J Am Oil Chem Soc (2007) 84:845–851
DOI 10.1007/s11746-007-1115-8

ORIGINAL PAPER

Does Soil Salinity Affect Yield and Composition of Cottonseed Oil?

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Received: 12 February 2007 / Revised: 14 July 2007 / Accepted: 20 July 2007 / Published online: 15 August 2007

Abstract Variation in the yield and composition of oil of cotton (*Gossypium hirsutum*) seed collected from two different sites (saline and non-saline) of Pakistan was examined. Hexane-extracted oil content of cottonseed from saline and non-saline areas was found to be 17.7 and 18.6%, respectively. No significant ($P > 0.05$) differences in the refractive index (40 °C), color, specific gravity (24 °C), iodine, free fatty acid, peroxide, unsaponifiable matter, saponification, conjugated diene and triene and *p*-anisidine values of the oils of cotton plants from saline and non-saline habitats were observed. Fatty acid compositional analysis showed the concentration of stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acids to be significantly ($P \leq 0.05$) higher, whereas that of linoleic ($C_{18:2}$) acid was lower in cottonseed oils from the saline area than those from the non-saline habitat. Tocopherol contents of cottonseed oils were significantly ($P \leq 0.05$) higher from the saline area than those from the non-saline area. The results of the present study showed that soil salinity did not affect the oil yield of cottonseed, however, it significantly ($P \leq 0.05$) affected the tocopherol and fatty acid profiles of the oils examined.

Keywords Cottonseed · Characterization · Salinity · Tocopherols · Fatty acid composition · Oil quality

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Introduction

Soil salinity is the major abiotic stress that drastically affects crop productivity, particularly in the arid and semi-arid regions of the world. Due to continuous build up of salinity in the soil, millions of hectares of arable land have now become unfit for cultivation [1]. It is projected that more than 1 million ha of land are subjected to salinization every year [2, 3].

The effects of salinity on plant growth include modification of a number of morphological, physiological, and biochemical processes as well as anatomical changes [4]. Furthermore, soil salinity is known to affect lipid metabolism [5]. A change in lipid composition, in response to salinity, was observed in sunflower [6].

Oilseed crops, if found suitable for irrigation with saline water and/or treated wastewaters, may be a highly economic alternative to traditional field crops. In view of some reports in the literature it is evident that soil salinity alters yield and fatty acid compositions of vegetable oils [6–8]. Flagella et al. [6] found that oleic acid increased, whereas linoleic acid progressively decreased in sunflower oil in response to salt stress.

Cotton is not only the most important fiber crop in the world, it is also the second best potential source for plant proteins after soybean and the fifth best oil-producing crop

after soybean, palm, colza and sunflower [9]. Although cotton is considered as being fairly tolerant to salinity [10, 11], yields are drastically reduced due to poor germination and subsequent abnormal plant development under saline conditions. For example, a decrease of about 41% in seed yield in cotton grown on slightly salty soils has been reported earlier [12].

Because abiotic stresses alter the physical and chemical characteristics of oilseeds, there is a need to gather information on the composition of different oilseed crops exposed to such stresses. There have been no reports available in the literature on the detailed characterization and comparison of the oil from cottonseeds grown on saline and non-saline soils. Thus, the present investigation was carried out in order to examine and quantify the effects of salinity on the yield and physico-chemical composition of cottonseed oil.

Materials and Methods

Materials

The seeds of the cotton variety NIAB-111 were procured from the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan, (harvested at NIAB Agricultural farm, Faisalabad, a non-saline area) and Saline Agriculture Farmer Participatory and Development Project (SAFPDP) site Lodharan (a saline area), Punjab, Pakistan. The average temperature and rainfall of the entire growth season in Lodharan were 33.2 ± 2.35 °C and 71 mm and those of Faisalabad were 32.3 ± 2.88 °C and 44 mm, respectively. Samples of cottonseed were collected from five different locations of the trial field. Each of the five samples contained 2.0 kg of the seeds. The five samples were extracted separately and analyzed individually in triplicate. All reagents (analytical and HPLC) used were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Pure standards of tocopherols [DL-α-tocopherol, (+)-δ-tocopherol, (+)-γ-tocopherol] and fatty acid methyl esters (FAMEs) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Oil Extraction

After removal of the seed coat, a sub-sample (200 g) was taken from each cottonseed sample, crushed and then the oil was extracted with *n*-hexane in a Soxhlet unit for 6 h. The solvent was removed at 45 °C under vacuum in a rotary evaporator (N-N Series, Eyela, Rikakikai Co. Ltd., Tokyo, Japan).

Analysis of Oilseed Residues

After oil extraction, the cottonseed residue was analyzed for protein, fiber and ash contents. Protein contents were determined according to AOAC method 976.06 [13]. Fiber content was estimated according to an ISO method 749 [14]. The sample (2.5 g) of sample was boiled with sulfuric acid (0.255 mol L^{-1}), followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol L^{-1}), followed by separation, washing, and drying. The dried residue was weighed and ashed in a muffle furnace (TMF-2100, Eyela) at 600 °C, and the loss in mass was determined.

Ash content was determined according to ISO method 749 [14]. Two grams of the sample was taken and carbonized by heating in a gas flame. The carbonized material was then ashed in an electric muffle furnace (TMF-2100, Eyela) at 550 °C, until a constant mass was achieved.

Analysis of Extracted Oils

Physical and Chemical Parameters of Oils

Determination of density, refractive index, iodine value, peroxide value, acidity, saponification value and unsaponifiable matter of the extracted oil was carried out following standard AOCS methods Cc 10a–25, Cc 7–25, Cd 1–25, Cd 8–53, F 9a–44, Cd 3–25, and Ca 61–40, respectively [15]. Color of the oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, UK), using a 1 inch cell. Specific extinctions at 232 and 270 nm were determined using a spectrometer (Hitachi, U-2001, model 121-0032). Samples were diluted with iso-octane to bring the absorbance within limits (0.2–0.8) and ($\varepsilon^{1\%}_{1\ cm(\lambda)}$) was calculated following IUPAC method II D.23 [16].

Tocopherol Content

Tocopherol (α, γ, and δ) analysis was carried out by HPLC following the Current Protocols in Food Analytical Chemistry method [17]. Oil (0.1 g) and 0.05 g ascorbic acid were placed in a 16 × 125 mm test tube. Five ml of 90.2% ethanol and 0.5 ml of 80% aqueous KOH solution were added to the test tube and vortexed for 30 s. The test tube was flushed with nitrogen, capped and incubated in a water bath (70 °C) for 30 min with periodical vortexing. The tubes were placed in an ice bath for 5 min, then 3 ml deionized water and 5 ml *n*-hexane were added and vortexed for 30 s followed by centrifugation at 1,000×*g* for 10 min at room temperature. The upper hexane layer was transferred to another test tube. The aqueous layer and the

residue were re-extracted by repeating the same procedure. The upper hexane layers from both the extractions were combined and evaporated to dryness under nitrogen. One milliliter (exact to 0.01 mL) mobile phase was added to the tube and vortexed for 30 s to re-dissolve the extract and then transferred to an HPLC sample vial. A 20 μL sample was injected into a Supelcosil LC-Si column (250 × 4.6 mm, Supelco Inc.,). A mobile phase of ethyl acetate/acetic acid/hexane (1:1:198, v/v/v) was used at the rate of 1.5 mL min^{-1}. Detection was performed at 295 nm. Tocopherols were identified by comparing the retention times with those of pure standards of α-, β-, γ-, and δ- tocopherols, and were quantified on the basis of peak areas of the pure standards (Sigma Chemical Co.). Quantification was based on an external standard method. A D-2500 Hitachi Chromatointegrator model with a built-in computer program for data handling was used for quantification.

Fatty Acid Composition

Fatty acid methyl esters were prepared by standard IUPAC method 2.301 [16] and analyzed on a SHIMADZU gas chromatograph model 17-A, fitted with a SP-2330 (SUPLECO, inc., Bellefonte, PA, USA) methyl lignoserate coated (film thickness 0.20 μm) polar capillary column (30 m × 0.32 mm) and a flame ionization detector. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3 mL min^{-1}. Other conditions were as follow: initial oven temperature, 180 °C; ramp rate, 5 °C/min; final temperature, 220 °C; injector temperature, 230 °C; detector temperature, 240 °C. FAMEs were identified by comparing their relative and absolute retention times with those of authentic standards of FAMEs (Sigma Chemical Co. (St Louis, MO, USA). The quantification was done by a Chromatography Station for Windows (CSW32) data handling software (Data APEX Ltd., Pague 5, The Czech Republic). The fatty acid composition was reported as a relative percentage of the total peak area.

Soil Analysis

Sampling and Pretreatment

Five soil samples were collected from each trial area at a depth of 15–30 cm during the course of experiment. A sub sample (200 g) was taken from each soil sample and dried in an oven at 90 °C for 5 days. An oven-dried soil subsample (100 g) was made into a paste with distilled water following the procedure described in US Salinity Laboratory Staff [18]. On an average 26–32 mL water was obtained from each extracted soil sample. The soil extract was obtained after filtering the extract using a vacuum pump.

Determination of Various Parameters of Soil

Nutrient Analysis The determination of potassium, sodium and calcium in the soil saturated extract was carried out using a flame photometer model Jenway PFP-7. The concentration of magnesium in the soil extract was determined using a Perkin Elmer atomic absorption spectrophotometer model Analyst-300. Chloride content was determined by Sherwood Chloride Analyzer model 920. Electrical conductivity (ECe) and pH of the soil extract were measured by pH/Cond (Inolab), level 1 m. Sodium adsorption ratio (SAR) was also determined by equation given in US Salinity Laboratory Staff [18].

Water Analysis

Five irrigation water samples were collected from each trial area during the course of experiment. The measurements for electrical conductivity (EC), SAR, residual sodium carbonate (RSC) and inorganic elements were made according to the methods described by the US Salinity Lab Staff [18].

Statistical Analysis

Five cottonseed (2.0 kg each) samples were assayed from each of the saline and non-saline areas. Data is reported as mean ± SD of five cottonseed samples from each area, analyzed individually in triplicate. For all investigated parameters, the analysis of variance (ANOVA) was performed using the Minitab Statistical Software (Version 13.20). A probability value of $P \leq 0.05$ was considered to denote a statistical significance difference.

Results and Discussion

Properties of Soil and Water

The chemical properties of soil samples from the investigated saline and non-saline areas are shown in Table 1. The values of ECe, SAR and the contents of Na^+ and Cl^- were found to be significantly higher in saline areas as compared with those in non-saline habitat. Furthermore, the concentrations of K^+, Ca^{2+} and Mg^{+2} were also significantly higher in the soil samples of saline areas as

Table 1 Physical and chemical characteristics of soil from saline and non-saline areas

Characteristics	Saline soil	Non-saline soil	ANOVA	
			F value	*P*
Saturation (%)	38.0 ± 1.90	32.4 ± 1.61	11.9*	0.02
pH	8.3 ± 0.33	7.9 ± 0.31	3.8^{NS}	0.12
ECe (dS/m)	11.0 ± 0.55	1.5 ± 0.02	1,081.3*	0.00
$Ca^{2+} + Mg^{2+}$ (mEq L^{-1})	29.6 ± 2.98	8.6 ± 1.02	132.1*	0.00
K^+ (mEq L^{-1})	4.1 ± 0.15	1.5 ± 0.01	1,902.0*	0.00
Na^+ (mEq L^{-1})	82.1 ± 2.70	7.0 ± 0.55	3,978.0*	0.00
Cl^- (mEq L^{-1})	92.0 ± 2.10	13.0 ± 1.15	3,650.2*	0.00
SAR	21.3 ± 0.93	3.4 ± 0.17	927.0*	0.00

Values are means ± SD for five soil samples from each of the saline and non-saline areas, analyzed individually in triplicate

ECe electrical conductivity of soil extract, *SAR* sodium adsorption ratio, *NS* non significant, * significant

Table 2 Physical and chemical properties of water from saline and non-saline areas

Characteristics	Saline water	Non-saline water	ANOVA	
			F value	*P*
pH	7.9 ± 0.39	7.4 ± 0.37	2.0^{NS}	0.23
EC (dS/m)	1.5 ± 0.07	0.6 ± 0.03	437.8*	0.00
CO_3^{2-} (mEq L^{-1})	2.0 ± 0.10	ND	–	–
HCO_3^- (mEq L^{-1})	10.2 ± 0.51	6.0 ± 0.30	159.4*	0.00
$Ca^{2+} + Mg^{2+}$ (mEq L^{-1})	3.9 ± 0.19	7.8 ± 0.39	228.1*	0.00
Na^+ (mEq L^{-1})	14.5 ± 0.72	4.4 ± 0.22	295.4*	0.00
Cl^- (mEq L^{-1})	5.4 ± 0.27	3.5 ± 0.17	81.6*	0.00
SAR	10.4 ± 0.90	2.2 ± 0.30	185.1*	0.00
RSC	8.3 ± 0.42	0.0 ± 0.00	1,652.0*	0.00

Values are means ± SD for five water samples from each of the saline and non-saline areas, analyzed individually in triplicate

EC electrical conductivity of water, *SAR* sodium adsorption ratio, *RSC* residual sodium carbonate, *NS* non-significant, * significant

compared with those of its counterpart. Higher levels of these attributes in soil from saline area might be attributed to the high concentration of NaCl due to which the area was found to be heavily salinized.

The physical and chemical analyses of irrigation water from saline and non-saline regions are presented in Table 2. The values of EC, SAR and residual sodium carbonate (RSC) were found to be significantly higher in saline water as compared with those of non-saline water. The contents of Na^+ and Cl^- were also significantly higher in the saline water samples as compared with those of its counterpart. The higher values of EC and RSC of water samples from the saline region indicate that the water from the saline region was also saline.

Proximate Composition and Physico-Chemical Properties of Cottonseed Oils

The oil yield of cottonseeds was not significantly ($P > 0.05$) different for samples collected from both areas (Table 3). The mean values of oil yield were 17.7 and 18.6% (w/w) in the samples collected from the saline and non-saline areas, respectively. In contrast to our present analysis, however, somewhat different results were reported by Flagella et al. [6], who demonstrated a significant effect of salinity on oil yield of a hybrid variety of sunflower seed. Royo et al. [19] reported a minor effect of soil salinity on the oil content of Arbequina olive oil. However, Heuer et al. [20] reported no change in the oil content of *Matthiola indica* due to the effect of salinity.

The analysis of oilseed residue revealed no significant ($P > 0.05$) variation in the contents of protein (34.0 and 36.2%), fiber (21.8 and 22.1%), ash (4.7 and 4.7%) and moisture (7.1 and 7.2%) in the cottonseed from saline and non-saline areas, respectively. In contrast to our present analysis, Singla and Grover [21] reported that salt stress caused protein level reductions.

Table 3 Proximate analysis of cotton seeds (cv. NIAB-III) grown on saline and non-saline areas

Constituents (%)	Saline	Non-saline	ANOVA	
			F value	*P*
Oil content	17.7 ± 1.06	18.6 ± 1.22	0.94^{NS}	0.38
Protein	34.0 ± 1.70	36.2 ± 1.81	2.35^{NS}	0.20
Fiber	21.8 ± 0.87	22.1 ± 1.37	0.00^{NS}	0.94
Ash	4.7 ± 0.23	4.7 ± 0.23	0.04^{NS}	0.84
Moisture	7.1 ± 0.28	7.2 ± 0.30	3.07^{NS}	0.15

Values are mean ± SD of five cotton seed samples from each of the saline and non-saline areas, analyzed individually in triplicate

NS non-significant

The results of various physical and chemical characteristics of the extracted cottonseed oils from saline and non-saline areas of Pakistan are presented in Table 4. No significant ($P > 0.05$) differences were observed in the values of density (0.9216 and 0.9292 g cm^{-3}) at 24 °C, refractive index (1.4643 and 1.4643) at 40 °C, iodine number (102.2, 103.0 g of I 100 g^{-1} of oil) and unsaponifiable matter (0.612, 0.532%) of cottonseed oils from saline and non-saline areas, respectively. However, there were significant ($P \leq 0.05$) differences in color (15.00R + 70.00Y and 12.80R + 60.00Y), free fatty acid (0.6 and 4.6% as oleic acid) and saponification (170.2 and 183.3 mg of KOH/g of oil) values of oils from saline and non-saline

Table 4 Physical and chemical characteristics of cottonseed (cv. NIAB-III) oil from saline and non-saline areas

Constituents	Saline	Non-saline	ANOVA	
			F value	*P*
Color (1″ cell) (red unit)	15.0 ± 0.60	12.8 ± 0.51	23.34*	0.01
Yellow unit	70.0 ± 2.80	60.0 ± 2.40	22.13*	0.01
Density (24 °C, g/cm^3)	0.9216 ± 0.04	0.9292 ± 0.04	0.11^{NS}	0.75
Refractive index (40 °C)	1.4643 ± 0.05	1.4643 ± 0.05	0.00 ^{NS}	1.00
Acidity (% as oleic acid)	0.6 ± 0.36	4.6 ± 0.27	90.65*	0.00
Iodine value (g of I/100 g of oil)	102.2 ± 3.06	103.0 ± 3.09	0.11^{NS}	0.75
Saponification value (mg of KOH/g of oil)	170.2 ± 5.11	183.3 ± 5.49	9.16*	0.03
Unsaponifiable mater (%)	0.612 ± 0.02	0.532 ± 0.21	0.42^{NS}	0.55

Values are mean ± SD of five cottonseed oils from each of the saline and non-saline areas, analyzed individually in triplicate

NS non-significant, * significant

regions, respectively. These findings are in good agreement with our previous findings on *Moringa oleifera* oil [22].

There was a significant ($P \leq 0.05$) variation in the oxidation values of specific extinctions at 232 and 270 nm (Table 5), which reveal the oxidative deterioration and purity of the oils [23]. The values of conjugated diene of cottonseed oils from the saline and non-saline areas were 2.1 and 3.1 and those of conjugated triene 1.0 and 0.9, respectively. Peroxide values of the oil, which measures hydroperoxides products of the oils and fats [24], was found to be significantly ($P \leq 0.05$) higher in cottonseed grown in the saline areas (3.0 mEq kg^{-1} of oil) as compared with that from the non-saline areas (2.0 mEq kg^{-1} of the oil). There are no earlier reports showing the effects of salinity on oxidation parameters of vegetable seed oils.

The levels of α-tocopherols of cottonseed oils from the saline and non-saline areas were 380.1 and 265.2 and those of γ-tocopherols 590.5 and 333.6 mg kg^{-1}, respectively, whereas, the contents of β- and δ-tocopherols were not detected in the present analysis (Table 6). The contents of α- and γ- tocopherols of cottonseed oils from saline and non-saline areas were in good agreement with those reported in the literature [25]. The levels of α-tocopherol, which has the greatest vitamin E potency [25], and γ-tocopherol, of cottonseed oils from the saline area were significantly ($P \leq 0.05$) higher as compared to those from the non-saline area, which could be attributed to the effect of salinity stress. The high α-tocopherol contents of *Moringa oleifera* seed oil from saline areas has also been reported [22].

Total saturates, i.e., palmitic (16:0) and stearic (18:0) acids, in the seed oils from the saline and non-saline habitats were 34.4 and 30.7%, respectively (Table 7). The contents of oleic (18:1) and linoleic acids (18:2n-6) in cottonseed oils were 23.3, 42.0 and 18.0, 50.6% from the saline and non-saline areas, respectively. The only significant ($P \leq 0.05$) differences between saline and non-saline areas were observed for stearic, oleic and linoleic acids. The contents of stearic and oleic acids were found to be higher from the saline area as compared with those from the non-saline area. However, contents of linoleic acid were found to be lower from the saline areas than those from the non-saline areas. The variation in the content of palmitic acid from both saline and non-saline areas was found to be non-significant ($P > 0.05$). This variation in the concentration of stearic, oleic and linoleic acids of cottonseed oils from

Table 5 Determination of oxidative state of cottonseed (cv. NIAB-III) oil from saline and non-saline areas

Constituents	Saline	Non-saline	ANOVA	
			F value	*P*
Conjugated diene $\varepsilon^{1\%}_{1\ cm}$ (λ232)	2.1 ± 0.04	3.1 ± 0.06	499.0*	0.00
Conjugated triene $\varepsilon^{1\%}_{1\ cm}$ (λ270)	1.0 ± 0.02	0.9 ± 0.01	43.3*	0.00
Peroxide value (mEq/kg of oil)	3.0 ± 0.04	2.0 ± 0.03	1,018.0*	0.00

Values are mean ± SD of five cottonseed oils from each of the saline and non-saline areas, analyzed individually in triplicate

* significant

Table 6 Tocopherol contents of cottonseed (cv. NIAB-III) oils from saline and non-saline areas

Tocopherol (mg kg^{-1} oil)	Saline	Non-saline	ANOVA	
			F value	*P*
α-tocopherol	380.1 ± 8.66	265.2 ± 7.70	299.7*	0.00
β-tocopherol	ND	ND	–	–
γ-tocopherol	590.5 ± 15.32	333.6 ± 6.45	759.2*	0.00
δ-tocopherol	ND	ND	–	–

Values are mean ± SD of five cottonseed oils from each of the saline and non-saline areas, analyzed individually in triplicate

ND Not detected, * significant

Table 7 Fatty acid composition of cottonseed (cv. NIAB-III) oils from saline and non-saline areas

Fatty acids (%)	Saline	Non-saline	ANOVA	
			F value	*P*
C 16:0	27.5 ± 0.55	27.0 ± 0.54	1.5^{NS}	0.28
C 18:0	6.8 ± 0.13	3.7 ± 0.07	1322.5*	0.00
C 18:1	23.3 ± 0.46	18.0 ± 0.36	247.0*	0.00
C 18:2	42.0 ± 0.84	50.6 ± 1.01	127.7*	0.00

Values are mean ± SD of five cottonseed oils from each of the saline and non-saline areas, analyzed individually in triplicate

* significant

NS non-significant

the investigated areas of Pakistan could be due to salt stress. The results of fatty acid composition in the present analysis of cottonseed oils followed the same trend those of our previous findings of *Moringa oleifera* seed oils [22] from saline and non-saline areas of Pakistan.

The literature also revealed the effects of salinity on the fatty acid composition of vegetable oils. For example, Samaoui and Cherift [26] reported that the amount of linoleic acid in cottonseeds decreased in plants grown under salt stress and that of oleic acid remained unchanged. In crambe (*Crambe abyssinica* Hochst.), a decrease in oleic and linoleic acids was observed in field trials carried out under saline irrigation having electrical conductivity ranging from 3.7 to 7.9 dS m^{-1}; however, no significant changes in erucic acid were observed [27]. The increase in the oleic/linoleic acid ratio observed under saline conditions was also evident under water stress conditions occurring during grain filling for high oleic hybrids grown in field [28, 29]. Flagella et al. [6] reported that salinity promoted the increase in oleic acid and a decrease in the linoleic acid content of sunflower oil. In another study, Heuer et al. [7] reported changes in the oil composition of *Matthiola tricuspidata* due to salinity. Similarly, Parti et al. [8] reported a gradual increase in oleic acid in the oil of mustard seeds with increasing salinity levels.

From the present study, it can be concluded that salinity does not affect the oil yield. In contrast, the fatty acid and tocopherol profiles of the cottonseed oils were affected by salinity. Therefore, this cultivar of cotton (NIAB-III) can be effectively grown in the saline and other cotton growing areas of Pakistan without significant changes to the edible oil.

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