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Refining of *Psophocarpus tetragonolobus* (L.DC.) Seed Oil

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Abstract

Degumminig, alkali refining and bleaching (series of refining process) of *Psophocarpus tetragonolobus* (L.DC.) seed oil and coconut fruit oil were done and the oils were then evaluated for different physico-chemical parameters like free fatty acid, iodine value, peroxide value, saponification value, unsaponiable matter and fatty acid composition (both before and after refining) and compared together to assess the effect of refining on these oils and to observe their edible and bio-fuel utilities. It was observed that the oil qualities in both cases were improved after the refining process.

Key words : Degumming, Alkali refining, Bleaching, *Psophocarpus tetragonolobus* seed oil.

P. tetragonolobus belongs to order Leguminosae, family Papilionaceae, is a perennial climber with tuberous roots. It grows naturally in India, Burma, Sri Lanka, Bangladesh, Indonesia, Malaysia, and some other South East Asian countries (1, 2). In India it is mainly grown in Maharashtra, West Bengal, Madras and Goa. Its local names are Goa Bean, Winged Bean, Four angled bean. Winged bean is presently grown only as field crop in Papua New Guines where a yield of 5,420 kg seed/hact has been reported (3). However, its all parts are edible viz. seeds, immature pods, leaves, flowers and tubers but it is mainly grown for its seed and tubers (4, 1). The entire plant of winged bean can be consumed because each part of the plant has nutritional and medicinal properties. The dry seeds contain 32—37% protein, 15 to 18% oil with 71% unsaturated fatty acids. The mature seeds are either cooked or roasted and never eaten raw. The flowers and leaves of the winged bean are eaten raw or cooked, added to salad, fish, and prawn soups. It could be used as green fodder, as forage crop for animals (4—7). Gandjar (8), Okezie and Martin (9) reported that winged bean dry seeds could be converted into temple by using Rhizopus mold. *P. tetragonolobus* and coconut oil has good nutritional profile due to their high fat and protein content (10, 11), but seed and oil of *P. tetragonolobus* are found found toxic in nature, due to presence of behenic acid and parinaric acid (12, 13). The oil cake is found rich in NPK, and can be considered as good organic fertilizer, but as such, it cannot be used as animal feed due to its toxic principles. According to Rockland et al. (14), Ekpenyong, and Borchers (15), the toxicological studies have revealed that no mortality of test animals was observed with refined/treated oil samples. Although there is no clear evidence yet on possible toxic properties of behenic acid, its high amount in winged bean seed oil must be regarded with caution. Nevertheless, despite of poor digestibility of this acid, on untoward effects were recorded in malnourished children who were fed full fat winged bean flour which supplied 1.2 g of the seed oil, i.e. 160—180 mg of behenic acid per kg body weight per day (16). The presence of behenic acid together with high level of tochopherols can be expected to act favorably by increasing resistance to the development of rancidity of the full fat winged bean flour. The usual way of preparing legumes at the household level i.e. by cooking the beans after shocking overnight, has been shown too adequate for detoxifying the seeds (17). Methods for maximum elimination of the toxic factors, without causing damage to the essential amino acids, will have to be developed yet.

Fatty oils, when extracted from vegetable and animals tissues, contain a number of impurities like free fatty acids, phosphatides, metal ions, waxes, oxi-

Table 1. Oil yield, protein in defatted cake and lecithin in oil of *P. tetragonolobus* and coconut oil.

	Oil (%)	Lecithin (%) in oil	Protein (%) in defatted cake
P. tetragonolobus	19.71	2.60	35.23
Coconut oil	36.82	2.30	14.70

Table 2. Effect of refining process on different bhysico-chemical properties of *P. tetragonolobus oil.*

	Before refining	After refining
Free fatty acid (mg KOH/g oil)	0.91	0.23
Iodine value (g/100 g oil)	91.72	80.20
Peroxide value (meq/kg oil)	9.23	3.83
Saponification value (mg KOH/g oil)	188.54	154.48
Unsaponifiable matter (%)	12.71	2.43

dation products, color bodies, which should be removed from the oil make it suitable for human consumption. Removal of such kinds of impurities is done in a series of processes, which include water degumming, alkali refining and bleaching of oil, which result in production of high quality oil having a good color, no taste or smell and remains fit for consumption, storage and transportation for long time (18, 19). These pretreatment processes also solved the problem of oil deterioration and incomplete combustion, as fuel. Further, it also increased the susceptibility of vegetable oils for trans-esterification during their conversion to biodiesel. Six different physico-chemicals properties like free fatty acids, iodine value, peroxide value, saponification value, unsaponifiable matter and fatty acid composition of *P. tetragonolobus* and coconut oil were studied before and after the refining process. Coconut oil is considered as one of the major edible oil from long time all over the world due to its good nutritional profile. Keeping these in view the results were compared together to assess the effect of refining on *P. tetragonolobus* (an underutilized plant) oil, so as to provide useful information on the possible uses of this under exploited food items for human consumption, food industry, bio-fuel and other technological uses.

Methods

The study was conducted at Department of Chemistry, Chaudhary Devilal University, Sirsa; Department of Chemistry and Physics; Department of Biochemistry and Department of Forestry, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Seed Material. The seeds of *P. tetragonolobus* were procured from R.M.D. College of Agriculture and Research Station, Krishi Nagar, Ambikapur, Chhattishgarh. The coconut fruits were procured from local market, Hisar.

Chemicals. The commercially available chemicals from Qualigens, Merk and Ranbaxy, (LR grade), of highest purity, were used for various experimental procedures.

Oil Extraction. The *P. tetragonolobus* seed were sun dried. The *P. tetragonolobus* seeds were deshelled by hand. The dried seeds kernels were blended and mixed thoroughly in Sujata food grinder. Similarly the coconut fruit was cut into small pieces and then grinded in the Sujata food grinder. The powdered sample was then stored in an airtight jar and kept in the refrigerator prior to the analysis. Oil was extracted by solvent extraction method AOAC (20). The oil was studied for various physico-chemical parameters both before and after degumming, alkali refining and bleaching. Defatted oil cake of *P. tetragonolobus* and coconut oil were also studied for crude protein estimation. Percent oil yield and lecithin were also estimated. Nitrogen was estimated by the micro-Kjeldahl method and the percentage nitrogen was converted to crude protein by multiplying with 6.25 as reported by Pearson (21).

Physico-Chemical Parameters. The physico-chemical properties i.e. iodine value, fatty acid value, peroxide value and unsaponifiable matter, were determined by the methods of AOAC (22). Saponification value content of the oil was obtained by refluxing the alcoholic potassium hydroxide solution of the oil and then titrated with 0.5 M HCL using phenolphthalein indicator. The iodine value of the oil was determined by titrating the chloroform and potassium iodine solution of *P. tetragonolobus* and coconut oil with sodium thiosulfate solution using starch indicator. The peroxide value of *P. tetragonolobus* and coconut oil were obtained by dissolving the oil in a solvent mix-

Table 3. Effect of refining process on different physico-chemical properties of coconut oil.

	Before refining	After refining
Free fatty acid (mg KOH/g oil)	1.32	0.81
Iodine value (g/100 g oil)	8.42	6.23
Peroxide value (meq/kg oil)	2.67	0.66
Saponification value (mg KOH/g oil)	253.77	233.21
Unsaponifiable matter (%)	0.90	0.52

ture of acetic acid and carbon tetrachloride, warmed with potassium iodide, and then titrated with sodium thiosulfate solution using strach indicator. The unsaponifiable matter content of the oil was estimated by dissolving the oil in alcoholic potassium hydroxide and refluxed. The homogeneous soda was then extracted with diethyl ether, the extract was filtered, and then oven dried to a constant weight. Fatty acid spectrum was estimated by method of Luddy et al. (23). The oils were converted into methyl esters using KOH/MeOH. The extracted fatty acid methyl esters (FAME) were dissolved in hexane for GC analyses.

Fractionation of Methyl Esters by GC. GC analysis were performend on a Chemito 8610 HT gas chromatograph equipped with FID and a BPX70, 0.25 mμ fused silica column (SGE Pvt. Ltd., Ringwood, Victoria, Australia) was used. The carrier gas was hydrogen and injection was operated in the split mode, the split ratio being approximately 50:1. Injector and detector temperature were 270 and 280 C respectively. The oven temperature was held at 70 C for 1 min and then programmed at 30 C/min to 170 C followed by further programming at 30 C/min to 200 C and held at this temperature for 6 min. Data were captured and analyzed with Chemito 5000 integrator (Tashniwal Instruments, India Ltd.).

Degumming. It was done by the method of Sartoretto (24) and Erickson et al. (25). Crude oil was mixed with 2—3% water and it was agitated gently for 30—60 minutes at a temperature of 70 C. Precautions were taken in order to prevent the introduction of air and subsequent oxidation of oil (26). Due to this, phosphatides and other impurities were settled down and were centrifuged (7,000 rpm for 15 minutes) out from the degummed oil. This process thus resulted in recovery of lecithin and other materials that can settle

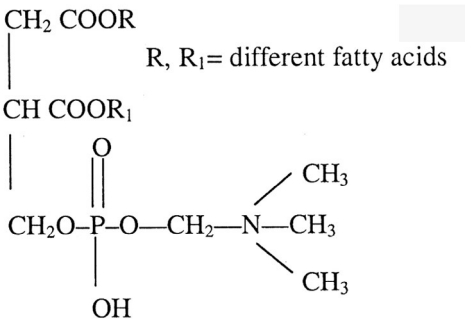

Figure 1. Lecithin phospholipids.

out during shipment or storage of pure oil.

Alkali Refining. It was done by the method of Erickson et al. (25). For alkali refining caustic soda around 0.1% was added and thoroughly mixed to ensure saponification of free fatty acids, hydration of phosphatides, albuminous and mucilaginous matter, and reactions with colord pigments. The mixture was then heated to 80 C and centrifuged (7,000 rpm for 15 minutes) to separate out the caustic from refined oil. Next the refined oil was heated to 88 C mixed with 10—20% soft water and was again centrifuged to separate into heavy and light phases.

Bleaching. It was done by the method of Erickson et al. (25). To the oil obtained after centrifugation was added 1% activated charcoal. It was stirred for half an hour and heated to 100 C, the slurry was then filtered and cooled, and then it was subjected to GLC analysis. The identification of the peaks was achieved by retention times and by comparing them with authentic standards analyzed under the same conditions.

Results and Discussion

Oil Yield

Oil yield in *P. tetragonolobus* and coconut oil were observed as 19.71% and 36.82% respectively (Table 1). Percent oil yield in *P. tetragonolobus* was found to be low with the percent oil yield of coconut oil, so *P. tetragonolobus* can be used as a potential oil yielding crop if its oil yield can be increased to appreciable amount by genetic modification or some other method. Comparable oil yield in *P. tetragonolobus* and coconut oil were also observed by Stephenson (27), Aletor and Aladetimi (28); Keay

Table 4. Fatty acid composition before and after oil refining in *P. tetragonolobus* and coconut oil. *Lauric acid (12:0) 55.65% (before refining) and 54.23% (after refining).

	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Total saturated fatty acids (%)	Total unsaturated fatty acids (%)
P. tetragonolobus	9.10	6.48	47.25	29.58	1.91	15.58	78.74
Coconut oil*	8.51	3.76	8.63	1.94	0.40	67.92	10.97
Composition (%) after refining							
P. tetragonolobus	8.13	6.19	49.20	30.02	1.11	14.32	80.33
Coconut oil*	8.06	4.20	9.25	1.63	0.79	66.49	11.67

et al. (29) and Anonymous (30). Crude protein in defatted cake of *P. tetragonolobus* was found to be 35.23%. Its value in defatted cake of coconut oil was 14.70%. Therefore, it can be said that *P. tetragonolobus* contained high protein as compared with those of some conventional defatted seed cakes like coconut oil. There are reported that *P. tetragonolobus* seed cake cannot be used for feeding purposes due to its toxicity but being good in NPK contents, it can be used as good manure (31). Seed oil contained phosphatides in the form of lecithin. Most of the phosphatides in the crude oils are hydratable and can be removed by water degumming (32, 33). Variations in lecithin yield were found as 2.60 and 2.30% in *P. tetragonolobus* and coconut oil, respectively.

It was observed that once the degumming is done then no further gums or waxes appeared in the oil, which results in the production of oil having good color, no taste, or smell, and remains fit for storage and transportation for long time (34). The gum did not combust completely, resulting in carbon deposits and lubricating oil thickening (35). Degumming of crude oil can solve this problem. Commercially obtained lecithin (Fig. 1) has wide industrial applications in food and beverages, medicines, cosmetics, tobacco, lubricants, gasket and cork products, urethan polymer, soap production (36, 37).

Physico-Chemical Parameters

Free fatty acid values of *P. tetragonolobus* and coconut oil before refining were found to be 0.91 and 1.32 mg KOH/g oil respectively (Tables 2 and 3). These values compared favorably with the results obtained by Ali and McKay (38), Sathe and Salunkhe (39) and Sathe et al. (40). There was a marked decrease in free fatty acid content after refining in the *P. tetragonolobus* and coconut oil to 0.23 and 0.81 mg KOH/g oil respectively. Release of short chain fatty acid such as butyric, caproic and capric acid, causes particularly disagreeable odors and flavor whereas the long chain fatty acids (C_{12} and above) produce candle like or, at alkaline pH, soapy flavor (41). Crude vegetable oils have abnormally high free fatty acid levels as enzyme lipase are activated by moisture in such cases which results in hydrolysis initiation and increase in the free fatty acid content, whereas in processed oils lipase activity is minimal which results in decrease in free fatty acid content in such oils (42).

Iodine value is the number of grams of iodine required to saturate 100-gram oil. Its value in *P. tetragonolobus* and coconut oil before refining were found out to be 91.72 and 8.42 g/100 g oil, respectively (Tables 2 and 3). These values compared favorably with the results obtained by Armour (31), Spricigo and Miguel (43). Iodine value gets reduced after refining for the *P. tetragonolobus* and coconut oil to 80.20 and 6.23 g/100 g oil, respectively. Decrease in iodine value after refining of oil shows that unsaturation of oil decreased and it is beneficial in the sense that lower the unsaturation of oils and fats, greater will be the oxidative stability of oils. Therefore, *P. tetragonolobus* oil having iodine value lower than coconut oil is best in this regard. Further, lower iodine value in oil produce biodiesel with high cloud and pour point that have poor cold performance. Peroxide value is measured in terms of milliequivalents

of peroxide per 1000 grams of the oil that oxidizes potassium iodide to iodine. The peroxide value before refining for *P. tetragonolobus* and coconut oil were observed as 9.23 and 2.67 meq/kg oil, respectively which got reduced to 3.83 and 0.66 meq/kg oil, respectively (Tables 2 and 3). The results are comparable with the results obtained by Hill (44).Low peroxide value of coconut oil (after refining) make it superior over *P. tetragonolobus* oil as it increases its suitability for the long time storage because of having low level of oxidative and lipolytic activities. Saponification value is a measure of the alkali reactive groups in fats and oils and is useful in predicting the type of glycerides in a sample. Glycerides containing short chain fatty acids have higher saponification value. *P. tetragonolobus* and coconut oils have comparable saponification value before refining i.e. 188.54 and 253.77 mg KOH/g oil, respectively. Nearly similar reduction trends of saponification value in *P. tetragonolobus* and coconut oil were observed after refining i.e. 154.48 and 233.21 mg KOH/g oil, respectively. It strengthens the practical utility of *P. tetragonolobus* and coconut oil for trans-esterification process and bio-fuel production. Lower the value of unsaponifiable matter in oil, higher will be purity because lower the unsaponifiables in the oil indicates low amount of secondary metabolites like compesterol, stigmasterol, β-sitosterol, δ-7 stigmasterol, natural fibers, gums, metal complexes, phosphatides like phosphotidyl choline, phosphotidyl inosetol, phosphotidic acid, phosphotidyl ethanolamine and other components. *P. tetragonolobus* and coconut oils have low unsaponification matter i.e. 12.71 and 0.90% respectively before refining, which further reduced to 2.43 and 0.52% (after refining) respectively. Coconut oil having low unsaponification matter, thus found to be superior than *P. tetragonolobus*. Biodiesel derived from oil with high unsaponifiable matter cause exhaust emissions during burning in the engine.

Palmitic acid which is considered as major saturated fatty acid was found (before refining) as 9.10 and 8.51% which got reduced to 8.13 and 8.06% in *P. tetragonolobus* and coconut oil, respectively (Table 4). From results, it can be concluded that refining can cause reduction of palmitic acid value. Nearly similar trend of reduction in stearic acid (another major saturated fatty acid) value was also observed, after refining process. *P. tetragonolobus* oil has relatively high stearic acid value, got reduced after refining from 6.48 to 6.19%, which makes it beneficial for its use as edible oil because low value of stearic acid can prevent from anthrosclerotic plaque (45). *P. tetragonolobus* oil has low saturated fatty acid count which indicates their utility for edible purpose. High degree of saturation in coconut oil is due to presence of lauric acid (12:0) 55.65% (before refining) and 54.23% (after refining). Total unsaturated fatty acid got increased after refining process in both oils. Oleic acid, which is considered as major unsaturated fatty acid got increased from 47.25 to 49.20% in *P. tetragonolobus* and 8.63 to 9.25% in coconut oil. Since the oleic acid value in *P. tetragonolobus* is high than in coconut oil. This indicates its edible utility. Higher levels of oleic acid are desirable to impart stability to oil during storage and deep fat frying. Linolenic acid which is considered to be a potential anti-nutritional compound, was found to be in *P. tetragonolobus* oil i.e. 1.91% (before refining) as compared to coconut oil (0.40%). Linolenic acid concentration further reduced (after refining) to 1.11% in *P. tetragonolobus* oil but remains high (0.79%) in coconut oil. In this respect coconut oil was found to be superior over *P. tetragonolobus* oil. Small variation in fatty acid composition of both crude and refined oil was noticed due to slight oxidation of linolenic acid during process of refining (46). Further, total unsaturated fatty acid in *P. tetragonolobus* and coconut oil were found to be increased after refining. It makes the oil favorable for edible purpose as it can reduce plasma triglycrides and is found to be anti-thrombogenic too.

So it can be concluded that *P. tetragonolobus* seed oil has good nutritional profile and other physicochemical properties which could be improved after the process of refining; therefore it can be used as a potential oil seed resource for both edible oil (if a suitable method to remove the toxicity is adopted) and bio-fuel production.

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