J Am Oil Chem Soc (2010) 87:1179–1187
DOI 10.1007/s11746-010-1599-5

ORIGINAL PAPER

Seed Composition and Seed Oil Antioxidant Activity of Maize Under Water Stress

Qasim Ali · Muhammad Ashraf · Farooq Anwar

Received: 19 November 2009 / Revised: 20 April 2010 / Accepted: 27 April 2010 / Published online: 19 May 2010

Abstract The changes in seed composition and seed oil quality and antioxidant capacity in two maize cultivars Agaiti-2002 (drought tolerant) and EV-1098 (drought sensitive) grown under field drought conditions were assessed. Both maize varieties used in the present study are widely cultivated in Pakistan and are an important source for developing different maize hybrids. At the early vegetative stage, plants of both maize cultivars were subjected to normal irrigation and water stress conditions. Overall, seed oil, α-, γ-, δ- and total tocopherols, and flavonoids increased considerably due to water stress in both cultivars. However, oil phenolics carotenoid and DPPH scavenging activity were decreased due to water stress. By drawing on the relationship between different components of seed oil such as oil lipophilic antioxidant compounds, seed oil phenolics carotenoid and DPPH scavenging activity; it is clear that oil antioxidant activity was positively associated with oil phenolic and carotenoid contents.

Keywords Maize · Seed composition · Phenolics · Flavonoids · Carotenoids · Antioxidant activity

Q. Ali · M. Ashraf
Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan
e-mail: qasimbot_uaf@yahoo.com

M. Ashraf
e-mail: ashrafbot@yahoo.com

F. Anwar (✉)
Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad 38040, Pakistan
e-mail: fqanwar@yahoo.com

M. Ashraf
King Saud University, Riyadh, Saudi Arabia

Introduction

Cereal grains contribute more than 60% to the total world food demand [1]. Grains are predominantly composed of carbohydrates, mostly in the form of starch, along with considerable amounts of proteins as well as some lipids, vitamins, and minerals [2]. Both genetic and environmental effects create a significant variation in the amount and quality of each of these constituents [3, 4]. Among cereals, maize not only has sufficient amounts of carotenoids, tocopherols, and oil but also has comparable amounts of starch and protein compared with other major food crops such as rice and wheat. Although corn (*Zea mays* L.) is principally cultivated for carbohydrate production, in the last few years, it has gained great significance as a source of vegetable oil for the food industry.

Maize oil has a large amount of unsaturated fatty acids such as oleic and linoleic acids which are in the range from 65 to 85% [5]. In addition, corn oil is a rich source of phenolics, carotenoids, flavonoids, and tocopherols. The interest in phenolic contents is related to their potential health benefits such as anti-allergenic, anti-atherogenic, anti-inflammatory and cardio protective effects [6, 7].

Tocopherols exist as a family of four derivatives (α-, β-, γ- and δ-), which differ in the number and position of methyl substitutions in the chromanol ring. Tocopherols are well recognized as antioxidants in vegetable oils, and their presence increases the stability of lipids against autoxidation [5].

It is well known that genetic and environmental factors play an important role in the economic importance of a crop and can affect the oil yield and quality of its seed/grain production [8]. Some earlier studies have shown that quality of seed composition depends on the type of cultivar, amount of irrigation used for production [9] and period taken to full maturity of cultivars [10].

Trials have shown that unfavorable conditions, especially drought, might alter the seed composition and related qualities such as oil physicochemical properties [11–13]. It has been reported that lack of water during all stages of growth and development is the limiting factor for seed growth that can influence its composition [13, 14]. For example, severe drought has been shown to decrease seed protein and oil contents in soybean [9, 15, 16].

In some earlier studies it has been reported that water deficit can affect seed chemical composition by reducing CO_2 assimilation [17] or through an alteration in the metabolic processes of seed chemical composition [18, 19]. Yang et al. [17] reported linear relationships between photosynthetic characteristics and seed chemical composition at different water availabilities. They also reported a positive relationship between plant photosynthetic characteristics and protein and starch contents in grass pea.

There are reports that shortage of water has a significant effect on oil fatty acid and tocopherol contents [13], but very little work has been reported about the effect of drought stress on maize kernel oil composition in relation to oil tocopherol, phenolic and flavonoids contents and oil antioxidant activity in different cultivars of maize. Therefore, the objective of the study was to determine the effects of drought stress on seed and seed oil composition in relation to oil fatty acids, oil phenolic, flavonoid and carotenoid contents in two maize cultivars. In addition, the phytochemical composition was evaluated for antioxidant activity.

Materials and Methods

Two maize cultivars, Agaiti-2002 (drought tolerant) and EV-1098 (drought sensitive) were used for the present study. The seeds of both lines were obtained from the Maize and Millet Research Institute, Yousafwala (Sahiwal), Pakistan. The effect of drought on seed and seed oil composition of both maize cultivars was assessed under field conditions at the Research Area in the New Botanical Garden, of the Department of Botany, University of Agriculture, Faisalabad, Pakistan (latitude 30°30 N, longitude 73°10 E and altitude 213 m), where the average photosynthetically available radiation (*PAR*) measured at noon ranged from 794 to 1,154 μmol m^{-2} s^{-1}, day/night relative humidity 33.1/75.1%. The average day and night temperatures were 38.28 ± 4 °C and 22.82 ± 3.6 °C, respectively. The climatic data presented here is the average of the entire growth period.

The soil was sandy clay (average 65% clay content, 22% sand and 13% silt). The soil texture was determined with the hygrometer method [20]. The soil had a saturation percentage of 31%, organic matter 0.78% and NO_3-N 6.5, NH_4-N 3.00, available phosphorous 5.6, potassium 187 and calcium 109 (all values of the nutrients in mg/kg of dry soil). The soil pH was 8.1 and soil electrical conductivity (EC_e) 2.1 dS/m. Electrical conductivity, pH and inorganic nutrients of the soil saturation extract were determined following the methods of Jackson [21].

The experiment was laid out in a completely randomized design (CRD) with four replications of each experimental unit. The main plot was divided into two sub-plots on the basis of water stress treatments. In one sub-plot, normal irrigation was applied and in the second sub-plot, drought stress was imposed at the early growth stage of crop growth. The pre-planting irrigation was applied 15 days before sowing. When the soil came into field capacity condition, the field was well prepared for sowing. Seeds (10 kg/ha) of both maize cultivars were hand drilled with a row-to-row distance of 75 cm and plant-to-plant distance of 30 cm. Thinning of plants was done 15 days after germination.

Drought stress was applied at the early vegetative stage of plant growth by controlling the irrigation schedule. The first irrigation was applied 8 days after the emergence to all the plots. The other irrigations were applied at 15 days interval after the first one except the plants that were subjected to drought stress; however, the irrigations to the water stressed plants were applied at 21 days intervals. Harvesting occurred on 29 and 30 September 2007. Before harvest, ears were removed. The ears were dried for 3–4 days in air during day time. After proper drying. the kernels were separated from the ears and used for further analysis. All the reagents used (Analytical and HPLC grades) were purchased from Merck (Darmstadt, Germany) or Sigma–Aldrich (Buchs, Switzerland).

Characterization of Maize Kernel and Kernel Oil

Proximate Analysis

Maize kernels were analyzed for moisture, ash, crude protein, crude fat, crude fiber and starch using their respective methods as described in AACC [22], i.e., Methods No. 44-15A, 08-01, 46-30, 30-25, and 32-10, respectively.

Oil Extraction

After proper drying, the seeds (200 g) of each treatment were crushed into approximately 80 mesh particle size. Then the properly weighed crushed seed material was packed in paper thimbles and thease were placed in a Soxhlet collector fitted with a 500-mL volumetric flask.

The extraction was carried out using *n*-hexane as a solvent for 8 h.

Chemical Parameters of Oil

Determinations of the iodine value, saponification value, unsaponifiable matter, peroxide value, density and free fatty acids contents of the extracted oil were carried out according to the standard AOCS methods Cd 1-25, Cd 3-25, Ca 61-40, Cd 8-53, Cc 10a-25 and F9a-44, respectively [23]. Specific extinctions [$\varepsilon^{1\%}$ 1 cm (λ)] at 232 and 270 nm were determined following the IUPAC method II. D. 23 [24]. Samples of the oil were diluted with iso-octane and spectra were recorded in the ultraviolet region and absorbance values were noted at 232 and 270 nm using a spectrophotometer (Hitachi, U-2001, model 121-0032). The determination of the *p*-anisidine value was made following IUPAC method II. D. 26 [23]. The oil samples dissolved in isooctane were allowed to react with *p*-anisidine for 10 min to produce colored complexes and the absorbance values were determined at 350 nm λ, using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan).

Oil Fatty Acid Composition

Fatty acid methyl esters (FAMEs) were prepared following the IUPAC standard method 2.301 and analyzed on a Perkin Elmer gas chromatograph model Clarus 500 fitted with a Rt-2340 NB (RESTEK, Corp., 800-356-1638, USA) methyl-lignoserate-coated (film thickness 0.20 μm), polar capillary column (60 m × 0.25 mm) and an FID detection. Nitrogen gas at a flow rate of 5 mL/min was used as the mobile phase. Other conditions were as follows: initial oven temperature, 80 °C; ramp rate, 3 °C/min; final temperature, 210 °C; injector temperature 210 °C; and detector temperature, 220 °C. FAMEs were identified by comparing their relative and absolute retention times with those of authentic standards purchased from Sigma–Aldrich (Buchs, Switzerland).

Tocopherol Content

The tocopherol content in the kernel oil was analyzed by HPLC (Sykam GmbH, Kleinostheim,Germany) following the method of Lee et al. [25]. The HPLC system was equipped with S-1122 dual piston solvent delivery system, S-3210 UV/VIS diode array detector. About 20 μl of the extract was injected into a Hypersil ODS reverse phase (C_{18}) column (5 μm particle size, 250 mm × 4.6 ID Thermo Hypersil GmbH, Germany) fitted with a C_{18} guard column and methanol: acetonitrile: methylene chloride (50: 44: 6, v/v) mobile phase at 1 mL min^{-1} flow rate. The peak areas were recorded and calculated by a computer with SRI peak simple chromatography data acquisition and integration software (SRI instrument, Torrance, CA) at 295 nm. The quantification of amounts was based on the pure standards purchased from Sigma–Aldrich (Buchs, Switzerland).

Total Phenolic Contents

The amount of total phenolics was assessed using Folin-Ciocalteu reagent [26]. Briefly 10 g oil was mixed with 60 mL hexane and extracted with 60% methanol. The solvent was evaporated to dryness (40 °C) using a rotary evaporator (Heidolph, model Laborota 4001, Germany) to obtain the crude extract. Then the crude extract (50 mg) was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL deionized water. The mixture was kept at room temperature for 10 min, and then 1.5 mL of 20% sodium carbonate (w/v) added. The mixture was heated in a water bath at 40 °C for 20 min and then cooled in an ice bath; absorbance was measured at 755 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Amounts of TP were calculated using a gallic acid calibration curve within the range of 10–100 ppm. The results were expressed as gallic acid equivalents (GAE) g/100 g of dry matter.

Total Flavonoids Content

Total flavonoids were determined following the procedure of Dewanto et al. [27]. The known quantity of crude extract obtained from oil was dissolved in methanol. About 1 mL of aqueous extract containing 0.01 g/mL of dry matter was placed in a 10-mL volumetric flask, then 5 mL of distilled water were added followed by 0.3 mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ solution was added. After another 5 min, 2 mL of 1 *M* NaOH was added and the volume made up to 10 mL with distilled water. The solution was mixed and absorbance read at 510 nm. TF concentrations were expressed as catechin equivalents g/100 g of dry matter.

Total Carotenoid Content

Total carotenoids were estimated following the method of Gao et al. [28]. Oil solutions in hexane (1 g/100 mL) were read at 460 nm in the spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Quantification of amounts was based on the carotene standard and amounts of carotenoids were expressed in μg g^{-1} of oil.

DPPH Scavenging Activity

The free radical scavenging activity of extracts was assessed using a procedure reported earlier [29, 30]. About

5 mL of a freshly prepared solution of 1,1′-diphenyl-2-picrylhydrazyl (DPPH) at a concentration of 0.025 g/L was added to 1.0 mL of the extract containing 25 μg/mL of dry matter in methanol. The mixture was incubated in the dark for 30 min at room temperature. Then the decrease in absorbance was measured against a blank at 515 nm (the changes in color from deep-violet to light-yellow). Radical-scavenging activity was calculated by the following formula:

$$\text{DPPH radical scavenging } (\%) = [(\text{AB} - \text{AA})/\text{AB}] \times 100$$

where AB—absorbance of blank sample ($t = 0$ min); AA—absorbance of tested extract solution after 15 min of incubation ($t = 30$ min).

Statistical Analysis

Analysis of variance of the data for each attribute was computed using the CoStat Computer Program (version 6.303, PMB 320, Monterey, CA, 93940 USA). The LSD at a 5% level of probability was used to test the differences among mean values [31].

Results and Discussion

Proximate analysis of corn kernels in the present study revealed that water stress reduced the kernel sugar, protein and moisture contents with a subsequent increase in the seed fiber and ash contents, however, no effect was observed on seed starch contents of both maize cultivars (Table 1). No significant difference between the cultivars was observed in relation to these parameters both under well-watered and water deficit conditions. Changes in seed chemical composition could have been due to the reason that low water supply during the plant life affects many enzymes such as invertases whose activity is reduced under water stress conditions [32] and metabolic activities [33] that result in altered translocation of assimilates to seeds during stress conditions [34]. Similarly, Schussler and Westgate [6] showed that sugars that are the main transported compounds are absorbed less rapidly in embryos under drought stress than that in controls. Furthermore, such changes in seed chemical composition due to water deficit conditions were reported in grass pea by Yang et al. [17]. They also reported that such drought-induced changes in seed chemical composition could have been due to the adverse effects of drought on plant photosynthetic parameters. Furthermore they found a positive correlation of *Pn* or *E*, with seed protein and starch contents.

Drought stress significantly reduced the seed oil content of both maize cultivars, however, the more drought induced reduction in seed oil contents was observed in cv. Agaiti-2002 though this cultivar had higher seed oil content under non-stress conditions (Table 1). Such changes in seed oil contents depend on different factors such as intensity of drought stress and temperature [8, 16], type of genotype [8, 35, 36], and timing of full maturity of cultivars [10]. In agreement with our present findings a marked decline in seed oil content of maize with water deficit was reported in both maize cultivars [37]. Furthermore, Carvalho et al. [38] reported that drought is a major factor responsible for any significant decrease in oil content in two lupin cultivars. Similarly, Asbagh et al. [39] reported a significant decrease in sunflower seed oil contents under water deficit condition.

The fatty acid composition of corn oil is important in determining nutritional quality and the possible uses of oil in industrial applications. The contents of oleic acid in the kernel oil increased due to water stress with a subsequent decrease in linoleic acid caused an increased oleic/linoleic ratio of kernel oil from drought stressed plants. However, the total saturated and unsaturated fatty acids remained unchanged in kernel oil of drought stressed plants (Table 2). Similar changes in the contents of oil oleic and linoleic acids as a result of drought stress have already been demonstrated in some other reports in different plant species, e.g. sunflower [40, 41] and *Moringa oleifera* [12]. In the former study, it was reported that shortage of water

Table 1 Proximate composition of the kernels of two maize cultivars when grown under water stressed and non-stressed conditions

		Sugar content	Oil content	Starch content	Protein content	Moisture content	Fiber content	Ash content
% Age								
Agaiti-2002	Control	1.70 ± 0.04^{a}	3.92 ± 0.12^{a}	61.33 ± 8.28^{a}	7.77 ± 0.48^{a}	8.74 ± 0.30^{a}	2.82 ± 0.22^{b}	1.89 ± 0.15^{b}
	Drought	1.14 ± 0.01^{b}	2.39 ± 0.04^{d}	67.00 ± 5.35^{a}	6.69 ± 0.45^{b}	$5.33 \pm .0.32^{b}$	3.42 ± 0.18^{a}	$2.11 \pm 0.18^{a,b}$
EV-1098	Control	1.73 ± 0.09^{a}	3.55 ± 0.25^{b}	58.33 ± 7.10^{a}	8.16 ± 0.71^{a}	8.74 ± 0.78^{a}	3.14 ± 0.23^{a}	2.02 ± 0.07^{b}
	Drought	1.13 ± 0.01^{b}	3.01 ± 0.12^{c}	63.67 ± 3.90^{a}	6.59 ± 0.45^{b}	5.84 ± 0.31^{b}	3.37 ± 0.31^{a}	2.28 ± 0.09^{a}
LSD at 5% level		0.09	0.29	11.98	1.00	0.89	0.45	0.24

Data are presented as means ± standard error

Means within the same column sharing the same superscript letters do not differ significantly at the 5% level

Table 2 Composition of seed oil fatty acids of two maize (*Zea mays* L.) cultivars when grown under water stressed and non-stressed conditions

		Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Oleic/linoleic	Saturated fatty acids	Unsaturated fatty acids
% Age									
Agaiti-2002	Control	10.51 ± 0.93[a]	2.02 ± 0.09[a]	28.12 ± 3.89[a]	57.74 ± 4.91[a]	0.28 ± 0.02[a]	0.50 ± 0.12[b,c]	12.52 ± 0.88[a]	86.14 ± 1.02[a]
	Drought	10.12 ± 0.54[a]	2.08 ± 0.08[a]	35.33 ± 2.49[a]	49.67 ± 2.95[b]	0.34 ± 0.02[a]	0.72 ± 0.09[a,c]	12.33 ± 0.62[a]	85.34 ± 0.73[a]
EV-1098	Control	10.62 ± 1.52[a]	1.95 ± 0.12[a]	31.63 ± 4.62[a,b]	54.67 ± 5.73[a,b]	0.31 ± 0.02[a]	0.60 ± 0.15[a,c]	12.57 ± 1.60[a]	86.61 ± 1.40[a]
	Drought	10.25 ± 0.94[a]	2.02 ± 0.19[a]	36.42 ± 3.34[a]	48.14 ± 3.66[b]	0.34 ± 0.03[a]	0.77 ± 0.14[a]	12.14 ± 1.11[a]	84.90 ± 0.53[a]
LSD at 5% level		1.95	0.24	5.71	7.66	0.04	0.22	2.09	2.60

Data are presented as means ± standard error

Means within the same column sharing the same superscript letters do not differ significantly at the 5% level

enhanced the amount of oleic acid in sunflower seeds compared with that in well-watered plants and further described that this change could have been due to the activity of the enzyme Δ-12 desaturase responsible for conversion of oleic to linoleic, which acts for a short time under water stress. However, other studies indicate some different trends of oil fatty acid composition under water deficit conditions. For example, a drought-induced increase in linoleic and a decrease in oleic acid contents of sunflower achene have been reported by Petcu et al. [42]. In contrast, a decrease in seed oil linoleic acid and oleic acid content in canola under drought stress was observed by [43].

Various hydrophilic and lipophilic antioxidant compounds (ascorbic acid, salicylic acid, tocopherols) play an important role in counteracting the reacting oxygen species (ROS), in plants. As the final phase of seed development in most crop species, especially in cereals, is prone to desiccation [44]. Under desiccation, accumulation of AOS and free radicals has often been considered as one of the most important factors during seed development [45, 46]. The ability of seeds to withstand desiccation during maturation is related, at least partly, to their ability to scavenge AOS to avoid deleterious events such as lipid peroxidation caused by these compounds [47, 48]. So the production of dehydrins [48] and activation of antioxidant defense systems [49] is a pre-requisite to avoid the deleterious effects of AOS. In the present study, α, γ, δ and total tocopherol contents in the kernel oils increased in both maize cultivars due to imposition of water stress. Of different determined tocopherols, γ-tocopherol occurred abundantly in the seed oil of both maize cultivars under stressed and non-stressed conditions. Significantly higher values of different tocopherols were observed in cv. EV-1098 as compared to those in Agaiti-2002 (Fig. 1). These results are similar to those found in soybean where drought stress caused a two to threefold increase in α-tocopherols [50]. An increase in tocopherol content in seed oil of water stressed maize plants may have been due to an increase in HTP activity [51], one of the most important tocopherol biosynthetic enzymes [52, 53]. Tocopherols protect the polyunsaturated fatty acids from peroxidation [54]. In the present study, a positive association has been found between unsaturated fatty acids such as oleic acid and each of α, γ, δ and total tocopherol contents. These findings are similar to those of Kriese et al. [55] who found a positive correlation between some fatty acids, and γ-tocopherols and total tocopherols.

The results of various physical and chemical parameters of the extracted maize kernel oils from drought stressed and well-irrigated plants shows that water stress caused a significant reduction in oil sponification value and increased the unsaponifiable matter (Table 3). However, the oil iodine value, a measure of oil unsaturation, remained unchanged (Table 3). These results are in agreement with the findings of some earlier studies of Anwar et al. [12] with *Moringa oleifera* and Ali et al. [13] with sunflower. They reported that drought stress affected the physico-chemical properties of *Moringa oleifera* and sunflower seed oil, respectively.

There were significant variations in the values of specific extinctions at 232 and 270 nm, which reveal the oxidative deterioration and purity of the oils [56]. The peroxide value and *p*-anisidine values, which measure hydroperoxides and aldehydic secondary oxidation products of oils and fats, respectively [57], were higher in kernel oils from water stressed maize plants (Table 3). The high oxidative stability of maize oil in drought stressed plants compared with non-stressed could be attributed to the high level of monoenoic FA, particularly, 18:1, which is less prone to oxidation than polyenoics.

Seed oil total phenolic content is indicative of the total antioxidative activity [58] due to the availability of the phenolic hydrogens, as hydrogen-donating radical scavengers [59]. In the present study, water stress caused a significant decrease in the kernel oil phenolic contents of both maize cultivars (Fig. 1). Similar findings were observed in some earlier studies on olive oil [60, 61] and rape seed [62] in which the effect of irrigation was found to

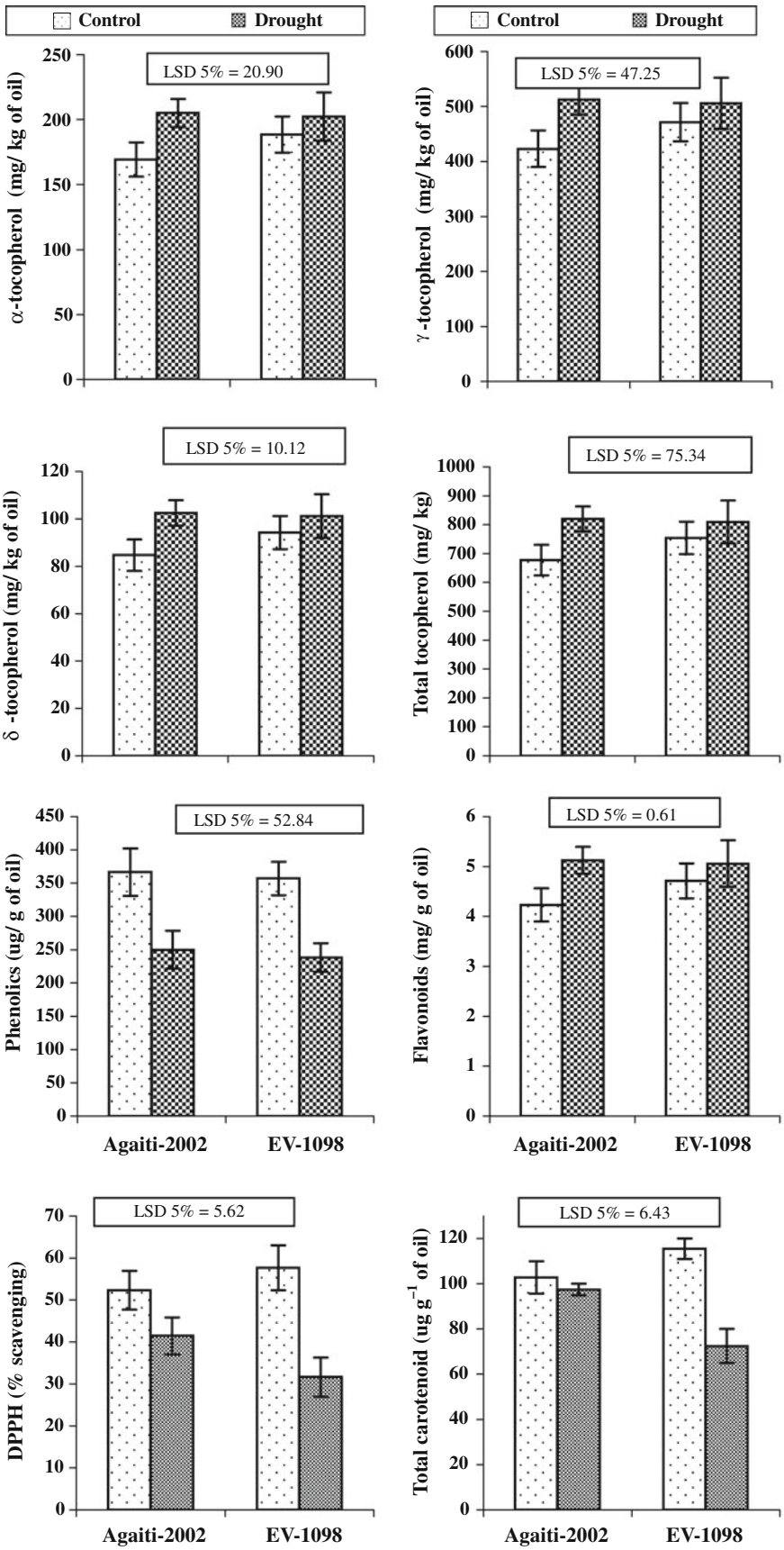

Fig. 1 Tocopherol, phenolic, flavonoid and carotenoid contents and antioxidant activity of seed oil of two maize cultivars when grown under water stress and non-stress conditions ($n = 4 \pm$ SE)

Table 3 Physicochemical properties of the kernel oil of two maize (*Zea mays* L.) cultivars when grown under water stressed and non-stressed conditions

		Saponification value (mg of KOH/g of oil)	Unsaponifiable matter (%)	Iodine value	FFA	PV (mequiv/kg)	*p*-Ansidine value	Density (25 °C) (g/cm^3)	Dienes [$\varepsilon_{1\ cm}(\lambda_{232\ nm})$]	Trienes [$\varepsilon_{1\ cm}(\lambda_{268\ nm})$]
Agaiti-2002	Control	194.82 ± 6.02^a	2.11 ± 0.17^b	111.61 ± 5.14^a	1.36 ± 0.06^b	6.06 ± 0.45^b	2.08 ± 0.10^b	0.920 ± 0.004^b	1.91 ± 0.01^a	1.76 ± 0.01^b
	Drought	180.55 ± 10.95^{ab}	2.48 ± 0.11^a	115.49 ± 3.81^a	1.66 ± 0.10^a	7.21 ± 0.59^a	2.37 ± 0.05^a	0.930 ± 0.002^a	1.83 ± 0.04^b	1.84 ± 0.01^a
EV-1098	Control	193.07 ± 8.12^a	2.14 ± 0.18^b	116.01 ± 6.21^a	1.43 ± 0.05^b	6.21 ± 0.40^b	1.89 ± 0.03^c	0.920 ± 0.002^b	1.64 ± 0.01^c	1.54 ± 0.01^c
	Drought	174.55 ± 4.03^b	2.45 ± 0.19^a	114.59 ± 4.47^a	1.67 ± 0.08^a	7.51 ± 0.45^a	2.17 ± 0.04^b	0.920 ± 0.003^b	1.65 ± 0.01^c	1.53 ± 0.01^c
LSD at 5% level		14.49	0.31	9.36	0.14	0.90	0.11	0.005	0.04	0.01

Data are presented as means ± standard error

Means within the same column sharing the same superscript letters do not differ significantly at the 5% level

be significant on the concentration of oil phenolic compounds. All these reports support our results for phenolic content that shortage of water decreased the concentration of phenolic compounds. This decrease in oil phenolic contents could have been due to the reason that water deficit at early stages of rape development and during the flowering process may have strongly reduced the accumulation of phenolic compounds [62].

Water stress significantly increased the kernel oil flavonoids contents of both maize cultivars. The cultivars differed significantly in oil flavonoid contents under non-stressed conditions because higher values of oil flavonoids were observed in cv. EV-1098 than those in Agaiti-2002 (Fig. 1). In some earlier studies it was observed that total flavonoid content increased throughout the seed development in two Korean soybean cultivars [58, 63]. Similarly, the drought induced increase in flavonoids contents of maize kernel oil in the present study may have been due to their enhanced synthesis during seed development and seed maturity. Kumar et al. [58] reported that seeds picked at early reproductive stages possess low concentrations of flavonoids as compared to those picked at maturity. The increase in flavonoids in kernel oil of drought stressed plants could have been due to the early maturity of seeds in the stressed plant.

The seed oil carotenoid contents of both maize cultivars were reduced significantly due to the imposition of water stress. This reduction in oil carotenoids due to water stress was greater in cv. EV-1098 as compared with that in cv. Agaiti-2002. Both cultivars differed significantly in relation to oil carotenoid contents both under stress and non-stress conditions. Under non-stress conditions significantly higher carotenoid contents were observed in cv. EV-1098 with those in cv. Agaiti-2002, but the reverse was true under water deficit conditions (Fig. 1). Similarly, in some earlier studies on different pea cultivars, it was observed that drought stress significantly reduces the seed total carotenoid contents of all pea cultivars, but this decrease in carotenoid contents was not uniform in all pea cultivars [64]. This decrease in oil carotenoid contents under water deficit conditions could have been due to the fact that seed lipid metabolism is sensitive to moisture contents especially during seed ripening which influences the lipid biosynthesis in seeds during ripening [65].

In the present study, the DPPH radical scavenging activity in maize kernel oil decreased significantly due to water stress. Furthermore, this reduction in oil DPPH radical scavenging was positively correlated with the oil phenolic and carotenoid contents and negatively with that of oil flavonoids and tocopherol contents (Fig. 1). This strong positive correlation between total phenolics carotenoid and DPPH radical scavenging was also observed in cereals [66] and soybean [58], which suggests that this

decrease in oil DPPH radical scavenging is contributed by the presence of large amounts of carotenoid and phenolic compounds. Furthermore, Weidner et al. [67] reported that phenolics are more potent DPPH scavengers than flavonoids. As in rye, they observed that a decline in total phenolic content was associated with a decline in free radical scavenging activity and total antioxidative capacity despite the increase in tocopherols and flavonoids.

Conclusion

From the results of the present comprehensive maize kernel and kernel oil analyses, it can be concluded that drought might be considered as one of the most visible factors which affected the chemical composition of maize kernel and kernel oils. Oil yield, degree of unsaturation, fatty acid composition, oxidative stability, oil phenolics, flavonoids and carotenoid are the parameters affected most by water deficit. Of different antioxidant bioactive compounds, the oil DPPH radical scavenging was associated with total phenolic and carotenoid contents of the oil.

References

1. La′sztity R (1999) Cereal chemistry. Akade′miai Kiado′. Budapest, Hungary, pp 11–51
2. Baye TM, Pearson TC, Settles AM (2006) Development of a calibration to predict maize seed composition using single kernel near infrared spectroscopy. J Cereal Sci 43:236–243
3. Baenziger PS, Shelton DR, Shipman MJ, Graybosch RA (2001) Breeding for end-use quality: reflections on the Nebraska experience. Euphytica 119:95–100
4. Dunlap FG, White PJ, Pollak LM, Brumm TJ (1995) Fatty acid composition of oil from adapted, elite corn breeding materials. J Am Oil Chem Soc 72:981–987
5. Goffman FD, Böhme T (2001) Relationship between fatty acid profile and vitamin E content in maize hybrids (*Zea mays* L.). J Agric Food Chem 49:4990–4994
6. Schussler JR, Westgate ME (1991) Maize kernel set at low water potential. 1. sensitivity to reduced assimilates during early kernel growth. Crop Sci 31:1189–1195
7. Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 99:191–203
8. Triboi E, Triboi-Blondel AM (2002) Productivity and grain or seed yield: a new approach to an old problem. Invited paper. Eur J Agron 16:163–186
9. Bellaloui N, Mengistu A (2008) Seed composition is influenced by irrigation regimes and cultivar differences in soybean. Irrig Sci 26:261–268
10. Zhang M, Kang MS, Reese PF, Bhardwaj HL (2005) Soybean cultivar evaluation via GGE biplot analysis. J New Seed 7:37–50
11. Nel AA (2001) Determination of sunflower seed quality for processing. Ph.D. Thesis. Department of Plant Production and Soil Sciences, University of Pretoria, Pretoria, South Africa, pp 40–56
12. Anwar F, Zafar SN, Rashid U (2006) Characterization of *Moringa oleifera* seed oil from drought and irrigated regions of Punjab, Pakistan. Grasas Y Aceites 57:160–168
13. Ali Q, Ashraf M, Anwar F (2009) Physicochemical attributes of seed oil from drought stressed sunflower (*Helianthus annuus* L.) plants. Grasas Y Aceites 60:475–481
14. Monotti M (2003) Growing non-food sunflower in dry land conditions. Ital J Agron 8:3–8
15. Dornbos DL, Mullen RE (1992) Soybean seed protein and oil contents and fatty-acid composition adjustments by drought and temperature. J Am Oil Chem Soc 69:228–231
16. Specht JE, Chase K, Macrander M, Graef GL, Chung J, Markwell JP, Orf HH, Lark KG (2001) Soybean response to water: a QTL analysis of drought tolerance. Crop Sci 41:493–509
17. Yang HM, Zhang XY, Wang GX (2004) Relationships between stomatal character, photosynthetic character and seed chemical composition in grass pea at different water availabilities. J Agric Sci 142:675–681
18. Xing GS, Cui KR, Li J, Li YF, Wang ZX (2001) Water stress and accumulation of β-N-Oxalyl-L-α, β -diaminopropionic acid in grass pea (*Lathyrus sativus*). J Agric Food Chem 49:216–220
19. Zhou GK, Kong YZ, Cui KR, Li ZX, Wang YF (2001) Hydroxy radical scavenging activity of β-Noxalyl-L-α, β-diaminopropionic acid. Phytochemistry 58:759–762
20. Dewis J, Freitas F (1970) Physical methods of soil and water analysis. FAO Soil Bull 10:39–51
21. Jackson ML (1962) Soil chemical analysis. Prentice-Hall Englewood Cliffs
22. Anonymous (2000) Approved methods of the American association of cereal chemists, 10th edn. AACC, St. Paul, MN
23. American Oil Chemist's Society (AOCS) (1997) Official and recommended practices of the American oil chemists society, 5th edn. AOCS Press, Champaign
24. International Union of Pure and Applied Chemistry (1987) In: Paquot C, Hautfenne A (eds) Standard methods for the analysis of oils, fats and derivatives, 7th Rev. Blackwell Scientific, London, UK, Jackson
25. Lee BI, New AL, Ong CN (2003) Simultaneous determination of tocotrienols, tocopherols, retinols and major carotenoids in human plasma. Clin Chem 49:2056–2066
26. Chaovanalikit A, Wrolstad RE (2004) Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. Food Chem Toxicol 69:67–72
27. Dewanto V, Wu X, Adom KK, Liu RH (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 50:3010–3014
28. Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V (2000) Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophaë rhamnoides* L.) during maturation. J Agric Food Chem 48:1485–1490
29. Sultana B, Anwar F, Przybylski R (2007) Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating. Food Chem 104:997–1005
30. Iqbal S, Bhanger MI (2007) Stabilization of sunflower oil by garlic extract during accelerated storage. Food Chem 100:246–254
31. Steel RGD, Torrie JH (1986) Principles and procedures of statistics. McGraw Hill, New York
32. Zinselmeier C, Westgate ME, Schussler JR, Jones RJ (1995) Low water potential disrupts carbohydrate metabolism in maize (*Zea mays* L.) ovaries. Plant Physiol 107:385–391
33. Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic Press, San Diego
34. Carvalho IS, Ricardo CP, Chaves M (2004) Quality and distribution of assimilates within the whole plant of lupins (*L. albus* and *L. mutabilis*) influenced by water stress. J Agron Crop Sci 190:205–210

35. Maestri DM, Labuckas DO, Meriles JM, Lamarques AL, Zygadlo JA, Guzman CA (1998) Seed composition of soybean cultivars evaluated in different environmental regions. J Sci Food Agric 77:494–498
36. Piper EL, Boote KJ (1999) Temperature and cultivar effects on soybean seed oil and protein concentrations. J Am Oil Chem Soc 76:1233–1242
37. Champolivier L, Merrien A (1996) Effects of water stress applied at different growth stages to *Brassica napus* L. Var. *oleifera* on yield, yield components and seed quality. Eur J Agron 5:153–163
38. Carvalho IS, Chaves M, Ricardo CP (2005) Influence of water stress on the chemical composition of seeds of two lupins (*Lupinus albus* and *Lupinus mutabilis*). J Agric Crop Sci 191:95–98
39. Asbagh FT, Moghaddam AF, Gorttapeh AH (2009) Influence of water stress and sowing date on sunflower yield and oil percentage. Res J Biol Sci 4:165–170
40. Flagella Z, Rotunno T, Caterina RD, Simone GD, Caro AD (2000) Effect of supplementary irrigation on seed yield and oil quality of sunflower (*Helianthus annuus* L.) grown in a sub-arid environment. In: Proceedings of XV international sunflower conference, pp 139–144, 1, Toulouse
41. Flagella Z, Rotunno T, Tarantito E, Caterina RD, Caro AD (2002) Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water regime. Eur J Agron 17:221–230
42. Petcu E, Arsintescu A, Stanciu D (2001) The effect of drought stress on fatty acid composition in some romanian sunflower hybrids. Romanian Agric Res 15:39–43
43. Pritchard F (2007) Challenges for irrigated canola in 2007. Information sourced from IREC farmers newsletter, No. 175
44. Taize L, Zeiger E (2006) Plant physiology, 4th edn
45. Hendry GAF (1993) Oxygen, free radical processes and seed longevity. Seed Sci Res 3:141–153
46. McDonald MB (1999) Seed deterioration: physiology, repair and assessment. Seed Sci Technol 27:177–237
47. Leprince O, Hendry GAF, McKersie BD (1993) The mechanisms of desiccation tolerance in developing seeds. Seed Sci Res 3:231–246
48. Vertucci CW, Farrant JM (1995) Acquisition and loss of desiccation tolerance. In: Kigel J, Galili G (eds) Seed development and germination. Marcel Dekker, New York, pp 237–271
49. Bailly C, Audigier C, Ladonne F, Wagner MH, Coste F, Corbineau F, Côme D (2001) Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. J Exp Bot 52:701–708
50. Steven JB, Diane FK (2002) Warm temperature and drought during seed maturation increase free α-tocopherol in the seeds of soybean. J Agric Food Chem 50:6058–6063
51. Collakova E, DellaPenna D (2003) The role of homogentisate phytyltransferase and other tocopherol pathway enzymes in the regulation of tocopherol synthesis during abiotic stress. Plant Physiol 133:930–940
52. Schledz M, Seidler A, Beyer P, Neuhaus G (2001) A novel phytyltransferase from *Synechocystis sp.* PCC6803 involved in tocopherol biosynthesis. FEBS Lett 499:15–20
53. Savidge B, Weiss JD, Wong YH, Lassner MW, Mitsky TA, Shewmaker CK, Post-Beittenmiller D, Valentin HE (2002) Isolation and characterization of homogentisate phytyltransferase genes from synechocystis sp. PCC6803 and arabidopsis. Plant Physiol 129:321–332
54. Kamal-Eldin A, Appelqvist LA (1996) The chemistry and antioxidants properties of tocopherols and tocotrienols. Lipids 31:671–701
55. Kriese U, Schumann E, Weber WE, Beyer M, Bruhl D, Matthäus B (2004) Oil content, tocopherol composition and fatty acid patterns of the seeds of 51 *Cannabis sativa* L. Genotypes. Euphytica, pp 339–351
56. Yoon SH, Kim SK, Kim MG, Kim KH (1985) Comparative study of physical methods for lipid oxidation measurement. J Am Oil Chem Soc 68:1487–1489
57. McGinely L (1991) Analysis and quality control for processing and processed fats. In: Rosell JBP, Ritchard JLR (eds) Analysis of oilseeds, fats and fatty foods. Elsevier, New York, pp 460–470
58. Kumar V, Rani A, Dixit AK, Bhatnagar D, Chauhan GS (2009) Relative changes in tocopherols, isoflavones, total phenolic content, and antioxidative activity in soybean seeds at different reproductive stages. J Agric Food Chem 57:2705–2710
59. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med 20:933–956
60. Maurizio S, Sonia E, Enrico L, Roberto S, Agnese T, Stefania U, Gianfrancesco M, Matteo S, Riccardo G (2007) Irrigation effects on quality, phenolic composition, and selected volatiles of virgin olive oils cv. Leccino. J Agric Food Chem 55:6609–6618
61. Greven M, Neal S, Green S, Dichio B, Clothier B (2009) The effects of drought on the water use, fruit development and oil yield from young olive trees. Agric Water Manag 96:1525–1531
62. Bouchereau A, Clossais-Besnard N, Bensaoud A, Leport L, Renard M (1996) Water stress effects on rapeseed quality. Eur J Agron 5:19–30
63. Kim JA, Chung IM (2007) Change in isoflavone concentration of soybean (*Glycine max* L.) seeds at different growth stages. J Sci Food Agric 87:496–503
64. Nemeskéri E (2006) Breeding strategy for improvement of colour quality and carotenoid levels in dry pea seeds. Commun Biometry Crop Sci 1:49–55
65. Mohapatra SC, Pattee HE (2006) Lipid metabolism in dehydrating peanut kernels. Physiol Plant 28:320–326
66. Dykes L, Rooney LW (2007) Phenolic compounds in cereal grains and their health benefits. Cereal Foods World 52:105–111
67. Weidner S, Amarowiczb R, Karamac M, Fraczek E (2000) Changes in endogenous phenolic acids during development of *Secale cereale* caryopses and after dehydration treatment of unripe rye grains. Plant Physiol Biochem 38:595–602