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Physical and Chemical Evaluation of Oils of Two Varieties of *Carthamus tinctorius* Grown in Pakistan

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Abstract. On evaluation of oils of two spineless varieties of *Carthamus tinctorius*, Thori-78 and Pawari-95 growing in Sindh, Pakistan, the quality of the oil was found to be similar, only the oil content differed. The hexane-extracted oil content of Thori-78 and Pawari-95 was 28.33±1.15 and 33.07±1.12, respectively. The oils contained 90.97% and 89.55% unsaturated fatty acids and 8.44% and 9.69%, saturated fatty acids, respectively. Linoleic acid was 75.42±0.59% and 76.40±1.0% and oleic acid was 15.55±0.30% and 13.15±0.49% by weight, respectively, and were the predominant fatty acids present in the oil.

Keywords: safflower oil, Thori-78, Pawari-95, linoleic acid, oleic acid

Introduction

Safflower (*Carthamus tinctorius*) is an annual herb belonging to the family Compositae. It is widely distributed throughout the world such as in Pakistan, India, Bangladesh, Afghanistan, Middle East, Thailand, China, Japan, Ethiopia, Sudan, Tanzania, Keneya, Tunisia, Europe, Argintina, USA, Canada and Australia (Knights *et al.,* 2001). *C. tinctorius* flowers, seeds and oil have wide range of medicinal uses in different countries. Flowers are used for the preparation of dyes and drugs which are used for treating a number of disorders such as for dilation of arteries, reduction of hypertension, increasing blood flow, decreasing blood cholesterol, in treatment of rheumotoid arthritis, menstrual problems, skin diseases, urinary problems and jaundice etc. (Kaffka *et al*., 2001; Sastri, 1950). Seed decoction is used as laxative in Pakistan. The oil is used in Iran to treat liver and heart ailments and in charred state, used in India in treatment of sores and rheumatism. In Northern America, it is cultivated for using as bird feed, animal meal and for industrial applications (Oyen *et al*., 2007; Mündel *et al.,* 2004; Oplinger *et al.,* 1992).

Safflower is used as a substitute for saffron; its flowers are commonly mixed with rice, pickles and other foods to give an attractive colour (Sastri, 1950). America, India and Africa are the main producers of safflower oil. Its seeds are edible and are eaten after roasting. The seed oil content varies from 24 to 36%, depending on the variety of safflower, soil texture, climate and other conditions (Pritchard, 1991; Swern, 1964a). There are two types of safflower oil: high oleic (high in mono-unsaturated fatty acids) and high linoleic (high in polyunsaturated fatty acids). Gas chromatography has been an indispensable analytical technique ever since its first use in the fatty acid determination of plant seed oil (Echard *et al.,* 2007; Peris-Vicente *et al.,* 2006; Seppänen-Laakso *et al.,* 2002). High performance liquid chromatography (HPLC) with ultraviolet and fluorescence detectors are the alternative methods for separation of volatile short chain and long chain fatty acids (Peris-Vicente *et al.,* 2005; 2004; Chen and Chuang, 2002).

Safflower oil can be used in cosmetics, foods, nutritional supplements, personal care products, soaps and shampoos. Cold press oil is golden yellow and is used for culinary purposes. The oil obtained by dry hot distillation is dark and sticky and is used only for greasing ropes and leather goods which are exposed to water. Developed countries have created the most significant market for safflower oil for use as salad oil and cooking oil and in making margarine; being non-allergenic, it is considered to be one of the healthiest oils for human consumption because it has a high ratio of polyunsaturated/saturated fatty acids.

Safflower was introduced as oilseed crop in Pakistan in 1960. It is mainly cultivated in Sindh and Baluchistan provinces. Being a drought-tolerant crop, it is recommended for planting in rainfed areas. In Sindh it is cultivated after the rice crop on residual moisture. Due to the increasing interest in the safflower oil for edible purposes based on its high content of linoleic acid, our studies are mainly focused on the content and physical and chemical evaluation of the oils of two spineless varieties of safflower, Thori-78 and Pawari-95, grown in

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Sindh, Pakistan. Proximate analysis of oils were carried out for the content, glycerides composition and physical and chemical parameters such as free fatty acid, acid value, peroxide value, iodine value, refractive index, saponification value, unsaponifiable matter, specific gravity and colour; the fatty acid composition of both the varieties of oils were investigated as methyl esters by gas chromatography.

Materials and Methods

Plant material. Two varieties of *Carthamus tictorius* seeds, Thori-78 and Pawari-95, were collected from Tandojam, Sindh, Pakistan. The fruit is an achene (dry, one seeded with a thin hull) and resembles sunflower seed but is smaller in size and creamish in colour. It is irregularly pear-shaped, smooth and shiny up to 10 mm long.

Reagents. Solvents and chemicals such as *n*-hexane (95%), *n*-heptane (99%), ethanol (95%), carbon tetrachloride (95.5%), chloroform (99.5%), methanol (98.8%), sulphuric acid (95.98%), hydrochloric acid (37%), acetic acid (100%), glacial acetic acid (99.5%); sodium hydroxide (98%), potassium hydroxide (98%), sodium thiosulphate pentahydrate (R.G), oxalic acid (extra pure), potassium dichromate (extra pure), potassium iodide (extra pure), iodine monochloride (R.G) and anhydrous sodium sulphate were purchased from E. Merck (Damstadt, Germany) and Labscan (Bankok, Thialand). Standards of fatty acid methyl esters were purchased from Supelco (Bellefonte, PA, USA) and Sigma Aldrich Co. (St. Louis, MO, USA).

Apparatus. The apparatus used included gas chromatograph with flame ionization detector, model Clarus 500, from Perkin Elmer Instruments LLC, (Shelton, CT, USA), capillary column Rtx-2330 (60 × 0.25 mm × 0.20 ìm, film thickness) from Supelco (Bellefonte, PA, USA), Lovibond model E tintometer (Salisbury, UK), Abbé refractometer model 2W (Shijiazhuang, China), Gallen Kamp air oven (West Mildlands, UK) and vacuum oven (Melrose Park, IL, USA).

Oil extraction. Safflower, Thori-78 and Pawari-95, seeds (500 g each) were crushed and finely ground to flour and then subjected to extraction with *n*-hexane (0.5 litre) in a one litre Soxhlet extractor for 8 h (AOCS, 2004). The fat was recovered using a rotary evaporator. The extracted fat was placed in an oven at 60 °C for 1 h, transferred to a capped reagent bottle and stored at 4 °C until required.

Quantitative separation of tri-, di- and mono-acylglycerols of oil. The lipid class composition, comprising of TAGs, DAGs, and MAGs mixture in *C. tinctorius* seed oil was determined by solid-liquid adsorption chromatography (SLAC), using silica gel as the adsorbent and eluted with different solvent systems by following the AOCS method (AOCS, 2004) with little modification. The effectiveness of separation was verified by thin layer chromatography, using solvent system (petroleum-ether and acetone: 9:1).

Fatty acid composition. Methyl esters of fatty acids were prepared according to standard IUPAC method 2.301 (IUPAC, 1987). The chemical composition of fatty acid methyl esters was accomplished with a Perkin Elmer gas chromatograph model Clarus 500 fitted with a polar capillary column Rtx-2330 (60×0.25 mm×0.20 µm, film thickness) and a flame ionization detector. Nitrogen was used as carrier gas at a flow rate of 3 mL/min. Other conditions were as follows: initial oven temperature, 70 °C was maintained for 5 min then ramped at 10 °C/min to 180 °C, followed by 3 °C/min to final temperature of 220 °C, where it was held for 15 mins; injector temperature and detector temperature was 270 °C. A sample volume of 0.3 µL was injected (splitless). Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of authentic standards of fatty acid methyl esters purchased from Supelco Sigma-Aldrich Co. Quantification was done by a built-in data-handling programme, provided by the manufacturer of the gas chromatograph. Analyses were performed in triplicate.

Physical and chemical analysis of the extracted oils. The following tests for refractive index, specific gravity, colour, free fatty acid, acid value, peroxide value, iodine value, saponification value and unsaponifiable matter of the extracted oils were performed by the standard methods of AOCS (2004). Colour of the oils was determined by a Lovi bond tintometer (Tintometer Ltd., Salisbury, UK) using a one inch cell.

Results and Discussion

Hexane extracted oil content of the two varieties of *C. tinctorius*, Thori-78 and Pawari-95, seeds was found to be 28.33±1.15 and 33.07±1.12%, respectively; the high percentage of oil gives these varieties distinct potential for the oil industry, because the average oil content of the seeds exceeds those of conventional oil seeds i.e., cotton (15.0-24.0%), canola (17-21%), soyabean (17-21%), olive (20-25%) which are grown in the USA, Brazil and Asia (Pritchard, 1991) but oil content is slightly lower than that of sunflower (25-35%).

Physical and chemical parameters of the oils are depicted in Table 1. At room temperature, both varieties of seed oil were present in a liquid state. The refractive index and specific gravity of Thori-78 and Pawari-95 oils were determined at 40 °C, which were concordant with the reported value and comparable with other vegetable oils (Rossell, 1991a; Swern, 1964a; 1964b). The values determined for free fatty acids as

OA and acid values are comparable with the reported values of the crude oil (Dhellot *et al.*, 2006; El-Adawy and Taha, 2001). Very low value of free fatty acid and acid value in the present analysis is an indication of the good quality of crude oil. Peroxide values (Table 1), indicating the presence of hydroperoxides in oils, were high, thus showing low resistance to oxidation (Onyeike and Acheru *et al.*, 2002); thus oils could be used after slight refining. The analyzed crude oils were high in colour index 2.13R + 45.4Y + 0.71N (Thori-78) and 2.48R + 46.16Y + 0.81N (Pawari-95). Intense colour of vegetable oils depend mainly on the presence of various colouring pigments of plants such as carotenoids, chlorophyll etc., which are effectively removed during refining and bleaching steps of oil processing. Vegetable oils with minimum values of colour index are good for edible purpose.

Table 1. Proximate and physicochemical characteristics of *C. tinctorius* oils

Parameters	Thori-78	Pawari-95
Oil content	28.33±1.15 (27.11-29.88)	33.07±1.12 (31.5-34.0)
Free fatty acid (% as OA)	0.52±0.012 (0.51-0.54)	0.53±0.004 (0.53-0.54)
Acid value (mg/kg)	1.12±0.15 (0.99-1.34)	1.06±0.02 (1.04-1.09)
Peroxide value (Meq/kg)	17.2±0.25 (16.94-17.54)	20.75±0.38 (20.2-21.1)
Iodine value (g of I/100 g of oil)	134.82±0.68 (133.99-135.67)	136.16±0.96 (134.89-137.21)
Saponification value (mg of KOH/ g of oil)	187.56±2.34 (184.89-190.60)	188.96±2.18 (186.5-191.8)
Unsaponifiable matter (%)	0.41±0.06 (0.32-0.46)	0.57±0.05 (0.50-0.62)
Refractive index at 40 °C	1.4734±0.0005 (1.4730-1.4742)	1.4679±0.0007 (1.4672-1.4689)
Specific gravity at 40 °C	0.9064±0.002 (0.9031-0.9085)	0.9240±0.0004 (0.9234-0.9245)
Colour (Red unit)	2.13±0.04 (2.1-2.2)	2.48±0.08 (2.4-2.6)
(Yellow unit)	45.4±0.29 (45.0-45.7)	46.16±0.62 (45.5-47.0)
(Blue unit)	0	0
(Neutral unit)	0.71±0.06 (0.65-0.80)	0.81±0.01 (0.79-0.83)

Iodine values were comparatively high due to the presence of high content of unsaturated fatty acids and are comparable with the values of poppy, soybean and sunflower oils (Rossell, 1991a). High iodine value shows that both the varieties of seed oils have good qualities of oils, required for edible and drying purposes (Eromosele *et al.*, 1994). Values of saponification and unsaponifiable matter of Thori-78 and Pawari-95 oils are concordant with the values of sunflower, poppy seed and soybean oils (Rossell, 1991a; Swern, 1964a; 1964b), indicating them to be good source of industrial oil which can be used in the manufacture of soap and liquid soap.

Table 2 shows glyceride composition of oils of both the varieties of *C. tinctorius*. The content of triacylglycerides is over 83%. Comparison of free fatty acid value, acid value, saponification value, unsaponifiable matter, iodine value, refractive index, specific gravity and colour of the studied oils with those of the known edible oils reveal that the quality of both the varieties of oil have great potential for edible usage.

Table 2: Glyceride composition of *C. tinctorius* seed oils (wt. %)

C. tinctorius variety	Monoglyceride	Diglyceride	Triglyceride
Thori-78	5.70±0.55	7.62±0.87	85.61±0.61
Pawari-95	5.77±0.50	8.72±0.53	83.93±0.88

Values are means ± SD, analyzed in triplicate.

Fatty acid composition of the oils of the two varieties was determined using gas chromatography (Fig. 1 and 2 and Table 3). The principal fatty acid components in Thori-78 and Pawari-95 were palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids. Linoleic acid is predominantly present in both the varieties as compared to other varieties, U.S.-10, S.-208 and V. F.-stp (53-1) grown in Pakistan (Table 3) (Raie, 2008). Fatty acid composition was more or less similar to that of sunflower, soybean, corn, and cotton seed oils and safflower oil originating from different geographic regions (Cosge *et al.*, 2007; Rossell, 1991b). These results suggest that these varieties of *C. tinctorius* can serve as potential dietary sources of mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA).

Present studies revealed that seed oils of *C. tinctorius* varieties, Thori-78 and Pawari-95, indigenous to Pakistan have very good potential for edible and industrial usage and also for use in developing nutritionally balanced formulations blended with other high stearic or high oleic oils. These Oils are used in the food and pharmaceutical industries to produce cooking oils,

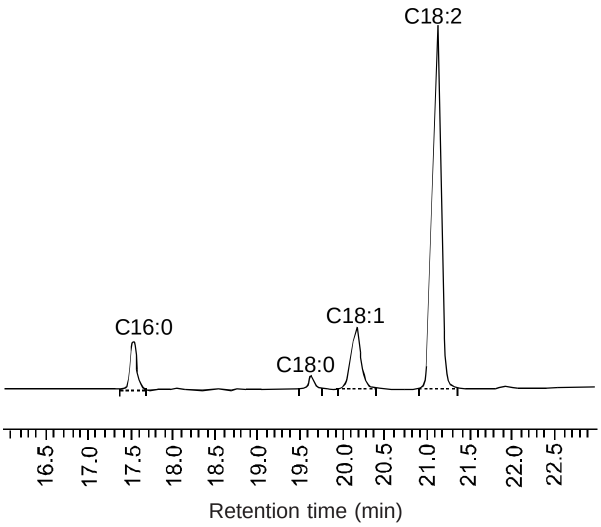

Fig. 1: Gas chromatogram of fatty acids of safflower (Thori-78) seed oil; major components are labelled.

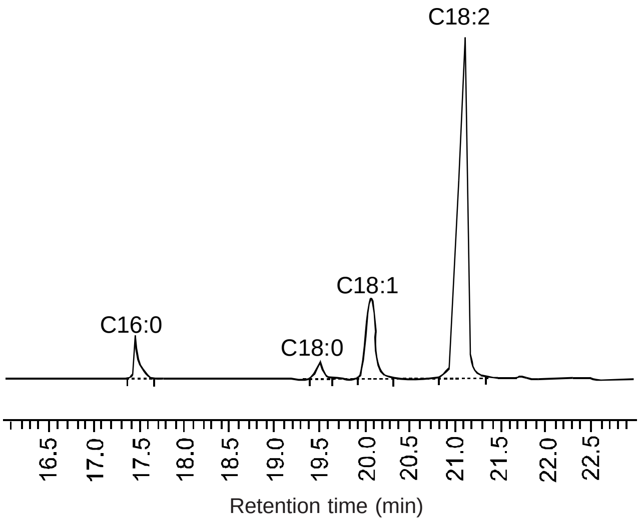

Fig. 2: Gas chromatogram of fatty acids of safflower (Pawari-95) seed oil; major components are labelled.

food supplements and skin care products. The good drying property and high content of linoleic acid and absence of linolenic acid and wax and low content of free fatty acid, colour and unsaponifiable compounds make them suitable for use in the production of high quality paints, alkyd resins, coatings, varnishes and linoleum. They can also be used in the production of biodiesel.

Pakistan imports huge amount of palm oil and soybean from foreign countries to fulfil the increasing demand of oil in the country. Moreover, Pakistan has suitable atmosphere for cultivating all the conventional and non-conventional oilseed crops. Cultivation of safflower varieties at larger scale could fulfil the requirements of the country and save enormous amount of foreign exchange spent otherwise.

Table 3. Fatty acid composition of high linoleic *C. tinctorius* varieties grown in Pakistan (wt. %)

Fatty acids	Thori-78	Pawari-95	US-10	S.-208	V.F.-stp (53-1)
Myristic Acid ($C_{14:0}$)	-	-	3.1	0.9	2.8
Palmitic Acid ($C_{16:0}$)	6.45±0.57 (5.66-7.02)	6.92±0.37 (6.41-7.28)	10.2	9.4	12.0
Stearic Acid ($C_{18:0}$)	1.99±0.09 (1.89-2.12)	2.77±0.49 (2.24-3.42)	5.5	2.3	3.6
Oleic Acid ($C_{18:1}$)	15.55±0.30 (15.31-15.98)	13.15±0.49 (12.67-13.81)	14.4	14.0	15.7
Linoleic Acid ($C_{18:2}$)	75.42±0.59 (74.65-76.11)	76.40±1.0 (75.01-77.32)	66.8	73.4	65.9
Others	0.59±0.04 (0.54-0.64)	0.76±0.06 (0.68-0.83)	-	-	-
Total saturated fatty acids	8.44	9.69	18.8	12.6	18.4
Total unsaturated fatty acids	90.97	89.55	81.2	87.4	81.6

Values are mean ± SD, analyzed in triplicate.

Conclusion

These studies were focussed on the yield and physical and chemical evaluation of seed oils of *C. tinctorius* varieties, Thori-78 and Pawari-95, cultivated in the region of Sindh, Pakistan. It was revealed that oils of both the varieties have very good potential for developing nutritionally blended formulations balanced with other high saturated fatty oils, as well as for different industrial usage due to the presence of high percentage of polyunsaturated fatty acids. These can also be used in the production of biodiesel and thus in different industries in Pakistan.

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