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ORIGINAL PAPER

Effect of germination on enzymatic, functional and bioactive attributes of different Pakistani legume cultivars

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Abstract Legume belonging to the family of *Fabaceae* or *Leguminosae*. Three legume cultivars green gram (*Vigna radiata*), lentils (*Lens culinaris*) and black gram (*Vigna mungo*) were selected to study the effect of germination. Germination was carried out by soaking legumes in water for 12 h, followed by spreading them on water soaked jute bag for 24 h. After sprouting, samples were dried at 40 °C in an oven for 3 days followed by storage in refrigerator before further analysis. The legume flours obtained from these germinated and non-germinated samples (control) were subjected to different analysis. Results showed a significant increase in the proximate composition as well as amylase, protease and lipase activity of the germinated legumes compared to non-germinated legumes. Hydration, foaming and emulsifying characteristics were greatly enhanced after germination. Bioactive components including total phenolic content and total antioxidant activities also positivity correlated with the germination of legumes. The results of the study revealed improvement in functional characteristics due to germination of legumes and suggested the use of selected legumes in the development of different nutraceutical products.

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Introduction

Legumes hold a significant position in human nutrition, especially among the lower income group people of developing countries [1]. The legumes are crops of the family *Leguminosae* also called *Fabaceae* [2], and is consumed in many ways, such as whole legumes called grains or dehusked and split legumes called "dhals" [3]. Inclusion of legumes in diet could play beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer [4]. Legume is an important source of protein, starch, dietary fiber, vitamins, especially niacin, riboflavin, thiamine, minerals such as calcium, iron, manganese, and zinc, as well as antioxidants and polyphenols [5]. However, their wider use is somehow limited by the presence of non-nutrient compounds in the seeds which may have adverse effects for human nutrition [1]. Some examples of these compounds are protease inhibitors, lectins, phenolics, phytates, and α-galactosides [6]. Therefore, it is important to develop new derived products for the consumers that could improve the nutritional quality of legumes and provide health benefits [1].

Germination increases the activity of many enzymes such as amylase, protease and lipase [3]. Furthermore, moisture, total ash and total protein contents have reportedly increased due to germination [7]. Legumes are rich in phenolic compounds which are considered as natural antioxidants that could prevent many diseases such as cancer, atherosclerosis [8]. These phenolic compounds do not only

prevent oxidation in foods, but also have impact on human health [9]. Because of these positive effects, scientists and consumers are demanding functional foods [5]. It is also reported that germination improves functional properties such as water absorption capacity, protein solubility index, and emulsification capacity [10]. Functional properties of proteins affect the behavior of protein in food systems as judged by the quality of the final product [11]. However, little work has been done on the effect of germination on the functional properties of legumes.

Thus, the aim of this work was to compare the proximate composition, enzymatic activities, and functional properties of germinated and non-germinated lentils, green gram and black gram grown in Pakistan. Furthermore, the influence of germination on the bioactive components such as antioxidant activity and phenolic compounds of the selected legumes was also evaluated.

Materials and methods

Raw materials

The legumes from a single cultivar namely green gram (*Vigna radiata*), black gram (*Vigna mungo*) and lentils (*Lens culinaris*) were procured from PARC (Pakistan Agriculture Research Council), Karachi, Pakistan.

Germination

Legume seeds (100 g) were soaked in distilled water for 12 h and then spread on a cleaned water soaked jute bag for 24 h to germinate. The seeds were rinsed with 0.7% sodium hypochlorite solution [5] for 6 h to reduce fungal contamination and were dried at 40 °C in an oven for 3 days. Dried seeds were ground to a fine powder (particle size 0.5 mm) using hammer mill.

Proximate composition of legumes

Moisture content, protein, fat and ash contents were determined according to AOAC method [12] whereas the total carbohydrate content was calculated by difference method i.e. by deducting the sum of all constituents from total weight.

Preparation of extracts for determination of enzymatic activity

For amylase and protease assay, extract of germinated and non-germinated legumes were prepared according to the method of Elkhalifa [13]. Briefly, two grams of germinated and non-germinated legume flours were separately suspended in 10 mL of double distilled water for 1 h at room temperature (27 ± 2 °C) with intermittent shaking and centrifuged at 10,000×*g* for 10 min. The supernatant thus obtained was then used for determining amylase and protease activity.

For lipase assay, extract was prepared by the method described by Rahman [14]. Hundred grams of germinated and non-germinated legume flours were separately suspended in 40 mL of 30% (v/v) acetone. After occasional gentle stirring for 3 h at 4 °C, the suspension was filtered and centrifuged at 10,000×*g* for 15 min. This supernatant was used for determination of lipase extract activity.

Analysis of enzymes activity

Amylase activity was assayed by the method of Ezeji [15]. One millilitre of extract was incubated for 1 h with 0.5 mL of 1% (w/v) soluble starch and 0.5 mL of 0.1 M acetate buffer, pH 4.5 in a water bath (37 °C). Dinitrosalicylic acid (2 mL) was added and boiled for 20 min in a water bath at 100 °C. After cooling the volume was made to 25 mL with distilled water and the absorbance of the solution was measured at 550 nm.

Protease activity was determined by employing the method of Cupp-Enyard [16]. Casein solution (0.65%, w/v) was prepared in 50 mM Tris–HCl buffer (pH 7.4) and was used as a substrate. The reaction mixture was made up of 5 mL of the casein substrate and 1 mL of the centrifuged extract previously prepared. After 30 min of incubation at 37 °C, the reaction was terminated by adding 5 mL of 110 mM trichloroacetic acid (TCA). The tubes were allowed to stand for 30 min at 37 °C. Then, each test solution was filtered and 1 mL of the above filtered solution was taken into another test tube. Each test tube was incubated for 30 min at 37 °C after adding 5 mL of 0.5 M sodium carbonate solution and 1 mL of Folins reagent and absorbance was measured at 660 nm using UV spectrophotometer. Concentration of tyrosine was measured with the help of standard graph of tyrosine obtained in the range of 10–100 µg/mL. One unit of protease activity was defined as the amount of the enzyme resulting in the release 1 µg/mL of tyrosine per minute.

The lipase activity was examined essentially as described by Sugihara [17]. Extract 50 µL was mixed with 1 mL of olive oil, 4.5 mL of 50 mM acetate (pH 5.6), 0.5 mL of 100 mM $CaCl_2$, and was incubated for 30 min at 30 °C with stirring at 500 rpm, and the lipase reaction was stopped by adding 20 mL of absolute ethanol. The amount of fatty acids released during the incubation was determined by titrating the mixture with 50 mM KOH. One unit of lipase activity is defined as the activity which liberates 1 µL of fatty acids under the specified conditions.

Water holding capacity

Water holding capacity was determined by the method of Robertson [18]. One gram of dried sample was taken into a graduated test tube and 30 mL of water was added followed by hydration for 18 h. The supernatant was removed by passing through a sintered glass crucible (G4) under vacuum. The hydrated residue weight was recorded and it was dried at 105 °C for 2 h to obtain the residual dry weight. Water holding capacity was expressed as the amount of water retained by per gram of dried sample (g/g dry weight).

$$\text{Water holding capacity (g/g)} = \frac{\text{residue hydrated weight} - \text{residue dry weight}}{\text{residue dry weight}}$$

Water retention capacity

Water retention capacity was determined by taking one gram of dried sample in a graduated test tube followed by addition of 30 mL distilled water. It was then allowed to hydrate for 18 h followed by centrifugation at 3000×*g* for 20 min. The supernatant solution was removed by passing through a sintered glass crucible (G4) under an applied vacuum. The hydrated residue weight was recorded and sample was then dried at 105 °C for 2 h [18].

$$\text{Water retention capacity (g/g)} = \frac{\text{residue hydrated weight after centrifugation} - \text{residue dry weight}}{\text{residue dry weight}}$$

Swelling capacity

Swelling capacity was measured by taking 0.2 g sample in a graduated test tube. Water (10 mL) was added and was allowed to hydrate for 18 h. After 18 h, the final volume attained by the sample was measured [18].

$$\text{Swelling capacity (mL/g)} = \frac{\text{volume occupied by sample}}{\text{original sample weight}}$$

Fat absorption capacity

Fat absorption capacity (FAC) was determined by the method of Sosulski [19]. Sample (4 g) was mixed with 24 mL corn oil in a 50 mL centrifuge tube. The contents were stirred for 30 s after every 5 min for a total time period of 30 min. The tubes were centrifuged at 1600×*g* for 25 min. The free oil was decanted and percentage of absorbed oil was determined by measuring weight difference.

Foaming properties

Foaming capacity and foaming stability was determined according to the method of Okaka [20]. Flour (0.5 g) was mixed with 25 mL of distilled water, in a centrifuge tube by shaking vigorously for 5 min followed by immediate pouring into a 250-mL graduated cylinder. The volume of the foam formed was recorded as the foam capacity (mL/100 mL). Foam stability (mL/100 mL) was recorded after 60 min of storage time.

Emulsion activity and emulsion stability

Emulsion properties (emulsion activity and emulsion stability) were determined by the method of Yasumatsu [21]. Flour (0.5 g) was suspended in a graduated tube containing 3 mL of distilled water followed by addition of 3 mL of vegetable oil. The contents were then shaken vigorously for 5 min. The resulting emulsion was centrifuged at 2000×*g* for 30 min.

$$\text{Emulsifying activity (mL/100 mL)} = \frac{\text{volume of emulsified layer}}{\text{volume of flour slurry}} \times 100$$

To determine the emulsion stability, the homogenized mixture of flour, water, and oil was heated at 80 °C for 30 min and subsequently centrifuged at 2000×*g* for 30 min. The emulsifying stability was then calculated as the volume of the emulsifying layer divided by that of the heated slurry multiplied by 100. Values were reported as mL/100 mL.

Car's index and hausner ratio

The bulk (ρ_B) and tapped (ρ_T) densities were determined by the method of Chinta [22]. Three grams (3 g) of each sample (m_0) was poured through a funnel into the cylinder. Then, the cylinder was slightly tapped to collect the powder sticking to the wall of the cylinder. The volume (V_0) was read directly from the cylinder and was used to calculate the bulk density ($\rho_B = m_0/V_0$). For tapped density ($\rho_T = m_0/V_n$), the cylinder was tapped until a constant volume (V_n) was reached.

The powder flowability was evaluated using the Carr's Index or "percent compressibility" (C) and the hausner ratio (HR) tables were used by the method of Turchiuli [23]. The Carr's Index and the Hausner Ratio were calculated using the following equations, respectively:

$$C = \frac{\rho B - \rho T}{\rho B} \times 100$$

$$HR = \frac{\rho T}{\rho B}$$

Preparation of extracts for determination of total phenolic content and antioxidant activity

The extract was prepared according to the method of Lin [24]. Five gram of each sprouted powder was suspended in 80% (v/v) methanol solution (100 mL) and extracted at 60 °C in a water bath with continuous shaking for 2 h. The extract was filtered through Whatman no. 541 filter paper and the filtrate was subsequently used for determination of total phenolic content and radical scavenging activity.

Determination of total phenolic content

Total phenols were evaluated by the method of Waterhouse [25]. The extract sample (20 µL) was diluted with distilled water (1580 µL) followed by addition of 100 µL of Folin–Ciocalteu (FC) reagent. The sample was mixed and kept for 8 min. Twenty-five percent sodium carbonate solution (300 µL) was added to the extract and the solution was then incubated for 2 h in dark at room temperature. The sample absorbance was measured at 765 nm using UV–visible spectrophotometer (JASCO model V670, JASCO Corporation, Tokyo, Japan). Quantification was performed with respect to the standard curve of gallic acid. The results were expressed as milligram of gallic acid equivalent per gram of legumes.

Determination of free radicals scavenging activity by DPPH

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay was used to determine free radical scavenging activity according to the method of Marinova [26]. The ethanolic diluted sample (1.5 mL) was placed in a test tube followed by addition of DPPH solution (0.5 mL) and was stored in dark for 30 min. Absorbance was determined at 517 nm using UV–visible spectrophotometer JASCO, Model V670 (JASCO Corporation, Tokyo, Japan) against diluted blank. The results were calculated from calibration curves, prepared with ascorbic acid (AEAC—ascorbic acid equivalent antioxidant capacity).

Statistical analysis

Analyses were performed in triplicate. The data was analyzed by analysis of variance (ANOVA) using SPSS (Version 17.0. Inc, Chicago, USA) statistical program. Duncan's multiple range tests were carried out to test any significant differences among the treatments employed. Significance levels were defined using $P \leq 0.05$.

Results and discussion

Proximate composition

Proximate compositions of non-germinated and germinated legumes are given in Fig. 1a–e.

Moisture content

According to the Fig. 1a, germination resulted in 31% increase in moisture content of raw legumes. These values were observed because dry seeds absorbed water rapidly during the process of germination and the kinetics of uptake is influenced by the structure of a seed and may not enter all regions equally [27]. Usually during sprouting, water is absorbed by seeds by a process called "imbibitions" [28]. Initially, imbibition results in the hydration of matrices, such as the cell walls and reserve polymers within the cells. The increase in water uptake of a seed with time depends on the quantity of cells inside the seed to be hydrated [28].

Carbohydrate content

According to Fig. 1b, carbohydrate content of germinated legumes decreased significantly compared to the non-germinated counterparts which is in agreement with the study of El-Adawy [10]. Inyang [29] and Nonogaki [28], observed that during the process of sprouting, carbohydrate was used as source of energy for embryonic growth which led to decline in carbohydrate content after sprouting. Moreover, starch was hydrolyzed into simpler carbohydrates by amylase activity so as to provide energy for cell division while the seeds mature and grow [30].

Crude protein content

The crude protein content of non-germinated legumes was in the range of 20.9–22.8%. Germination resulted in increase of protein content as shown in Fig. 1c. The crude protein content of germinated legumes ranged from 27.1 to 29%. Apparent increase in protein content could be attributed to loss of dry matter, particularly carbohydrates through respiration during sprouting. There is reawakening of protein synthesis upon imbibitions [28] which leads to increase in protein content in sprouted seeds. The

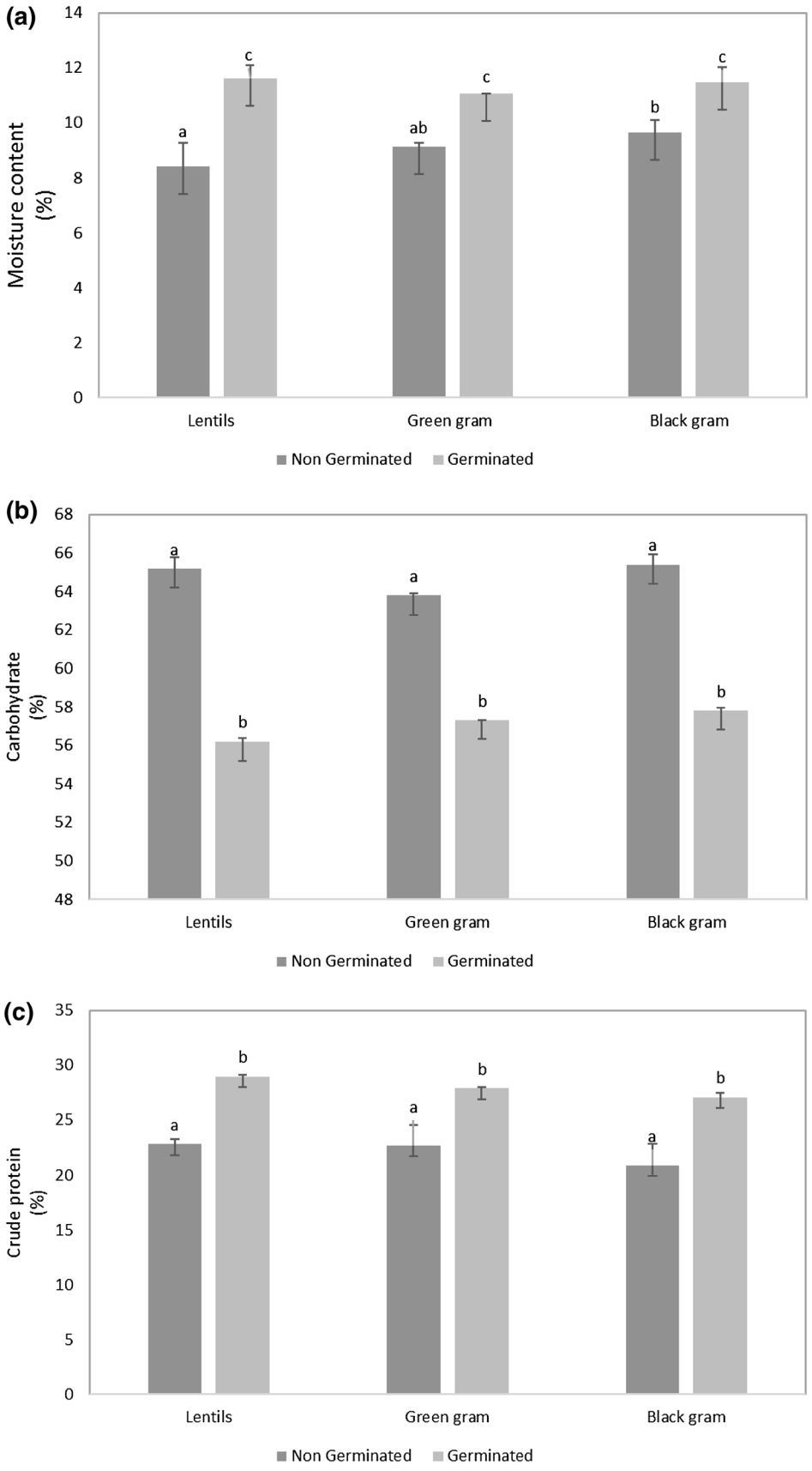
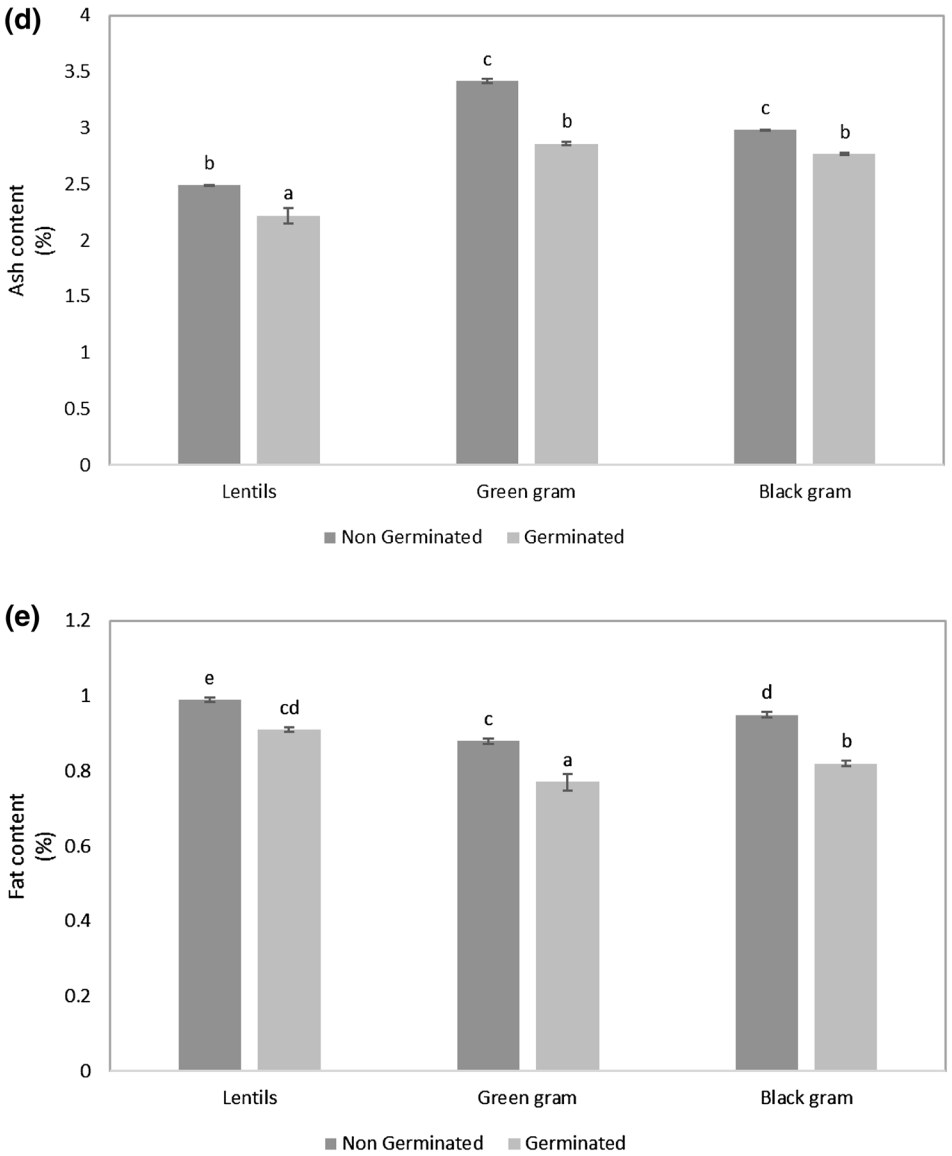


Fig. 1 **a** Effect of germinated on moisture content of legumes. **b** Effect of germinated on carbohydrate of legumes. **c** Effect of germinated on crude protein of legumes. **d** Effect of germinated on ash content of legumes. **e** Effect of germinated on fat content of legumes

Fig. 1 (continued)



synthesis of protease enzymes during germination could be another reason for increase in protein content [31].

Ash content

Ash contents of non-germinated and germinated legumes is shown in Fig. 1d. The decrease in ash content could be due to the rootlet and the removal of hull portion that have some amounts of minerals [32].

Fat content

The fat content decreased during germination as shown in Fig. 1e. Reduction of fat content could be due to total solid loss during soaking prior to germination. Moreover, fat is also used as an energy source during sprouting process [33]. Stored fat is used in catabolic activities of the seeds during sprouting [34]. Kornberg and Beevers [35] reported that degradation of reserve nutrients (lipids and carbohydrates) during sprouting is a process which is essential to provide the energy required for protein synthesis in plant growth. Kwon [36] suggested that fatty acids are oxidized to CO_2 and H_2O to generate energy for synthesis of certain structural constituents in young seedlings.

Enzymatic activities

Seed imbibitions is the initial stage of germination and is characterized by the hydration of tissues and cells and the activation of the enzymes. A growth hormone gibberellin acid is activated and transported from the embryo to the aleurone layer. Active gibberellin acid triggers amylase and protease activity secreted from the aleurone layer into

the endosperm to catalyze the hydrating reaction of stored starch and protein, respectively. Whereas, lipases during the process of germination hydrolyzes stored fats in the cotyledons [37, 38]. The results of amylase, protease and lipase activities are shown in Fig. 2a–c, respectively. The non-germinated seeds of the legumes exhibited low amylase activity that ranged from 0.28 to 0.40 glucose U/mL of extract. The amylase activity increased significantly ($P < 0.05$) in all legumes on germination which ranged from 3.69 to 5.99 glucose U/mL of extract. Whereas, the control samples had low protease activity which significantly increased in germinated seeds. The highest value was observed for germinated black gram 1.77 U/mg. Among the treated legumes, germinated black gram showed the highest (4.00 U/mL) and the lowest (1.57 U/mL) lipase activities. Enzymes activities were found to increase tremendously during 24 h of germination. Our results agree with those reported by Rahman [14], Lasekan [39] and Vanderstoep [40]. These studies explained, that seed reserves are broken down during germination which simultaneously increases the enzymatic activity.

Water holding capacity, water retention capacity and swelling capacity

Water holding capacity (WHC), water retention capacity (WRC) and swelling capacity (SC) are important in food industries when developing formulations for bakery products such as breads, cakes and biscuits because these are associated with the amount of water retained by sample for example during kneading of dough [41]. The ability to hold water physically against gravity is water holding capacity while the quantity of water that remains bound to the hydrated sample following the application of an external force such as pressure or centrifugation is water retention capacity [42]. As shown in Table 1, germinated legumes showed significant increase in water holding capacity and water retention capacity compared to non-germinated counterparts. Increase could be due to the rise of insoluble dietary fiber and starch [43]. The increase in water holding capacity and water retention capacity could be useful for products like sausages, custards and doughs. Since these are supposed to imbibe water without protein dissolution and ultimately attain higher viscosity [44]. Whereas, swelling capacity is the parameter which indicates how much the sample matrix is swollen in terms of volume when the water is absorbed [41]. Germinated legumes exhibited lower swelling properties than non-germinated ones. This fact could be due to losses of starch, since swelling capacity is related to the amount of starch. The hydrolysis of amylopectin chains as a consequence of metabolic activity during germination reduces the swelling capacity of germinated legumes [43].

The fat-absorption capacity

The fat-absorption capacity (FAC) of germinated legumes increased significantly ($P < 0.05$) as shown in Table 1. The highest value of FAC was observed for germinated black gram i.e. 1.77 mL/g. The improvement in FAC of germinated samples could be due to increase in amino acids or due to unmasking of the non-polar residues from the interior of protein molecules [19]. Oil binding property depends on the surface availability of hydrophobic amino acids. This is attributed to the physical entrapment of oil [13]. It is an important factor in food formulation where oil holding properties and flavor retention of food are important considerations [11].

Foaming properties

During whipping, protein gets denatured and aggregates forming a large surface area at the liquid/air interface [45]. The foaming capacity (FC) and foaming solubility (FS) were determined by the loss of liquid resulting from destabilization or leakage which is measured in terms of decrease in volume [13]. Foaming of flours is due to the proteins forming a continuous cohesive film around air bubbles in the foam. However, germination increased the amount of solubilized proteins resulting in improved foaming capacity and foaming stability. It also reduced the surface tension of molecules by denaturation of proteins and thus increased the foaming capacity [13]. The foaming property of germinated legumes is presented in Table 2. The study showed that germinated legumes had significantly higher foaming capacity and foaming stability compared to non-germinated legumes. The better FC of germinated legumes implies incorporation of more air bubbles into the product. Germinated black gram markedly showed higher FC and FS than the remaining legumes. It could be due to its higher protease activity among other germinated legumes. Determination of foaming properties are important to understand the ability of materials to maintain whip as long as possible [46].

Emulsion activity and emulsion stability

The emulsion properties of germinated legumes are presented in Table 3. The emulsion activity (EA) and emulsion stability (ES) of non-germinated legumes ranged from 27 to 35.5 mL/100 mL and 24 to 32 mL/100 mL, respectively. Control black gram had the highest EA and ES which increased by 10 and 8%, respectively after germination. However, after germination, green gram and lentils exhibited better emulsion activity and stability compared to black gram. Whereas, control lentils had

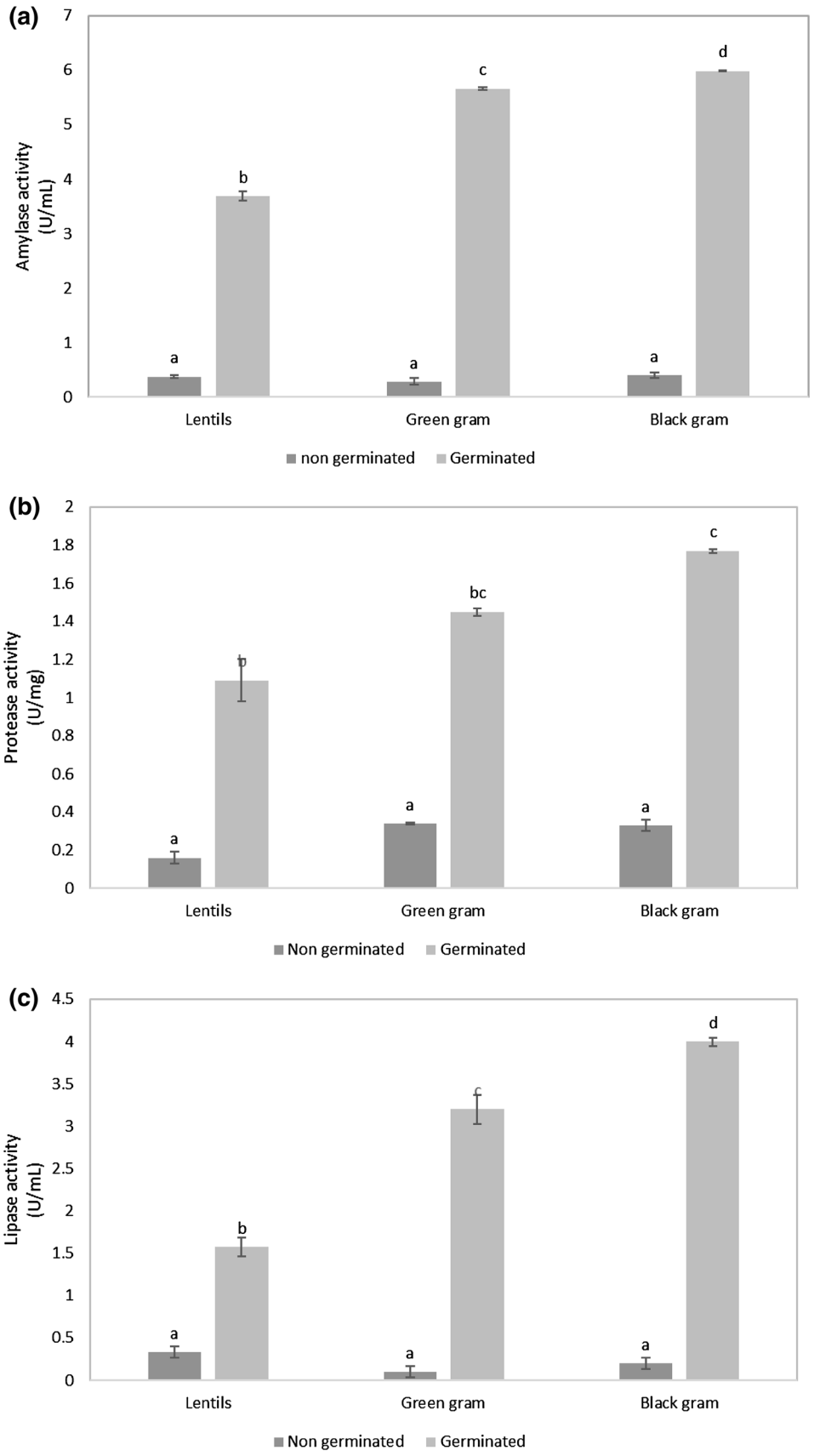


Fig. 2 **a** Effect of germination on amylase activity of legumes. **b** Effect of germination on protease activity of legumes. **c** Effect of germination on lipase activity of legumes

Table 1 Effect of germination on hydration properties and fay absorption capacity of legumes

Samples	Water holding capacity (g/g)	Water retention capacity (g/g)	Swelling capacity (mL/g)	Fat absorption capacity (mL/g)
CL	3.03 ± 0.22^{b}	1.24 ± 0.01^{ab}	$4.13 \pm 0.32c$	1.28 ± 1.19^{a}
GL	3.98 ± 0.39^{c}	3.08 ± 0.13^{c}	2.43 ± 0.40^{b}	1.50 ± 0.03^{b}
CG	2.82 ± 0.17^{a}	1.88 ± 0.03^{b}	$5.83 \pm 0.28d$	1.31 ± 0.02^{a}
GG	4.05 ± 0.09^{c}	2.79 ± 0.17^{c}	1.44 ± 0.09^{a}	1.71 ± 0.05^{c}
CB	2.31 ± 0.42^{a}	0.62 ± 0.20^{a}	5.33 ± 0.76^{d}	1.21 ± 0.11^{a}
GB	4.26 ± 0.50^{c}	3.07 ± 0.89^{c}	2.16 ± 0.28^{ab}	1.77 ± 0.12^{c}

All values are mean of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$

CL non germinated lentil, *GL* germinated lentil, *CG* non germinated green gram, *GG* germinated green gram, *CB* non germinated black gram, *GB* germinated black gram

Table 2 Effect of germination on foam capacity and foam stability of legumes

Samples	Foam capacity (mL/100 mL)	Foam stability (mL/100 mL)
CL	49.66 ± 0.5^{a}	45.31 ± 1.5^{a}
GL	50.66 ± 0.5^{b}	48.33 ± 0.5^{b}
CG	48.66 ± 0.5^{a}	48.00 ± 0.1^{b}
GG	50.66 ± 0.5^{b}	49.00 ± 1.0^{b}
CB	52.00 ± 0.5^{c}	51.00 ± 0.5^{c}
GB	55.33 ± 1.0^{d}	54.33 ± 1.0^{d}

All values are mean of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$

CL non germinated lentil, *GL* germinated lentil, *CG* non germinated green gram, *GG* germinated green gram, *CB* non germinated black gram, *GB* germinated black gram

Table 3 Effect of germination on emulsion activity and emulsion stability of legumes

Samples	Emulsion activity (mL/100 mL)	Emulsion stability (mL/100 mL)
CL	27.17 ± 2.35^{a}	24.61 ± 1.54^{a}
GL	44.61 ± 1.54^{d}	42.79 ± 1.61^{c}
CG	27.68 ± 4.07^{a}	24.61 ± 5.54^{a}
GG	44.15 ± 1.80^{d}	42.80 ± 1.71^{a}
CB	33.39 ± 1.70^{b}	31.79 ± 2.30^{b}
GB	37.67 ± 0.77^{c}	34.35 ± 0.88^{b}

All values are mean of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$

CL non germinated lentil, *GL* germinated lentil, *CG* non germinated green gram, *GG* germinated green gram, *CB* non germinated black gram, *GB* germinated black gram

Table 4 Effect of germination on flow properties of legumes

Samples	Bulk density (g/cm^3)	Hausner ratio	Carr's index (CI)
CL	0.62 ± 0.00^{c}	1.41 ± 0.06^{b}	29.1 ± 3.60^{c}
GL	0.52 ± 0.00^{b}	1.40 ± 0.06^{b}	28.9 ± 3.00^{c}
CG	0.50 ± 0.00^{b}	1.39 ± 0.05^{b}	28.3 ± 2.88^{c}
GG	0.60 ± 0.02^{c}	1.22 ± 0.05^{a}	18.0 ± 3.46^{a}
CB	0.50 ± 0.02^{b}	1.33 ± 0.04^{ab}	24.8 ± 2.82^{bc}
GB	0.41 ± 0.01^{a}	1.26 ± 0.07^{a}	20.7 ± 4.73^{ab}

All values are mean of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$

CL non germinated lentil, *GL* germinated lentil, *CG* non germinated green gram, *GG* germinated green gram, *CB* non germinated black gram, *GB* germinated black gram

Table 5 Powder flowability from the Carr's index and Hausner ratio [23]

Carr's index	Flowability	Hausner ratio
≤10	Excellent	1.00–1.11
11–15	Good	1.12–1.18
16–20	Fair	1.19–1.25
21–25	Passable	1.26–1.34
26–31	Poor	1.35–1.45
32–37	Very poor	1.46–1.59
>38	Awful	>1.60

the lowest EA and ES that is 27.17 and 24.6 mL/100 mL, respectively. Germination caused dissociation and partial unfolding of polypeptides, exposing hydrophobic sites of amino acids, which in turn facilitated hydrophobic association of the peptide chains with the lipid droplets, resulting in increased volume/surface area of protein, and enhancing emulsification properties [11]. Germinated legumes could be suggested in food systems as it confers a stabilized colloidal emulsion because of its higher EA and ES properties [13].

Car's index and hausner ratio

Bulk density, hausner ratio and Carr's index of germinated and non-germinated legumes are presented in Table 4. The bulk density of non-germinated legumes ranged between 0.50 to 0.62 g/cm^3. There was significant decrease ($P < 0.05$) in bulk density of germinated legumes ranging from 0.40 to 0.52 g/cm^3. The reduction in the bulk density on germination could be due to the activity of alpha-amylase enzyme which was activated and dextrinifies starch into its constituent sub-units [47]. Hausner ratios of germinated green gram and black gram showed the least value that is 1.22 and 1.25, respectively which falls into the category of

"fair flowability" as shown in Table 1. Flours with hausner ratio <1.4 could assist in conveying, blending and packaging operations [48]. The Carr's index determines the flowability of the product. Lower the value, higher the flowability as shown in Table 5. The Carr's Index decreased in germinated legumes which showed better flowability in contrast to non-germinated legumes.

Total phenolic content and antioxidant activity

The total phenolic content and percent antioxidant activity of germinated and non-germinated legumes is illustrated in Table 6. Both parameters dramatically increased ($P < 0.05$) in all legumes after germination of 24 h. Total phenols are produced naturally during the development and growth of plants [49]. The total phenolic content of non-germinated legumes ranged from 1.16 to 2.48 mg gallic acid/L which were enhanced significantly in germinated legumes. The rise of phenolic compounds during germination could have an influence on antioxidant activity. Therefore, percent antioxidant activity was also evaluated which showed that germination could improve antioxidant activity. The highest antioxidant activity was observed in germinated lentils (96.8%) which increased significantly after germination. Phenols are produced naturally during the growth and development of plants in response to biotic stresses such as diseases, insects and environmental stresses [49]. The dramatic change in phytochemicals during germination process is considered a natural phenomenon in plants. The present study supports this theory as phenolic content increased for all legumes after germination [50]. The increase in total phenolic content during germination process also has an influence on the free radical scavenging capacity [51]. The Pearson coefficient of correlation between total phenolic content and antioxidant activity was found to be $R^2 = 0.525$ at $P < 0.05$ for different legumes used in the study which suggested the fact that phenols are a major contributor towards antioxidant activity.

Table 6 Effect of germination on total phenolic content and antioxidant activity of legumes

Samples	Total phenols (mg gallic acid/L)	Antioxidant activity (%)
CL	1.16 ± 0.05^{a}	84.06 ± 0.20^{b}
GL	1.61 ± 0.08^{bc}	96.85 ± 0.54^{f}
CG	1.29 ± 0.00^{a}	81.86 ± 0.30^{a}
GG	1.72 ± 0.10^{c}	94.45 ± 0.20^{e}
CB	1.48 ± 0.08^{a}	85.48 ± 0.15^{c}
GB	2.82 ± 0.06^{d}	92.62 ± 0.63^{d}

All values are mean of triplicate determinations. Means within a column with different superscripts are significantly different at $P < 0.05$

CL non germinated lentil, *GL* germinated lentil, *CG* non germinated green gram, *GG* germinated green gram, *CB* non germinated black gram, *GB* germinated black gram

Conclusion

The germination of lentils, green gram and black gram modified their nutritional, enzymatic and functional properties. The present study also revealed the increase in total phenolic content and antioxidant activity after 24 h of germination. Due to which germinated legumes could be incorporated into human food products such as weaning foods, baked goods, dairy products and soups with enhanced nutritional profile.

References

1. L. Trugo et al., Effect of heat treatment on nutritional quality of germinated legume seeds. J. Agric. Food. Chem. **48**(6), 2082–2086 (2000)
2. A. Iqbal et al., Nutritional quality of important food legumes. Food. Chem. **97**(2), 331–335 (2006)
3. R. Tharanathan, S. Mahadevamma, Grain legumes—a boon to human nutrition. Trends Food Sci. Technol. **14**(12), 507–518 (2003)
4. N.A. Shehata et al., Supplementation of wheat flour with some local legumes. Food/Nahrung **32**(1), 1–8 (1988)
5. M. López-Amorós, T. Hernández, I. Estrella, Effect of germination on legume phenolic compounds and their antioxidant activity. J. Food Compos. Anal. **19**(4), 277–283 (2006)
6. S. Deshpande et al., Dry beans of Phaseolus: a review. Part 3. Crit. Rev. Food Sci. Nutr. **21**(2), 137–195 (1984)
7. T. El-Adawy, Chemical and technological studies on faba bean seeds. MSc thesis, Faculty of Agriculture, University of Menofiya, Shibin El-Kom, Egypt, 1986
8. J. Formica, W. Regelson, Review of the biology of quercetin and related bioflavonoids. Food Chem. Toxicol. **33**(12), 1061–1080 (1995)
9. H. Kikuzaki et al., Antioxidant properties of ferulic acid and its related compounds. J. Agric. Food. Chem. **50**(7), 2161–2168 (2002)
10. T. El-Adawy et al., Nutritional potential and functional properties of germinated mung bean, pea and lentil seeds. Plant Foods Hum. Nutr. **58**(3), 1–13 (2003)
11. J.E. Kinsella, N. Melachouris, Functional properties of proteins in foods: a survey. Crit. Rev. Food Sci. Nutr. **7**(3), 219–280 (1976)
12. K. Helrich, *Official Methods of Analysis of the AOAC*, vol. 2 (Association of Official Analytical Chemists Inc, Arlington, 1990)
13. A.E.O. Elkhalifa,, R. Bernhardt, Influence of grain germination on functional properties of sorghum flour. Food Chem. **121**(2), 387–392 (2010)
14. M. Rahman et al., Enzyme activities and degradation of nutrients in chickpea (*Cicer arietinum* L.) seeds during germination. J. Bio-Sci. **16**, 29–34 (2008)
15. T.C. Ezeji, H. Bahl, Purification, characterization, and synergistic action of phytate-resistant α-amylase and α-glucosidase from Geobacillus thermodenitrificans HRO10. J. Biotechnol. **125**(1), 27–38 (2006)

16. C. Cupp-Enyard, Sigma's non-specific protease activity assay-casein as a substrate. J. Vis. Exp. **19**, 899 (2008)
17. A. Sugihara, Y. Shimada, Y. Tominaga, Separation and characterization of two molecular forms of *Geotrichum candidum* lipase. J. Biochem. **107**(3), 426–430 (1990)
18. J.A. Robertson et al., Hydration properties of dietary fibre and resistant starch: a European collaborative study. LWT Food Sci. Technol. **33**(2), 72–79 (2000)
19. F. Sosulski et al., Functional properties of rapeseed flours, concentrates and isolate. J. Food Sci. **41**(6), 1349–1352 (1976)
20. J.C. Okaka, N.N. Potter, Functional and storage properties of cowpea powder-wheat flour blends in breadmaking. J. Food Sci. **42**(3), 828–833 (1977)
21. K. Yasumatsu et al., Whipping and emulsifying properties of soybean products. Agric. Biol. Chem. **36**(5), 719–727 (1972)
22. D.D. Chinta et al., Spray-dried chitosan as a direct compression tableting excipient. Drug Dev. Ind. Pharm. **35**(1), 43–48 (2009)
23. C. Turchiuli et al., Oil encapsulation by spray drying and fluidised bed agglomeration. Innov. Food Sci. Emerg. Technol. **6**(1), 29–35 (2005)
24. P.-Y. Lin, H.-M. Lai, Bioactive compounds in legumes and their germinated products. J. Agric. Food. Chem. **54**(11), 3807–3814 (2006)
25. A.L. Waterhouse, Determination of total phenolics. In *Current Protocols in Food Analytical Chemistry*, ed. by J. Whitalcer (John Wiley and Sons, Inc. Davis, California, 2002) online unit 11.1
26. G. Marinova, V. Batchvarov, Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulg. J. Agric. Sci. **17**(1), 11–24 (2011)
27. H. Nonogaki, Seed germination and reserve mobilization. eLS (2008)
28. H. Nonogaki, G.W. Bassel, J.D. Bewley, Germination—still a mystery. Plant Sci. **179**(6), 574–581 (2010)
29. C. Inyang, U. Zakari, Effect of germination and fermentation of pearl millet on proximate, chemical and sensory properties of instant "fura"—a Nigerian cereal food. Pak. J. Nutr. **7**(1), 9–12 (2008)
30. C.F. Onwuka et al., Investigation on the effect of germination on the proximate composition of African Yam bean (*Sphenostylis stenocarpa* Hochst ex A Rich) and fluted Pumpkin (*Telferia occidentalis*). J. Appl. Sci. Environ. Manag. **13**(2), 59–61 (2010)
31. H.M. Bau et al., Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (*Glycine max*) seeds. J. Sci. Food Agric. **73**(1), 1–9 (1997)
32. R.A. Ghavidel, J. Prakash, The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT Food Sci. Technol. **40**(7), 1292–1299 (2007)
33. T.A. El-Adawy, Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods Hum. Nutr. **57**(1), 83–97 (2002)
34. I. Onimawo, S. Asugo, Effects of germination on the nutrient content and functional properties of pigeon pea flour. J. Food Sci. Technol. **41**(2), 170–174 (2004)
35. H. Kornberg, H. Beevers, The glyoxylate cycle as a stage in the conversion of fat to carbohydrate in castor beans. Biochim. Biophys. Acta **26**(3), 531–537 (1957)
36. T. Kwon, Changes in rutin and fatty acids of buckwheat during germination. Korean J. Food Nutr. **7**, 124–127 (1994)
37. M. Kaneko et al., The α-amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in epithelium. Plant Physiol. **128**(4), 1264–1270 (2002)
38. S. Gujjaiah, C. Kumari, Evaluation of changes in α-amylase, β-amylase and protease during germination of cereals. Int. J Agric. Sci. Res. **3**(3), 55–62 (2013)
39. O. Lasekan, Effect of germination on alpha-amylase activities and rheological properties of sorghum (*Sorghum bicolor*) and acha (*Digitaria exilis*) grains. J. Food Sci. Technol. **33**(4), 329–331 (1996)
40. J. Vanderstoep, Effect of germination on the nutritive value of legumes. Food Technol. **26**, 3121–3125 (1981)
41. M. de Escalada Pla et al., Composition and functional properties of enriched fiber products obtained from pumpkin (*Cucurbita moschata* Duchesne ex Poiret). LWT Food Sci. Technol. **40**(7), 1176–1185 (2007)
42. S. Raghavendra et al., Grinding characteristics and hydration properties of coconut residue: a source of dietary fiber. J. Food Eng. **72**(3), 281–286 (2006)
43. V. Benítez et al., Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes. Food Res. Int. **50**(1), 64–69 (2013)
44. S. Seena, K. Sridhar, Physicochemical, functional and cooking properties of under explored legumes, Canavalia of the southwest coast of India. Food Res. Int. **38**(7), 803–814 (2005)
45. H. Belitz, W. Grosch, *Food Chemistry*, 2nd edn. (Springer, Berlin, 1999)
46. M.J.-Y. Lin, E. Humbert, F. Sosulski, Certain functional properties of sunflower meal products. J. Food Sci. **39**(2), 368–370 (1974)
47. F. Hook, Physico-chemical properties of complementary food from malted quality protein maize (*Zea mays* L.) and defatted fluted pumpkin flour (*Telfairia occidentalis*). Food Pub. Health **3**(6), 323–328 (2013)
48. B.S. Ogunsina, C. Radha, R.S. Govardhan Singh, Physicochemical and functional properties of full-fat and defatted *Moringa oleifera* kernel flour. Int. J. Food Sci. Technol. **45**(11), 2433–2439 (2010)
49. A.M. Nderitu et al., Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated in vitro gastrointestinal digestion. Food Chem. **141**(3), 1763–1771 (2013)
50. D.T. Khang et al., Phenolic profiles and antioxidant activity of germinated legumes. Foods **5**(2), 27 (2016)
51. M. Duenas et al., Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). Food Chem. **117**(4), 599–607 (2009)