
Comparative study of four safflower oils (*Carthamus tinctorius*) varieties grown in eastern of Morocco

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ABSTRACT

In this study, four safflower varieties, originating from Spain (*Rancho*), India (*Sharda*) and Morocco (*Cartamar* and *Cartafri*) cultivated at the experimental station of OUJDA (semi-arid region of Eastern Morocco) were evaluated for their oil yield and seed oil quality. The analysis of results revealed significant differences among varieties for all traits. Indeed, our result show that seeds of the four varieties have important oil content ranged between 35.38% (*Rancho*) and 28.84% (*Cartamar*). Characterization of these oils showed a low value of acidity index less than 0.7% for all the varieties but significant differences in their peroxide values ranged between 4.5 meq O₂/Kg (*Cartamar*) and 31.21 mEqO₂/Kg (*Rancho*) and high value of phenolic content between 143 ppm (*Rancho*) and 97 ppm (*Sharda*). Analysis of the fatty acids shows that linoleic acid is the main fatty acid ranged between 77.94% (*Cartamar*) and 79.98% (*Sharda*). Oils of those four varieties can be classified in the linoleic acid group. The analysis of triglycerides by HPLC shows 10 molecular species of TAG (LLL, LPL, LLO, PLO, LLS, POP, OOO, POO, SOO, PPL). The trilinoleate (LLL) is the main molecular species with more than 50%. Safflower seed oil cultivated in eastern Morocco presents good physicochemical proprieties (polyphenols and Omega 6 richness); therefore, safflower culture could be suggested for all areas of Morocco, including the areas of low pluviometry, while the potential for culture of other oilseeds is more limited.

1 Introduction

Safflower (*Carthamus tinctorius*) is an oilseed crop, which, for many years, has been grown on a relatively small scale in parts of North Africa and Middle East (Purdy and al., 1959). Safflower is a tap-rooted annual crop which can tolerate environmental stresses including salinity and water stress (Lovelli and al., 2007). Therefore it has a good yield potential in Morocco's semiarid areas where research work, particularly breeding, started early in the 60's (Nabloussi and Boujghagh, 2006). Safflower was originally grown for the flowers that were used in making red and yellow dyes for clothing and food preparation. Safflower is an excellent forage plant, which is palatable and is feeding value (crude value and total digestible nutrients) and yields are similar to or better than cereal or alfalfa. Safflower stubble is highly desired by cattle, sheep and goat. (Smith and al. 1996; Landaua and al. 2004). In recent years, considerable attention has been generated in the consumption and development of safflower seed oil as an excellent health care product and health benefits derived from it include prevention and treatment of hyperlipaemia, arteriosclerosis, coronary heart disease (Han and al., 2009).

Safflower oil has been characterized in different regions of the world and several experiences have demonstrated a variability of composition functions of varieties, soil and climate conditions. So the introduction of a new crop to a regional cropping system requires information concerning its performance under local environmental conditions (Çamaş and al., 2007)

The aim of this present work is to complete our precedent investigation laboratory by characterization of safflower seeds produced in North Eastern of Morocco (Zraibi and al., 2012). The objective is to determine the chemical composition of four safflower seeds varieties cultivated in this region. Parameters like quality (acidity index, peroxide value) fatty acid, triacylglycerols and phenols composition of their lipid fraction are compared.

2 Material and Methods

2.1 Plant Material

Plant material consisted of four *Carthamus tinctorius* varieties (pure lines) from different origins: 'Cartafri' and 'Cartamar' (Morocco), 'Rancho' (Spain) and 'Sharda' (India). Seeds of all these varieties were kindly provided by the National Institute for Agricultural Research (INRA), Regional Research Center of Meknes (Morocco).

2.2 Experimental Procedures

The trial was conducted at the Center for Agricultural Qualification Bouchtat in Oujda (East Northern Morocco). Sowings were performed on February 2009, Climatic data for the research areas are given in Table 1.

Table 1. Climatic data for the experimental area during 2009

	value
Total rainfall	249.93 mm
annual average Temperature	18.2°C
Maxima Temperature mediates annual	25.6°C
minimum Temperature annual average	11.6°C
annual average Humidity	59.7%

Soil types of the station are silty clay loam to a low active lime. The experimental design was a randomized complete block design with three replications. Capitula were harvested manually on june at maturity (browning of all leaves and bracts of the last capitula), with three plants for each genotype or by line.

All analytical determinations were performed at least in triplicate. Values were expressed as the mean \pm standard deviation.

2.3 Chemical analysis

Fat content: The seeds were ground by using a coffee grinder, Soxhlet system according to the AOCS method (AOCS Ag 1-65; AOCS, 1993). This grinder was used to extract 30g of each of the ground seed flours with 100% n-hexane for 5 hours, the final volume of each extract was made up to 250 ml. Using rotary evaporator, oils and meals were recovered and stored in the dark at 6 °C. The result was expressed as the percentage of lipids in the dry matter of seed powder.

Fatty acid composition analysis: Fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 10 mg oil on 0.2 ml of hexane with 0.5 ml of solution A (solution A : 55 ml of methanol sec + 20 ml of pentane + 25 ml of BF₃ at 14% weight in methanol), placed in a water bath at 75 °C for 90 min, then 0.6 ml of saturated NaCl and 0.2 ml of 10% H₂SO₄ (V:V) were added.

Fatty acids were analyzed by a HP 6890 series GC System chromatograph, equipped with a capillary column (Supelcowax: 30.0 m * 250 μ m * 0.25 μ m) and an FID detector. The carrier gas was nitrogen, at a flow of 1.7 ml/min. The temperatures of the injector and detector were set at 150 and 250 °C, respectively and the oven temperature was set at 210 °C. The injection volume was 1 μ l.

HPLC analysis of triacylglycerols: The chromatographic system consisted of Shimadzu model LC-6AD, CBM 20A controller and refractive index detector RID 10A. HPLC analyses were conducted using C18 reversed- phase column (ODS C18: 250x 5mm, 5 μ m). The mobile phase consisted of acetone/acetonitile (60/40; V/V). Prior to use the solutions were degassed in ultrasonic bath and filtered through 0.45 μ m membranes. Elution was carried out at 1 ml/min in isocratic conditions. Seed oils were dissolved in acetone (9%) and filtered through 0.45 μ m membranes. The injection volume is 20 μ l. All separations are performed at ambient temperature.

Titration acidity: is the free fatty acid content of safflower oil, expressed as % of linoleic acid. Titration acidity was determined by titration of a solution of oil dissolved in ethanol with 0.1 M potassium hydroxide ethanolic solution, using phenolphthalein as indicator. (ISO 660:2009)

Peroxide value: expressed in milliequivalents of active oxygen per kilogram of oil (meq O₂ kg⁻¹ oil), and determined as follows: safflower oil dissolved in acetic acid/chloroform mixture (3:2 V/V) was left to react with a saturated potassium iodide for 5 minutes in darkness. Then 75 ml of deionized water was added, followed by two drops of starch solution; the free iodine was titrated with 0.01N sodium thiosulfate solution to complete bleaching. (ISO 3960:2007)

Colorimetric determination of total phenols: Safflower oil phenols content was determined following the method described by Ollivier and al., (2004), according to the Folin–Ciocalteu colorimetric method using

caffeic acid as a standard. A mixture of safflower oil (10 g) and 10 ml methanol solution (methanol/water 80/20, V/V) placed in a centrifuge tube, after a vigorous mixing for 10 min, was centrifuged (3800 g/15 min) and the collected methanol phase was transferred to a 50 ml flask. Total phenols compounds were isolated by double extraction. To 2 ml extracted solution, 5 ml distilled water, 1 ml Folin-Ciocalteu reagent and 5 ml Na₂CO₃ (10%) were added. After agitation, the solution was placed in darkness before measuring the absorbance at 750 nm.

3 Results and discussion

Safflower seeds oil content of the four varieties ranged from 28,84 g/100g (*Cartamar*) to 35,38 g/100g (*Rancho*). The observed values for oil contents were close to those reported by (Smith 1996, Gecgel and al, 2007; Ashrafi and Razmjoo 2010)

Free fatty acid (FFA) content or acidic index is one of the most important parameters featuring the quality of oil and it is often determined to classify and/or evaluate oil (Mariotti and Mascini, 2001). It is a measurement of hydrolytic breakdown of the fatty acid chains from triglycerides into diglycerides and monoglycerides, liberating free fatty acids (Vossen, 2007). All the analyzed oils showed very low values of free fatty acid (Table 2), *Rancho*, *Cratafri* and *Sharda* with 0.7% and *Cartamar* with 0.63%.

The factors that contribute to an increase in FFA are those that bring the triacylglycerols into contact with endogenous lipase enzymes which can break the molecules down. Other contributions may be due to a reduction in moisture content to a level where desiccation caused cell breakdown and an increase in free fatty acids (Boskow, 1996). The high acidic index which is the basis of olive oil classification also indicates bad seed conservation. Compared to this classification, oils extracted from safflower seeds grown in climatic conditions of eastern Morocco can be considered as a very good quality.

Peroxide value (PV) is a crude indicator of the amount of primary oxidation of lipids (Vossen, 2007). The PV is in fact a measure of the amount of the hydroperoxide formed through oxidation during storage (Cosio and al., 2007).

The peroxide value recorded for the oil samples ranged between 4.58 and 31.21 meq O₂ per Kg (Table 2). Three of the studied oil samples were noted to exhibit a peroxide value that was lower than the maximum limit of 20 established for extra virgin olive oils. Higher peroxide value (31.21 meq O₂/Kg) was however, recorded for the Spanish variety *Rancho*

The PV results were found to be relatively high as compared to those already reported in literature by (Rafiqzaman and al., 2006). These results do not necessarily indicate a deterioration of oils in seeds but can be explained by the method of oil extraction. Indeed, Soxhlet method used in this work presents the drawback of a hot extraction that promotes lipid peroxidation. A cold extraction (Bligh and Dyer 1958) gives a value of peroxide to *Cartafri* 6.6 meq O₂/Kg (data not shown) instead of 15.2 meq O₂/Kg

According to varieties, significant differences in peroxide and free fatty acid values were found ($p < 0.05$). The comparison of the free acidity and peroxide values of the four oil varieties shows that *Rancho* oil presents the higher free acidity and peroxide values while *Cartamar* presents the most lower values.

The hydroperoxides accumulate in safflower oil at the beginning of the reaction of oxidation. They are relatively stable, but in the presence of metallic ion or high temperatures and other factors present at the same time like chlorophyll and pheophytin, they decompose fast into radical alkoxy and hydroxy. Sensory perception is not affected by the accumulation of hydroperoxide. The presence of substances like phenols, tocopherols, β -carotene etc. opposes the spread of oxidation (Amelio, 2003).

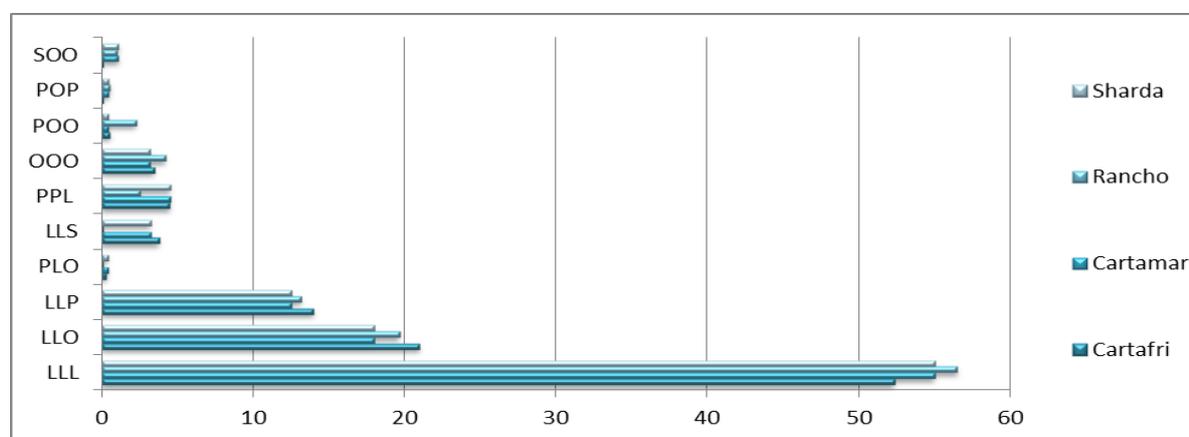
Stability parameters such as total phenols are shown in table 2. Phenols are natural antioxidants that can be found in the natural oils and correlate with stability and flavor. These compounds showed changes in the studied oils according to the cultivar. The level of total phenolic compounds in the four varieties studied were showed to be highly in *Rancho* with 143.65 ppm, *Cartamar* with 125.78 ppm followed by *Cartafri* 118.15 ppm and *Sharda* with 97.46 ppm, the observed value of total phenolic compound were close to those reported by Mailer (2008). Significant difference in the values of total phenols according to varieties were found ($p < 0.05$).

Table 2 Physicochemical quality of safflower oils samples.

Physicochemical parameters	Varieties			
	<i>Rancho</i>	<i>Cartamar</i>	<i>Cartafri</i>	<i>Sharda</i>
Free Acidity (% of Linoleic acid)	0,70±0,03 ^b	0,63±0,05 ^a	0,67±0,04 ^{ab}	0,70±0,02 ^b
Peroxide value (meq O ₂ /Kg)	31,21±1,41 ^c	4,58±1,04 ^a	15,64±1,59 ^{ab}	15,81±3,73 ^{ab}
Total phenolic compounds (mg/Kg)	143,64±27,92 ^b	125,78±5,28 ^{ab}	118,14±15,76 ^{ab}	97,47±15,87 ^a

Values are the means of the four different safflower oils samples (n=3) ± standard deviations. Significant differences in the same row are shown by different letters (a–c) varieties (P<0.05).

Composition of safflower seed oil corresponds approximately to the TAG compounds. Analysis of molecular species of triacylglycerols by HPLC/IR (Fig.1) allow to distinguish essentially ten molecular species TAG (LLL, LPL, LLO, PLO, LLS, POP, OOO, POO, SOO, PPL). For the four varieties studied, there are three major species which represent more than 80% of total TAG of all varieties. The major peak occurred at ECN (equivalent carbon number) 42 corresponding to trilinolein (LLL). Indeed the amount of this molecular species is ranged from 52.36% (*Cartafri*) to 56.5% (*Rancho*).

**Fig.1** Relative percentages of the main molecular species of triacylglycerol of different samples

Fatty acid compositions of safflower oil are presented in Table 3. We note a clear predominance of unsaturated fatty acids compared to saturated fatty acids. For safflower oils analyzed total based on fatty acids, the proportion of saturated fatty acids (SFA) slightly varies between 10.76% and 9.67%, the unsaturated fatty acids (UFA) varies between 90.2% and 89.23%.

Safflower seed oils analyzed show that palmitic, oleic, stearic and linoleic esters are the main FAME identified. Palmitic acid (C16:0) was the major saturated fatty acid (7.2-8.6%) followed by stearic acid (2-2.39%). Linoleic acid (C18:2 ω-6) is the principal fatty acid (77.94-79.49%) followed by oleic acid (C18:1 ω-9) as the second main fatty acid (9.5-11.29%). The relative proportions of these two major fatty acids determine relevant technological and nutritional properties of edible oils. In the past six decades, breeders have exploited safflower's natural genetic diversity to modify the oleate/linoleate ratio for particular end use purposes. Numerous breeding lines with high levels of either oleic acid (75-84%) or linoleic acid (71-89%) have been selected (Cao and al 2013), but it is known that environmental temperature also modulates oleic and linoleic acid in developing seed through regulated FAD2-1 gene expression (Byfield and Upchurch, 2007). The high linoleic acid content could be explained by the high omega-6 fatty acid desaturase (FAD2-1) activity during pod fill. Study of the acidic composition of various samples of safflower oils analyzed showed no difference according to particular varieties.

The fatty acid composition of safflower oils analyzed is similar to those observed by other authors (Smith 1996; Rafiqzaman, and al 2006; Bozan and al., 2008, Yeilaghi and al., 2012)

Table 3. Fatty acid composition (% GC area) of safflower oils

Fatty acid	<i>Rancho</i>	<i>Cartamar</i>	<i>Cartafri</i>	<i>Sharda</i>
C14 :0	0,12 ± 0,01	0,13 ± 0,02	0,16 ± 0,02	0,15 ± 0,04
C15 :0	ND	0,07 ± 0,03	ND	ND
C15 :1	ND	0,44 ± 0,07	ND	ND
C16 :0	7,36 ± 0,54	7,20 ± 0,73	8,60 ± 0,64	8,12 ± 1,26
C16 :1	0,08 ± 0,01	0,09 ± 0,01	ND	0,10 ± 0,02
C17 :1	ND	ND	ND	ND
C18 :0	2,19 ± 0,01	2,39 ± 0,13	2,00 ± 0,20	2,15 ± 0,08
C18 :1	10,63 ± 0,13	11,29 ± 0,19	10,61 ± 0,43	9,50 ± 0,53
C18 :2	79,49 ± 0,67	77,94 ± 0,47	78,62 ± 0,03	79,98 ± 1,77
C18 :3	ND	0,09 ± 0,00	ND	ND
C20 :0	ND	0,19 ± 0,06	ND	ND
C20 :1	ND	ND	ND	ND
C22 :0	ND	0,16 ± 0,06	ND	ND
ΣSFAs	9,67	10,14	10,76	10,42
ΣMUFAs	10,71	11,82	10,61	9,60
ΣPUFAs	79,49	77,94	78,62	79,98

Values are the means of the four different safflower oils samples (n=2) ± standard deviations. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0, margaric acid; C17:1, margaroleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid and C20:1, gadoleic acid. C22:0, behenic acid.

4 Conclusions

The present study shows that the four safflower varieties cultivated in eastern of morocco could be a good source of oil rich in linoleic and phenolic acid. The varietal differences are not significant regard to the fatty acid and triglycerides molecular species. But we can note that *Rancho* cultivar presents the most amount of LLL, high amount of insaturated fatty acids, free fatty acids (Acid value), peroxide value (PI), and phenolic content. The high value of IP could be result of degradation of insaturated fatty acids which is promoted by high phenolic contents. Phenols could act as prooxydants (Aruoma and al, 1993; Kobayashi and al., 2004.) if some conditions are combined, in this case high temperature during oil extraction (soxhlet) and high phenolic and unsaturated fatty acids content.

The high linoleic acid and phenolic acid contents make safflower oil nutritionally valuable and usable as cooking oil in eastern Morocco where only source of oil is olive which the price is high and can not satisfy the needs in oils area.

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