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Physicochemical characteristics, protein analysis, and antioxidant properties of defatted *Lagenaria siceraria* (Molina) Standley and *Cucumeropsis mannii* (Naudin) seed kernel flours

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Abstract

Defatted *Lagenaria siceraria* seeds and those of *Cucumeropsis mannii*, obtained after lipid extraction using food-grade hexane, were converted into flours (defatted *Lagenaria siceraria* seeds flours, LSDSF; defatted *Cucumeropsis mannii* seed flours, CMDSF), and analysed for their chemical and amino acid (AA) compositions, protein fractions, protein molecular weight distribution, granular surface morphology by scanning electron microscopy, and thermal properties by differential scanning calorimetry. In addition, their antioxidant activities were evaluated using DPPH radical scavenging and phosphomolybdate reducing power assays. LSDSF and CMDSF contained mainly globular shaped proteins with high thermal stability. Composition wise, these proteins primarily consisted of globulins, glutelins, and albumin. AA analysis of the total protein identified 18 amino acids including all the essential AA. These flours thus could be potential sources of antioxidant compounds with higher activity in aqueous than in methanolic extract. Based on their composition and physicochemical characteristics, LSDSF and CMDSF are potentially good ingredients usable in food systems with low lipid oxidation.

Introduction

Cucurbitaceous vegetables are a large family in the Plantae kingdom, consisting of 118 genera and 825 species, widely distributed in the warmer regions of the world (Milind and Satbir, 2011), either wild or cultivated. The family is predominantly distributed around the tropics, where those with edible fruits and seeds were amongst the earliest cultivated plants. Many of them are economically important, and some have tremendous nutritional and therapeutic potentials. In the Cucurbitaceae family, *Lagenaria* (Milind and Satbir, 2011) and *Cucumeropsis* genera are amongst the most popular.

L. siceraria and *C. mannii* are cultivated almost all year round, mainly in African and Asian countries, because they require a warm and humid climate. They possess high genetic diversity in fruit shapes characteristics, thus resulting in a wide variety in food systems. In some African countries such as Nigeria and Cameroon, *L. siceraria* and *C. mannii* are cultivated mainly for their seed kernels which are used to prepare dough and some local dishes (Djiogue *et al.,* 2017). In some other cases, the ground kernels are used as a thickener or to improve flavour of soups, or are consumed directly as a snack in many cultures throughout the world, and the fleshes are used for human consumption such as soup or purées. In Nigeria, the seeds are used in the preparation of fermented products (*ogiri*), fried cake (*robo*), or pudding (*igbalo*). Data on the nutritive value of *L. siceraria* and *C. mannii* seed kernels from different regions of Cameroon revealed that they have a high nutritional potential, especially as excellent sources of protein and oil (Achu *et al.,* 2005). In this respect, they contain about 50% of lipids (with about 70% of unsaturated fatty acids) and 37.8 - 45.4% of proteins. Furthermore, their defatted cakes contain up to 74% of protein (Achu *et al.,* 2005). *L. siceraria* and *C. mannii* seed kernels are therefore interesting raw materials for plant-based high-protein products, and can be used to supply the growing needs for proteins, both as functional food ingredients and nutritional supplements.

Seed proteins are drawing increased attention as they are relatively abundant with bioactivity such

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as antioxidant activity or protective effects against some chronic degenerative diseases (Krajcovico-va-Kudlackova *et al.,* 2005). In this respect, one of the most widely used plant proteins is that of soy beans, which has been shown to improve the functional and nutritional properties of diverse industrial food products (Henk, 2005). However, the use of these rich plant protein source (Cengiz and Gokoglu, 2007) has been found to be limited by their poor flavour and high content of antinutritional factors (Das *et al.,* 2008).

Given the high nutritional profile of *L. siceraria* and *C. mannii*, their use as potential sources of plant dietary proteins in industrial food process remains unexploited. Due to the immense importance and use of plant proteins in the food industry, also the need to bridge our knowledge gap on their potential as ingredients in industrial food process, the present work was carried out to investigate the chemical composition, thermal properties, and antioxidant activities of the defatted flour of *L. siceraria* and *C. mannii* seed kernels. It was hoped that the use of defatted flours of *L. siceraria* and *C. mannii* seed kernels could serve as good sources of low-cost and high-protein food ingredients for product formulation in the food industry, and contribute to improving the protein requirements of consumers, especially in the developing countries of the world, where dietary protein intake levels are known to be very low.

Materials and methods

Materials

Previous studies revealed that the proximate composition of these seeds produced in Cameroon is not significantly affected by their agro-ecological origin (Achu *et al.,* 2005). Thus, the samples used in the present work were collected from their main production localities in Cameroon. *L. siceraria* and *C. mannii* seeds were collected in June 2016 at Garoua town (North region) and Bafia town (Centre region), Cameroon, respectively. The seeds were immediately transported to the laboratory in polyethylene bags, and there manually decorticated. Decorticated seeds were sorted to eliminate the impurities and damaged seeds, washed with tap water, rinsed with distilled water, drained on trays, and dried at 45°C for 24 h in a ventilated hot air dryer (CK2000AUF, ENSAI, Cameroon). The *L. siceraria* defatted seed flour (LSDSF) and *C. mannii* defatted seed flour (CMDSF) were produced from defatted cake following lipid extraction according to Noumo *et al.* (2016) protocol.

All the chemicals and solvents used were of analytical grade, except for the food-grade hexane that was used for lipid extraction.

Chemical analysis

The chemical analysis, moisture, ash, total fibre (AOAC, 1990), total polyphenol (Marigo, 1973), phytate (Vaintraub and Lapteva, 1988), and vitamin C (Tomohiro, 1990) contents were evaluated. The total protein was obtained from nitrogen content using 6.25 conversion factor (AOAC, 1990), and the sugar content was determined according to Dubois *et al.* (1956). The mineral contents (P, Ca, and Mg) were determined using atomic absorption spectrophotometer AAS 1100 (Perkin-Elmer, USA). Rodier (1978) method was used for iron (Fe) analysis.

For amino acid analysis, 10 mg of sample were digested using 3 mL of 6 M HCl containing 0.1% phenol at 110°C for 24 h under nitrogen. Amino acid (AA) analysis was carried out in a SYKAM system (SYKAM S433 Amino Acid Analyser, Germany) with ninhydrin (Moore and Stein, 1951) post-column derivatisation at 135°C. Briefly, 50 µL of sample were injected into an ion exchange column at 48°C, and derivatised AA detected at 570 nm, except for proline, at 440 nm, with integrated dual length photometer. For tryptophan analysis, the method described by Sastry and Tammuru (1985) was followed with few modifications. Briefly, 50 mg of sample were hydrolysed with 3 mL of 4 M NaOH at 110°C for 24 h in a sealed tube under nitrogen. The hydrolysate was neutralised to pH 7.0 with 6 N HCl using phenolphthalein indicator. The volume was made up to 50 mL with distilled water, and filtered through Whatman No. 1 filter paper. Solution of 2.5% of sucrose (0.1 mL) and 0.1 mL of 0.6% thioglycolic acid were added to test tube containing 4 mL of 50% H_2SO_4, incubated for 5 min in a water bath at 50 ± 1°C, and cooled at 30 ± 2°C. An aliquot of the sample (0.15 mL) was then added to the test tubes. Tryptophan solution (10 µg/mL, 0.1 mL to 0.8 mL) was used as standard. The volume was made up to 5 mL with 0.1 N HCI, and allowed to stand for 10 min for the development of colour. The absorbance was measured against a reagent blank at 500 nm.

The amino acid composition was expressed as g of amino acid per 100 g of protein. The amino acid score or chemical score (Eq. 1) was calculated from essential amino acid using the amino acid composition. The protein used as reference was the standard protein composition proposed by WHO (2007).

$$\text{Chemical score (\%)} = \frac{mg\ of\ amino\ acid\ in\ 1\ g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1\ g\ of\ reference\ protein} \quad \text{(Eq. 1)}$$

Colour

Instrumental colour readings for LSDSF and CMDSF were carried out using a Konica Minolta CM5 Spectrophotometer (Konica Minolta Optics, Inc, Japan) with a built-in SpectraMagic NX software, and equipped with a D65 circumferential optical sensor. The CIE LAB colour coordinate system L*, a*, and b* (L* = whiteness, a* = redness, b* = yellowness) values were recorded. The L*, a*, and b* values of the standard white calibration plate were 97.19, -0.21, and -0.13, respectively.

Scanning electron microscopy

Dried samples were mounted on the specimen holder and coated with gold (3 nm) in a sputter coater (POLARON E5100, Watford, UK), and transferred to the microscope (LEO 435 VP, Leo Electron Microscopy Limited, UK) where it was observed at 10 kV under high vacuum. The digital images were generated, filtered, and recorded in a computer.

Fractionation of seed proteins

The fractionation of *L. siceraria* and *C. mannii* seed proteins was carried out as described by Sauvaire *et al.* (1984) with some modifications. Four solvents were used consecutively to extract the proteins from LSDSF and CMDSF: MilliQ water for albumin, 5% (w/v) NaCl for globulin, 70% ethanol for prolamin, and 0.05 M NaOH for glutelin. Sample (5 g) was suspended in 50 mL of solvent (1:10 w/v), and the protein was extracted by mechanical stirring (60 min at 100 rpm). The extract was centrifuged at 8,000 *g* for 20 min, and the clear supernatants were collected. The residue was mixed with the solvent, and extractions were repeated two more times using the same conditions. Finally, the supernatants from three extractions with the same solvent were pooled. The percentages of protein extracted in each fraction were calculated from nitrogen content using 6.25 conversion factor (AOAC, 1990), with respect to the total extractable protein from defatted flour.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses were performed according to Laemmli (1970). Briefly, 3 mg of the sample were added to 1.5 mL of the buffer containing 0.0625 M Tris-HCl (pH 6.8), 0.025% bromophenol blue, 10% glycerol, 2% SDS, and 5% 2-mercaptoethanol (2-ME). Samples were heated in a water bath at 100°C for 3 min, cooled to room temperature, centrifuged at 8,000 rpm for 15 min, and the supernatant were used for analysis. For SDS-PAGE analyses under non-reducing conditions, the reducing agent 2-ME was omitted in the sample buffer. A discontinuous gel system (1.5 mm of thickness) was prepared with 10 and 4% of acrylamide/N and N'-methylene bisacrylamide (30/0.8, w/w), for resolving and stacking gel, respectively. Electrophoresis was carried out on protein samples (15 µL) added onto the gel using vertical Midi Gel System (Genie, Bengaluru, India) at 21 mA for 12 h. The reservoir buffer contained a mixture of 0.025 M Tris, 0.192 M glycine, and 0.1% SDS to a final pH of 8.3. Proteins were fixed and stained in the gel using 0.1% (w/v) Coomassie Brilliant Blue R250 in a mixture of methanol, acetic acid, and distilled water (5:2:5; v/v/v). Extra dye was removed by repeated washings in a solution of methanol/glacial acetic acid/water (3/1/3; v/v/v), and preserved in 7% acetic acid. The molecular weight standard was the broad range molecular weight markers (6.5 to 200 kDa) (Sigma-Aldrich Co., USA). The molecular weights of proteins in test samples were computed by employing a regression equation expressing molecular weight as a function of relative mobility (Rm) using Eq. 2.

$$Rm = Dp / Dd \quad \text{(Eq. 2)}$$

where, Dp = distance covered by the protein in the resolving gel, and Dd = distance covered by the dye in the resolving gel.

Differential scanning calorimetry

The thermal denaturation of dried and hydrated (10% of the flour in distilled water, w/v) samples was studied by differential scanning calorimetry (DSC 800, Perkin Elmer, USA). The experiment was done as follows: 3 - 5 mg of sample was introduced in an aluminium pan, and the pan was sealed. An empty sealed pan was used as a reference. The heat-denaturation temperature parameters: onset temperature (Tm), thermal stability or peak temperature (Td), and enthalpy of denaturation (DH) were calculated from the thermogram measured with a programmed rate of increase of 5°C/min from 30 to 200°C using indium as a standard.

Determination of antioxidant properties

As the flours were obtained after lipid extraction with hexane, the polar solvent, water, and methanol were used to evaluate antioxidant properties of the flours. For this, the following codes were used for different samples: LS MeOH = methanolic extract of LSDSF; LS H_2O = aqueous extract of LSDSF; CM MeOH = methanolic extract of CMDSF; and CM H_2O = aqueous extract of CMDSF.

Free radical scavenging activity essay

The simple, rapid, and sensitive 2.2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) scavenging method by Brand-Williams *et al.* (1995) was used to evaluate the radical scavenging activity. The tested samples were prepared at different concentrations (from 5 to 100 mg/mL in methanol, 1 to 20 mg/mL in water) under stirring using magnetic stirrer at 500 rpm for 10 min, then centrifuged at 2,500 rpm for 10 min. For the test, 4 mL of DPPH solution (0.2 mM in methanol) were mixed with 1 mL of the corresponding supernatant. The reaction mixture was vortexed and incubated in the dark at room temperature for 30 min. Absorbance was measured at 536 nm against the blank (solvent). DPPH radical scavenging activity of extracts was calculated as the percentage of DO reduction as compared to control. The IC_{50} value corresponding to the amount of antioxidant necessary to halve the initial DPPH concentration was calculated from the results, and butylated hydroxyanisole (BHA) (5 - 100 µg/mL) was used as the standard.

Determination of total antioxidant capacity by phosphomolybdenum method

The total antioxidant activity of the flours was evaluated by the phosphomolybdenum method (Prieto *et al.,* 1999). The tested samples were prepared at 8 mg of flour/mL of solvent in methanol and water using a magnetic stirrer at 500 rpm for 10 min, then centrifuged at 2,500 rpm for 10 min. A 0.3 mL of supernatant was combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. The absorbance of the reaction mixture was measured at 695 nm after cooling at room temperature. The antioxidant activity was expressed as the number of µg equivalents of ascorbic acid.

Statistical analysis

Except the DSC, SDS-PAGE, and amino acid analysis, the experiment was carried out in triplicate. Differences between means were tested using the Duncan Multiple Range in Stagraphic® Centurion XVI, and graphs were plotted using SigmaPlot version 12 software.

Results and discussion

Chemical composition

The results of the chemical composition presented in Table 1 show that LSDSF and CMDSF contained 65.51 ± 1.53 and 69.43 ± 1.19 g/100 g DM of proteins, respectively, and as such they constituted good potential and valuable sources of protein. The high protein content of LSDSF and CMDSF could be due to the initial high protein content of seeds (34.19 ± 0.85 and 40.49 ± 2.75 g/100 g DM for *L. siceraria* and *C. mannii*, respectively (Achu *et al.,* 2005)) and oil extraction, since *L. siceraria* and *C. mannii* seed kernels content were 56.6 ± 0.40 and 55.48 ± 1.10 g/100 g DM of lipid, respectively. Similar results have been reported by other studies (Achu *et al.,* 2005; 2013). The high protein content can enhance the use of LSDSF and CMDSF as sources of protein for human consumption, and could be a good substitute of animal protein to meet the increasing demand of protein due to the fast-growing world population. These flours can also be used for the improvement of nutritive value of some foods.

Also shown in Table 1 are the sugar contents of LSDSF (23.04 ± 1.35 g/100 g DM) and CMDSF (18.78 ± 2.74 g/100 g DM), which varied significantly ($p \leq 0.05$) with the species. These values are higher than those reported on defatted seed flour of five species of Cucurbitaceae from different regions of Cameroon (7 to 11%) by Achu *et al.* (2005). However, the crude fibre contents of these flours (3.90 ± 0.79 to 4.75 ± 0.39 g/100 g DM) are close to those found in some Cucurbita seeds from Nigeria (2.43 - 6%) (Blessing *et al.,* 2011) and in *C. maxima* seeds collected in Cameroon (3.81 ± 0.64%) (Achu *et al.,* 2005). LSDSF and CMDSF also contained vitamin C (43.02 ± 5.49 and 35.74 ± 4.59 mg/100 g DM), polyphenols (598.17 ± 35.36 and 420.02 ± 45.98 mg/100 g DM), and phytates (233.87 ± 7.74 and 236.49 ± 4.78 µg/100 g DM) (Table 1). The vitamin C contents of LSDSF and CMDSF are higher than the value reported in some Nigerian *Cucurbita* seeds (3.473 - 4.387 mg/100 g) (Blessing *et al.,* 2011). The polyphenol contents of LSDSF and CMDSF are close to those defatted flours of seed kernels from five species of Cucurbitaceae from different regions in Cameroon (0.34 to 0.43%) (Achu *et al.,* 2013), but higher than

Table 1. Proximate composition, colour, and protein fractions of *L. siceraria* and *C. mannii* defatted seed flours.

Parameter	LSDSF	CMDSF
Proximate composition		
Dry mater (DM) (%)	94.09 ± 0.15[a]	93.7 ± 0.34[a]
Protein (g/100 g DM)	65.51 ± 1.53[a]	69.43 ± 1.19[b]
Total carbohydrate (g/100 g DM)	23.04 ± 1.35[b]	18.78 ± 2.74[a]
Total fibre (g/100 g DM)	3.90 ± 0.79[a]	4.75 ±0.39[a]
Ash (g/100 g DM)	8.06 ± 1.24[a]	7.04 ± 1.51[a]
Vitamin C (mg/100 g DM)	43.02 ± 5.49[a]	35.74 ± 4.59[a]
Polyphenol (mg/100 g DM)	598.17 ± 35.36[b]	420.02 ± 45.98[a]
Calcium (mg/100 g DM)	48.03 ± 0.28[a]	49.72 ± 0.28[b]
Magnesium (mg/100 g DM)	21 ± 0.21[b]	18 ± 0.14[a]
Iron (mg/100 g DM)	41.14 ± 1.46[b]	16.68 ± 1.51[a]
Phosphorus (mg/100 g DM)	291.89 ± 1.57[b]	284.45 ± 0.64[b]
Phytate (µg/100 g DM)	233.87 ± 7.74[a]	236.49 ± 4.78[a]
Protein fraction (%, w/w)		
Total extractable protein	77.89 ± 2.17[a]	88.38 ± 1.02[b]
Albumin	12.64 ± 0.46[a]	15.18 ± 1.18[b]
Globulin	55.06 ± 3.18[a]	72.78 ± 3.45[b]
Prolamin	2.89 ± 0.41[b]	1.81 ± 0.56[a]
Glutelin	21.29 ± 0.41[b]	9.50 ± 0.77[a]
Colour parameter		
L*	83.27 ± 0.05[a]	85.27 ± 0.22[b]
a*	00.15 ± 0.03[a]	0.12 ± 0.01[a]
b*	13.82 ± 0.06[b]	11.65 ± 0.06[a]

LSDSF = *L. siceraria* defatted seed flour; CMDSF = *C. mannii* defatted seed flour.

the value reported by Karaye *et al.* (2013) in some Nigerian *Cucurbita* seeds (1.1 to 3.70 mg/100 g DM). The phytate contents found in LSDSF and CMDSF are similar to those reported by Karaye *et al.* (2013) in five species of Cucurbitaceae from Nigeria (0.2 to 0.4 mg/100 g DW). LSDSF and CMDSF are also important sources of minerals (8.06 ± 1.24 and 7.04 ± 1.51 g/100 g DM of ash) (Table 1). In decreasing order of occurrence, the mineral content of the samples were as follows: phosphorus > calcium > iron > magnesium. Similar results have been reported by Blessing *et al.* (2011) on *Cucurbita* sp. collected in Nigeria with potassium, magnesium, and calcium as the most prevalent minerals. The presence of polyphenols and vitamin C in these flours can favour their use in food products, since polyphenols and vitamin C can act as antioxidants thus enhancing the storage ability of food products in which these flours might be incorporated.

Based on the composition and physicochemical properties of these flours, their fibre could provide physiological advantages by protecting against colon cancer, while their polyphenols and vitamin C have protective role potential against free

radical damage and related diseases in the body. Moreover, phytates present in these flours are one of the bioactive compounds that are being intensively studied as a good anti-cancer agent and antioxidant compound. The differences in chemical compositions obtained by other authors could be due to the agro-ecological differences of the analysed species, seed maturities, harvest periods, or seed treatments.

Amino acid composition

Access to sufficient protein of adequate quality is fundamental to maintaining health. One of the basic methods to assess nutritional qualities of proteins is the evaluation of their amino acid composition. The results of the amino acid composition presented in Table 2 reveal a similarity in the amino acid profile of proteins in LSDSF and CMDSF. They both contained all the essential amino acids with aspartate/asparagine, arginine, and glutamate/glutamine being the predominant amino acids. Except for lysine, the essential amino acid contents of LSDSF and CMDSF proteins were within the levels of essential amino acid content of the "ideal protein" (Table 2) for human nutrition from 1 year old. Based on the values of chemical score, lysine was the limiting amino acid with the value of 59.23% (*L. siceraria*) and 58.85% (*C. mannii*). The ratio of essential amino acids to total amino acids (33.70 and 35.08%, respectively, for *L. siceraria* and *C. mannii*) was above 27.7%, which was considered adequate for the adults ideal protein (WHO, 2007). Hence, LSDSF and CMDSF could be useful sources of protein in food supplementation.

The lysine to arginine ratio is used to assess the effect of protein on lipid metabolism. Protein with a lower ratio of lysine to arginine has lesser lipidemic and atherogenic effects (Kaushik *et al.,* 2016). The low value of lysine to arginine ratio obtained in the present work (0.20) showed that LSDSF and CMDSF protein may be better proteins for cardiac health than several other seed protein such as flax seed (Linum usitatissimum) protein isolate (0.25) (Kaushik *et al.,* 2016), soy protein isolate (SPI) (0.71) (Tang *et al.,* 2006), as well as white (0.74) and brown (0.85) Bambara groundnut (*Voandzeia subterranean*) protein isolates (Adebowale *et al.,* 2011). Thus, even though lysine is the limiting amino acid for these seeds' proteins, the low lysine content can enhance the dietary use of LSDSF and CMDSF to improve lipid metabolism, or in high fat food products, to improve their nutritional value, and reduce the effect of their consumption on some diseases related to fat metabolism.

The results obtained in the present work are different from those reported in other two varieties of *L. siceraria*, where the first limiting amino acid in both raw seed flours and their protein fractions was methionine, followed by valine (Ogunbusola *et al.,* 2011). This difference may be due to the different methods of hydrolysis and derivatisation as sulphur-containing amino acid can be oxidised (lost) during either the acid hydrolysis (when it is not under nitrogen atmosphere) or the alkaline hydrolysis, with subsequent reduction in their concentration.

Amino acid composition of SPI was also used for comparison (Table 2), since it is considered as a good source of essential amino acid, mainly for the infant nutrition. The essential amino acid ratio of LSDSF and CMDSF protein was similar to that reported in SPI (Tang *et al.,* 2006). Thus, as SPI, *L. siceraria* and *C. mannii* seed protein can be used in infant's food formulation. Moreover, the total sulphur-containing amino acids of LSDSF and CMDSF proteins are higher than the value reported in SPI by previous authors, thus suggesting that LSDSF and CMDSF can find specific applications in food formulations like baking products, in which development of protein network through sulphide bounds is required.

In general, the tryptophan content in proteins is always less as compared to many other amino acids. However, it is a nutritionally important amino acid, since it is a precursor for important metabolites such as serotonin and nicotinamide, in the latter case, giving it vitamin-like properties through its ability to replace dietary niacin (WHO, 2007). As shown in Table 2, the tryptophan content of LSDSF (2.09 g/100 g of protein) and CMDSF (2.45 g/100 g of protein) were higher than the WHO (2007) recommended value (0.6 g/100 g of protein). These results suggested that *L. siceraria* and *C. mannii* seed protein can be considered as important sources of tryptophan.

Colour

The L*, a*, and b* values of LSDSF and CMDSF were measured and observed as 83.27 ± 0.05 and 85.27 ± 0.22; 0.15 ± 0.03 and 0.12 ± 0.01; 13.82 ± 0.06 and 11.65 ± 0.06, respectively (Table 1). There were very few differences between the colour parameters of LSDSF and CMDSF; CMDSF being more whitish. The L*, a*, and b* values of LSDSF and CMDSF were similar to the values reported in kidney bean (*Phaseolus vulgaris*) protein isolates (Wani *et al.,* 2015). The whitish colour of LSDSF and CMDSF could be due to the removal of

Table 2. Amino acid composition (g/100 g protein), soy protein isolate (SPI), WHO protein standard, and chemical scores (%) of *L. siceraria* and *C. mannii* defatted seed flours.

Amino acid	LSDSF	CMDSF	SPI	Standard		Chemical score	
				≥ 1 year	Adult	LSDSF	CMDSF
Essential amino acid							
Threonine	2.55	2.68	4.10	2.7	2.3	94.44	99.26
Lysine	3.08	3.06	5.39	5.2	4.5	59.23	58.85
Valine	4.44	5.20	4.41	4.2	3.9	123.33	144.44
Methionine	2.14	1.90	0.93		1.6		
Isoleucine	3.85	4.12	4.48	3.1	3.0	124.19	132.90
Methionine + cysteine						129.55	118.18
Leucine	6.43	6.65	7.00	6.3	5.9	102.06	105.56
Phenylalanine	5.83	5.99	5.30		2.2		
Phenylalanine + tyrosine						247.11	251.84
Histidine	3.29	3.12	2.90	1.8	1.5	182.78	173.33
Tryptophan*	2.09	2.45	-	0.74	0.6	282.43	331.08
Total EAA	**33.70**	**35.08**	**34.51**		**27.7**		
Non-essential amino acid							
Serine	2.29	2.09	5.48				
Cysteine	0.71	0.70	0.06		0.6		
GLX	18.53	19.44	21.29				
Glycine	5.73	6.24	3.86				
Alanine	4.81	5.16	3.83				
Tyrosine	3.56	3.58	3.71				
Arginine	15.53		7.57				
Proline	5.64	3.53	5.29				
ASX	9.50	8.78	11.81				
Total NEAA	**66.30**	**64.92**	**62.9**				
TSAA	**2.85**	**2.61**	**0.99**	**2.6**	**2.2**		
TArAA	**11.48**	**12.02**		**4.6**	**3.8**		
LYS/ARG	**0.20**	**0.20**	**0.71**				

*analysed according to Moore and Stein (1951) method. LSDSF = *L. siceraria* defatted seed flour; CMDSF = *C. mannii* defatted seed flour; SPI = soy protein isolates (Tang *et al.,* 2006); Standard = adult indispensable amino acid requirements (WHO, 2007); TEAA = total essential amino acids; ASX = aspartic acid + asparagine; GLX = glutamic acid + glutamine; TNEAA = total non-essential amino acids; TSAA = total sulphur-containing amino acids; and TArAA = total aromatic amino acids.

fat-soluble pigments during defatting of seed flour or the small particle size of the flour as shown in Figure 1, as the reduction of flour particle size increases the brightness (Drakos *et al.,* 2017). The whiteness of the flour is an important parameter for the acceptability by the consumers, where a* and L* values are critical; a low a* and high L* value are required (Drakos *et al.,* 2017) for many applications. Based on colour parameters, LSDSF and CMDSF can be used in a wide range of food products without

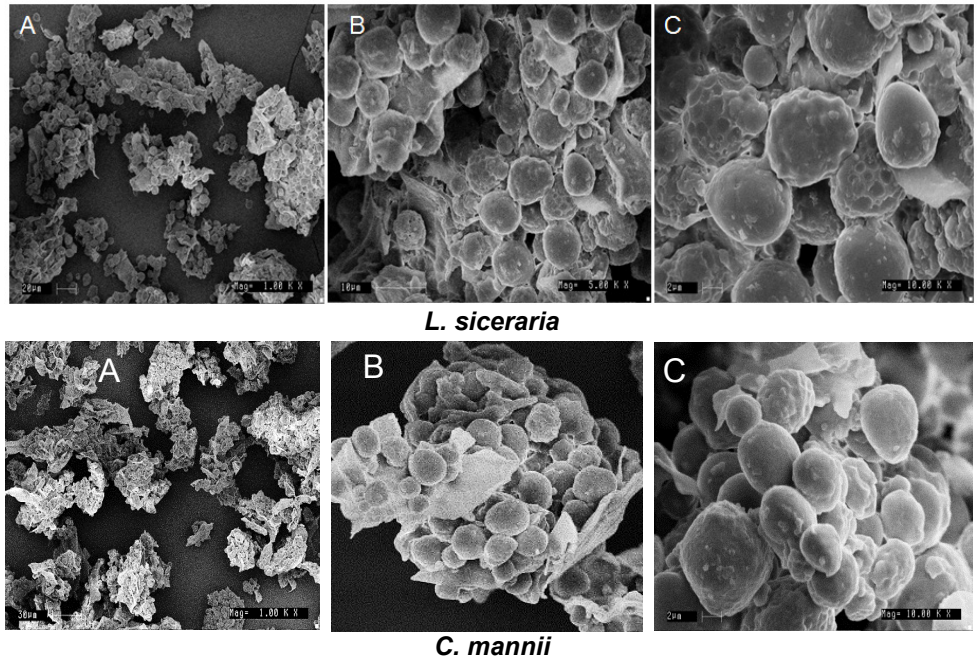


Figure 1. Scanning electron micrographs of *L. siceraria* and *C. mannii* defatted seed flours. (A) at 1,000× magnification; (B) at 5,000× magnification; and (C) at 10,000× magnification.

adverse effect on colour.

Scanning electron microscopy

Granule sizes and shapes are an important parameters in flours, which can affect their overall physiochemical and functional properties, thus determining the use of flours in different processes. Studies on surface topographies of LSDSF and CMDSF by scanning electron microscope revealed granules of globular shape with slightly rough surfaces and heterogeneous size, with a diameter less than 6 µm, and clumped together in a flake-like structure (Figure 1). Similar results have been reported on pea and sesame seed protein bodies (Tai *et al.,* 2001), with a diameter around 3 and 5 µm, respectively. LSDSF and CMDSF had almost no damage granules, which is positive for storage purpose. Moreover, the cluster structure of these flour particles increases their porosity and improves their wettability, dispersibility, and solubility (Turchiuli, 2013). LSDSF and CMDSF showed sheet-like structures which could be a cellular wall as reported in soybean by Wolf and Baker (1975).

Protein fractions

Of the three types of protein commonly associated with seeds, *i.e.* the structural, metabolically active, and storage-type proteins; the storage-type proteins are found in highest abundance, and are considered responsible for the nutritional as well as technological properties of the whole grain (Marcone *et al.,* 1998). The total extractable proteins in LSDSF and CMDSF were 77.89 ± 2.17 and 88.38 ± 1.02% (w/w) of total protein, respectively.

Based on the protein solubility in various solvents, seed proteins can be classified into four groups. The distribution of protein fractions is significantly ($p \leq 0.05$) affected by plant species. The different fractions of *L. siceraria* and *C. mannii* seed proteins (Table 1) indicated that globulins were the most dominant fraction (55.06 ± 3.18 and 72.78 ± 3.45% for *L. siceraria* and *C. mannii*, respectively), whereas prolamin was the least (2.89 ± 0.41 and 1.81 ± 0.56% for *L. siceraria* and *C. mannii*, respectively). Similar results were reported by Dash and Ghosh (2017) in *C. moschata* seed protein. Indeed, in dicotyledonous seeds, globulins are known as the major protein (Marcone *et al.,* 1998). Since sodium chloride solution (5%, w/v) resulted in maximum protein extraction, it was found to be the solvent of choice for *L. siceraria* and *C. mannii* seed proteins isolation. Moreover, *L. siceraria* and *C. mannii* seed proteins can find particular importance in the food industry mainly in the meat product characterised by high salt content. The properties of protein vary with fractions; globulins of certain seed protein are associated with hypolipidemic effect (Marcone, 1999), and the sesame seed protein 2S albumin was found to be resistant to human proteolytic enzymes, whereas the globulin fraction was relatively labile to pepsin (Orruño and Morgan, 2011). Thus, *L. siceraria* and *C. mannii* seed proteins

have potential biological activity, and could have good digestibility in connection to their high globulin content. However, in contrast to CMDSF protein (Table 1) and many other seeds where albumin tend to be the second most dominant group of proteins (Tai *et al.,* 2001), glutelin is the second major protein in LSDSF.

Electrophoretic characterisation

To characterise the seed proteins, SDS-PAGE analysis of total seed protein was carried out. To distinguish between free polypeptide chains and the chains linked by disulphide bridges, the analysis was performed in the presence and absence of a reducing agent, 2-mercaptoethanol (2-ME) (Figure 2). Based on molecular weight, the results showed that *L. siceraria* and *C. mannii* had similar seed proteins distribution. In non-reducing conditions (NRC) (Figure 2), three polypeptides bands were detected with a double-like band at 54 - 57 kDa, and the third at 8.8 kDa. In reducing condition (RC) (Figure 2), *L. siceraria* and *C. mannii* seed protein showed more complex structure containing six different bands (8.8 - 52 kDa); the band at 8.8 kDa was detected in reducing and non-reducing conditions, whereas the bands at 57 kDa disappeared in reducing condition. These results showed that the polypeptides bands at 54 - 57 kDa are oligomers containing subunits linked by disulphides bounds. The presence of disulphide cross-linked polypeptides in LSDSF and CMDSF proteins suggested their propensity toward intermolecular disulphide cross-linking with other proteins containing sulphur amino-acid like wheat gluten, thus enabling their applications in baking products.

Thermal properties of L. siceraria and C. mannii defatted seed flour

One of the main parameters affecting proteins and other nutrients in a food system during processing, manufacturing, storage, and preparation is heat. DSC analysis was carried out in dry condition to assess the effect of heat treatment on LSDSF and CMDSF. Moreover, after adding water, information was obtained on gelatinisation temperatures. The results presented in Figure 3 show that in dry or wet conditions, LSDSF and CMDSF showed a single endothermic peak, whereas two peaks have been reported in soy protein isolate (Adebowale *et al.,* 2011) and kidney bean (*Phaseolus vulgaris* L.) protein (Wani *et al.,* 2015). In the dried conditions, LSDSF and CMDSF had similar thermal stability (Tb) with respective peak temperatures

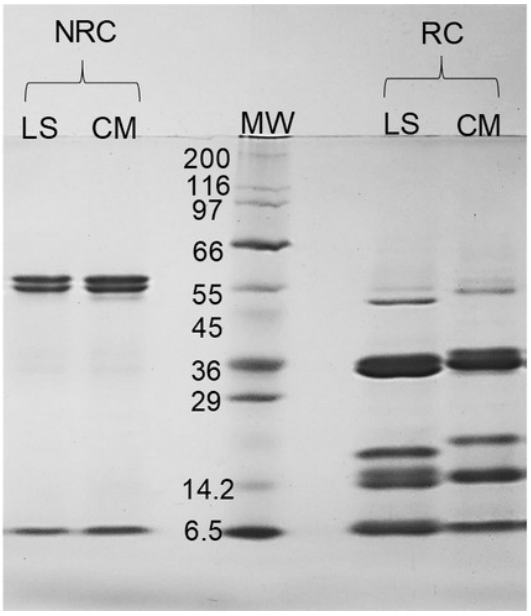
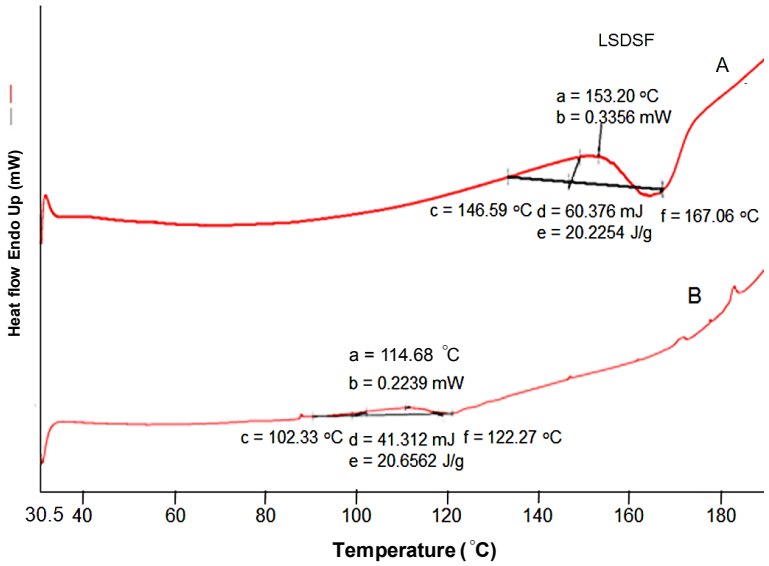


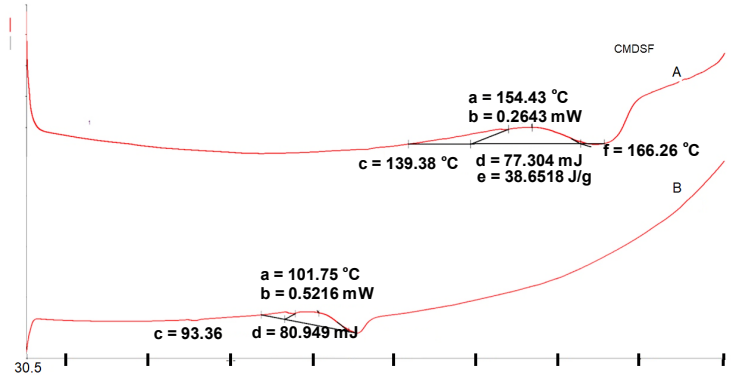
Figure 2. Electrophoresis profile of *L. siceraria* and *C. mannii* seed protein. NRC = non-reducing condition; RC = reducing condition; MW = molecular weight (KDa); LS = *L. siceraria*; and CM = *C. mannii*.

of 153.20 and 154.43°C, respectively. The Tm of LSDSF and CMDSF were 139.38 and 146.69°C; and DH were 38.65 and 20.23 J/g, respectively. These values are higher than those reported in flax seed protein of 83.4°C, 105°C, and 8.25 J/g for Tm, Td, and DH, respectively (Kaushik et al., 2016) in soybean glycinin; and *b*-conglycinin with Td values approximately 82 and 68°C, respectively (Liu *et al.,* 2007). These differences could be attributed to the nature of the protein, varying extraction methods, moisture content, presence of non-protein components (Kaushik *et al.,* 2016) or heating rate. The high thermal stability of LSDSF and CMDSF may be attributed to sulphide bonds between their protein's subunits or a high degree of crystallisation of flour granules. From this result, LSDSF and CMDSF can be used in a process using temperature above 100°C and low moisture content such as roasting, frying, and some extrusion processes.

Tm and Td decreased respectively in wet sample. The values of Tm were 102.33°C (LSDSF) and 93.36°C (CMDSF), while Td values were 114.68°C (LSDSF) and 101.75°C (CMDSF) (corresponding to gelatinisation temperatures). The decrease of onset temperature and Td could be due to the rapid unfolding of protein in the presence of water. As water enhances molecular mobility, the increase in water content will lead to a decrease in the glass transition temperature. Between the two flours, LSDSF had the higher thermal stability in wet



L. siceraria



C. mannii

Figure 3. Differential scanning calorimetry of *L. siceraria* and *C. mannii* defatted seed flours. A = dried flour; B = wet flour (10% w/w of dried material); a = peak; b = peak height; c = onset; d = area; e = delta H; f = end; CMDSF = *C. mannii* defatted seed flour; and LSDSF = *L. siceraria* defatted seed flour.

condition. This could be mainly due to the presence of more sulphide bonds in *L. siceraria* than in *C. mannii* seed proteins. Indeed, there were positive and strong correlations ($p \leq 0.05$) between sulphur-containing amino acids and Td ($r = 0.86$). The increase in Td with the decrease in water content has been reported in the case of soybean protein during DSC analysis (Kitabatake *et al.,* 1990). The DH increase in wet samples were 40.47 and 41.31 J/g for LSDSF and CMDSF, respectively. The high value of DH could be due to simultaneous denaturation of different protein subunits in a close temperature range, and the presence of non-protein components with similar thermal stability as LSDSF and CMDSF proteins.

Antioxidant properties

Nowadays, leguminous seeds are considered not only as sources of valuable proteins, but also as sources of bioactive compounds with antioxidant activities. Due to the variety of oxidation processes and reactions, different methods involving reducing power and DPPH radical scavenging activities were used to determine the antioxidant activity of LSDSF and CMDSF.

Radical scavenging activity

DPPH assay is widely used to determine the free radical-scavenging activity of various extracts or pure compounds. In the present work, the DPPH$^{\cdot}$ inhibition percentage values were dose-dependent, where it increased in the range of the tested concentration for LSDSF, CMDSF, and the control

(BHA).

In general, the radical scavenging activity of flour is significantly affected by the type of solvent. The DPPH$^{\bullet}$ IC_{50} values of LS MeOH, LS H_2O, CM MeOH, CM H_2O, and BHA were 29.64 ± 0.03 mg/mL, 3.19 ± 0.31 mg/mL, 34.56 ± 2.51 mg/mL, 3.45 ± 0.61 mg/mL, and 14.01 ± 0.34 µg/mL, respectively. A lower IC_{50} value indicates its high antioxidant capacity. The radical scavenging activity is approximately 10-fold higher in aqueous extract than in methanolic extract, and the LSDSF extracts had the highest activities. The high radical scavenging activity in aqueous extracts might be due to the higher solubility of LSDSF antioxidant compounds in water than in methanol.

The radical scavenging activities of these flours could be attributed to their phytochemical contents, mainly phenolic compounds and vitamin C, as well as peptides (Figure 2). There was a significant ($p \leq 0.05$) and negative correlation (r = -0.64) between polyphenolic contents of flours and the IC_{50} of their aqueous extracts. There were also significant and negative correlations between vitamin C content and IC_{50} (r = -0.77 for DPPH radical scavenging activity of aqueous extracts). The radical scavenging activities found in the present work were lower than those reported in hexane extract (IC_{50} = 149.09 µg/mL), chloroform extract (IC_{50} = 315.50 µg/mL), and ethanolic extract (IC_{50} = 183.52 µg/mL) of *Citrullus lanatus* seed (Rahman *et al.,* 2013). This low activity could be due to the low LSDSF and CMDSF phytochemical contents, suggesting that some antioxidant compounds present in Cucurbitaceae seeds could be lipophilic, and were eliminated during lipid extraction. These results demonstrated that LSDSF and CMDSF could convert free radicals to more stable products but less than BHA.

Total antioxidant activity

The total antioxidant activity (TAA) shows the reducing capacity of a compound, and is used as a significant indicator for the whole antioxidant activity. The TAA of the LSDSF and CMDSF were evaluated and expressed as the concentration of vitamin C having the equivalent antioxidant activity (EAA: equivalent ascorbic acid) on dry matter basis. The TAA was significantly affected by type of solvent and Cucurbitaceae species ($p \leq 0.05$). The TAA (in µg EAA/mg) of extracts in LSDSF and CMDSF ranged as follows: LS MeOH (25.90 ± 0.73) < CM MeOH (80.76 ± 0.59) < LS H_2O (112.89 ± 10.51) ≤ CM H_2O (106.47 ± 1.64); where the highest activity recorded with aqueous extract of *L. siceraria* defatted seed flour (LS H_2O). The reducing power activity correlated positively with the results obtained with the DPPH assay (r = 0.91 and 0.75 for aqueous and methanolic extracts, respectively) using the DPPH IC_{50} values, thus indicating that almost the same compounds were involved both in radical scavenging activities and in the TAA. Therefore, the antioxidant activities of LSDSF and CMDSF could be due to the hydrogen-donating ability of its phytochemicals or some amino acids and peptides. One of the most active dietary antioxidants belongs to the family of phenolic and polyphenolic compounds due to hydroxyl groups in their chemical structure (Doss *et al.,* 2010). Polyphenolic compounds express their antioxidant activity through their redox properties that allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. However, there was a low correlation between phenolic content of flour and their TAA (r = -0.71 and -0.25 for aqueous and methanolic extracts, respectively) in the present work. This suggested that not only the phenolics of LSDSF and CMDSF were involved in the TAA, but also other substances like proteins and vitamin C. As for the radicals scavenging activity, the TAA may also be due to the presence of peptides in the LSDSF and CMDSF. Antioxidant activity was also confirmed for other peptides from other plant sources such as wheat gliadin, pea, and soy proteins (Malaguti *et al.,* 2014).

Conclusion

The defatted flours of *L. siceraria* and *C. mannii* seed kernels contained substantial quantities of high-quality proteins (most of which were globular), with a complete essential amino acid profile, and lysine as the limiting amino acid. The observed amino acid profile revealed importance of these plant foods as sources of good proteins for heart health. *L. siceraria* and *C. mannii* also contained adequate amounts of important minerals (calcium, magnesium, and iron), fibre, phenolic compounds, and relatively low levels of phytates. Their defatted flours had high thermal stability and antioxidant activity. The composition and characteristics of these seeds make them good sources of plant proteins with potential use in food formulations in the food industry. In conclusion, the defatted *L. siceraria* and *C. mannii* seed flours could have the potential as alternative and economic sources of proteins in the fight against malnutrition.

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