THE FATTY ACID COMPOSITION OF LEUCAENA LEUCOCEPHALA OIL

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Abstract. *The fatty acid composition of* Leucaena leucocephala *as determined by GLC, containing 9.0% oil, is* $C_{16:0}$ *(18.3%),* $C_{18:0}$ *(1.5%),* $C_{18:1}$ *(15.3%),* $C_{18:2}$ *(61.0%),* $C_{18:3}$ *(2.1%) and* $C_{22:0}$ *(1.8%).*

Introduction. The major natural resources for the production of oils and fats among the vegetable kingdom are crops (1) (sunflower, safflower, soybean, mustardseed, rapeseed, groundnut etc.), fruit trees (2) (Olive, coconut etc.). agricultural and forest waste/by product materials (3-7) (rice bran, mango stone kernel, tobacco seeds, *Sapium sebiferum*, *Coriandrum sativum*, cotton seed, *Leucaena leucocephala* etc.). The present study, carried out first time in Pakistan, on *Leucaena leucocephala* (ipil ipil) is under the continuous programme of the utilization of agricultural and forest waste materials for the procurement of oils and fats to meet the requirements of the country.

The plantation of *Leucaena leucocephala* even in deserts is possible by pitcher technique. Its pods, are approximately 17.5 Cm long and 2.0 Cm wide, usually contain over 23 dark brown coloured hard seeds. The seeds of *Leucaena leucocephala* are about 7 mm long and 4 mm abroad. They were processed after grinding for the extraction of oil by solvent. The oil consists of mainly triglycerides, low percentage of colouring material and free fatty acids. The fatty acid composition of the oil was determined by the application of chromatography in addition to the physico-chemical characteristics of the oil. The fatty acid composition of the oil under study has been compared with those containing higher percentage of linoleic acid to realize the importance of this oil.

Materials and Methods. 1. *Extraction of the oil.* The dark brown coloured seeds (100 g) were extracted, after grinding and drying, with distilled hexane in an soxhlet apparatus. The solvent with oil was dried over anhydrous sodium sulphate, filtered and the dark yellow coloured oil (9.0 g) after distillation of hexane was obtained.

2. *Saponification of the oil, Liberation and Methylation of the Fatty acids.* The oil (6 g) was refluxed with 0.5 N ethanolic potassium hydroxide solution (90 ml) for 3 hrs. The soap solution was reacted with 2N sulphuric acid to liberate fatty acids after the separation of unsaponifiable matter by diethyl ether. The fatty acids (800 mg) were converted into methyl esters by refluxing with dry methanol (12 ml) and 1% w/w sulphuric acid for 2 hrs. (8-9).

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3. *Thin Layer Chromatography*. The slurry of silicagel (180 g) was prepared with distilled water (150 ml) to prepare fifteen thin layer chromatograms (20 cm x 20 cm) of thickness (0.5 mm) for the purification of methylated acids (600 mg) after the activation of chromatograms by heating at 105° for 60 min. The developing solvent used was ether-hexane mixture (1:9 v/v). The non-destructive locating reagent 2, 7 dichlorofluorescein was used to have purple yellow coloured bands under an ultraviolet light at 254 mu. The bands of purified methyl esters were scratched out and the material from silicagel was eluted by ether which was distilled to get methyl esters of the fatty acids. The silver nitrate (16.7%) impregnated thin layer chromatography, applied by using hexane: ether (9: v/v) for the separation of purified methyl esters into saturates, monene and diene shows pink colouration with the same locating reagent.

4. *Gas Liquid Chromatography*. The instrument (Pye Unicam 204 Series) was used for the identification of methyle esters by the comparison of their retension times with the known methyl esters by using a column (152.4 cm x 0.95 cm) prepared by coating polydiethylene glycol succinate (10%) on diatomite 'C' (80-100 mesh) at 200° with the flow rate of 40 ml per min. of nitrogen as a carrier gas. The percentage of each component fatty acid was determined by triangulation.

Results and Discussion. The dark brown coloured seeds of *Leucaena leucocephala* being very hard were not decorticated. The seeds after grinding were extracted with hexane to yield (9.0%) brownish yellow coloured oil. The procurement of oil is done either by a mechanical (Expeller) or a chemical (Hexane extraction) method. In the present study of *Leucaena leucocephala* due to the low yield of oil in the kernel and hard shell of seeds, the process of solvent extraction is preferable to the mechanical one. The yield of oil (9.0%) of *Leucaena leucocephala* in comparison with other agricultural and forest waste/ by-product materials, for example, rice bran oil (18.7%), mango stone kernel fat (10.8%), *Nicotiana tabacum* oil (18.0%), *Sapium sebiferum* oil (69.5%), *Coriandrum sativum* oil (11.0%) and Cottonseed oil (17.5%) shows lowest yield of oil among these materials studied so far. However, the yield of *Leucaena leucocephala* oil is significant to exploit it on an industrial scale.

After the extraction of the oil it was checked up qualitatively to determine the major class of lipid compounds of the oil under study. It was observed that the oil mainly consists of triglycerides, low quantity of free fatty acids and colouring material. The physico-chemical characteristics of the plant and its seed oil such as refractive index by Abbe's Refractometer at 40°, unsaponifiable matter, iodine value etc., are given in Table-I)

Table I

Physico-chemical Characteristics of Leucaena leucocephala and its Seeds Oil.

A.	PLANT CHARACTERISTICS		
	1.	Height	25-60 Ft.
	2.	Flowers	Sep.-Nov.
	3.	Seeds	Apr.-Jun.
	4.	Moisture content	21%
	5.	Density	564 Kg/m^3
	6.	Fibre length	0.57-1.24 mm
	7.	Bark thickness	0.62 mm
B.	OIL CHARACTERISTICS		
	1.	Oil yield (% age)	9.0
	2.	Seeds moisture (% age)	7.9
	3.	Non-saponifiable (% age)	2.8
	4.	Free fatty acids (% age)	1.1
	5.	Saponification number	196
	6.	Iodine number	132.8
	7.	Refractive Index at 40°	1.4782

The chromatography has extensively been applied for the purification and identification of fatty acids as methyl esters comprising of ($C_{16:0}$-$C_{22:0}$) (10-11). Among the saturated and unsaturated fatty acids, the highest percentage is of palmitic acid (18.3%) and linoleic acid (61.0%) respectively (Table II). The fatty acid composition of an oil or fat is considered to be the most important and useful provided it contains linoleic acid ($C_{18:2}$) which is a precursor of prostaglandins essential for reproduction, skin permeability and normal growth. It has been studied that seeds of different plants belonging to different families have different percentage of linoleic acid such as sunflower (32.0%), safflower (66.8%) of Compositeae family, tobacco (61.7%) of Solanaceae family and *Leucaena leucocephala* (61.0%) of Leguminosae family, The safflower oil due to the highest percentage of linoleic acid is known as one of the best oil, but there is no remarkable difference in the percentage of linoleic acid of *Leucaena leucocephala* and safflowerseed oil. The quality of an oil is also determined by the absence of linolenic acid ($C_{18:3}$) which, being highly unsaturated, is susceptible to oxidation and degradative products resulting in rancidity (12) and flovour reversion of the oil. The *Leucaena leucocephala* oil contains linolenic acid (2.1%) which is very low as compared to the different varieties of soybean oil containing linolenic acid (5.1-9.2%). So the quality of *Leucaena leucocephala* oil on the basis of low percentage of linolenic acid is better than soybean oil.

Table II

The Fatty Acid Composition Comparison of Leucaena leucocephala, Sunflower, Safflower (U.S-10) and Tobacco (Nicotiana tabacum) Oils

Fatty Acids	Leucaena Leucocephala	Sunflower	Safflower (U.S-10)	Tobacco (Nicotiana Tabacum)
Lauric	—	—	—	3.0
Myristic	—	1.5	3.1	2.1
Palmitic	18.3	11.5	10.2	9.7
Stearic	1.5	2.3	5.5	6.2
Oleic	15.3	14.0	14.4	17.3
Linoleic	61.0	73.4	66.8	61.7
Linolenic	2.1	—	—	—
Behenic	1.8	—	—	—

These studies highlight that the fatty acid composition of *Leucaena leucocephala* oil is one of the best composition and therefore, this oil can be strongly recommended for edible purposes after the usual removal of free fatty acids and colouring material by refining and bleaching respectively.

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