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RESEARCH ARTICLE

Characterization and Functional Properties of Watermelon (*Citrullus lanatus*) Seed Protein Isolates and Salt Assisted Protein Concentrates

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Abstract Watermelon (*Citrullus lanatus*) seed meal contains significant amount of extractable protein which can be used as nutritional and functional ingredients in food formulations. Alkali (0.1 M) and NaCl (0.5 M) were used to prepare protein isolates and concentrates from the defatted watermelon seed meals. Protein isolates reported protein yield of 35.15-38.27% and protein content (79.05-83.79%) which was significantly ($p \leq 0.05$) higher than the protein concentrates. SDS-PAGE of protein isolates and concentrates showed major polypeptides in the range of 74.72-110.42 kDa. Also, *in vitro* pepsin digestibility showed that most of the proteins were readily digested within 30 min of hydrolysis. Amino acids were dominated by arginine, aspartic, and glutamic acid. DSC results indicated that protein concentrates had significantly ($p \leq 0.05$) higher denaturation temperatures than protein isolates. The functional properties of concentrates in terms of solubility and surface properties were better than respective isolates. The results indicated that NaCl extracted proteins had comparatively better functional properties but their yield is significantly lower than respective protein isolates.

Keywords: watermelon seed, protein isolate, concentrate, analysis, functional property

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Introduction

The global demand for new sources of proteins has been focused mostly on oilseeds and their agro-industrial residuals, defatted oil cakes. Besides the most frequently cultivated oilseeds (soybeans, rapeseed, cottonseed, groundnuts, and sunflower seeds), watermelon (*Citrullus lanantus*) seeds have excellent potential as a source of proteins. The main proteins of watermelon seed are composed from storage salt-soluble globulins, accompanied by albumins and glutelins (1). Watermelon seeds could be used as raw material for production of high quality protein products for food formulations as nutrition supplements, and functional ingredients (2,3). Additionally, many proteins from cucurbit seed reported pharmacological activities, including anti-diabetic, anti-fungal, antibacterial, anti-inflammatory, and antioxidant activity (4-7). Exploiting underutilized food crops can boost economic development, especially in the developing countries, where protein intake is less than desirable (8).

Watermelon being one of the major underutilized fruit crops has received attention in the recent past because of the potent antioxidant lycopene in its red flesh. The fruits also contain substantial quantities of seeds which are excellent source of dietary oil and their defatted meals are exceptionally high in proteins (3,9). Watermelon proteins have been reported to contain significant quantities of glutamic acid, aspartic acid, arginine, and leucine (2,3,10). Limited reports are available on biological value, true digestibility, protein efficiency ratio, and net protein utilization of watermelon seeds (1,3). In India and some African countries the fresh watermelon seeds are mainly used for oil production. Up till now, the defatted watermelon oil cake, left as the residual after oil pressing, represents poorly utilized industrial byproducts, used mainly for animal feeding. Since this byproduct still contains many

beneficial components remained from watermelon seed, the development of techniques for value-addition e.g., protein concentrates has great economical interest, as well environmental significance (3,11). The procedures for its extraction are complicated and not commercially viable. Several factors including pH, temperature, ionic strength, solvent type, extraction time, and solid-liquid ratio have been reported to affect protein extraction of melon and watermelon seed meals (2,12). Based on end use requirements, various extraction, isolation, and fractionation procedures are followed. However, the extraction of protein rich material in alkaline solution followed by isoelectric precipitation is commonly followed for production of protein isolates or concentrates. Protein isolates are significantly more expensive when compared to concentrates. This is mainly because they are 'more pure', as they consist of about 80% protein. Isolates also have the fat content significantly lower than those of concentrates. They are also great for people on dieting, because of their fat-free and cholesterol-free contents. Protein concentrate are filtered from aqueous solutions to separate the protein from the water, and other organic materials. The acquired protein is then made into powder, or protein concentrate. However, acid precipitation and neutralization steps result in loss of solubility. This in turn affects important physico-chemical and functional properties of alkali extracted proteins. NaCl extraction can be used to manufacture purified protein ingredients from watermelon seed meal, resulting in improved physico-functional properties.

Therefore, the present study was designed to utilize watermelon seed meal for protein recovery, and to compare the quality and functional properties of proteins extracted by 2 different methods.

Materials and Methods

Materials Certified watermelon fruits (*Citrullus lanatus*) of cv. Sugar baby was procured from the Department of Horticulture, Punjab Agricultural University, Ludhiana, India while cv. Mateera was procured from Central Institute for Arid Horticulture, Bikaner, India. SDS-PAGE, pI standard protein markers, and ampholytes were purchased from Amersham Biosciences (Little Chalfont, UK). All other chemicals were of analytical reagent (AR) grade from Sisco Research Laboratories (Mumbai, India).

Preparation of defatted seed meal Ripe fruits were cut by a sharp knife and the juice was expelled using burr mill, seeds were separated from the pomace by pilot scale sedimentation system according to the methods of Kaur *et al*. (13). Seeds were dried in a cabinet dryer, dehulled, grinded, and defatted to obtain 212 μ mesh meal (10).

Preparation of protein isolates and concentrates Protein isolates were prepared with slight modification to the methods of El-Adawy *et al*. (14). Defatted meal was mixed with deionized distilled (DIDI) water in a ratio of 1:20 and adjusted to pH 10.0 using 0.5 M NaOH. The slurry was stirred for 1 h at 40ºC and then centrifuged at 10,000×g for 15 min. The supernatant was filtered through Whatman No. 1 filter paper and adjusted supernatant pH to 4.5 (isoelectric pH) using 1 M HCl. It was then allowed to settle down into 2 phases supernatant and the sediment. The supernatant was siphoned off and the precipitate was centrifuged at 5,000×g for 15 min. The pellet was collected, neutralized by 0.1 M NaOH, washed, freeze dried (LL3000; Jouan Nordic, Allerod, Denmark), milled, and sieved (60 mesh).

Defatted meal was mixed with 0.5 M NaCl in a ratio of 1:20 and the slurry was stirred for 1 h at 40ºC, and then centrifuged at 10,000×g for 15 min (Fig. 1). The supernatant (albumin and globulins) were filtered through Whatman No. 1 filter paper, dialyzed in a membrane with pore size 204 nm (Hi-Media Laboratories Ltd., Mumbai, India). After 12 water changes, the proteins were recovered from the membrane, dried on a freeze dryer (LL3000; Jouan Nordic), milled, and finally sieved (60 mesh).

Physico-chemical characteristics Protein content of the lyophilized protein samples was measured according to Kjeldhal method (15). Extracted protein was calculated from the ratio of protein fractionate to the total extracted protein. Product yield expressed as percentage was determined as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of lyophilised protein}}{\text{Weight of seed meal}} \times 100$$

Non-protein nitrogen was determined according to the methods of Naczk *et al*. (16). Surface hydrophobicity was measured fluorometrically using 8-anilino-1-naphthalene sulfonic acid magnesium salt (ANS) as a hydrophobic probe according to the methods of Paulson and Tung (17). Bulk density (g/mL) was measured as tapped mass of protein isolates or concentrates using a measuring cylinder. The total color difference (ΔE) was determined using Hunter color lab (Hunter Associates, Reston, VA, USA). Prior to analysis, calibration with black and white tiles was performed and ΔE was calculated as:

$$\text{Total color difference } (\Delta E) = [(\Delta L)^2 + (\Delta a)^2 (\Delta b)^2]^{1/2}$$

where, $\Delta L = (L_{sample} - L_{std})$, $\Delta a = (a_{sample} - a_{std})$, and $\Delta b = (b_{sample} - b_{std})$

pH solubility profile Protein solubility was determined with a slight modification to the methods of Sze-Tao and Sathe (18). Sample (2 g) was extracted with 20 mL of 0.1 M NaOH at 25ºC for 1 h under constant magnetic stirring,

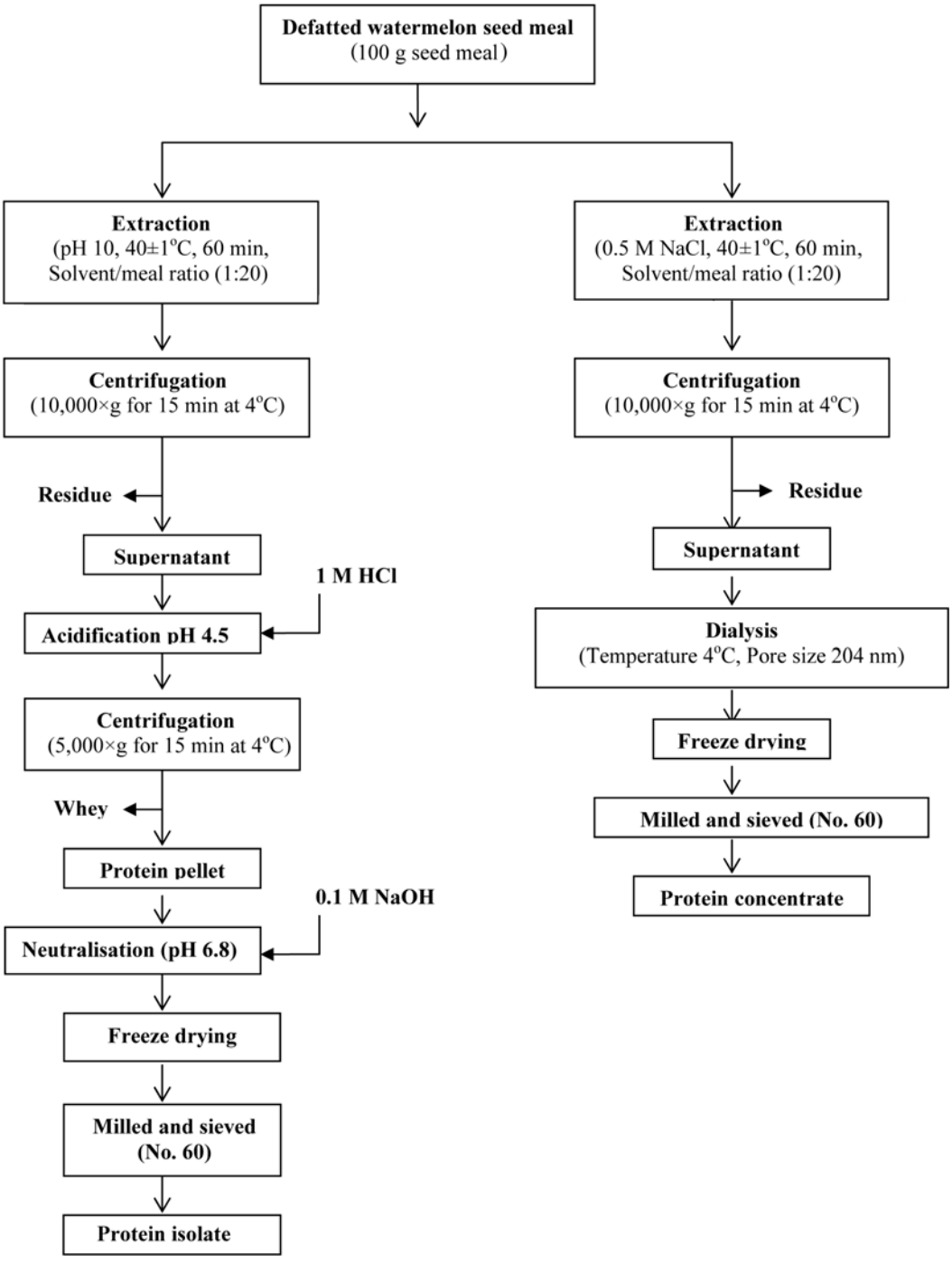

Fig. 1. Flow sheet for the preparation of protein isolates and concentrates.

centrifuged (12,600×g, 4ºC, 15 min). The supernatants were filtered through Whatman No. 1 and diluted 10-fold with DIDI water. The pH of 5 mL aliquots was adjusted to pH 1-12 with 1.0 M NaOH or 1.0 M HCl. Samples were magnetically stirred on a stirrer for 30 min (maintaining the desired pH) and then centrifuged (12,600×g, 4ºC, 15 min). The supernatants filtered through Whatman No. 1 and the protein was determined according to the methods of AOAC (15).

Gel electrophoresis SDS-PAGE was carried out with 11.2% acrylamide gels according to the procedures of Laemmli (19). Approximately 15 mg of protein samples were loaded into each well and the electrophoresis (Mini-PROTEAN 3; Bio-Rad Laboratories, Hercules, CA, USA) was allowed to run at 100 V until the tracking dye reached the bottom of the gel, removed and stained. Gels were stained with silver nitrate, employing a slight modification of Amersham Biosciences procedure (20). The stained gels

were scanned with carge-coupled device (CCD) camera (Ultra Lum, Inc., Claremont, Canada) and the molecular weights were determined using Gel Pro Analyser 3.1 (Media Cybernetics, Silver Spring, MD, USA).

Amino acid analysis Amino acid composition was determined after hydrolysis with 6 N HCl in the presence of nitrogen at 110°C for 24 h. The hydrolysates were analysed with amino acid analyser (118BL; Beckman Instruments, Fullerton, Canada) and reported as g amino acid/100 g proteins. Tryptophan was determined calorimetrically according to the methods of Spies and Chembers (21). The amino acid composition was used to calculate the nutritional value

***Essential to total amino acid*:** The proportion of essential amino acids (E) to the total amino acids (T) of the protein was calculated as:

$$\text{E/T (\%)} = \frac{\left(\begin{array}{c}Ile+Leu+Lys+Met+Cys+Phe\\+Tyr+Thr+Trp+Val+His\end{array}\right)}{\left(\begin{array}{c}Ala+Asp+Arg+Gly+Glu+His+Ile+Leu+Lys\\+Met+Cys+Phe+Tyr+Pro+Ser+Thr+Trp+Val\end{array}\right)} \times 100$$

***Amino acid score*:** The amino acid composition of test protein is compared with the standard protein

$$\text{Amino acid score} = \frac{\text{mg of amino acid/g test protein}}{\text{mg amino acid/g of FAO/WHO standard pattern}} \times 100$$

Essential amino acid (g amino acid/16 g N) pattern of the FAO/WHO standard protein is Ile-4.00, Leu-7.04, Lys-5.44, Met+Cys-3.52, Phe+Tyr-6.08, Thr-4.00, Trp-0.96, and Val-4. 96.

Differential scanning calorimetry (DSC) The thermal properties were determined according to the methods of Meng and Ma (22) using a Dupont DSC fitted with a graphic plotter and a thermal analyst 2100 system (TA Instruments, New Castle, DE, USA). Approximately (1 mg) of protein was weighed into the aluminium pan and 10 μL of 0.01 M phosphate buffer, pH 7.4, was added. The pan was hermetically sealed and heated from 25 to 200°C at a rate of 5°C/min. A sealed empty pan was used as a reference. Onset temperature (T_m), peak transition temperature or denaturation temperature (T_d), and enthalpy of denaturation (ΔH) were computed from the thermograms using Thermal Analyst 2100 system.

***In vitro* protein digestibility** *In vitro* protein hydrolysis was determined with a slight modification to the procedures of Thomas *et al*. (23). Microfuge tube containing 1.52 mL of pepsin solution (4,000 EU; 0.1% in 0.084 M HCl and 35 mM NaCl; pH 2.0) was preheated to approximately 37°C and then 0.08 mL of test protein solution (5 mg/mL) was added. The contents were mixed and immediately placed in a 37°C water bath (TC-2000; Brookfied Inc, Lorch, Germany). Samples (200 μL) were periodically removed at 0.5, 2, 5, 10, 20, 30, and 60 min and then mixed with of 70 μL $NaHCO_3$ (200 mM, pH 11) to quench enzyme activity. Electrophoresis was carried out according to determine the extent of digestion (19).

Functional properties

***Water/oil absorption capacity*:** Sample (2 g) was dispersed in 25 mL of DIDI water or oil. The contents were mixed 6 times using vortex shaker for a period of 30 min and then centrifuged at 4,000×g for 15 min. The supernatant was carefully decanted and the contents of the tube were allowed to drain at a 45° angle for 20 min and then weighed. Average gain in weight of the 4 samples was expressed as water absorption/oil capacity.

***Protein dispersibility index*:** Sample (0.5 g) was mixed in 25 mL water stirred for 30 min and centrifuged at (300×g for 10 min). The supernatant was dried (110°C for 12 h) and then weighed.

$$\text{Dispersibility (\%)} = \frac{\text{Weight of dish after drying–Weight of empty dish}}{\text{Weight of sample}} \times 100$$

***Foaming properties*:** Sample (1 g) was suspended in 50 mL phosphate buffer at pH 7 and then stirred in a mixer blender (HL1606; Phillips, Mumbai, India) for 2 min. The contents were transferred into a 250-mL measuring cylinder and the volume of foam was measured after 30 s for foam capacity. The foam stability was measured as decrease in foam volume with time. Average of 4 replicates was expressed as mean values.

***Emulsifying properties*:** Emulsifying capacity (EC) and emulsion stability (ES) were determined according to the method procedures of Pearce and Kinsella (24). Samples (0.1% protein) were prepared in 6 mL (w/v) of 10 mM phosphate buffer (pH 7.0) and 2 mL of soya oil were homogenized for 1 min in a high speed homogeniser (Yorco Scientific Industries, Delhi, India) at a speed of 10,000 rpm. Emulsion samples (50 μL) at time intervals of 0-20 min were pipetted into 5 mL of SDS solution (0.1%, w/v) and absorbance was read at 500 nm with spectrophotometer (Model UV 1601; Shimadzu, Kyoto, Japan). Absorbance at 0 min time was expressed as emulsifying capacity of protein, and decrease in absorbance was represented as emulsifying stability.

Statistical analysis All experiments were replicated at least 3 times. Mean values and standard deviations (SD) were reported when and where necessary. Analysis of

Table 1. Yield, protein, non-protein nitrogen, and functional properties of watermelon protein isolates and concentrates

Parameter	'Mateera'		'Sugar baby'	
	Protein isolate	Protein concentrate	Protein isolate	Protein concentrate
Yield (%)	$38.27^{d}\pm0.76$[1)]	$25.21^{a}\pm0.57$	$35.15^{c}\pm0.15$	$27.41^{b}\pm0.89$
Protein content (% d.b.)	$83.79^{c}\pm0.68$	$72.26^{a}\pm0.52$	$79.05^{b}\pm0.53$	$71.38^{a}\pm1.15$
Non-protein nitrogen (%)	$3.62^{ab}\pm0.09$	$3.65^{ab}\pm0.03$	$3.49^{a}\pm0.07$	$3.76^{b}\pm0.16$

[1)]Values expressed are mean±SD (n=4); Means in the same rows with different letters are significantly different at $p\leq0.05$.

variance (ANOVA) was performed and differences in mean values were determined using Duncan's multiple range test at $p\leq0.05$ level of significance using commercial statistical package SPSS 16.0 (SPSS, INC., Chicago, IL, USA).

Results and Discussion

Yield and physico-chemical composition The yield of 'Mateera' protein isolate (38.27%) was significantly ($p\leq0.05$) higher than 'Sugar baby' protein isolate (35.15%), concentrate (27.41%) and 'Mateera' protein concentrate (25.21%) (Table 1). Significant ($p\leq0.01$) decrease in yield of protein concentrates may be attributed poor solubility of prolamin and glutelin type proteins in NaCl (0.5 M) solution which resulted in their lower yield. Khalil (2) reported 8.84% yield for melon protein isolates which is considerably lower than our results. However, Giami and Isichei (25) had reported protein yield of 24.5, 28.2, and 29.4% respectively for raw, geminated, and fermented fluted pumpkin seeds. The higher yield of protein isolates and concentrates is probably due to high protein content of the watermelon seed meal (10,11).

The protein content of isolates and concentrates varied from 71.38-83.79%. Protein isolates showed significantly ($p\leq0.05$) high protein content than their respective protein concentrates. Khalil (2) reported 89.6% protein content in the melon protein isolates, whereas 61.5-70.8% protein content has been reported for fluted pumpkin protein concentrates (25). Non-protein nitrogen contents were 3.46-3.76% for watermelon protein isolates and concentrates. Results indicated significant ($p\leq0.05$) differences in the non-protein nitrogen content of protein isolates and concentrates. Protein concentrates reported high values of non-protein nitrogen than protein isolates. Differences in non-protein nitrogen have been reported in a number of plants including legume species (26). Germination significantly ($p\leq0.05$) increased non-protein nitrogen of fluted pumpkin seed protein from 0.37 to 1.48% (27).

pH solubility profile Protein isolates and concentrates of watermelon seeds had typical solubility profile with minimum solubility (10.45-15.21%) at pH 4.0 (Fig. 2).

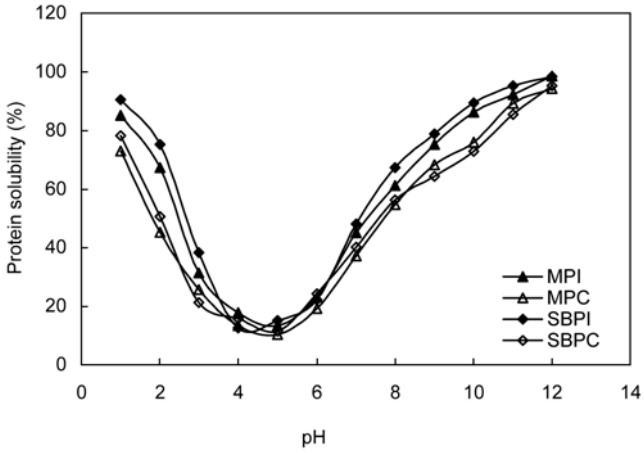

Fig. 2. pH solubility profile of 'Mateera' protein isolate (MPI), 'Mateera' protein concentrate (MPC), 'Sugar baby' protein isolate (SBPI), 'Sugar baby' protein concentrate (SBPC).

Irrespective of isolation method, protein solubility of both protein isolates and concentrates dramatically increased below pH 3 and above pH 5. These results are in agreement with the previous studies on watermelon seed protein fractionates (28). The low solubility in this pH range is due to the isoelectric pH of these proteins thus indicating acidic nature of these proteins.

Increase in protein solubility under acidic (pH<3) and alkaline (pH >7) conditions was due to gain in net negative or positive charge in proteins, resulting in interaction with water molecules (29). At pH 11, protein solubility of 95% was achieved for isolates and concentrates. Increased solubility in alkaline conditions may be attributed to the higher amounts of aspartic and glutamic acid in the protein isolates and concentrates (29). Similar reports are available on pH dependent water solubility of watermelon seed protein isolates (28).

Electrophoresis SDS-PAGE of 'Mateera' protein isolate under non-reducing conditions indicated complex polypeptide banding pattern in the range of 10.7-110.42 kDa with heavy staining in the region of 74.72-110.42 kDa (Fig. 3, lane 1). 'Sugar baby' protein isolate (Fig. 3, lane 2) resolved into a number of bands (12.63-107.66 kDa), mostly similar to polypeptides of 'Mateera' protein isolate. However, 'Mateera' protein isolate showed additional

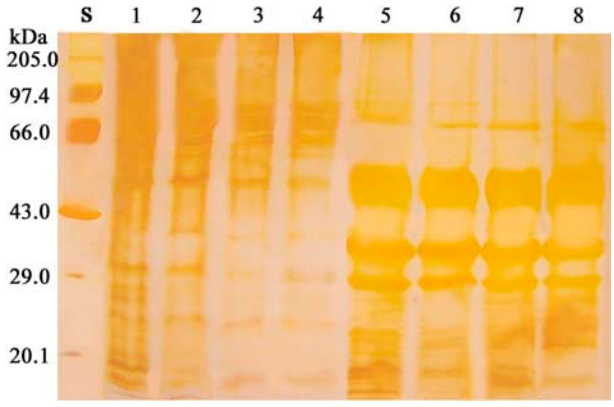

Fig. 3. SDS-PAGE (11.2%) under non-reduced (without β-merceptoethanol) and reducing (with 2% β-merceptoethanol) conditions of watermelon seed proteins. Lane S represents standard proteins, lane 1-'Mateera' protein isolate (unreduced), lane 2-'Sugar baby' protein isolate (unreduced), lane 3-'Mateera' protein concentrate (unreduced), lane 4-'Sugar baby' protein concentrate (unreduced), lane 5-'Mateera' protein isolate (reduced), lane 6-'Sugar baby' protein isolate (reduced), lane 7-'Mateera' protein concentrate (reduced), lane 8-'Sugar baby' protein concentrate (reduced)

bands with molecular weight of 27.71, 26.73, 21.73, and 17.17 kDa. SDS-PAGE of 'Mateera' protein concentrate under unreduced conditions resolved into 5 major bands (54.10-84.41 kDa) and several minor bands were found in the range of 10.66-34.50 kDa (Fig. 3, lane 3). Similar banding pattern in the range of 10.85-87.31 kDa was found for 'Sugar baby' protein concentrate (Fig. 3, lane 4). The SDS-PAGE of protein isolates and concentrates under unreduced conditions showed that a portion of protein remained at the top of the separating and stacking gel, indicating polypeptides possibly stabilized by disulfide linkages.

SDS-PAGE under reduced conditions showed that the polypeptides of protein isolates and concentrates dissociated into smaller polypeptides due to breakage of disulfide linkages by β-merceptoethanol. Under reduced conditions 'Mateera' protein isolates (Fig. 3, lane 5) resolved into a number of polypeptides ranging from 7.70-74.72 kDa with strong bands at 51.65, 29.29, and 12.04 kDa. 'Sugar baby' protein isolate (Fig. 3, lane 6) resolved into a similar banding pattern with sharp bands at 51.30, 28.59, and 24.17 kDa. The banding pattern of protein isolates showed that the polypeptides of both cultivars were similar except some minor bands. 'Mateera' protein concentrate (Fig. 3, lane 7) also resolved into a number of polypeptides with molecular weight from 6.91-70.84 kDa. 'Sugar baby' protein concentrates (Fig. 3, lane 8) showed similar polypeptide banding pattern with 4 major bands in the range of 16.17-28.94 kDa. Except few additional polypeptides (4-20 kDa) of protein concentrates, major polypeptides were similar to protein isolates of the 2 cultivars. The electrophoretic results showed that most of the polypeptide pattern was similar in different protein types under reduced and unreduced conditions. Previous studies on electrophoretic banding pattern under reduced conditions of watermelon proteins have showed that the principal bands were in the molecular weight range of 12.10-58.80 kDa (1,30). Four major bands of watermelon albumin and globulins constitute most of the melon seed protein had molecular weights of 33.7, 29.6, 21.6, and 12.0 kDa (1).

***In vitro* pepsin digestibility** *In vitro* digestibility of watermelon protein isolates and concentrates with pepsin was evaluated (Fig. 4). SDS-PAGE of 'Mateera' protein isolate (Fig. 4A, lane 1) showed that with increased digestion times of pepsin, the number of bands and their density progressively decreased with time. However, more than 90% digestion of 'Mateera' protein isolate took place in 45-60 min (Fig. 4A, lane 4-8). 'Sugar baby' protein isolate showed that pepsin readily digested major polypeptides within 2 min of digestion time (Fig. 4B, lane 1-8). This indicated that 'Sugar baby' protein isolate was more susceptible to pepsin hydrolysis than 'Mateera' protein isolate. 'Sugar baby' protein isolate polypeptides were resistant upto 20 min of digestion time, but further increase in digestion times considerably reduced band intensity (Fig. 4B, lane 2-8). Previous studies have also reported differences in the protein digestibility for several proteins (18,30). Protein digestibility has been reported to be affected by tannins, heat, pressure, pH, salts, linkages with carbohydrates and protein confirmations (28).

Electrophoresis of 'Mateera' protein concentrate showed rapid hydrolysis to pepsin (Fig. 4C). Major polypeptides with estimated molecular weights of 50.22-33.47 kDa were hydrolyzed within 20 min. The polypeptide with molecular weight of 12.32 was most resistant to pepsin hydrolysis even at 60 min digestion time (Fig. 4C, lane 8). 'Sugar baby' protein concentrates showed that most of the polypeptides were resistant to pepsinolysis (Fig. 4D). However, polypeptide measuring molecular weight of 34.26 kDa (Fig. 4D, lane 5) was hydrolysed at digestion time of 20 min, whereas, polypeptides with molecular weights 22.18 and 18.24 were hydrolysed at 30 min (Fig. 4D, lane 6-8). Bands with molecular weights of 22.18 and 18.24 on hydrolysis produced polypeptides of relatively lower molecular weights.

Amino acid analysis The amino acid composition of watermelon protein isolates and concentrates is presented in Table 2. Glutamic acid, aspartic acid, and arginine constituted more than 40% of the total amino acids in these protein isolates and concentrates. Leucine, cystine, aspartic, and glutamic acids were significantly ($p \leq 0.05$) higher in both protein isolates while arginine was significantly

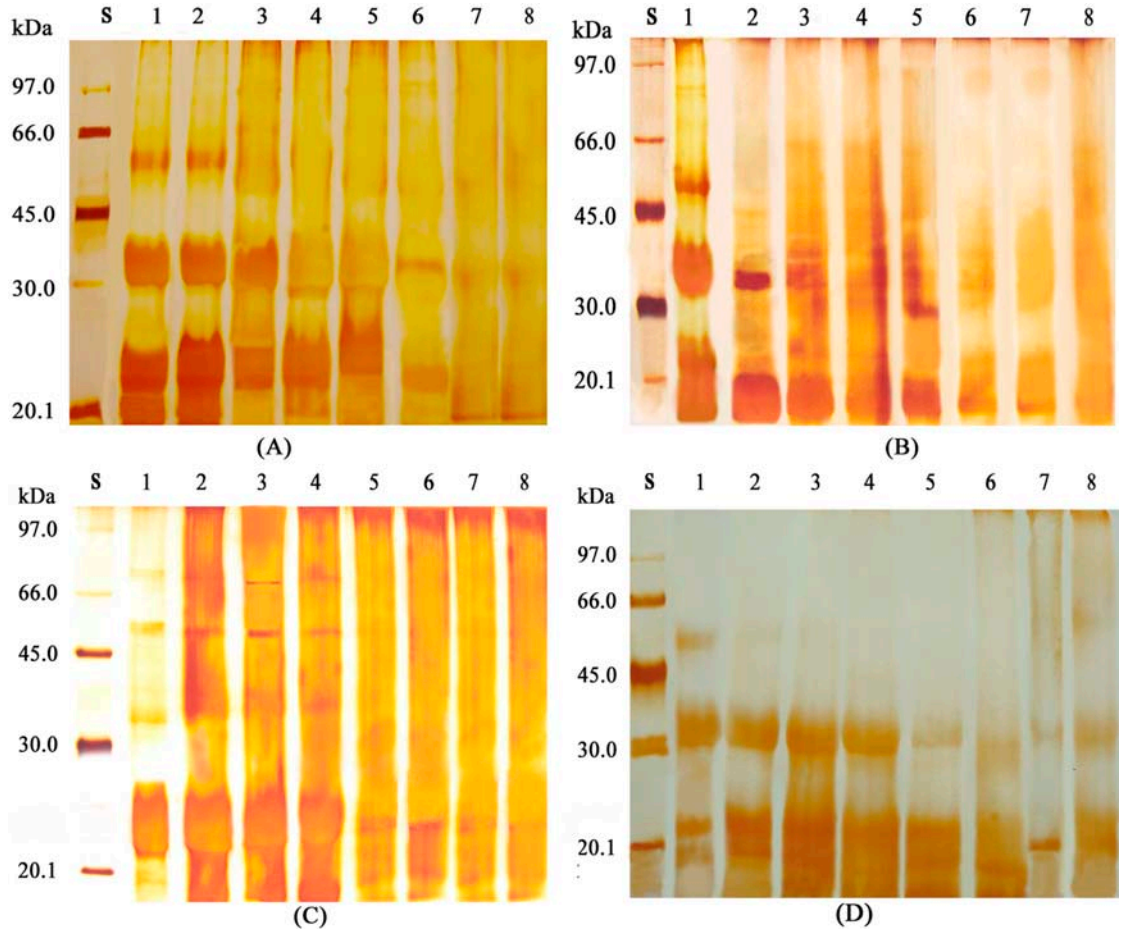

Fig. 4. SDS-PAGE (11.2%) *in vitro* pepsin digestibility of watermelon protein isolates and concentrates in the presence of 2%(v/v) β-merceptoethanol; 'Mateera' protein isolate (A), 'Sugar baby' protein isolate (B), 'Mateera' protein concentrate (C), 'Sugar baby' protein isolate (D). Protein: pepsin ratio was 500:1 (w/w). Lane S: protein Mw standards (97.0-20.1 kDa); lane 1-8: 0, 0.5, 2, 10, 20, 30, 45, and 60 min digestion, respectively (25 mg protein load in each lane)

($p \leq 0.05$) higher in protein concentrates. King and Onuora (30) also reported arginine (13.0-15.2%), aspartic acid (7.0-8.0%), and glutamic acid (14.7-16.7%) in substantial quantities of different melon seeds. Khalil (2) also reported the dominance of glutamic, aspartic acid, and arginine in melon protein isolates. When compared with the recommended FAO amino acid pattern, methionine and lysine were the most limiting amino acid followed by tryptophan and threonine, (31). Longe *et al*. (32) reported lysine and threonine as limiting amino acids in fluted pumpkin (*Telfaria occidentails*) based on amino acid score. The low level of lysine has been previously reported for melon seed proteins (3,30).

The ratio for essential to total amino acids (E/T) was in the range of 42.19-42.69 and 41.35-41.54% respectively for protein isolates and concentrates. E/T ratio above 40% is considered adequate for an ideal protein (31). The amino acid score was in the range of 79.43-85.10 and 95.57-95.61 for protein isolates and concentrates, respectively. Similar reports have been reported for fluted pumpkin (32), watermelon seed meal (3), and melon seed meal (9). The results indicated that the overall quality of the protein concentrates was significantly ($p \leq 0.05$) higher than protein isolates. The E/T ratio and amino acid score was comparatively lower to that of egg protein but was comparable to other studies reported on watermelon protein isolates, melon seed meal, and fluted pumpkin proteins (2,3,9,32). Both protein isolates and concentrates had lower lysine and leucine contents than the FAO reference pattern (31). Therefore, the protein isolates and concentrates require supplementation with complementary protein.

Thermal properties DSC thermograms of watermelon protein isolates and concentrates showed 2 distinct endothermic peaks (Table 3). Endothermic peak I of 'Mateera' protein concentrate showed onset temperature (T_o) of 72.62°C, peak denaturation temperature (T_p) of 98.17°C, offset temperature (T_c) of 103.52°C, and enthalpy (ΔH) of 4.48 J/g. However, polymer melting of 'Sugar baby' protein concentrate started at 71.64°C (T_o), with peak

Table 2. Amino acid composition of watermelon seed protein isolates and concentrates

Amino acid (%)	'Mateera'		'Sugar baby'		FAO/WHO profile
	Protein isolate	Protein concentrate	Protein isolate	Protein concentrate	
Essential					
Isoleucine	5.17^{c}±0.03[1)]	4.93^{b}±0.04	5.21^{c}±0.02	4.82^{a}±0.03	4.00
Leucine	7.09±0.13	7.27±0.07	7.19±0.09	7.22±0.11	7.04
Lysine	3.21^{d}±0.03	3.13^{c}±0.02	2.92^{a}±0.04	3.04^{b}±0.03	5.44
Methionine	0.97^{b}±0.01	1.19^{c}±0.03	0.88^{a}±0.02	1.25^{d}±0.05	2.20
Phenylalanine	5.36^{a}±0.04	5.65^{b}±0.03	5.83^{c}±0.05	5.97^{d}±0.04	2.80
Threonine	3.49^{c}±0.04	3.08^{b}±0.06	3.17^{b}±0.07	2.95^{a}±0.03	4.00
Tryptophan	0.96^{a}±0.02	1.14^{b}±0.02	1.17^{b}±0.04	1.29^{c}±0.02	0.96
Valine	4.15^{b}±0.07	3.89^{a}±0.05	4.23^{b}±0.09	3.99^{a}±0.03	4.96
Non-essential					
Arginine	14.53^{a}±0.09	19.29^{b}±0.15	15.21^{c}±0.08	18.16^{b}±0.11	
Alanine	4.89^{a}±0.07	5.18^{c}±0.02	5.05^{b}±0.06	5.35^{d}±0.04	
Aspartic acid	9.14^{c}±0.05	8.05^{a}±0.03	10.39^{d}±0.04	8.33^{b}±0.02	
Cysteine	6.31^{d}±0.09	5.43^{b}±0.04	6.09^{c}±0.04	5.22^{a}±0.07	
Glutamic acid	17.69^{d}±0.06	14.54^{a}±0.11	16.75^{c}±0.04	15.13^{b}±0.14	
Glycine	4.86^{a}±0.03	5.13^{c}±0.02	5.04^{b}±0.06	5.21^{d}±0.04	
Histidine	1.86^{c}±0.05	1.52^{a}±0.04	2.15^{d}±0.08	1.67^{b}±0.05	
Proline	4.21^{c}±0.06	4.05^{ab}±0.05	3.95^{a}±0.08	4.09^{b}±0.05	
Serine	4.87^{a}±0.04	5.08^{c}±0.05	4.96^{b}±0.04	5.14^{c}±0.06	
Tyrosine	3.89^{a}±0.03	4.12^{b}±0.06	3.96^{a}±0.04	4.07^{b}±0.07	
E/T %	42.69	41.54	42.19	41.35	
Amino acid score	79.43	95.57	85.10	95.61	

[1)]Values expressed are mean±SD (n=4); Means in the rows with different superscript are significantly different at $p \leq 0.05$.

Table 3. Thermal denaturation of watermelon seed protein isolates and concentrates (n=2)

Product	Peak-I[1)]				Peak-II			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
'Mateera' protein isolate	59.05	81.53	87.90	3.95	108.64	122.85	139.67	13.07
'Mateera' protein concentrate	72.62	98.17	103.52	4.48	125.64	139.60	148.29	14.21
'Sugar baby' protein isolate	64.57	97.48	105.09	3.37	104.22	133.66	157.25	13.78
'Sugar baby' protein concentrate	71.64	88.62	98.74	4.27	109.44	154.12	175.24	14.78

[1)]T_o, onset denaturation; T_p, peak denaturation; T_c, conclusion temperature, ΔH, enthalpy of denaturation

denaturation of 88.62°C (T_p), peak conclusion temperature of 98.74°C (T_c), and enthalpy of 4.27 (J/g). The endothermic peak I of 'Mateera' protein isolate showed T_o of 59.05 °C, T_p of 81.53°C, T_c of 87.90°C, and ΔH of 3.95 J/g whereas, 'Sugar baby' protein isolate showed T_o of 64.60°C, T_p of 97.48°C, T_c of 105.09°C, and ΔH of 3.37 J/g. These results indicated that the protein concentrates had higher enthalpy and melting temperatures than their respective protein isolates.

Similar trend was followed for the endothermic peak II of protein isolates and concentrates under this study. The endothermic peak II of 'Mateera' protein isolates showed T_o (108.64°C), T_p (122.85°C), T_c (139.67°C), and ΔH (13.07 J/g). However, 'Sugar baby' protein isolate showed higher values for T_o (104.22°C), T_p (133.66°C), T_c (157.25°C), and ΔH of 13.78 J/g. 'Mateera' protein concentrate showed T_o (125.64°C), T_p (139.60°C), T_c (148.29°C), and ΔH (14.21 J/g) whereas, for 'Sugar baby' protein concentrate showed T_o (122.78°C), T_p (154.12°C), T_c (175.24°C), and ΔH (14.87 J/g). It is evident from the results that the enthalpies of denaturation (ΔH) of 'Sugar baby' protein isolate (14.78 J/g) and 'Sugar baby' protein concentrate were higher than 'Mateera' protein isolate (13.07 J/g) and concentrate (13.78 J/g). King and Onoura (30) reported T_p (83°C) and ΔH (6.7 J/g) for melon protein whereas, melon globulin reported T_p (90°C) and ΔH (12.1 J/g). Uruakpa and Aluko (33) reported that egusi (*Colocynthis citrullus* L) proteins had T_p of 93°C and ΔH of 8.03 J/g. The variation in thermal protein denaturation is dependent on several factors including, ionic strength, type of protein, and processing conditions (29). Similar reports are available on red bean globulins (22).

Table 4. Functional properties of watermelon protein isolates and concentrates

Parameter	'Mateera'		'Sugar baby'	
	Isolate	Concentrate	Isolate	Concentrate
Dispersibility (%)	18.06^{a}±0.32[1)]	23.06^{b}±1.21	19.49^{a}±0.71	25.63^{c}±1.22
Water absorption capacity (g/g)	3.13^{a}±0.19	3.92^{ab}±0.21	3.57^{ab}±0.36	4.17^{b}±0.73
Oil absorption capacity (g/g)	2.37^{a}±0.07	2.97^{a}±0.08	2.49^{a}±0.13	3.12^{a}±0.04
Bulk density (g/mL)	0.45^{a}±0.17	0.53^{a}±0.05	0.47^{a}±0.12	0.51^{a}±0.19
Surface hydrophobicty (S_o)	98.63^{c}±1.45	73.29^{a}±1.84	88.49^{c}±1.30	68.14^{b}±1.65
Total color difference (ΔE)	27.25^{a}±1.56	33.17^{b}±1.49	25.04^{a}±1.37	32.25^{b}±1.23

[1)]Values expressed are mean±SD (n=4); Means in the same rows with different letters are significantly different at $p \leq 0.05$.

Functional properties The data pertaining to functional properties of watermelon seed protein isolates and concentrates is presented in Table 4. Dispersibility index of 25.63 and 23.06% was observed respectively for 'Sugar baby' and 'Mateera' protein concentrates. The dispersibility of protein concentrates was significantly ($p \leq 0.05$) high than 'Sugar baby' (19.49%) and 'Mateera' (18.06 %) protein isolates. The high dispersibility of concentrates is attributed to increased proportion of albumins (water soluble) in their composition. 'Sugar baby' protein concentrate showed water absorption capacity of 4.17 g/g protein, followed by 'Mateera' protein concentrate (3.92 g/g), 'Sugar baby' protein isolate (3.57 g/g) and 'Mateera' protein isolate (3.13 g/g). The water absorption capacity (WAC) of 'Sugar baby' protein concentrate and 'Mateera' protein isolate were significantly ($p \leq 0.05$) high than other protein types in this study. The WAC of melon isolates (1.44 g/g) was comparatively lower than our results (2), however Giami and Isichei (25) reported WAC of fluted pumpkin concentrates (5.6 g/g) was higher than our results. The results were comparable to tomato protein isolates and concentrates 3.11-4.09 g/g (34). These results suggest that both protein isolates and concentrates could be used as functional ingredients in food systems such as bakery products which require hydration to improve handling characteristics. Oil absorption capacity (OAC) was observed in the range of 2.37-3.12 g/g for isolates and concentrates. The results on OAC revealed non-significant ($p > 0.05$) differences between the protein isolates and concentrates of the 2 cultivars. OAC for melon protein isolates (1.12 g/g) and fluted pumpkin concentrates (0.65 g/g) were comparatively lower than our results (2,25). Similar results are also available for almond protein isolate (2.92 g/g) (18). Bulk density was in the range of 0.45-0.53 g/mL for protein isolates and concentrates, but there were no significant ($p \geq 0.05$) differences in the bulk density for protein isolates or concentrates.

Surface hydrophobicity (S_o) of 'Mateera' protein isolate (98.63) and 'Sugar baby' protein isolate (88.49) were significantly ($p \leq 0.05$) higher than their respective protein concentrates. The differences in S_o are primarily dependent on the presence of hydrophobic/hydrophilic amino acids on the protein surface. Voutsinas *et al*. (35) reported surface hydrophobicity in the range of 47-128 of different plant protein isolates which clearly shows that surface hydrophobicity varies among different protein types. The total color difference (ΔE) of isolates and concentrates were in the range of 25.04-33.17. The results indicated that protein concentrates had significantly ($p \leq 0.05$) higher color index than respective protein isolates. The dark color of protein isolates may be attributed to the alkylation of amino acids resulting in the diminished color values. Moreover, the membrane dialysis of protein concentrates removes the water soluble colouring compounds which resulted in high color value of protein concentrates.

The foaming properties of protein isolates and concentrates was determined and presented in Fig. 5A. Foaming capacity of 89.9 mL was obtained for 'Sugar baby' protein concentrate, followed by 'Mateera' protein concentrate (81.0 mL), 'Sugar baby' protein isolate (78.19 mL) and 'Mateera' protein isolate (74.38 mL). Protein isolate or concentrate from 'Sugar baby' showed higher foaming capacities than 'Mateera' protein isolate or concentrate. Khalil (2) reported foaming capacity of 65.6% for melon protein isolates whereas, Giami and Isichei (25) reported foaming capacity of 37, 36.5, and 40 mL for protein concentrates prepared from raw, fermented, and germinated fluted pumpkin. In the present study, protein concentrates reported better foam stability than their respective protein isolates. The foam stability of protein isolates or concentrates was comparable to melon and almond protein isolates (18,28).

Emulsifying properties of protein isolates and concentrates is presented in Fig. 5B. Emulsifying activity varied significantly between protein isolates and concentrates. Emulsifying activity of 0.188 was observed for 'Mateera' protein isolate, 'Sugar baby' protein isolate (0.182), 'Mateera' protein concentrate (0.253) and 0.260 for 'Sugar baby' protein concentrate. This indicated that protein concentrates had significantly ($p \leq 0.05$) higher emulsifying activity than protein isolates. Similar results were followed for emulsifying stability of 'Sugar baby' protein isolates

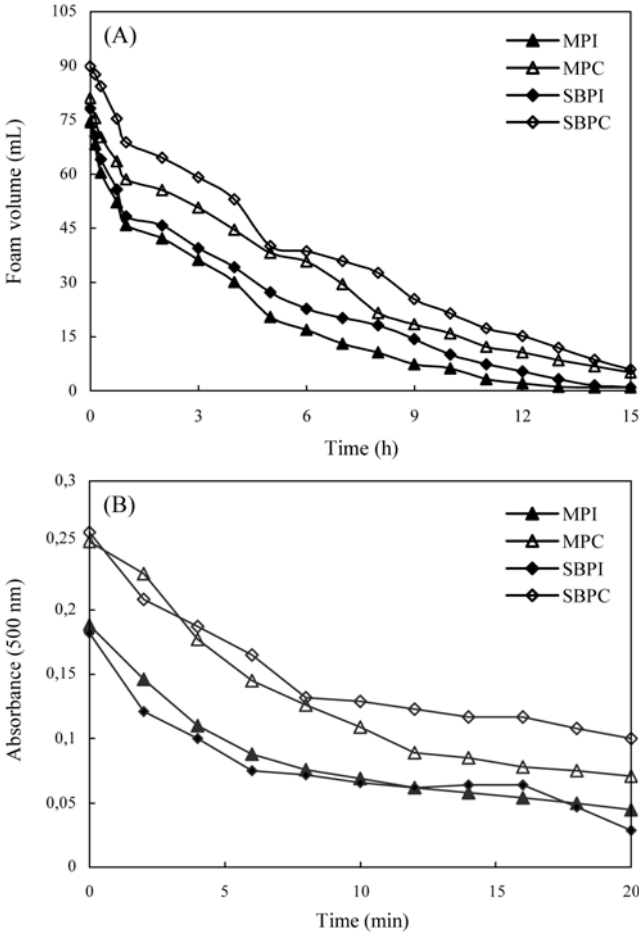

Fig. 5. Foaming (A) and emulsifying properties (B) properties of protein isolates and concentrates of 'Mateera' (MPI and MPC) and 'Sugar baby' (SBPI and SBPC) cultivars.

and concentrates. The factors affecting the emulsion properties are molar mass, hydrophobicity, confirmation stability, charge, and environmental conditions (29). Emulsifying properties of watermelon seed protein isolates or concentrates were comparable to almond and melon seed proteins (18,28).

The present study on the use of 2 different methods for the preparation of protein isolates and concentrates showed differences in the yield, and protein functionality. From the economical or industrial perspective, the alkali extraction method proved to be effective; however the protein quality of protein concentrates was comparatively higher than protein isolates. Alternatively, both of the methods may be integrated and optimised for yield and protein quality from industrial perspective. The present study revealed that the proteins are of high quality amino acids and may be used as a protein source in a number of food products in the developing countries. At the same instance, the approach of extracting proteins may help in by product utilization and providing protein rich diet in least developed countries. Further work may be carried on the utilization of protein isolates and concentrates in different food systems and to check the bioavailability in different animal models.

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