

Physicochemical Characterization of Seed and Seed Oil of *Jatropha curcas* L. Collected from Bardoli (South Gujarat)

(Ciri-ciri Fizikokimia Biji dan Minyak Biji *Jatropha curcas* L.
diambil dari Bardoli (Selatan Gujarat))

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ABSTRACT

The seed of *Jatropha curcas* was collected from the outskirts region of the Bardoli (Gujarat) and it was utilized for determination of seed characterization. The *Jatropha curcas* oil was extracted using light petroleum ether (60-80°C) by Soxhlet apparatus. The physicochemical properties of *Jatropha curcas* oil were evaluated. The result showed that the seeds consist of 46.31% (dry w/w) oil, moisture and volatilities (5.8% v/w) and protein content (22.50%). The physicochemical properties shows acid value (36.46), iodine value (106.00 mg/g) and saponification value (194.70 mg/g). The unsaponifiable matter was 1.02%. Negative Halphen test indicated the absence of cyclopropanoid acids in seed oil. GC analysis of *J. curcas* oil showed presence of palmitic acid (16.69%), stearic acid (7.67%), oleic acid (40.39%) and linoleic acid (33.09%).

Keywords: *Jatropha curcas*; oil characterisation; physiochemical properties; seed characterisation

ABSTRAK

Biji *Jatropha curcas* telah dikumpulkan dari kawasan luar Bardoli (Gujarat) dan telah dicirikan. Minyak *Jatropha curcas* telah diekstrak menggunakan eter petroleum ringan (60-80°C) dan alat Soxhlet. Sifat fizikokimia minyak *Jatropha curcas* telah dinilai. Keputusan menunjukkan biji tersebut mengandungi 46.31% (w/w kering) minyak, kelembapan dan kemeruapan (5.8% v/w) dan protein (22.50%). Sifat fizikokimia menunjukkan nilai asid (36.46), nilai iodin (106.00 mg/g) dan nilai saponifikasi (194.70 mg/g). Jirim yang tak tersaponifikasi ialah 1.02%. Ujian Halphen negatif menunjukkan ketidakhadiran asid siklopropanoid di dalam minyak biji ini. Analisis GC minyak *J. curcas* menunjukkan kewujudan asid palmitik (16.99%), asid steanik (7.67%), asid oleik (40.39%) dan asid linolik (33.09%).

Kata kunci: Ciri-ciri biji; ciri-ciri minyak; *Jatropha curcas*; sifat fizikokimia

INTRODUCTION

Jatropha curcas is a shrub belonging to the Euphorbiaceae family. It is cultivated in central and South America, South East Asia, India and Africa (Gübitz et al. 1999). *J. curcas* can grow well under such adverse climate because of its low moisture demands, fertility requirements and tolerance to high temperatures (Kaushik et al. 2007). All parts of *J. curcas* plant have their own uses. Like many other *Jatropha* species, *J. curcas* is a succulent tree that sheds its leaves during the dry season. It is a well adaptor to arid and semi-arid conditions and often used for erosion control. The leaves are used in traditional medicine against coughs or as antiseptics after birth, and the branches are chewing sticks (Gübitz et al. 1999). The latex produced from the branches is useful for wound healing and other medical uses. Each fruit contains 2 to 3 oblong black seeds which can produce oil. The seed kernel oil contained 40-60% (w/w) oil (Makkar et al. 1997). The seed oil extracted is found useful in medicinal and veterinary purposes, as insecticide, for soap production and as fuel substitute (Gübitz et al. 1999).

The composition of *J. curcas* oil from Nigeria consists of main fatty acid such as palmitic acid (13%), stearic acid (2.53%), oleic acid (48.8%) and linoleic acid (34.6%) (Martínez-Herrera et al. 2006). *J. curcas* oil contains high percentage of unsaturated fatty acid which is about 78-84%. This made the oils suitable for biodiesel production. However, the chemical compositions of the oil vary according to the climate and locality. To date, Bardoli (Gujarat) varieties of *J. curcas* oil have yet to be characterised. In this paper, we report the physicochemical properties of Bardoli (Gujarat) wild *J. curcas* seed and seed oil.

MATERIAL AND METHOD

J. curcas seeds were obtained from rural area of Bardoli (Gujarat) of India. The ripe seeds were collected and the damaged seeds were discarded. The seeds were cleaned, de-shelled and dried in an oven at 105°C for 30. The seeds were ground to powder using a grinder prior to oil extraction. All chemicals used in the study were analytical grade and used without further purification.

CHARACTERISATION

SEED INDEX

Weight and volume occupied by 1000 seeds was determined.

MOISTURE AND VOLATILITIES

About 5 to 6 g of seeds were accurately weighed in a petridish and kept in hot-air oven maintained at 110°C for 4 hrs. After cooling in a dessicator, the loss in weight was recorded in each case. This procedure was repeated till constant weight was obtained.

PROTEIN CONTENT

Deoiled meal (about 1 g) was weighed accurately by transfer method into a Kjeldhal's digestion flask. Sodium sulphate (10 g), copper sulphate (0.1g), a pinch of selenium metal and 20 mL of concentrated sulphuric acid were added to the flask and the mixture was heated gently in fume chamber for 15 min and then strongly for 2 to 3 until the mixture in the flask became colourless. This point indicates complete conversion of nitrogen into ammonium sulphate. The flask was cooled and the contents dissolved in 200 mL distilled water and made to 250 mL in the measuring flask. From this, 25 mL solution was taken in round bottom flask fitted with cork carrying a dropping funnel and delivery tube. A 25 mL of 50% sodium hydroxide taken in the dropping funnel. The delivery tube was connected via condenser to conical flask. The flask contains 100 mL of 20 % boric acid in which condenser tip just dipped. Round bottom flask was cooled by ice cold water and sodium hydroxide, about 20 mL was added immediately. The flask was heated for 20 min so that contents of Kjeldhal's flask were distilled to expel ammonia in conical flask in the form of bubbles. Top of funnel containing sodium hydroxide was opened. Flask was cooled, assembly dismantled, washed condenser and receiver with little quantity of distilled water. The contents of flask were titrated with 0.1N sodium hydroxide using bromophenol blue indicator. End point is from blue to greenish yellow. A blank experiment was also done side by side (Vogel 1975).

OIL CHARACTERISATION

EXTRACTION OF OIL

The collected ripe seeds were collected and the damaged seeds were discarded. The seeds were cleaned and dried in an oven at 105°C for 30 minutes. The seeds were powdered and extracted thoroughly with light petroleum ether (60-80°C) in a soxhlet extractor for 24-48 h in each case. Once more the remaining powdered seed was extracted to collect all oil in the seeds. Combined petroleum ether (60-80°C) extract was dried over anhydrous sodium sulphate and solvent was removed in vacuum at 40°C by using rotary evaporator to recover oil (Link 1975). The

seed oils were filtered through Whatman filter paper No.1 to remove any foreign particles and pure oil preserved in cold storage properly. Official and tentative methods (1993) of AOCs Chicago were followed for the determination of physicochemical characteristics of seed oil (Mukherjee 2002).

REFRACTIVE INDEX

Refractive index was determined on Abbe's refractometer. The prisms were cleaned with xylene and dried. Place few drops of oil on the prism, close the prisms and allow to stand for 1-2 min, adjusted the instrument and light to obtain the most distinct reading and determine the refractive index. Refractive index of oil increases with the increase in unsaturation and also chain length of fatty acid (Singhal & Sekiya 2003).

ACID VALUE

Two gram of the pure oil was weighed accurately by transfer method into a 250 mL conical flask. Neutral ethanol (20 mL) was added by means of a pipette and the flask heated on a steam bath for 3-min. Then the flask was cooled and the contents titrated with 0.1N alcoholic potassium hydroxide solution using phenolphthalein as an indicator. A blank titration was also conducted side by side.

IODINE VALUE

Oil (0.2 g) was weighed accurately by transfer method into a 250 mL iodine flask and dissolved in chloroform (20 mL). Wij's reagent (20 mL) was added by means of a pipette. The flask was stoppered and kept in darkness for one hr. with intermittent shaking. Then 15% of potassium iodide solution (10 mL) and 50 mL of distilled water were added to the flask and mixture was shaken well. The liberated iodine was titrated with 0.1 N sodium thiosulphate solution using fresh starch solution as indicator. A blank titration was also conducted side by side.

SAPONIFICATION VALUE AND SAPONIFICATION EQUIVALENT

Two gram of oil was weighed accurately by transfer method into a 250 mL round bottom flask. Freshly prepared 0.5 N alcoholic potassium hydroxide solution (25 mL) was added to the sample by means of pipette and the mixture gently refluxed on a water bath using an air-condenser for one hr. Then the flask was cooled, the condenser tip washed with little distilled water and the contents were titrated with 0.5 N hydrochloric acid solution using phenolphthalein as indicator. A blank titration was carried out simultaneously.

UNSAPONIFIABLE MATTER

After the titration of the sample for saponification value was completed, the contents of the flask were made

alkaline and extracted with light petroleum ether (60-80°C) and ether twice. The combined ethereal solution was washed thoroughly with distilled water, dried over sodium sulfate, solvent evaporated and the residue weighed. It was dissolved in neutral alcohol and the free acid titrated with 0.02 N alcoholic potassium hydroxide solution using phenolphthalein as indicator.

ESTIMATION OF CYCLOPROPENOID FATTY ACIDS

The Halphen test was originally developed as an empirical method of testing the adulteration of various vegetables oils by cotton seed oil (Halphen 1897). Though many modifications of the reagent and reaction conditions have been described (Carter & Frampton 1964). The method involves heating the oil with a 1.0% solution of sulphur in carbon disulphide combined with one part of amyl alcohol. If oil contain cotton seed oil a pink colour develops. The reaction is now believed to be specific for the cyclopropane ring (Nordby et al. 1962). The method is quick and easy for checking cyclopropenoid fatty acids in a mixture of oils. It is possible to use the reagent as TLC sprays (Morris & Hall 1967). Under controlled conditions the reaction can also be used as a colorimetric method of estimating the total cyclopropane fatty acid content of oil (Bailey et al. 1965; Deutschman & Klaus 1960).

GAS CHROMATOGRAPHY OF SEED OIL

The GC-FID analysis was performed with Shimadzu, GC-14B series gas chromatograph equipped with FID detector and the capillary column DB-23 (30 m × 0.25 mm; 0.5 µM). The column temperature was initially maintained at 160°C for 2 min, increased to 180°C at 6°C/min., maintained for 2 min at 180°C, then further increased to 230°C at 4°C/min and finally maintained for 10 min at 230°C. The carrier gas was nitrogen at a flow rate of 1.5 mL/min. The injector and detector temperature were maintained at 230 and at 250°C, respectively and split ratio was 50:1.

RESULTS AND DISCUSSION

Dull brownish black colour *Jatropha curcas* seed was evaluated for physical properties. Physical properties of 1000 seeds are given in Table 1. Chemicals are contained

either in pulp or kernel that directly affected by the physical parameters. The average weight and volume of seed directly relate to the hardness of seeds that directly affects process of analysis. Physical properties of one seed to another seed could distinct the product quality of *J. curcas* seed and its chemical. The *Jatropha* seed contains 46.31% oil and 22.50% protein. It has been reported that the toxicity and the disagreeable odor of seed is due to protein. The 4.56 %w/w total ash content of seeds indicates presence of abrasive solids, soluble metallic soaps, and silica residue in the seed.

The property of different fats and oils depends upon characterization of the degree of unsaturation or saturation with respect to hydrogen. Hence different oils are less or more saturated according as they contain greater or lesser proportion of the saturation in fatty acids. Therefore, it is important for researcher to know degree of unsaturation present in sample. The various number of test parameters like: iodine value (IV), saponification value (SV), and acid number (AN) that what we already applied to our sample. Results are presented in Table 2. The IV is a measure of the average amount of unsaturation of fats and oils and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample (Gerhard 2002). The oil shows a high iodine value due to its high content of unsaturated fatty acids (Table 3). The IV has found applications to various chemical and physical properties of fats and oils, having physiological applications, and serving as a quality control method for hydrogenation, these applications include use in standards for biodiesel and in assessing oxidative stability. *Jatropha* collected from rural area of Bardoli (Gujarat) has nearer iodine value 106.00 then that reported 105.20 in Nigerian and 135.85 in Malaysian (Salimon & Abdullah 2008).

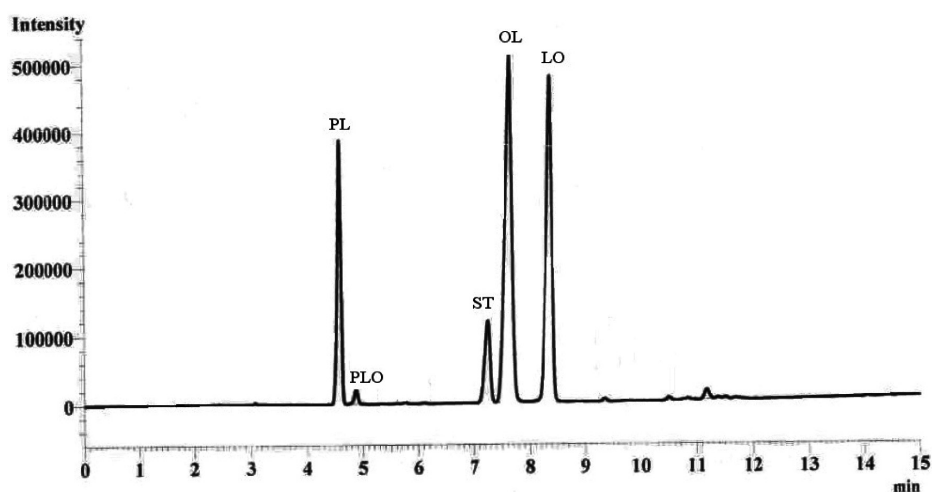
The hydroxyl value (HV) is applicable to fatty compounds containing hydroxy groups. The hydroxyl content of the oils and fats has long been recognised as a characteristic comparable in the determination of IV and SV. The saponification value (SV) is expressed as the number of milligrams of potassium hydroxide (KOH) required to saponify 1 g of sample. The saponification value of Malaysian *J. curcas* seed oil (208.50 mg/g) was higher compared to the Nigerian *J. curcas* seed oil (198.85 mg/g). While it is less for African *Jatropha* reported in the

TABLE 1. Characterisation of *Jatropha curcas* Seed

Sr. No.	Analytical parameter	Values
1	Weight of 1000 seeds	540.51 g
2	Volume of 1000 seeds	730.00 mL
3	Oil content (% v/w)	46.31
4	Moisture and volatilities (% w/w)	05.80
5	Ash content (% w/w)	4.56
6	Colour	Dull brownish black
7	Odour	Disagreeable
8	Taste	Bitter
9	Protein % w/w (on dry basis)	22.50

TABLE 2. Physicochemical characterisation of *Jatropha curcas* seed oil

Sr. No.	Physicochemical parameters	Values for oil
1	Specific gravity	0.913 at 32°C
2	Refractive index	1.496 at 32°C
3	Acid number (mg KOH ^m /g)	36.461
4	Iodine value (mg/g)	106.00
5	Saponification value (mg/g)	194.70
6	Unsaponifiable matter (%)	1.02
7	Halphen test	-ve

FIGURE 1. GC chromatogram of *Jatropha curcas* seed oilTABLE 3. Fatty acids composition of *Jatropha curcas* seed oil

Fatty Acids	%
Palmitic (PL)	16.69
Palmitoleic (PLO)	0.96
Stearic (ST)	7.67
Oleic (OL)	40.39
Linoleic (LO)	33.09
ΣSaturated Fatty Acid	24.36
ΣUnsaturated Fatty Acid	75.64

study (Adebowale & Adedire 2006). Negative Halphen test indicated the absence of cyclopropanoid acids in the seed oil.

The fatty acid composition of *J. curcas* oil was analysed by gas chromatography (Figure 1). Table 3 shows major long chain fatty acids present in the *J. curcas* oil which are palmitic acid (16.69%), stearic acid (7.67%), oleic acid (40.39%) linoleic acid (33.09%) and Linolenic acid (0.28%). *J. curcas* oil contains high percentage of unsaturated fatty acid which is about 75.64%.

CONCLUSION

J. curcas oil contains high percentage of unsaturated fatty acid which is about 75.64%. The study shows that fatty acids composition of the *J. curcas* oil is rich in oleic and linoleic acids and the oil can be classified as unsaturated oil. Hence the *J. curcas* oil has a great potential for oleochemical application such as surface coating and low pour point biodiesel. Therefore, it is amiable to have more research on *J. curcas* seed oil in the future to explore its potentials for future industrial oilseeds crop.

REFERENCES

- Adebowale, K.O. & Adedire, C.O. 2006. Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil. *African Journal of Biotechnology* 5(10): 901-906.
- Bailey, A.V., Pittman, R.A., Magne, F.C. & Skau, E.L. 1965. Methods for the determination of cyclopropanoid fatty acids: a spectrophotometric method for cotton seed oils based upon the Halphen-test reaction. *J. Am. Oil Chem. Soc.* 42: 422-424.
- Carter, F.L. & Frampton, V.L. 1964. Adverse effects of cyclopropanoid fatty acids. *Chem. Rev.* 64: 497-525.
- Deutschman, A.J. & Klaus I.S. 1960. Adverse effects of cyclopropanoid fatty acids. *Anal. Chem.* 32: 1809-1810.

- Gerhard Knothe. 2002. Structure indices in FA chemistry. How relevant is the iodine value? *J. Am. Oil Chem. Soc.* 79(9): 847-854.
- Gübitz, G.M., Mittlebach, M. & Trabi, M. 1999. Exploitation of the tropical oil seed plant *Jatropha curcas*. L. *International of Biosource Technology* 58: 77-82.
- Halphen, G. 1897. Adverse effects of cyclopropenoid fatty acids. *J. Pharm.* 6: 390-392.
- Kaushik, N., Kumar, K., Kumar, S., Kaushik, N. & Roy, S. 2007. Genetic variability and divergence studies in seed traits and oil content of *Jatropha (Jatropha curcas L.)* accessions. *Biomass and Bioenergy* 31: 497-502.
- Link, W.E. 1975. *Official and tentative methods of American oil and Chemists Society*. 3rd edition. Champaign, USA: AOCS.
- Makkar, H.P.S., Becker, K., Sporer, F. & Wink, M. 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *J. Agr. Food Chem.* 45: 3152-3157.
- Martínez-Herrera, J., Siddhuraju, P., Francis, G., Dávila-Ortíz, G. & Becker K. 2006. Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas L.* from Mexico. *Food Chemistry* 96: 80-89.
- Morris L.J. & Hall, S.W. 1967. Fatty acid content of *Pachira insignis*, Bombacaceae. *Chem. Indi.* 32: 25-29.
- Mukherjee P.K. 2002. *Quality Control of Herbal Drugs*. (1st ed) New Delhi: Business Horizon.
- Nordby, H.E., Heywang, B.W., Kircher, H.W. & Kemmerer, A.R. 1962. Sterculic derivatives and pink egg formation. *J. Am. Oil Chem. Soc.* 39: 183-185.
- Salimon Jumat & Abdullah Rozaini. 2008. Physicochemical properties of Malaysian *Jatropha curcas* seed oil. *Sains Malaysiana*. 37(4): 379-382.
- Singhal, S.C. & Sekiya J. 2003. *Modern Technology in the Oils and Fats Industry*. (2nd ed.) New Delhi: AOCS-OTA13.
- Vogel, A.I. 1975. *A Text Book of Quantitative Inorganic Analysis* 3rd Edition. London: Longman.
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