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Food Chemistry 95 (2006) 493–502
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of variety and crude protein content on nutrients and anti-nutrients in lentils (*Lens culinaris*) ☆

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Received 10 December 2004; received in revised form 27 January 2005; accepted 1 February 2005

Abstract

Protein content was used as an indicator of environmental conditions for a study on varietal and environmental variation of proximate composition, minerals, amino acids and anti-nutrients of lentils. Four lentil varieties, each with three levels of protein content, were selected. Crude protein content ranged from 24.3% to 30.2%. Analysis of variance showed that both varietal and environmental conditions had a significant effect on starch content. Significant varietal differences were found in acid detergent fibre (ADF), neutral detergent fibre (NDF), fat, ash, calcium (Ca), copper (Cu), potassium (K), manganese (Mn), phosphorus (P) and Zinc (Zn). Protein showed significant effects on the amino acids, arginine and tryptophan. Variety had a significant effect on sucrose, stachyose, phytic acid, tannins and trypsin inhibitor activity (TIA). Major lentil components, protein and starch content were inversely correlated. K, Mn, P and Zn were negatively correlated with protein content. Tryptophan was the most deficient amino acid and the sulphur-containing amino acids were the second limiting amino acid in lentils. Raffinose was positively correlated with starch while negatively correlated with ADF.

Keywords: Lentils; Nutrients; Anti-nutrients; Minerals; Amino acids

1. Introduction

Lentil (*Lens culinaris*) is one of the most important pulse crops in western Canada, and Canada has become a world leader in lentil exports. As a result of this, there is an increased demand for information on nutritional data for Canadian lentils. Nutritional quality and composition are important to consumers, both of food and animal feed, but there is little information available on composition of Canadian lentils other than crude protein content.

Although proximate composition of lentil has been widely reported in the literature (Dhindsa, Sood, & Chaudhary, 1985; Naivikul & D'Appolonia, 1979; Sosulski, Garratt, & Slinkard, 1976), the data are not always comparable due to differences in genotypes, environments and methods of analysis. In many cases the analysis consisted of only single sample. Protein content seems to be particularly sensitive to environmental stress such as rainfall, light intensity, length of growing season, length of day, temperature as well as agronomic factors such as plant density, weeds, or soil fertility (McLean, Sosulski, & Youngs, 1974; Robertson, Highkin, Smydzuk, & Went, 1962; Singh, Campbell, & Salunkhe, 1972). Detailed data on the chemical composition and nutritive values of Australian lentils have been reported (Petterson, Sipsas, & Mackintosh, 1997). Aside from limited information published by Bhatty (1984), mostly with respect to protein content, information on variations in composition of Canadian lentils due to varieties and growing conditions is not

☆ Paper 880 of the Grain Research Laboratory, Canadian Grain Commission, Manitoba, Canada.

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doi:10.1016/j.foodchem.2005.02.001

readily available. Knowledge of the variability in composition will benefit the pulse industry by providing a database which can be used to enhance the use of lentils in human consumption in both domestic and export markets as well as a reference for nutritional labeling of food products. Knowledge of the variability in composition will also benefit plant agronomists and physiologists in effort to improve lentil quality.

The annual mean crude protein content of lentils grown in western Canada between 1998 and 2003 ranged from 25.8% to 27.1%. The range of crude protein content in the individual samples submitted by producers within each year ranged from 21.4% to 30.0% (Wang & Daun, 2003). While the overall average crude protein content did not vary greatly from year to year, the large variation among individual samples within a year suggests a large impact of the combination of environmental conditions, agronomic practice and genetic factors. Large differences in protein content were noted among samples of the same variety. This suggested that, within a variety, crude protein content could be used as an indicator of a general "environmental" effect. This general environmental effect would include both uncontrolled weather effects such as temperature and rainfall and partially controlled agronomic effects such as planting density, fertilization and inoculation.

Pulses contain anti-nutritional components that limit their utilization. These components include trypsion inhibitors, phytic acid, tannins and oligosaccharides. Trypsin inhibitors are low molecular weight proteins capable of binding to and inactivating the digestive enzyme, trypsin (Salunkhe & Kadam, 1989). Phytic acid lowers the bioavailability of minerals (Reddy, Sathe, & Pierson, 1988), and oligosaccharides are responsible for flatulence (Fleming, 1981). One of the major disadvantages of tannins in lentils is a discolouration of the seed (Nozzolillo & De Bezada, 1984). In addition to seed discolouration, tannins bind to proteins through hydrogen binding and hydrophobic interactions, thereby reducing their nutritional quality (Hahn, Rooney, & Earp, 1984). While some efforts have been directed to minimize their contents in the seeds or to minimize their effects through processes, little information on the effect of varietal and environmental conditions on these anti-nutritional factors is available.

The objectives of this work were to study varietal and environmental effects on nutrients and anti-nutrients of Canadian lentils and to determine the relationship between chemical components of commercially grown lentils. Crude protein content within a variety was used as a broad indicator of environmental effect as discussed above.

2. Materials and methods

2.1. Sample preparation

Samples were selected from Canadian Grain Commission (CGC)'s harvest survey of the commercially grown crop of lentils. For this survey, samples were submitted to the CGC by producers from across western Canada. Producers in this survey were selected at random from known lentil producers. About 1000 envelopes were distributed with a return rate of 35%. Crude protein content was determined on each sample in the survey using a NIRSystems 6500 analyzer calibrated against the Dumas method (AOAC, 1998). Four lentil varieties, each with three levels of crude protein content were selected from producer samples grown in 1999 (Table 1). Broken and damaged seeds and foreign material were handpicked from the sample before testing commenced.

Table 1
Protein content of lentil samples studied[a]

Variety	Protein content			Mean[b]	CV
	Level 1	Level 2	Level 3		
Crimson	25.5	26.1	28.9	26.9	6.3
	25.1	26.8	29.0		
Eston	26.8	28.5	30.1	28.7	5.0
	27.2	29.1	30.2		
Laird	25.5	27.4	28.4	27.3	5.9
	25.3	28.0	29.3		
Richlea	24.3	25.7	27.4	25.9	5.1
	24.7	25.7	27.4		
Mean[c]	25.6	27.2	28.8		
CV	3.9	4.8	3.7		

[a] Crude protein = $N \times 6.25$.
[b] From six individual samples for each variety.
[c] From eight individual samples for each mean value.

2.2. Chemical analysis

2.2.1. Proximate analysis

Nitrogen was determined by the Dumas combustion method using a Leco FP-428 nitrogen analyzer (AOAC, 1998). Moisture and ash content were determined gravimetrically by AACC methods 44-15A and 08-16 (AACC, 2000), respectively. Starch was determined colorimetrically by the method AACC 76-13 (AACC, 2000). Fat content was determined gravimetrically by cold extraction of the ground sample with petroleum ether in a ball mill for 1 h and then by hot extraction of the miscella with petroleum ether for 1 h on a Soxtec™ extraction unit (Foss-Tecator, Höganäs, Sweden) (AOCS, 1998). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the method of Ankom Technology (1998) using the Ankom fibre analyzer. Minerals were determined by atomic absorption spectrophotometer (Gawalko, Nowicki, Babb, & Tkachuk, 1997).

2.2.2. Amino acids

Total amino acids were determined on a Beckman 7300 High Performance Amino Acid Analyzer (Beckman Instruments, Inc., Palo Alto, CA) from hydrolysates obtained by hydrolysis of 15–25 mg sample with 2.0 ml of 6.0 N HCl in an evacuated sealed tube at 110 °C for 24 h. Cystine was measured as cysteic acid and methionine as methionine sulfone after performic acid oxidation prior to hydrolysis in 6 N HCl, and tryptophan analysis was performed on a hydrolysate obtained by alkaline hydrolysis of a sample as described by Tkachuk and Irvine (1969).

2.2.3. Trypsin inhibitor activity, phytic acid and tannins

Trypsin inhibitor activity (TIA) was determined colorimetrically using a spectrophotometer at 410 nm (Smith, Megen, Twaalfhoven, & Hitchcock, 1980). Phytic acid was extracted and separated by ion-exchange chromatography according to the method of AOAC (1998). The phytic acid was then determined colorimetrically using a spectrophotometer at 500 nm (Latta & Eskin, 1980). Tannins were assayed according to the modified vanillin-HCl method of Price, Van Scoyoc, and Butler (1978), and (+) Catechin was used as the reference standard.

2.2.4. Oligosaccharides

Oligosaccharides were determined by high performance anion exchange chromatography (HPAE) with pulsed amperometric detection (PAD) (Wang, Daun, & Malcolmson, 2003). One gram of lentil flour was extracted with 10 ml of 80% EtOH at 70 °C for 30 min. Lactose was added as an internal standard in the sample before extraction. The suspension was then centrifuged at 12,000 rpm at 20 °C for 10 min. The supernatant was decanted, and an aliquot was centrifuged at 10,000 rpm for 5 min. A sample of the centrifuged supernatant was diluted with deionized water and passed through a 0.45 μm filter. The filtrate was injected onto the HPAE column without further treatment. Samples (20–50 μl) were analyzed by HPAE using a modular system (Dionex Corp., Sunnyvale, CA), comprised of a DX500 solvent delivery pump, an ED40 chemical electrode detector and an AS3500 autosampler (Dionex Corp., Sunnyvale, CA). Sugars were separated on a PA1 analytical anion exchange column (4 × 250 mm) (Dionex Corp., Sunnyvale, CA) connected to a Carbopac™ PA1 guard column (4 × 50 mm) (Dionex Corp., Sunnyvale, CA). The mobile phase was 150 mM sodium hydroxide, and the flow rate was 1.0 ml/min.

2.3. Statistical analyses

Data were assessed by analysis of variance (ANOVA) (SAS, 2002). The Duncan multiple range test was used to separate means and significance was accepted at $P \leqslant 0.05$.

3. Results and discussion

Crude protein content ($N \times 6.25$) varied from 24.3% to 30.2% with a mean of 27.2% (Table 2). This was within the range reported by Bhatty (1984). These wide variations in protein content were due to a combination of genetic and environmental factors (Reichert & MacKenzie, 1982). Starch content ranged from 41.5% to 46.5%, which was within the range of 35–53% reported for lentils (Reddy, Pierson, Sathe, & Salunkhe, 1984). The mean ADF and NDF values were 5.6% and 8.3%, respectively. ADF values were similar to, whereas NDF values were lower than the range reported for Australian lentils (Petterson et al., 1997). Crude fat content was in the range of 1.0–1.3%. Ash content varied from 12.3% to 3.5%. Both fat and ash values in this study were within the range reported by Petterson et al. (1997). Analysis of variance showed a strong varietal and environmental (as indicated by crude protein content) effect on starch whereas ADF, NDF, fat and ash content were only affected by variety (Table 3). There was a significant effect of variety × environmental conditions on fat content. Correlations among the main components are summarized in Table 4. Starch content was negatively correlated with protein content ($r = -0.73$, $p < 0.01$), which is expected since these two constituents form the bulk of lentil constituents in inverse proportions. This result was similar to that reported by Borowska, Zadernowski, and Konopka (1996). ADF was negatively correlated with starch. ADF and NDF were directly correlated with each other. Ash content was positively related with NDF.

Table 2
Proximate and mineral composition of different lentil varieties[a]

	Varietal means[c]				Mean[d]	Range[e]
	Crimson	Eston	Laird	Richlea		
Chemical composition (%)						
Protein ($N \times 6.25$)	26.9	28.7	27.3	25.9	27.2	24.3–30.2
Starch	42.7	43.4	44.0	44.8	43.7	41.5–46.5
ADF[b]	6.0	5.4	5.7	5.4	5.6	5.0–6.5
NDF[b]	9.0	8.3	8.7	8.1	8.3	7.7–9.5
Fat	1.0	1.2	1.2	1.0	1.1	1.0–1.3
Ash	3.0	2.6	3.0	3.0	2.9	2.3–3.5
Minerals (mg/100 g)						
Calcium (Ca)	97.3	76.4	64.0	81.3	79.7	48.4–107.7
Copper (Cu)	1.0	0.8	1.2	1.0	1.0	0.8–1.3
Iron (Fe)	7.3	8.5	8.0	7.7	7.9	6.6–9.8
Potassium (K)	1134.6	992.4	976.4	1116.9	1055.1	550.8–1286.5
Magnesium (Mg)	138.8	130.7	136.1	147.1	138.2	121.5–167.1
Manganese (Mn)	2.4	1.5	1.7	1.9	1.9	1.2–2.9
Phosphorus (P)	541.6	462.0	465.5	568.4	509.4	344.7–725.8
Zinc (Zn)	4.3	3.3	4.4	4.3	4.0	2.9–5.9

[a] Results are expressed as a dry weight basis.
[b] ADF = acid detergent fibre, and NDF = neutral detergent fibre.
[c] From six individual samples for each variety.
[d] From twenty four individual samples for each mean value.
[e] From twenty four individual samples.

Table 3
Analysis of variance of the effect of variety and crude protein content on nutritional composition of lentils[a]

	Variety (V)	Crude protein (E)	$V \times E$
Chemical composition (%)			
Starch	4.87**	15.13**	–
ADF[b]	0.47*	–	–
NDF[b]	1.04**	–	–
Fat	0.02**	–	0.01*
Ash	0.29**	–	–
Minerals (mg/100 g)			
Calcium (Ca)	1133.9**	–	–
Copper (Cu)	0.13**	–	–
Iron (Fe)	–	–	2.09*
Potassium (K)	40,528*	–	40,766*
Magnesium (Mg)	–	–	–
Manganese (Mn)	1.07**	–	–
Phosphorus (P)	17,381*	–	–
Zinc (Zn)	1.68**	–	0.74*

**, *=significant at $P < 0.01$, and $P < 0.05$, respectively.
– Blank spaces indicate no significance.
[a] Values are the mean square.
[b] ADF = acid detergent fibre, and NDF = neutral detergent fibre.

Potassium (K) was the most abundant element in lentils with a mean value of 1055.1 mg/100 g (Table 2). Calcium (Ca) level ranged from 48.4 to 107.7 mg/100 g. Copper (Cu) ranged from 0.8 to 1.3 and iron (Fe) from 6.6 to 9.8 mg/100 g. Magnesium (Mg) varied from 121.5 to 167.1, manganese (Mn) from 1.2 to 2.9, phosphorus (P) from 344.7 to 725.8 and zinc (Zn) from 2.9 to 5.9 mg/100 g. The mineral contents for Canadian lentils were in the range reported for Australian lentils (Petterson et al., 1997) except that Canadian lentils had higher K and Mg content than Australian ones. Results obtained in this study were also comparable with those reported for other pulses (Jagadi, Rundgren, & Ogie, 1987; Salunkhe & Kadam, 1989). According to this study, consumption of 260 g of lentils per day provides sufficient Mg to meet the recommended daily allowance of 350 mg per person, and 160 g supplies the recommended adult daily allowance of P (800 mg) and Fe (10 mg). Consumption of 10 g of lentils per day provides sufficient K to meet the recommended daily allowance of

Table 4
Correlation coefficients among chemical composition of lentils[a]

	Protein	Starch	ADF	NDF	Fat	Ash	Ca	Cu	Fe	K	Mg	Mn	P
Chemical composition (%)													
Starch (%)	−0.73**												
ADF (%)	–	−0.52*											
NDF (%)	–	–	0.67**										
Fat (%)	–	–	–	–									
Ash (%)	–	–	0.62**	0.40*	–								
Minerals (mg/100 g)													
Ca	–	–	0.46*	–	−0.55**	–							
Cu	–	–	–	–	–	0.48*	–						
Fe	–	–	–	–	–	–	–	–					
K	−0.39*	–	–	–	−0.40*	0.41*	0.48*	–	–				
Mg	–	–	–	–	−0.58**	0.48*	–	–	–	–			
Mn	−0.46*	–	0.47*	–	−0.47*	0.56*	0.66**	–	–	0.74**	0.44*		
P	−0.40*	–	–	–	−0.46*	0.46*	0.46*	–	–	0.47*	0.50*	0.57**	
Zn	−0.67**	–	–	–	–	0.63**	–	–	–	0.48*	–	0.63**	0.40*

**, *=significant at $P < 0.01$, and $P < 0.05$, respectively.
– Blank spaces indicate no significance.
[a] ADF = acid detergent fibre; NDF = neutral detergent fibre.

99 mg per person. A strong variety effect on Ca, Cu, K, Mn, P and Zn was found (Table 3). There was an effect of variety × environmental conditions on Fe, K and Zn. Ca was positively correlated with ADF, but negatively related with fat (Table 4). Cu was positively correlated with ash content. K was directly correlated with ash content and Ca, but negatively correlated with protein and fat content. Mg was directly related with ash, but negatively related with fat content. Mn was positively correlated with ADF, ash, Ca, K and Mg but negatively correlated with protein and fat content. P correlated directly with ash, Ca, K, Mg and Mn whereas negatively correlated with protein and fat content. Zn was positively correlated with ash, K, Mn and P whereas negatively correlated with protein content.

The contents of amino acids (Table 5) were similar to those of mungbean (Abdus, Durrani, Mahmood, Ahmad, & Khan, 1989) and lentils (Petterson et al., 1997). Nutritive value of protein is determined by the pattern and quantity of essential amino acids present. The presence of one or more of the essential amino acids in adequate amounts would increase the nutritive value of the protein. Hence, the seed protein as a source of amino acids can usually be assessed by comparison with the FAO/WHO (1991) suggested pattern of essential amino acids. The data on the amino acid pattern of total seed protein showed that tryptophan was the first limiting amino acid in lentils (amino acid score 64) (Table 5), followed by the sulphur-containing amino acids (methionine and cystine) with amino acid score of 83. It had been reported that sulphur-containing amino acids and tryptophan were the most limiting amino acids in pulses (Peace, Keith, Sarwar, & Botting, 1988). However, histidine, isoleucine, leucine, lysine, phenylalanine, tyrosine, threonine and valine contents were found to be higher than FAO/WHO requirement patterns.

Analysis of variance showed that variety had a significant effect on histidine and serine (Table 6). Environmental conditions had a strong influence on arginine and tryptophan. There was an interactive effect of variety × environment on tryptophan. Oshodi, Ipinmoroti, Adeyeye, and Hall (1995) reported that environmental factors under which pulses were grown influenced their amino acid composition. It was found that lysine was negatively correlated with the protein content (Table 7). Holt and Sosulski (1979) obtained similar correlation for amino acids for field peas. Alanine, glycine, histidine, leucine, proline, threonine, tyrosine and valine were positively correlated with ADF. Histidine and valine were positively correlated with NDF. Serine and threonine were correlated negatively with fat content. Alanine, glycine, threonine, tyrosine and valine were negatively related with ash content.

The relationships among essential amino acids were also studied (Table 7). The sulphur-containing amino acid (cystine) was related positively with methionine. Histidine had a positive correlation with alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine. Similar relationships for other essential amino acids were found (Table 7). We have established no physiological bases for the observed relationships. Some might be coincidental but other might be the result of some biochemical mechanisms.

The major soluble sugars (Table 8) found in the lentils were sucrose and oligosaccharides (raffinose, stachyose and verbascose), confirming the work of others (Vose, Basterrechea, Gorin, Finlayson, & Youngs, 1976). The mean sucrose, raffinose, stachyose and verbascose contents were 1.9%, 0.42%, 1.87% and 0.49%, respectively, in the same range as reported (Reddy

Table 5
Amino acid composition of different lentil varieties

Amino acid (g/16 g N)	Varietal means[b]				Mean[c]	Range[d]	Amino acid score[a]	FAO/WHO (1991) pattern (g/16 g N)
	Crimson	Eston	Laird	Richlea				
Alanine	4.3	4.0	4.2	4.2	4.2	3.9–5.0		
Arginine	7.2	7.3	7.5	7.0	7.2	6.7–8.8		
Aspartic acid	11.5	11.2	11.2	11.2	11.3	10.6–13.8		
Glutamic acid	15.5	14.7	15.0	15.0	15.1	14.4–18.3		
Glycine	4.1	3.8	3.9	3.9	3.9	3.7–4.8		
Histidine	3.0	2.7	2.7	2.8	2.8	2.6–3.3	147	1.9
Isoleucine	4.7	4.5	4.6	4.7	4.6	4.4–5.5	164	2.8
Leucine	7.4	7.0	7.3	7.2	7.2	6.8–8.7	109	6.6
Lysine	7.0	6.5	6.7	6.9	6.8	6.3–8.2	117	5.8
Methionine + cystine	2.8	3.0	2.7	2.9	2.9	2.2–4.2	83	3.5
Phenylalanine + tyrosine	8.0	7.6	7.8	7.9	7.8	7.4–9.4	124	6.3
Proline	4.0	3.7	3.8	3.8	3.8	3.6–4.6		
Serine	4.6	4.2	4.1	4.4	4.3	3.8–5.3		
Threonine	3.8	3.5	3.6	3.7	3.6	3.4–4.4	106	3.4
Tryptophan	0.7	0.7	0.7	0.7	0.7	0.6–0.9	64	1.1
Valine	5.2	4.8	5.1	5.0	5.0	4.7–6.1	143	3.5

[a] Amino acid score = grams of essential amino acid in 100 g of test protein/grams of essential amino acid in 100 g of FAO/WHO reference pattern × 100.
[b] From six individual samples for each variety.
[c] From 24 individual samples for each mean value.
[d] From 24 individual samples.

Table 6
Analysis of variance of the effect of variety and crude protein content on amino acid composition of lentils[a]

Amino acid (g/16 g N)	Variety (V)	Crude protein (E)	$V \times E$
Alanine	–	–	–
Arginine	–	0.97*	–
Aspartic acid	–	–	–
Cystine	–	–	–
Glutamic acid	–	–	–
Glycine	–	–	–
Histidine	0.07*	–	–
Isoleucine	–	–	–
Leucine	–	–	–
Lysine	–	–	–
Methionine	–	–	–
Phenylalanine	–	–	–
Proline	–	–	–
Serine	0.27*	–	–
Threonine	–	–	–
Tryptophan	–	0.01*	0.01*
Tyrosine	–	–	–
Valine	–	–	–

**, *=significant at $P < 0.01$, and $P < 0.05$, respectively.
– Blank spaces indicate no significance.
[a] Values are the mean square.

et al., 1984). Among the sugars, oligosaccharides predominated in most pulses and were a major cause of flatulence (Fleming, 1981). There was a strong variety effect on sucrose and stachyose content (Table 9). Environmental conditions had a little effect on sugar contents. Sucrose was positively correlated with protein and fat content. Raffinose was positively correlated with starch and fat content, but negatively correlated with ADF and ash content (Table 10). Stachyose was positively related with fat and raffinose content, but negatively related with ADF and ash content. Verbascose was positively correlated with protein content.

Phytic acid levels ranged from 0.30% to 1.20% with an average value of 0.91% (Table 8). Phytic acid level was comparable with that in peas, lentils and beans (Bhatty & Slinkard, 1989; Lolas & Markakis, 1975; Petterson et al., 1997). Variety showed a significant effect on phytic acid content (Table 9). Similar results had been

Table 7
Correlation coefficients among chemical and amino acid composition of lentils[a]

Amino acid (g/16 g N)	Chemical composition						Essential amino acid (g/16 g N)								
	Protein	Starch	ADF	NDF	Fat	Ash	Cys	His	Ile	Leu	Lys	Phe	Thr	Try	Val
Alanine	–	–	0.46*	–	–	0.49*	–	0.78**	0.95**	0.93**	0.96**	0.94**	0.94**	0.92**	0.98**
Arginine	–	–	–	–	–	–	–	–	0.60**	0.69**	0.45*	0.64**	0.45**	0.62**	0.62**
Aspartic acid	–	–	–	–	–	–	–	0.67**	0.87**	0.92**	0.81**	0.90**	0.82**	0.78**	0.86**
Cystine	–	–	–	–	–	–	1.00	–	–	–	–	–	–	–	–
Glutamic acid	–	–	–	–	–	–	–	0.80**	0.93**	0.98**	0.89**	0.94**	0.92**	–	0.93**
Glycine	–	–	0.47*	–	–	0.41*	–	0.85**	0.95**	0.97**	0.96**	0.94**	0.97**	–	0.98**
Histidine	–	–	0.48*	0.40*	–	–	–	1.00	0.79**	0.78**	0.80**	0.72**	0.85**	–	0.79**
Isoleucine	–	–	–	–	–	–	–	0.79**	1.00	0.97**	0.96**	0.98**	0.93**	–	0.94**
Leucine	–	–	0.43*	–	–	–	–	0.78**	0.97**	1.00	0.94**	0.98**	0.93**	–	0.97**
Lysine	–0.40*	–	–	–	–	–	–	0.80**	0.96**	0.94**	1.00	0.95**	0.96**	–	0.94**
Methionine	–	–	–	–	–	–	0.86**	–	–	–	–	–	–	–	–
Phenylalanine	–	–	–	–	–	–	–	0.72**	0.98**	0.98**	0.95**	1.00	0.92**	–	0.94**
Proline	–	–	0.50*	–	–	–	–	0.79**	0.91**	0.98**	0.91**	0.93**	0.92**	–	0.96**
Serine	–	–	–	–	–0.52*	–	–	0.81**	0.70**	0.73**	0.75**	0.69**	0.86**	–	0.66**
Threonine	–	–	0.40*	–	–0.40*	0.44*	–	0.85**	0.93**	0.93**	0.96**	0.92**	1.00	–	0.92**
Tryptophan	–	–	–	–	–	–	–	–	–	–	–	–	–	1.00	–
Tyrosine	–	–	0.47*	–	–	0.42*	–	0.82**	0.92**	0.93**	0.90**	0.90**	0.90**	–	0.91**
Valine	–	–	0.80*	0.41*	–	0.43*	–	0.79**	0.94**	0.97**	0.94**	0.94**	0.92**	–	1.00

**, *=significant at $P < 0.01$, and $P < 0.05$, respectively.
– Blank spaces indicate no significance.
[a] ADF = acid detergent fibre; NDF = neutral detergent fibre; Cys = cystine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Phe = phenylalanine; Thr = threonine; Try = tryptophan; Val = valine.

Table 8
Sugars, phytic acid, tannins and trypsin inhibitor activity (TIA) in lentils[a]

	Varietal means[c]				Mean[d]	Range[e]
	Crimson	Eston	Laird	Richlea		
Sugars (%)						
Sucrose	1.79	2.03	2.00	1.81	1.90	1.67–2.48
Raffinose	0.40	0.44	0.42	0.43	0.42	0.33–0.48
Stachyose	1.81	2.01	1.87	1.80	1.87	1.66–2.20
Verbascose	0.48	0.55	0.48	0.45	0.49	0.36–0.74
Phytic acid (%)	1.10	0.72	0.89	0.92	0.91	0.30–1.20
Tannins (%)	0.68	0.73	0.49	0.65	0.64	0.40–1.01
TIA (mg/g sample)[b]	2.64	2.11	2.58	2.64	2.49	1.94–3.07

[a] Results are expressed as a dry weight basis.
[b] TIA = trypsin inhibitor activity.
[c] From six individual samples for each variety.
[d] From 24 individual samples for each mean value.
[e] From 24 individual samples.

reported for chickpeas (Dahan, Chauhan, Punia, & Kapoor, 1989). There was a little effect of environmental conditions on phytic acid content. There was significant effect of variety × environmental conditions on phytic acid content. Phytic acid level was positively correlated with ADF and ash content, but negatively correlated with starch and fat content (Table 10). The high content of phytic acid is of nutritional significance as not only is the phytate phosphorus unavailable to the human, but it also lowers the availability of many other essential minerals. Phytic acid may interfere with the utilization of proteins due to the formation of phytate–protein and phytate–mineral–protein complexes and also inhibit the digestive enzymes (Reddy, Sathe, & Salunkhe, 1982). The phytic acid, however, could be substantially eliminated by processing methods such as soaking and cooking (Reddy et al., 1982).

The tannins level varied from 0.40% to 1.01% with an average of 0.64% (Table 8), which was slightly higher than that reported by Vailancourt, Slinkard, and Reichert (1986). Variety had a significant effect on tannins content whereas environmental conditions had a little effect (Table 9). Tannins content was positively correlated with verbascose content (Table 10). Tannins can be eliminated by decortication, soaking, heat treatment, or cooking (Singh, 1988).

Table 9
Analysis of variance of the effect of variety and crude protein content on anti-nutritional composition of lentils[a]

	Variety (*V*)	Crude protein (*E*)	*V* × *E*
Sugars (%)			
Sucrose	0.09*	–	–
Raffinose	–	–	–
Stachyose	0.05*	–	–
Verbascose	–	–	–
Phytic acid (%)	0.14**	–	0.08*
Tannins (%)	0.07*	–	–
TIA (mg/g sample)[b]	0.40**	–	–

**, * = significant at $P < 0.01$, and $P < 0.05$, respectively.
– Blank spaces indicate no significance.
[a] Values are the mean square.
[b] TIA = trypsin inhibitor activity.

Table 10
Correlation coefficients among chemical composition, sugars, phytic acid and trypsin inhibitor activity in lentils[a]

	Protein	Starch	ADF	NDF	Fat	Ash	SUC	RAF	STA	VERB	PA
Sugars (%)											
Sucrose	0.52**	–	–	–	0.41*	–					
Raffinose	–	0.55**	−0.53**	-	0.40*	−0.46*	–				
Stachyose	–	–	−0.54**	–	0.58**	−0.69**	–	0.68**			
Verbascose	0.43*	–	–	–	–	–	–	–	–		
Phytic acid (%)	–	−0.42*	0.56**	–	−0.52**	0.88**	–	−0.48*	−0.69**	–	
Tannins (%)	–	–	–	–	–	–	–	–	–	0.53**	–
TIA (mg/g sample)	−0.55**	–	0.65**	–	–	0.79**	–	–	−0.63**	–	0.58**

**, * = significant at $P < 0.01$, and $P < 0.05$, respectively.
– Blank spaces indicate no significance.
[a] ADF = acid detergent fibre; NDF = neutral detergent fibre; SUC = sucrose; RAF = raffinose; STA = stachyose; VERB = verbascose; PA = phytic avcid (%) and TIA = trypsin inhibitor activity (mg/g sample).

The trypsin inhibitor activity (TIA) values ranged from 1.94 to 3.07 mg/g sample (Table 8). The mean TIA value was 2.49% which was comparable with that of various edible legumes (Giffith, 1984; Petterson et al., 1997). There was a strong variety effect on TIA (Table 9). TIA was positively correlated with ADF, ash and phytic acid content but negatively related with protein and stachyose content (Table 10). Trypsin inhibitors are low molecular weight proteins capable of binding to and inactivating the digestive enzyme, trypsin (Salunkhe & Kadam, 1989). Cooking and autoclaving had been reported to be effective in inactivating protease inhibitors in pulses (Khokhar & Chauhan, 1986; Wang, Lewis, Brennan, & Westby, 1997; Wang et al., 2003).

In conclusion, lentils showed a high range in seed protein content from 24.3% to 30.2%. Starch accounted for 41.5–46.5%, while the remainder consisted of acid detergent fibre (ADF) (5.0–6.5%), neutral detergent fibre (NDF) (7.7–9.5%), soluble sugars (4.3–5.4%), fat (1.0–1.3%) and ash (2.3–3.5%). Lentils were good sources of minerals such as calcium, potassium, magnesium, phosphorus and zinc. The proteins of lentils were rich in lysine (6.3–8.2 g/16 g N), but deficient in sulphur-containing amino acids and tryptophan. Since cereals are most deficient in lysine but contain sufficient methionine and cystine (Khalil & Rahman, 1984), lentils can serve as a good protein and lysine supplement in cereal diets for human. Oilgosaccharides varied from 2.5% to 3.3%, phytic acid from 0.3% to 1.2%, tannins from 0.4% to 1.0% and trypsin inhibitor activity from 1.9 to 3.1 mg/g sample. Cooking can substantially eliminate/inactivate these anti-nutritional factors in pulses (Khokhar & Chauhan, 1986; Wang et al., 1997, 2003).

Variety and environmental conditions as indicated by crude protein content had a significant effect on starch, arginine and tryptophan content. Significant differences in ADF, NDF, fat, ash, calcium (Ca), copper (Cu), potassium (K), manganese (Mn), phosphorus (P), zinc (Zn), histidine, serine, sucrose, stachyose, phytic acid, tannins and TIA were found among varieties. Starch, arginine and tryptophan had significant environmental differences. K, Mn, P, Zn and amino acid lysine were negatively correlated with protein content. Sucrose and verbascose were positively correlated with protein but TIA was negatively correlated with protein. Phytic acid was negatively related with starch. We have established no physiological bases for the observed relationships. Some might be coincidental but other might be the result of some biochemical mechanisms. Information gathered from this study could be useful to plant agronomists and

physiologists in effort to improve potential new cultivars that enhance the nutritional quality with respect to protein contents, improving the amino acid patterns and reducing the levels of anti-nutritional factors.

Acknowledgements

We gratefully acknowledge the technical assistance of R. Toews, Canadian Grain Commission, Grain Research Laboratory. We are also grateful to E. Gawalko, Canadian Grain Commission, for the determination of mineral content, S. Lowe, Canadian Grain Commission, for amino acids analyses, and E. Nilmalgoda, summer student, for fat determination.

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