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Composition, Physical Properties and Drying Characteristics of Seed Oil of *Momordica charantia* Cultivated in Sri Lanka

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Abstract Karawila (*Momordica charantia*), also known as bitter gourd, is widely used as a food and a medicine in Asian countries. Representative samples of the seeds of the most abundant cultivar (MC43) in Sri Lanka were collected. The kernel represented 60 ± 4.7% of the seed by dry weight basis. The oil content of the dry kernel was 40.45 ± 3.12%. The seed oil was rich in α-eleosteric acid (50.04 ± 4.80%) and three other geometrical isomers of 9,11,13-octadecatrienoic acid that constituted 6.55%. The acid value, the saponification value and the iodine value were 2.73 ± 0.876, 190.70 ± 1.82 mg/g and 115.96 ± 3.46 cg/g, respectively. The set-to-touch drying time of 3 h observed for the seed oil of MC43 was significantly less than that of linseed oil (13 h). The presence of a high amount of conjugated octadecatrienoic acids, low acid value, high saponification value, moderate iodine value and the low set-to-touch drying time are promising indicators of the potential of karawila seed oil as a good drying oil for the paint and coating industry.

Keywords *Momordica charantia* · Bitter gourd · Drying oil · Eleosteric acid

Introduction

The plant *Momordica charantia* commonly known as the bitter gourd or Chinese melon belongs to the Cucurbitaceae family. It is widely cultivated in tropical climates for use as a vegetable and a medicine. In Sri Lanka, it is known as karawila and two cultivars of karawila are available. They are MC43 and Thinnaveli white, the former being the most abundant. The average annual production of both cultivars was about 20,000 metric tons during 1997–2005 (Source: Department of Census and Statistics, Sri Lanka). The young fruits of both cultivars have distinct warty looking exteriors and oblong shapes. The two cultivars can be distinctly identified from the dentate ridges of MC43 and smooth continuous ridges of Thinnaveli. Leaves and fruits of the karawila plant are a source of nutritional substances such as iron, calcium, phosphorous, vitamin C, protein, riboflavin, carotene, thiamine and some pharmacologically active compounds [1, 2]. Recent studies have confirmed the pharmacological actions and potential use of karawila in the treatment of diabetes and its complications; nephropathy, cataract and insulin resistance. Other important pharmacological activities of karawila are its antibacterial activity, antiviral activity (including HIV infection), anthelmintic activity, abortifacients and antifertility activity, immunomodulatory activity, analgesic and anti-inflammatory activity, hypotensive and anti prothrombin activity, hypocholesterolemic and anti-oxidant potential, antipsorisis activity, anticancer activity and anti-ulcer activity [1]. The use of karawila in decolourisation and removal of textile dyes and phenolic compounds from effluents are other potential applications [3, 4].

In contrast to most of the commonly available seed oils, the seed oil of *M. charantia* contains a large amount of eleosteric acid [5]. The presence of eleosteric acid in substantial amounts improves the drying rates of thin films of fatty oils when exposed to air. Seed oil of *M. charantia* had been identified as a good material for making wrinkle varnish [6]. In a study on Chinese melon (*M. charantia* L.),

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its potential to become an important industrial crop was discussed in detail with reference to the composition of the seed oil and the defatted seed meal [2]. However, a detailed analysis on the important parameters such as iodine value, saponification value, acid value and drying properties in formulating alkyd resins using the seed oil of *M. charantia* was not carried out in these studies. Furthermore, the physico–chemical properties of the seed oil of the most abundant variety in Sri Lanka, *M. charantia* (MC43), had not been reported. The present study analyses the composition and the physical properties of the seed oil of MC43 and examines its potential use as a drying oil for manufacturing alkyd resins.

Experimental Procedures

Seed Material

Karawila fruits were obtained from the main wholesale market in Colombo which generally receives karawila from almost all the cultivation areas in Sri Lanka. Ten batches of ripen fruits were obtained at different time intervals throughout the year in order to have a representative sample of the annual production of karawila. Seeds of fruits in each batch were separated from the fleshy part and were cleaned to remove the mucilaginous materials. The weight of cleaned seeds in one batch was approximately 150 g.

Oil Extraction

The cleaned seeds were dried in an oven at 105 °C for nearly 6 h until the weight reached a constant value. Dried seeds were then crushed to form a powder. Fatty oil from a known weight of powdered samples from each batch of seeds was extracted using hexane for 8 h by using a soxhlet extractor [7, 8]. A stream of pure nitrogen gas was purged into the container to repel the air in order to prevent oxidation. Desolventisation of the hexane in the extracted fatty oil was carried out using a rotary evaporator.

Analytical Methods

Analytical determinations of each sample were achieved in triplicate for all the samples. The mean values of the triplicate analysis for each sample were used to find the overall mean ± standard deviation of the entire population represented by ten batches.

Physical Analysis of the Seed Oil

The moisture content of the seed was determined gravimetrically by placing samples of 5 g each from the ten batches in an oven at 105 °C for 6 h until a constant weight was achieved [9]. The kernels were separated by hand and the weight percentage of kernel to the seed on a dry basis was estimated. The oil content of the seed was found by the solvent extraction technique. The colour and the state of the oil at room temperature were noted by visual inspection and the specific gravity was determined according to ASTM D1475. The refractive index of the oil at room temperature was estimated using an Abbe refractometer.

Chemical Analysis of the Seed Oil

The seed oil of MC43 was analysed to determine the acid value (ASTM 1639-70), saponification value (ASTM D 1962-85) and iodine value by the Wijs method (ASTM 1959-85).

Fatty Acid Analysis

The fatty acid composition was determined by the instrumental methods; GLC, GC-MS, FTIR and UV spectroscopy. GLC analysis and GC-MS analysis were performed using a capillary column (Supelcowax 10, 15 m × 0.10 mm). The chemicals used were of chromatographic grade and were products of Merck Ltd. Methyl esters of the seed oil were used in chromatographic analysis and they were prepared by transesterification of 0.5 g of the seed oil of MC43 with 2 ml of 0.5 M methanolic sodium methoxide, 1 ml of benzene and 2 ml of methanol in a 50 ml screwed cap type test tube. A stream of nitrogen was first used to repel air and the test tube was submerged in a water bath which was maintained at 100 °C for 45 min during the transesterification. Thereafter, the reaction mixture was cooled to room temperature and 10 ml of distilled water was added to separate water soluble compounds. The methyl esters were then extracted with *n*-hexane by three successive extractions, each with 5 ml of *n*-hexane. Hexane in the extract was evaporated to concentrate the methyl ester solution until the final volume reached 0.5 ml.

Helium was used as the carrier gas for the GLC analysis at a pressure of 0.15 kg cm^{-2}. The pressure of H_2 gas and air were set to 0.15 kg cm^{-2} and the initial temperature in the oven was set to 140 °C. The capillary column was initially conditioned over 1 h at 140 °C. The temperature of both detector and injector ports was set to 250 °C and the temperature of the capillary column was programmed to maintain the initial temperature at 140 °C for 1 min followed by increasing at a rate of 5 °C per minute until the temperature reached 190 °C. The temperature programme was started immediately after injecting 1 µl of concentrated methyl ester solution to the injector port and once the temperature reached 190 °C, it was maintained until the

analysis was completed. In the GC-MS analysis, the flow rate of carrier gas was set to 1 ml per minute and, the injector and the detector temperatures were set to 250 and 280 °C, respectively. A temperature programme similar to GLC analysis was used in GC-MS analysis also.

The GLC analysis was carried out in triplicate for each sample of methyl ester solution. Compounds were identified by comparison of their GC retention times with those of reference solutions of 1% (w/v) of the methyl esters of the fatty acids and also by comparison of their mass spectra with either known compounds analysed under the same conditions or published spectra [10, 11]. The relative percentage of fatty acids in methyl ester solutions were calculated from the total area under the peaks [12] using the software of the instrument.

Drying Property

The drying property of the seed oil of MC43 was examined by the method of set-to-touch drying time. A uniform film of seed oil was first prepared by drawing down a film on a glass panel with an applicator bar. The film was allowed to dry in a horizontal position under ambient conditions, namely a temperature 27 °C and a relative humidity of 80%. The drying characteristic of the film was tested at regular intervals by lightly touching with the fingers. The "set-to-touch" time is that time at which the film does not transfer to the finger [13].

Results and Discussion

The physico–chemical properties of seed oil of MC43 are given in Table 1. The seed oil of MC43 is a reddish-brown coloured liquid at room temperature (27 ± 3 °C) with a specific gravity of 0.92 ± 0.01. The average moisture content of fresh seeds was found to be 53 ± 13% (w/w). The high value of standard deviation of the moisture content might be due to different levels of maturity of the fruits at the time of collection. The kernel represented 60 ± 4.7% (w/w) of the seed on a dry basis. The oil content of the seed, given as a percentage to the dry weight of the kernel, was found to be 40.45 ± 3.12%. It was well in agreement with the range of oil content (41–45%) for four different cultivars of *M. charantia* reported in the literature [2]. The refractive index which was fairly constant at 1.4895 ± 0.0050 for the investigated oil samples, indicated the consistency of the texture of the seed oil of MC43. Further, it is comparable with the values of the refractive index of most of the drying oils [14]. The refractive index is not a useful property for specifying drying oils but it is useful in detecting the adulteration of oils that contain substantial amounts of conjugated double bonds. Therefore, the average value of the refractive index given in Table 1 can be used as a quality parameter in preliminary testing of the seed oil of MC43.

Table 1 Physical and chemical properties of oil extracts from seeds of MC43

Properties	
Moisture content of fresh seeds (% w/w)	53 ± 13
Kernel to seed dry basis (% w/w)	60 ± 4.7
Specific gravity	0.92 ± 0.01
Refractive index at 25 °C	1.4895 ± 0.0050
Colour	Reddish brown
Oil content of the kernels (% w/w)	40.45 ± 3.12
Acid value (mg/g)	2.73 ± 0.876
Saponification value (mg/g)	190.70 ± 1.82
Iodine value (cg/g)	115.96 ± 3.46

The saponification value is defined as the number of milligrams of potassium hydroxide that will react with one gram of sample. It is an indication of the average molecular mass of fatty acids present in oil. The high saponification value of seed oil of MC43 (190.70 ± 1.82 mg/g) indicated that it contained mainly fatty acids of high molecular mass. This result compares favourably with saponification values of common drying oils; soy oil (190 mg/g), linseed oil (190 mg/g) and tung oil (192 mg/g) [14]. The low standard deviation of the saponification value suggested that the total amount of fatty acids per unit weight of seed oil of MC43 was fairly constant. However, the acid value (2.73 ± 0.876 mg/g) which depends on the degree of lipase activity that hydrolyses glycerides to form free fatty acids in seed tissues, varied significantly among batches. This result indicated that the time period from harvesting to deactivation of lipase activity by oven drying of the seeds had a significant effect on the amount of free fatty acids in the oil. A separate experiment was carried out to verify this observation. A sample of the seeds from ripen fruits of MC43 was kept under ambient conditions of 27 °C and a relative humidity of 80% for 1 week after collecting them from the market. The acid value and the saponification value for this oil sample were found to be 8 and 190 mg/g, respectively. Comparison of these values with the results given in Table 1 indicated that the acid value was increased from about 2.7 to 8.0 mg/g while the saponification value was constant at about 190.0 mg/g. This result confirmed that the lipase activity only changed the form of fatty acid from triglycerides to free fatty acids without affecting the total amount of fatty acids in the oil with aging of the seeds.

In contrast to most of the IR spectra for natural fatty oils, the FTIR spectrum of the seed oil of MC43 (Fig. 1) showed a characteristic doublet at wave numbers 991 and 962 cm^{-1}. A previous study on isomers of eleosteric acid

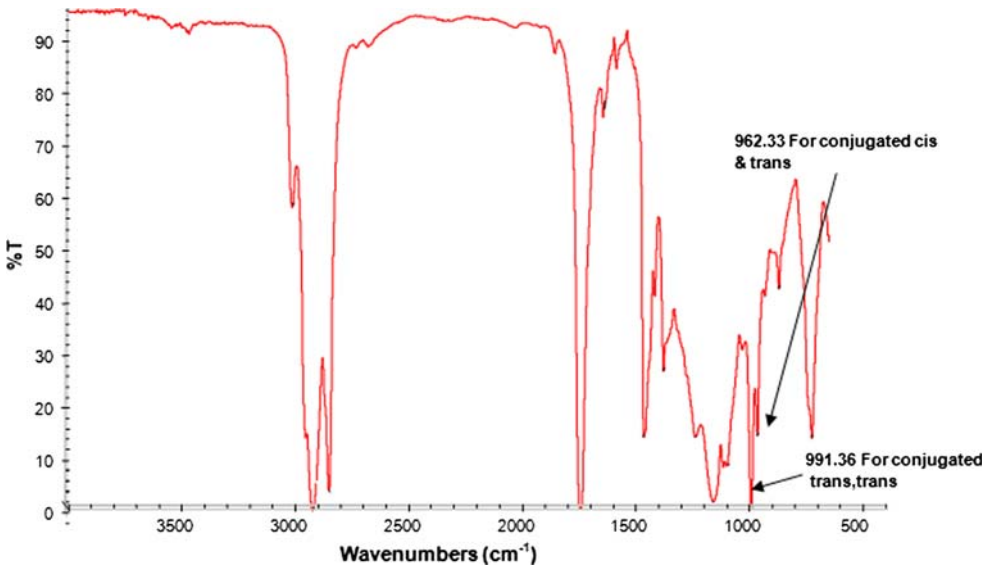

Fig. 1 FTIR spectrum of the seed oil of MC43

identified a strong spectral band at 993 cm^{-1} corresponding to β-eleosteric acid and a doublet having a strong band at 991 cm^{-1} and a weaker band at 963 cm^{-1} corresponding to *trans*:*trans* and *cis*:*trans* conjugated double bonds in the α-eleosteric acid, respectively [15]. Therefore, it could be concluded that the corresponding doublet in Fig. 1 was an indication of the presence of α-eleosteric acid in the seed oil of MC43. The effect of the lipase activity on the hydrolysis of glycerides in seed tissues with time was confirmed by the presence of an additional peak at 3,468 cm^{-1} (characteristic peak to OH group) in the FTIR spectrum of the seed oil of MC43 which was extracted 1 week after collecting the seeds.

Three spectral bands around 260, 269 and 280 nm were observed in the UV spectrum and the band appearing at 269 nm showed the highest absorbance. In early studies on the isomers of eleosteric acid, similar set of peaks were identified as characteristic peaks for β-eleosteric acid [16, 17]. This observation confirmed the presence of β-eleosteric acid in the seed oil of MC43.

The GC analysis of the methyl esters of seed oil of MC43 revealed the presence of usual fatty acids with a substantial amount of stearic acid, identified by comparing the retention times of the standard compounds. Furthermore, four significant GC peaks with retention characteristics that are not usually found in seed oils were observed. The mass spectrum corresponding to these methyl esters showed a molecular ion at *m*/*z* 292 corresponding to an empirical formula $C_{19}H_{32}O_2$ suggesting that these methyl esters consisted of a C_{18} chain with three double bonds. In addition to the base peak at *m*/*z* 292, the peak observed at *m*/*z* 91 which might be due to tropylium ions, is typical for conjugated double bond systems [18]. These observations confirmed that these four compounds were isomers of conjugated octadecatrienoic acids. The comparison of mass spectra of methyl esters of conjugated octadecatrienoic acids available in the literature [10, 11], with the mass spectra of these compounds suggested that the predominant unsaturated fatty acid (50.04%) in the seed oil of MC43 was α-eleosteric acid. The mass spectra of methyl esters of the other three isomers of conjugated octadecatrienoic acids were very similar to the mass spectrum of α-eleosteric acid which is given in Fig. 2. This suggested that the conjugated triene system might have appeared in the same position in the chemical structures of these four compounds. Hence, they were considered to be geometrical isomers of 9,11,13-octadecatrienoic acid namely α-eleosteric acid, β-eleosteric acid, punicic acid and catalpic acid. The sequence of the resolved GC peaks corresponding to these isomers were identified by comparing the GC resolutions of conjugated octadecatrienoates for mixture of fatty acids using capillary columns reported in a previous study [19]. The results of GC analysis of the fatty acid composition, given in Table 2 were in agreement with those of other varieties of *M. charantia* [2]. The predominant fatty acid in the other verities of *M. charantia* was also α-eleosteric acid with a percentage of 56–67%. They also had substantial amount of stearic acid (14–26%) but comparatively less than that in the seed oil of MC43 (35.08 $\pm$ 1.83).

A moderate iodine value, 115.96 $\pm$ 3.46 mg/g, was observed for the seed oil of MC43 (Table 1) due to the presence of a considerable amount of conjugated octadecatrienoic acids. Tung oil which also has a high amount of conjugated octadecatrienoic acids (80%) and a high iodine number (170 cg/g) is a fast drying oil. Eleosteric acid enhances efficient drying but it promotes yellowing of the dried film with time and rapid gelling at high temperatures when present in very large amounts. Therefore, tung oil is generally used as a blend with another drying oil. The seed

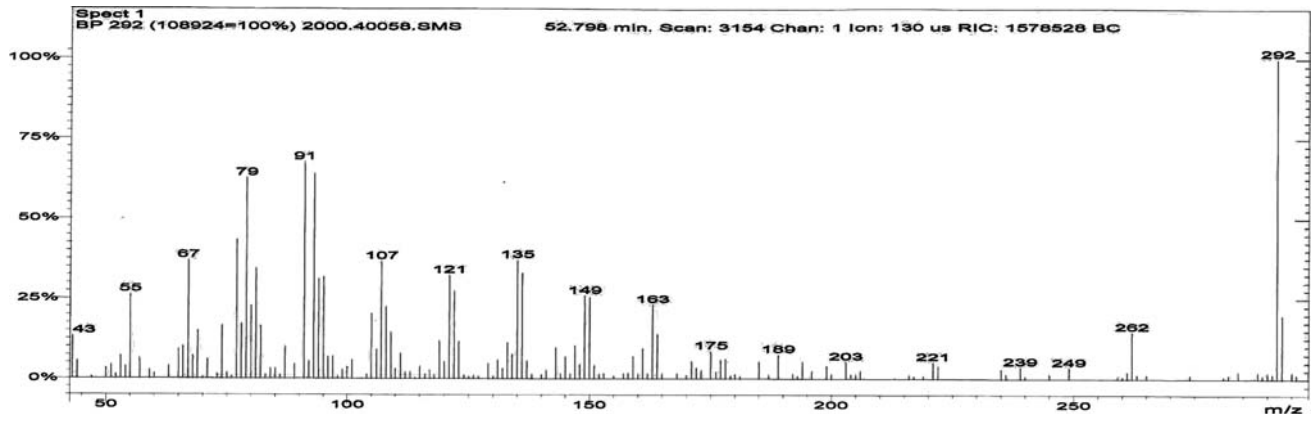

Fig. 2 Mass spectrum of methyl ester of α-eleosteric acid in the seed oil of MC43

Table 2 Chemical composition of seed oil of MC43

Fatty acids	Composition (%)
Caprilic acid (C:8-0)	0.11 ± 0.20
Capric acid (C:10-0)	0.04 ± 0.10
Lauric acid (C:12-0)	0.23 ± 0.49
Myristic acid (C:14-0)	0.13 ± 0.19
Palmitic acid (C:16-0)	2.15 ± 0.32
Stearic acid (C:18-0)	35.08 ± 1.83
Oleic acid (C:18-1)	1.98 ± 0.36
Linoleic acid (C:18-2)	3.70 ± 0.25
Punicic acid (ctc, 9,11,13–18:3)	1.31 ± 0.39
α-Eleosteric acid (ctt, 9,11,13–18:3)	50.04 ± 4.80
Catalpic acid (ttc, 9,11,13–18:3)	1.30 ± 0.93
β-Eleosteric acid (ttt, 9,11,13–18:3)	3.94 ± 3.62

oil of MC43 also had a moderately high amount of conjugated octadecatrienoic acids (56%), but the ratio of stearic to eleostearic in seed oil of MC43 was 20 times greater than that in tung oil. As a result, the seed oil of MC43 had the characteristics of being a good drying oil with a lower gelling risk and a yellowing tendency. The excellent drying characteristic of seed oil of MC43 was confirmed by the observation of low set to touch drying time of 3 h which was significantly less than that of linseed oil (13 h) under similar conditions (27 °C and a relative humidity of 80%) without using any drying agents. These results indicate that seed oil of MC43 has the potential to become a new source for the paint and coating industry.

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