Pak. J. Bot., 43(3): 1563-1567, 2011.

COMPOSITIONAL STUDIES OF LENTIL (*LENS CULINARIS* MEDIK.) CULTIVARS COMMONLY GROWN IN PAKISTAN

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Abstract

Four improved lentil cultivars viz., Masoor 85, Masoor 93, NIAB Masoor 2002 and NIAB Masoor 2006 grown and consumed in Pakistan have been systematically analyzed to determine and compare their nutritional and compositional properties. Proximate analysis, anti-nutritional contents, amino acid and fatty acid profiles of the oil extracted along with mineral content from all four cultivars were investigated. Mineral composition showed that sufficient amounts of Ca, P, K, Cu, Zn and Mg were present to meet the macro and micro-nutrients demand in human diets. Despite variations, potassium and manganese were noted as being present in highest and lowest concentrations, respectively, in all cultivars. The distribution patterns of various amino acids in these cultivars suggested sulfur containing amino acids as limiting amino acids. Fatty acid profile indicated unsaturated fatty acids as major fatty acids in all cultivars. The data show that, in terms of both quality and quantity, all four lentil cultivars can serve as a significant source of essential amino acids, essential fatty acids and trace minerals to meet the demand of population of Pakistan.

Introduction

Lentil (*Lens culinaris* Medik.) is predominantly grown in South East Asia and commonly consumed as thick soup made from whole grain or split pulse commonly referred to as 'dhal'. Seeds can be fried and seasoned for consumption; flour is used to make soups, stews purees, and mixed with cereals to make bread and cakes, and as a food for infants (Williams & Singh, 1988). It is used in culinary dishes in the Indo-Pakistan sub-continent and in the Middle East and incorporated into soups in Europe and North America. In Western countries, lentils may be used in casseroles and as meat substitutes in vegetarian diets. Lentil although called as a 'poor man's meat', is equally liked by all socioeconomic groups in South East Asia (Bhatty, 1988).

Lentils are excellent source of protein and also rich in important vitamins, minerals, soluble and insoluble dietary fiber. The unsaponifiable lipid fraction of lentil is a potential source of bioactive components such as phytosterols, squalene and tocopherols (Ryan *et al.*, 2007). Lentils contain saponins (triterpene glycosides), which have been implicated in hypercholesterolemia in animals (Savage, 1991) and phenolic compounds with high antioxidant activity (Amarowicz & Pegg, 2008; Amarowicz *et al*., 2009, 2010). Besides this, it is a valuable green manure and used as a forage crop. Husks, dried leaves, and stems are used as livestock feeds (Anon., 2000). All these factors have contributed to place the cultivation of lentils at the same economic level as that of cereals with the additional value that its cultivation is more environment-friendly, as it adds to soil fertility by symbiotic nitrogen fixation.

Lentil is the second largest grown legume crop of *Rabi* season in Pakistan after chickpea (*Cicer arietinum* L.) both in quality and quantity (Ayub *et al*., 2001). In 2006 lentil was grown on 43,4000 ha with 25,9000 tones production and average yield of 597 kg/ha (MINFAL, 2006). In recent years, lentil production in Pakistan has increased substantially. This has been brought about by the development of new lentil cultivars with higher yields, improved adaptation to local agroclimatic conditions and better acceptability through improved nutritional status such as fatty acid and ANF (Anti-nutritional factor profiles), by the expansion of export markets, and through a keener appreciation of the benefits of crop rotation and alternative cropping systems. In perspective of nutritional benefits and nutraceutical attributes of lentil, characterization and compositional analysis of its seed are of great importance. Current food databases contain limited or dated compositional data and antioxidant activity by different assay procedures on different lentil cultivars. As part of our studies to explore the flora of Pakistan (Ahmad *et al.,* 2010; Zia-Ul-Haq *et al.,* 2007; 2008 a, b; 2009 a, b; 2010 a, b; Nisar *et al.,* 2010 a, b, c) we have determined the chemical composition of four lentil cultivars commonly grown and consumed in Pakistan.

Materials and Methods

The seeds of four lentil (*Lens culinaris* Medik.) cultivars, Masoor 85, Masoor 93, NIAB Masoor 2002, and NIAB Masoor 2006 were procured from Department of Agronomy, Bahauddin Zakariya University, Multan. Seeds of all the varieties were divided into groups for storage in stainless-steel containers at 4°C prior to analysis.

Proximate analysis: Moisture, lipids, ash, protein and carbohydrates were determined according to AOAC methods (Anon., 1990).

Minerals analysis: The samples were incinerated at 450°C for 12 h in a muffle furnace and acid digest was prepared by oxidizing each sub-sample with a nitric/perchloric acid (2:1) mixture. Aliquots were used to estimate Na and K by flame photometer (Flame Photometer Model-EEL). The minerals, such as calcium, manganese, magnesium, zinc, iron and copper were determined with an atomic absorption spectrophotometer (Perkin–Elmer Model 5000) while phosphorus was determined by the phosphovanado-molybdate (yellow) method (Anon., 1990). The samples were quantified against standard solutions of known concentration that were analyzed concurrently.

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Amino acid analysis: Samples (300 mg), in triplicate from each cultivar, were hydrolyzed with 6 M HCl in an evacuated test tube for 24 h at 105°C. The dried residue was dissolved in citrate buffer (pH 2.2) after flash evaporation. Aliquots were analysed in an automatic amino acid analyser (Hitachi Perkin–Elmer Model KLA 3B), using the buffer system described earlier (Khalil *et al.*, 1990). Methionine and cystine were analysed separately after performic acid treatment and subsequent hydrolysis with HC (Khalil *et al.*, 1990). Tryptophan was determined after alkali (NaOH) hydrolysis by the colorimetric method (Freidman & Finely, 1971). Essential amino acids score was calculated with reference to the FAO/WHO reference amino acid pattern (Anon., 1985).

$$\text{Amino acid score} = \left(\frac{\text{Test amino acid}}{\text{Reference amino acid}} \right) \text{x } 100$$

Fatty acid (FA) composition: Fatty acid methyl esters (FAMEs) were prepared according to the standard of IUPAC method 2.301 (Anon., 1987) and analyzed on a Shimadzu 17-A gas chromatograph with flame ionization detector (FID). Separation was done on a capillary column SP 2330 (30 m $\bar{7}$ 0.32 mm $\bar{7}$ 0.25 μm; Supelco; Bellefonte, Pa., U.S.A.). Nitrogen was used as a carrier gas at a flow rate of 3.0 mL/min. Column temperature was programmed from 180 to 220 °C at the rate of 3 °C/min. Initial and final temperatures were held for 2 and 10 min, respectively. Injector and detector were kept at 230 and 250 °C, respectively. A sample volume of 1.0 µL was injected with the split ratio of 1:75. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards. The quantification was done by a Chromatography Station for Windows (CSW32) data handling software (Data Apex Ltd. CZ-158 00 Prague 5, the Czech Republic). The fatty acid composition was reported as a relative percentage of the total peak area and the results were calculated as mg/100 g of dry lentil seeds.

Antinutritional factors: Trypsin inhibitor activity was estimated according to the method of Kakade *et al*., (1969) using benzoyl-DL-arginine- *p*-nitroanalide hydrochloric as substrate.

Statistical analysis: Analyses were performed in triplicate. Data analysis was carried out using the analysis of variance and LSD test using the "MSTATC" statistical computer package.

Results and Discussion

At the national level, efforts are being made to develop higher yielding varieties of pulses to increase their production and per capita availability and to improve the nutritional status of the people. However, information is needed on the biochemical composition of cultivars to understand their nutritional profiles from production and consumption points of view.

The results of the proximate composition are reported in Table 1. The observed range for protein was 28.80 % for Masoor 93 to 30.60% for NIAB Masoor 2006 and the results are in agreement for protein content in lentils that have been reported by several other workers. Iqbal *et al*., (2006) reported the content of protein for lentil of 26.1 g/100 g. According to Boyle *et al*., (2010) the contents of protein in green and red lentils were 23.03 and 25.88 g/100 g respectively. The crude fiber content ranged from 6.99% to 8.14%. The range observed for lipid content was between 1.93 and 2.15% while carbohydrates showed a range from 54.08% to 55.81%. The results are in agreement to those reported earlier (Solanki *et al*., 1999; El-Adawy *et al*., 2003). Seeds of NIAB Massor 2002 were characterized by the highest content of ash (5.72 mg/100g) where Massor 85 exhibited the lowest one (4.16 mg/100g). In the study of Wang *et al*., (2009) the content of ash of lentils ranged from 2.48 to 2.84 mg/100g.

Mineral constituents of lentil seeds (Table 2), varied among the cultivars, but potassium constituted the major mineral. Potassium content ranged from 875 mg/100 g in NIAB Masoor2006 to 872mg/100 g in Masoor 85. Sodium was found in lower quantity in NIAB Masoor2002 (76mg/100 g) while NIAB Masoor2006 had the highest iron (3.2 mg/100 g) content. All cultivars contained good amounts of calcium, zinc and copper. The results correspond to those already reported for lentil in Pakistan (Amjad *et al.*, 2006). These results revealed that lentils may provide a sufficient amount of minerals to meet the human mineral requirement.

The amino acid composition of the four lentil cultivares indicated little variation in the content of essential and non-essential amino acids (Table 3). However, significant variation existed in the individual amino acids, for example the content of isoleucine varied from 3.9 (Masoor 93) to 4.4 g/16 g N (NIAB Masoor 2006); cystine from 0.5 (NIAB Masoor 2006) to 0.9 g/16 g N (Masoor 93). Glutamic acid and aspartic acid were found to be the major non-essential amino acids in the sample tested. The lowest essential to nonessential AA ratio was noted for Masoor 93 (0.78) and the highest for NIAB Masoor 2006 (0.84). The results obtained are in fair agreement with those reported for lentil by Iqbal *et al*., (2006) and Boyle *et al*., (2010). Iqbal *et al*., (2006) reported a value of 0.81 as an essential to nonessential AA ratio for lentil. The chemical score and amino acid index are widely used for screening potential protein foods. Essential amino acid score was computed with reference to the FAO/WHO (Anon., 1985), standard amino acid profiles established for humans. The data in Tables 3 and 4 indicate that all essential amino acids, except S-containing types and tryptophan are present in excessive amounts in all the cultivars analyzed. Amino acid profile showed methionine and cystine as the limiting amino acids. Results are comparable to those of earlier workers (Amjad *et al.,* 2006, Boyle *et al.*, 2010). Amino acid deficiency can be met by consuming large amounts of legumes or by taking a mixture of legumes or by employing the complementarity that exists between high sulfur amino acid cereals and legumes, especially the soybean.

Data about the qualitative and quantitative composition of fatty acids are summarized in Table 5. Fatty acid profile of all lentil cultivars reveals the lipids as a good source of the nutritionally essential linoleic and oleic acids. Linoleic acid, palmitic acid and oleic acid were the dominating fatty acids. Most of the fatty acids were unsaturated fatty acids, while saturated fatty acids mainly palmitic acid contributed little of the total fatty acids content. The fatty acid composition and high amounts of unsaturated fatty acids make lentil a special legume suitable for nutritional applications. The presence of high levels of unsaturated fatty acids in all the presently studied cultivars is nutritionally desirable and results are comparable with some edible legumes.

Statistical analysis did not show any significant differences in the content of phytic acid in four lentil varieties (Table 6). In the study of Wang *et al*., (2006) phytic acid content in Canadian lentil seed was a bit lower and varied from 6.2 to 8.8 mg/g with a mean of 7.7 mg/g. Seeds of NIAB Masoor 2002 were characterized by the lowest content of trypsin inhibitors (29.37 TIU/mg protein). The highest content of trypsin inhibitors was

found in seeds of NIAB Masoor 2006 (33.86 TIU/mg protein). The results agree with the data reported for lentil by Wang *et al*., (2009) and Champ (2002). Germination, extrusion cooking, dehulling and hydroythermal processing are common commercial processes used to inactivate protease inhibitors in pulse crops (Roy *et al.*, 2010). Usually proximate composition of plants and crops seeds varies depending on cultivars, agrogeoclimatological conditions, maturity and collection time of seed, water and fertilizers application as well as acceptability, selectivity and sucking up of nutrients by plants and crops. This study will pave path for future detailed investigations on this legume crop.

Table 1. Proximate chemical composition (g/100 g) of seeds.

Components	Masoor 85	Masoor 93	NIAB Masoor 2002	NIAB Masoor 2006
Crude protein	30.41 ± 1.71^{a}	28.80 ± 1.66^{a}	29.37 ± 1.60^{a}	30.60 ± 1.72^{a}
Total lipids	2.15 ± 0.05^{a}	2.09 ± 0.05^{a}	1.93 ± 0.09^{a}	2.08 ± 0.09^{a}
Total carbohydrates	54.08 ± 0.09^{a}	55.43± 1.73^{a}	54.74 ± 1.10^{a}	55.81 ± 1.75^{a}
Crude fiber	7.74 ± 1.7^{b}	8.14 ± 1.6^{a}	8.14 ± 1.6^{a}	6.99 ± 1.6^{b}
Ash	4.16 ± 0.19^{a}	5.54 ± .18^{ab}	5.72 ± 0.19^{b}	4.52 ± 0.18^{a}

Data are expressed as means ± standard deviations on dry weight basis; values having different letters differ significantly ($p<0.05$)

Table 2. Content on mineral compounds (mg/100 g) of seeds.

Mineral	Masoor 85	Masoor 93	NIAB Masoor 2002	NIAB Masoor 2006
Sodium	79 ±2.65^{a}	79 ±2.65^{a}	76 ± 1.33^{b}	30.60 ± 1.72^{a}
Potassium	874 ± 6.43^{a}	872 ± 3.78^{a}	873 ± 4.08^{a}	875 ± 0.09^{a}
Phosphorus	294 ± 3.61^{a}	293 ± 2.13^{a}	292 ± 3.08^{a}	294 ± 2.92^{a}
Calcium	120 ± 6.24^{a}	119± 5.48^{a}	121± 4.73^{a}	118± 5.10^{a}
Iron	3.1 ± 0.26^{ab}	2.9 ± 0.69^{bc}	2.7 ± 0.52^{c}	3.2 ± 0.19^{a}
Cooper	9.9 ± 0.10^{a}	8.9 ± 0.07^{b}	9.5 ± 0.04^{a}	9.6 ± 0.09^{a}
Zinc	4.4 ± 0.20^{ab}	3.9 ± 0.17^{c}	4.6 ± 0.11^{a}	4.2 ± 0.07^{bc}
Manganes	1.6 ± 0.03^{ab}	4.3 ± 0.07^{bc}	1.4 ± 0.06^{ab}	1.7 ± 0.05^{a}
Na:K ratio	0.09	0.08	0.08	0.09
Ca:P ratio	0.40	0.41	0.40	0.40

Data are expressed as means ± standard deviations on dry weight basis; values having different letters differ significantly ($p<0.05$)

Table 3. Amino acid composition of seeds of lentil cultivars (g/ 16 gN).

Amino acid	Masoor 85	Masoor 93	NIAB Masoor 2002	NIAB Masoor 2006
Essential AA				
Isoleucine	4.1± 0.05^{bc}	3.9 ± 0.07^{a}	4.3 ± 0.05^{ab}	4.4 ± 0.07^{a}
Leucine	7.8 ± 0.05^{ab}	7.3 ± 0.03^{c}	7.5 ± 0.04^{bc}	7.9 ± 0.01^{a}
Lysine	7.0 ± 0.03^{a}	6.9 ± 0.01^{a}	6.8 ± 0.08^{a}	7.2 ± 0.03^{a}
Methionine	0.8 ± 0.02^{a}	0.9 ± 0.05^{a}	0.9 ± 0.09^{b}	0.6 ± 0.02^{b}
Phenylaniline	5.0 ± 0.12^{ab}	4.8 ± 0.06^{ab}	4.3 ± 0.07^{b}	5.0 ± 0.08^{a}
Threonine	3.5ab± 0.04^{ab}	3.2b± 0.04^{b}	3.7a± 0.03^{a}	3.4ab± 0.04^{ab}
Tryptophan	0.7ab± 0.03^{ab}	0.7ab± 0.03^{ab}	0.8b± 0.02^{b}	0.8a± 0.05^{a}
Valine	5.0ab± 0.05^{ab}	4.8b± 0.08^{b}	5.3a± 0.04^{a}	4.9ab± 0.07^{ab}
Arginine	7.8 ± 0.03^{a}	7.5 ± 0.04^{a}	7.6 ± 0.03^{a}	7.6 ± 0.03^{a}
Histidine	2.2 ± 0.05^{ab}	2.3 ± 0.02^{ab}	1.9 ± 0.01^{b}	2.5 ± 0.02^{a}
Non-essential AA				
Alanine	4.3 ± 0.03^{a}	4.2 ± 0.07^{a}	4.6 ± 0.05^{a}	4.0 ± 0.01^{a}
Aspartic acid	11.2 ± 0.07^{a}	11.8 ± 0.08^{a}	11.4 ± 0.07^{a}	11.4 ± 0.07^{a}
Cystine	0.7 ± 0.08^{ab}	0.9 ± 0.04^{a}	0.5 ± 0.03^{c}	0.5 ± 0.08^{bc}
Glutamic acid	22.0 ± 0.05^{a}	21.5 ± 0.07^{a}	20.9 ± 0.09^{a}	21.3 ± 0.09^{a}
Glycine	3.2± 0.04^{a}	3.6 ± 0.05^{a}	3.7± 0.04^{a}	3.0± 0.04^{a}
Proline	3.9 ± 0.02^{a}	3.5 ± 0.03^{ab}	3.1 ± 0.01^{b}	3.8 ± 0.07^{ab}
Serine	4.9 ± 0.03^{a}	5.2 ± 0.05^{a}	5.4 ± 0.08^{a}	5.0 ± 0.03^{a}
Tyrosine	3.0 ± 0.01^{a}	3.2 ± 0.06^{a}	3.3 ± 0.02^{a}	3.27 ± 0.05^{a}
Essential to nonessential AA ratio	0.82	0.78	0.81	0.84

Data are expressed as means ± standard deviations; values having different letters differ significantly ($p<0.05$)

Table 4. Essential amino acid score of protein of lentil cultivars.

Amino acid	Masoor 85	Masoor 93	NIAB Masoor 2002	NIAB Masoor 2006
Isoleucine	146	139	153	157
Leucine	118	110	113	119
Lysine	120	118	117	124
Methionine	60	72	56	56
Phenylaniline + tyrosine	126	126	120	131
Threonine	102	94	108	100
Tryptophan	63	81	72	72
Valine	142	137	151	140
Histidine	115	121	100	131
Limiting amino acid	Sulfur amino acids	Sulfur amino acids	Sulfur amino acids	Sulfur amino acids

Table 5. Content of individual fatty acids (mg/100 g of dry matter) of seeds.

Fatty acid	Masoor 85	Masoor 93	NIAB Masoor 2002	NIAB Masoor 2006
16:0	14.57 ± 0.03^{a}	13.67 ± 0.05^{a}	13.67 ± 0.05^{a}	13.67 ± 0.05^{a}
16:1	0.09 ± 0.02^{a}	0.03 ± 0.04^{a}	0.07 ± 0.03^{a}	0.05 ± 0.09^{a}
17:0	0.13 ± 0.09^{ab}	0.09 ± 0.02^{bc}	0.17 ± 0.04^{a}	1.17 ± 0.01^{b}
18:0	1.17 ± 0.01^{b}	1.32 ± 0.08^{a}	1.32 ± 0.08^{a}	1.17 ± 0.01^{b}
18:1	22.65 ± 0.08^{a}	22.65 ± 0.08^{a}	21.87 ± 0.08^{a}	22.11 ± 0.07^{a}
18:2	47.21 ± 0.05^{a}	46.98 ± 0.03^{a}	47.01 ± 0.05^{a}	46.89 ± 0.05^{a}
18:3	11.77 ± 0.07^{a}	11.21 ± 0.02^{c}	10.99 ± 0.01^{d}	11.43 ± 0.06^{b}
20:0	0.44 ± 0.04^{a}	0.19 ± 0.01^{c}	0.31 ± 0.05^{b}	0.27 ± 0.04^{bc}
20:1	0.70 ± 0.01^{a}	0.51 ± 0.03^{b}	0.44 ± 0.07^{b}	0.65 ± 0.08^{a}
22:0	0.28 ± 0.07^{a}	0.31 ± 0.05^{a}	0.19 ± 0.06^{b}	0.13 ± 0.09^{b}

Data are expressed as means ± standard deviations on dry weight basis; values having different letters differ significantly ($p<0.05$)

Table 6. Content of antinutrient factors in seeds of lentil cultivars.

Compounds	Masoor 85	Masoor 93	NIAB Masoor 2002	NIAB Masoor 2006
Phytic acid (mg/g dry matter)	11.45 ± 0.31^{a}	10.99 ± 0.32^{a}	11.18 ± 0.28^{a}	11.31 ± 0.19^{a}
Trypsin inhibitors (TIU/mg protein)	31.30 ± 1.25^{ab}	27.93 ± 0.33^{b}	29.37 ± 0.35^{b}	33.86 ± 0.09^{a}

Data are expressed as means ± standard deviations; values having different letters differ significantly ($p<0.05$)

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(Received for publication 15 February 2010)