



TARIM BİLİMLERİ DERGİSİ 2009, 15(1) 25-30
ANKARA ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ
DOI: 10.1501/Tarimbil_0000001068

Effects of Cultivar and Row Spacing on Tocopherol and Sterol Composition of Chickpea (*Cicer arietinum* L) Seed Oil

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Geliş Tarihi: 22.09.2008

Kabul Tarihi:17.12.2008

Abstract: Effect of cultivars and row spacing (RS) on tocopherol and sterol composition of chickpea oil from chickpea, grown under semi-arid conditions of Thal, Pakistan has been studied. The tocopherol and sterol contents were significantly affected by cultivars during 2005 and 2006 ($P < 0.05$). Among sterols; campesterol, Δ^5 -avenasterol, stigmasterol, Δ^7 -avenasterol, and clerosterol were significantly affected by cultivars while β -sitosterol did not differ significantly. Tocopherols, including α -, γ - and total tocopherol contents were not significantly affected by cultivars but β - and δ -tocopherols differed statistically among cultivars CM 72, Bittal 98, Punjab 91 and Punjab 2000. The results of present study showed that seed oil constituents significantly varied among chickpea cultivars.

Keywords: Chickpea, cultivars, Pakistan, row spacing, Semi-arid, sterol, tocopherol.

Çeşit ve Sıra Aralığının Nohut (*Cicer arietinum* L) Yağının Tokoferol ve Sterol Bileşimine Etkileri

Öz: Pakistan'ın Thal bölgesinde yarı kurak şartlarda, nohuttan elde edilen nohut yağının tokoferol ve sterol içeriği üzerine nohut çeşitlerinin ve sıra aralığının etkisi araştırılmıştır. Tokoferol ve sterol içeriği 2005 ve 2006 yıllarında nohut çeşitlerinden önemli derecede etkilenmiştir ($P < 0.05$). Sterollerden Kampesterol, Δ^5 –avenasterol, stigmasterol, Δ^7 -avenasterol ve kolesterol nohut çeşitlerinden önemli derecede etkilenmiş, ancak β -sitosterol etkilenmemiştir. α , β içeren tokoferoller ve toplam tokoferol içeriği çeşitlerden etkilenmemiş, ancak β ve δ - tokoferoller yönünden CM72, Bittal98, Punjab 91 ve Punjab 2000 nohut çeşitleri arasında istatistiksel olarak önemli farklılıklar tespit edilmiştir. Bu çalışma sonucunda nohutun yağ içeriğinin farklı nohut çeşitlerinden etkilendiği görülmüştür.

Anahtar Kelimeler: Nohut, çeşitler, Pakistan, sıra aralığı, yarı kurak, sterol, tokoferol.

Introduction

Chickpea (*Cicer arietinum* L.) is the world's third largest food legume crop with a total annual production of 8.8 million tons. The cultivated area is over 10 million hectares (Anonymous 2007). It is cultivated in about 50 countries in the arid or Semi-arid regions. About 90% of world's chickpea is grown under rainfed conditions (Kumar and Abbo 2001) where the crop grows and matures on a progressively depleting soil moisture profile (Ludlow and Muchow

1990, Krishnamurthy et al. 1999). In Pakistan, it is the most important *rabi* pulse crop, grown in rain fed areas of country especially the successfully, accounts for 96 % cultivated area of "Thal" on poor, sandy soils (Saleem et al. 2007, Jamil et al. 2000). Thal, where no other crop grows chickpea (Ali et al. 2003, Rehana et al. 2003) and has typical semiarid environment. The area is roughly triangular in shape, with the base to the north and the apex to the south.

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The average annual rainfall varies between 261 mm to 385 mm in the north-east and 169 mm in the south, following a bimodal pattern with more than 69% falling in summer (June to September). The climate is hot and windy in summer and mild in winter. It is the major cool season crop of Thal, and has been sown in this region for half a century (Habib et al. 1991).

In Pakistan, chickpeas are consumed locally, and about 56% of the crop is retained by growers (Khattak et al. 2006). Indigenous people of Thal report chickpea use both as food and medicine. Its green seeds are used as a vegetable. Chickpea starch is used in textile industry and in the manufacture of plywood. Indigo like dye is obtained from chickpea leaves. The stems and leaves have high concentration of maleic, malonic, citric and oxalic acids (Shanmugasundaram 2000). They are low in sodium and fat, and can be used in gluten-free, diabetic, low salt, low calorie, low cholesterol, and high fiber diets (Khalil et al. 2007).

Oil contents are the third important organic component of chickpea (Gul et al. 2007). In contrast to most other pulses and cereals, chickpeas have a relatively high fat content. This makes them an important energy source for vegans (vegetarians) and those without regular access to meat and dairy products – the fat is mostly poly-unsaturated, with less than 1 % saturated fat. Tocopherols and sterols are important constituents of chickpea oil (Akihisa et al. 1992, Gopala Krishna et al. 1997). All these oil constituents have their own nutritional and medicinal importance. Sterols are common components of cellular membranes, the main ones in plants being β -sitosterol, campesterol and stigmasterol (Benveniste 1986). These are correlated with oil quality and are used to verify its authenticity. Plant sterols also have an increasing interest due to their multiple biological activities. Phytosterols have a lowering effect on cholesterol levels in humans (Jones et al. 1997) and they also display anti-inflammatory, antibacterial, antifungal, anti-ulcerative and antitumor activities (Arisawa et al. 1985, Ling and Jones 1995, Akihisa et al. 2000).

Area, production and productivity of chickpea in Pakistan have begun to show positive growth and farmers are recognizing chickpea as important cash crop and also its export potential, which results in a spillover effect of increased domestic supply. The compositional variation of seeds and oil among cultivars are attributed to agro-climatic, agronomic and genetic factors and also varies with the interaction of more than one factors. Despite published work on chickpea sterol and tocopherol composition and agroclimatic effects on tocopherol

and sterol contents and composition (Akihisa et al. 1992, Gul et al. 2008) there is little information available about effects of cultivar and row-spacing on oil attributes of chickpea from Thal region of Pakistan, a potential area for the establishment of domestic production to provide chickpea. The objectives of the study were to evaluate the effects of cultivars and row spacing on the phytosterols and tocopherols oil constituents of chickpea. The information obtained will be useful for consumers as well as producers and the derived chemical and biochemical data will also be helpful for the researchers in future for further studies.

Materials and Methods

Plant Material and Cultural Practices: The seeds of four Desi chickpea (*C. arietinum* L.), cultivars namely CM 72, Bittal 98, Punjab 91, and Punjab 2000 were procured from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The seeds were grown under rainfed conditions during 2005/2006 at a farmer's field in Thal, latitude 31° 22' °N and longitudes 73° 4' °E, altitude 179 m, Pakistan. The soil was sandy loam in nature with pH of 7.2, organic matter content 0.65 %. A randomized complete block design was employed in the experiment. Treatments included in the study were four desi chickpea cultivars, viz., V₁ = CM 72, V₂ = Bittal 98, V₃ = Punjab 91 and V₄ = Punjab 2000 and three row spacing, i.e. R₁ = 20 cm, R₂ = 30 cm and R₃ = 40 cm. Weeds were controlled by hand. At maturity, pods from plants were harvested manually. After removing immature and damaged seeds, seeds of all the cultivars were divided into groups and stored in stainless-steel containers at 4 °C prior analysis.

Extraction: The solvents (Fisher Scientific, Loughborough, United Kingdom) used were of analytical grade and were not further purified. The seed powders were extracted with a mixture of *n*-hexane/2-propanol (3:1, V/V) in a Soxhlet apparatus (6 h).

Sterol Composition: The determination of sterols was made following the official method of the Association of Official Analytical Chemists (AOAC, 1984). Analysis was carried out on a Perkin Elmer gas chromatograph model 8700, equipped with methylphenyl polysiloxane coated capillary column OV-17 (30m × 0.25mm, 0.20 μ m film thickness) and a Flame Ionization Detector (FID). The column was isothermally operated at temperature of 255 °C. Injector and FID temperatures were set at 275 and 290 °C, respectively. Extra pure N₂ at a flow rate of 3 ml min⁻¹ was used as a carrier gas. The internal standard used

was 5- α -cholestane. Identification and quantification of unknown sterol components were made using a pure sterol standard mixture.

Tocopherol Contents: Tocopherol (α -, β -, γ - and δ -) analysis was performed by HPLC following the method described by Lee (Lee et al. 2003). An HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with an S-1121 dual piston solvent delivery system and S-3210 diode array detector was used. One gram of oil was accurately weighed and made up to volume with acetonitrile in a 10 mL volumetric flask wrapped in foil to inhibit oxidation. A 20- μ L of filtered sample was injected into an analytical Hypersil (Thermo Hypersil, GmbH, Germany) ODS reverse phase (C18) column (250 \times 4.6 mm; 5 μ m particle size) fitted with a C18 guard column. The mobile phase consisted of mixture of HPLC grade methanol and acetonitrile (65:35 V/V). The chromatographic separation was performed by isocratic elution of the mobile phase at a flow rate of 1.3 ml min⁻¹ at 30 °C. Detection was performed at wavelength of 292 nm. Tocopherols were identified by comparing the retention times and quantified on the

basis of peak area percent of the unknowns with those of pure standards of (α -, β -, γ - and δ - tocopherols (Sigma Chemical Co. St. Louis, MO). The peak areas were recorded and calculated by a computer with SRI peak simple chromatography data acquisition and integration software (SRI Instrument, Torrance, California, USA).

Statistical analysis: Analyses were performed in triplicate and values marked by the similar letter in same row and class were not significantly different ($P < 0.05$). Data were analyzed by using the "MSTATC" statistical computer package (Freed et al. 1991).

Results and Discussion: In perspective of global industrialization, ever increasing demand and interest of people for low cholesterol diets, and scientific awareness regarding the nutritional and functional properties of food, compositional characterization of legume crops like chickpea is of great importance in Pakistan, where it occupy a major area of cultivation.

Table 1. Sterol Composition (%) of Chickpea Oil as affected by Cultivars.

Sterols	CM72	Bittal 98	Punjab 91	Punjab 2000
Campesterol	13.37 \pm 0.40 ^{ab}	12.06 \pm 0.85 ^c	12.89 \pm 0.29 ^b	13.67 \pm 0.10 ^a
Δ^7 -avenasterol	1.21 \pm 0.17 ^a	1.07 \pm 0.69 ^b	0.96 \pm 0.50 ^c	0.79 \pm 0.43 ^d
Stigmasterol	5.38 \pm 0.40 ^a	4.92 \pm 0.75 ^b	5.00 \pm 0.21 ^b	5.29 \pm 0.06 ^a
β -sitosterol	73.46 \pm 0.23	76.10 \pm 0.61	72.52 \pm 0.28	73.12 \pm 0.51
Δ^5 -avenasterol	3.33 \pm 0.15 ^b	1.94 \pm 0.77 ^d	2.67 \pm 0.19 ^c	4.01 \pm 0.39 ^a
clerosterol	3.30 \pm 0.13 ^c	3.91 \pm 0.42 ^b	5.72 \pm 0.59 ^a	3.12 \pm 0.52 ^c

Data are expressed as means \pm standard deviations ($n = 3$) on dry weight basis, values marked by the same letter in same column of same class are not significantly different ($P < 0.05$).

Table 2. Tocopherol contents of chickpea oil as affected by cultivars

Tocopherols (mg/100g of oil)	CM72	Bittal 98	Punjab 91	Punjab 2000
α	34.82 \pm 0.70	32.99 \pm 0.36	33.81 \pm 0.83	34.52 \pm 0.19
β	1.89 \pm 0.28 ^a	1.67 \pm 0.61 ^b	1.79 \pm 0.33 ^{ab}	1.85 \pm 0.41 ^{ab}
γ	186.02 \pm 0.63	185.79 \pm 0.13	185.97 \pm 0.41	185.08 \pm 0.57
δ	8.70 \pm 0.31 ^a	7.93 \pm 0.71 ^b	8.38 \pm 0.17 ^{ab}	8.88 \pm 0.31 ^a
Total	231.43 \pm 1.92	228.38 \pm 1.81	229.95 \pm 1.74	230.33 \pm 1.48

Data are expressed as means \pm standard deviations ($n = 3$) on dry weight basis, values marked by the same letter in same column of same class are not significantly different ($P < 0.05$).

Table 3 Sterol and tocopherol (%) of chickpea oil as affected by row spacing

Treatments	Row spacing (cm)		
	20	30	40
Sterol composition			
Campesterol	13.32ns	13.39	13.44
Δ^7 -avenasterol	0.99ns	1.03	1.05
Stigmasterol	5.01ns	5.04	5.07
β -sitosterol	73.11ns	74.12	73.83
Δ^5 -avenasterol	3.65ns	3.92	3.86
Clerosterol	3.12ns	3.19	3.17
Tocopherol (mg/100g of oil)			
α	32.55ns	33.11	32.92
β	1.65ns	1.74	1.76
γ	185.56ns	186.05	186.01
δ	8.85ns	8.94	8.90
Total	231.65ns	232.12	231.97

Data are expressed as means \pm standard deviations ($n = 3$) on dry weight basis, values marked by the same letter in same column of same class are not significantly different ($P < 0.05$).

Effects of cultivars and row spacing on sterol and tocopherol are presented in Tables 1-3. Among sterol contents campesterol were significantly affected by chickpea cultivars, which varied from 12.06 to 13.67 %, with maximum percentage from cv. Punjab 2000. Δ^7 -avenasterol also differed statistically among cultivars and higher value of 1.21 % was obtained by CM72. The maximum stigmasterol (5.38 %) was observed in cv. CM 72, followed by cv. Punjab 2000 (5.29 %) but both were statistically similar. Non significant results were found for β -sitosterol which ranged 72.52 to 76.10 % among four chickpea cultivars. Δ^5 -avenasterol varied significantly among cultivars and least quantity of 1.94 % was recorded by cv. Bittal 98. Clerosterol percentage also differed significantly among cultivars and the encouraging results (5.72 %) were obtained in case of Punjab 91. In case of tocopherol contents both significant and non-significant results were obtained during the study with respect to chickpea cultivars. Data revealed that α -, γ - and total tocopherol contents were not significantly affected by cultivars and higher values of these were found in case of cultivar CM72, i.e. 34.82 %, 186.02 % and 231.43 %, respectively. Significant results were achieved in case of β - and δ -tocopherols. The highest values of β - and δ -tocopherols were recorded by cultivars CM72 (1.89 %), and Punjab 2000 (8.88 %), respectively. The effect of variation of row spacing (RS) on sterols and tocopherols is presented in Table 3. Averaged over the two years data, the results revealed that RS did not significantly affect the sterols and tocopherols

composition in all treatments of desi chickpea cultivars. However the treatments of wider row spacing (30 cm and 40 cm) performed better than the narrow row spacing (20 cm). Higher values of sterols i.e. campesterol (13.44 %), Δ^7 -avenasterol (1.05 %), and stigmasterol (5.07 %) and β -tocopherols (1.76 %) were recorded in case of 40 cm a part crop rows. Whilst in the similar fashion higher values of sterols i.e. β -sitosterol (74.12 %), Δ^5 -avenasterol (3.92 %) clerosterol (3.19 %) and tocopherols i.e. α - (33.11 %), γ - (186.05 %), δ - (8.94 %), and total (232.12 %) were obtained in case of RS 20 cm (narrow row spacing) a part crop rows. Over all lower values of sterols and tocopherols were found in narrow row spacing treatment (20 cm a part crop rows). Similar results were obtained by Bhardwaj et al, 2004 in which they mentioned that row spacing effects on protein and sugar concentration were non significant in case of white lupin (winter grain legume crop). But contradictory results are reported earlier (Boydak et al. 2002) for soybean in which they mentioned that row spacing (RS) affected protein and oil contents significantly.

Sitosterol was the principal sterol in all of the four investigated chickpea cultivars which is in line with reported literature while a minute amount of brassicasterol and cholesterol as reported was not found. Most of the plants belonging to family leguminosae so far examined are reported to contain campesterol, sitosterol and stigmasterols as the

Table 4. Correlation Coefficients between Tocopherols and Sterols in Oil from Chickpea Cultivars in Pakistan

Characteristics	Correlation coefficient	Characteristics	Correlation coefficient
Sterols-total tocopherols		Sterols-Sterols	
Campesterol and total tocopherols	+0.8	Campesterol and β -sitosterol	-0.7
Δ^7 -avenasterol and total tocopherols	+0.2	Campesterol and Δ^5 -avenasterol	+0.9
Stigmasterol and total tocopherols	+0.8	Campesterol and Δ^7 -avenasterol	-0.3
β -sitosterol and total tocopherols	-0.7	β -sitosterol and Δ^5 -avenasterol	-0.6
Δ^5 -avenasterol and total tocopherols	+0.7	β -sitosterol and Clerosterol	-0.2
Clerosterol and total tocopherols	-0.3	Stigmasterol and Δ^5 -avenasterol	+0.8

dominant sterols (Akihisa et al. 2000). Regional and cultivars variations for the distribution of campesterol, stigmasterol, β -sitosterol, Δ^5 , avenasterol and clerosterol are already reported in the literature (Norman 1979, Rossell 1991). Plant sterols possess a broad spectrum of therapeutic effects in animals and humans. In humans, consumption of plant-derived sterols, particularly β -sitosterol, reduces blood pressure serum cholesterol levels, and the risk of chronic heart diseases (Ling and Jones 1995, Clark 1996, Moreau et al. 2002). Phytosterols also serve as intermediates for the synthesis of hormonal sterols and other related pharmaceuticals (Clark 1996). Furthermore, phytosterols, especially, β -sitosterol, exhibit significant anti-inflammatory effects and antitumor properties (Moreau et al. 2002, Ling and Jones 1995). In addition, phytosterols are known as antipolymerization factors and as antioxidants, especially those containing an ethylidene group in the aliphatic side chain (Δ^7 - and Δ^5 -avenasterols), in vegetable oil at frying temperature (Wang et al. 2002). Tocopherol contents are well in line to those reported earlier (Gopala Krishna et al. 1997). The contents of α -tocopherol, which has greatest vitamin E potency (Rossell 1991) was relatively higher in chickpea oils. The concentration of δ -tocopherol which has a greater antioxidant activity than either γ - or α -tocopherol (Tsaknis 1998) is comparable to the values reported (Gopala Krishna et al. 1997) These relatively higher values would be expected to contribute to excellent oxidative stability and protection to chickpea oil during storage and processing.

Table 4 showed the correlation coefficients that describe the association between (sterols and total tocopherols) and (sterols-sterols) at 5% of probability level. Campesterol has positively association (high) with total tocopherols (+0.8). Low positive correlation

exists between Δ^7 -avenasterol and total tocopherols (+0.2). Stigmasterol was also positively correlated with the content of total tocopherols (+0.8). The content of β -sitosterol has high negative association with the total tocopherols (-0.7). Δ^5 -avenasterol was positively (+0.7), while clerosterol was negatively (-0.3) correlated with the total tocopherols. The contents of campesterol (Table 4) exhibited a high negative correlation with the content of β -sitosterol (-0.7), low negative with Δ^7 -avenasterol (-0.3) and highly positive with Δ^5 -avenasterol (+0.9). β -sitosterol showed (both high and low) negative association with Δ^5 -avenasterol (-0.6) and clerosterol (-0.2), respectively, but stigmasterol exhibited a high positive correlation with Δ^5 -avenasterol (+0.8). So, the above mentioned results revealed that values of sterols and total tocopherols varied among different cultivars of chickpea used in the study. The positive association between the two variables reflect that change move in the same direction, while the negative association between two variables reflect that change move in the opposite direction or Correlation coefficient significant at 5% probability level.

Conclusion

Phytosterol and tocopherol content and composition were measured for four cultivars of chickpea seeds. These results suggest that cultivars affected the distribution of phytosterols and tocopherols. Furthermore, the results indicated that oil from chickpea seeds was comparable in phytosterol and tocopherol contents to that grown in other locations in the world. Continued strong support for both basic and applied research on chickpea, are required at the national level to maintain momentum generated by current improvements.

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