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Original Research Article

Proximate and lipid composition of cowpea (*Vigna unguiculata* L.) cultivated in Bulgaria

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ABSTRACT

The seeds of four accessions of cowpea (*Vigna unguiculata* L.) from the collection of the Institute of Plant Genetic Resources, Sadovo, Bulgaria were analyzed for their chemical composition and a detailed study of their lipids was carried out. Chemical composition values were as follows: protein content ranged from 22.5 to 25.6%, starch 28.3 to 36.2%, fat 1.3 to 1.9%, insoluble fiber 1.7 to 3.0% and minerals 3.2 to 3.7%. The oil content was relatively low, but an extremely high content of biologically active compounds (tocopherols in the oils range from 3838 to 11,475 mg/kg, phospholipids 12.2 to 27.4%) was noted. In oils from seeds of cowpea palmitic (35.1–47.1%) and linoleic acid (21.7–30.9%) dominated, followed by linolenic (7.3–16.8%) and oleic acid (6.9–10.6%). The main component in sterols composition was stigmasterol (42.1–43.3%), followed by β-sitosterol (27.6–39.5%). In the tocopherol fraction of oils from seeds of cowpea the main component γ-tocopherol varied from 44.0 to 66.6%, followed by δ-tocopherol (30.3–52.8%). Phospholipids in oils and seeds of various accessions of cowpea had similar qualitative and quantitative composition. Phosphatidylcholine was predominant – 34.5–46.0% of total phospholipids.

1. Introduction

Cowpea (*Vigna unguiculata* L.) is grown as a main legume crop in Africa (Egypt, Nigeria), South America (Colombia), USA, Mexico, Asia (China, Pakistan, Japan) and in South and Southeast Europe (Spain, Italy, Portugal, Greece, Cyprus). Its seeds represent an important source of protein. Also, its green pods and leaves are used as green vegetables, and the dry plant parts as animal feed. According to Food and Agriculture Organization of the United Nations, world cowpea production is 5.0 million tons and the cultivated area 10.5 million hectares (FAO, 2011).

In Bulgaria, cowpea is grown mainly in the southeast (Svilengrad, Haskovo, Dimitrovgrad) and southwest (Melnik and Petrich) regions, close to the boundaries of Turkey and Greece, respectively. Increasing occurrences of drought and heat stress and the modifications in season duration due to climate changes have lead to an enhanced interest by farmers for drought-tolerant crops. Because of its drought tolerance cowpea often becomes an alternative crop for bean, the main legume in Bulgaria. Its yield is more stable under abiotic stress conditions (high temperatures and low rainfall), and the crop requires less input during the vegetation period (Lobato et al., 2009; Stoilova et al., 2003; Watanabe et al., 1997). Scientific data also confirm the unique ability of cowpea to fix soil nitrogen under stressed conditions and to grow in poor soils (Berova et al., 2001; Hall et al., 1997; Singh et al., 2003). Cowpea seeds have almost the same nutritional value as bean seeds. The chemical composition (amino-acid and fatty acid profile, minerals content) and assessment of the nutritional value of cowpea seeds is now the focus of many scientific investigations (Onwuliri and Obu, 2002; Thangadurai, 2005; Frota et al., 2008; Islam et al., 2008; Sabeva and Stoilova, 1998, 2008; Stoilova and Sabeva, 2008; Zia-Ul-Haq et al., 2010; Carvalho et al., 2012). Proximate composition of cowpea range as follows: protein, 17.4–31.7%; fat, 1.00–3.03%; carbohydrates, 35.7–65.7%; dietary fiber (including insoluble fiber), 19.5–35.6% (1.7–16.6%); and mineral content 2.6–4.6%. According to Thangadurai (2005) the seeds of *V. unguiculata ssp. cylindrica* L. (*Fabaceae*) have higher lipid content, 10.0%. Vasconcelos et al. (2010) reported data of chemical and nutritional values of new high-yielding cowpea cultivars and

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all cultivars showed the usual compositional characteristics of *V. unguiculata*, but the content of antinutritional factors differed among the cultivars.

The information about lipid content and composition in cowpea seeds is scarce. There is information for the fatty acid composition of glyceride oil cowpea seeds. In the composition of triacylglycerols the long-chain unsaturated fatty acids prevail (60.0–70.7%). They are composed of 35.4–41.0% linoleic acid, 10.0–23.2% linolenic acid and 9.5–16.2% oleic acid (Frota et al., 2008; Islam et al., 2008; Thangadurai, 2005). Among saturated fatty acids, palmitic (24.4–31.6%) and stearic (14.0%) acids dominate. The ratio of unsaturated fatty acids to saturated fatty acids varies from 1.80 to 2.21 across the different cowpea species (Islam et al., 2008). A more extensive survey on lipid content (physicochemical characteristics, fatty acid, sterol and tocopherol composition) of four cowpea (*V. unguiculata* (L.) *Walp.*) cultivars commonly grown in Pakistan showed that basic components of sterols were stigmasterol, followed by β-sitosterol and campesterol (Zia-Ul-Haq et al., 2010). Presence of the basic classes of tocopherols (α-, β-, γ- and δ-tocopherols) was found with α-tocopherol (50.0–60.0%) dominating in all oils, followed by δ-tocopherol (30.0%). Similar lipid composition was reported in the seeds of other cowpea species (*V. radiata* (L.) *Wilczek*) grown in Pakistan (Zia-Ul-Haq et al., 2008), but tocopherol analysis demonstrated highest content of γ-tocopherol among its isomers.

The information on the lipid composition (fatty acid composition of triacylglycerols, content and composition of sterols, tocopherols and phospholipids) of cowpea seeds grown in Bulgaria is rather limited. The purpose of the present study was to investigate the chemical composition of the seeds of two local and two introduced accessions of cowpea as well as to characterize the isolated lipids, the content of biologically active substances (essential fatty acids, tocopherols, sterols and phospholipids). Hence, this work determines nutritional value of cowpea and as a potential source of bioactive components.

2. Materials and methods

2.1. Plant material

The seeds of four cowpea accessions (*V. unguiculata* L.) (marked with the following catalog numbers 87209007, 95210073, A4E008 and A4E007) from the collection of the Institute of Plant Genetic Resources (IPGR), Sadovo are included in the present study. The first two were obtained from IITA-Ibadan, Nigeria (1987 and 1995, respectively) and the rest were acquired by national collecting missions in the southern part of the country during 1990 and 1992. The selected accessions are characterized by early maturation cycle and high yield potential. Bulgarian landraces were the first cowpea accessions collected and registered at the Documentation Department of the Institute with Cat. Nos: A4E007 and A4E008.

The seed material was stored in National Genebank, belonging to the same Institute under short-term storage conditions. The seed material used was obtained from plants grown in the experimental field at the Institute of Plant Genetic Resources, Sadovo, Bulgaria during 2010 and 2011. The Institute is situated in the Thracian plane (Central Southern Bulgaria) on cinnamonic-forest soil with a neutral pH. The four cowpea accessions were sown manually in plots containing 4 rows 2 m long with 0.7 m inter-row, with 50 seeds per row in order to obtain 40 plants. Each accession was sown in three replications. The plants reached maturity at the end of July and middle of August. The plants were harvested and pods were picked, crushed and cleaned manually in order to obtain seeds. The seed sample to be analyzed from each replication was chosen individually, and 200 g from each accession and each replication were taken for analysis. The necessary seed quantity was chosen randomly.

Prior to use for analysis, the cowpea seeds were air dried for 72 h at 25 °C. The moisture content of accessions nos. 87209007, 95210073, A4E008 and A4E007 were 10.1 ± 0.1 (mean of three replications ± SD), 9.8 ± 0.3, and 10.1 ± 0.2 and 9.7 ± 0.1%, respectively.

2.2. Chemical composition of seeds

Moisture was determined according to AOAC (1995). Crude proteins were calculated from the nitrogen content by Kjeldahl method using factor 6.25 (AOAC, 1996, Method 945). Insoluble fiber was determined by the gravimetric procedure of AOAC (1995). Ash content was evaluated by incinerating at 550 °C in a muffle furnace for 6 h (AOAC, 1995). The method for determining the content of starch is based on the treatment of the plant material with alcoholic KOH solution and additional acid hydrolysis of starch into glucose. The quantity of glucose is determined by the oxidation with a bivalent copper from a copper reagent and then it is converted into starch (BS 13488, 1976).

2.3. Isolation of glyceride oil and determination of oil content

The seeds (100 g sample) were air-dried at room temperature and were ground to powder by mechanical mill and the oil was extracted with *n*-hexane in Soxhlet apparatus for 8 h. The solvent was partly removed in a rotary vacuum evaporator (Buchi, Donau Lab., Switzerland), the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under a stream of nitrogen to a constant weight to determine the oil content (ISO 659, 2009).

2.4. Analysis of fatty acids

The fatty acid composition of oils was determined by gas chromatography (GC) after transmethylation of the respective sample with 2% H_2SO_4 in absolute CH_3OH at 50 °C (ISO 5509, 2000). Fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) on 20 × 20 cm plates covered with 0.2 mm silica gel 60 G (Merck, Darmstadt, Germany) layer with mobile phase *n*-hexane:diethyl ether (97:3, v/v). GC was performed on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph equipped with a 30 m × 0.25 mm (I.D.) × 25 μm (film thickness) capillary EC™-Wax column (Alltech Associates, Inc., Deerfield, IL 60015, USA) and a flame ionization detector. The column temperature was programmed from 130 °C (4 min), at 15 °C/min to 240 °C (5 min); injector and detector temperatures were kept at 250 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL/min; split was 1:50. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions (ISO 5508, 2004). The internal standard undecanoic acid was used for quantitation of fatty acids. The analytical standard of fatty acid methyl esters (SUPELCO F.A.M.E. Mix C4–C24) and undecanoic acid methyl ester (purity ~99%, GC, Sigma–Aldrich, Switzerland) were from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All solvents and reagents were of analytical grade from Merck (Darmstadt, Germany) and were used without additional purification. Iodine value (gI_2/100 g fat) was calculated on the basis of fatty acid composition of the oil (AOCS, 1999).

2.5. Analysis of sterols

Unsaponifiables were determined after saponification of the glycerides oil and extraction with hexane (ISO 18609, 2000). The