the proximate compositions in percentage (%) for the two varieties of watermelon seed (Charleston gray and Crimson red) were shown. In this analysis, the percentage of crude protein (27.21±0.01%) and crude fat (36.61±0.01%)were found higher in the seed of Crimson red variety than seed of Charleston gray (22.51±0.01 % and 30.65±0.01%) respectively. On the other hand, the seed of Charleston gray contained higher amount of crude fiber (29.85±0.01%) than the fiber content (21.55±0.01%) of the seed of Crimson red. The difference in values of the proximate compositions for the two varieties of watermelon seed, aside moisture content were significant, ($p < 0.05$).

Table 4.1: Proximate Composition of Watermelon Seed

Proximate Parameters (%)	Sample (Watermelon Seed)		P value
	Charleston gray	Crimson red	
Moisture	3.65±0.01	3.71±0.01^{ns}	>0.05
Ash	2.61±0.01	3.11±0.01**	< 0.001
Crude Protein	22.51±0.01	27.21±0.01**	< 0.001
Crude Fat	30.65±0.01	36.61±0.01**	< 0.001
Crude Fiber	29.85±0.01	21.55±0.01**	< 0.001
Carbohydrate	10.75± 0.01	7.85±0.01**	< 0.001

Legend: Result values are given in mean value ± SD of triplicate estimations, ns: the mean difference is not significant at P> 0.05; **the mean difference is significant P< 0.05.

4.2 Energy Value of Watermelon Seed

As shown in below figure 4.2, the energy value for the two varieties of watermelon seed was found 408.85±0.01 Kcal/100g for Charleston gray and 469.60±0.01 Kcal/100g for Crimson red. This analytical study revealed that the seed of Crimson red variety contained higher amount of energy than the seed of Charleston gray variety.

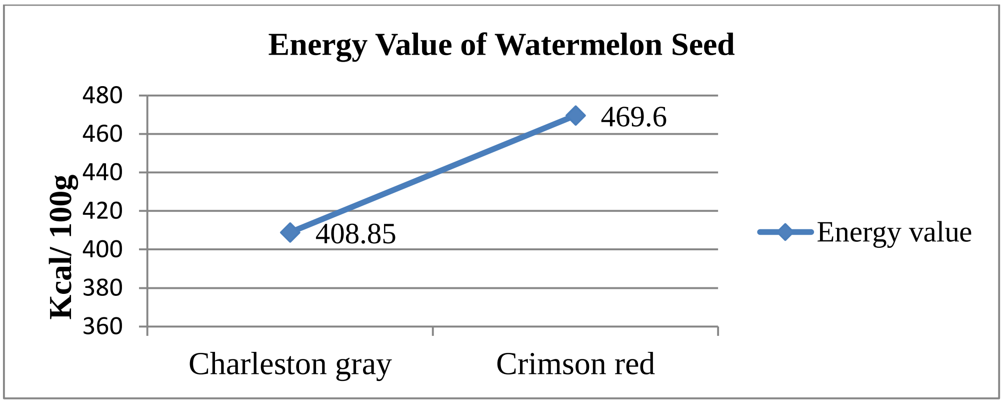

Figure 4.2: Comparison of Energy Value between Two varieties of Watermelon Seed

4.3 Minerals Content of Watermelon Seed

In Table 4.3, the minerals content in mg/ 100g for the two varieties of watermelon seed (Charleston gray and Crimson red) were shown. In this study, the result obtained from minerals analysis revealed that the minerals (mg/100g) such as calcium (124.33±0.57), Phosphorus (540.33±0.57), magnesium (360.33±0.57), Iron (20.13±0.006), potassium content (20.67±0.006) were found to be high in amount in the seed of Crimson red variety when compared to the seed of Charleston gray variety (28.33± 0.57, 320.33±0.57, 310.33±0.57, 14.24±0.006 and 6.64±0.006) respectively. The difference in value of the minerals content for the two varieties of seed, aside copper and zinc were significant ($p < 0.05$).

Table 4.3: Minerals Content of Watermelon Seed

Minerals Content (mg/ 100g)	Sample (Watermelon Seed)		P value
	Charleston gray	Crimson red	
Calcium	28.33± 0.57	124.33±0.57**	< 0.001
Phosphorus	320.33±0.57	540.33±0.57**	< 0.001
Magnesium	310.33±0.57	360.33±0.57**	< 0.001
Copper	0.04±0.006	0.04±0.006ns	1.000
Iron	14.24±0.006	20.13±0.006**	<0.001
Zinc	0.05±0.007	0.053±0.006**	0.778
Potassium	6.64±0.006	20.67±0.006**	<0.001

Legend: Result values are given in mean value ± SD of triplicate estimations, ns: the mean difference is not significant at P> 0.05; **the mean difference is significant P< 0.05.

4.4 Phytochemical Compounds of Watermelon Seed

In Figure 4.4, the phytochemical compounds in percentage (%) for the two varieties of watermelon seed (Charleston gray and Crimson red) were shown. The crude alkaloid content (14.05±0.006 %) in the seed of Crimson red variety was found higher than in the seed of Charleston gray (10.56±0.006 %). The difference in value of the crude alkaloid content for the two varieties of seed was significant ($p < 0.05$) and the value of saponin content was not significant ($p > 0.05$).

Table 4.4: Phytochemical Compound of Watermelon Seed

Phytochemical compound (%)	Sample (Watermelon Seed)		P value
	Charleston gray	Crimson red	
Crude Alkaloid	10.56±0.006	14.05±0.006**	<0.001
Saponin	0.04±0.006	0.04±0.006ns	1.000

Legend: Result values are given in mean value ± SD of triplicate estimations, ns: the mean difference is not significant at P> 0.05; ** the mean difference is significant P< 0.05

4.5 Functional Properties of Watermelon Seed

In Table 4.5, the functional properties in percentage (%) for the two varieties of watermelon seed (Charleston gray and Crimson red) were shown. The water absorption capacity (133.33±0.006 %), emulsion activity (23.31±0.006 %) and foaming stability (45.21±.006 %) were found higher in the seed of Charleston gray variety than in the seed of Crimson red. On the other hand, the oil absorption capacity (122.51±0.006 %) and Foam capacity (6.31±0.006 %) were present in the seed of Crimson red variety high in amount when compared to the seed of Charleston gray variety. The result of functional properties for two varieties of watermelon seed revealed that the seed of both varieties watermelon having good functional properties. The difference in value of the functional properties for the two varieties of seed, aside emulsion stability were significant ($p < 0.05$).

Table 4.5: Functional Properties of Watermelon Seed

Functional Parameter (%)	Sample (Watermelon Seed)		P value
	Charleston gray	**Crimson red**	
WAC	133.33±0.01	120.33±0.01**	< 0.001
OAC	118.51±0.01	122.51±0.01**	< 0.001
EA	23.31±0.01	21.41±0.01**	< 0.001
ES	11.51±0.01	11.50±0.01^{ns}	1.000
FC	4.61±0.01	6.31±0.01**	< 0.001
FS	45.21±0.01	39.61±0.01**	< 0.001

Legend: Result values are given in mean value ± SD of triplicate estimations, ns: the mean difference is not significant at P> 0.05; ** the mean difference is significant P< 0.05, WAC: Water absorption capacity, OAC: Oil absorption capacity, EA: Emulsion activity, ES: Emulsion stability, FC: Foam capacity, FS: Foam stability.

Chapter 5: Discussions

5.1 Proximate Composition of Watermelon Seed

The present research study evaluated the nutritional composition and functional properties of the Crimson red and Charleston gray varieties of watermelon seed to attain a scientific basis for the potential use of watermelon seed in formulation of food products, manufacturing of medicine and industrial purpose. The proximate component in percentage for the two varieties of watermelon seed was shown in Table 4.1.In this study, the moisture content was found for the two varieties of watermelon seed was 3.65 % for Charleston gray and 3.71% for Crimson red. This result was similar to that studies reported by Mehra et al. (2015) and Egbuonu (2015). This moisture content was lower than that reported by Akusu and Kinn-Kabiri (2015); Tabiri et al. (2016); Hannah and Krishnakumari (2015) and Jacob et al. (2015) who found the moisture content range of 7.1 to 9.59%. The disparity observed in result might be due to difference in varieties, drying process, and methods of analysis etc. Moisture content is considered an important factor for storage quality of product because high moisture facilitates the rapid growth of microorganism which ultimately spoils the product.

In present study, the result was found for ash content of the two varieties of watermelon seed was 2.61% for (Charleston gray) and3.11 % for Crimson red. This result showed that the ash content of the seed of Crimson red variety was found higher than the seed of Charleston gray. The result for ash content was consistent with the result reported by Egbuonu (2015); Tabiri et al. (2016); Jacob et al. (2015) and Akusu and Kinn-Kabiri (2015) who found the moisture content range of 2.48 to 3.63% respectively.

The percentage of protein found in this analysis for the two varieties of watermelon seed was 22.51% for Charleston gray variety and 27.21% for Crimson red variety. This analysis revealed that the Crimson red variety of watermelon seed contained higher amount of protein than that of the Charleston gray variety. The protein percentage found in this study was compatible with that study reported by Akusu and Kinn-Kabiri (2015) and Egbuonu (2015) who showed the protein percentage range of 21.46 to 25.33% and higher than that reported by Tabiri et al. (2016) and Mogotlane et al. (2018) who found the protein content range of 14.90 to 17.75% and lower than the result (34.22%)

reported by Mehra et al. (2015). The distinction observed might be due to difference in variety, geographical location, method of analysis etc.

In this experimental study, the fat content of two varieties watermelon seed was found 30.65% for Charleston gray variety and 36.61% for Crimson red variety. The result showed that both varieties of watermelon seed contained high amount of fat or lipid but the fat content of the seed of crimson red variety was higher than Charleston gray variety. This result is consistent with Mogotlane et al. (2018) and Mehra et al. (2015) who reported a ranged of 31.6 to 34.4%, lower than Akusu and Kinn-Kabiri (2015) **r**eported 45.66% fat content in watermelon seed flour and higher than Tabiri et al. (2016) and Hannah and Krishnakumari (2015) who reported a ranged of 26.4 to 27.8% lipid content in watermelon seed extract.

The fiber and carbohydrate content were found in this analytical study for two varieties of watermelon seed were 29.85% of fiber and 10.75% of carbohydrate for Charleston gray variety and 21.55% of fiber and 7.85% for carbohydrate Crimson red variety. This study revealed that the seed of Charleston gray variety contained higher amount of fiber and carbohydrate than the Crimson red variety. The result found for carbohydrate content is similar to that study reported by Akusu and Kinn-Kabiri (2015), higher than that reported by Mogotlane et al. (2018) who showed the carbohydrate content range of 3.16 to 5.26% and lower than the studies reported by Mehra et al. (2015) and Egbuonu (2015) who reported a range of 26.57 to 28.07 % of carbohydrate content in watermelon seed flour. The fiber content obtained in this study was higher than the study analysed by Jacob et al. (2015); Akusu and Kinn-Kabiri (2015) and Mehra et al. (2015) who reported a range of 0.1 to 6.4% of fiber content in watermelon seed.

In this analytical study the energy value found for the two varieties of watermelon seed was shown in figure 4.2 where found408.85±0.01 Kcal/100g for Charleston gray variety and469.60±0.01Kcal/100gfor Crimson red variety. The result showed that the seed of Crimson red variety contained higher amount of energy than the seed of Charleston gray variety. The energy value obtained in present study was consistent with that study conducted by Hannah and Krishnakumari (2015) who found 465.68 Kcal /100g for watermelon seed extract, lower than that analytical study reported by Egbuonu (2015) and Mehra et al. (2015) who showed that a range of 531.15 to 574.58 kcal/ 100g energy value for watermelon seed and higher than the result reported by

Tabiri et al. (2016) who found a range of 354.05 to 369.11 Kcal/ 100g. The disparity observed in energy value of watermelon seed among the studies might be due to difference in varieties, geographical location, soil, method of analysis etc.

5.2 Minerals Content of Watermelon Seed

The minerals content in mg per 100g for the two varieties of watermelon seed were shown in Table 4.3. This study revealed that two varieties of watermelon seed (Charleston gray and Crimson red) contained a good amount of phosphorus (320.33±0.57, 540.33±0.57), magnesium (310.33±0.57, 360.33±0.57), calcium (28.33± 0.57, 124.33±0.57), iron (14.24±0.006, 20.13±0.006) and potassium (6.64±0.006, 20.67±0.006) respectively. The seed of Crimson red variety contained these minerals comparatively higher than the seed of Charleston gray variety. The zinc and copper content were found same in two varieties of watermelon seed. The result of mineral analysis in this present study was compatible with the studies reported by Mehra et al. (2015); Tabiri et al. (2016) and Hannah and Krishnakumari (2015) who found that watermelon seed was rich in potassium, iron, calcium, magnesium, copper, zinc and phosphorus.

5.3 Phytochemical Compound of Watermelon Seed

The Phytochemical components in percentage (%) for the two varieties of watermelon seed were shown in Table 4.4. In this research study, result showed that two varieties of watermelon seed contained phytochemical compound such as crude alkaloid in high amount and saponin in negligible amount. The seed of Crimson red variety comparatively contained higher amount of crude alkaloid (14.05 %) than the amount of crude alkaloid content (10.56%) in the seed of Charleston gray variety. The saponin content (0.04%) was found same in two varieties of watermelon seed. The result of this study was consistent with that study conducted by Mehra et al. (2015).

5.4 Functional Properties of Watermelon Seed

The functional properties in percentage (%) for the two varieties of watermelon seed were shown in Table 4.5. The functional properties are the intrinsic factors which are important for food processing and storage. In present study, the result showed that the functional properties including water absorption capacity, emulsion activity and foam stability were found higher 133.33±0.01%, 23.31±0.01% and 45.21±0.01% respectively in the seed of Charleston gray variety than the seed of Crimson red variety (120.33±0.01%, 21.41±0.01% and 39.61±0.01%) respectively. On the other hand, the seed of Crimson red variety contained higher amount of oil absorption capacity (122.51±0.01%) and foam capacity (6.31±0.01%) than the seed of Charleston gray variety (118.51±0.01 and4.61±0.01%) respectively. Foam stability (11.51±0.01%) was found same in two varieties of seed. The result for functional properties of this study was in contrast to the result of the research work reported by Egbuonu (2015) and corresponded to that reported by Marie et al. (2015). The distinction observed might be due to difference in varieties of watermelon, agro-ecological condition, geographical location, climate, propagation method, method of analysis etc.

Chapter 6: Conclusion

The present study evaluated the nutritional, phytochemical and functional properties of two varieties of watermelon seed. In this study, the result obtained from the analysis of nutritional composition of the two types of watermelon seed showed that they were rich in energy, crude fat, crude protein, fiber and minerals such phosphorus, magnesium, calcium, iron and potassium. The phytochemical compound analysis of the watermelon seed indicated that the both varieties of seed contained a good amount of valuable phytochemical compound such crude alkaloid. The result found for the analysis of functional properties of the watermelon seed revealed that the two varieties of watermelon seed having good functional characteristics. This study is an indication that the great potential opportunities for the utilization of the unutilized watermelon seed to develop as medicine, cosmetics, value added product and dietary supplement instead of throwing them away as waste after consuming the fruit pulp.

Chapter 7: Recommendations & future perspectives

The current study was conducted to analyze the nutritional profile, phytochemical compound and functional properties of watermelon seed. The findings of the present study suggest watermelon seed as potential source of nutrients in the diet and may have potential health and economic benefits due to its high amount of protein, fat, fiber, minerals and alkaloid contents and good functional characteristics.

In this research, the result obtained that watermelon seed was a good source of fat. So it is considered as a potential source of edible oil. In future, research study can be conducted on oil extraction from watermelon seed and analysis of its parameters such as antioxidant activity, fatty acid content, phenolic profile, physio-chemical and functional properties.

In further study, protein based food product can be formulated by using watermelon seed flour as it is a good source of protein and analysis the parameters such as nutritional composition, antimicrobial activity, shelf life and acceptability of the product. The seed flour also incorporated in infant diet for improvement of infant nutrition.

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Appendices: Photo Gallery

Appendix I: Processing of Watermelon Seed

Watermelon Seed

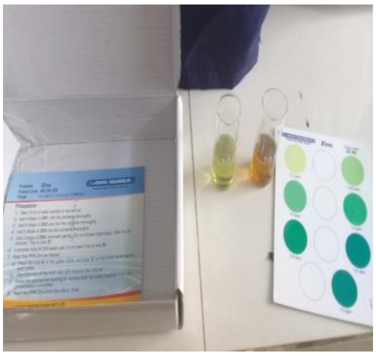
Cabinet Drying

Watermelon Seed Powder

Blending

Appendix II: Experimental Analysis of Watermelon Seed


Proximate Composition Analysis


Minerals Content Analysis



Phytochemical Compound and Functional Properties Analysis

Brief Biography

Jainal Akter Japu passed the Secondary School Certificate (SSC) Examination in 2009 with Grade Point Average (GPA) 5.00 followed by Higher Secondary Certificate (HSC) Examination in 2011 with GPA 4.40. She received the B.Sc. (Hons.) in Food Science and Technology in 2016 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, CVASU. Author has immense interest in research regarding Food Safety, New Food Product Development from Unutilized Parts of Fruits and Vegetables, Malnutrition and Improve the Food Pattern of Population of Under-Developing Countries by Awareness Building.