European Journal of Nutrition & Food Safety

10(4): 224-241, 2019; Article no.EJNFS.2019.024
ISSN: 2347-5641

Amino Acid Profile, Functional Properties and *In-vitro* Antioxidant Capacity of *Cucurbita maxima* and *Cucurbita mixta* Fruit Pulps and Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. Author AWO designed the study, carried out the chemical analysis and performed the statistical analysis. Author DTO managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Author OMO supervised the study and managed the analyses of the study. Author NVE designed and supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2019/v10i430117
Editor(s):
(1) Dr. Diego A. Moreno-Fernández, Food Science and Technology Department, Campus Universitario de Espinardo – Edificio 25, E30100-Espinardo, Murcia, Spain.
Reviewers:
(1) Gayatri Gawade, Bharati Vidyapeeth Dental College and Hospital, India.
(2) K. Manorama, PJTS Agricultural University, India.
(3) Ahmed Emam, Egypt.
Complete Peer review History: http://www.sdiarticle4.com/review-history/51899

Original Research Article

Received 12 August 2019
Accepted 15 October 2019
Published 23 October 2019

ABSTRACT

The study aimed at evaluating the amino acid profile, fatty acid profile, anti-nutritional factors, functional properties and *in-vitro* antioxidant activities of *Cucurbita maxima* and *Cucurbita mixta* fruit pulps and seeds. Freshly harvested *Cucurbita mixta* and *Cucurbita maxima* fruit were processed into flour as; Pa: *Cucurbita maxima* pulp flour, Pi: *Cucurbita mixta* pulp flour, Sa: *Cucurbita maxima* seed flour and Si: *Cucurbita mixta* seed flour and were evaluated for amino acid profile, fatty acid profile, anti-nutritional factors, functional properties and *in-vitro* antioxidant activities. Triplicate data were analysed and means were separated using New Duncan Multiple Range Test (NDMRT) at $p<0.05$. The protein content of the fruit pulps and seeds flour samples were 12.77 g/100 g (Pi), 13.22 g/100 g (Pa), 15.37 g/100 g (Sa) and 16.86 g/100 g (Si). Total

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essential amino acid was 5.33 mg/100 g of protein (Pa) 6.62 mg/100 g of protein (Pi), 9.85 mg/100 g of protein (Sa) and 14.61 mg/100 g of protein (Si). Total essential amino acid of Pi and Si are significantly higher ($p>0.05$) than Pa and Sa. Statistically, saturated fatty acid and polyunsaturated fatty acid of the Pi and Si were significantly lower ($p<0.05$) than Pa and Sa respectively. Antioxidant activities against ABTS* (Pi) is significantly higher ($p>0.05$) than Pa and with no significant differences ($p<0.05$) between ABTS values of Si and Sa. While there is a significant difference ($p<0.05$) between Si and Sa as well as Pi and Pa antioxidant activities against DPPH* respectively. In conclusion, *Cucurbita* seeds and pulps flour contains high protein content, appreciable amount of essential minerals, lower Na/K molar ratio of less than one and they also exhibit a good free radical scavenging abilities against DPPH*, ABTS* and ability to reduce Fe^{3+} to Fe^{2+} with high content of total phenol and flavonoid.

Keywords: Cucurbita; amino acid profile; fatty acid profile; anti-nutrient factor; antioxidant.

1. INTRODUCTION

The *Cucurbitaceae* family, also known as Cucurbits represents a large group of plants, which consists of approximately 130 genera and 800 species [1]. They grow in tropical, subtropical, arid deserts and temperate locations. Most commonly cultivated species are musk or sweet melon (*Cucumis melo L.*), cucumber (*Cucumis sativus L.*), watermelon (*Citrullus lanatus T*) and summer or winter squash (*Cucurbita ficifolia B.*, *Cucurbita mixta P.*, *Cucurbita moschata P.*, and *Cucurbita maxima D.*) [2]. The *Cucurbita* genus is regarded as a major vegetable crop in many regions of the world [3]. In 2005, it altogether accounted for about 20.4 million tonnes of crop production [4]. *Cucurbita maxima* and *Cucurbita mixta* belong to the same family *Cucurbitaceae* with the common name winter squash or pumpkin [5]. China is the world's leading producer of *Cucurbita maxima* and *Cucurbita mixta*, contributing about 30% of the world's production [6]. In West Africa, the seeds are planted in March to April. They are planted directly into the beds at about 120-200 cm between rows and between seeds. 3 seeds are planted per hole at a depth of 3 cm and they mature after 5 months [7]. In many parts of Nigeria, *Cucurbita maxima* and *Cucurbita mixta* are grown mainly for their fruits and leaves, which are consumed as a vegetable [8]. A previous study on the nutrient composition of the leaves of *Cucurbita maxima* and *Cucurbita mixta* by Duke and Ayensu [9], showed that the leaves contain 43.8% protein, which is comparable with that of soybean [10]. *Cucurbitaceae* seed oils also have anti-helminthic properties [11]. Considering this high nutritional value of *Cucurbitaceae* seeds, they are still underexploited industrially in Nigeria and other developing countries. Hence, the present study aimed at evaluating the amino acid profile, fatty acid profile, anti-nutritional factors, functional properties and *in-vitro* antioxidant activities *cucurbita maxima* and *cucurbita mixta* fruit pulps and seeds.

2. MATERIALS AND METHODS

2.1 Samples Collection

Cucurbita mixta seeds were obtained from Gbongan junction market, Ayedade Local Government Area (LGA), Osun state, Nigeria while *Cucurbita maxima* was obtained from Owena market, Ifedore LGA, Ondo state, Nigeria. The seeds of the two *Cucurbita* species were cultivated at Ponpola village, Ede-south LGA, Osun state, Nigeria.

Both fruits were harvested separately at the point of maturity prior for sample preparation for various analysis. The food materials were authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Nigeria.

All chemical reagents used were of analytical grade.

2.2 Sample Preparation

2.2.1 Preparation of *Cucurbita mixta* and *Cucurbita maxima* fruits pulps and seeds flour

The freshly harvested matured *Cucurbita mixta* and *Cucurbita maxima* fruits were processed into pulps and seeds flour. *Cucurbita* fruits were manually peeled (using stainless table knife), sliced into smaller pieces and the pulp was separated from the seeds, oven-dried at 60°C for 15 h (using hot-air oven; Plus 11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire,

UK), milled using laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and sieved using a 60 mm mesh sieve (British Standard) to obtain fine flour of pulp and seeds powder respectively. The flour was packed in a plastic container, sealed and stored at room temperature (~27°C) until required for use.

2.2.2 Determination of proximate composition of *Cucurbita* flour samples

The proximate composition (moisture content, crude fibre, crude fat, total ash, and crude protein contents) of the flour were determined using standard methods as described by AOAC [12]. Carbohydrate content was determined by difference thus:

$$Carbohydrate(\%) = 100 - (\%\ protein + \%fat + \%fibre + \%ash + \%\ moisture)$$

2.2.3 Determination of mineral composition of *Cucurbita* flour samples

Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu) and zinc (Zn) were determined using Atomic Absorption Spectroscopy (AAS Model SP9). Sodium (Na) and potassium (K) in the flour samples were determined using flame emission photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) with NaCl and KCl as the standards [12]. Phosphorus was determined using Vanado-molybdate colorimetric method. The Na/K, Ca/P, Ca/Mg, molar ratios were also calculated.

2.2.4 Determination of amino acid profile of *Cucurbita* of flour samples

The amino acid profiles of the *Cucurbita* Flour Samples were determined using the method described by Spackman et al. [13]. The sample (2.0 g) was oven-dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into Technicon Sequential Multi-Sample Amino Acid Analyser (TSM-1). Defatting of the sample was done using soxhlet extraction apparatus as described by AOAC [12]. Each defatted sample was hydrolysed and loaded into TSM-1 analyser. The analyser then separated and analysed free acidic, neutral and basic amines, which lasted for 76 hours. Norleucine was employed as the internal standard. Ten micro-litters (10 µL) of the standard solution mixture of the amino acid were also loaded into the analyser. Values of both the standard and samples were recorded and printed out as chromatogram peaks by the chart recorder. The net height of each peak produced on the chromatogram (each representing amino acid) was measured. The half-height of each peak was located and the width of the peak at half-height was accurately measured. Approximate area of each peak was then obtained by multiplying the height with the width of the half height. All measurements were in millimetre (mm). The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated as:

$$NE = \frac{Area\ of\ peak}{Area\ of\ each\ amino\ acid\ in\ the\ standard\ mixture}$$

A constant (Sstd) for each amino acid in the standard mixture was then calculated.

$$Sstd = NEstd\ x\ mol.weight\ of\ amino\ acid\ x\ \mu mol\ AAstd$$

Then the amount of each amino acid (in g/ 100 g protein) in each diet sample was calculated as follows

$$Concentration\ of\ amino\ acid\ \left({}^{g}/_{100g\ protein}\right) = NH\ x\ \frac{NH}{2}\ x\ Sstd\ x\ \left(\frac{dilution\ x\ 160}{sample\ weight\ x\ \%N\ x\ 10\ x\ vlume\ loaded\ x\ NHx\ W_{nleu}}\right)$$

$NH = net\ height;$

$W = width\ at\ half\ height\ and\ nleu = norleucine$

Tryptophan was estimated by the ninhydrin method [14]. And the concentration of tryptophan was calculated and expressed as g/100 g protein.

2.2.5 Determination of *Cucurbita* protein fraction (globulin and albumin)

Protein globulin and albumin of defatted samples of *Cucurbita maxima* and *Cucurbita mixta* pulps and seeds flour were obtained through the process of dialysis as described by Markwell et al. [15] with some modifications. Briefly, Methylsufonylmethane (MSM) was dispersed in 0.5 M NaCl for 1 h with continuous stirring followed by centrifugation (8000 x g, 60 min at 4°C). The supernatant was clarified with Whatman No 1 filter paper and the residue discarded. The filtrate was dialyzed for 5 days at 4°C using the 6-8 kDa MWCO dialysis tube and the dialysis water was changed at least 3 times

daily. Thereafter, the content of the dialysis tube centrifuge (8000 x g, 60 min at 4°C) and the supernatant was collected as the albumin fraction. The precipitate was washed with distilled water and centrifuged under similar conditions. The precipitate was collected as the globulin protein fraction. Both fractions were freeze dried and the protein contents determined by the modified Lowry method [15].

2.2.6 Determination of fatty acids profile of *Cucurbita* oil samples

The oil samples were extracted with chloroform:methanol (2:1v/v) and non-liquid material was removed by filtration. The total extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were re-dissolved in anhydrous chloroform/methanol (19:1 v/v), and clarified by centrifugation at 10,000 x *g* for 10 min. Tranmethylation was performed using 14% (w/v) boron triflouride (BF_3) in methanol [16]. Fifty nanograms of heptadecanoic acid (internal standard) and 1 mL aliquot of each sample were transferred to a 15 mL Teflon-lined screw-cap tube. After removal of the solvent by nitrogen gassing, the samples were mixed with 0.5 ml of BF_3 reagent (14% w/v), placed in a warm bath at 100°C for 30 min and cooled. After the addition of saline solution, the transmethylated fatty acids were extracted into hexane. A calibration mixture of fatty acid standards was processed in parallel. Aliquots of the hexane phase were analyzed by gas chromatography. Fatty acids were separated and quantified using a Hewlett-Packard gas chromatograph (5890 Series II) equipped with a flame-ionization detector. Two microliter aliquot of the hexane phase was injected in split-mode onto a fused silica capillary column (Omegawax: 30 m x 0.32 mm ID, Supleco, Bellefonte, PA). The injector temperature was set at 200°C, detector at 230°C, oven at 120°C initially, then 120-205°C for 18 min. The carrier gas was helium and the flow rate was approximately 50 cm/sec. Electronic pressure control in the constant flow mode was used. The internal standard (heptadecanoic acid, C17:0) and calibration standards (NuCheck, Elysian, MN) were used for quantitation of fatty acids in the lipid extracts. The fatty acids reported representing the average of three determinations. Other fatty acid parameters calculated were total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), PUFA/SFA, n-6/n-3, LA/ALA, and MUFA/SFA [12].

2.2.7 Determination of anti-nutrient composition of *Cucurbita* flour samples

Determination of tannin content was done using the method of Medoua et al. [17]. Determination of oxalate content was done using the method described by Ukpabi and Ejidoh [18]. Determination of phytate content was done using the method described by Abulude [19]. Determination of saponin content was determined using the method described by Obadoni and Ochuko [20].

2.2.8 Determination of functional properties *Cucurbita* flour samples

Water absorption capacity (WAC) was determined using a modified method by Adebowale et al. [21]. Oil absorption capacity (OAC) was determined using the procedure of Sathe and Salunkhe, [22]. Foaming capacity (FC) of the flour samples were determined according to the methods described by Deshpande et al. [23]. Emulsion capacity (EC) was determined using the procedure described by AOAC [12]. Least gelation (LG) properties of the samples were determined by employing the method of Adebowale et al. [24]. Packed bulk density was determined according to the method of Asoegwu et al. [25]. Loose bulk density of each sample was determined by the method of Onweluzo and Nwabugwu [26].

2.2.9 Determination of *In-vitro* antioxidant capacity of *Cucurbita* of flour samples

2.2.9.1 DPPH radical scavenging assay

The scavenging effect of the samples on 2, 2-Diphenyl-1-picryhydrazyl (DPPH) free radical was measured according to the method of Aluko and Monu [27]. Each sample (10 mg) was dissolved in 1 mL of buffer (0.1 M sodium phosphate buffer, pH 7.0 containing 1% (w/v) Triton X-100). DPPH was dissolved in methanol to a final concentration of 100 µM. *Cucurbita* flour samples (100 µl) were mixed with 100 µL of the DPPH solution in the 96-well plate to a final assay concentration of 1 mg/mL and incubated at room temperature in the dark for 30 min. The absorbance values of the blank, Glutathione (GSH) (control) and samples were measured at 517 nm. The control consisted of sodium phosphate buffer in place of the protein fractions sample while Glutathione (GSH) was used as the positive control. The percent DPPH radical

scavenging activity of the samples was determined using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{A}_{517}\ of\ sample}{\text{A}_{517}\ of\ blanc}\right) \text{x } 100$$

2.2.9.2 Ferric-reducing antioxidant property (FRAP)

The ferric reducing power of *Cucurbita* flour samples was determined according to the modified method of Zhang et al. [3]. Experimental sample or Glutathione (GSH) was dissolved in 0.2 M phosphate buffer, pH 6.6; an aliquot (250 µL) was mixed with 250 µL of the buffer and 250 µL of 1% potassium ferricyanide solution. The mixture was thoroughly mixed using a vortex machine and heated at 50°C for 20 min. After incubation, 250 µL of 10% trichloroacetic acid (TCA) was added followed by 50 µL of 0.1% ferric chloride dissolved in double distilled water and then 200 µL of double distilled water was added. The solution was allowed to stand for 10 min at room temperature, after which it was centrifuged at 1000 × g for 10 min. An aliquot (200 µL) of the supernatant was transferred to a clear bottom 96-well plate and the absorbance was measured at 700 nm.

2.2.9.3 ABTS radical scavenging activity

The ABTS scavenging ability of *Cucurbita* flour samples was determined according to the method described by Re et al. [28]. The ABTS was generated by reacting a (7 mM) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/L, final concentration) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. About 0.2 of the appropriate dilution of the extract was then added to 2.0 mL of ABTS solution and the absorbance was read at 732 nm after 15 minutes. The ABTS scavenging activity was calculated using the following equation:

$$ABTS^{*}\ scavenging\ ability\ (\%) = \frac{Abs._{ref} - Abs._{sample}}{Abs._{ref}} x\ 100$$

2.2.9.4 Determination of total phenol content

The total phenol content (TPC) was determined by Folin–Ciocalteu assay [29] using gallic acid as standard. Fifty microliter of aqueous extract solution containing 0.5 mg of aqueous extract was dispensed into a test tube, 50 µL of distilled water and 500 µL of Folin–Ciocalteu reagent was added respectively into the test tube and shaken thoroughly, after 3 min, 400 µL of 7.5% sodium carbonate solution was added and the mixture was incubated at 45°C in a water bath for 40 min. Absorbance was measured at 765 nm against blank. The same procedure was repeated to all standard gallic acid solution (0.1 mg/mL). The blank is a mixture of 100 µL of distilled water, 500 µL of Folin-Ciocalteu reagent and 400 µL of 7.5% sodium carbonate. The total phenolic content was expressed as gallic acid equivalent per gram of sample (mg of GAE/g sample) through the calibration curve of gallic acid and calculated as follows;

$$Total\ phenolic\ content\ (mg\ GAE/g) = \frac{Abs._{sample}\ x\ Conc._{standard}\ \left(\frac{mg}{ml}\right)}{Abs._{standard}\ x\ Conc._{sample}\left(\frac{mg}{g}\right)}$$

2.2.9.5 Determination of total flavonoid

Total flavonoid content of *Cucurbita* flour samples was determined by aluminum chloride colorimetric assay [30] with slight modification. About 500 µL of methanol was added to 10 mL flask containing 500 µL of aqueous extract. To this 50 µL 10% $AlCl_3$ and 50 µL of 1M CH_3COOK was added respectively. The total volume was made up to 2500 µL with distilled water. The solution was then incubated at room temperature for 30 min. Absorbance was read against blank at 415 nm with spectrometer. (JENWAY 6305, United Kingdom). The flavonoid was calculated using quercetin as standard [30].

$$Total\ flavonoid\ content\ (mg\ QE/g) = \frac{Abs._{sample}\ x\ Conc._{standard}\ (mg/ml)}{Abs._{standard}\ x\ Conc._{sample}(mg/g)}$$

$Abs_{standard}$ is the absorbance of the solution containing 500 µL quercetin, About 50 µL 10% $AlCl_3$ and 1M CH_3COOK. Blank is the mixture of 500 µL of distilled water, 500 µL of methanol, 50 µL distilled water and 1M CH_3COOK.

2.3 Statistical Analysis

All determinations were done in triplicates and data generated was analyzed by one-way analysis of variance (ANOVA) using SPSS (21.0) software. Means were compared by the New Duncan's Multiple Range Tests (NDMRT); significance was accepted at the 5% level. Graphs were plotted using GraphPad Prism 6.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of *Cucurbita mixta* and *Cucurbita maxima* Pulp and Seed Flours

The proximate chemical compositions of *Cucurbita mixta and Cucurbita maxima* pulps and seeds are presented in Table 1. The moisture content of the samples was 8.06 g/100 g (Pi), 7.13 g/100 g (Pa), 5.91 g/100 g (Si) and 6.47 g/100 g (Sa). The moisture content of the *Cucurbita mixta* pulps were significantly ($p>0.05$) higher when compared with the *Cucurbita maxima* pulp. Also, the moisture content of the *Cucurbita* pulp was significantly ($p>0.05$) higher when compared with the *Cucurbita* seeds. Comparatively, the moisture content of the *Cucurbita* flour samples of this present study were within the acceptable limit recommended for flour (<10%) by the FAO [31]. Moisture content is a function of the drying time and the loading depth during the drying operation. It is well established that high moisture content of flour sample indicates increased susceptibility to spoilage and thus reduce shelf life [31]. In comparison with other findings, it was observed that the moisture content of the *Cucurbita* species in this present study were lower to the values (25.51 g/100 g – 74.06 g/100 g) reported for *Cucurbita pepo, moschata* and *maxima* species [32,33]. In contrast, the values were higher than 4.40 g/100 g reported by Karanja et al. [34] for *Cucurbita maxima* seeds and pulp. The differences between the moisture contents observed in the present study and other reports could be attributed to the relative humidity, soil nature and processing methods. The low moisture contents observed in these *Cucurbita mixta and Cucurbita maxima* pulp and seed flour samples could enhance the storage stability of the flour by reducing biochemical reactions, preventing mould growth, and microbial activities in the flour samples. This finding agreed with the reports of Aremu et al. [35] and Anuonye et al [36] who reported that low moisture content of flour prevents food spoilage.

The crude fiber content of the *Cucurbita* flour samples was 3.63 g/100 g (Pi), 4.15 g/100 g (Pa), 3.75 g/100 g (Si) and 5.77 g/100 g (Sa). From this research, the crude fiber contents of *Cucurbita mixta* pulp flour were significantly ($p<0.05$) lower than *Cucurbita maxima* pulp. However, the crude fiber content of *Cucurbita* seed was significantly ($p>0.05$) higher than *Cucurbita* pulp. Comparatively, the values of crude fibers reported in this present study were lower compared with values (16.84 g/100 g) reported by Alfawaz et al. [37] and higher than the reports (0.47 - 2.1 g/100 g) of Karanja et al. [34] for *Cucurbita maxima* seeds. Consumption of food rich in fibre are recommended for the young population to avoid insulin resistance syndrome and to decrease the incidence of other metabolic diseases such as obesity and cardiovascular diseases [38]. The pulps and seeds of *Cucurbita mixta* and *Cucurbita maxima* species in the present study could serve as good sources of dietary fibre. Fibre content of vegetables varies owing to many factors including growth conditions (climate, soil), time of harvest and species [39].

The protein content of the *Cucurbita* flour samples was 12.77 g/100 g (Pi), 13.22 g/100 g (Pa), 15.37 g/100 g (Sa) and 16.86 g/100 g (Si). From this research, the protein contents of *Cucurbita mixta* pulp flour were significantly ($p>0.05$) higher than *Cucurbita maxima* pulp. Also, the protein contents of the *Cucurbita* seeds flour samples were significantly ($p>0.05$) higher than *Cucurbita* pulp flour samples. In comparison with other study, the protein content of this study *Cucurbita* seed flour samples was similar when compared with the report (16.54 g/100 g) of Kim et al. [32] for *Cucurbita maxima* peel. Nutritionally, food with the high protein contents is reported to be suitable for the growth and development of infants and repair of tissues in adults [40].

3.2 Mineral Composition of *Cucurbita mixta* and *Cucurbita maxima* Pulp and Seed Flours

The mineral compositions of *Cucurbita mixta* and *Cucurbita maxima* pulps and seeds flour samples are presented in Table 2. The mineral compositions of the *Cucurbita* species flour samples had phosphorous as the most abundant elements, with values ranging from 0.20 to 0.30 mg/100g, while sodium had the least concentration in both *Cucurbita* species flour as well as in seed and pulp samples respectively. Statistically, the concentration of sodium, phosphorus, calcium, iron, zinc and manganese in *Cucurbita mixta* pulp flour (Pi) were significantly higher ($p>0.05$) than in *Cucurbita maxima* pulp flour (Pa): and also were significantly higher ($p>0.05$) in *Cucurbita mixta* seeds flour (Si) than in *Cucurbita maxima* seeds flour (Sa). Hence, in this present study, it could be inferred that both species of *Cucurbita* pulp and seeds contain an appreciable amount of essential minerals like magnesium, calcium and iron which are very essential for the body.

Magnesium content is a component of chlorophyll and it is an important macro-mineral element in connection with ischemic heart disease and calcium metabolism in bones, in addition to its coenzyme activity [41]. Calcium plays an important role in bone formation and maintenance of healthy teeth. It is noteworthy that, the calcium content in both species of *Cucurbita* pulp and seed of the present study was relatively high thus could supply the required RDA [42].

The Na/K ratio in the body is of great importance for prevention of high blood pressure. Na/K ratio of less than one is recommended [43]. In the present study, both species of *Cucurbita* pulp and the seed had Na/K ratio lower than one, which is within the recommended standard. Both these two ions aid in maintaining the water balance in the body and blood composition [44]. Children, women of reproductive age and pregnant women need food with high iron content since they are most vulnerable to micronutrient deficiency and anaemia.

Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats. In this study, it is evident that both the pulp and seed could supply the required RDA of 8 mg Fe/day for men (19 years and older) and women over 50 years, 18 mg/day for the girls and women of about 11 - 50 years old [43]. The high content of iron in both species of *Cucurbita mixta* pulp and seeds makes them a potential source of iron for the vulnerable groups.

3.3 Amino Acids Composition of *Cucurbita mixta* and *Cucurbita maxima* Pulp and Seed Flours

The results of the amino acid profile and predicted nutritional qualities of *Cucurbita mixta,* and *Cucurbita maxima* pulps and seeds flour samples are presented in Tables 3 and 4. The total essential amino acid for *Cucurbita maxima* pulps and *Cucurbita mixta* pulp was 5.33 g/100 g of protein (Pa) and 6.62 mg/100 g of protein (Pi). Statistically, the total essential amino acid of Pi is significantly higher ($p>0.05$) than Pa. Also, the total essential amino acid between *Cucurbita maxima* seed (Sa) and *Cucurbita mixta* seed (Si) was 9.85 mg/ 100 g of total protein (Sa) and 14.61 mg/100 g of protein (Si). Statistically, the total essential amino acid of Si is significantly higher ($p>0.05$) than Sa. Comparatively, the *Cucurbita* pulp and seed of species understudy has a good essential amino acid profile as these values are significantly higher ($p>0.05$) than the total essential amino acid value of 2.68 mg/ 100 g recommended for children under two years [45]. This increase implies that *Cucurbita* pulp and species under study can be used in enrichment of diets to avoid Protein-Energy-Malnutrition (PEM) in the infant which has continued to pose challenges in developing countries. This, according to other researchers, is due to poor feeding practices and low-quality protein commonly associated with plant-based single diets [46,47,48].

The predicted nutritional qualities of *Cucurbita mixta, Cucurbita maxima* pulps and seeds shows that the total sulphur-containing amino acid, total aromatic amino acids, total branch chain amino acid, total essential amino acid, predicted biological value and total amino acid of *Cucurbita mixta* is significantly ($p>0.05$) higher than *Cucurbita maxima* and a similar trend was observed for seeds sample. This implies that the *mixta* species contains more predicted nutritional qualities than *maxima* species. Comparatively, the results of the present study were similar to the findings of Ojiako et al. [49] who showed that *Cucurbita* species are rich sources of arginine, isoleucine, leucine and valine (branch chain amino acid) which helps in production of nitric oxide thereby lowering blood pressure and production of insulin which helps in management of diabetes mellitus respectively [50].

Table 1. Proximate chemical composition (g/100 g) of *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Samples	Moisture	protein	Fat	Fibre	Ash	Carbohydrate
Pi	8.06^{a} ±0.01	13.22^{a} ±0.01	3.74±0.01^{a}	3.63±0.01^{b}	5.13±0.02^{b}	66.28±0.03^{b}
Pa	7.13^{b} ±0.02	12.77^{a} ±0.03	3.67^{b} ±0.01	4.15^{a} ±0.04	5.26^{b} ±0.03	67.04^{a} ±0.02
Si	5.91^{b} ±0.01	16.86^{a} ±0.03	4.02^{a} ±0.01	3.75^{b} ±0.01	6.15^{b} ±0.03	63.33^{a} ±0.02
Sa	6.47^{a} ±0.02	15.37^{a} ±0.01	3.86^{b} ±0.01	5.77^{a} ±0.01	6.85^{a} ±0.03	62.07^{a} ±0.02

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

Table 2. Mineral composition (mg/ 100 g) of *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Samples	Na	K	Ca	Mg	P	Fe	Zn	Na/K	Ca/P	Ca/Mg
Pi	0.08 ±0.02[a]	0.20 ±0.01[a]	0.13 ±0.04[a]	0.24 ±0.02[a]	0.30 ±0.04[a]	0.14 ±0.03[a]	0.11 ±0.01[a]	0.40[a]	0.43[b]	0.54[b]
Pa	0.02 ±0.01[b]	0.11 ±0.01[b]	0.11 ±0.01[a]	0.12 ±0.01[b]	0.20 ±0.02[b]	0.08 ±0.02[b]	0.01 ±0.00[b]	0.18[b]	0.55[a]	0.92[a]
Si	0.10 ±0.03[a]	0.31 ±0.01[a]	0.19 ±0.03[a]	0.39 ±0.07[a]	0.42 ±0.01[a]	0.21 ±0.01[a]	0.15 ±0.02[a]	0.32[a]	0.45[b]	0.49[b]
Sa	0.08 ±0.02[b]	0.29 ±0.02[b]	0.17 ±0.03[b]	0.26 ±0.01[b]	0.34 ±0.02[b]	0.22 ±0.01[a]	0.12 ±0.02[b]	0.28[b]	0.50[a]	0.65[a]

Means (±SEM) with different alphabetical superscripts in the same row are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima sedes

Table 3. Amino acids composition (g/ 100 g of Protein) of *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Amino Acids	*Cucurbita* pulps		*Cucurbita* seeds		*Adult	*Children
	Pi	Pa	Si	Sa		
Essential Amino Acid (EAAs)						
Leucine	0.83[a]	0.72[b]	1.84[a]	1.65[b]	3.9	7.3
Isoleucine	0.76[a]	0.67[b]	1.23[a]	1.06[b]	2.0	3.1
Methionine	0.32[a]	0.25[b]	0.87[a]	0.57[b]	1.5	2.7
Lysine	0.28[a]	0.15[b]	0.58[a]	0.37	3.0	6.4
Phenylalanine	0.96[a]	0.85[b]	2.55[a]	1.46[b]	2.5	6.9
Valine	1.26[a]	1.19[b]	3.47[a]	2.27[b]	2.6	3.8
Tryptophan	0.82[a]	0.67[b]	1.46[a]	0.93[b]	0.4	1.25
Threonine	0.66[a]	0.37[b]	1.35[a]	0.68[b]	1.5	3.7
Histidine	0.73[a]	0.46[b]	1.26[a]	0.86[b]	-	1.0
ΣEAAs +Histidine	6.62[a]	5.33[b]	14.61[a]	9.85[b]	-	-
Non-Essential Amino Acid (NEAAs)						
Alanine	0.42[a]	0.37[b]	1.28[a]	1.15[b]	-	-
Arginine	0.66[a]	0.56[b]	0.75[b]	0.78[a]	1.0	-
Cysteine	1.27[a]	1.23[b]	2.26[a]	1.46[b]	-	-
Glutamic acid	1.36[b]	1.97[a]	3.70[a]	2.47[b]	-	-
Serine	1.45[a]	1.25[b]	2.74[a]	2.18[b]	-	-
Proline	1.13[a]	0.93[b]	2.58[a]	1.76[b]	-	-
Tyrosine	0.93[a]	0.86[b]	4.68[a]	1.24[b]	-	-
Glycine	0.65[a]	0.58[b]	1.18[a]	0.74[b]	-	-
ΣNEAA	7.87[a]	7.75[b]	19.17[a]	11.78[b]	-	-

Means (±SEM) with different alphabetical superscripts in the same row are significantly different at P<0.05
*Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds*RDA of essential amino acids ((mg/100g b.w)) for Adult and Children (<5 yrs.) [62]*

Table 4. Predicted nutritional qualities of *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Amino Acids	*Cucurbita* pulps		*Cucurbita* seeds	
	Pi	Pa	Si	Sa
Predicted nutritional qualities				
ARG/LYS	2.36[b]	3.73[a]	1.29[b]	2.10[a]
TSAAs	1.59[a]	1.48[b]	3.13[a]	2.03[b]
TArAAs	1.89[a]	1.71[b]	7.23[a]	2.71[b]
TBCAAs	2.85[a]	2.58[b]	6.54[a]	4.98[b]
TEAAs	6.62[a]	5.33[b]	14.6[a]	9.85[b]
TNEAAs	7.87[a]	7.75[b]	19.17[a]	11.78[b]
EAA/NEAA	0.84[a]	0.69[b]	0.76[b]	0.84[a]
BV	70.22[a]	75.81[b]	75.92[a]	72.84[b]
TAAs	14.49[a]	13.08[b]	33.78[a]	21.63[b]

Means (±SEM) with different alphabetical superscripts in the same row are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds. ARG /LYS: Arginine/ Lysine; TSAAs: Total Sulphur Containing Amino Acids (Methionine + Cysteine); TArAAs: Total Aromatic Amino Acids (Phenylalanine + Tyrosine); TBCAAs: Total branch chain amino acids (Valine + Leucine + Isoleucine); TEAAs: Total essential amino acid; TNEAAs: Total non-essential amino acid; BV: Biological value; TAAs: Total amino acids

3.4 Fatty Acids Profile of *Cucurbita mixta* and *Cucurbita maxima* Pulp and Seed Flours

Fatty acid profiles of *Cucurbita mixta* and *Cucurbita maxima* pulps and seeds flour sample are presented in Table 5. The results showed that the total saturated fatty acid (SFA) of *Cucurbita* pulp species under study was 26.01 mg/100 g (Pi) and 30.07 mg/100 g (Pa), while that of the seed samples was 34.44 mg/100 g (Si) 36.68 mg/100 g (Sa) respectively. For total polyunsaturated fatty acid, the values were 5.94 mg/100 g (Pi) and 7.10 mg/100 g (Pa) for *Cucurbita* pulp species, while that of the *Cucurbita* seed species samples was 9.41 mg/100 g (Si) and 9.74 mg/100 g (Si) respectively. The total monounsaturated fatty acid values of the *Cucurbita* pulp species flour samples were 8.64 mg/100 g (Pi) and 9.61 mg/100 g (Pa), while that of the *Cucurbita* seed species samples was 11.06 mg/100 g (Sa) and 11.12 mg/100 g (Si), respectively. Statistically, saturated fatty acid and polyunsaturated fatty acid of the *Cucurbita mixta* pulp (Pi) flour samples and *Cucurbita mixta* seed (Si) flour samples were significantly lower ($p<0.05$) than *Cucurbita maxima* pulp (Pa) flour samples and *Cucurbita maxima* seed (Sa) flour samples respectively. Comparatively, PUFA+MUFA/SFA ratio are all greater than 0.5 and are tending toward one (1.0) approximately. This implies the samples under study contain more good fats than the bad fats [50].

Table 5. Fatty acid profile (%) of oil *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Fatty acids	*Cucurbita* pulps		*Cucurbita* seeds	
	Pi	Pa	Si	Sa
Saturated (SFA)				
Caprylic acid (C8:0)	0.18^{b}	0.33^{a}	0.34^{b}	0.84^{a}
Capric acid (C10:0)	0.17^{b}	0.29^{a}	0.26^{b}	0.53^{a}
Lauric acid (C12:0)	5.22^{b}	6.38^{a}	6.79^{b}	7.57^{a}
Myristic acid (C14:0)	0.35^{b}	0.44^{a}	0.84^{a}	0.62^{b}
Palmitic acid (C16:0)	5.18^{b}	6.23^{a}	7.25^{b}	7.95^{a}
Margaric acid (C17:0)	0.68^{a}	0.85^{a}	1.48^{a}	0.96^{b}
Stearic acid (C18:0)	14.23^{b}	15.37^{a}	17.36^{a}	17.85^{b}
Behenic acid (C22:0)	0.06^{b}	0.18^{a}	0.12^{b}	0.36^{a}
∑SFA	26.01^{b}	30.07^{a}	34.44^{b}	36.68^{a}
Monounsaturated (MUFA)				
Palmitoleic acid (C16:1)	1.07^{b}	1.28^{a}	2.44^{a}	1.55^{b}
Oleic acid (C18:1)	7.57^{b}	8.33^{a}	8.68^{b}	9.51^{a}
∑MUFA	8.64^{b}	9.61^{a}	11.12^{a}	11.06^{b}
Polyunsaturated (PUFA)				
Linolenic acid (C18:3)	3.27^{b}	4.24^{a}	5.32^{b}	6.14^{a}
Linoleic acid (C18:2)	2.53^{b}	2.78^{a}	3.63^{a}	3.33^{b}
Arachidonic acid (C20:4)	0.14^{a}	0.08^{b}	0.46^{a}	0.27^{b}
∑PUFA	5.94^{b}	7.10^{a}	9.41^{b}	9.74^{a}
PUFA/MUFA	0.69^{b}	0.74^{a}	0.85^{b}	0.88^{a}
PUFA/SFA	0.23^{b}	0.24^{a}	0.24^{b}	0.27^{a}
(PUFA+MUFA)/SFA	*0.56^{b}*	*0.56^{a}*	*0.60^{b}*	*0.57^{a}*

Means with different alphabetical superscripts in the same row are significantly different at $P<0.05$
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

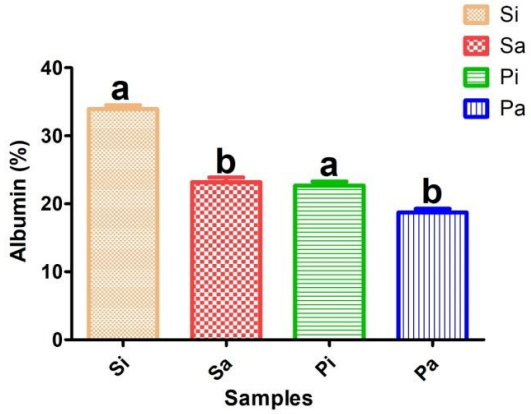

Fig. 1(a). Albumin fraction of *Cucurbita* pulps and seeds flour samples
Means with different alphabetical superscripts are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

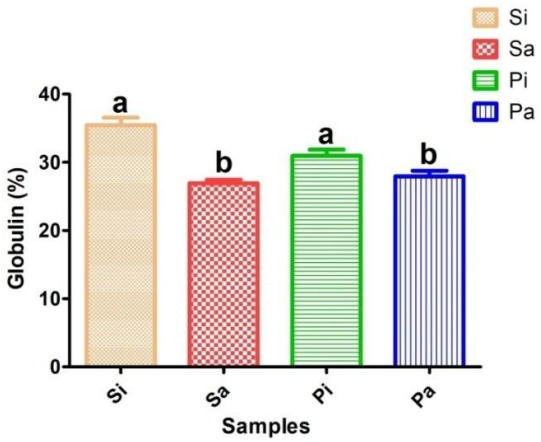

Fig. 1(b). Globulin fraction of *Cucurbita* pulps and seeds flour samples
Means with different alphabetical superscripts are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

3.5 Protein Fraction of *Cucurbita mixta* and *Cucurbita maxima* Pulp and Seed Flours

The protein fractions (albumin and globulin) of *Cucurbita mixta* and *Cucurbita maxima* pulp and seeds flour samples are presented in Fig. 1 (a-b). The globulin content was 28.77% (Pa), 31.86% (Pi), 27.43% (Sa) and 36.56% (Si). Statistically, the globulin content in *Cucurbita mixta* pulp and seed flour samples are significantly ($p>0.05$) greater than *Cucurbita maxima* pulp and seed flour samples. A similar trend of the increase was also observed in the albumin content of both *Cucurbita* species under study. And this implies that *Cucurbita mixta* contains more globulin and albumin content than *Cucurbita maxima* species.

3.6 Anti-nutritional Factors of *Cucurbita mixta* and *Cucurbita maxima* Pulp and Seed Flours

Table 7 shows the results for the anti-nutritional content of *Cucurbita mixta* and *Cucurbita maxima* pulps and seeds flour samples. The use of *Cucurbita mixta* and *maxima* species as food for man and animal has limiting factors such as storage and presence of anti-nutritional factors. Anti-nutritional factors lower the nutritional value

of a food by lowering the digestibility or bioavailability of nutrients. The anti-nutritional factors found in *Cucurbita mixta* and *maxima* species include phytates, oxalates, tannins and saponin. Some of these do serve as a defensive mechanism against pests and diseases. The oxalates are a defence mechanism and a storage reserve for calcium [51].

The anti-nutritional content of the *Cucurbita mixta* and *maxima* species were generally low and below critical values. Phytate values were 0.16 mg/100 g (Pa) and 0.18 mg/100 g (Pi). Comparatively, there is no significant difference ($p<0.05$) between Pa and Pi. However, the values of tannin were 0.03 mg/100 g (Pi) and.20 mg/100 g (Pa). Comparatively, the values of Pa were significant ($p>0.05$) higher than Pi. Similarly, the same trend was observed among *Cucurbita mixta* and *maxima* seed flour samples. It has been reported by authors that processing decreases the anti-nutritional content of foods. Tannins are known for their ability to form insoluble complexes with proteins thereby reducing the digestibility of food proteins [52,53]. The presence of tannins in food can, therefore, lower feed efficiency, depress growth, decrease iron absorption, damage the mucosal lining of the gastrointestinal tract, alter excretion of cations, and increase excretion of proteins and essential amino acids). Tannins also decrease palatability, cause damage to intestinal tract, and enhance carcinogenesis [36].

3.7 Functional Properties of *Cucurbita mixta* and *Cucurbita maxima* Pulp and Seed Flours

The packed bulk density was 0.64 g/ml (Pi) and 1.83 g/ml (Pa) for the pulp of both species of *Cucurbita* under study and 0.63 g/ml (Sa) and 0.68 g/ml (Si) (Table 7). Statistically, the bulk density of Pa is significantly ($p>0.05$) higher than Pi and Si is greater than Sa. These values are low and low bulk density has both nutritional and economic significance as more of the products thereof can be eaten resulting in high energy and nutrient density [54,55]. Bulk density is also important in the packaging requirement and material handling of the complementary diet [56]. The result of the present findings is similar with the report of Thierry et al. [57] for *Cucurbita maxima* seeds.

The result of Water Absorption Capacity (WAC) was 1.45 ml/g (Pa), 2.35 ml/g (Pi), 2.20 ml/g (Si) and 2.29 ml/g (Sa). Comparatively, Pi is significantly ($p>0.05$) greater than Pa and Sa is greater than Si. While water absorption was 1.45 ml/g (Pa), 1.92 ml/g (Pi), 1.67 ml/g (Si) and 1.73 ml/g (Sa). Statistically, Pi is significantly ($p>0.05$) greater than Pa and Sa is greater than Si. Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC) are index of the maximum amount of water that a food product can absorb and hold or retain. WAC gives an indication of the amount of water needed to form a gruel that results to gelatinization. Lower water absorption is desirable for making thinner gruels that will enhance more in-take of nutrients [58]. Flours with both high WAC values hold large amounts of water during preparation into gruels and thus become voluminous with low energy and nutrient density [59]. The result showed that *Cucurbita mixta* WAC and OAC where both lower in pulp compared with *Cucurbita maxima*. However, a different trend of reduction was observed in seed samples as *Cucurbita maxima* WAC and OAC where greater than *Cucurbita mixta* seeds sample. The result of the present findings is similar with the report of Thierry et al. [57] for *Cucurbita mixta* seeds.

Foaming Capacity (FC) was 10.28% (Pi) and 18.93% (Pa) for *Cucurbita mixta* and *Cucurbita maxima* pulp flour while the seeds value was 11.85% (Si) and 13.35% (Sa) respectively. Comparatively, the foaming capacity of Pa and Sa are significantly ($P>0.05$) higher than that of Pi and Si respectively. The ability of the flours to form foam depends on the presence of the flexible protein molecules which may decrease the surface tension of water [23,57] and the solubility of protein. Foam stability is important since success of a whipping agent depends on its ability in maintaining the whip as long as possible. There is a positive correlation between foaming capacity and foaming stability. These results are different to those reported by Kempka et al. [60] stipulating that in general, proteins that exhibit low foaming capacity show good stability and vice versa. This could be due to the presence of other foaming agent like saponin which can increase the stability of the foam. The use of flours as food ingredients depends on the water-flour interaction, which determines the rehydration.

3.8 *In-vitro* Antioxidant Activities of *Cucurbita mixta* and *Cucurbita maxima* Pulp and Seed Flours

Antioxidant properties of *Cucurbita mixta, Cucurbita maxima* pulps and seeds flour samples

is presented on Fig. 2(a – e). The ABTS values shows that there is no significant different ($p<0.05$) between ABTS values of Si and Sa (0.02 mMol/ g). However, the ABTS values of pulp were 0.02 mMol/ g (Pa) and 0.03 mMol/ g (Pi). The ABTS values of Pi is significantly higher ($p>0.05$) than Pa. And this implies that Pa have a better free radical scavenging abilities than Pi. Antioxidant is compounds that protect cells from free radicals. Free radicals, although being natural by-products of cellular metabolism, can attach to healthy cells, leading to disease in the body [61]. The generation of radical ABTS+ is the basis of one of the spectrophotometric methods that have been applied to measure the antioxidant activity of solutions of pure substances, aqueous solutions and beverages [62]. The DPPH free radical scavenging activities of seed was 39.08% (Sa) and 41.20% (Si) while the DPPH free radical scavenging activities of pulp was 45.64% (Pa) and 47.10% (Pi). There is a significant difference ($p<0.05$) between Si and Sa as well as Pi and Pa respectively. Also it was observed that Cucurbita *mixta* contains more DPPH than *Cucurbita maxima* for both seeds and pulp respectively. The total phenol content was 6.80 mg GEA/ g (Sa) 13.01 mg GEA/ g (Si), 33.04 mg GEA/ g (Pi) 1.34 mg/g, 42.71 mg GEA/ g (Pa). The total phenol content of Si was significantly higher ($p>0.05$) than Sa, also the total phenol content of Si is significantly higher ($p>0.05$) than Si. A similar trend was observed for flavonoid. Ferric reducing antioxidant potential of Si is significantly higher ($p>0.05$) than Sa. This finding could be attributed to phytochemicals, antioxidant and bioactive compounds that were significantly presents in these experimental samples [50,66].

Table 6. Anti-nutrients (mg/ 100 g) in *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Samples	Pi	Pa	Si	Sa	*CV
Phytate	0.18^{a}±0.01	0.16^{a}±0.02	0.19^{a}±0.01	0.20^{a}±0.02	5-6 g/100g
Oxalate	0.12^{a}±0.01	0.11^{a}±0.03	0.14^{a}±0.03	0.13^{a}±0.01	0.25 g/100g
Tannin	0.03^{b}±0.01	0.20^{a}±0.01	0.40^{a}±0.01	0.27^{b}±0.01	-
Saponin	0.23^{a}±0.01	0.21^{a}±0.04	0.24^{b}±0.01	0.33^{a}±0.01	-
Phytate/mineral (Ca, Zn & Fe) molar ratios					
*Phytate/calcium	0.084	0.088	0.061	0.071	0.24
*Phytate/zinc	0.161	1.576	0.125	0.164	15.00
*Phytate/iron	0.109	0.169	0.076	0.077	>1.00
*Phy*Ca/Zn	0.001	0.004	0.001	0.001	200.00

Means (±SEM) with different alphabetical superscripts in the same row are significantly different at P<0.05
*Key: Pi: Cucurbita mixta pulps; Pa: Cucurbita maxima pulps; Si: Cucurbita mixta seeds; Sa: Cucurbit maxima sedes *Critical molar ratios Phytate:calcium = 0.24 [63]. Phytate:zinc = 15 [62] Phytate:iron = > 1 [64] phytate: calcium/zinc > 200 [65]*

Table 7. Functional properties of *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Samples	Loose B. density (g/ml)	Packed B. density (g/ml)	Emulsion capacity (%)	Foaming capacity (%)	Least Gelation (%)	Protein solubility (%)	OAC (ml/g)	WAC (ml/g)
Pi	0.45^{b}	0.64^{b}	19.57^{b}	10.28^{b}	8.75^{a}	11.73^{a}	1.92^{a}	2.35^{a}
	±0.00	±0.00	±0.01	±0.01	±0.01	±0.06	±0.02	±0.03
Pa	0.93^{a}	1.83^{a}	56.30^{a}	18.93^{a}	0.90^{b}	8.47^{b}	1.45^{b}	1.45^{b}
	±0.48	±0.01	±0.10	±0.55	±0.00	±0.15	±0.00	±0.00
Si	0.46^{a}	0.68^{a}	21.26^{ab}	11.85^{b}	9.85^{ab}	12.36^{a}	1.67^{b}	2.20^{b}
	±0.00	±0.00	±0.02	±0.01	±0.01	±0.03	±0.01	±0.01
Sa	0.44^{b}	0.63^{b}	24.79^{a}	13.35^{a}	10.38^{a}	13.37^{b}	1.73^{a}	2.29^{a}
	±0.00	±0.01	±0.01	±0.01	±0.01	±0.15	±0.02	±0.01

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds. OAC: Oil absorption capacity; WAC: Water absorption capacity; Loose B. Density: Loose bulk density; Packed B. density: Packed bulk density

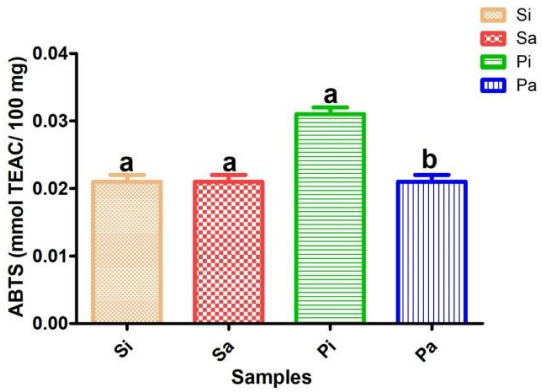

Fig. 2(a). ABTS Activities of *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Means with different alphabetical superscripts are significantly different at P<0.05 Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

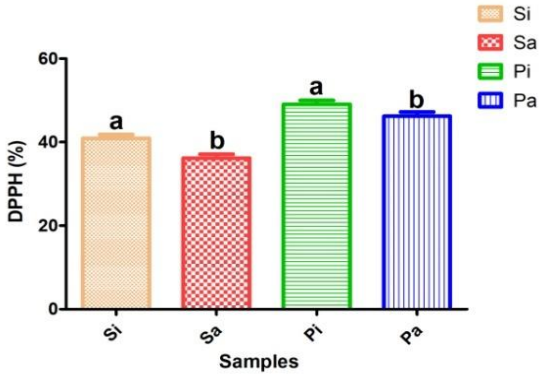

Fig. 2(b). DPPH free radical scavenging ability *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Means with different alphabetical superscripts are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

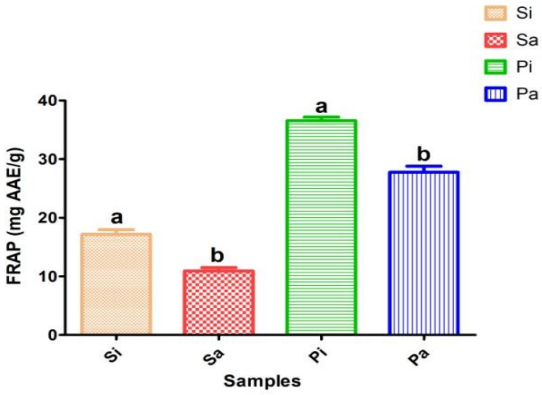

Fig. 2(c). FRAP inhibitory activities *Cucurbita mixta, Cucurbita maxima* pulp and seed flours

Means with different alphabetical superscripts are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

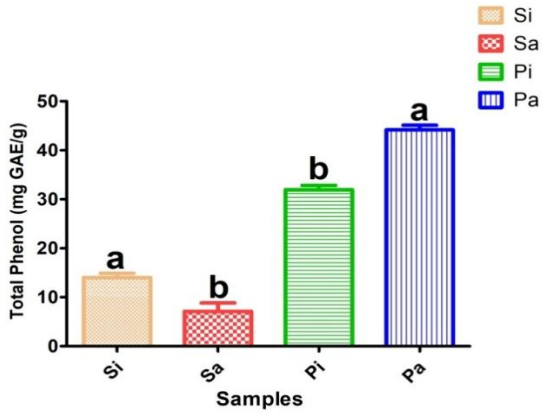

Fig. 2(d). Total phenol content *Cucurbita mixta, Cucurbita maxima* pulp and seed flours
Means with different alphabetical superscripts are significantly different at P<0.05 Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

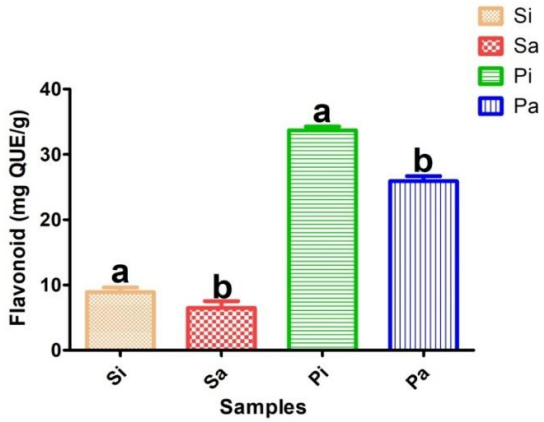

Fig. 2(e). Flavonoid content *Cucurbita mixta, Cucurbita maxima* pulp and seed flours
Means with different alphabetical superscripts are significantly different at P<0.05
Key: Pi: Cucurbita mixta pulp; Pa: Cucurbita maxima pulp; Si: Cucurbita mixta seeds; Sa: Cucurbita maxima seeds

4. CONCLUSION

Cucurbita mixta and *Cucurbita maxima* fruit pulps and seeds contains high protein content, appreciable amount of essential minerals, lower Na/K molar ratio of less than one, which makes it suitable for hypertensive patient. High content of essential amino acid profile and they also exhibit a good free radical scavenging abilities against DPPH, ABTS and ability to reduce Fe^{3+} to Fe^{2+} with high content of total phenol and flavonoid which may help in prevention of cardiovascular diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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