Physicochemical and functional properties of seed flours obtained from germinated and non-germinated *Canavalia gladiata* and *Mucuna pruriens*

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ABSTRACT

C. *gladiata* and *M. pruriens* are underutilized legumes available in Sri Lanka. The physicochemical and functional properties of germinated and non-germinated flours from these two legumes were investigated. Protein contents of the flours of *C. gladiata* and *M. pruriens* increased by 17.04% and 14.69% respectively while fat and carbohydrate contents decreased due to germination. Higher Fe, Cu, Zn, Mn, and Se content was observed in both germinated flour types. Glutamic acid is the highest non-essential amino acid whereas leucine is the highest essential amino acid found in both flour types. The majority of amino acids in 100 g of seed flour of both legumes increased due to germination. Moreover, some of the functional properties of legume flours were changed such as swelling power, water and oil holding capacities because of the germination. In conclusion, the functional and nutritional properties of flour can be altered by the germination process making them ideal for utilization as ingredients for functional food formulations.

1. Introduction

Legumes, belonging to the family Fabaceae are considered an affordable source of protein for the human diet in most of the developing countries worldwide. These dicotyledonous seeds are much sought after nowadays as a favorable protein source since the consumption of red meat and processed meat has provoked numerous health problems including hypertension, colorectal cancer and cardiovascular diseases. Many researchers have emphasized the important health benefits of legume consumption such as reducing cardiovascular diseases by lowering cholesterol levels, reducing diabetic conditions and cancer due to the presence of nutritive and non nutritive constituents [1–3]. However, utilization of uncommon varieties of legumes such as *Mucuna pruriens* and *Canavalia gladiata* is limited by the presence of antinutritional constituents such as protease inhibitors [4]. *Mucuna pruriens*, commonly known as "velvet bean" is reported to have antiparkinson, antioxidant, anti-diabetic, anti-inflammatory, anti-cancer and anti-cholesterolemic activities [5]. *Canavalia gladiate* is another legume commonly called "sword bean", having high protein according to Ref. [6].

The functional properties of legume flour are mostly attributed to the seed storage protein, including albumins, globulins and prolamins. The conformation of proteins, chemical and heat stability, amino acid composition and sequence, structure, surface electrostatic charge, and effective hydrophobicity have a major influence on the functional properties such as Least gelation concentration, foaming capacity and foam stability, emulsifying activity and emulsion stability, water and oil holding capacities [7]. The

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swelling power of starch granules in the presence of water and thermal energy and solubility index due to leaching amylose, gelatinization properties are based on the carbohydrate, especially the starch fraction of legume seeds. The functional properties of a flour convey how the different compounds in a food system can contribute to increasing their shelf stability, texture, appearance and palatability. Additionally, the process of germination may have a significant impact on the physicochemical and functional properties of legumes seed flour. Many nutrients are increased after the germination process while at the same time, many macromolecules are involved in the metabolism [8].

The germination process has been considered an effective and inexpensive means to enhance the quality of legumes, by improving the protein quality [9] and reducing the contents of various antinutritional factors [10]. Moreover, germination significantly affects the seed's biochemical and nutritional composition, sensory and physical characteristics since it utilizes seed reserves by degrading compounds to generate new constituents [11]. It not only affects the anti-nutrients but also affects nutrients such as amino acids, carbohydrates and dietary fiber content. The starch content, fat content, anti-nutrients such as phytate and tannin, dry matter content and bulkiness are reduced during the germination process [12]. Protein, fat and starch digestibility are improved during germination, as reported in previous studies [8,12]. Increments could be seen in amino acids such as asparagine and glutamine in germinated legume seeds [13]. However, legumes are considered incomplete protein sources due to the deficiency in sulfur-containing amino acids and tryptophan. Even so, they are excellent sources of aspartic acid, glutamic acid, leucine, lysine and arginine [14]. Germination effect on known anti-nutritional factors and nutritional constituents in common legumes such as soybean and green gram has been widely studied. Changes in the physicochemical properties of flour proteins affect their functional properties including protein solubility, oil and water holding capacity and foaming ability [15]. However, there are limited discoveries in the published literature about the germination on physicochemical and functional properties to explore the possible applications of underutilized legumes such as *Mucuna pruriens* and *Canavalia gladiata* in food. Therefore, this study aimed to evaluate the effect of germination on physicochemical and functional properties in the flour from two underutilized legumes, *Mucuna pruriens* and *Canavalia gladiata*.

2. Materials and methods

2.1. Materials

Seeds of *C. gladiata* and *M. pruriens* were collected from mature plants in Horticultural Crop Research and Development Institute (HORDI) Gannoruwa, Kandy, Sri Lanka. All the procured chemicals used for the study were of analytical grade.

2.2. Germination of C. gladiata and M. pruriens seeds

Cleaned legume seeds were immersed in distilled water (seed to water ratio 1:5 w/v) overnight in the dark at room temperature (30 ± 2 °C) and spread on trays lined with cheesecloth. Water was sprayed frequently onto trays kept in shade and seeds were allowed to germinate. After 06 days, the germinated seeds were collected separately and processed into flour.

2.3. Production of germinated C. gladiata and M. pruriens seed flour

The germinated seeds were winnowed and oven-dried (Model:32706 TSM products, Buffalo, NewYork, USA) at 50 °C for 12 h and then ground into flour using an electric sample grinder (Jaipan Grinder model IS:4250, India) and sifted through a 250 μm sieve. Non-germinated flours were prepared similarly with cleaned legume seeds separately. Thus prepared flour samples were packed in air-tight containers at −18 °C until further analysis.

2.4. Physicochemical properties of legume flours

2.4.1. Proximate composition
The proximate composition (Moisture, ash, crude protein and crude fat) of germinated and non-germinated legume flour was determined using standard AOAC methods [16]. The crude fiber was determined according to the Weendy method as described in Ref. [17] with some modifications, using the Fibertec™ M6 fiber analysis system (Model: Fibertec TM M6, Denmark). About 0.10 g of flour was weighed into pre-weighed crucibles and mixed with 1.25% (v/v) H_2SO_4 followed by adding 1 mL of Octanol into each after placing it in a fiber analyzer. After 45 min of runtime, 1.25% (w/v) of KOH was added and continued for another 45 min, before washing the residues with acetone and drying at 105 °C to a constant weight. Then the content in the crucible was incinerated at 550 °C for 3 h and crude fiber content was calculated.

2.4.2. Mineral content analysis
Selected minerals were analyzed using AOAC methods using the Atomic Absorption Spectrophotometer (Model 205, Buck Scientific, USA). Following AOAC procedures were used for different elements; Iron (as Fe) and Copper (as Cu)-AOAC 999.10: 2012 [18], Zinc (as Zn) and Manganese (as Mn)-AOAC 2011.14: 2012 [18], Selenium (as Se)-AOAC 986.15: 2012 [18].

2.4.3. Amino acid profile analysis - LFOD-TST-SOP8512 (Ref. ISO 13903:2005)
Total amino acid profiles were analyzed according to a previously validated method of the European Commission (Commission Directive 98/64/EC of September 3, 1998) by using reversed-phase high-performance liquid chromatography (Agilent 1200 series,

Agilent Technologies Inc., Santa Clara, CA, USA) equipped with EC 125/4.6 Nucleodur® C18 Pyramid, 5 μm column (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). Amino acid reference standards (Biopharma, Santa Clara, CA 95051, United States) were used in quantitation. The reproducibility of the results was approximately 3%. Duplicate samples were hydrolyzed with 6 N hydrochloric acid at 110 °C for 24 h. The hydrolyzed flour samples were then analyzed as outlined below according to ISO 13903:2005 as described in Ref. [19].

a Cystine and methionine: Oxidative hydrolysis, amino acid analyzer (Amino Acid Analyzer, Biochrom 30, Laborservice Onken, Gründau, Germany) with ninhydrin (ISO 13903:2005; EU 152/2009) [19].

b Tryptophan: Alkaline hydrolysis, quantification by high-performance liquid chromatography (HPLC) techniques (ISO 13903: 2005; EU 152/2009) [19].

c. Other Amino acids: Acid hydrolysis, amino acid analyzer (Amino Acid Analyzer, Biochrom 30, Laborservice Onken, Gründau, Germany) with ninhydrin (ISO 13903: 2005; EU 152/2009) [19].

To ensure that quality data were gathered, several approaches were practiced as mentioned in ISO 1390: 2005 [19]. Briefly, a site-specific protocol was developed based on the analytical techniques used in the laboratories and also incorporated the appropriate QA/QC with blanks, standards, and replicates.

2.4.4. Differential Scanning Calorimetry (DSC)

DSC was conducted according to the method described in Ref. [20] using the instrument DSC (DSC Q200 TA Instruments, India). Flour samples of known moisture content were made to give a paste of 60% moisture content by adding the appropriate amount of water. Samples were weighed into DSC aluminum standard pans, sealed and left to equilibrate for 24 h at room temperature before DSC analysis. A sample size of 10 mg was used. Samples were reweighed to check for weight loss before scanning. Samples with weight changes beyond 0.01 were not used for analysis. Thermal transitions were defined in terms of T_o (transition onset temperature), T_p (transition peak temperature) and ΔH (transition enthalpy). Peak height index (PHI) was calculated as the ratio of $\Delta H/T_r$, where T_r is the range of temperature in which transition occurred and is the difference between T_p and T_o. Three determinations were made per sample. Sample slurries were heated from room temperature (32 °C) up to 130 °C at a constant heating rate of 5 °C/min and samples were scanned over a scan range of 40 °C-130 °C. A sealed empty pan was used as the reference. Thermal transitions were obtained and recorded as thermograms.

2.5. Functional properties

2.5.1. Swelling power and solubility index

Swelling power and solubility were determined according to the method described in Ref. [17] by dispersing 0.5 g of flour samples in 20 mL of distilled water in pre-weighed centrifuge tubes. Then the slurries were heated at 60, 70, 80 and 90 °C for 30 min in a thermostatically controlled water bath while shaking every 5 min to keep the flour granules suspended and then cooled to room temperature (30 ± 2 °C). Then the prepared slurries were centrifuged (EBA 20, Hettich, Tuttlingen, Germany) at 30 g for 10 min to separate the gel and supernatant and then the decanted supernatant was used for subsequent analysis of the solubility pattern. The weight of swollen flour was determined and swelling power was calculated as the ratio of the weight of the swollen flour particles to the weight of the flour sample and expressed as a percentage.

To determine the solubility index, supernatants were transferred into evaporating dishes (pre-weighed) and dried in an air oven at 105 °C for 4 h and the weight of the residue was measured. The water solubility index was calculated using the amount of dried solids obtained by evaporating the supernatant. Water solubility index presented as gram dried solids in 100 g of sample on a dry weight basis.

$$Solubility\ index = \left\{ \frac{(weight\ of\ dried\ solid)}{weight\ of\ sample} \right\} * 100\%$$

2.5.2. Water and oil holding capacity

The water holding capacity (WHC) and Oil holding capacity (OHC) of the legume flours were determined as described in Ref. [17]. Briefly, 1 g of legume flour was mixed with 10 mL of water/oil in a centrifuge tube, vortexed (ZX3, Thomas Scientific,

United States) and kept at 30 ± 2 °C for 1 h. Then the suspension was centrifuged (EBA 20, Hettich, Tuttlingen, Germany) at 20 g for 30 min and the volume of water/oil on the sediment water was measured. WHC and OHC were calculated as mL of water/oil absorbed per gram of legume flour.

2.5.3. Least gelation concentration (LGC)

LGC was determined by the method of 21 [21]. Test tubes containing suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) of legume flours in 5 mL distilled water was heated for 1 h in a boiling water bath. The heated slurries were subjected to rapid cooling under running tap water and then the tubes were refrigerated to cool further at 4 °C for 2 h.

The LGC was recorded by observing the samples which did not fall/slip from the tube, when inverted.

2.5.4. Foaming capacity and foam stability

The foam capacity (FC) and foam stability (FS) of the legume flours were determined as described by Ref. [3] with some modifications. Briefly, 2 g of legume flour were added to 50 mL distilled water at 30 ± 2 °C and the suspension prepared in the graduated measuring cylinder was mixed and properly shaken manually to foam and the volume of the foam after the 30s was recorded. Then the changes in the foam volume in the cylinder were measured at 0, 30 and 60 min intervals to obtain FC of the legume sample. The foam volume recorded after 1 h was used to determine the FS as a percentage of the initial foam volume. The procedure was triplicate and the mean values were calculated.

$$FC = \left\{ \frac{initial\ foam\ volume}{foam\ volume\ after\ 30s} \right\} * 100\ \%$$

$$FS = \left\{ \frac{foam\ capacity\ after\ 1\ hour}{(foam\ capacity\ after\ 30s)} \right\} * 100\%$$

2.5.5. Emulsifying activity and emulsion stability

Emulsifying properties of the legume flours were determined according to the method given by Ref. [22] with slight modifications. Briefly, a 3.5 g flour sample in 50 mL water was homogenized for 30s at 10000 rpm (Omni General Laboratory homogenizer, GLH 850, Georgia, USA). Soybean oil (25 mL) was added and then the mixture was homogenized for 30 s. Thereafter, 25 mL soybean oil was added to the same mixture and the mixture was homogenized again for another 90 s. Finally, the prepared emulsion was divided equally into two 50 mL centrifuge tubes and then centrifuged (EBA 20, Hettich, Tuttlingen, Germany) at 30 g for 5 min.

EA was computed as follows;

$$EA = \left\{ \frac{Volume\ of\ emulsion\ layer\ cream\ fraction}{volume\ of\ emulsion\ before\ centrifuging} \right\} * 100\%$$

The ES of the legume flour was measured using the samples prepared for emulsifying activity measurement. Samples were heated at 85 °C for 15 min and then cooled and centrifuged for 5 min at 1100 g. The ES was presented as the % of emulsifying activity left after heating.

2.6. Statistical analysis

Significant differences between the results (except least gelation concentration and results obtained from DSC thermograms) were calculated using ANOVA (General linear model and Tukey test) with the help of Minitab 18. Differences at $p < 0.05$ were considered to be significant. Complete Randomized Design (CRD) was used for the study. Results were expressed as mean $\pm$ Standard deviations. Values were the average of triplicate experiments.

3. Results and discussion

3.1. Physicochemical properties

3.1.1. Proximate composition

Germination of legume seeds had a significant effect on the proximate composition of legume flours as indicated in Table 1. Significant differences ($p < 0.05$) were observed in protein content between germinated and non-germinated two legume flour samples. Protein contents of the flours of *C. gladiata* and *M. pruriens* increased by 17.04% and 14.69% respectively due to germination, suggesting the nutritional advantage of the use of germinated legume flour in food application. The significant increase in protein content of both germinated flour types could be related to the respiration process of seed during sprouting that consumed most carbohydrate and fat molecules and yielded new protein compounds [8]. An increase in the protein content may be due to the synthesis

Table 1

Proximate composition and selected mineral content of flour obtained from germinated and non-germinated under-utilized legume seeds.

Scientific Name	Flour type	Moisture %	Ash %	Crude Protein %	Crude Fat %	Crude Fiber %	Mineral composition (mg/kg)				
							Fe	Cu	Zn	Mn	Se
Canavalia gladiate	Non-germinated	6.06 ± 0.05[b]	2.27 ± 1.13[a]	22.29 ± 1.55[a]	1.37 ± 0.00[b]	3.73 ± 0.01[b]	26.00 ± 0.01[a]	5.50 ± 0.00[a]	22.00 ± 0.00[a]	7.30 ± 0.00[a]	2.00 ± 0.00[c]
	Germinated	7.44 ± 0.21[c]	2.10 ± 0.31[a]	26.09 ± 0.35[b]	1.28 ± 0.08[a]	5.00 ± 0.85[c]	28.00 ± 0.02[b]	6.10 ± 0.02[b]	26.00 ± 0.07[b]	8.80 ± 0.05[b]	2.00 ± 0.01[c]
Mucuna pruriens	Non-germinated	5.79 ± 0.04[a]	2.71 ± 0.84[a]	24.31 ± 1.09[a]	2.59 ± 0.01[d]	1.46 ± 0.50[a]	57.00 ± 0.09[c]	10.00 ± 0.01[c]	28.00 ± 0.01[c]	17.00 ± 0.02[d]	1.10 ± 0.01[b]
	Germinated	8.61 ± 0.07[d]	3.25 ± 0.45[a]	27.89 ± 0.86[c]	2.48 ± 0.06[c]	2.18 ± 0.47[a]	63.00 ± 0.03[d]	17.00 ± 0.08[d]	32.00 ± 0.02[d]	14.00 ± 0.00[c]	0.73 ± 0.00[a]

Average values of three measurements (For n = 3 ± SD), All data reported on a dry basis, Values followed by the same letter in each column are not significantly different ($p < 0.05$) by Tukeys test.

of enzyme protein by germinating legume seeds resulting in the production of more amino acids [23]. A similar trend also was reported in tiger nuts 21 [21].

The fat content of the flour samples was within the range of 1.28%–2.59% (Table 1) and there were significant differences in the fat content of the samples. The fat content of the germinated legume flour samples was significantly lower ($p < 0.05$) compared with the flours from non-germinated seeds. This could be due to the higher activities in lipolytic enzymes during the germination process which hydrolyses fat in the seeds into fatty acids. A similar observation was also reported in Ref. [21]. The ash content of the flours from both germinated and non-germinated two legumes ranged from 2.10% to 3.25%. However, there were no significant differences ($p < 0.05$) among the flour in ash content. The crude fiber content of the flour is within the range of 1.46%–5.00% (Table 1). It was found that the crude fiber content increased significantly ($p < 0.05$) during the germination process in *C. gladiata*. However, the increase of fiber content in *M. pruriens* was insignificant ($p < 0.05$). A similar trend was also found in Ref. [24]. An increase in fiber content in *C. gladiata* may be due to the increased production of cellulose and pectic polysaccharides during germination. The moisture content of both germinated and non-germinated legume flour samples varied from 5.79% to 8.61%. Carbohydrate content (calculated by the difference) ranged between 55.59% and 64.28%. Decreased carbohydrate contents were found in both varieties during the germination process and this could be due to the breakdown of more complex carbohydrates into simple forms to facilitate the growing seedlings to utilize more efficiently. A similar trend was also reported in some conventional legumes such as *Lablab purpureus* (L.), *Vigna unguiculata* (L.), *Stizolobium niveum* (L.) and *Canavalia ensiformis* (L.) in Ref. [24].

3.1.2. Mineral content

Table 1 represents the effect of germination on selected minerals (Fe, Cu, Zn, Mn, Se) in both *C. gladiata* and *M. pruriens* legume flour types. *C. gladiate* had the highest content (2.0 mg/kg) of Selenium, which is considered a primary antioxidant [25]. Consumption of 25 g of *C. gladiata* seed flour may fulfill the Recommended Dietary Allowance (RDA) (55 μg/day) of Selenium for an adult. However, higher quantities of Fe, Cu, Zn and Mn were reported in Ref. [6] compared to mineral contents reported in this study for *C. gladiata*. *M. pruriens* seed flour had the highest contents of Iron (57 mg/kg), Copper (10 mg/kg), Zinc (28 mg/kg) and Manganese (17 mg/kg), which are trace minerals, beneficial as cofactors for antioxidant enzymes. Consuming 100 g of *M. pruriens* seed flour may account for 35% of the RDA (16–18 mg/day) of Iron, 100% fulfillment of the RDA (900 μg/day) of Copper, (1.8–2.3 mg/day) of Manganese, 25% of the RDA (8–11 mg/day) of Zinc for an adult. However, all minerals analyzed were reported in increased quantities for germinated seed flour samples, than in the non-germinated. Germination has delivered a positive impact on the availability of these essential minerals, reducing the anti-nutrient factors such as phytate which binds minerals making them not readily available for absorption [26,27]. Phytic acid is hydrolyzed during the process of germination, making micro elements more bioavailable. During germination, a significant increase ($p < 0.05$) in studied mineral levels was observed in both varieties except Selenium and Manganese in *M. pruriens*. As reported in Ref. [21], the increase of minerals such as Calcium, Magnesium, Potassium in *Vigna subterranean* (Bambara groundnut) flours had been due to germination.

3.1.3. Amino acid profile

Legumes contain 20–45% of protein with both essential and non-essential amino acids [28]. As extracted from the chromatograms

Table 2
Amino acid composition of selected Non-germinated and germinated legume seed flour.

	Amino acid composition (mg/100 g)			
	Canavalia gladiate		Mucuna pruriens	
	Non-germinated	Germinated	Non-germinated	Germinated
4-Hydroxy proline	Not detected LOD = 2.6	Not detected LOD = 0.87	Not detected LOD = 0.87	Not detected LOD = 0.87
Alanine	819.00 ± 0.01^h	914.00 ± 0.04^i	853.00 ± 0.02^e	840.00 ± 0.02^e
Arginine	850.00 ± 0.02^k	924.00 ± 0.00^j	1429.00 ± 0.01^n	1381.00 ± 0.00^m
Aspartic acid	1973.00 ± 0.05^p	2392.00 ± 0.01^P	2689.00 ± 0.13^p	2893.00 ± 0.00^p
Cystine + Cysteine (sum)	177.00 ± 0.00^a	164.00 ± 0.10^a	302.00 ± 0.02^b	315.00 ± 0.05^b
Glutamic acid	2262.00 ± 0.12^q	2556.00 ± 0.08^q	2997.00 ± 0.13^q	2969.00 ± 0.00^q
Glycine	742.00 ± 0.10^e	768.00 ± 0.02^e	1107.00 ± 0.17^g	1058.00 ± 0.01^g
Histidine	540.00 ± 0.00^d	557.00 ± 0.12^d	621.00 ± 0.06^c	617.00 ± 0.03^d
Isoleucine	797.00 ± 0.01^g	860.00 ± 0.10^f	1169.00 ± 0.01^j	1150.00 ± 0.02^j
Leucine	1481.00 ± 0.02^o	1622.00 ± 0.05^o	1394.00 ± 0.02^m	1770.00 ± 0.04^o
Lysine	1055.00 ± 0.10^n	1051.00 ± 0.11^m	1546.00 ± 0.20^o	1484.00 ± 0.20^n
Methionine	228.00 ± 0.01^b	216.00 ± 0.09^b	280.00 ± 0.01^a	284.00 ± 0.02^a
Phenylalanine	842.00 ± 0.00^j	929.00 ± 0.01^l	1175.00 ± 0.01^k	1175.00 ± 0.04^k
Proline	761.00 ± 0.03^f	883.00 ± 0.20^g	1383.00 ± 0.02^l	1283.00 ± 0.01^l
Serine	1014.00 ± 0.01^m	1128.00 ± 0.01^n	1137.00 ± 0.05^h	1148.00 ± 0.03^i
Threonine	829.00 ± 0.02^i	903.00 ± 0.30^h	1004.00 ± 0.01^f	959.00 ± 0.01^f
Tyrosine	264.00 ± 0.20^c	301.00 ± 0.15^c	657.00 ± 0.03^d	600.00 ± 0.20^c
Valine	866.00 ± 0.10^l	926.00 ± 0.01^k	1143.00 ± 0.01^i	1155.00 ± 0.06^h

Average values of three measurements (For $n = 3 \pm SD$), All data reported on a dry basis, Values followed by the same letter in each column are not significantly different ($p < 0.05$) by Tukeys test.

in Fig. 2, Table 2 represents the amino acid composition of non-germinated and germinated seed flour of two legume varieties; *C. gladiata* and *M. pruriens*. Seed flour of *M. pruriens* contains significantly higher levels of all amino acids, except leucine, compared with *C. gladiata flour*. Tyrosine, arginine, proline, cysteine, lysine and glycine content in flour of *M. pruriens* were more than 40% higher compared with that of *C. gladiata.* The majority of the amino acids in seeds of both legumes increased due to germination except lysine, cysteine and methionine in *C. gladiata* and arginine, lysine, proline and tyrosine in *M. pruriens* (Table 2). Glutamic acid, which is one of the non-essential amino acids, had scored the highest content in the amino acid composition of germinated and non-germinated legume flour of both legume varieties. Aspartic acid had been the second most abundant amino acid in all flour samples. Leucine had the highest concentration among essential amino acids present in tested legume flour samples and was recorded in the range of 1394–1770 mg/100 g. Leucine also showed an increased concentration in both germinated seed flour samples compared to the non-germinated of the same legume variety. Essential sulfur-containing amino acids such as methionine, cystine and cysteine were present in considerably lower contents in all tested flour samples. Cystine was a limiting amino acid in *C. gladiata* whereas methionine in *M. Pruriens*. Except for soy protein, most other legumes are considered incomplete sources of proteins due to the limited availability of essential sulfur-containing amino acids [28].
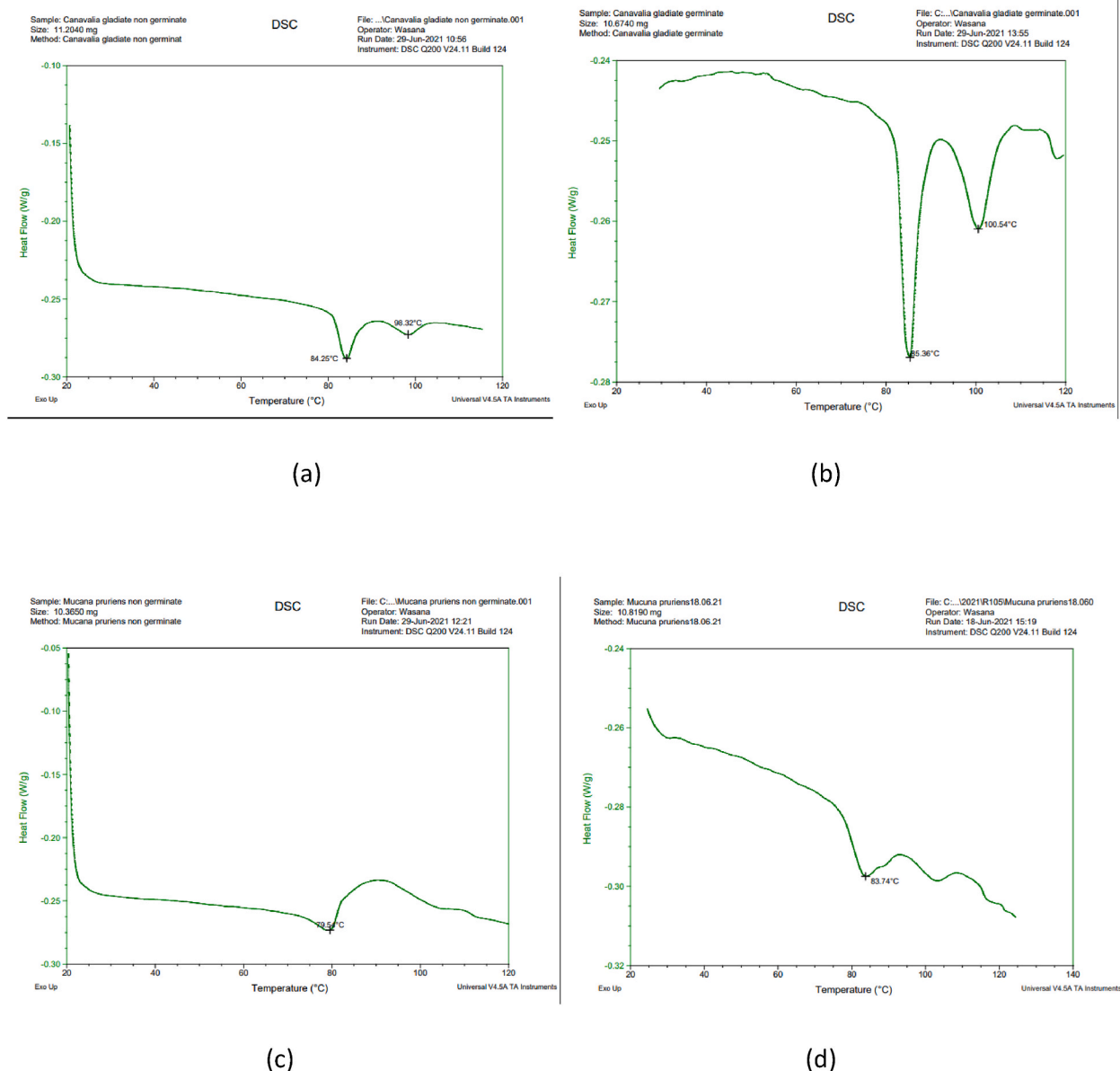

(a)

(b)

(c)

(d)

Fig. 1. DSC thermograms of (a) Non-germinated *C. gladiate* seed flour (b) Germinated *C. gladiate* seed flour (c) Non-germinated *M. Pruriens* seed flour (d) Germinated *M. Pruriens* seed flour.

Nonetheless, underutilized legume varieties are emerging cheap sources of protein for catering the protein-energy malnutrition in developing countries, whilst in developed countries, they are being studied for their potential in treating cardiovascular diseases and related disorders. Obesity risk can be reduced by the increased consumption of dietary amino acids, both essential and non-essential [29]. Administration of histidine through diet has decreased inflammation; body mass index and fat mass [29]. The seed flour of *M. Pruriens* contained about 621 mg/100 g histidine and it is more than 12% higher than that of *C. gladiata*. Green gram (*Vigna radiate*) contains about 150–200 mg/100 histidine content [30].

Although an insufficient amount of research has been carried out in humans, rodent model-based studies proved the positive influences of branched-chain amino acids, methionine and glutamic acid on the complications related to obesity [31]. In addition, the
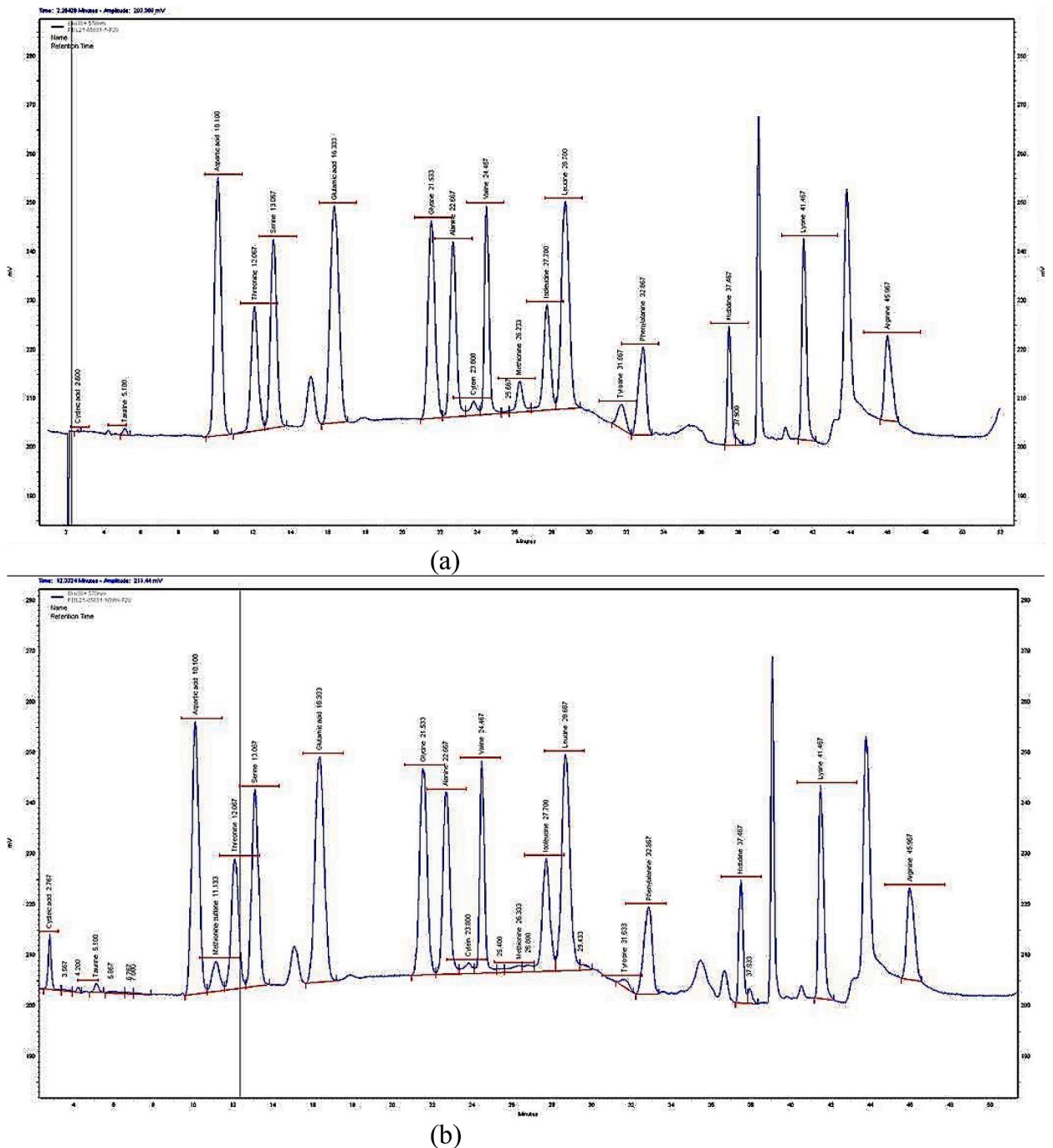

(a)

(b)

Fig. 2. Illustrative chromatograms (a-unoxidized sample, b-oxidized sample) of Amino acid composition analysis of selected Non-germinated and germinated legume seed flour.

therapeutic effects of glutamic acid as a neuromodulator for the regulation of appetite and adiposity control have been studied [10]. In contrast, researchers have stated the importance of administering a controlled diet with specific amino acids in the management of obesity-related disorders such as non-alcoholic fatty liver disease and insulin resistance, since higher consumption of specific amino acids may cause negative impacts on liver health and glucose metabolism [32].

3.1.4. Thermal properties

Fig. 1 shows the thermal properties of both non-germinated and germinated *C. gladiata* and *M. Pruriens* seed flour evaluated using Differential Scanning Calorimetry. Table 3 shows the DSC parameters, transition enthalpy (ΔH), transition onset temperature (T_o), transition peak temperature (T_p) and Peak Height Index (PHI) extracted from the thermograms. The thermal properties were recorded under the scanning temperature range between 20 °C and 130 °C. The temperature is of utmost importance to the performance of a product process, as protein functionality can be modified by heat and also some proteins get denatured during the process. Nevertheless, each flour sample was hydrated up to 60% moisture content since complete gelatinization could be observed in the gelatinization endotherm only when the flour: water mixtures have 60% or higher water content [33]. The water content greatly affects the gelatinization behavior of starch where starch granules undergo the transition from a crystalline state to a gel state by absorbing water at higher temperatures. Water also affects the thermal stability of proteins [20]. In the current study, all experimental samples indicated peak gelatinization temperature within the range of 79.5–85.4 °C. It can be aligned with the gelatinization temperature ranges (78.1–82.2 °C) obtained for different varieties of Cowpea (*Vigna unguiculata*) in the study conducted by Ref. [20]. The peak gelatinization temperature of both *C. gladiata* and *M. pruriens* germinated seed flour samples was increased when compared to their non-germinated seed flour samples. This may be indicative of the presence of more stable starch crystallites and amylose-lipid complexes in germinated seeds irrespective of the fact that most starch granules undergo decomposition during the germination process of legume seeds. Besides, having a higher peak gelatinization temperature and wide gelatinization temperature range is sometimes unacceptable for industrial purposes in terms of energy cost. The transition enthalpies of all samples deviate within 0.88–1.75 J/g, showing an increased enthalpy value in the germinated sample when compared to the non-germinated sample of each variety. The enthalpy values denote the balance of all heat exchanges associated with the starch gelatinization, protein denaturation and protein-starch interactions. Peak Height Index (PHI) indicates the sharpness of the transition range which is related to the structural relationship between the amorphous and crystalline regions of the starch granules which directly influences the starch gelatinization [20]. Narrower transition range results in higher PHI. The present study reported narrower transition ranges with higher PHI for germinated seed flour samples. The thermogram obtained for non-germinated *M. Pruriens* seed flour (Fig. 1c) had a narrow transition range where it could be assumed that peak/melting temperature (T_p) recorded in this case is an apparent endothermic melting peak and there is a great possibility to get the correct endothermic melting peak beyond 120 °C. The thermogram of germinated *M. Pruriens* flour also indicated a similar apparent endothermic melting peak in which there was no conclusion temperature to be detected at 120 °C. The concluding temperature of the thermogram may have existed beyond 120 °C, which authors were unable to observe due to lack of heating facilities in DSC for temperatures beyond 120 °C. Both *C. gladiata* (Fig. 1a) and *M. Pruriens* (Fig. 1c) non-germinated seed flour samples had thermograms with a large endothermic startup hook. Both germinated and non-germinated *C. gladiata* seed flour had double endothermic melting peaks (Fig. 1a & b). It is possible to have double peaks since during starch gelatinization the most stable crystallites undergo fusion later than already gelatinized starch which caused the appearance of the first peak [34]. On the other hand, peaks can be shifted to higher temperatures if the water content in the mixture decreases.

3.2. Functional properties

3.2.1. Swelling power

Functional characteristics of legume seed flour are mostly attributed to its protein, starch and fiber fraction [35]. During germination seed physiology changes with the metabolic reactions and part of the macromolecules, being reactants and degrade to simple compounds to provide the energy needed for sprouting [36]. Swelling power depends on the hydration capacity of starch available as it consists of three-dimensional branched structures to facilitate holding water [37]. With the increment of temperature starch molecules absorb water and swell, leading to a subsequent disintegration of starch granules and leaching to the solution [17]. Table 4 shows the swelling power and solubility index of non-germinated and germinated *C. gladiata* and *M. Pruriens* legume seed flours measured at 60, 70, 80 and 90 °C. Results revealed that the swelling power of both legume flours was reduced during germination. Losses of starches due to the consequence of metabolism-related activities during the process of germination, resulted in decreased swelling power as it is

Table 3
Thermal properties of flour obtained from germinated and non-germinated under-utilized legume seeds.

Scientific Name	Flour type	ΔH (J/g)	T_o (°C)	T_p (°C)	PHI
Canavalia gladiate	Non-germinated	1.07 ± 0.00^b	81.00 ± 0.02^d	84.25 ± 0.00^c	0.33 ± 0.00^c
	Germinated	1.75 ± 0.01^d	80.40 ± 0.00^c	85.36 ± 0.00^d	0.35 ± 0.00^d
Mucuna pruriens	Non-germinated	0.88 ± 0.00^a	73.40 ± 0.01^a	79.54 ± 0.01^a	0.14 ± 0.00^a
	Germinated	1.41 ± 0.00^c	77.00 ± 0.00^b	83.74 ± 0.00^b	0.21 ± 0.00^b

Values extracted from thermograms (Fig. 1), Average values of three measurements (For n = 3 $\pm$ SD), Values followed by the same letter in each column are not significantly different (p < 0.05) by the Tukeys test.

Table 4

Swelling power and Solubility index of flour obtained from germinated and non-germinated under-utilized legume seeds.

Scientific Name	Flour type	Temperature (°C)	Swelling power (%)	Solubility Index (%)
Canavalia gladiate	Non-germinated	60	5.55 ± 0.05[b]	19.31 ± 1.54[a]
		70	5.49 ± 0.03[b]	21.59 ± 0.58[a]
		80	9.66 ± 0.01[d]	22.42 ± 0.23[a]
		90	9.12 ± 1.40[d]	24.99 ± 1.04[a]
	Germinated	60	3.54 ± 0.22[a]	22.59 ± 0.15[a]
		70	3.63 ± 0.05[a]	21.76 ± 1.61[a]
		80	4.18 ± 0.35[a]	20.12 ± 1.10[a]
		90	6.32 ± 0.07[c]	22.49 ± 1.31[a]
Mucuna pruriens	Non-germinated	60	4.38 ± 0.02[a]	27.01 ± 0.76[b]
		70	4.15 ± 0.13[a]	23.03 ± 1.01[a]
		80	7.26 ± 0.11[c]	26.29 ± 0.05[b]
		90	9.03 ± 0.16[d]	23.89 ± 4.77[a]
	Germinated	60	3.02 ± 0.20[a]	18.71 ± 3.53[a]
		70	3.26 ± 0.13[a]	20.02 ± 1.02[a]
		80	3.78 ± 0.06[a]	20.12 ± 0.65[a]
		90	5.47 ± 0.28[b]	16.75 ± 0.04[a]

Average values of three measurements (For n = 3 ± SD), All data reported on a dry basis, Values followed by the same letter in each column are not significantly different (p < 0.05) by Tukeys test.

related to the amount of starch available, especially amylopectin [24].

The highest swelling power was recorded for non-germinated *C. gladiata* seed flour at 80 °C while its germinated fraction showed a reduction in swelling power at all temperatures compared to non-germinated flour. Also, the germinated fraction of *C. gladiata* seed flour showed the highest swelling power at 90 °C indicating an increment in the gelatinization temperature range. A trend of increasing swelling power with increasing temperature within the range was also observed in *Voandzeia subterranean* (Bambarra groundnut) by 5 [4]. This may be due to the strengthening of the bonding forces within the granules of starch due to higher temperatures. Results revealed that both non-germinated and germinated *M. Pruriens* seed flour had their highest swelling powers at 90 °C, while the germinated fraction clearly showed a lower swelling power compared to the non-germinated fraction. There was a decrease in the amount of starch leached out to the solution during gelatinization at 80 and 90 °C, in the germinated seed flour of both legume varieties. The amylose fraction of starch may be decreased during the germination process of legume seeds due to the degradation of macromolecules in metabolic reactions that occurred at seed germination. Water solubility index is related to the solubility of molecules and it varied from 16.75% to 27.01% (Table 4) in both legume flours, studied and no significant difference (p < 0.05) was observed due to germination and the temperature. A similar range of solubility index is also presented in lima and pinto beans by Ref. [3] and the formation of protein-starch complex and amylose-lipid complex in the heating process may affect the water solubility index of the legume flour.

3.2.2. Water and oils holding capacities

The water-holding (WHC) and oil holding capacities (OHC) of flour are critically a function of protein and starch. The fiber aid in trapping water or oil. The OHCs of the studied two legume flours are presented in Table 5. WHC of non-germinated *C. gladiata* flour was significantly higher (p < 0.05) than that of *M. Pruriens* flour. The WHC of flour is important physical property as it influences other functional and sensory properties of food. It was reported that many legume seeds contain hydrophilic molecules like polysaccharides and globular proteins and therefore generally they have high WHC [22]. Furthermore, the protein quality of the legume seeds also affects their WHC [3]. The conformation of protein molecules, their polarity and amino acid composition are some intrinsic factors affecting WHC and OHC [38]. The OHC of legume flour plays an important role in improving the texture and flavor of food. The OHCs of the legume flours ranged from 0.85 mL/g to 1.34 mL/g and significantly higher OHC was found in *C. gladiata* flour (Table 4). The

Table 5

Water Holding Capacity, Oil Holding Capacity, Least Gelation Concentration, Foaming Capacity, Foam stability, emulsifying activity and Emulsion stability of flour obtained from germinated and Non-germinated under-utilized legume seeds.

Scientific Name	Flour type	WHC (mL/g)	OHC (mL/g)	Least Gelation Concentration (%)	Foaming Capacity (%)	Foam stability (%)	Emulsifying activity (%)	Emulsion stability (%)
Canavalia gladiate	Non-germinated	2.62 ± 0.31[c]	1.22 ± 0.20[b]	20	26.50 ± 2.12[c]	71.71 ± 0.40[a]	48.91 ± 1.88[c]	98.23 ± 1.28[a]
	Germinated	2.24 ± 0.26[c]	1.34 ± 0.07[b]	16	31.33 ± 1.15[d]	70.14 ± 4.34[a]	50.25 ± 2.53[c]	98.36 ± 4.42[a]
Mucuna pruriens	Non-germinated	1.97 ± 0.01[a]	0.85 ± 0.12[a]	16	18.00 ± 0.00[b]	80.56 ± 11.79[a]	45.78 ± 0.51[b]	98.66 ± 8.55[a]
	Germinated	2.13 ± 0.07[b]	1.29 ± 0.14[b]	18	12.00 ± 2.00[a]	78.25 ± 6.14[a]	39.17 ± 4.32[a]	99.85 ± 1.97[a]

Average values of three measurements (For n = 3 ± SD), All data reported on a dry basis, Values followed by the same letter in each column are not significantly different (p < 0.05) by Tukeys test.

mechanism of oil absorption by legume flour involves capillary interaction to retain the absorbed oil in the matrix and also hydrophobic proteins present in the flour play a significant role in the absorption of oil [3]. Different OHC of the studied two legume flours may be due to differences in starch content, protein content, types of protein and particle sizes. As shown by Ref. [3], legume flour exhibits generally a higher OHC as protein molecules in the flour contain significant levels of non-polar side chains. Germinated *M. pruriens* flour exhibited a significantly higher ($p < 0.05$) WHC and OHC compared with its non-germinated flour. As indicated in the amino acid composition of legume seed flour (Table 2), the polar amino acid content has increased in both legume seeds with germination paving the way to hold more water in the flour matrix. However, both germinated and non-germinated *M. pruriens* flour had higher quantities of polar amino acids when compared to *C. gladiata* flour. The ability to hold more oil in macromolecules may lead to better flavor-retaining ability and is highly acceptable in food systems where optimum oil absorption is desired.

3.2.3. Least gelation capacity

The Least Gelation Concentration (LGC) measures the ability of the protein fraction to form a gel and a lower LGC indicates a good gelling capacity. LGC of studied germinated and non-germinated flours of two legume varieties varied from 16% to 20% as shown in Table 5. In a straight sense, the gelation ability of *M. pruriens* seed proteins was improved with the germination and a similar trend was also reported in flours of *Cyperus esculentus* by 21]. However, the gelation ability of *C. gladiate* seed flour was decreased with germination. A similar range of LGC has been previously reported in *Pithecellobium jiringa* legume flour by Ref. [39] and they have reported that the presence of carbohydrates in the system enhances the gelling capacity as it reduces the protein's thermodynamic affinity for the aqueous solution and strengthens the interaction among protein molecules.

3.2.4. Foaming capacity and stability

Foaming capacity (FC) and foam stability (FS) are other functional attributes of protein globules highly desired in a colloidal food system. With the interfacial area created by a protein to accommodate air bubbles, foaming ability increases and if the protein films are less flexible and easily collapsed the air bubbles thus formed cannot retain, leading to lower foam stability [12]. Table 5 represents the FC and FS of non-germinated and germinated seed flours. There was a significant difference among FC of all tested samples and the highest FC was recorded for germinated *C. gladiata* seed flour. However, there was no significant difference among the FS of both non-germinated and germinated *C. gladiata* seed flour. The FC of *M. Pruriens* seed flour had lowered with germination. As reported in Ref. [3], proteins and carbohydrates which are present in the flour are responsible for the foaming properties.

3.2.5. Emulsifying activity and stability

Emulsifying activity (EA) and emulsion stability (ES) indicate the ability to keep food systems stable without phase separation. It is highly dedicated to protein molecules which act as surface-active agents for creating electrostatic repulsion on oil droplet surfaces [40]. The EA reflects the ability of the proteins in the system to give strength to an emulsion to withstand stress and the ES is related to the consistency of the interfacial area in a given period [41]. According to Table 5, significantly higher ($p < 0.05$) EA was found in both non-germinated and germinated *C. gladiata* seed flour whereas *M. Pruriens* seed flour had lower EA which further decreased after germination of seeds. EA of the studied flour remained within the range of 39.70–50.25%. However, relatively higher EAs were reported in chickpea (61.14%) and red kidney beans (92.20%) by Ref. [3]. ES did not change significantly after germination in both *C. gladiata* and *M. Pruriens* seed flour and the ES values remained within the range of 98.23%–99.85%. Pinto bean flour and navy bean flour have shown about 84.15% and 96.9% ES values respectively [3]. Differences in the composition especially protein content, protein quality and starches present in legume flours may contribute to the differences in emulsion activities.

4. Conclusion

The physicochemical and functional properties of germinated *C. gladiata* and *M. Pruriens* seed flour showed overall significant variations in comparison to non-germinated *C. gladiata* and *M. Pruriens* seed flour. This may be ascribed to changes in seed physiology during the germination process that involves several metabolic reactions, degrading macromolecules to provide energy. The resultant decrease in some amino acids (Lysine, Methionine), swelling power, solubility indices and increase in LGC may have been caused due to the breakdown of starch, fat and protein. In addition, all experimental samples indicated peak gelatinization temperature within the range of 79.54 °C–85.36 °C. The peak gelatinization temperature of both *C. gladiate* and *M. Pruriens* germinated seed flour samples was increased when compared to their non-germinated seed flour samples. Nevertheless, the new compounds that could have been synthesized by metabolic reactions including respiration during the germination process, may have resulted in improving the protein content, mineral content, some amino acids such as glutamic acid, aspartic acid and leucine and functional properties such as WHC, OHC, FC, EA and thermal properties. Therefore, in light of the aforementioned, the germinated *C. gladiata* and *M. Pruriens* seed flour are much preferred for functional food product development.

Author contribution statement

H. A. C. O. Hettiarachchi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials analysis tools or data; Wrote the paper. K. D. P. P Gunathilake: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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