

Article

A Comparative Study on the Nutritional, Antioxidant, Thermal, Morphological and Diffraction Properties of Selected Cucurbit Seeds

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Abstract: Cucurbit seeds are highly nutritious but generally discarded as a waste by various processing sectors. This study aims to investigate the nutritional profile of seeds of ash gourd, pumpkin, watermelon and musk melon. The techniques, such as mineral, vitamin, amino acid and fatty acid profiling, will provide the nutritional status of the selected seed samples, while the antinutritional accounts will assess the safety of the food application. The thermal behavior of the seeds will guide the decision of the food application of the selected seeds. These seeds were found to be rich in protein and fat, minerals, vitamins and amino acids. The antinutritional compounds in the studied seeds were within the safety limits. Ash gourd seeds had the highest antioxidant activity at 85.11%, with the highest values of total phenols at 176.07 mg GAE/100 g and flavonoids at 159.16 mg QE/100 g. The peaks of the functional group at 3008 cm⁻¹, 1651 cm⁻¹, 1528 cm⁻¹ and 1233 cm⁻¹ denote the presence of polyunsaturated fatty acids, and amide I, II and III, respectively. The thermal analysis of the seeds revealed that the seeds were thermally stable and could be used in product development. The surface morphology was attributed to the interaction of fat, protein and carbohydrates. The semicrystalline or amorphous nature of the seeds resulted in an A-type pattern in the XRD analysis. The results obtained here supports the food application of the selected seeds.

Keywords: cucurbits; fatty acid; amino acid; thermal; seed morphology; diffraction properties



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1. Introduction

Ash gourd (*Benincasa hispida*), pumpkin (*Cucurbita moschata*), watermelon (*Citrullus lanatus*) and musk melon (*Cucumis melo*), commonly known as cucurbits, are the members of the Cucurbitaceae family. They have several health-promoting properties such as antidepressant, anti-inflammatory, antidiabetic, antiobesity, antibacterial, antidiarrheal, anticancer, anticomulsive, anti-histaminic, muscle relaxant and antiulcer properties, as well as being nephroprotective, etc. With rising awareness about these health benefits, these crops are gaining interest worldwide, which has increased their global consumption for food and medicinal applications [1,2]. This has encouraged the production of cucurbits. In the year 2018, the global production of watermelon was 103.9 million metric tons, while musk melon was 27.3 million metric tons and pumpkins, squash and gourds collectively accounted for 27.6 million metric tons. The gross production value of about USD 34 billion and USD 13.2 billion was estimated for watermelon and musk melon, respectively, whereas the collective gross production of pumpkins, squash and gourds was USD 8.5 billion. In 2018, China was the leading producer of watermelon, musk melon, pumpkins, squash and gourds, whereas India was the second-largest producer of pumpkins, squash and gourds, and held the fourth rank in the production of watermelon and musk melon (FAOSTAT 2018) [2]. The processing of these crops into value-added products produces a huge quantity of unused flesh, seeds, peels and rinds as waste or byproducts, which are usually discarded.

The waste generated by agroindustries throughout the world exceeds 2 billion tons [3]. According to the FAO, these byproducts are food losses and waste (FLW) that causes a decline in the successive food production supply chain. There is worldwide concern regarding agroindustrial residues, which cause environmental pollution and economic losses. The global economic losses arising from agroindustrial waste are estimated at around USD 990 billion [4]. These losses in the food production chain could be overcome by applying the circular economy model which will, in turn, help to manage waste utilization. Musk melon seed generated as waste during processing in 2015 was 738 thousand tons, which increased to 984 thousand tons in 2018 (FAOSTAT, 2015 and 2018), and the amount of seed waste is increasing every year. This seed (waste) can be considered a renewable source of nutrition, as they still consist of many beneficial compounds which can be derived for further usage. Since the last decade, seeds and nuts have increasingly gained attention due to the numerous health benefits of their phytonutrients [5]. The seed contents of ash gourd, pumpkin, watermelon and musk melon are 8–10%, 3.4–4%, 2% and 3.4–7%, respectively [6,7]. Cucurbit seeds are good sources of protein, fats, carbohydrates, minerals, vitamins and a wide range of phytonutrients [1,8]. Various phytochemicals exist in the cucurbit seeds, and they include antiobesity, anti-inflammatory, antiangiogenic, antipyretic, antinociceptive, antifungal, antibacterial and soporific properties. They can be used to treat chronic or acute eczema, and also inhibit the angiotensin-converting enzyme and the oxidation of linoleic acid [1,8]. The utilization of seed waste for food purposes can help to overcome nutritional security. Given these facts, this research work aims to investigate the nutritional, antioxidant, thermal, morphological and diffraction properties of ash gourd, pumpkin, watermelon and musk melon seeds. These observations can help to evaluate the industrial application for food fortification, as well as for the extraction of oil, phytonutrients (phytosterols, phenolic compounds, tocopherols, carotenoids, etc.) and the isolation of proteins, which have numerous applications in food and the cosmetic and pharmaceutical industries. In this study, the seeds of different species of the cucurbit family were chosen, rather than different varieties within the same crop, because the data on the nutritional composition of ash gourd seeds is scanty, while the nutritional profile of the other three crops is well known. So, the detailed comparison of these crops becomes necessary to utilize these seeds for successful food fortification. The selected crops are common in the day-to-day life of people, and the awareness of the consumption of these crops is increasing consistently. The seeds of these crops are generated as a waste by the processing sector. For instance, the ash gourd vegetable, which is used for the preparation of *Petha* (an Indian sweet that holds nutritional and historical importance), gives rise to a large amount of seed waste, and government agencies are trying their best to utilize this seed waste. The current study will help to utilize the waste for fortification, and thus can overcome the nutritional security.

2. Materials and Methods

2.1. Material

2.1.1. Sample Collection and Preparation

The seeds of the ash gourd (variety, TIL, PAG-3), pumpkin (variety, Pusa Viswesh), watermelon (variety, Sugar Queen) and musk melon (variety, Hara Madhu) were used in this research. A required quantity of fruits was purchased from the local market in Delhi. Fruits were cut into two halves and the seeds were separated manually, washed to remove adhering flesh and eventually air-dried in a forced circulation oven (Macro Scientific Workers, MAC, ATC-222, India) at 40 ± 0.5 °C for 48 h and stored in air-tight jars at ambient temperature until further used for analysis (Figure 1). About 15 kg of ash gourd vegetables, 25 kg of pumpkin vegetables, 50 kg of watermelon fruits and 25 kg of musk melon fruits were purchased from Azadpur Mandi, Delhi, India. After cleaning, washing, drying and finally dehulling, the dehulled seeds were sorted from the damaged or spoiled seeds on the basis of quality parameters like color, appearance any off flavor or smell, etc. Samples of this study were collected from a research study center of the Indian government,

and this research center is working with scientists only. Punjab Agricultural University Ludhiana has recognized the plant material.

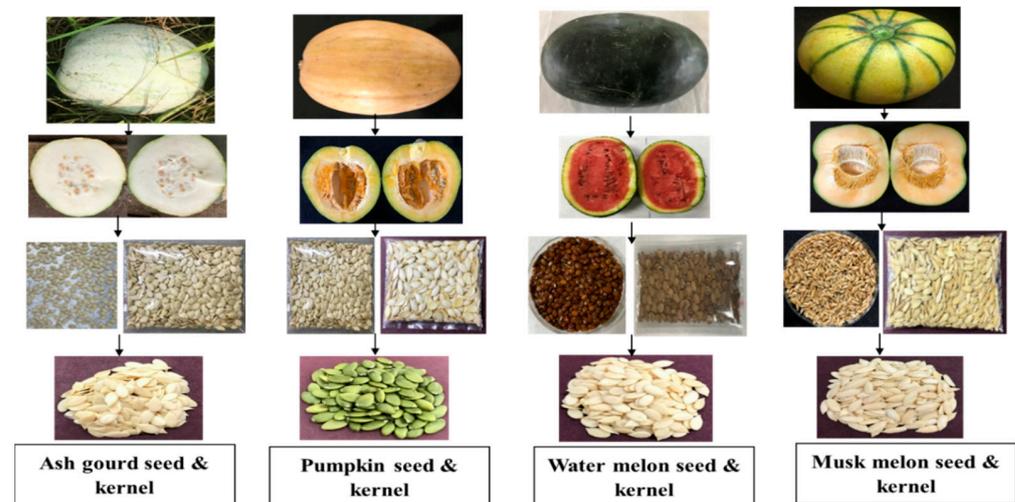


Figure 1. Preparation of seed samples.

2.1.2. Chemicals and Reagents Used

All analytical and HPLC-grade chemicals and reagents (Sigma Aldrich Research Laboratories Pvt. Ltd., Mumbai (Maharashtra, India)) were used in the analysis of seed samples.

2.2. Methods

2.2.1. Determination of Nutritional Composition

The proximate composition of the cucurbit seeds was determined by the Association of Official Agricultural Chemist (AOAC) methods. Determination of moisture content was carried out by drying the seed samples in a hot air oven at 105 ± 1 °C to a constant weight (AOAC, 925.09). Total fat content was determined by using the Soxhlet extraction method for 6 h, and hexane was used as a solvent (AOAC, 960.36). Ash content was estimated by incinerating the seed samples for 8 h at 550 °C in a muffle furnace (AOAC, 923.03). Nitrogen content was determined by using the Kjeldahl method (AOAC, 978.04), and crude protein was calculated by multiplying with a factor 6.25. The gravimetric procedure of (AOAC, 920.860) was used to determine the crude fiber content of the seed samples. The carbohydrate was determined by the difference method, as reported by [5], as the % Carbohydrate = $100 - (\% \text{ moisture} + \% \text{ crude fiber} + \% \text{ ash} + \% \text{ crude fat} + \% \text{ crude protein})$. The calorific value, that is, the total energy content, was determined by multiplying the values of the crude protein, crude fat and total carbohydrates by their respective Atwater factors: 4, 9 and 4. The sum of the products is expressed in kilocalories per 100 g sample, as given in Equation (1), which is reported by [8]. All analyses were conducted in triplicate.

$$\text{Total energy (Kcal/100 g)} = 4 \times (\text{Protein} + \text{Carbohydrate}) + 9 \times (\text{Lipid}) \quad (1)$$

2.2.2. Determination of Water Activity

The water activity (a_w) of the seed samples was measured at 25 °C using an electronic dew point water activity meter (Aqualab Series 4TE, Decagon Devices Inc., Washington, WA, USA).

2.2.3. Determination of PH

The pH of the seed samples was determined using the method reported by [9] with little modification. Briefly, 10% (w/v) suspension of powdered seeds was prepared using distilled water and allowed to stand for 30 min. The solution was filtered and the pH of the filtrate was measured using a digital pH meter (Ecphtutor D, Singapore).

2.2.4. Determination of Color

The color evaluation of the seed samples was performed with a handheld Minolta chromameter (Konica Minolta CR-400, Japan). At the beginning of the evaluation, the colorimeter was calibrated using a white tile (no. 13333104; $L^* = 94.89$, $a^* = 0.38$, $b^* = 4.04$); subsequently, the seeds were placed on the optical glass bottom to determine the color: L^* (lightness), a^* (redness) and b^* (blueness). The values of chroma (C), hue angle (h°), the total color difference (ΔE) and the color index (CI) were calculated using Equations (2)–(5), as reported by [6,10].

$$C = \sqrt{[(a^*)^2 + (b^*)^2]} \quad (2)$$

$$H^\circ = \tan^{-1}[(b^*)/(a^*)] \quad (3)$$

$$\Delta E = \sqrt{[(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2]} \quad (4)$$

$$C = (1000 a^*)/[(L^* - B)^2] \quad (5)$$

2.2.5. Determination of Minerals

The mineral content of the seed samples was determined according to the method described by [11] using inductively-coupled plasma–optical emission spectrometry (ICP-OES).

2.2.6. Determination of Vitamin Content

Water-soluble vitamins

Water-soluble vitamins such as C, B1, B2, B3, B5, B6 and B9 were determined by following the methods described by [12] using an HPLC and UV detector.

Fat-soluble vitamins

The normal-phase HPLC fluorescence technique (λ excitation 290 nm/ λ -emission 330 nm for vitamin E) reported by [13] was used to determine the content of vitamin A and vitamin E.

2.2.7. Determination of Amino Acids

The amino acid profiling was done by following the procedure given by [14] using the amino acid analyzer.

2.2.8. Fatty Acid Profiling

Methylation of oil was done using the method of (AOCS, 2009), followed by fatty acid profiling, as earlier reported by [14].

2.2.9. Determination of Antinutritional Properties

The phytate, saponin, alkaloid, tannin, oxalate and cyanide contents of the seeds were determined using the method reported by [15].

2.2.10. Determination of Total Antioxidant Activity, Phenolic and Flavonoids Content

The method of [5] was adopted with some modifications for the preparation of the seed extract of each sample for analyzing the functional properties of the seed samples. Briefly, 100 mL ethanol (50%, *v/v*) was poured into a 250 mL conical flask containing 5 g of the ground seed sample. The flask was sealed and shaken continuously at 30 °C and 100 rpm (Rotation per minute) for 24 h on an orbital shaker (New Brunswick™ Innova 42R, SI42DM301834, Eppendorf, Enfield, CT, USA). Subsequently, the extract was filtered through Whatman no.1 filter paper. Then, the extract was evaporated using a rotary vacuum evaporator (EYELA, Muttentz, Switzerland) to dryness. The resulting residue was extracted once in the same way and combined, and the extracts were stored at −20 °C. The prepared ethanolic seed extract was used for the determination of the total phenolic and flavonoid content in the seed samples. The method given by [16] was modified to obtain 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity. Briefly, 0.5 mL extract was mixed with 3.5 mL DPPH reagent and the absorbance was measured at 517 nm using

ethanol as blank. The % DPPH was calculated using the following Equation (6). Ascorbic acid was used as standard to compare the DPPH activity of the seed samples.

$$\text{DPPH radical scavenging (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (6)$$

where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

The total phenolic content (TPC) was measured following the procedure given by [16] in a spectrophotometer, and the results were defined on a dry weight basis as mg of gallic acid equivalents/100 g. The total flavonoid content (TFC) was measured using calorimetry as described by [5] with some modifications. The TFC was calculated using the standard quercetin curve, and the results were expressed on a dry weight basis as mg equivalents/100 g.

2.2.11. Functional Group Analysis

The functional groups of the seeds were investigated using the Fourier Transform Infrared (FTIR) Spectrophotometer (Alpha Bruker, Berlin, Germany). To measure the infrared spectra of the seed samples, a small quantity of priorly ground seed samples was transferred to the FTIR sample holder. The scan rate of the FTIR spectrometer was 24 per min and the resolution of 4 per cm in the wavenumber region range of 4000–600 cm^{-1} at a room temperature of 30 °C, and an RH of 65% was documented in the transmittance mode as a function of the wave.

2.2.12. Thermal Characterization

Thermal analysis by differential scanning calorimetry (DSC).

About 2.5 mg priorly ground sample was sealed hermetically in the aluminum crucible and then analyzed by differential scanning calorimeter (NETZSCH DSC-200 F3, Upper Franconia, Germany). Samples were heated from 30 to 180 °C at 5 °C/min. Enthalpy, onset, peak and conclusion temperature were recorded.

Thermal degradation profile by Thermogravimetric and derivative thermogravimetric analysis (TGA and DTG).

About 2.5 mg priorly ground sample was weighed in a crucible and placed into the TG (NETZSCH TG-209 F1, Upper Franconia, Germany) furnace and heated from 30 to 800 °C with a heating rate of 10 °C/min. Analysis was carried out under a nitrogen atmosphere with a 20 mL/min flow rate using an alumina crucible. Obtained TG curves, as well as their derivative curves (DTG), were used to analyze the thermal properties of the samples.

2.2.13. Morphological Analysis

The seed sample was coated with platinum using a sputter coater and then observed by a Scanning Electron Microscope (SEM) system (FEI, Quanta-200, Hillsboro, OR, USA) under high-vacuum conditions.

2.2.14. X-ray Diffraction Properties

The degree of crystallinity of the seed samples was investigated using an X-ray diffractometer (D 8 Advance Bruker, Karlsruhe, Germany). To carry out the X-ray diffraction (XRD) measurements, 1 g of the powdered seed sample was tightly packed in silicon cell. The radiation employed was Cu K α ($\lambda = 1.54060 \text{ \AA}$), operated at 30 kV and 15 mA. Data was taken for the scanning region of the angles in the 2θ range of 10° to 80° at a scanning speed of 0.02°/min and a scanning rate of 5°/min.

2.2.15. Statistical Analysis

The results of three replicates are presented as mean \pm SD (standard deviation). One-way analysis of variance (ANOVA) was used to evaluate the significant difference among parameters ($p < 0.05$) using SPSS 23 version (SPSS, Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Nutritional Composition

Data on the proximate composition of the cucurbit seeds are summarized in Table 1. Significant variations ($p < 0.05$) were found among all the seed samples in their proximate composition.

Table 1. Nutritional composition of seeds.

Component	Ash Gourd	Pumpkin	Watermelon	Musk Melon
Proximate Composition (%)				
Moisture	5.2 ± 0.020 ^c	5.6 ± 0.010 ^b	5.1 ± 0.010 ^d	5.85 ± 0.010 ^a
Protein	18 ± 0.026 ^d	23.55 ± 0.030 ^b	35.43 ± 0.020 ^a	19.14 ± 0.020 ^c
Fat	56.66 ± 0.003 ^a	26.4 ± 0.020 ^d	39.8 ± 0.020 ^c	42 ± 0.010 ^b
Ash	6.28 ± 0.010 ^a	3.83 ± 0.017 ^d	5.31 ± 0.020 ^b	4.46 ± 0.010 ^c
Fiber	10.96 ± 0.010 ^c	18.4 ± 0.010 ^a	6.69 ± 0.010 ^d	15.67 ± 0.020 ^b
Carbohydrate	2.89 ± 0.030 ^d	22.22 ± 0.026 ^a	7.67 ± 0.034 ^c	12.88 ± 0.026 ^b
Calorific Value (Kcal/100 g)	593.55 ± 0.093 ^a	420.68 ± 0.053 ^d	530.6 ± 0.156 ^b	506.08 ± 0.115 ^c
Water activity	0.349 ± 0.001 ^b	0.353 ± 0.003 ^a	0.347 ± 0.001 ^b	0.356 ± 0.002 ^a
pH	6.52 ± 0.030 ^a	6.28 ± 0.036 ^c	6.11 ± 0.026 ^d	6.41 ± 0.020 ^b
Minerals (mg/100 g)				
Potassium	1286 ± 0.050 ^a	1146.46 ± 0.0173 ^b	920 ± 0.026 ^d	994.73 ± 0.020 ^c
Phosphorus	1198 ± 0.045 ^a	93.69 ± 0.025 ^c	769 ± 0.050 ^b	65 ± 0.030 ^d
Calcium	87.60 ± 0.020 ^a	75.15 ± 0.020 ^b	40.58 ± 0.010 ^d	61.73 ± 0.020 ^c
Magnesium	1330.95 ± 0.038 ^a	1012.73 ± 0.026 ^b	282 ± 0.030 ^d	528.15 ± 0.045 ^c
Sulphur	319.71 ± 0.097 ^a	120.63 ± 0.020 ^b	116.35 ± 0.030 ^c	81.3 ± 0.020 ^d
Sodium	98.2 ± 0.020 ^a	82.2 ± 0.036 ^b	22.03 ± 0.040 ^d	53.36 ± 0.037 ^c
Iron	13.06 ± 0.015 ^b	20 ± 0.030 ^a	10.22 ± 0.017 ^c	5.51 ± 0.015 ^d
Zinc	29.49 ± 0.037 ^a	10.25 ± 0.020 ^b	7.65 ± 0.030 ^c	6.73 ± 0.015 ^d
Manganese	4.17 ± 0.025 ^c	7 ± 0.037 ^a	3.49 ± 0.020 ^d	5.24 ± 0.030 ^b
Copper	0.49 ± 0.010 ^d	2.13 ± 0.026 ^b	1.36 ± 0.017 ^c	3.57 ± 0.026 ^a
Vitamins (mg/100 g)				
Vitamin C	80.62 ± 0.025 ^a	79.38 ± 0.040 ^b	61.17 ± 0.075 ^d	75.37 ± 0.075 ^c
B1	0.21 ± 0.040 ^c	0.28 ± 0.010 ^{b,c}	2.26 ± 0.079 ^a	0.33 ± 0.040 ^b
B2	0.19 ± 0.020 ^c	0.16 ± 0.010 ^c	1.07 ± 0.052 ^a	1 ± 0.043 ^b
B3	4.8 ± 0.020 ^b	5.10 ± 0.086 ^a	3.29 ± 0.070 ^d	4.11 ± 0.030 ^c
B5	0.68 ± 0.030 ^a	0.75 ± 0.050 ^a	0.12 ± 0.017 ^b	0.17 ± 0.050 ^b
B6	1.4 ± 0.040 ^c	1.42 ± 0.043 ^c	11.29 ± 0.072 ^a	1.6 ± 0.040 ^b
B9	1.1 ± 0.040 ^c	2.1 ± 0.050 ^b	10.1 ± 0.050 ^a	1.12 ± 0.035 ^c
Vitamin A	86.34 ± 0.060 ^c	80.17 ± 0.070 ^d	89.11 ± 0.070 ^b	98.47 ± 0.070 ^a
Vitamin E	496.72 ± 0.080 ^c	517.66 ± 0.060 ^b	470.26 ± 0.047 ^d	523.14 ± 0.045 ^a
Color				
L*	70.81 ± 0.149 ^c	38.33 ± 0.280 ^d	72.54 ± 0.231 ^b	73.41 ± 0.298 ^a
a*	2.44 ± 0.110 ^b	−1.39 ± 0.153 ^c	2.38 ± 0.207 ^b	2.97 ± 0.227 ^a
b*	20.09 ± 0.578 ^a	12.67 ± 0.159 ^b	20.11 ± 0.132 ^a	20.24 ± 0.199 ^a
Chroma	20.44 ± 0.586 ^a	12.75 ± 0.175 ^b	20.24 ± 0.150 ^a	20.45 ± 0.192 ^a
Hue Angle (°)	83.06 ± 0.155 ^a	−83.71 ± 0.601 ^c	83.23 ± 0.548 ^a	81.63 ± 0.654 ^b
ΔE	7.37 ± 0.103 ^b	7.87 ± 0.038 ^a	7.07 ± 0.052 ^c	6.94 ± 0.083 ^c
Color Index	1.71 ± 0.041 ^{a,b}	−2.87 ± 0.293 ^c	1.63 ± 0.132 ^b	2 ± 0.158 ^a

Values with different letters are significantly different $p < 0.05$. Values are means ± standard deviations (SD) of three determinations.

The moisture content of all the seed samples corresponded to their water activity. The authors of [17] reported a similar range of moisture content for the watermelon seeds. Watermelon seeds possess the highest protein content (35.43%), followed by pumpkin (23.55%), musk melon (19.14%) and ash gourd (18%). The high-protein-content seeds could be used for fortification to meet protein malnutrition. The highest fat content (56.66%) was found in ash gourd seeds, while the lowest was recorded in pumpkin seeds (26.4%). The high amount of fat in ash gourd makes it potentially useful in the oil industry [18]. The cucurbit seeds examined in this study also exhibited good amounts of ash and fiber. The intake of dietary fibers is found to have positive effects on human health and enhance

normal bodily function. The consumption of dietary fibers in the required amount can reduce the risk of common health issues such as constipation, obesity and diabetes [5]. The variability in the proximate composition among the seeds was generally observed due to the differences in the species of cucurbit seeds. The major findings were in agreement with [8], who reported compositional differences in the seeds due to growing in different areas, climate conditions, soil types, agricultural practices and genetic diversity. The proximate seed refers to its high lipid content and an appreciable amount of protein, ash and fiber. Thus, the seeds could be considered a good source of nutrients and could be used for value addition.

3.2. PH

The value of the pH for the seeds of the ash gourd, pumpkin, watermelon and musk melon was 6.52, 6.28, 6.11 and 6.41, respectively. The observed pH value of all seed samples was found to be significantly different from each other. The pH values were slightly in the acidic range. The authors of [19] observed a lower pH value compared to the pumpkin seeds investigated in this study.

3.3. Color

The examined seed samples differed ($p < 0.05$) from each other in all color parameters (L^* , a^* , b^* , C^* , h° , ΔE and color index) (see Table 1). Luminosity (L^*) towards 0 indicates darkness, while 100 indicates lightness. The positive and negative a^* arise if the a^* values are near red and green, respectively. The value that is near blue has a negative b^* and the value that is near yellow has a positive b^* . A dark green tone in the pumpkin seeds was confirmed by the lowest L^* (38.33) and b^* (12.67) values, with negative a^* values (-1.39). The musk melon seeds had the highest L^* value (73.41) compared to watermelon (72.54) and ash gourd (70.81), but all the values lie in the lighter tone. The seed sample with a higher b^* value shows a strong tendency toward a yellowish tone, which was observed in the seeds of the musk melon ($b^* = 20.24$), then watermelon ($b^* = 20.11$) and ash gourd ($b^* = 20.09$). The color of the musk melon seed studied by [20] shows a little dark yellowish tone, with ($L^* = 66.31$), ($a^* = 3.25$) and ($b^* = 20.63$) compared to the musk melon seeds studied in this study. In terms of the color intensity evaluated by the magnitude of the chroma, the result showed that the pumpkin seed (12.75) color was less intense than the other seed samples. The amount of redness and yellowness is expressed by the hue angle, where $0^\circ/360^\circ$ represents redness/magenta, 90° represents yellowness, 180° represents green and 270° represents the blue or purple color, or the immediate color between the adjacent pair of these basic colors [21]. The hue angle was significantly lower for the musk melon seeds (81.63), while similar values were recorded for the seeds of the ash gourd (83.06), watermelon (83.23) and pumpkin (83.71). The values of the hue angle were situated in the edge of red but near yellow, indicating an orangish–yellow color, probably due to the carotenoid content of the seeds. Delta E or ΔE is used to represent the color difference between two samples, and at what distance they are in the visual color ‘sphere’. The smaller color difference which is identical to the visual observation has a ΔE value from 0 to 1.5. The color difference that can be visually distinguished has the range of ΔE between 1.5 and 5.0. If the value of ΔE is less than 3.0, the color difference cannot be easily noticed by the naked eye. However, if the value of ΔE is more than 5, the color difference is quite obvious [22]. The recorded values of ΔE were 7.73, 7.87, 7.07 and 6.94 for the seeds of the ash gourd, pumpkin, watermelon and musk melon, respectively. The ΔE values of more than 5 demonstrate that all the studied seed samples show evidence of visual color difference which could be detected by the human naked eye [22]. The value for the color index (CI) for the musk melon seeds was (2), which was higher than the ash gourd (1.71) and watermelon (1.63) seeds, but lower than the pumpkin (-2.87) seeds. The authors of [20] reported that the factors that mainly influence the color of seeds are the growing conditions, environmental factors, genotype and postharvest practices.

3.4. Mineral Content

Minerals are inorganic substances necessary in minute quantities for the normal growth and functioning of the human body. They are classified as major (potassium, magnesium, calcium, etc.) and minor (manganese, zinc, iron, etc.) minerals. Edible seeds are an excellent source of minerals vital for human beings, as their deficiency leads to malfunctioning in the human body [23]. Because minerals are an inescapable constituent of many syntheses' pathways of metabolic compounds, such as vitamins, enzymes and hormones, they are necessary in regulating water and acid-base, the transmission of impulses and muscle functioning. The mineral content of our studied seed samples is reported in Table 1. A significant difference ($p < 0.05$) was found in the mineral content of all the studied seeds. Among the major minerals, the highest content recorded was of magnesium in the seeds of the ash gourd (1330.95 mg/100 g), while the lowest content was recorded in the seeds of the watermelon (282 mg/100 g). In the case of the minor minerals, the highest value was observed for zinc in the ash gourd seeds (29.49 mg/100 g), while the lowest value was observed in the musk melon seeds (6.73 mg/100 g). Kamble et al. [17] reported a higher amount of potassium (2116.83 mg/100 g) and a lower amount of manganese (2.05 mg/100 g) compared to all the seeds investigated in the present study. The values reported by [19] (pumpkin seeds) for manganese (327.7 mg/100 g) and copper (95 mg/100 g) were much higher than the observed results of manganese and copper in all the seed samples investigated in the present study.

3.5. Vitamin Content

The result of the vitamin analysis exhibited that the seed samples were rich in vitamin C, A and E.

Other vitamins are present in appreciable amounts, as shown in Table 1. Among the water-soluble vitamins, the highest content was of vitamin C. The observed content of vitamin C in the ash gourd (80.62 mg/100 g) seeds did not vary much from the vitamin C content of the pumpkin (79.38 mg/100 g) seeds, but it was slightly higher than the musk melon (75.37 mg/100 g) seeds, and the lowest content of vitamin C was recorded in the watermelon (61.17 mg/100 g) seeds. The content of vitamin C reported by [17] for watermelon seeds (0.37 mg/100 g) was much lower than our observed result for all the seeds. The niacin (B3) content of the seed samples was also found in a notable amount. Seeds of pumpkin (5.10 mg/100 g) recorded higher niacin content, followed by ash gourd (4.8 mg/100 g), musk melon (4.11 mg/100 g) and watermelon (3.29 mg/100 g) seeds. The niacin content of the seed samples was corroborated by [24], with slightly similar niacin content in the seeds of the pumpkin (4.8 mg/100 g). In the case of fat-soluble vitamins, vitamin E was the prevalent vitamin (tocopherol). The seeds of the musk melon (523.14 mg/100 g) observed the highest amount compared to the pumpkin (517.66 mg/100 g), ash gourd (496.72 mg/100 g) and watermelon (470.26 mg/100 g) seeds. The vitamin A (in the form of carotenoids) content of the seed samples was also in a considerable amount. The recorded value of vitamin A (descending order) was 98.47 mg/100 g (musk melon), 86.34 mg/100 g (ash gourd), 80.17 mg/100 g (pumpkin) and 70.11 mg/100 g (watermelon) in the seeds. A comparably lower amount of carotenoid (8.94 mg/100 g) was found by [19] in pumpkin seeds. The seeds of the Cucurbitaceae family are well known for their good quality of fat-soluble fraction, which is supported by the results of the fat-soluble vitamin (A and E) of the current study.

3.6. Amino Acid Content

The amino acid profile of the seed samples is presented in Table 2. A total of 18 amino acids were found in the seed samples among the 22 amino acids in nature.

In the ash gourd seeds, the highest amount of amino acids were glutamic acid (9.48 mg/100 g) and aspartic acid (6.14 mg/100 g), while the lowest amount was methionine (1.01 mg/100 g) and cystine (1.06 mg/100 g). The result of the pumpkin seeds shows that arginine (6.28 mg/100 g), leucine (5.75 mg/100 g) and phenylalanine (5.39 mg/100 g)

were the prevalent, and a low amount of methionine (1.08 mg/100 g) and histidine (1.69 mg/100 g) were also present. The abundant quantity of amino acids in the watermelon seeds were found to be arginine (8.73 mg/100 g), and the lowest content was observed to be cystine (1.13 mg/100 g) and glycine (1.16 mg/100 g). As for the musk melon seeds, the amino acid that dominated the whole composition was glutamic acid (8.77 mg/100 g) and arginine (7.24 mg/100 g), whereas the minimum amount was found to be methionine (1.04 mg/100 g) and histidine (1.14 mg/100 g). A much higher content of amino acids was reported by [25,26] for watermelon and ash gourd seeds, respectively.

Table 2. Amino acid content of seeds (mg/100 g).

Amino Acids	Ash Gourd	Pumpkin	Watermelon	Musk Melon
Alanine	2.47 ± 0.080 ^d	4.76 ± 0.070 ^a	3.17 ± 0.060 ^c	3.51 ± 0.050 ^b
Arginine	4.79 ± 0.055 ^d	6.28 ± 0.052 ^c	8.73 ± 0.050 ^a	7.24 ± 0.050 ^b
Aspartic acid	6.14 ± 0.050 ^a	2.17 ± 0.036 ^d	2.23 ± 0.036 ^c	5.71 ± 0.040 ^b
Cystine	1.06 ± 0.036 ^c	2.54 ± 0.062 ^b	1.13 ± 0.040 ^c	3.43 ± 0.052 ^a
Glutamic acid	9.48 ± 0.040 ^a	3.93 ± 0.052 ^d	4.91 ± 0.040 ^c	8.77 ± 0.065 ^b
Glycine	1.11 ± 0.026 ^c	3.27 ± 0.036 ^a	1.16 ± 0.050 ^c	2.68 ± 0.06 ^b
Histidine	1.16 ± 0.020 ^c	1.69 ± 0.050 ^a	1.43 ± 0.050 ^b	1.14 ± 0.040 ^c
Isoleucine	2.57 ± 0.055 ^d	3.18 ± 0.040 ^c	4.37 ± 0.060 ^a	3.76 ± 0.060 ^b
Leucine	3.86 ± 0.030 ^c	5.75 ± 0.050 ^a	4.41 ± 0.050 ^b	3.38 ± 0.070 ^d
Lysine	1.14 ± 0.030 ^d	3.62 ± 0.0655 ^a	1.81 ± 0.040 ^b	1.53 ± 0.050 ^c
Methionine	1.01 ± 0.010 ^b	1.08 ± 0.026 ^b	1.23 ± 0.060 ^a	1.04 ± 0.030 ^b
Phenylalanine	3.17 ± 0.036 ^d	5.39 ± 0.051 ^a	3.27 ± 0.060 ^c	3.84 ± 0.050 ^b
Proline	2.11 ± 0.020 ^d	3.51 ± 0.051 ^a	3.14 ± 0.052 ^b	2.73 ± 0.52 ^c
Serine	2.41 ± 0.055 ^d	4.68 ± 0.045 ^a	3.21 ± 0.030 ^c	4.18 ± 0.030 ^b
Threonine	1.17 ± 0.034 ^d	2.46 ± 0.062 ^b	1.42 ± 0.050 ^c	2.86 ± 0.030 ^a
Tryptophan	1.01 ± 0.010 ^b	1.03 ± 0.020 ^b	1.03 ± 0.020 ^b	1.12 ± 0.017 ^a
Tyrosine	1.13 ± 0.030 ^d	2.86 ± 0.045 ^c	3.41 ± 0.055 ^a	3.16 ± 0.060 ^b
Valine	2.71 ± 0.036 ^d	4.13 ± 0.036 ^b	4.87 ± 0.070 ^a	3.24 ± 0.045 ^c

Values with different letters are significantly different $p < 0.05$. Values are means ± standard deviations (SD) of three determinations.

3.7. Fatty Acid Profile

The fatty acid profile of the seed samples is illustrated in Table 3. The result revealed that the analyzed seeds were significantly different ($p < 0.05$) for the content of saturated acid such as palmitic, stearic and arachidic acid, and unsaturated acid such as oleic and linoleic acid.

These variations lead to major differences in the content of total polyunsaturated, monounsaturated and saturated fatty acids. The amounts of the total unsaturated fatty acids of all the examined seeds were high, which mainly contained linoleic and oleic acid. They represented 86.45% for the ash gourd, 79.11% for the pumpkin seeds, 83.66% for the watermelon seeds and 81.1% for the musk melon seeds. The result of the ash gourd was slightly higher than [27]. The results reported by [18] were lower than the seeds of the pumpkin and musk melon, but higher than the watermelon seeds compared to the seeds investigated in the present work. The seed oil contained an appreciable quantity of linoleic and oleic acids, and thus the examined seeds may be considered as linoleic–oleic oil-containing seeds. Linoleic acid is nutritious and helpful to prevent coronary heart disease and cancer [5]. All the investigated seed samples could be used for cooking, salad dressing and the manufacturing of margarine [18]. Polyunsaturated fatty acids were about 85% of the total unsaturated fatty acids in the investigated seed samples. Polyunsaturated fatty acids enhance immune response, insulin sensitivity and help to reduce both total and LDL cholesterol [5]. So, the seeds could be considered a source of oil of good quality. The quantity of α -linolenic acid in the seed samples was an appreciable amount. The highest content of linolenic acid was recorded in the ash gourd seeds (0.73%), followed by the watermelon seeds (0.26%), musk melon seeds (0.21%) and pumpkin seeds (0.14%).

The linolenic acid content of the watermelon seeds was lower than those found by [17]. The result of the musk melon seeds was similar to [18]. The major saturated fatty acids, such as palmitic and stearic acid, were observed in all the investigated seeds. The total saturated fatty acids content of the pumpkin seeds was higher (20.89%) than the musk melon (18.9%), watermelon (16.34%) and ash gourd (13.55%) seeds. These results were in range with [5,17,18].

Table 3. Fatty acid composition of seeds (%).

Fatty Acids		Ash Gourd	Pumpkin	Watermelon	Musk Melon
Myristic acid	C14:0	0.01 ± 0.052 ^d	0.21 ± 0.070 ^a	0.13 ± 0.030 ^c	0.16 ± 0.048 ^b
Palmitic acid	C16:0	8.59 ± 0.027 ^b	12.78 ± 0.010 ^a	10.37 ± 0.034 ^d	11.26 ± 0.020 ^c
Palmitoleic acid	C16:1	0.7 ± 0.030 ^a	0.66 ± 0.050 ^c	0.34 ± 0.010 ^b	0.11 ± 0.048 ^c
Margaric acid	C17:0	0.09 ± 0.020 ^c	0.13 ± 0.061 ^a	0.14 ± 0.060 ^a	0.8 ± 0.010 ^b
Stearic acid	C18:0	4.63 ± 0.028 ^d	7.21 ± 0.054 ^a	5.33 ± 0.060 ^c	6.28 ± 0.071 ^b
Oleic acid	C18:1	12.26 ± 0.024 ^d	12.38 ± 0.015 ^b	17.12 ± 0.045 ^a	10 ± 0.036 ^c
Linoleic acid	C18:2	72.75 ± 0.054 ^a	65.77 ± 0.060 ^d	65.7 ± 0.021 ^b	70.69 ± 0.040 ^c
Linolenic acid	C18:3 α - 3	0.73 ± 0.036 ^a	0.14 ± 0.010 ^d	0.26 ± 0.046 ^b	0.21 ± 0.031 ^c
Arachidic acid	C20:0	0.21 ± 0.026 ^c	0.31 ± 0.070 ^a	0.29 ± 0.040 ^b	0.3 ± 0.010 ^d
Eicosanoic acid	C20:1	0.01 ± 0.057 ^d	0.16 ± 0.040 ^b	0.24 ± 0.010 ^a	0.09 ± 0.070 ^c
Behenic acid	C22:0	0.01 ± 0.056 ^d	0.14 ± 0.048 ^a	0.03 ± 0.073 ^b	0.02 ± 0.054 ^c
Lignoceric acid	C24:0	0.01 ± 0.015 ^d	0.11 ± 0.023 ^a	0.05 ± 0.045 ^c	0.08 ± 0.060 ^b
Total saturated fatty acid	Σ SFA	13.55 ± 0.031 ^c	20.89 ± 0.024 ^a	16.34 ± 0.054 ^d	18.9 ± 0.026 ^b
Total unsaturated fatty acid	Σ UFA	86.45 ± 0.012 ^a	79.11 ± 0.054 ^b	83.66 ± 0.026 ^b	81.1 ± 0.031 ^b
Total monosaturated fatty acid	Σ MUFA	12.97 ± 0.032 ^d	13.2 ± 0.015 ^a	17.7 ± 0.053 ^b	10.2 ± 0.030 ^c
Total polysaturated fatty acid	Σ PUFA	73.48 ± 0.054 ^a	65.91 ± 0.025 ^d	65.96 ± 0.028 ^c	70.9 ± 0.031 ^b

Values with different letters are significantly different $p < 0.05$. Values are means ± standard deviations (SD) of three determinations.

3.8. Antinutritional Factors

The antinutritional factors of the seed samples are presented in Table 4. It was observed that the examined seed samples contained a high amount of alkaloids, saponins and phytate content.

Table 4. Antinutritional compounds of seeds (mg/100 g).

Component	Ash Gourd	Pumpkin	Watermelon	Musk Melon
Phytates	2.15 ± 0.050 ^d	2.48 ± 0.045 ^c	9 ± 0.040 ^a	3.17 ± 0.045 ^b
Saponins	7.13 ± 0.026 ^b	2.18 ± 0.030 ^d	3.16 ± 0.060 ^c	8.18 ± 0.050 ^a
Alkaloids	65 ± 0.030 ^a	31.16 ± 0.050 ^d	43 ± 0.050 ^c	52 ± 0.040 ^b
Tannins	1.11 ± 0.020 ^b	1.46 ± 0.040 ^a	0.82 ± 0.030 ^c	0.7 ± 0.026 ^d
Oxalate	0.13 ± 0.062 ^b	0.21 ± 0.060 ^{a,b}	0.18 ± 0.020 ^{a,b}	0.24 ± 0.045 ^a
Cyanide	0.015 ± 0.004 ^a	0.019 ± 0.004 ^a	0.021 ± 0.006 ^a	0.018 ± 0.004 ^a

Values with different letters are significantly different $p < 0.05$. Values are means ± standard deviations (SD) of three determinations.

Phytate binds to form a complex with protein and minerals that affect the digestibility, solubility and functionality of protein and the absorption of minerals (iron and calcium) [25,28]. So, the low content of phytate in seeds is appreciated. The lowest amount of phytate was found in the ash gourd (2.15 mg/100 g) compared to the pumpkin (2.48 mg/100 g), musk melon (3.17 mg/100 g) and watermelon (9 mg/100 g) seeds. The result obtained was much lower than those reported by [25] for the watermelon and [29] for musk melon seeds. The saponin content of the ash gourd (7.13 mg/100 g) and musk melon (8.18 mg/100 g) seeds was quite similar to each other but varied from the pumpkin (2.18 mg/100 g) and watermelon (3.16 mg/100 g) seeds. Saponins are extremely poisonous, as they cause hemolysis

of the blood by reacting with the sterols of the erythrocyte membrane, and also interfere with iron absorption [25,28,29]. A comparatively lower content of saponin was reported for the seeds of the pumpkin (3.42 mg/100 g) by [28] and musk melon (6.8 mg/g) by [29]. The content of alkaloids was found to be higher in the ash gourd seeds (65 mg/100 g), followed by the musk melon (52 mg/100 g) and watermelon (43 mg/100 g) seeds, and the least alkaloid content was recorded in the pumpkin seeds (31.6 mg/100 g). If the alkaloid content is above 20 mg/100 g, it can lead to gastrointestinal and neurological disorders [28]. The tannin content of the seed samples was 1.11 mg/100 g, 1.46 mg/100 g, 0.82 mg/100 g and 0.7 mg/100 g for the seeds of the ash gourd, pumpkin, watermelon and musk melon, respectively. The tannin content of the musk melon seeds was found to be similar to [29]. Protein bioavailability gets affected in the presence of tannins, as it forms insoluble complexes and also limits the activity of digestive enzymes. Moreover, a high tannin content is attributed to the poor palatability of food [25]. The observed level of tannins in all the seed samples was very low, and thus could not limit the absorption of the vital nutrients [28]. Oxalate interferes with mineral availability by forming insoluble salts; for example, oxalate binds to calcium and forms calcium oxalate, which causes throat irritation and the formation of kidney stones [28–30]. A minor amount of oxalate was found in the current seed samples. The lowest content was recorded in seeds of the ash gourd (0.13 mg/100 g) watermelon (0.18 mg/100 g), pumpkin (0.21 mg/100 g) and musk melon (0.24 mg/100 g). The level of oxalates in all the examined samples was lower, and thus could not lead to any health threat [28]. Among the antinutritional compounds, cyanide is considered extremely hazardous. The lowest value was for seeds of the ash gourd (0.015 mg/100 g) compared to the musk melon (0.018 mg/100 g), pumpkin (0.019 mg/100 g) and watermelon (0.021 mg/100 g). Tropical ataxic neuropathy (TAN) is a neurodegenerative disease that refers to the consumption of cyanide-containing-rich food [25,30]. Food containing 200 mg/100 g of hydrocyanic acid is considered harmful [28]. The cyanide content of our investigated seed samples was in a much lower range than the safe limit. So, all the seeds investigated in the present work could be considered safe for consumption.

3.9. Antioxidant Activity, TPC and TFC

The observed results show that the investigated seed samples had a good amount of phytochemicals. Table 5 represents the data of the percent of the DPPH radical scavenging activity (% DPPH inhibition) of the seeds with the total phenolic content (TPC) and total flavonoid content (TFC).

Table 5. Antioxidant activity, TPC and TFC of seeds (mg/100 g).

Component	Ash Gourd	Pumpkin	Watermelon	Musk Melon	Ascorbic Acid
Antioxidant activity (% DPPH)	85.11 ± 0.062 ^b	81.55 ± 0.003 ^d	80.02 ± 0.036 ^e	82.31 ± 0.055 ^c	98.09 ± 0.053 ^a
TPC (mg GAE/100 g)	176.07 ± 0.010 ^a	175.11 ± 0.020 ^c	170.86 ± 0.030 ^d	175.28 ± 0.030 ^b	
TFC (mg QE/100 g)	159.16 ± 0.020 ^a	142.82 ± 0.030 ^c	140.12 ± 0.035 ^d	148.57 ± 0.040 ^b	

Values with different letters are significantly different $p < 0.05$. Values are means ± standard deviations (SD) of three determinations.

The antioxidant capacity of any substance or compound could be evaluated by using the DPPH inhibition method. In the present work, the highest percentage of the DPPH scavenging activity was recorded for the seeds of the ash gourd (85.11%), followed by the musk melon (82.31%), pumpkin (81.55%) and watermelon (80.02%), whereas the used standard (ascorbic acid) showed the highest inhibition (98.09%). When compared with standard (ascorbic acid), the DPPH scavenging activity of all the selected seed samples was found to be highly appreciable (see Table 5). Phenolic compounds protect from cellular damage, mutagenesis and carcinogenesis via their antioxidant effect against free radicals, and this effect is due to the presence of diverse chemical structures. The phenolic

compounds can scavenge free radicals due to the presence of hydroxyl groups [5]. The highest value of total phenols was recorded for the seeds of the ash gourd (176.07 mg GAE/100 g) compared to the musk melon (175.28 mg GAE/100 g), pumpkin (175.11 mg GAE/100 g) and watermelon (170.86 mg GAE/100 g). It was earlier reported that the phenols are responsible for a higher scavenging activity than other phytochemicals [31]. Flavonoids are the most common phytochemical under the phenolic compounds in plants. They are very good scavengers due to the presence of hydroxyl groups [5]. Ash gourd seeds were found to have the highest flavonoid content (159.16 mg QE/100 g) when compared to musk melon (148.57 mg QE/100 g) and pumpkin (142.82 mg QE/100 g), whereas watermelon seeds contained the lowest content of flavonoids (140.12 mg QE/100 g) among all the seed samples. The results of the antioxidant activity of all the studied samples followed the trends of the TPC and TFC. The higher availability of the phenolic and flavonoid compounds in the musk melon seeds results in a higher antioxidant activity in terms of free radical scavenging activity. The results of this study were confirmed by the previous investigation done by [32] on different Cucurbitaceae family seeds, who reported that the higher antioxidant activity was due to the presence of higher phenolic and flavonoid contents in the seed composition.

3.10. Functional Group Analysis

FTIR is an important technique for the identification of functional groups in organic compounds. In this technique, structural differences among the samples could be compared based on the functional groups [16].

Figure 2 shows the % Transmittance versus wavelength (cm^{-1}) graph of the FT-IR spectra of seeds from 4000 to 600 cm^{-1} . Each peak represents a specific functional group present in the seeds. The observed spectrum of all the seeds did not differ significantly from each other. The absorption peaks in the range from 3850 to 3600 cm^{-1} for all seed samples were due to the O-H stretching vibration, which indicates the presence of the alcohol and phenol or polysaccharides content of the seeds [16,33]. All the seeds investigated in the present study showed the peak at 3008 cm^{-1} comes from the C-H stretching vibration of the cis allylic C = CH, denoting the presence of polyunsaturated fatty acid. The observed peaks were similar to the peak reported by [34] for pumpkin seed oil. The strong peak at 2924 cm^{-1} and the medium peak at 2856 cm^{-1} (for each studied seed sample) were due to asymmetric and symmetric stretching vibrations of the C-H bonds of the methylene ($-\text{CH}_2$) group [33]. The carbonyl (C = O) esters' stretching vibrations for lipids was observed at 1743 cm^{-1} [33]. This strong peak is related to saturated aldehyde functional groups observed in each seed sample. The higher the intensity at 1743 cm^{-1} , the more carbonylic compounds present in seeds. A similar peak was observed by [34] and [33] for pumpkin seed oil and inaja (*Maximilia maripa*) fruit, respectively. The IR spectrum presented a typical protein band: amide I (1600–1700 cm^{-1}), amide II (1500–1580 cm^{-1}) and amide III (1200–1400 cm^{-1}), referred to as the stretching and bending vibrations of the protein backbone [35]. The presence of a small peak at 1651 cm^{-1} was due to the amide I band, which is attributed to the C = O stretching vibration and some NH stretching characteristics. The medium peaks at 1528 cm^{-1} support the carbonyl group and the presence of the α -helices of amide II, which come from the C-N stretching vibrations and the N-H bending in a plane. Similar peaks for amide I and amide II were observed by [36] for musk melon seed flour and [35] for black cumin seed. The bending vibrations of methylene (CH_2) were generated at wavenumbers of 1456 cm^{-1} , while the bending vibrations of methyl (CH_3) were observed at wavenumbers of 1370 cm^{-1} [34]. The peak at 1233 cm^{-1} was for amide III. The rise of the peak is considered a complex mix of N-H bending and C-N stretching, along with a minor contribution from C-O in a plane bending and the C-C stretching vibration. The observed peak was confirmed by comparing with the peak reported by [35] for black cumin seed. The peak at 1159 cm^{-1} shows the stretching vibration of the C-O alkynes. The authors of [37] reported a similar peak for pumpkin seed oil. The peak at 717 cm^{-1} represents the overlapping of the CH_2 rocking vibration and the out-of-plane

vibration of cis-disubstituted olefins. A similar peak was reported by [37] for pumpkin seed oil.

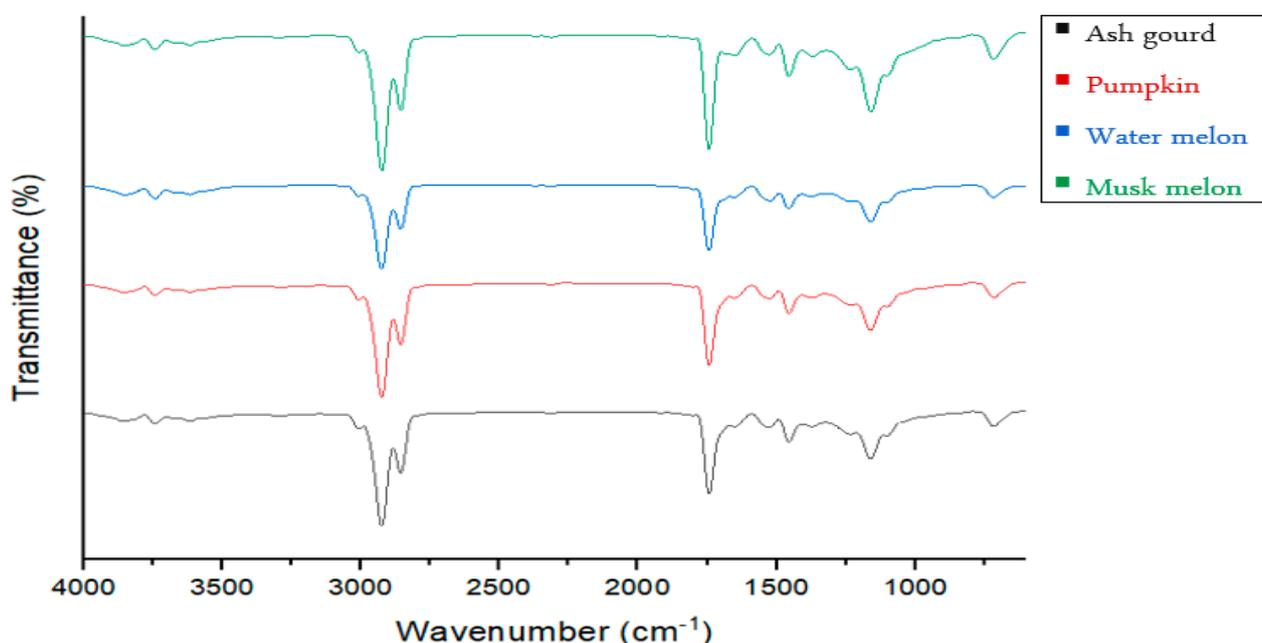


Figure 2. Fourier transform infrared spectra of seeds.

3.11. Thermal Properties

3.11.1. Thermal Transition Behavior

In differential scanning calorimetry (DSC) analysis, the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpies (ΔH) of the seed samples were recorded and are depicted in Table 6 and Figure 3.

Table 6. Thermal transition behavior of seeds.

Name of Samples	Onset (T_o) °C	Peak (T_p) °C	End (T_c) °C	Enthalpy ΔH (J/g)
Ash gourd	40.5	66.2	110.1	345.5217
Pumpkin	30.8	49.4	103.3	120.4836
Watermelon	29.8	47.0	93.3	111.4839
Musk melon	42.4	47.1	109.7	106.6942

Numerous researchers have documented the thermal changes of the isolated protein, defatted flour and starch fractions of seeds because seeds have a complex composition (fat, protein, carbohydrate, minerals, etc.). The thermal profile of our investigated seeds is quite different, since they contain a large amount of fat, which forms lipid–amylose and lipid–protein complexes [38]. During our investigation, the lowest onset temperature (T_o) ($p < 0.05$) was exhibited by watermelon seeds (29.8 °C), followed by the pumpkin (30.8 °C), ash gourd (40.5 °C) and musk melon seed (42.4 °C).

The fat content of seeds enhances the hydrophobic binding between lipids and protein molecules and hinders the hydrophobic interactions between protein molecules, thus reducing the thermal stability [39]. It was confirmed from the chemical composition that watermelon seeds contain a good amount of fat, with the highest amount of protein among the seeds. The highest T_p was credited to ash gourd (66.2 °C), which indicates that the stability and heat resistance levels of its matrix was superior to those of pumpkin (49.4 °C), musk melon (47.1 °C) and watermelon (47.0 °C) ($p < 0.05$). The stability of protein gets enhanced by a high mineral content, mainly Na, K and Ca, because the denaturation temperature gets shifted toward a slightly higher temperature. For example, Ojo Negro

seeds showed a lower peak temperature due to the presence of a lower Na content, whereas Pinto seeds showed a higher peak temperature due to the presence of higher Na and K contents [38]. Enthalpy is related to the energy required for a transition, while T_p is the temperature at which 50% of the molecules have undergone thermal transition [38]. The enthalpy (ΔH) of the seed samples was observed to be considerably dissimilar ($p < 0.05$). The highest and lowest values of enthalpy (ΔH) were recorded for ash gourd (345.5217 J/g) and musk melon (106.6942 J/g), respectively.

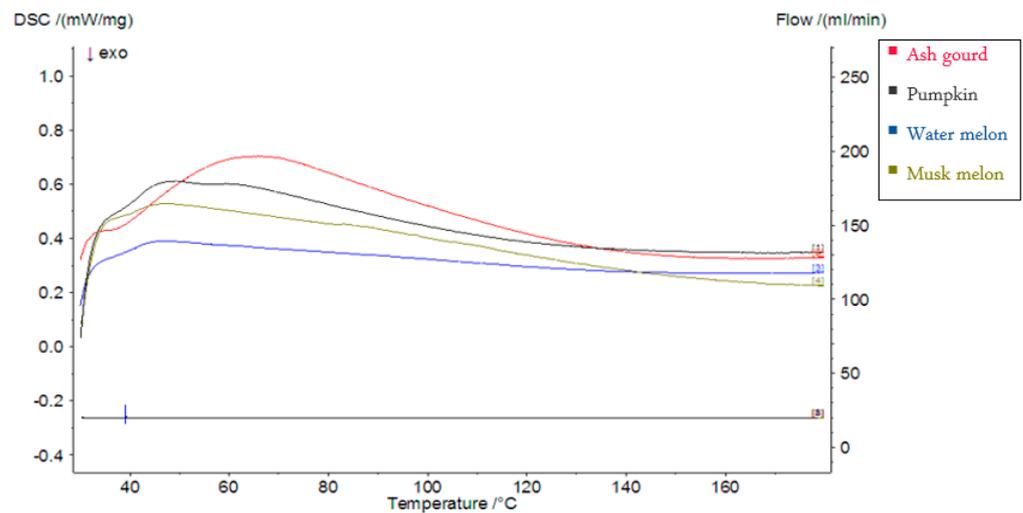


Figure 3. Thermal transition behavior of seeds.

3.11.2. Thermal Degradation Profile by Thermogravimetric and Derivative Thermogravimetric Analysis (TGA and DTG)

The TGA test was used to investigate the thermal degradation of the seeds. The recorded thermograms are shown in Figure 4, and the profile of the thermal degradation is shown in Table 7. The curve in the thermogram displays the typical reverse s-shaped curve that is characteristic of thermal degradation [40]. The TG-DTG curves indicate that the thermal degradation of the seed samples occurred in three events, namely drying, devolatilization and char degradation. The peak of the first event was recorded at 341.7, 332.8, 319.3 and 389.6 °C with a percent mass loss of 7.02, 7.41, 6.66 and 7.81% for ash gourd, pumpkin, watermelon and musk melon seeds, respectively.

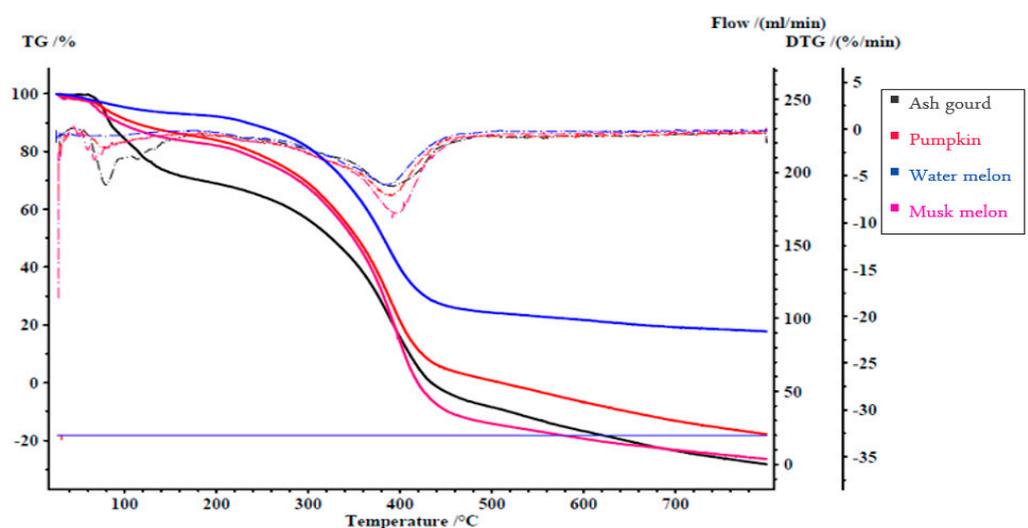


Figure 4. Thermal degradation profile by thermogravimetric and derivative thermogravimetric analysis (TGA and DTG).

Table 7. Thermal degradation profile by thermogravimetric and derivative thermogravimetric analysis (TGA and DTG).

Name of Samples	Stage	TGA Peak Temperature °C (Tp)	Mass Loss during Decomposition Stage (%)	Residue Mass at 800 °C (%)	DTG Peak Temperature °C (Tp)
Ash gourd	1	210	7.02	29	406.4
	2	390	55.61		
	3	700	8.37		
Pumpkin	1	214	7.41	11.02	386.5
	2	395	57.07		
	3	700	24.50		
Watermelon	1	218	6.66	27	385.4
	2	390	54.58		
	3	700	11.76		
Musk melon	1	210	7.81	19	393.7
	2	390	53.73		
	3	700	19.46		

The first event was the drying stage, as the mass loss observed here was due to evaporation of moisture [36,40,41]. During the drying stage, the recorded mass loss was slightly higher than the moisture content of the seed samples. The difference in mass loss might be from a mass loss during the TG drying stage, which is not only due to the loss of surface-bounded moisture in the seeds, but also due to the loss of light volatile compounds [40]. The peak observed of the second event for ash gourd, pumpkin, watermelon and musk melon seeds were at 406.4, 386.5, 385.4 and 393.7 °C with a percent mass loss of 55.61, 57.07, 54.58 and 53.73%, respectively. The second event is commonly attributed to a major mass loss due to the thermal decomposition of the organic matter in seeds [40]. This could be due to the volatilization of fragments of protein produced by thermally activated reactions (degradation) and the degradation of carbohydrates [42]. For the third and final event, the highest mass loss was observed for the seeds of the pumpkin at 24.50%, with the peak at 426.1 °C, followed by the musk melon at 19.46%, with the peak at 403.1 °C and watermelon at 11.76%, with the peak at 428.1 °C, while the lowest mass loss (8.37%) was for the ash gourd seed, with the peak at 443.6 °C. Mass loss during this event could be mainly due to the char degradation (carbonization and formation of the fixed mineral residues) of the organic matter and the liberation of HCN, the nitriles of aromatics, carbon dioxide and ethers, and which is typically denoted by the long tailing [40,41]. At the temperature of 700 °C, the residue mass of the seed samples was recorded, which was the lowest for pumpkin (11.02%), followed by the musk melon (19%), watermelon (27%) and ash gourd (29%). This might be due to the presence of ash (inorganic oxides) [40]. The DTG curve represents the rate of change of mass with respect to time. The peak of DTG was recorded at 406.4, 386.5, 385.4 and 393.7 °C for the ash gourd, pumpkin, watermelon and musk melon seeds, respectively. Mass loss was similarly the result reported by [40] for the *Citrullus Colocynthis* L. seed husk.

3.12. Morphological Analysis

The selected seed samples are classified as dicotyledon and could be divided into two parts, namely the seed coat (husk/hull) and cotyledons. The cotyledons or kernel is further divided into the endosperm and aleurone layers; the endosperm consists of a cytoplasmic network, stored protein and starch granules [5,20]. The findings of the SEM of the current investigation (See Figure 5) showed that all the seeds contained a high amount of fat, protein and fiber, and little amount of starch granules.

Smoother bodies intact to the surface confirm the presence of an abundance of fat in all seeds [43]. The results indicated that the microstructure of the pumpkin and watermelon seeds showed a homogenous, globular, cellular dense structure with a smooth surface, which was probably due to the high concentration of protein and an appreciable amount of fat. The presence of protein helps to prevent the formation of particles with a uniform wall [44]. The complex of the ash gourd seed showed a less homogenous complex, with

macromolecules of fat embedded into to the protein surface. These irregular-shaped fat bodies present on the protein matrix in a large amount indicate the highest fat content among all the seeds. The musk melon seed showed a closely related surface, with an irregular-shaped and gritty structure, which is probably due to the high carbohydrates in comparison to the other seeds. On the other side, the structure of the watermelon kernel (C) was found to be uniform, with higher agglomerates due to interparticle adhesion as compared to the other seeds. This might be because of the presence of a high protein content or a low fiber content, and their interaction with the fat constituent. The microstructure of all seeds indicated the presence of protein associated with fat and fiber constituents. On the other side, the seed of the ash gourd, pumpkin and watermelon appeared as a coarse protein matrix compared to the musk melon, probably due to the development of a thick fat layer [45]. The pumpkin and musk melon seeds showed starch granules and high dietary fiber with a cytoplasmic network [46]. Starch granules are spherical or ball-like, appearing on the surface, and such gatherings were much less in all other seeds, indicating the presence of less starch content compared to the other constituents in the seeds [33].

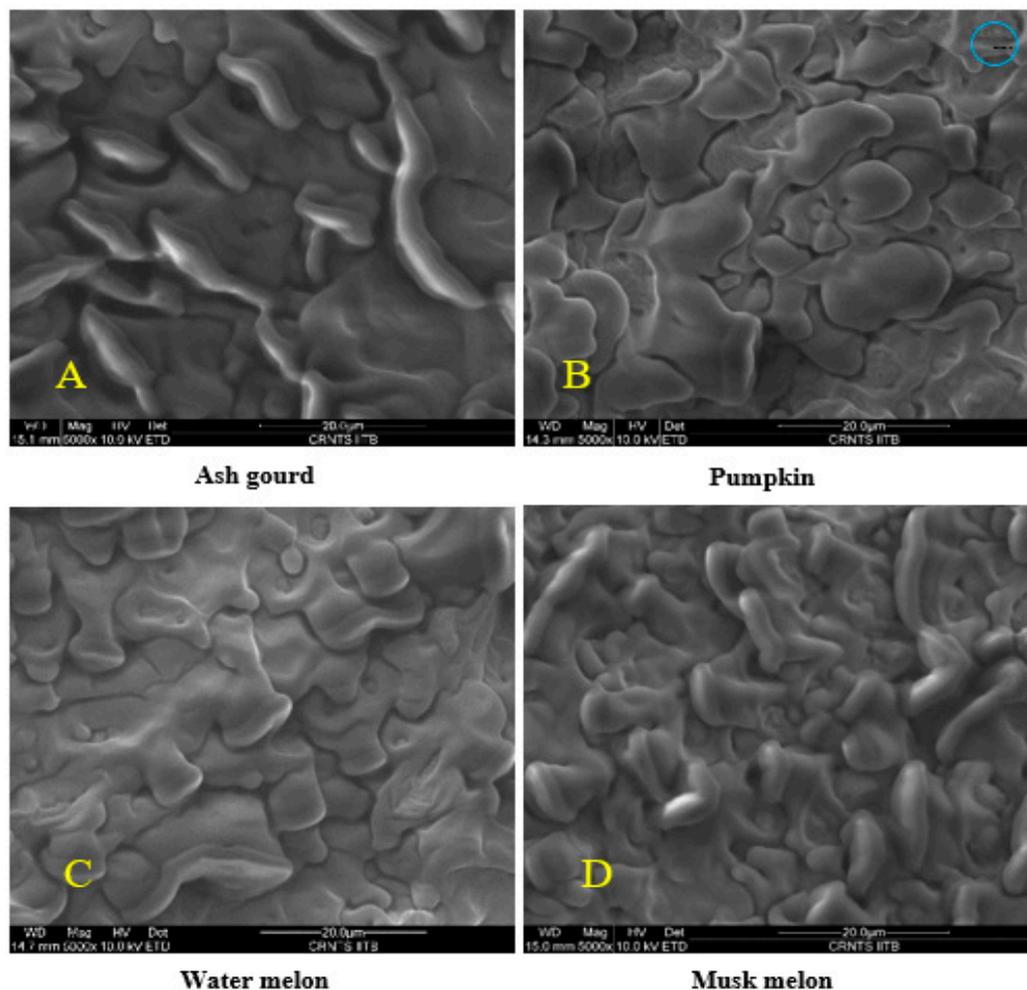


Figure 5. Microstructure (SEM) of seeds: (A) ash gourd, (B) pumpkin, (C) watermelon, (D) and musk melon at 5000 \times .

3.13. X-ray Diffraction Properties

The X-ray diffraction technique allows to distinguish the food sample as crystalline or amorphous based on the peak pattern. A, B, C and V are the four major types of X-ray diffraction patterns [33,47].

The atom's orientation in crystalline material is found to be in particular directions, thus giving sharp and definite peaks, whereas the amorphous material has no orientation

of atoms that lead to the wider and diffuse peak. The diffractogram (Figure 6) of all seed samples showed a characteristic A-type pattern, which indicates that the seeds have an amorphous or semicrystalline nature. The peak of the ash gourd, pumpkin, watermelon and musk melon seeds were at 20.81, 19.90, 20.25 and 19.75, respectively. The comparison of the peaks shows a decreased pattern of crystallinity and a significant difference in the main peaks. The degree of crystallinity of the seed samples is depicted in Table 8.

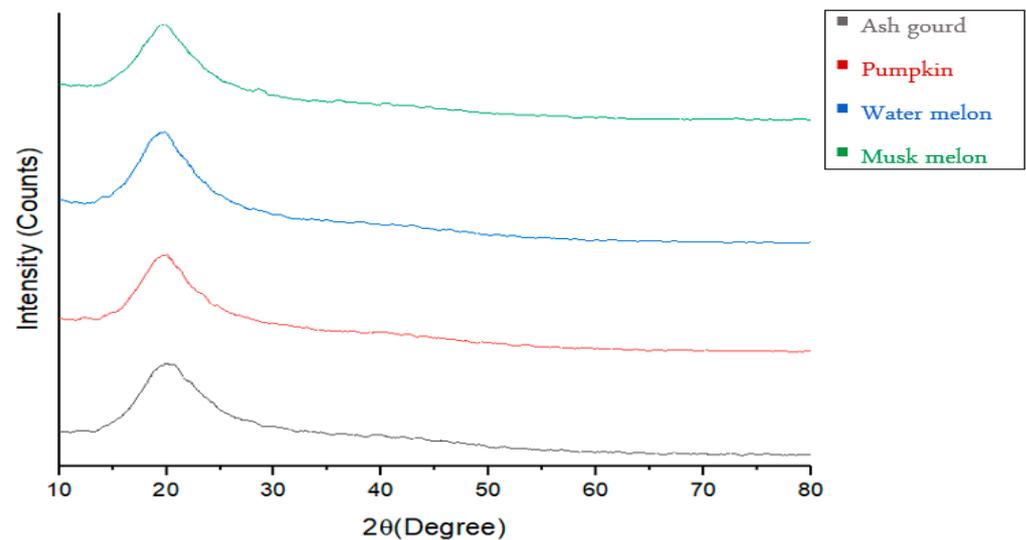


Figure 6. Diffractogram of seeds.

Table 8. Details of 2θ peaks and degree of crystallinity obtained from XRD diffraction of cucurbit seeds.

Sample	2θ (Degree)	θ (Degree)	θ (Radians)	$\cos \theta$ (Radians)	$\beta = \text{FWHM}$ (Degree)	$\beta = \text{FWHM}$ (Radians)	$D = 0.9\lambda/\beta \cos \theta$ (Radians)	Degree of Crystallinity
Ash gourd	20.8143	10.4071	0.18164	0.98355	11.2243	0.1959	0.7198	41.2415
Pumpkin	19.9067	9.95334	0.17372	0.98495	12.7034	0.22172	0.63509	36.3878
Watermelon	20.2507	10.1254	0.17672	0.98443	12.8226	0.2238	0.62952	36.0688
Musk melon	19.7523	9.87617	0.17237	0.98518	12.6097	0.22008	0.63966	36.6496

The highest value was for the seeds of the ash gourd (41.24), followed by the musk melon (36.65), pumpkin (36.39) and watermelon seeds (36.07). The findings agreed with the amorphous nature of the musk melon seed flour with an A-type pattern of [36]. The formation of glycoprotein occurs when the protein binds specially and reversibly to monosaccharides, which hinders the crystallization, and thus the peak is not sharp and definite, thereby affecting the degree of crystallinity [36,47]. The observed change in the degree of crystallinity only specifies the difference in the structural order. The observed amorphous nature of the seed samples confirms the presence of a good amount of fat and protein, with a smaller amount of carbohydrates. The amorphous structure of the investigated seeds was in agreement with [33], who studied the structural pattern of inaja flours.

4. Conclusions

The cucurbit seeds are generally discarded as waste, but today's requirement of waste management and new and cheaper sources of nutrition has provoked the world's interest in these seeds. The present investigation revealed that the selected cucurbit seeds had nutrients indicating their application-oriented use in supplementary food. Protein was found in a good range (from 18 to 35.43%), so the seeds could be used further for protein extraction and characterization. The selected seeds also contained a high amount of fat in the range from 26.4 to 56.66%. The highest amount was present in the seeds of the ash gourd (56.66%). Cucurbit seeds may be exploited as an oil source for daily

consumption. The highest content of unsaturated fatty acids was in the seeds of the ash gourd (86.54%). The high UFA content of seed oils enables the prevention of coronary heart-related diseases. In addition, cucurbit seeds contain adequate quantities of minerals and amino acids required for normal human growth. Cucurbit seeds are found to be a potentially rich source of vitamins, especially vitamin C, B3, A and E. The range of vitamins was from 61.17 to 80.62 mg/100 g, 3.29 to 5.10 mg/100 g, 80.17 to 98.47 mg/100 g and 470.26 to 523.14 mg/100 g for vitamin C, B3, A and E, respectively. The seeds could be used for the preparation of protein isolate and concentrate, as the seeds contain a good amount of protein. A significant amount of antioxidants, especially in the ash gourd seeds (85.11%), may play role in the prevention of diseases. The other selected seeds also had good antioxidant activity (above 80%) and could be a high-value source of natural antioxidants. The seeds as a whole could be used for fortification because they had thermal stability, while the presence of protein, fat and fiber, as well as the amorphous nature of the seeds, was confirmed by SEM and XRD, respectively. The degree of crystallinity obtained from the XRD analysis was found to be the highest for the ash gourd seeds among all the selected seeds, which supports the presence of a high amount of fat and protein in the seeds. The literature revealed that the *Cucurbitaceae* seeds are an excellent source of phytonutrients. Further studies are required to optimize the oil extraction for the better retention of the phytonutrients at a cheaper rate so that common people can also afford the application.

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Abbreviations

ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemist
Aw	Water Activity
C	Chroma
CI	Color Index
ΔE	Total Color Difference
DTG	Derivative Thermogravimetric
DSC	Differential Scanning Calorimetry
DPPH	1,1-diphenyl-2-picrylhydrazyl
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FLW	Food Losses and Waste
FTIR	Fourier Transform Infrared

HPLC	High-Performance Liquid Chromatography
H°	Hue Angle
RH	Relative Humidity
SEM	Scanning Electron Microscope
SD	Standard Deviation
TFC	Total Flavonoid Content
TGA	Thermogravimetric Analysis
TPC	Total Phenolic Content
US\$	United States of America Dollar
USF	Unsaturated Fatty Acids
XRD	X-ray Diffraction

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