

## THE SANDAL SEED, ITS OIL AND PROTEINS.

*By Motnahalli Sreenivasaya and Nuggchalli Narayana.*

In addition to the valued essential oil from its heartwood, the sandal plant yields an oil-seed containing on an average a fixed oil to the extent of 50 to 55 per cent. Under favourable conditions of growth, the plant commences to bear fruit in its third year and continues to yield two crops a year during the entire period of its life history. June-July and December-January are roughly the two principal seasons during which sandal seeds can be collected from sandal areas in general. It is, however, not uncommon to find sandal plants in all stages of physiological activity, vegetating, flowering and fruiting in almost all parts of the year. The December-January seeds enjoy the reputation of being more fertile and possessing greater vitality and are accordingly used as seed for propagation of sandal. Oil-seeds which mature during the winter usually contain higher percentages of oil and the oil is reputed to possess a higher degree of unsaturation. A comparative study of the yield and "quality" of the oil from the seeds collected during the two seasons of the year, has not been carried out.

The yield of seed by a plant depends upon season, soil conditions, age and associated host plants. Pot culture experiments have shown that sandal plants deprived of their host plants seed only once a year, usually in the months of November-December and yield seeds with practically no germinating power. A study of the yield of seeds in relation to the floristic composition of the host groups nourishing the sandal plants, is highly desirable in view of the possible economic importance of the seed.

The endosperm of the seed is edible and is usually consumed by the cow-boys in the forest. Rodents in general and certain kinds of birds, feed on the seed. Cattle can be made to feed on crushed sandal seeds, if properly prepared. At the moment, the seeds are collected more for purposes of propagation than for commercial exploitation. It is, therefore, difficult to estimate even roughly, the quantity of seeds that could be made available, should any industrial demand for this seed arise.

A preliminary analysis of the seeds showed that they contain a high percentage of a fixed oil which could be estimated by extraction

with any of the organic solvents like ether, petrol-ether, carbon tetrachloride or benzene. The petrol-ether gives the lightest coloured extract and yields on an average about 50 to 55 per cent. of a thick and viscid oil. The oil is associated with a highly unsaturated resin which renders an expression of the oil from the seed extremely difficult. With a view to find out an economical method of expressing the oil from the seeds on a commercial scale, a number of well-known methods were tried. The country "Ghani" did not yield any oil while experiments with the Anderson Oil Expeller, which were conducted through the kind courtesy of Mr. B. K. Garudachar, were not successful in spite of several trials and various pre-treatments given to the seed meal.

The process of "rendering" involving the extraction of the fried and ground seeds with boiling water, yields only about 40 per cent. of the oil present in the seed. The cake cannot be recovered in the usual form and the gruel-like residue left over, is difficult to dispose of. The method, while being thus wasteful and uneconomical, yields an oil whose properties differ in many respects, from the oil obtained by solvent extraction. The oil obtained by "rendering" is thicker, has a darker colour and solidifies to a semi-solid at a temperature at about 20°C., yielding crystalline glycerides.

The next trial at expressing the oil was carried out with a hot "castor oil" press, with more encouraging results. The seeds after frying are ground to a fine meal which is subsequently packed in gunny cloth and hot-pressed, the heat being supplied either by steam or by an open charcoal fire. The oil thus obtained is dark red, thick and highly viscous and when exposed in thin films dries up in a shorter time than the oil obtained by solvent extraction. By this method about 60 per cent. of the oil can be obtained and the cake that is obtained can be used either as manure or as cattle food after suitable treatment. The gunny cloth once used cannot be employed for another batch of expression and the frequent use of the fresh cloth raises the cost of expression. The oil can now be produced at about Rs. 450 to Rs. 500 per ton—a value which is not economical unless a special industrial use is found.

With a view to discover some special uses for this oil, a detailed examination of the oil was carried out. It is thicker than castor oil and further thickens either on long exposure to sunlight, or on continued heating. The higher the temperature, the more rapid the rate of thickening. In presence of catalysts like the lead, cobalt and manganese oxides or "Driers", the process of thickening is greatly accelerated and if the heating and the proportion of the catalyst or the "drier" to the oil, is not carefully controlled, the oil rapidly decomposes yielding a soft, sticky and porous mass.

At about 220°C., the oil reacts violently with flowers of sulphur evolving fumes which smell of burnt rubber and yielding a dark sticky rubber-like product. This property of dissolving sulphur possessed by the oil places it in the class of "vulcanisable" oils.

If the oil is heated up with 1.0% zinc chloride to about 180°C., the oil thickens to a dark plastic solid highly sticky and resinous. This product dissolves in benzene and petroleum, forming an ideal base for the manufacture of insulation tape. The oil probably undergoes some kind of polymerisation under these conditions. A similar change can be brought about at the room temperature by shaking the oil with about 2.0% strong sulphuric acid. On repeatedly washing the reaction mixture with hot water the sticky mass can be freed from all traces of acid. This product also can find employment in the manufacture of sticky tapes.

Resins like colophony, dammar and copal can be made to dissolve in the oil at a temperature between 180° and 200°C., yielding an orange coloured bright varnish which, on drying in thin films, leaves a glossy elastic and hard film. The varnish has been found to possess good insulating properties and can be used for the impregnation of field coils.

The varnish can also be used for the manufacture of pigmented enamels. The preliminary trials with the oil in manufacturing these various products have shown that the oil possesses very valuable properties which can be utilised for the manufacture of varnishes and sticky compositions. Since the oil is vulcanisable, its employment in the rubber industry may be worth serious consideration.

Sandal seed oil is a drying oil and compares favourably with the other well-known drying oils such as linseed and tung oils (Table I).

TABLE I.

	Sandal seed Oil	Linseed Oil	Tung Oil
Percentage of Oil in seed	50—55	37—45	37—44
Specific gravity 25° C.	0.9304	0.932—0.941	0.9488
Refractive index 60° C.	1.4790	1.4805	1.5210
Iodine value (Hams)	145—155	175—196	137—162

TABLE II.

*Sandal seed Oil—*

Specific gravity 25° C.	..	0.925—0.950
Refractive index 60° C.	..	1.476—1.480
Iodine value (Winkler's)	..	170— 175
„ (Hubli's)	..	130— 135
„ (Hanus's)	..	145— 155
Saponification value	..	185— 195
Acid value	..	9— 15
Acetyl value	..	21.3— 23.4
Reichert-Meissel No.	..	2.00— 3.11
Polansky Number	..	0.21— 0.30

*Mixed fatty acids—*

Mean molecular weight	..	287.5—288.5
Iodine value	..	120— 125
Titre	..	34.5°—35.8° C.

Table II incorporates the results of analysis of a number of samples of sandal seed oil prepared by various methods.

The iodine value of the oil varies with the method employed for its determination. During saponification of the oil with alcoholic potash, a small quantity of white resinous solid separates and sticks to the bottom of the flask. On separating and washing with water, a determination of the iodine value of the resin was carried out. The substance was found to possess an iodine value of 290.

The free fatty acids were prepared by saponifying the oil with alcoholic potash. The alcohol was evaporated off and the soap dispersed on filter paper pulp and extracted with petrol-ether to remove the sterols. The filter paper pulp was extracted with hot water and the soap solution thus obtained, was acidified to liberate the fatty acids.

The solid acids were prepared from the mixed fatty acids by the lead-alcohol method. We could not obtain solid acids having no or a low iodine value. Repeated separations still gave a high iodine value suggesting the presence of elaidic and iso-oleic acids. The nature and composition of the solid and liquid fatty acids have not yet been fully investigated.

### PROTEINS OF THE SANDAL SEED.

The whole seed meal has an average nitrogen content of 1.81 per cent. while that of the residue freed from its oil amounts to 3.52 per cent. on the weight of the meal. For the extraction of the proteins a 4 per cent. solution of sodium chloride was found to be the best solvent, nearly 60 per cent. of the total nitrogen of the meal being extracted in about an hour. 500 gms. of the oil-free seed meal was treated with 5 litres of the saline solution and shaken for 4 hours. The mixture on filtration gave an opalescent light brown extract, which was subjected to hydro-dialysis. As in the case of the globulins of horse gram, the proteins did not completely precipitate even after the dialysate gave no test for Cl. But, on addition of a few drops of dilute acetic acid, the proteins were completely precipitated including the albumins. The proteins were recovered by centrifugation, washed several times with water, dehydrated by treatment with graded strengths of alcohol and the preparation finally washed with ether and dried.

The preparation was a light powder, slightly brownish in colour, yielding all the usual colour reactions, characteristic for proteins. It contained sulphur, tyrosine and tryptophane. Phosphorus was, however, absent. On analysis, it gave:—

Moisture	..	5.02 per cent.	
Ash	..	1.11	„
Nitrogen	..	16.03	„
Sulphur	..	0.76	„
			} <sup>a</sup> Ash & Moisture free.

Results of a Van Slyke analysis of two preparations are given in Table III.

TABLE III.

*Analysis of sandal seed globulin.  
Results expressed in per cent. of total Nitrogen.*

		(1) %	(2) %	Average %
Melanin N.	Insoluble ..	1.21	1.10	1.11
	Soluble ..	0.98	1.18	1.08
Amide N. ..	..	8.17	7.99	8.08
Basic N. ..	..	31.12	32.22	31.67
Non-basic N.	Amino ..	56.52	56.90	56.71
	Non-amino ..	2.21	2.49	2.35
	Total ..	100.21	101.88	101.00

*Distribution of the basic nitrogen.*

Arginine N. ..	21.04	21.66	21.35
Histidine N. ..	1.92	1.78	1.85
Cystine N. ..	0.49	0.65	0.57
Lysine N. ..	7.67	8.13	7.90

*Direct estimation of arginine in solutions of hydrolysed protein.*

Arginine N.	25.84
-------------	-------

*Free Amino Nitrogen in native proteins.*

Amino N.	4.22
Half lysine N.	3.95

The above Table shows that arginine as estimated by the direct method is 4.5 per cent. higher than when estimated by the basic fraction. Free amino nitrogen is nearly equal to half the lysine nitrogen.

*Estimation of Tyrosine and Cystine.*

Tyrosine and cystine were estimated by the methods of Zuwerkalow (*Zeits. Physiol. Chem.*, 1926, 163, 185) and Folin and Looney (*J. Biol. Chem.*, 1922, 51, 421) respectively.

TABLE IV.

*Estimations of Tyrosine and Cystine in the proteins of the sandal seed.*

	Tyrosine %	Cystine %
N. of amino acid in per cent. of total nitrogen .. .. .	2.29	1.42
Amino acid in per cent. of protein ..	5.99	2.04

Cystine sulphur in per cent. of total S = 71.3%

Table V gives the content of the several essential amino acids in the sandal seed proteins as percentages of the total protein.

TABLE V.

	N. of amino acid in per cent. of total Nitrogen %	Amino acid in per cent. of protein
Ammonia .. .. .	8.03	1.57
Arginine (Van Slyke Analysis) ..	21.35	10.63
Arginine (direct estimation) ..	25.84	12.86
Histidine .. .. .	1.85	1.09
Cystine .. .. .	1.42	2.04
Lysine .. .. .	7.90	6.60
Tyrosine .. .. .	2.29	5.99
Tryptophane .. .. .	present	present

The composition of the proteins agrees with that of the globulins from other oil-seeds like cocoanut and sesamum. There appears to be nothing abnormal or striking revealed by the present analysis, which might be characteristic of proteins from a seed of parasitic origin.

## SUMMARY AND CONCLUSIONS.

1. Attention is drawn to the economic importance of sandal seeds borne by the sandalwood tree which is at present known for its valuable essential oil.

2. The seeds contain a drying oil to the extent of 50-55 per cent., 60 per cent. of which can be recovered by hot pressing. The oil has some valuable properties—dissolves the varnish "gums", is vulcanisable, and polymerises on heating in presence of zinc chloride, yielding a sticky and plastic mass. Some possible technical applications of the oil are indicated.

3. After extraction of the oil, the cake is valuable as a cattle food or fertiliser.

4. The predominant proteins of the seed have been isolated and their analysis has not revealed anything striking or abnormal, which might be characteristic of proteins from a seed of parasitic origin.

5. Our grateful thanks are due to Professor R. V. Norris for his kind encouragement and interest in this investigation. Our thanks are also due to Mr. B. V. Ramiengar, Chief Conservator of Forests in Mysore, and Mr. G. C. Robinson, Chief Forest Officer, Coorg, both of whom arranged for the collection of large quantities of seeds required for this investigation.

*Department of Biochemistry,  
Indian Institute of Science,  
Bangalore.*

[Received, 14-12-1935.]