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Some chemical properties, mineral content and amino acid composition of cowpeas (*Vigna sinensis* (L.) Savi)

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RESEARCH ARTICLE

Abstract

Chemical properties, mineral content and amino acid composition of three cowpea genotypes (Karagöz, Samandağ and Sarıkız) were determined. Almost all investigated characteristics (except for potassium, sulphur, copper, proline, and sarcosine) were revealed to be statistically important. Chemical properties of cowpeas showed that protein ranged from 27.6 to 30.1%, carbohydrate 56.3-60.0%, ash 3.8-4.2%, fat 2.0-2.3% and moisture 5.9-7.2%. The amounts of potassium, phosphorus, calcium, sulphur, magnesium, iron, zinc, manganese and copper were adequate to meet macronutrient and micronutrient demand in human diets. The ratios of 22 amino acids showed that methionine, tryptophan and tyrosine amino acids were limiting components. According to the results, the cowpea genotypes were rich in the essential amino acids and chemical composition.

Keywords: amino acid, cowpeas, minerals, proximate

1. Introduction

Cowpeas (*Vigna sinensis* (L.) Savi) are consumed in Western countries, but they are sources of protein and energy for people in Africa and Asia. Cowpea is a good alternative to expensive meat and fish protein. The long-shelf life, easy transportation and low cost benefits of cowpeas make them an appealing crop for farmers (Akcin, 1988). Cowpea is one of the most important crops in Turkey, particularly for people living in the southern and western regions. As a consequence, there is a growing demand for knowledge about the nutritional values of local cowpea genotypes.

Cowpeas are used as fresh pods, fresh seeds, canned food and dry grains. Cowpea is especially important among vegetable food products because it contains higher protein levels in the seeds (Sert and Ceyhan, 2012). Although it provides a good source of dietary protein and lysine (Juliano,1999; Uwaegbute *et al.*, 2000; Vasconcelos *et al.*, 2010), cowpea seed is primarily deficient in methionine and cysteine. (Mensa-Wilmot *et al.*, 2001; Saikia *et al.*, 1999; Vasconcelos *et al.*, 2010). Additionally, it contains anti-nutritional factors such as protease inhibitors, lectin, phytic acid, tannin, etc., which can cause several physiological effects when consumed by humans and pets (Maia *et al.*, 2000; Vasconcelos *et al.*, 2010). Cowpea soup is beneficial for coughs and colds (Akcin, 1988).

Worldwide there are numerous significant health problems due to poor nutrition. Former research has shown that the reasons for these health problems are caused by economic weakness and a lack of knowledge about good nutrition (Sert and Ceyhan, 2012). It is necessary to consume more plant proteins in addition to animal proteins for good health. Eating legumes which are protein sources is important for a well-balanced diet. Cowpea is nutritionally significant because of its similarity to animal proteins in the area of protein and amino acid composition, vitamin and mineral nutrition elements. For this reason, protein levels, mineral composition and amino acid compounds were measured in cowpeas (Akcin, 1988; Uwaegbute *et al.*, 2000; Vasconcelos *et al.*, 2010).

2. Materials and methods

Seed material and preparation of samples

A total of three local cowpea genotypes from Turkey were used as material. The genotypes (Karnıkara, Sarıgöbek, Samandağ) are grown widely in the southern Anatolian part of Turkey.

For the field trial, the cowpea genotypes were grown in Hatay (Turkey) in 2010. Agronomic practices were applied in the normal way. After the harvest, the samples of cowpea genotypes were prepared separate from each plot. The seeds were thrashed manually to remove all useless matter such as dust, stones and straw as well as immature and broken seeds. Before the chemical analysis of cowpea samples was performed, they were dried in an air heated oven at 70±1 °C for 48 h.

Chemical analysis

Samples of cowpea seeds were analysed for their crude protein, crude oil, total ash and moisture (in oven 105 °C) using standard methods (AACCI, 1999; Özdemir *et al.*, 1998). The Kjeldahl method was used to determine crude protein content with a conversion factor of 6.25 (Özdemir *et al.*, 1998). All tests were done in triplicate and the results were expressed on dry basis.

Mineral analysis

Grain samples were dried at 70 °C in a drying cabinet with air circulation until they reached stable weight. After that, about 0.3 g dried and collapsed sample was digested using 5 ml of 65% HNO_3 and 2 ml of 35% H_2O_2 in a closed microwave system (Cem-MARS Xpress, CEM Corporation, Matthews, NC, USA). The volumes of the digested samples were increased to 20 ml with ultra-deionised water, and mineral concentrations were determined by inductively coupled plasma atomic emission spectroscopy (Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia). Measurements of mineral concentrations were checked using the certified values of the related minerals in the reference plant samples received from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). The mineral contents of the samples were quantified against standard solutions of known concentrations which were analysed concurrently (Skujins, 1998). These values were expressed as mg/100 g dry matter.

Amino acid standards

Five different concentrations of amino acid standards were used to evaluate the precision of retention times and areas, and the limit of detection and linearity. The amino acid composition was determined according to the AOAC official method 982.30 E (AOAC, 2006). These were 10, 25, 100, 250 and 1000 pmol/µl (Agilent Technologies, Waldbronn, Germany). The standards contained the following compounds: aspartic acid, glutamic acid, serine, histidine, glycine, threonine, alanine, arginine, tyrosine, cystine, valine, methionine, phenylalanine, isoleucine, leucine, lysine and proline. In addition to these amino acids, other kits contain norvaline, sarcosine, asparagine, glutamine, tryptophan and hydroxyproline (Agilent Technologies, Germany).

Derivatisation reagents

The online derivatisation was performed using ortho-phthalaldehyde (Agilent Technologies, Germany) for the primary amino acids and 9-fluorenylmethyl chloroformate (Agilent Technologies, Germany) for the secondary amino acids. An amount of 0.4 N borate buffer (Agilent Technologies, Germany) was used with pH=10.4.

Mobile phases

Mobile phase A was composed of sodium acetate tri-hydrate, triethylamine, acetic acid and tetrahydrofuran (Merck, Darmstadt, Germany). Mobile phase B was composed of sodium acetate tri-hydrate, acetonitrile, acetic acid and methanol (Merck).

Extraction and derivatisation

Hydrolysis of protein and peptide samples is necessary for amino acid analysis of these molecules. Acid hydrolysis is the most common method for hydrolysing a protein sample before amino acid analysis. Hydrolysis solution was 6 N hydrochloric acid containing 0.1 to 1.0% of phenol. Samples (0.2 g) were weighed in test tubes with screw caps and were hydrolysed at 110 °C for 24 h in vacuum. After hydrolysis, samples were dried in vacuum to remove any residual acid. The hydrolysates were filtered and injected into a high-performance liquid chromatograph (HPLC).

High performance liquid chromatography analysis

The analysis was performed with an HPLC system consisting of an HP Agilent 1200 series quaternary pump with degasser, injector and photodiode array detector (Agilent Technologies, Germany). Samples were injected with an HP Agilent 1200 auto sampler with thermostated column compartment on a Hypersil 200×2.1 mm, 5 micron AA column and guard column Hypersil ODS (20×2.1) (Agilent Technologies, Santa Clara, CA, USA). The diode array detector was set at 338/10 and 262/16 nm, flow rate 0.5 ml/min and the oven temperature was 40 °C.

Calibration and calculation

System was controlled and data analyses were performed with Agilent Chem Station (Agilent technologies, Germany). All the calculations regarding the quantitative analysis were performed with external standardisation by measurement of peak areas. Amino acid composition was expressed as grams of amino acid per 100 g of protein.

Statistical analysis

Results were expressed as mean values of three separate determinations. Data were statistically analysed using JUMP, 5.0.1a (SAS Institute Inc., Cary, NC, USA; http://www.jmp.com). The significant differences between means were calculated by analysis of variance (ANOVA) using the least significant difference test at $P<0.05$ and $P<0.01$.

3. Results and discussion

The crude protein, carbohydrate, lipid, ash and moisture content of the cowpea seeds are shown in Table 1. Crude protein content ranged from 27.6% (Sarıkız) to 30.1% (Karagöz) with a mean of 28.7% (Table 1). This result was within the range reported by Unlu (2004) and Herken *et al.* (2007). Sert and Ceyhan (2012) reported that protein content of cowpea seeds was highly variable, and these differences might be attributed to genetic variations in genotypes and climatic and environment factors. Dietary proteins are needed for the synthesis of new cells, enzymes, hormones, antibodies and other substances required for the healthy functioning and development of the body as well as for its protection (Cheesebrough, 1987; Onwuliri and Obu, 2002). Furthermore, dietary proteins help to rehabilitate the protein energy malnutrition status of humans (Omoruyi *et al.*, 1994).

Carbohydrate content varied from 56.3% (Karagöz) to 60.0% (Samandağ), with an average of 58.47% (Table 1). The range of carbohydrate content in these genotypes was similar to that reported by Herken *et al.* (2007) and Asante *et al.* (2007). Carbohydrates generally function as a storage form of fuel and structural elements (Onwuliri and Obu, 2002), thus starch is an important caloric component.

Ash content varied from 3.8 to 4.2% with a mean of 4.02% (Table 1). The mean ash content in the genotypes was greater than that reported by Onwuliri and Obu (2002) and Herken *et al.* (2007) for cowpea. These differences are probably due to growing conditions and the higher skin content of cowpea grains.

The fat content of cowpea samples varied between 2.0% (Samandağ) and 2.3% (Karagöz). The results were similar to previous reports for cowpea in Turkey (Herken *et al.*, 2007). The moisture content ranged from 5.9% (Samandağ) to 7.2% (Karagöz). Moisture values were lower than the range reported for cowpea (Herken *et al.*, 2007). These differences were due to a combination of genetic structure, agronomic practice and environmental factors.

Cowpeas are good sources of minerals such as potassium, phosphorus, calcium, sulphur, magnesium, iron and zinc (Sert and Ceyhan, 2012). Mineral contents of cowpea (Table 2) showed significant differences among the varieties, but potassium and sulphur contents were found to be insignificant. Potassium was the most abundant element for cowpea with a mean value of 1,114.6 mg/100 g. Potassium content varied from 1,108.2 to 1,129.9 mg/100 g, phosphorus 532.1-577.2 mg/100 g, sulphur 247.6-249.5 mg/100 g, magnesium 202.1-223.8 mg/100 g and calcium from 70.7 to 140.7 mg/100 g. Iron varied from 3.9 to 8.4 mg/100 g, zinc 4.2-4.6 mg/100 g, manganese 1.7-2.1 mg/100 g, and copper 1.2-1.3 mg/100 g. The results correspond to those already reported for cowpea (Asante *et al.*, 2007). The results revealed that cowpea might provide a sufficient amount of minerals to meet human requirements of the National Research Council (1989).

Table 1. Chemical properties of three cowpea genotypes.

Chemical properties	Genotypes			Mean	LSD[1]
	Karagöz	Samandağ	Sarıkız		
Protein	30.1 a	28.0 b	27.6 b	28.7	$LSD_{0.05}$: 1.793
Ash	4.02 ab	3.84 b	4.21 a	4.02	$LSD_{0.05}$: 0.240
Moisture	7.16 a	5.88 c	6.25 b	6.43	$LSD_{0.01}$: 0.368
Crude oil	2.27 a	2.03 b	2.06 b	2.12	$LSD_{0.05}$: 0.165
Carbohydrate	56.3 b	60.0 a	59.1 ab	58.5	$LSD_{0.01}$: 2.913

[1] LSD = least significant difference test at $P<0.05$ and $P<0.01$; mean values of the same chemical property followed by different superscript letters differ significantly ($P<0.05$).

Table 2. Mineral contents of three cowpea genotypes.

Mineral contents (mg/100 g)	Genotypes			Mean	LSD[1]
	Karagöz	Samandağ	Sarıkız		
Phosphorus	560.7 ab	532.1 b	577.2 a	556.7	$LSD_{0.05}$: 43.68
Potassium	1,120.9 a	1,114.9 ab	1,108.2 b	1,114.6	ns
Calcium	140.7 a	70.7 c	73.5 b	95.0	$LSD_{0.01}$: 0.394
Magnesium	223.8 a	202.2 b	202.1 b	209.3	$LSD_{0.01}$: 4.522
Sulphur	249.5 a	247.7 b	247.6 b	248.2	ns
Iron	8.4 a	5.2 b	3.9 c	5.8	$LSD_{0.01}$: 0.697
Zinc	4.3 b	4.2 b	4.6 a	4.4	$LSD_{0.01}$: 0.205
Manganese	1.7 b	1.7 b	2.1 a	1.8	$LSD_{0.01}$: 0.192
Copper	1.2 b	1.2 b	1.3 a	1.2	ns

[1] LSD = least significant difference test at $P<0.05$ and $P<0.01$; mean values of the same mineral followed by different superscript letters differ significantly ($P<0.05$).

According to this study, consumption of 175 g of cowpea per day provides sufficient magnesium to meet the recommended daily allowance of 350 mg per person, and 160 g supplies daily allowance of phosphorus (800 mg) and iron (10 mg) for the adult. Consumption of 10 g of cowpea per day provides sufficient potassium to meet the recommended daily allowance of 100 mg. Amino acid composition generally indicates the nutritive value of a protein source (Bodwell *et al.*, 1980; Zia-Ul-Haq *et al.*, 2007). The chemical score and amino acid index are widely used for screening potential protein foods. The essential amino acid score was computed with reference to the FAO/WHO (1985) standard amino acid profile established for humans. Data indicated that all of the genotypes were rich in terms of the amounts of essential amino acids (Table 3).

Among the genotypes studied, the two essential amino acids (methionine and tryptophan) were found to be lower than for the other amino acids. Large variations result in statistically different groups with respect to arginine (Karagöz, 2.09 g/100 g protein; Sarıkız, 4.19 g/100 g protein), histidine (Karagöz, 7.47 g/100 g protein; Sarıkız, 8.57 g/100 g protein), isoleucine (Sarıkız, 3.07 g/100 g protein; Karagöz, 3.76 g/100 g protein), leucine (Sarıkız, 1.96 g/100 g protein; Karagöz, 2.37 g/100 g protein), lysine (Sarıkız, 1.68 g/100 g protein; Karagöz, 2.62 g/100 g protein), methionine (Karagöz, 0.46 g/100 g protein; Samandağ, 1.34 g/100 g protein), phenylalanine (Sarıkız, 4.17 g/100 g protein; Karagöz, 5.83 g/100 g protein), threonine (Karagöz, 4.88 g/100 g protein; Sarıkız, 5.92 g/100 g protein), tryptophan (Sarıkız, 0.44 g/100 g protein; Karagöz, 0.84 g/100 g protein), and valine (Sarıkız, 3.40 g/100 g protein; Karagöz, 4.97 g/100 g protein) (Table 3).

Table 3 shows the non-essential amino acid compositions of the three genotypes. Aspartic acid (12.69-14.04 g/100 g protein) and glutamic acid (13.26-13.30 g/ 100 g protein) were the most abundant amino acids in the samples. The high contents of these two amino acids might be due to the fact that they are the storage forms of nitrogen (Onwuliri and Obu, 2002). The amino acids of methionine, tryptophan and tyrosine were the limiting compositions in all the genotypes. The amino acid results were quite similar to previously reported results for pea (Baniel *et al.*, 1992), and also a recent report on the composition of *Vigna unguiculata* and *Phaseolus vulgaris* (Onwuliri and Obu, 2002) seeds. Legume grains in the formulation can be used as animal protein in human nutrition in addition to the traditional food product (Aluko and Yada, 1995; Eke and Akobundu, 1993; Idouraine *et al.*, 1991).

It has been reported that sulphur-containing amino acid and tryptophan were the most limiting amino acids in pulses (Onwuliri and Obu, 2002; Zia-Ul-Haq *et al.*, 2007). On the other hand, histidine, isoleucine, threonine and valine contents were determined as higher than FAO/WHO (1985) requirement patterns.

4. Conclusions

According to the results, the cowpeas, which are commonly grown in Turkey, are characterised by both high levels of essential amino acids and a rich chemical composition. The amino acids of histidine, isoleucine, threonine and valine were the dominant compounds in the essential amino acids. Therefore, we recommend a wide range of cultivation and consumption of the three cowpea genotypes due to their nutritional values.

Table 3. Amino acid composition (g/100 g protein) of three cowpea genotypes.

	Genotypes				LSD[1]	FAO/WHO pattern (1985)[2]
	Karagöz	Samandağ	Sarıkız	Mean		
Essential amino acid						
Arginine	2.09 c	3.11 b	4.19 a	3.13	$LSD_{0.01}$: 0.084	
Histidine	7.47 b	8.57 a	8.57 a	8.20	$LSD_{0.01}$: 0.065	1.9
Isoleucine	3.76 a	3.07 b	3.07 b	3.30	$LSD_{0.01}$: 0.038	2.8
Leucine	2.37 a	2.00 b	1.96 b	2.11	$LSD_{0.01}$: 0.084	6.6
Lysine	2.62 a	1.80 b	1.68 b	2.03	$LSD_{0.05}$: 0.682	5.8
Methionine	0.46 b	1.34 a	1.17 a	0.99	$LSD_{0.01}$: 0.419	3.5
Phenylalanine	5.83 a	4.18 b	4.17 b	4.73	$LSD_{0.01}$: 0.075	6.8
Threonine	4.88 b	5.79 a	5.92 a	5.53	$LSD_{0.01}$:0.415	3.4
Tryptophan	0.47 b	0.84 a	0.44 b	0.58	$LSD_{0.01}$: 0.075	1.1
Valine	4.97 a	3.42 b	3.40 b	3.93	$LSD_{0.01}$: 0.084	3.5
Non-essential amino acid						
Alanine	4.07 b	4.60 a	3.55 c	4.07	$LSD_{0.01}$: 0.065	
Aspartic acid	12.69 c	14.04 a	13.84 b	13.52	$LSD_{0.01}$: 0.136	
Glutamic acid	13.26 b	13.30 a	13.26 b	13.27	$LSD_{0.01}$: 0.137	
Cystine	2.89 a	2.16 c	2.41 b	2.49	$LSD_{0.01}$: 0.112	
Glycine	1.20 b	1.82 a	1.81 a	1.61	$LSD_{0.01}$: 0.065	
Proline	3.27 a	2.81 b	2.91 b	3.00	ns	
Serine	4.15 c	4.75 a	4.69 b	4.53	$LSD_{0.01}$: 0.037	
Tyrosine	0.36 b	0.54 a	0.51 a	0.47	$LSD_{0.01}$: 0.038	
Asparagine	1.69 a	1.08 c	1.45 b	1.41	$LSD_{0.01}$: 0.209	
Glutamine	2.17 c	2.90 a	2.68 b	2.58	$LSD_{0.01}$: 0.035	
Hydroxyproline	5.55 b	3.70 c	7.14 a	5.46	$LSD_{0.01}$: 0.862	
Sarcosine	1.75 a	1.61 b	1.70 a	1.69	ns	

[1] LSD = least significant difference test at $P<0.05$ and $P<0.01$; mean values of the same amino acid followed by different superscript letters differ significantly ($P<0.05$).
[2] FAO/WHO (1985) amino acid reference pattern of protein for children's (2-5 years old) diet. Values are percentage of protein. Each amino acid in the reference pattern was presumed to score a value of 100. Values for each cultivar are expressed in relation to the reference pattern.

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