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EFFECT OF GERMINATION TIME ON PROXIMATE ANALYSIS, BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF LENTIL (*LENS CULINARIS* MEDIK.) SPROUTS

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ABSTRACT

Background. The lentil plant, *Lens culinaris* L., is a member of the Leguminoceae family and constitutes one of the most important traditional dietary components. The purpose of the current study was to investigate the effects of sprouting for 3, 4, 5 and 6 days on proximate, bioactive compounds and antioxidative characteristics of lentil (*Lens culinaris*) sprouts.
Material and methods. Lentil seeds were soaked in distilled water (1:10, w/v) for 12 h at room temperature (~25°C), then kept between thick layers of cotton cloth and allowed to germinate in the dark for 3, 4, 5 and 6 days. The nutritional composition, protein solubility, free amino acids, antinutritional factors, bioactive compounds and antioxidant activity of raw and germinated samples were determined using standard official procedures.
Results. Sprouting process caused significant ($P \leq 0.05$) increases in moisture, protein, ash, crude fiber, protein solubility, free amino acids, total, reducing and nonreducing sugars. However, oil content, antinutritional factors (tannins and phytic acid) significantly ($P \leq 0.05$) decreased. Results indicated that total essential amino acids of lentil seeds protein formed 38.10% of the total amino acid content. Sulfur-containing amino acids were the first limiting amino acid, while threonine was the second limiting amino acid in raw and germinated lentil seeds. Sprouting process has a positive effect on the essential amino acid contents and protein efficiency ratio (PER) of lentil sprouts. Phenolics content increased from 1341.13 mg/100 g DW in raw lentil seeds to 1411.50, 1463.00, 1630.20 and 1510.10 in those samples germinated for 3, 4, 5 and 6 days, respectively. Sprouted seeds had higher DPPH radical scavenging and reducing power activities.
Conclusions. Based on these results, sprouting process is recommended to increase nutritive value, and antioxidant activity of lentil seeds.

Key words: sprouting, protein, minerals, phytate and antinational factors

INTRODUCTION

Legumes are cultivated all over the world as main or subordinate crop. Only 18 species of approximately 80 different legumes are widely cultivated worldwide, and among them, common bean (*Phaseolus vulgaris*) and lentil (*Lens culinaris*) are the most important species (Shehata, 1992). Lentil (*Lens culinaris* Medik.), a very important legume crop, is widely cultivated and its consumption is steadily increasing. The plants are grown for their small lens-shaped edible seeds, which are rich in protein (35–40%) and carbohydrates,

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and are a good source of calcium, phosphorus, iron and B vitamins (Giannakoula et al., 2012). It is one of the oldest known food crops exclusively used in human foods (Iqbal et al., 2006). It is also high in lysine and therefore a great complement to the amino acid content of cereal grains (Farzana and Khalil, 1999). On the other hand, legumes, in particular lentil, have been reported to contain adequate amounts of antinutrients such as lectins, protease inhibitors, non-protein amino acids (NPAAs), alkaloids, cyanogenic glycosides, pyrimidine glycosides, saponins, tannins, isoflavones, oligosaccharides, α-amylase inhibitors, erucic acid and phytates (Alonso et al., 2000). The anti-nutritional factors (ANFS) may be defined as those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms (e.g. inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed) which exert effects contrary to optimum nutrition (Kumar, 1992). The biological utilization of existing nutrients of these legumes is limited by the presence of various antinutritional substances (Liener, 1994). The mineral content of legumes is generally high, but the bioavilibility is poor due to the presence of phytate, which is a main inhibitor of iron and zinc absorption (Sanberg, 2002). Phytate not only decreases the bioavailability of essential minerals, it also decreases the bioavailability of proteins by forming insoluble phytate-mineral and phytate-protien complexes (Cheryan, 1980; Reddy and Salunkhe, 1981). Most of the toxic and antinutrient effects of these compounds in plants could be removed by several processing methods such as soaking, germination, sprouting, boiling, autoclaving, fermentation, genetic manipulation and other processing methods (Siddhuraju et al., 2002; Soetan, 2008). Sprouting is the practice of soaking and leaving seeds until they germinate and begin to sprout. This practice is reported to be associated with improvements in the nutritive value of seeds (Greiner et al., 2001; Kumar et al., 2010; Zanabria et al., 2006). At the same time, there are indications that germination is effective in reducing phytic acid (Kalpanadevi and Mohan, 2013). In case of white kidney beans, Faba beans and chickpeas; sprouting improved the protein digestibility by decreasing anti-nutritional factors (Mahmoud and El--Anany, 2014; Rubio et al., 2002). The aim of the current study was to investigate the effects of germination time on proximate analysis, bioactive compounds and antioxidative characteristics of lentil (*Lens culinaris* Medik.) sprouts.

MATERIAL AND METHODS

Materials

Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate and aluminum chlorid were purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA). Gallic acid, ethanol and methanol were purchased from E. Merck Co. (Darmstadt, Germany). Solvents used for extraction were purified by distillation prior to use. All other reagents were of analytical grade.

One batch (20 kg) of lentil brown seeds (*Lens culinaris*) namely Giza 1 was obtained from Leguminous Crops Research Department (LCRD), Field Crops Research Institute, Agriculture Research Center (ARC), Giza, Egypt.

Soaking and germination

Lentil seeds were cleaned by hand to remove the foreign materials and soaked in 70% ethanol solution for 15 min at room temperature for disinfection. After washing out of ethanol from the seeds with tap and distilled water, the seeds were soaked in distilled water (1:10, w/v) for 12 h at room temperature (~25°C), then kept between thick layers of cotton cloth and allowed to germinate in the dark for 3, 4, 5 and 6 days. They were watered every day with fresh distilled water. During this time, the radicle of the seed came out, and the seed coat was torn. Sprouted seeds (radicle with cotyledons) were frozen for 12 h to stop the germination process. After thawing at room temperature, the seeds were dried in an electric air draught oven (Isotemp Oven, Fisher Scientific) at 50°C for 48 hrs. Raw and sprouted seeds were ground in an electric grinder (Braun, Model 1021, Germany), passed through a 150 μm mesh sieve and stored in glass containers at 4°C for further analysis.

Analytical methods

Chemical composition. The raw and sprouted samples were analysed for moisture, crude oil, crude protein (N × 6.25), crude fiber and ash as described

in AOAC (2000). The nitrogen free extracts (NFE) was calculated by difference.

Total soluble, reducing and nonreducing sugars. Total soluble sugars were extracted by refluxing in 80% ethanol (Cerning and Guilbot, 1973). Quantitative determination of total soluble sugars was carried out according to the colorimetric method (Yemm and Willis, 1954). Reducing sugars were estimated by Somogyi's modified method (Somogyi, 1945). Nonreducing sugars were determined by calculating the difference between total soluble sugars and reducing sugars.

Determination of protein solubility. Protein solubility was determined by the method of Sathe and Salunkhe (1981). One gram of samples was dispersed in 25 ml of 1 M NaOH. The obtained suspensions were mixed and stirred in an orbital shaker at 150 rpm for 12 h at room temperature and then centrifuged at 3000 g for 20 min. Soluble proteins in supernatants were determined by Lowry et al. (1951). Bovine Serum Albumin was used as standard protein. Soluble protein was expressed as g/100 g DW sample.

Determination of free amino acids. Free amino acids were determined using the method outlined by Rosen (1957). Ninhydrin reagent used for the determination of free amino acids. The free amino acids were calculated as mg/g DW from the standard curve which prepared by using L-aspartic acid as standard.

Antinutritional factors. Total tannins were determined colorimetrically as described in AOAC (1990). Phytic acid was determined according to the method of Wheeler and Ferrel (1971).

Amino acids. Amino acids were determined according to method of Moore and Stein (1963). Hydrolysis of the samples was performed in the presence of 6M HCl at 110°C for 24 h under a nitrogen atmosphere. Sulfur-containing amino acids were determined after performic acid oxidation. Tryptophan was chemically determined by the method of Miller (1967). The amino acid score (AAS) was calculated for each essential amino acid using the FAO/WHO (1991) reference pattern as follows:

AAS = concentration of essential AA in the protein under test / concentration of essential AA in the FAO / WHO standard × 100.

Values for AAS lower than 100 indicate a deficiency of that amino acid. The limiting amino acid (LAA) was defined as that showing the lowest AAS value Chavan and Heigaard (1981). Protein efficiency ratio (PER) was estimated using the regression equation proposed by Alsmeyer et al. (1974):

PER = –0.468 + 0.454 (leucine) – 0.105 (tyrosine).

Determination of total phenolic content, total flavonoids content, DPPH and reducing power of raw and sprouted lentil seeds

Preparation of the extract. Extraction was carried out according to the method of Kim et al. (2006). The flour of raw and sprouted seeds (5 g) was extracted overnight with 100 mL of 70% ethanol solution in a shaking incubator (100 rpm) at room temperature. Then the extracts were centrifuged at 3500 rpm for 15 min. The supernatants were filtered through a Whatman No. 1 filter paper. Extract solutions were concentrated to dryness in a vacuum evaporator at 45°C. Dried extracts were kept in the dark at –25°C until further analyses.

Determination of total polyphenols. Total phenolic content of the extracts were determined using the Folin-Ciocalteu assay system (AOCS, 1990). A 0.5 ml extract was added with 2.5 ml of Folin-Ciocalteu reagent followed by addition of 2 ml sodium carbonate (Na_2CO_3) (75 g/l). The sample was then incubated for 5 min at 50°C. The absorbance was measured at 760 nm. Phenolic contents were calculated on the basis of the standard curve for gallic acid (GAL). The results were expressed as mg of gallic acid equivalent per 100 g DW.

Determination of total flavonoid. The total flavonoid content was determined using the Dowd method (Meda et al., 2005). 5 mL of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extracts solution (0.4 mg/mL). After ten minutes the absorbance was measured at 415 nm using Perkin-Elmer UV-VIS Lambda. Blank sample consisting of a 5 mL extract solution with 5 mL methanol without

AlCl3. The total flavonoid content was determined using a standard curve with catechin (0–100 mg/L) as the standard. Total flavonoids content is expressed as mg of catechin equivalents (CE)/100 g DW.

Determination of antioxidant efficiency

Free radical scavenging capacity. The free radical scavenging capacity of the extracts was measured by using 1, 1-diphenyl-2-picrilhydrazyl (DPPH) assay (Juntachote and Berghofer, 2005). Aliquot (100 µL) of extract was mixed with 5 ml of $6 \cdot 10^{-3}$ M methanolic solution of DPPH radical. The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then measured at 517 nm against a blank. The control was prepared, as above, without any extract and methanol was used for the base line correction. The radical-scavenging activity was expressed as percentage of inhibition and calculated using the following formula:

$$\% \text{ radical scavenging activity} = [(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100$$

where:

Abs control – the absorbance of DPPH radical + methanol, Abs sample – the absorbance of DPPH radical + sample extract/standard.

Determination of reducing power. The ability of the extracts to reduce Fe^{3+} was assayed by the method of Chou et al. (2009). Briefly, 1 ml of extract was mixed with 2.5 ml of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1 g/100 ml). After incubation at 50°C for 25 min, 2.5 ml of trichloroacetic acid (10 g/100 ml) were added and the mixture was centrifuged at 650 × g for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of aqueous $FeCl_3$ (0.1 g/100 ml). The absorbance was measured at 700 nm. BHT was used as reference standard. Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical analysis

All analyses were performed in triplicate and data reported as mean ±standard deviation (SD). Data were subjected to analysis of variance (ANOVA) ($P \leq 0.05$). Results were processed by Excel (Microsoft Office 2007) and SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Chemical compositions of raw and sprouted lentil seeds

Chemical compositions of raw and sprouted lentil seeds are presented in Table 1. Sprouting process caused significant increases in moisture content. The percentage

Table 1. Proximate composition of raw and sprouted lentil seeds, g/100 g DW basis

Sprouting period day	Moisture	Crude protein	Crude oil	Crude fiber	Ash	Total carbohydrate*
0	10.70^{a} ±0.40	25.63^{a} ±0.28	2.20^{a} ±0.13	21.70^{a} ±0.92	2.77^{a} ±0.16	48.70^{a} ±0.67
3	13.42^{b} ±0.31	27.51^{b} ±0.31	1.32^{b} ±0.15	22.30^{b} ±0.81	3.10^{a} ±0.10	46.27^{b} ±0.92
4	14.35^{c} ±0.52	27.90^{bc} ±0.91	1.24^{b} ±0.20	23.61^{b} ±0.82	3.19^{ab} ±0.21	44.56^{bc} ±1.05
5	14.62^{c} ±0.26	28.41^{bc} ±0.64	1.15^{b} ±0.17	24.29^{b} ±0.31	3.25^{ab} ±0.13	43.33^{cd} ±2.09
6	14.90^{c} ±0.39	28.86^{c} ±0.2	0.90^{b} ±0.09	25.40^{b} ±0.43	3.35^{b} ±0.11	41.69^{d} ±1.43
LSD at 0.05	0.85	1.09	0.29	0.84	0.41	2.35

Data are expressed as mean ±standard deviation (SD). Values given represent means of three determinations. Values followed by the same letter are not significantly different ($P < 0.05$).
*By difference.

of increments ranged from 25.42 to 39.25%. This increase was gradually and significantly increased with increasing sprouting time. Dry legumes absorb water rapidly, influenced by the structure of the legume. The increase in water uptake with time is due to the increasing number of cells within the seed becoming hydrated (Nonogaki et al., 2010). Protein content of raw lentil was 25.63%. Sprouting process caused significant ($P <$ 0.05) increases in protein content. These increases in protein content ranged from 7.33 to 12.60% in sprouted lentil seeds. The increase in protein content was attributed to loss in dry weight, particularly carbohydrates through respiration during germination (Mahmoud and El-Anany, 2014; Uppal and Bains, 2012). Bau et al. (1997) assumed that the increased was due to synthesis of enzyme proteins (for example, proteases) by germinating seed or a compositional change following the degradation of other constituents. A further explanation was done by Nonogaki et al. (2010) where they noted that protein synthesis occurred during imbibitions and that hormonal changes play an important role in achieving the completion of germination. Oil content of raw lentil seeds was 1.2%. Oil content of sprouted seeds was lower than that of raw seeds. Sprouting process caused significant reductions in oil content. Oil content decreased significantly from 2.2 (g/100 g) in raw samples to 1.32, 1.24, 1.15 and 0.90 (g/100 g) in those samples germinated for 3, 4, 5 and 6 days, respectively. These reductions in oil content may be attributed to the increased activity of lipolytic enzymes during germination, which hydrolyzed the fats into fatty acid and glycerol (Uvere and Orji, 2002). Germination process enhances the hydrolysis of complex organic compounds which are insoluble in the seeds and form more simple organic compounds that are water soluble. In addition, the decrease in fat content of seed could be due to total solid loss during soaking prior to germination (Wang et al., 1997) or use of fat as an energy source in sprouting process (El-Adawy, 2002). These results are agreement with Narsih et al. (2012) who observed significant fat reductions in sorghum seed germinated for 72 hours. Ash content of raw lentil seeds was 2.77%. Sprouting process significantly ($P \leq 0.05$) increased the ash contents of lentil seeds. These increases could be due to an increase in phytase enzyme activity during germination. The enzyme will hydrolyze the bond between the protein-enzyme minerals become free, therefore increasing the availability of minerals (Narsih et al., 2012). Crude fiber content of raw seeds was 21.70%. Crude fiber content of sprouted seeds was higher than that of raw seeds. Sprouting process caused significant increases in crude fiber content. Changes in fiber content may attribute to the fact that part of the seed fiber may be solubilized enzymatically during seed germination (El Maki et al., 1999). These results are in agreement with Chung et al. (1989) who found that the fibre content increased from 3.75% in unsprouted barley seed to 6% in 5-day sprouts. The carbohydrate content decreased from 48.70% in raw samples to 46.27, 44.56, 43.33 and 41.69 in seeds sprouted for 3, 4, 5 and 6 days, respectively. These decreases might be due to increase in alpha-amylase activity (Lasekan, 1996). The alpha-amylase breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination. The observed decreases in carbohydrate and oil contents with germination could be attributed to their utilization in the sprouting process as energy sources. The increase in respiration rate during germination brings about the release of energy from the breakdown of carbon compounds. Germination changes the stored insoluble nutrients in the cotyledons to soluble nutrients through the hydrolysis of macromolecules (Enujiugha et al., 2003).

Total soluble, reducing and nonreducing sugars

Table 2 shows total, reducing and nonreducing sugar contents of raw and sprouted lentil seeds. Total, reducing and nonreducing sugar contents in raw lentil seeds were 5.01, 1.52 and 3.49%, respectively. Sprouting process caused significant ($P \leq 0.05$) increases in the content of sugars (Table 2). Total, reducing and nonreducing sugars of sprouted seeds at the end of sprouting period were about 2.89, 3.10 and 2.80 times as high as that in raw seeds. These changes in sugar content may be due to mobilisation and hydrolysis of polysaccharides during soaking and germination processes (Hooda and Jood, 2003). During germination, there was a decrease in storage carbohydrates and an increase in total soluble and reducing sugars due to the energy needs of the growing plant (Colmenares De Ruiz and Bressani, 1990). A similar trend in sugar content during germination has been observed earlier (Ravi Kiran et al., 2012; Shakuntala et al., 2011).

Table 2. Total, reducing and non reducing sugar contents of raw and sprouted lentil seeds, g/100 g DW basis

Sprouting period day	Total sugar	Reducing sugar	Non-reducing sugar
0	5.01[a] ±0.21	1.52[a] ±0.09	3.49[a] ±0.30
3	8.60[b] ±0.14	2.76[b] ±0.15	5.84[b] ±0.42
4	10.42[c] ±0.23	3.24[c] ±0.20	7.18[c] ±0.19
5	12.27[d] ±0.17	3.90[d] ±0.31	8.37[d] ±0.92
6	14.50[e] ±0.36	4.72[d] ±0.21	9.78[e] ±0.86
LSD at 0.05	0.37	0.37	1.01

Values are means ±standard deviation (SD) of three determinations.
Values followed by the same letter are not significantly different ($P < 0.05$).

Protein solubility and free amino acids

The protein solubility and free amino acids (mg/g DW) of raw and sprouted lentil seeds are shown in Table 3. Free amino acids play essential roles in cellular biosynthesis and homeostasis and as nitrogen transporters (Mapelli et al., 2001). Free amino acids content raw lentil seeds was 1.86 mg/g DW. Free amino acids content of sprouted seeds was higher than that of raw seeds. Sprouting process caused significant increases in free amino acids content. The highest level of free amino acid 15.20 mg/g DW was observed for those samples sprouted for 6 days. These increases may be due to the activity of proteolytic enzymes (Afify et al., 2012; Müntz, 1996). In this sense, Afify et al. (2012) found that free amino acids content in germinated Shandaweel-6 sorghum (72 h) was 9.94 mg/g. Protein solubility characteristics are influenced by factors such as origin, processing conditions, pH, ionic strength and the presence of other ingredients (Elkhalifa and Bernhardt, 2010). Results indicated that solubility of raw lentil protein was 3.62 g/100 g DW. The protein solubility was significantly ($P \leq 0.05$) increased by sprouting process. This rise was gradually and significantly increased with increasing sprouting time. Protein solubility of lentil seeds sprouted for 3, 4, 5 and 6 days were about 1.40, 1.62, 1.65 and 1.68 times as high as that in unsprouted seeds. These increases may be due to the high proteolytic activity during germination, which will lead to an increase in the protein solubility resulting from hydrolysis of the storage proteins (Afify et al., 2012).

Table 3. Protein solubility, g/100 g DW, and free amino acids, mg/g DW, of raw and sprouted lentil seeds

Sprouting period day	Free amino acids mg/g DW	Protein solubility g/100 g DW
0	1.86[a] ±0.09	3.62[a] ±0.08
3	13.80[b] ±0.62	5.10[b] ±0.24
4	14.02[b] ±0.35	5.90[c] ±0.06
5	14.10[b] ±1.20	6.00[c] ±0.44
6	15.20[b] ±1.34	6.10[c] ±0.09
LSD at 0.05	1.34	0.48

Values are means ±standard deviation (SD) of three determinations.
Values followed by the same letter are not significantly different ($P < 0.05$).

Antinutritional factors

Antinutrients, commonly found in plant food, have both adverse effects and health benefit. One common example is phytic acid, which forms insoluble complexes with calcium, zinc, iron and copper. Another particularly widespread form of antinutrients are the flavonoids, which are a group of polyphenolic compounds that include phenolic compounds (tannins), saponins and enzyme (amylase and protease) inhibitors (Reyden and Sel Vendran, 1993). The antinutritional factors of raw and sprouted lentil seeds are shown in Table 4. Tannins and phytic acid content of raw seeds were 466.10 and 233.04 mg/100 g on dry weight basis, respectively. Sprouting process caused significant decreases in tannins and phytic acid content. These decreases were gradually and significantly increased with increasing sprouting time. Tannins (47.86–59.40%) and phytic acid (45.85–73.76%) in lentil seeds were significantly ($P \leq 0.05$) reduced by sprouting process. The highest reductions were caused by sprouting for 6 days. Germination significantly ($P \leq 0.05$) reduced the tannin contents of all studied legumes as previously observed by Ghavidel and Prakash (2007) in green gram, cowpea, lentil and chickpea. The reduction of

Table 4. Effect of sprouting process on the antinutritional factors of lentil seeds, mg/100 g DW basis

Sprouting period day	Phytic acid		Tanins	
	mg/100 g sample	reduction %	mg/100 g sample	reduction %
0	233.04[a] ±9.23	–	466.10[a] ±26.70	–
3	126.19[b] ±12.03	45.85	243.00[b] ±16.14	47.86
4	86.21[c] ±13.66	63.00	210.17[c] ±9.17	54.90
5	71.47[c] ±11.09	69.33	200.01[c] ±7.84	57.08
6	61.13[c] ±7.32	73.76	189.20[c] ±6.13	59.40
LSD at 0.05	20.47		29.25	

Values are means ±standard deviation (SD) of three determinations.
Values followed by the same letter are not significantly different ($P < 0.05$).

tannins of germinated seeds may due to the leaching of tannins into the water (Shimelis and Rakshit, 2007) and binding of polyphenols with other organic substances such as carbohydrate or protein (Saharan et al., 2002). In this respect, Saxena et al. (2003) and Khandelwal et al. (2010) showed that during the period of soaking prior to germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols. The reduction in phytates content during germination of different legume seeds apparently as a result of a large increase in phytase activity (El-Adawy, 2002; Khattak et al., 2007; Shimelis and Rakshit, 2007). Because germination is mainly a catabolic process that supplies important nutrients to the growing plant through hydrolysis of reserve nutrients, reduction in phytic acid was expected (Colmenares De Ruiz and Bressani, 1990).

Amino acids

The nutritional quality of a protein is principally governed by its amino acid composition. Data presented in Table 5 show the amino acid composition of raw and sprouted lentil seeds. Results indicated that total essential amino acids of lentil seeds protein formed 38.10% of the total amino acid content. Lentil protein was rich in essential amino acids such as isoleucine, leucine, lysine, total aromatic amino acids and tryptophan compared with the FAO/WHO (1991) reference. Therefore, lentil protein could very well complement those protein sources that are low in lysine and tryptophan. However, total sulfur amino acids and threonine were slightly deficient in lentil seeds protein compared with the reference pattern. Total amount of nonessential amino acids represented 61.90% of the total amino acid content. Glutamic, aspartic and arginine acids were found to be the major non-essential amino acids in lentil seeds protein 21.40, 13.70 and 7.60%, respectively. These results are in good agreement with the values reported by several authors (Carbonaro et al., 1997; Kavas and Nehir, 1992; Mahmoud and El-Anany, 2014; Rozan et al., 2000; Porres et al., 2002). Germination process has a positive effect on the essential amino acid contents in lentil seeds. Sprouting process caused significant ($P \leq 0.05$) increases in the content of leucine, lysine, phenylalanine and valine (Table 5). However, sprouting process caused significant decrease in the total sulfur amino acids, tyrosine, threonine and tryptophan content of lentil seeds. Several studies have reported that germination significantly increased the essential amino acids except for histidine and sulphur amino acids (Afify et al., 2012; Elemo et al., 2011). Germination of cereals and legumes has been shown to be generally advantageous as it also improves the nutritional qualities and bioavailability of amino acid of cereals and legumes (Correia et al., 2008; Egli et al., 2004; Gernah et al., 2011; Mubarak, 2005).Germination process caused significant decreases in the content of in non essential amino acids except

Table 5. Amino acid composition of raw and sprouted lentil seeds, g/100 g protein

Amino acid	Sprouting period, day					FAO/WHO pattern*
	0	3	4	5	6	
Isoleucine	3.80	3.70	3.70	3.65	3.60	2.8
Leucine	7.8	8.00	8.10	8.10	8.25	6.6
Lysine	7.3	7.54	7.55	7.55	7.59	5.8
Cystine	0.7	0.64	0.63	0.62	0.62	–
Methionine	0.8	0.52	0.50	0.47	0.42	–
Total sulfur amino acids	1.5	1.16	1.13	1.09	1.04	2.5
Tyrosine	3.3	3.20	3.22	3.19	3.16	
Phenylalanine	4.5	4.70	4.71	4.93	5.60	
Total aromatic amino acids	7.8	7.90	7.93	8.12	8.76	6.3
Threonine	3.0	2.89	2.85	2.79	2.54	3.4
Tryptophan	1.2	1.2	1.16	1.15	1.15	1.1
Valine	4.5	4.96	5.70	5.75	5.95	3.5
Total essential amino acids	36.9	37.35	38.10	38.20	38.88	
Histidine	2.5	2.56	2.31	2.45	1.96	1.9
Arginine	7.60	7.81	7.62	7.65	7.66	
Aspartic acid	13.7	13.8	14.01	14.02	14.02	
Glutamic acid	21.4	21.5	21.3	21.1	21.1	
Serine	3.5	3.5	3.5	3.5	3.5	
Proline	4.9	4.9	4.8	4.8	4.6	
Glycine	3.6	3.6	3.5	3.4	3.4	
Alanine	4.70	4.98	4.86	4.88	4.88	
Total non-essential amino acids	63.1	62.65	61.90	61.80	61.12	

*FAO/WHO (1991) reference pattern.

for alanine, serine and Aspartic acids. During the first 72 h of germination changes in the amino acid content could be related to protein hydrolysis, synthesis, and re-arrangement (Tarasevičienė et al., 2009). According to Rodrıguez et al. (2008), seeds germination involves mobilization of the protein reserves in cotyledons, coupled with the synthesis of new proteins, necessary for sprout's growth. Furthermore, the amino acids produced by hydrolysis of the protein reserves are not used solely in synthesizing new components, but may also be used as an energy source, especially in the early stages of the germination (Tarasevičienė et al., 2009).

Protein efficiency ratio (PER) is one of the quality parameters used for protein evaluation (FAO/WHO/UNU, 1985). Protein efficiency ratio (PER) of ungerminated lentil seeds was 2.70. Protein efficiency ratio was significantly ($P \leq 0.05$) improved

Table 6. Protein quality of raw and sprouted lentil seeds

Treatment	Protein efficiency ratio PER	Chemical score %	Limiting amino acids	
			first	second
Raw	2.70	60.00	methionine + cystine	threonine (88.23)
Sprouted				
3 days	2.82	46.40	methionine + cystine	threonine (85.00)
4 days	2.87	45.20	methionine + cystine	threonine (83.82)
5 days	2.87	43.60	methionine + cystine	threonine (82.05)
6 days	2.94	41.60	methionine + cystine	threonine (74.70)

by germination process. Natural biological processes such as germination and fermentation can be used to improve the protein quality of legumes (Chilomer et al., 2013). Protein efficiency ratio (PER) of lentil seeds sprouted for 3, 4, 5 and 6 days were about 1.04, 1.06, 1.06 and 1.08 times as high as that in raw seeds. The improvement of protein efficiency ratio (PER) is attributed to the increase in the levels of leucine amino acid and the reduction of tyrosine amino acid during germination process. Germination may affect the nutritive value of protein by improving availability of some essential amino acids (Khan and Ghafoor, 1978). Sulfur-containing amino acids were the first limiting amino acid, while threonine was the second limiting amino acid in raw and germinated lentil seeds. The results of the current study agree with the findings of Porres et al. (2002) who found that methionine + cysteine were the first limiting AAs in lentil seeds. The chemical score, an index of protein quality was estimated by comparing the essential amino acid contents of raw and germinated lentil seeds with a reference amino acid pattern (FAO/WHO, 1991). Chemical score of raw and germinated lentil seeds are given in Table 6. Chemical score of raw lentil seeds was 60.00. Sprouting process caused significant decreases in chemical score values. Chemical score decreased significantly from 60.00 in raw samples to 46.40, 45.20, 43.60 and 41.60% in those samples germinated for 3, 4, 5 and 6 days, respectively. These findings confirmed those reported by Mubarak (2005), who found that germination process reduced the sulfur-containing amino acids. These results are in good agreement with those reported by Alajaji and El-Adawy (2006) who reported that the chemical score and limiting amino acid varied considerably depending on treatment.

Total phenolic content, total flavonoids content, DPPH and reducing power of raw and sprouted lentil seeds

Total phenolic contents of raw and sprouted lentil seeds in Table 7. Phenolic content of ungerminated lentil seeds was 1341.13 mg/100 g DWb. Total phenolic contents was significantly ($P \leq 0.05$) increased by germination process. Phenolics content increased from 1341.13 mg/100 g DW in raw lentil seeds to 1411.50, 1463.00, 1630.20 and 1510.10 13 mg/100 g DW in those samples germinated for 3, 4, 5 and 6 days, respectively. These increases could be due to the biosynthesis and bioaccumulation of phenolic compounds as a defensive mechanism to survive under environmental stresses, like cold exposure (Randhir et al., 2004), and to degradation of polymerized polyphenols, specifically hydrolysable tannins, and the hydrolysis of other glycosylated flavonoids (Monagas et al., 2005).

The maximum level of Phenolic content (1630.20 ±17.30 mg/100 g DW) was observed for lentil seeds germinated for 5 days. However, these increases went down in the sixth day of germination and decreased to 1510.10 mg/100 g DW. This decrease might be due to mobilization of stored phenolics by the activation of enzymes like polyphenol oxidase during sprouting process (Vadivel and Biesalski, 2012). After germination, various changes in the phenolic compounds occur

Table 7. Total phenolic content, total flavonoids content, DPPH and reducing power of raw and sprouted lentil seeds

Sprouting period day	Total polyphenols	Flavonoids	DPPH radical scavenging %	Reducing power at 700 nm
0	1341.13[e] ±26.72	398.33[a] ±17.06	40.76[a] ±7.2	0.224[a] ±0.08
3	1411.50[d] ±13.61	429.50[c] ±15.10	49.26[ab] ±5.3	0.310[a] ±0.03
4	1463.00[c] ±14.02	461.38[b] ±12.12	54.73[b] ±6.1	0.320[a] ±0.06
5	1630.20[a] ±17.30	483.10[ab] ±10.91	61.31[b] ±5.3	0.510[b] ±0.04
6	1510.10[b] ±9.81	496.21[a] ±10.03	62.19[b] ±6.2	0. 550[b] ±0.02
LSD at 0.05	31.41	24.21	10.83	0.10

Values are means ±standard deviation (SD) of three determinations.
Values followed by the same letter are not significantly different ($P < 0.05$).
Total polyphenols are expressed as mg gallic acid 100 g of dry plant material.
Flavonoids are expressed as mg catechin/100 g of dry plant material.

which are not only dependent on the type of seeds but also on the process conditions, the presence or absence of light and germination time (Lopez-Amoros et al., 2006).

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in the plant kingdom (Grotewold, 2006). They are widespread in vegetables, fruits, flowers, seeds, and grains (Dragan et al., 2007). Total flavonoids content of raw and sprouted lentil seeds are presented in Table 7. Total flavonoids of ungerminated or raw lentil seeds was 398.33 mg/100 g DW. Flavonoids content in raw lentil seeds was significantly ($P \leq 0.05$) lower than sprouted seeds. Significant increase in the total flavonoids content of lentil seeds was observed as a result of sprouting process. As germination days progressed, the contents flavonoids were gradually increased. The highest level (496.21 mg/100 g DW) of flavonoids was recorded for lentil seeds sprouted for 6 days.

Antioxidant activities of raw and sprouted lentil seeds, as determined by the DPPH radical scavenging method, are shown in Table 7. DPPH radical-scavenging activity expressed in % inhibition of raw and sprouted lentil seeds ranged from 40.76 to 62.19%. Sprouted seeds had significantly ($P \leq 0.05$) higher DPPH radical-scavenging activity compared to raw seeds. Phytochemicals, particularly polyphenols, have high free radical scavenging activity, which helps to reduce the risk of chronic diseases, cancer and age related neuronal degeneration (Lako et al., 2007; Picchi et al., 2012; Teow et al., 2007). DPPH radical-scavenging activity increased significantly from 40.76% in raw samples to 49.26, 54.73, 61.31 and 62.19 in those samples sprouted for 3, 4, 5 and 6 days, respectively. The increase in antioxidant activity with sprouting is one of the many metabolic changes that take places upon sprouting of seeds, mainly due to an increase in the activity of the endogenous hydrolytic enzymes. Other common metabolic changes include improved protein and starch digestion, increased sugar and B vitamin content and decreased levels of phytates and proteases inhibitors (Chavan and Kadam, 1989; Alvarez-Jubete et al., 2009). This increase may also be due to the synthesis of compounds like vitamin C and tocopherols which are responsible for antioxidant activity (Sharma and Gujral, 2010).

Doblado et al. (2007) suggested that during germination, the hydrolytic enzymes modify the endosperm and may liberate some of the bound components that play a role in antioxidant activity. The reducing power is also an indicator of Antioxidant activiti (Lee et al., 2007). The electron donor compounds are considered as a reducing agent and can reduce the oxidized intermediates of the lipid peroxidation reactions; therefore, they may be primary or secondary antioxidants (Zhao et al., 2008). The reducing power activities of raw and

sprouted lentil seeds are presented in Table 7. Spouting process caused significant increases in reducing power activity. The reducing power of lentil seeds increased significantly from 0.22 in raw samples to 0.31, 0.32, 0.51 and 0.55 (absorbance value) in those samples sprouted for 3, 4, 5 and 6 days, respectively. The reducing power of certain compounds is associated with antioxidant activity (Jayaprakasha et al., 2003). Duh (1998) reported that the reducing properties of antioxidants are generally associated with the presence of reductones. Gordon (1990) reported that the antioxidant action of reductones is based on the breaking of the free-radical chain by donating a hydrogen atom.

CONCLUSIONS

Based on these results, sprouting process is recommended to increase nutritive value, and antioxidant activity of lentil seeds.

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