A STUDY ON THE THERMAL PROPERTIES AND SOLID FAT CONTENT OF MALAYSIAN RUBBER SEED OIL

(Satu Kajian Sifat Terma dan Kandungan Pepejal Lemak Dalam Minyak Biji Getah Malaysia)

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Abstract

This study was carried out to determine the characteristics, thermal properties and solid fat content of Malaysian rubber (*Hevea brasiliensis* (kunth. Muell)) seed oil (RSO). RSO was extracted using hexane and degummed using phosphoric acid. The RSO physicochemical properties such as free fatty acid (FFA %), acid value, saponification value, iodine value, and unsaponifiable matter gave values of 7.55±0.02%, 15.03±0.04, 182.12±0.27, 135.79±0.33 and 1.83±0.01 respectively. Gas chromatography (GC) was used to determine the fatty acid (FA) composition. RSO consisting of saturated FA (19.12±0.28%) such as palmitic (8.56±0.07%) and stearic (10.56±0.02%) acids and unsaturated FA (79.45±0.31%) such as oleic (22.95±0.15%), linoleic (37.28±0.10%) and linolenic (19.22±0.21%) acids. High performance liquid chromatography (HPLC) was used to determine the triacylglycerol (TAG). Polyunsaturated TAGs of LnLnL (7.7%), LnOO (7.5%), PPL (6.4%), LnLL (5.9%), OLLn (5.9%) and PPL (5.8%) were the major TAGs found in RSO. The differential scanning calorimetry (DSC) showed that crystallized TAG was observed at -9.95°C. The melting curves displayed two major exothermic regions of RSO the unsaturated TAG at -28.87°C and the saturated TAG at -10.22°C. The solid fat content (SFC) of RSO showed low percentages of solids (0.24%, 0.13%, 0.12%, 0% and 0%) at different temperatures of 0, 5, 10, 15 and 20°C. The results of this study showed that the RSO is plausible source of polyunsaturated fatty acid (PUFA) to be developed in the future after removing the free fatty acid to make it more acceptable.

Keywords: Rubber seed oil; Thermal properties; Solid fat content

Abstrak

Kajian ini dilakukan untuk menentukan sifat kimia, sifat terma dan kandungan lemak pejal minyak biji getah Malaysia (*Hevea brasiliensis* (kunth. Muell)) (RSO). RSO diekstrak menggunakan pelarut heksana dan dinyahgamkan dengan menggunakan asid fosforik. Sifat fisikokimia RSO seperti kandungan asid lemak bebas, nilai asid, nilai safonikasi, nilai iodin dan nilai taktersabun memberikan nilai masing-masing sebanyak 7.55±0.02%, 15.03±0.04, 182.12±0.27, 135.79±0.33 dan 1.83±0.01. Kromatografi gas (GC) digunakan untuk mennentukan komposisi asid lemak (FA). RSO mengandunggi asid lemak tepu sebanyak 19.12±0.28% seperti asid palmitik (8.56±0.07%) dan asid stearik (10.56±0.02%) sementara asid lemak taktepu sebanyak 79.45±0.31% seperti asid oleik (22.95±0.15%), linoleik (37.28±0.10%) dan asid linolenik (19.22±0.21%). Kromatografi cecair prestasi tinggi (HPLC) digunakan untuk menentukan kandungan triasilgliserol (TAG). TAGs politaktepu seperti LnLnL (7.7%), LnOO (7.5%), PPL (6.4%), LnLL (5.9%), OLLn (5.9%) dan PPL (5.8%) merupakan TAGs yang utama dalam RSO. Kalorimetri pengimbas kerbedaan (DSC) menunjukkan TAG RSO menghablur pada -9.95°C. Lengkuk peleburan RSO menunjukkan dua kawasan eksoterma bagi politaktepu TAG pada -28.87°C dan politepu TAG pada -10.22°C. Kandungan lemak pejal (SFC) minyak RSO menunjukkan kandungan yang rendah (0.24%, 0.13%, 0.12%, 0% and 0%) masing-masing pada suhu 0, 5, 10, 15 dan 20°C. Hasil kajian ini menunjukkan bahwa RSO menunjukkan potensi yang baik untuk menjadi sumber asid lemak politaktepu (PUFA) untuk dikembangkan dimasa depan selepas pengasingan asid lemak bebas untuk menjadikan ianya lebih diterima.

***Kata kunci**: Minyak biji getah, sifat terma, kandungan lemak pejal*

Introduction

The rubber tree (*Hevea brasiliensis*) is widely used as a source of natural rubber, and its seeds have been found to be rich in oil [1]. At the present time, the production of rubber seed oil (RSO) shows a huge increase in both quantity and quality in Malaysia. This is because of its important role in different industrial processes.

Rubber seed oil is yellow, semi-drying oil [1]. The oil does not contain any unusual fatty acids, and its rich source of essential fatty acids $C_{18:2}$ and $C_{18:3}$ that make up 52 % of its total fatty acid composition [2].

There are many uses of the rubber seed oil, such as its uses in the manufacturing of laundry soap, paints, varnishes, deoxidized oil. The RSO is suitable for the production of fat liquor for the tanning of leather industry and in the preparation grease. The rubber seed oil is used for the production factice and epoxidized. RSO is used in formulations for anticorrosive coatings and adhesives [1]. The rubber seed oil is used as a plasticizer and the fatty acid component of the activator in natural rubber and styrene butadiene rubber [3].

The most popular modern thermal analysis technique is the Differential Scanning Calorimetry (DSC). In the present study, the effect of heating rates on melting curves and cooling rates on crystallization curve oil were determined by DSC. The endotherms in each melting curve have been labeled in order of increasing temperature, while the exotherms in each crystallization curve have been marked in order of decreasing temperature [4].

DSC is a technique involves recording the energy flux necessary to establish a zero temperature difference between a substance against either time or temperature, as two specimens are subjected to identical temperature regimes in an environment heated or cooled at controlled rate. DSC also provides information on the excess specific heat over a wide range of temperatures [5]. It is well known that melting and crystallization behaviors of oils and fats are two of the important properties for the functionality in many prepared food products. These thermal properties are counterparts of the TAG profile in oils and fats [4].

Solid fat content (SFC) in a solid or semi-solid fat sample is measured based on the ratio of the number of detected protons in solid fat over the total number of detected protons in both solid and liquid phases. The solid fat content is dependent on tempering temperature, any deviation from specific condition will change the data obtained. The most important technique is NMR widely used in physics and chemistry to characterize materials. NMR is a microscopic method, in the sense that it probes the nuclei and their immediate surroundings.

The microscopic nature of the NMR measurement makes it extremely useful, and sometimes unique. The nuclei investigated in this experiment are hydrogen atoms (protons) and they have particularly strong signals. The decay rate gives physical information because it is modified by molecular moment. In solid-phase, motion is restricted and fast, yielding a slowly decaying signal. This is the basis for measurement of solid fat content [6].

In this paper, the characteristics, thermal properties and solid fat content of Malaysian RSO including the fatty acids and triacylglycerol (TAG) of the Malaysian RSO were carried out.

Experimental

Seed material and oil extraction

Rubber seeds were collected from Rubber Research Institute, Sungai Buloh. The seeds were shelled and dried in the oven at 105°C for 30 min. The rubber seeds were milled using the grinder. The seeds were kept in the refrigerator. RSO was extracted from the 500g rubber seeds by soxhlet extractor using hexane as solvent at 60°C for 6 hours.

Physicochemical characteristics

The physicochemical properties of RSO such as color, FFA%, acid value, saponification value, iodine value and unsaponifiable matter were determined according to AOCS.

Fatty acid composition

The fatty acid composition of RSO was determined using its fatty acid methyl esters. GC analysis was performed on shimadzu, GC equipped with flame ionization detector and capillary column (30m × 0.25mm × 0.25μm films). The detector temperature was programmed for 280°C with flow rate of 0.3 mL/min. The injector temperature was set at 250°C. Nitrogen was used as the carrier gas at a flow rate of 20 mL/min.

Triacylglycerol composition

TAG of RSO was determined by using HPLC from Waters model 1515 equipped with refractive index detector, and spherisorb C18 column, (150mm × 4.8μm ×3mm). The mobile phase was a mixture of acetone:acetonitrile

(63.5:36.5) set at flow rate of 1ml/min. The sample was dissolved in 10 ml of the mixture acetone:acetonitrile before 20 ml of the sample being injected into HPLC with total running time of 50 min.

Thermal properties

Differential scanning calorimetry (DSC) analysis (DSC 822e METTLER TOLEDO calibration) was used to study the melting temperature and the crystallization temperature of RSO, about 3-5 mg of the RSO was weighted into the 49 ml sealed aluminum pan, the RSO was put in oven for 30min and then was put in the freezer for 90 min, after that the RSO was left at room temperature for 48 hours, and then the RSO was put at 5 °C and was used directly to run at -60 °C to 60 °C to -60 °C with heating rate 10°C.

Solid fat content

Pulsed nuclear magnetic resonance (pNMR) spectrometer (Bruker minispec mq20) was used to study the solid fat contain of RSO using circulating refrigerated water bath with digital display (HAAKE K15) as a cooler system with different degrees of the temperature.

Results and Discussion

The physicochemical properties of RSO determined are given in (Table 1). The color of RSO present as pale yellow oil (33.98±0.08), and darker (lower L* value) than commercial oils (66.8-68.1) such as corn oil, Soya bean oil and sunflower oil [7]. The present FFA (7.55±0.02, as oleic acid) and acid value (15.03±0.04) for RSO show that the RSO has a high FFA due to the RSO was not neutralized. The RSO shows high iodine value (135.79±0.33) comparing with Iodine value of palm oil (52) [8] due to the high content of unsaturated fatty acids such as oleic acid (22.9%) as shown in (Table 2) which mean the RSO is semi-drying oil and can be used in paint industry.

The Sapoification value of RSO (182.12±0.27) is similar to the other typical seed oil such as sunflower and corn oil [9] and lower than the other vegetable oil such as, coconut, melon, groundnut, oil bean seed, and palm kernel seed, on the other hand its higher than castor [8] and *perah* oil [10] with an average range saponification number of 175 to 250 [11].

Table 1: The physicochemical properties of rubber seed oil (RSO)

Analysis	RSO
Crude Oil content	40%
Degummed oil content	37%
FFA% (as oleic)	7.55±0.02
Acid value	15.03±0.04
Iodine value	135.79±0.33
Saponification value	182.12±0.27
Unsaponifiable matter	1.83±0.01
Average M.wt of TAG	924.12±8.89
Color	
a*	0.86±0.01
b*	0.47±0.04
L*	33.98±0.08

The unsaponifiable matter is important to determine the quantity of substances present in the RSO and the quality of RSO. The value of unsaponifiable matter of RSO is 1.83±0.01% (Table 1). This value is in agreement with the value reported by Ghandhi [2].

The fatty acid composition of the RSO is shown in Table 2. The fatty acids of RSO consist from saturated FA (19.12±0.28%) and unsaturated FA (79.45±0.31%). The saturated FA are consisting mainly palmitic acid (8.56±0.07%) and stearic acid (10.56±0.02%) and unsaturated FA are consisting mainly of oleic acid

(22.95±0.15%), linoleic acid (37.28±0.10%) and linolenic acid (19.22±0.21%) (Table 2). The fatty acid composition of RSO can be used as an indicator for type of each fatty acid [2].

Table 2: Fatty acids composition of rubber seed oil (RSO)

Fatty acids composition	RSO%
Saturated	
Palmitic acid	8.56±0.07
Stearic acid	10.56±0.02
Total	19.12±0.28
Unsaturated	
Oleic acid	22.95±0.15
Linoleic acid	37.28±0.10
Linolenic acid	19.22±0.21
Total	79.45±0.31
Others	1.43±0.07

Table 3 shows the TAGs present in RSO. Major TAG peaks in the RSO were the monounsaturated TAG of PPL, followed by polyunsaturated TAGs of LnLnL and LnOO, whereas saturated TAG consist of PPP.

TAG composition of RSO was determined by comparing the retention times of the TAG in the RSO, and the retention time of *Perah* seed oil, Soybean oil, linseed oil, and palm oil chromatographs [10]. The comparison of TAG was determined as the percentage of the peak area. The mechanism in sparating the TAG involves the chain length and degree of instauration of the fatty acids [13].

Table 3: TAGs composition of rubber seed oil (RSO)

TAGs	ECNs	Relative composition (%)
Unsaturated		
LnLnLn	36	2.3
LnLnL	38	7.7
LnLL	40	5.9
LLL	42	4.1
OLLn	42	5.9
PLLn	42	3.9
PLL	44	5.8
LnOP	44	1.7
LnOO	44	7.5
POL + SOL	46	3.3
POO	48	1.9
PPL	46	6.4
POP	48	4.4
POS	50	1.0
Saturated		
PPP	48	3.9
PPS	50	0.5

Ln: linolenic acid, L: linoleic acid, O: oleic acid, P: palmitic acid, S: stearic acid. ECNs: equivalent carbon number

DSC provides information on the excess specific heat over a wide range of temperatures [5]. The crystallization curve displayed one major exothermic region of RSO (Fig. 1).

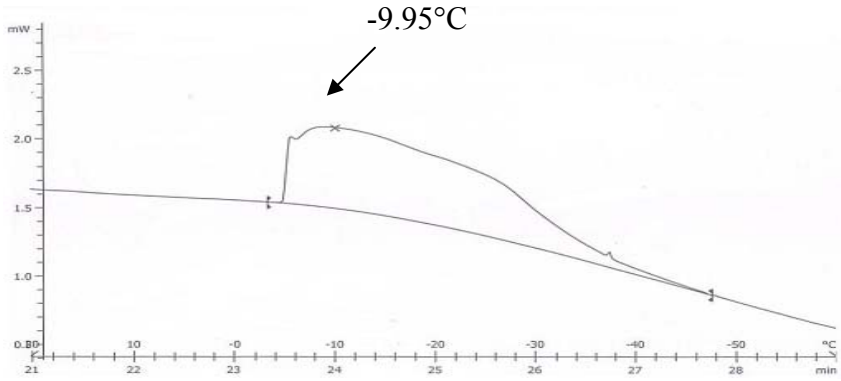

Fig. 1: DSC crystallization signals of RSO

The temperature of this regions started in (-4.56°C), in this area the crystallize started to change from liquid to solid and indicate the most saturated TAG crystallize, and when transferred the temperatures to (-9.95°C) in this area the energy released because of the crystallization and most number of TAG was crystallized and when the temperature transferred to (-36.01°C), in this area most unsaturated TAG was crystallized.

The melting curve displayed two major exothermic regions (Fig. 2), the first area (-28.87°C) shows that the RSO more unsaturated TAG and GC indicated that RSO contain unsaturated fatty acid linoleic (37.27%) [14], and the second area (-10.22°C) shows RSO contain saturated fatty acid TAG.

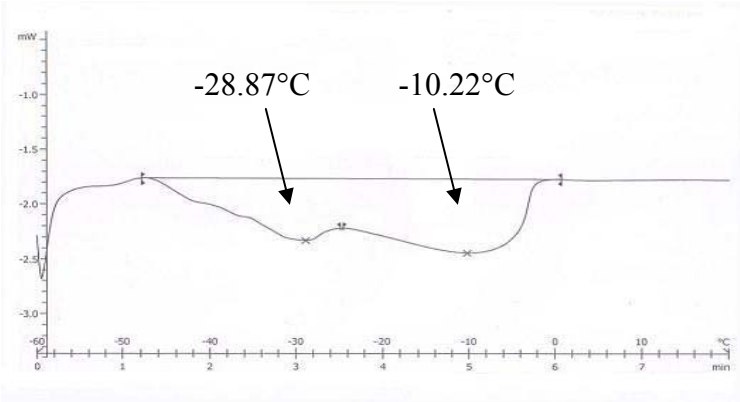

Fig. 2: DSC melting signals of RSO

Pulsed nuclear magnetic resonance spectroscopy pNMR was used to determine the percentage of solids in RSO as the ratio between the response from the hydrogen nuclei in the solid phase and that from all the hydrogen nuclei. Fig. (3) illustrates that the solid fat content profiles (SFC) decreases when the temperature increases. It was constant for temperatures above 20°C. At lower temperature, solid fat content was relatively high in RSO.

The solid fat content accompanies to the saturated fatty acid and phospholipids of rubber seed oil. This can be explained by the fact that RSO had saturated fatty acid (19.12±0.28%) and phospholipids (3%). The solid fat content profiles of RSO indicate that the RSO at different temperatures gave different percentages of solids (Fig. 3), its indicate RSO contain a high unsaturated fatty acid linoleic [14].

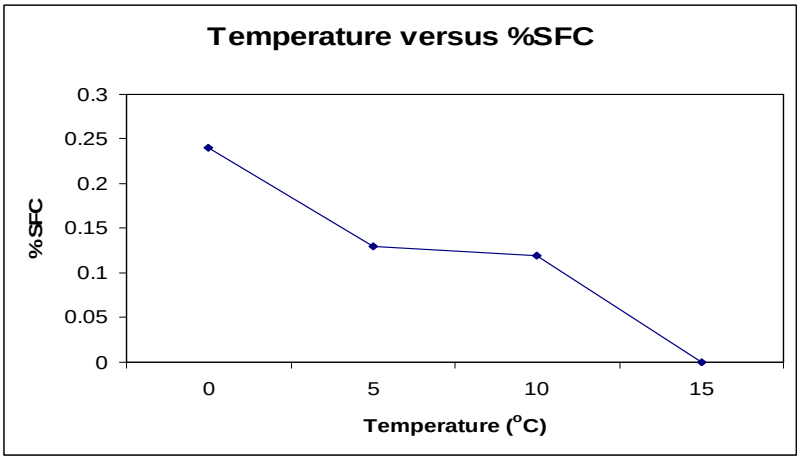

Fig. 3: Temperature versus SFC content of RSO

Conclusion

The fact that the thermal properties suggest the presence of mixed triacylglycerol groups with different melting and crystallization curves in the used conditions. On the basis of the results of this study, DSC can provide information about the nature of TAG interaction in RSO. Recognition of the pattern of interactions in TAG can be helpful in many physical processes in the RSO industry. The solid fat content shows that the RSO at different temperature degrees contain less saturated fatty acid.

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