

NOTE**Determination of Fatty Acids in Plant Seeds of Leguminosae Family from Arid Zone of Rajasthan**

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Some fatty acids viz., palmitic acid, oleic acid, linoleic acid and linolenic acid in three plant seeds were determined. The plant seeds samples analyzed were *Acacia nilotica*, *Cassia fistula*, *Tamarindus indica*. All samples were collected from arid zone of Rajasthan and have been identified and studied for their component fatty acids using TLC and HPLC techniques. Physico-chemical characteristics were determined by standard AOCS methods. The fatty acids were analyzed by high performance liquid chromatography with μ Bondapak C-18 column and UV/VIS. detector. The omega-6 or linoleic acid was found the highest value in *Tamarindus indica* and *Cassia fistula*. The palmitic acid, oleic acid and linolenic acids were also found in all samples.

Key Words: Chemical analysis of seed oils, Phenacyl ester, HPLC.

Fatty acid is a carboxylic acid with long aliphatic tail. Fatty acid is divided into saturated and unsaturated acid, depending on the presence of unsaturated double bond in the fatty acid chain. Essential fatty acids are polyunsaturated fatty acid. Linoleic acid (C18:2) is the parent compounds of the omega-6 (ω -6) fatty acid series¹. They are essential in the human diet since they cannot be synthesized by the body. The essential fatty acids are very important to human immune system, to help regulate blood pressure. The ω -6 fatty acid are found in some food such as fish, shellfish flaxseed (linseed), soya oil, canola (rapeseed) oil, hemp oil, chia seed, pumpkin seed, sunflower seed, cotton seed oil, leafy vegetables and walnut.

The significance of fatty acid analysis has gained much attention because of the nutritional and health implications. The most common procedure for the analysis is the conversion of fatty acid components to phenacyl ester in order to improve their volatility. The conversion of component fatty acid to phenacyl ester, which is separated and analyzed by high performance liquid chromatography. There are many papers focusing the analysis of fatty acid in plant seeds such as *Phaseolus vulgaris* seed, grape seed oil², Soybean seed oil³, *Zinnia elegans* seed (pink zinnia), *Melia azodirecta* (neem), *Jatropha curcas* (ratanjoat), *Tamaridus indica* L⁴. Therefore, the objective of this research is determination of palmitic acid, stearic acid, linoleic acid, linolenic acid and oleic acid in some plant seeds. The prime target of the present study is to perform chemical screening of the seed oil from lesser known family from the arid zone of Rajasthan.

Acacia nilotica, *Cassia fistula* and *Tamarindus indica* were brought from local place. The impurities were removed from the seeds and the cleaned seeds were ground using a ball mill into powder.

Oil extraction by different solvents: The seed oils are generally extracted with solvent like *n*-hexane and light petrol or petroleum ether (40-60 °C). About 15 g powder was used for extraction in Soxhlet. The solvent is removed under reduced pressure and the oils are stored under nitrogen atmosphere in a refrigerator until further use.

Fatty acid extraction and sample preparation: Seed oils were refluxed with ethanolic potassium hydroxide. The unsaponifiable matter was removed and free fatty acids were obtained in usual manner. Wherever required, the saponification was carried out under nitrogen and samples were stored at low temperature in a nitrogen atmosphere.

Esterification was carried out by usual procedure, except where specified. Phenacyl esters derivatives, which absorb strongly in the UV region of the spectrum, are of particular value for analysis by means of HPLC. Phenacyl esters were prepared by reaction of phenacyl bromide and a fatty acid in the presence of potassium carbonate and crown ether in acetonitrile solution.

For the HPLC analysis, a modified HPLC method (with gradient elution) was used. The equipment included Gilson HPLC with a degasser, a binary pump and a column (900 mm × 6.4 mm) was packed with μ Bondapack C-18 and was eluted with acetonitrile-water in the proportions 67:33 (by volume) initially and is gradually increased to 74:26 in 0.5 h then gradually increased in another 30 min which is also changed to 97:3 in another 0.5 h at flow rate of 2 mL/min and detection was completed with UV/VIS detector.

The analytical values of the oils and seeds and the physico-chemical characteristics like acid, saponification, iodine values of the oils were determined using standard AOCS methods are given in Table-1.

TABLE-1
ANALYTICAL AND PHYSICO-CHEMICAL
CHARACTERISTICS OF THE SEEDS AND OILS

Species	Oil (%)	Moisture (%)	Protein (%)	Acid value	Iodine value	Saponification value	Unsap. matter
<i>Acacia nilotica</i>	5.1	4.87	18.5	7.43	138	188	7.7
<i>Cassia fistula</i>	2.5	3.10	12.2	2.87	136	174	9.3
<i>Tamarindus indica</i>	2.4	4.50	24.3	16.3	149	114	7.4

HPLC analysis and oils characterization: The saturated fatty acids were identified as palmitic acid, stearic acid and arachidic acid. Palmitic acid was reported as major saturated acid (Table-2). It ranges 34.3, 15.8 and 18.3 % in *Acacia nilotica*, *Cassia fistula* and *Tamarindus indica*, respectively. Myristic acid was absent in all while stearic acid and arachidic acid were also absent in *Cassia fistula*.

TABLE-2
FATTY ACIDS CONTENTS IN PLANT SEEDS

Plant seed	Fatty acid composition (%) of oils					
	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid (ω -6)	Linolenic acid	Arachidic acid
<i>Acacia nilotica</i>	34.3	6.0	19.2	41.4	4.4	1.1
<i>Cassia fistula</i>	15.8	nil	31.1	48.1	5.3	nil
<i>Tamarindus indica</i>	18.3	4.9	10.5	55.5	12.2	nil

nil = not found

Oleic acid, linoleic acid (ω -6) and linolenic acid were present as unsaturated fatty acids. *Tamarindus indica*, *Acacia nilotica*, *Cassia fistula* were found in oleic acid in the range of 10.5-31.1 %. Linoleic acid (ω -6) concentration of the both *Acacia nilotica* and *Cassia fistula* were found similar as 41.4-48.1 %, respectively. High concentration *i.e.*, 55.5 % of the linoleic acid (ω -6) was found in *Tamarindus indica*. Linolenic acid was found in all the species but dominant amount was noticed also in *Tamarindus indica* as 12.2 %, respectively (Table-2).

Conclusion

Linoleic acid (ω -6) is an essential fatty acid for human. Fatty acids contents and their composition depended on the kinds of the plant seeds *Acacia nilotica*, *Cassia fistula*, *Tamarindus indica* seeds are found as the potential source of ω -6.

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