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Studies on the Physico-Chemical Properties of Siyal Kanta (*Argemone mexicana* linn) Seed Oil

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Abstract

The physico-chemical properties of the extracted oil were studied by the conventional methods. It was observed that Siyal Kanta grown under the soil and climatic condition of Bangladesh contains about 35% of pale yellow coloured oil. The total lipids were fractionated into three major lipid groups, neutral lipids, glycolipid and phospholipids by silicic acid column chromatography. Among the lipids, the neutral lipids were varied from 92.1-92.3%, glycolipid 5.5-5.8% and phospholipid 1.5-1.7% of the total oil of the lipid applied. The oil was also fractionated into mono-, di- and triglyceride by silicic acid column chromatography. The triglycerides were varied from 90.1-90.3%, diglycerides from 2.3-2.8% and monoglycerides from 1.5-1.8%. The saturated and unsaturated fatty acids present in the oil were separated and found to be 14.2-14.5% and 84.2-84.8% respectively depending on the areas in which the plant grows. The fatty acid compositions of the oil were analyzed by Gas Liquid Chromatography (GLC). The major fatty acids found in the oil were oleic acid (23%), linoleic acid (58%), palmetic acid (7%) and ricinoleic acid (10%).

Key words: Siyal kanta seed oil, Glyceride, Lipid and Fatty acid.

Introduction

Siyal kanta (*Argemone mexicana* linn.) is an annual herb plant with prickly stem which belongs to the family papaveraceae. The plant was originated in tropical America. Now it thrives well in India, Pakistan and Bangladesh. The plant reaches a height of 2-3 ft and generally it is grown mixed with other crops in sandy loom soil. The plant flowers during the month of March-April and sets fruits in the month of May-June. The juice of the plant is used as a remedy for dropsy, jaundice and skin diseases (Mukherjyee *et al* 1950). The plants bear a good number of bristly capsules containing a lot of seeds resembling black mustard seed which are demulcent, laxative, nauseat and expectorant (Goni 1998). Its seed contains about 34-38% non edible oil and possesses a semi drying property (Thorpe *et. al*.1949, Hill 1951). The compositions of oil vary with the source and depend on some factors such as climatic conditions, soil type and the maturity of the plant (Sallans 1964). The oil is used as an illuminate, lubricant and as fuel in lamps, medicines, ulcers and eruption (Anon 1948, Watt 1972).

The physico-chemical properties of the oil are directly related to their lipids and glyceride compositions (Rahman *et. al.*2002). So, knowledge on the compositional factors is very essential in connection with the properties. The present work had been undertaken to carry on the physico-chemical properties, lipids and glyceride composition of Siyal Kanta seed oil.

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Materials and Methods

Ripe and matured Siyal kanta seeds were collected from the district of Rajshahi, Pabna and Dinajpur. The seeds were then dishelled manually and the kernels thus obtained were crushed into smaller particles in a glass mortar and dried in the oven at a temperature of 105 ^{o}C for about ½ an hour to about 4-6% moisture. The moisture content in the fresh kernels was determined by IUPAC method (Anon 1979). The oil was then extracted with n-hexane in a Soxhlet apparatus for 8 hours. n-hexane as extracted solvent has been selected because the solvent has better effect over other polar solvents like alcohol, ketone, aldehyde, ether and ester etc (Ali *et. al.* 1996) . The oil was recovered by the evaporation of the solvent under reduced pressure using a rotary vacuum evaporator and the percentage of oil content was calculated. The percentage of free fatty acid (FFA), saponification value, peroxide value and unsaponifiable matters in the oils were determined by the standard AOCS method (Anon 1955). Hanus method was followed to determine the iodine value of the oils (Anon 1980).

Separation of Lipid Classes by Column Chromatography

The major lipids classes of the oil were fractionated by silicic acid (E. Merck, Darmastad, Germany, 70-230 mesh) column chromatography (Rouser *et al* 1966). The silicic acid

was washed with 5% (w/w) water and methanol to remove fines and impurities. It was activated at 120°C overnight and again for 1 hour immediately before the column was prepared. For each column 25 g. silicic acid was washed with 250 ml of chloroform/ methanol (7:1 v/v), 120 ml chloroform/methanol (15:1 v/v) and 160 ml chloroform. A slurry of 25 g. of silicic acid in chloroform was poured into the column (2.2 cm i. d). 150 mg total lipids were dissolved in 5ml eluting solvent and quantitatively transferred to the column.

The neutral lipid was eluted with chloroform, glycolipids with acetone and phospholipids with methanol (Ali *el. al.*1997). The elution was controlled with a flow rate of 1.5-2.0 ml/min. The elution of each fraction was monitored by micro-slide Thin Layer Chromatography (TLC) to ensure uniformity of separation of each lipid class during silicic acid chromatography and the eluted solvents were collected in a weight flask. The fractions thus obtained were evaporated in a rotary vacuum evaporator and were dried under reduced pressure before being weighed. The lipid classes were identified by R_f comparison with standard references. The percentages of these fractions were determined by gravimetric method.

Separation of glycerides

The whole oil was separated into mono-, di and triglycerides on silicic acid column (E.Merck, Darmstad, Germany, 70-230 mesh) column. The silicic acid was activated at 120°C over night and again for 1hr. immediately before the column was prepared. Then the silicic acid was hydrated with 5% water. A slurry of 25g. of silicic acid in chloroform was poured into the column (2.2cm. i.d). 1g. of oil was dissolved in 15ml. of chloroform and quantitatively transferred to the column. The triglyceride was eluted with 200ml. of benzene, diglyceride with 200ml. of a 1:9 v/v mixture of diethyl ether and benzene and monoglyceride with 200ml. of diethyl ether (Gafur *et. al.*1993). The elution was controlled at a flow rate of 1.5-2ml./min.

The elution of each fraction was monitored by micro-slide thin layer chromatography (TLC) to ensure uniformity of separation of each class of glyceride during silicic acid chromatography and the eluted solvents were collected in a weighed flask. The fractions thus obtained were evaporated in a rotary evaporator and were dried under reduced pressure before being weighted. The glyceride classes were identified by comparison of R_f values with standard references. The percentage of each glyceride class was calculated by gravimetric method.

Analysis of fatty acid composition

To know the nature of the fatty acid composition present in the siyal kanta seed oil of the sample of Rajshahi district were analyzed as their methyl esters, which were prepared by the Boron trifluride methanol method (Mondol *et.al.* 2006). A GCD Pye Unicam Gas chromatographed equipped with a flame ionization detector was used to determined the fatty acid methyl ester. Nitrogen carrier gas was used at a flow rate 30 ml/mm. Fatty acids were separated on a 1.8 m x 2 mm i. d. glass column packed with 6% BDS (Butanediol Succinate Polyesters) on solid support Anakrom ABS 100/120 mesh. Analysis was carried out at isothermal column temperature of 190 °C, injector and detector temperature for all GLC analysis were 230 °C. The peaks were identified by comparison with standard methyl esters for retention times by plotting the log of retention time against equivalent carbon length (ECL). The peak areas were determined by multiplying peak height by width at half height. The percentage of each peak was calculated as the percentage of the total area of all the peaks.

Separation of saturated and unsaturated fatty acids in Siyal kanta seed oil

Separation of saturated and unsaturated fatty acids was carried out by Lead-salt ether method (Das, 1989) on about 50g. of oil. The oil was saponified with alcoholic caustic soda to obtain soap solution. An excess of lead acetate solution was added to the soap solution to form a mixture of lead salts of fatty acids which were then separated by filtration. Diethyl ether was added to the mixture of lead salt and the whole mixture was boiled for 15 minutes and then cooled at 0°C for 24 hours. The precipitated lead salts of saturated fatty acids so formed were separated from the solution of lead salts of unsaturated fatty acids by filtration. The lead salts of unsaturated fatty acids were obtained by removing the ether from the ethereal solution. Each group of lead salts was suspended in water and treated with sufficient hydrochloric acid (35%) to form fatty acids and lead chlorides. The mixture was then extracted with ether to obtain the ethereal solution of each group. On evaporating the ether the fatty acids were obtained in separated group. Finally masses of saturated and unsaturated fatty acids were obtained by weighing them separately.

Results and Discussion

Siyal kanta seed kernels were collected from three different districts and the oil had been extracted by n-hexane to evaluate the physico-chemical characteristics, lipids and glyceride compositions. The physico-chemical characteristics of the extracted oils were determined by the conventional meth-

ods and the results were shown in Table I. The results (Table I) indicated that no significant difference in physico-chemical characteristics among the three samples collected in three different districts was observed. The saponification values and unsaponifiable matters of the oil more or less agree with the reported results of Tobacco seed oil (Gofur *et. al.* 1993). Specific gravity and refractive index of the oils were comparable with those of important vegetable oils (Hilditch, 1949).

were presented in Table II. From the result it was evident that diglycerides in the oil sample of Dinajpur district were found to be higher in comparison with other two samples. No appreciable changes of triglycerides was observed among the three samples and from the results it was observed that triglycerides in all the three samples irrespective of origin accounted for over 90% of the total weight of the lipid.

Table I: Physico-chemical properties of Siyal kanta seed oil ± SE

	Physical and chemical properties	Origin		
		Rajshahi	Pabna	Dinajpur
1.	Percentage of oil	35±0.0348	34.8±0.0345	34.5±0.0343
2.	Moisture and volatile matter (%)	4.5±0.047	4.2±0.042	4.3±0.045
3.	Specific gravity at 28°C	0.920± 0.001	0.918±0.001	0.921±0.001
4.	Refractive Index at 28°C	1.4580±0.005	1.4579±0.005	1.4581±0.005
5.	Free fatty acid as oleic (%)	5.2±0.0577	5.1±0.0570	5.2±0.0.75
6.	Iodine value	118±1.527	118±1.527	119±1.528
7.	Saponification value	188±0.5575	187.5±0.5573	187.8±0.5575
8.	Unsaponifiable matter (%)	1.5±0.115	1.2±0.112	1.3±1.11
9.	Peroxide value meqv./kg	1.20±0.112	1.18±0.110	1.21±0.112

Mean value of three experimental results

Total extracted Siyal kanta seed lipids were separated into neutral lipids, glycolipids and phospholipids by silicic acid column and results were depicted in Table II. The results (Table II) indicated that no significant change in the lipid composition among the three samples was noticed. But it is remarkable to note that the percentages of phospholipids (Table II) were found to be very higher (1.5-1.71) in comparison with other vegetable oils (Ali *et. al.* 1985, Uddin *et. al.* 2007, Rahman *et. al.* 2004, 2007). From results shown in Table II, it was observed that the neutral lipids were averaged to over 92% of the total weight of the lipid applied.

The whole oil was fractionated into mono-, di- and triglycerides by means of column chromatography and the results

Table II: Glycerides, lipids and fatty acids of Siyal Kanta seed oil (%) ±SE

Compositions	Rajshahi	Pabna	Dinajpur
Mono-glyceride	1.8±0.115	1.5±0.112	1.7±0.114
Di-glyceride	2.5±0.172	2.3±0.170	2.8±0.175
Tri-glyceride	90.3±0.057	90.1±0.054	90.2±0.055
FFA	5.2±0057	5.1±0.055	5.0±0.053
Neutral lipid	92.3±0.252	92.1±0.250	92.2±0.251
Glycolipid	5.8±0.117	5.5±0.113	5.7±0.115
Phospholipid	1.5±0.100	1.7±0.115	1.7±0.115
Saturated fatty acid	14.2±0.055	14.5±0.057	14.4±0.055
Unsaturated fatty acid	84.8±0.5775	84.2±0.5772	84.3±0.5773

The saturated and unsaturated fatty acids present in the oils were separated by Lead-Salt ether method and the results were shown in Table II. From the results, it was observed that the percentages of saturated and unsaturated fatty acids present in the oil samples of Pabna and Dinajpur districts were almost similar, but a slight variation was noticed in the sample of Rajshahi district which proves it to be slightly better than those of other two samples.

To know the nature of the fatty acid composition present in the Siyal kanta seed oil, one of the samples (sample of Rajshahi district) was analyzed by GLC and the results were shown in Table III. Gas chromatographic analysis showed that unsaturated fatty acids present in the siyal kanta seed oil were mainly linoleic (58%) and oleic (23%) acids, which altogether accounted for 81% of the total fatty acids.

Table III: Fatty acids compositions of Siyal Kanta seed oil ±SE

Fatty acids	Weight percent
Oleic acid	23.00±0.05877
Linoleic acid	58.00±0.05773
Palmetic acd	7.00±0.1877
Ricinoleic acid	10.00±0.02023

Conclusion

As Siyal kanta seed contains about 35% non-conventional oil, so it may be considered as one of the important sources of non-edible oil. At present, Bangladesh is facing acute shortage of edible and non-edible industrial oils. So, it is compelled to import edible and non-edible oils from abroad. Under these circumstances, Siyal kanta seed oil can play a vital role in bridging the vegetable oil gap in the country. Moreover the oil is suitable for the manufacture of paint, varnishes and soap as the oil rich in linoleic acid and higher saponification value.

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