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Quantitation of Fatty Acids by GLC and Separation of Omega-6 Nutraceutical Fatty Acid From *Carthamus tinctorius* L. Seed Oil Cultivated in Pakistan

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Abstract. The GLC analysis of *Carthamus tinctorius* (safflower) yielded average hexane extracted oil content of 28% (25-30%); the oil contained high level of linoleic acid (74%). Monounsaturated fatty acid, oleic acid amounted 12.94%, while the saturated fatty acids like palmitic acid and stearic acid were 9.43 and 1.81%, respectively. Iodine value of linoleic acid was found to be 160.1 while its purity was 93.1%.

Keywords: *Carthamus tinctorius*, linoleic acid, omega-6 fatty acids

Introduction

Carthamus tinctorius (safflower) is an annual species of the family compositae. This crop is adapted to dry land or irrigated cropping system. It is also known as false saffron, thistle saffron, cartame, saffron baturd in English, while Arab calls it Kazhirah (Eckey and Miller, 1954; George, 1892) and in Pakistan (Sindh) it is commonly known as Kushumba, Khusakdana or Powariji bij (Jafri, 1966). Seeds usually mature in September, about four weeks after the end of flowering (Oelke *et al*., 1989) with a seed oil content between 30-45%.

The plant is cultivated in California, Middle East, Africa and India (Eckey and Miller, 1954). It is an indigenous crop in Pakistan and is cultivated in Gilgit, Hunza, Kotri and Mirpur (Jafri, 1966). Normally two varieties of *C. tinctorius*, are found, one that produces oil with high amount of monounsaturated fatty acid (oleic acid) and the other with high concentration of polyunsaturated fatty acid (linoleic acid). *C. tinctorius* is a valuable source of one of the most important nutraceutical fatty acid, omega-6 (linoleic acid), an essential fatty acid which cannot be synthesized by the body but plays an important role in the control of joint ailments, skin, hair, nail and scalp disorders like eczema, acne, psoriasis etc.

The present study was made on the seeds of *C. tinctorius* variety producing linoleic acid and growing in Pakistan. The aim of the study was to assess the fatty acid composition of *C. tinctorius* in order to separate the linoleic acid quantitatively using urea adduct separation technique. This procedure is frequently applied to obtaina polyunsaturated or branched chain fatty acids in concentrated form. Toxicity studies were also carried out to check the feasibility of using the oil as a source of essential fatty acid. Such studies based on the application of urea complexation technique have not yet been reported by any author in Pakistan.

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Materials and Methods

All the reagents (analytical and GC) used, were purchased from E-Merck/Sigma/Aldrich. Pure standards of fatty acid methyl esters were obtained from Supelco Chemicals Co.

Extraction of oil. Seeds (one kg) were taken from the seed cultivars, cleaned to remove admixtures then grinded in an electrical grinder and fed to a soxhlet extractor fitted with a 2 litre round bottom flask and a condenser. The extraction was done on a water bath for 4-6 h with 1.5 litre *n*-hexane. Then the solvent was distilled off under vacuum in rotary evaporator. The oil was dried over anhydrous sodium sulphate, filtered and weighed. The procedure was performed in triplicate.

Fatty acid composition. Fatty acid methyl esters were prepared according to the standard IUPAC method (IUPAC, 1987) and analyzed on a Perkin-Elmer gas chromatograph model Clarus 500, fitted with a polar capillary column SP 2340 (60 m x 0.25 mm) and a flame ionization detector. Oxygen free nitrogen was used as a carrier gas at a flow rate of 3.5 ml/min.

Other operational conditions were as follows: initial oven temperature 70 °C for 5 min, increase in temp @ 10 °C/min to 180 °C and then @ 3 °C/min to 220 °C, held for 8 min; FID temperature: 275 °C, injector: 250 °C.

A sample volume of 1.0 µl was injected and the total analysis time was 37 min. Fatty acid methyl esters were identified by

comparing their relative retention time to those of authentic standard of fatty acid methyl esters obtained from Supelco Co. All of the quantification was done by a built-in data handling programme provided by the manufacturer of gas chromatograph. The data was transferred to HP Laser Jet-1300 printer attached to the instrument. The fatty acid composition was reported as a relative percentage of the total peak area.

Toxicity studies of *C. tinctorius* oil. The prescribed daily dose of the test material was 1-2 table spoon (5-10 ml) twice a day for an adult person. To ensure safe evaluation, the test group animals (albino rats) were administered 5 ml/kg body weight of the test drug. Olive oil was purchased from the local market in a sealed tin pack and used as standard reference oil for comparative study; it was also given in a dose of 5 ml/kg of body weight.

Before proceeding to toxicity studies, animals (males and females) were housed separately and kept under keen observation for a period of 20 days with free access to food and water. Any animal showing sluggish movement or any sign of illness was not included in the test.

Acute oral toxicity studies. Acute oral toxicity study was conducted in albino rats. The rats were divided into three groups, each group comprising of 10 animals (5 male and 5 female). Test material was administered in a single dose by means of a gavage to group A i.e., test group, according to the standard method of acute oral toxicity test (Loomis, 1978). Group B was given standard branded olive oil and group C was the control. Observations with reference to physicobehavioural changes and mortality within 24 h were made. Animals were further observed for a period of 72 h for changes in behavioural pattern and delayed mortalities.

Separation of omega-6 (linoleic) fatty acid from the oil. *C. tinctorius* oil was converted to its methyl ester by its methanolysis in presence of sodium as catalyst. The mixed fatty acid methyl esters were fractionated by urea adduct formation. The procedure was adopted as follows:

C. tinctorius oil (100 gm) was dried on steam bath, at controlled temperature of 80 °C. The mixed fatty acid methyl esters were prepared by transesterifiction process (IUPAC, 1987).

35 g Urea was added to a solution of 50 g of mixed ester in 300 ml methanol and dissolved while heating. The solution was kept overnight at -5 °C then filtered off. The precipitate was again washed with methanol, cooled to -5 °C and combined with the washed liquid and filtrate. Again 30 g urea was added to the filtrate and the mass was warmed until the urea was completely dissolved. The solvent was cooled and kept at 5 °C overnight; the crystallizate was filtered off and washed with 200 ml cold methanol. The washing and filtrate were combined and finally 20 g urea was added and the above mentioned procedure was again repeated. The last filtrate was concentrated to obtain linoleate. Pure linoleate was weighed to calculate the percentage recovery. The experiment was performed in triplicate.

Purity of the product was determined by iodine value and compared with the theoretical values, mentioned in the literature.

Results and Discussion

Oil content. The triplicate analysis of *C. tinctorius* seeds yielded oil content in the range of 25-30% with an average yield of 28%. This value complies with the values earlier reported by others (Hamrouni *et al*., 2004; Eckey and Miller, 1954). The oil content of *C. tinctorius* is comparatively higher than that of the other most commonly used oil seeds such as soybean seeds containing 20% oil (Eckey and Miller, 1954). Hence, in this consideration, *C. tinctorius* seeds may be used for commercial purposes.

Fatty acid composition. The fatty acid composition of oil from *C. tinctorius* seeds is summarized in Table 1; chromatogram is shown in Fig. 1. The oil contained fatty acids, generally present in seed oils such as saturated fatty acids including palmitic and stearic acids and unsaturated fatty acids including oleic, linoleic and linolenic acids. Average values are resultant of three analysis on GC. *C. tinctorius* was found to be an excellent source of pure linoleic acid (Parker *et al*., 1955) which is normally considered as one of the most important essential nutraceutical fatty acids of biomedical importance.

Oleic acid , 12.41%, found in the oil, is in close agreement with the earlier reported values (Swern, 1964) while the present value of omega-6 (linoleic acid) fatty acid of 74%, is also in good conformance with Gecgel *et al*. (2007) and Swern (1964). It means that the dependence of the fatty acid composition of *C. tinctorius* on the location of the plant is comparatively little. Total saturated acids were found to be 11.24%. Fatty acid composition determined in the present work is also in good agreement with the conventional *C.tinctorius* fatty acid profile reported by Knowles (1989).

Table 1. Fatty acid composition of *C. tinctorius* oil (av. wt %)

Palmitic acid C_{16}	Stearic acid $C_{18.0}$	Oleic acid $C_{18.1}$	Linoleic acid $C_{18.2}$	Linolenic acid $C_{18.3}$
9.43	1.81	12.94	73.98	0.93

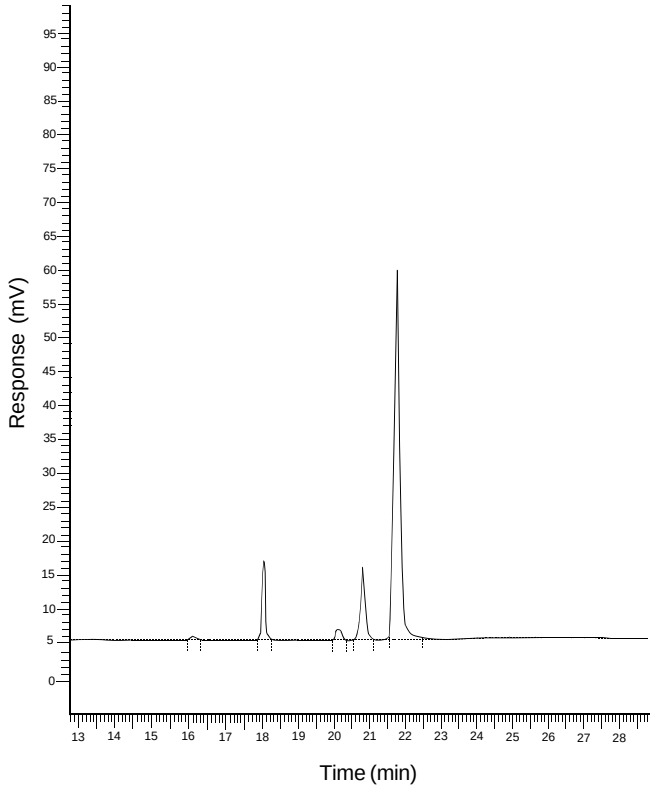

Fig. 1. Chromatogramme of *C. tinctorius* seed oil.

Toxicity studies. Oral administration of safflower oil for 42 days in a dose of 5.0 ml/kg body weight was not found to produce any toxic effect in both male and female animals in comparison with the standard and the control group (Table 2).

Table 2. Acute oral toxicity studies

Group	Av. wt. of animals (kg)	Dosage	Results
A	150-200	5 ml / kg body wt. (safflower oil)	- no mortality - all animals found normal in their activities - there was no sign of any untoward effects during said period of observation.
B	150-200	5 ml / kg body wt. (olive oil)	same as above
C	150-200	control (placebo)	same as above

Preparation of linoleic acid. The technique of separation of polyunsaturated fatty acid by urea adduct complexation was first described by Bengen (1940). The main findings of Bengen was that urea can be used to separate straight chain compounds from branched or cyclic compounds; later, numerous investigators confirmed this method. The new technique was used for the preparation of methyl oleate (Swern and Parker, 1952; Schlenk and Holman, 1950), for the preparation of concentrates of linoleic acid from safflower oil (Kim *et al*., 2003; Swern and Parker, 1953), for separation of docosahexaenoic acid from algal oil via urea complexation (Senanayak and Shahidi, 2000), gamma linolenic acid concentrate from borage oil (Spurvey and Shahidi, 2000) and preparation of omega-3 PUFA concentrate from fish oil via urea complexation (Ratnayake *et al*., 1988).

The reviews of Schlenk (1953) gives detailed explanation of the application of this technique. In the present findings about 21.7% linoleic acid (omega-6) was separated at the end of experiments performed in triplicate. Low yield of the inclusion compound was also observed earlier by Schlenk and Holman (1950), suggesting that the more highly unsaturated fatty acids may be even more difficult to bind as inclusion compounds.

The purity of linoleate in the present study was found to be 93.1%. The percentage yield, 21.76%, of the product is in good agreement with the findings of Keppler *et al*. (1959) while the purity shows some variation with the results reported by him. The urea complex of linoleic acid was analyzed by determination of iodine value of the complex (Knight *et al*., 1952). The results are reported in Table 3. The average iodine value of 160.1 shows a small variation from that reported by Keppler *et al*. (1959). It was been observed that the tendency of fatty acids and esters to combine with urea decreases with increasing unstauration.

Table 3. Percentage yield of linoleate (quantity and purity)

Iodine value	Purity of (%)	Yield of linoleate (%)
160.8	93.5	22.8
159.9	93.0	21.5
159.6	92.8	21.0
160.1*	93.1*	21.76*

* = average of triplicate analysis

It is thus concluded that omega-6 fatty acids, can well be extracted from the seeds of *C. tinctorius* and are suitable for use in nutritional products after purification.

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