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Studies on the physico-chemical properties, lipids, glycerides and fatty acid composition of mesta *(Hibiscus cannabinus* Linn*)* seed oil

A. Akhter[1], M. M. Rahman[1], M. M. M. Morshed[2], M. Ismail[2] and M. Saiful Islam[3]

[1]*BCSIR Laboratories, Binodpur Bazar, Rajshahi-6206, Bangladesh.*

[2]*Dept of Applied Chemistry and Chemical Engineering, University of Dhaka, Dhaka-1000, Bangladesh.*

[3]*DRICM, BCSIR Laboratories, Dhaka-1205, Bangladesh.*

E-mail: saifulacctdu@yahoo.com

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Abstract

The Physico-chemical properties, lipids, glycerides and fatty acid composition of mesta seed oil have been studied by the conventional methods. It was observed that the seed contains about 21% light yellow coloured oil. The total lipids were fractionated into three major lipid groups, neutral lipids, glycolipids and phospholipids by silicic acid column chromatography. The neutral lipid varied from 91.5-92.1%, glycolipid from 5.3-5.7 and phospholipid from 0.081-0.087% of the total oil of the lipid applied. The oil was also fractionated into mono-, di- and tri-glyceride by silicic acid column chromatography. The triglyceride varied from 91.5-92.8%, diglyceride from 2.0-2.8% and monoglyceride from 2.1-2.9%. The fatty acid composition of the oil was analysed by GLC. The percentage compositions of fatty acid were found to be oleic acid (44.9), linoleic acid (25.3), palmitic acid (14.7) and stearic acid (6.2).

Keywords: Mesta Seed oil, lipid and glyceride compositions, column chromatography, fatty acid, GLC.

1. Introduction

Mesta (*Hibiscus cannabinus* Linn) of family Malvaceae is an erect annual plant with prickly stem and reaches a height of 8-12 ft. It is generally grown mixed with other crops. The plant is cultivated from May to July and harvested in October-November. Its leaves are considered as purgative and aperients. The juice of the flowers mixed with sugar and black pepper is a remedy for biliousness. The seeds are stomachic, appetizing, aphrodisiac and fattening; they are also employed externally as poultice for pains and bruises [1]. It is extensively cultivated for its fibre [2] and is used as a substitute of jute in the manufacture of coarse canvas, gunny cloth, cordage, matting, fishing nets etc. [3]. The seed contains about 22% golden yellow coloured oil possesses semi-drying properties [4]. The oil can be used for cooking purposes after being refined [5]. The oil is also used as a lubricant and for lightening [6] and is suitable for manufacture of paints, varnishes, soaps, linoleum and other industrial products [1]. The physical and chemical properties of the oil are related to their lipid and glyceride compositions. So attempt has been taken to evaluate the physico-chemical properties, lipids and glyceride compositions of mesta seed oil.

2. Materials and Methods

Ripe and mature mesta seeds were collected from the district of Rajshahi, Natore and Nawabgang. The seeds were crushed into smaller particles in an iron mortar and dried in the oven at a temperature of 105°C to about 4-5% moisture. The oil was then extracted with n-hexane in a Soxhlet apparatus for about 8 hours. The extracting solvent was then removed by using rotary vacuum evaporator and the percentage of the oil content was calculated. The specific gravity of the oil was determined with the help of a pycnometer. The refractive index, moisture and volatile matters of the oil were determined by IUPAC method [7]. The free fattyacid (FFA), saponification value, peroxide value and unsaponifiable matters in the oil were determined by the standard AOCS method [8]. Hanus method [9] was followed to measure the iodine value of the oil.

2.1 Separation of lipids

The major lipids of the oil were separated by silicic acid (E.Merck. Darmstad, Germany, 70-230 mesh) column chromatography [10]. The silicic acid was washed with water and methanol to remove fines and impurities. It was then activated at 120°C overnight and again for 1 hours before the column was prepared. Slurry of 25g silicic acid in chloroform was poured into the column (2.2 cm dia.). 150 mg of the oil lipid was dissolved in a 5 ml eluting solvent and transferred to the column. Neutral lipid was eluted with chloroform, glycolipid with acetone and phospholipid with methanol [11]. The elution was controlled at a flow rate of 0.50 ml-1.0 ml/min. The elution of each fraction was monitored by micro-slide thin layer chromategraphy (TLC) to ensure uniformity and separation of each lipid class. The eluted solvents were collected in a weighed flask. The fraction thus obtained were evaporated in a rotary vacuum evaporator and dried under reduced pressure before being weighed. The percentage of these fractions were estimated by gravimetric method.

2.2 Separation of glycerides

The oil was separated into mono-, di- and triglycerides on silicic acid (E. Merck, Dermstad, Germany, 70-230 mesh) column chromatography. The silicic acid was activated at 120°C overnight and again for 1 hours before the column was prepared. Then the silicic acid was hydrated with 5% water. Slurry of 25 g of silicic acid in chloroform was

poured into the column (2.2 cm dia.). 1 g oil was dissolved in 15 ml of chloroform and quantitatively transferred to the column. The triglyceride was eluted with 200 ml. of benzene, diglyceride with 200 ml of a 1:9 v/v mixture of di-ethyl ether and benzene and monoglyceride with 200 ml of di-ethyl ether [12]. The elution was controlled at a flow rate of 1.5-2.0 ml/min. The elution of each fraction was monitored by micro-slide TLC to ensure uniformity of separation of each class of glyceride and the eluted solvents were collected in weighed flask. The percentages of these fractions were determined by gravimetric method.

2.3 Analysis of fatty acid compositions

Fatty acid compositions present in the oil of Rajshahi district sample was analysed as its methyl ester which was prepared by the Boron trifloride methanol method [13]. A GCD pye unicam gas chromatograph equipped with a flame ionization detector was used to determine the fatty acid methyl ester. Nitrogen carrier gas was used at a flow-rate of 30 ml/min. Fatty acids were separated on 1.8×2 mm dia. 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.

3. Results and Discussion

The physico-chemical properties of the extracted oil are given in Table-1. The data of three samples collected from three districts are almost similar. It is observed that the specific gravity, refractive index of the oil were comparable with other important vegetable oils [14]. The saponification and iodine values were almost similar with cotton seed oil [15] and other values were within the normal limits.

Table 1: Physico-chemical properties of mesta seed oil

Physical and chemical properties	Name of the districts from where seeds were collected		
	Rajshahi	Natore	Nawabgang
1. Percentage of oil	20.3	20.1	20.5
2. Moisture and volatile matters	5.2	5.1	5.0
3. Specific gravity at 28°C	0.915	0.917	0.914
4. Refractive index	1.465	1.462	1.467
5. Free fatty acid as oleic (%)	2.1	2.3	2.0
6. Acid value	4.3	4.5	4.1
7. Saponification value	193	191	190
8. Iodine value	99.3	98.7	101
9. Unsaponifiable matters (%)	1.5	1.7	1.8
10. Peroxide value (m.eq.kg)	1.25	1.23	1.22

The total lipids were separated into neutral lipid, glycolipid and phospholipid (Table 2). The whole oil was fractionated into mono-,di- and triglycerides and the results are shown in Table-2. The lipid & glyceride composition of mesta seed oil are almost similar to other vegetable oil like seasame seed oil [13].

Table 2: Lipid and glyceride composition of the oil (wt%)

Compositions	Rajshahi	Natore	Nawabgong
Neutral lipid	91.5	91.6	92.1
Glycolipid	5.7	5.6	5.3
Phospholipid	0.083	0.087	0.081
Monoglyceride	2.9	2.3	2.1
Diglyceride	2.8	2.5	2.0
Triglyceride	91.5	92.1	92.8

Mean value of three experimental results.

The fatty acid analysis of the oil of Rajshahi district (Table-3) showed that the unsaturated fatty acids present in the oil is mainly oleic acid (44.9%) and linoleic acid (25.3%) which more or less agrees with the reported result [16].

Table 3: Fatty acid compositions of the oil of Rajshahi district (wt%)

Fatty acids	Weight Percent
$C_{18:1}$ (oleic acid)	44.9
$C_{18:2}$ (linoleic acid)	25.3
$C_{16:0}$ (palmitic acid)	14.7
$C_{18:0}$ (stearic acid)	6.2

4. Conclusion

Mesta seed contains about 21% fatty oil which is non-conventional and unused. There is shortage of edible and non-edible industrial oils in our country which is met up by importing these oils from abroad. Mesta seed oil is suitable for the manufacture of paints, varnishes and soaps as it is rich in oleic and linoleic acid and has higher saponification value. Refined oil is edible and may be used for cooking purpose. So the oil can play a vital role in bridging the vegetable oil-gap in the country.

5. References

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