Rubber Seed Oil Properties, Authentication and Quality Assessment Using (Chloroform: Methanol) as Solvent

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Abstract

Physicochemical and instrumental characterization of rubber (*Hevea brasiliensis* (kunth. Muell.)) seed oil (RSO) were carried out for the purpose of quality assessment, identification, and authentication. Properties such as free fatty acid (FFA %), acid value, color, saponification value, iodine value, and unsaponifiable matter were determined. Fatty acid (FA) composition and TAG profiles were determined using gas chromatography (GC) and high performance liquid chromatography (HPLC), respectively. Structural features of RSO were also determined using FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy. The natural form of RSO is highly acidic (acid value=17.43±0.06 mg KOH/g). The saturated FAs are palmitic (9.10±0.06) and stearic (12.63±0.01), and the main unsaturated FAs are oleic (25.31±0.13), linoleic (36.31±0.09), and linolenic (15.78±0.18). The oil can be classified as semidrying. Polyunsaturated TAGs of LnLnL and LnOO were the major TAGs found in RSO. FTIR, ^{1}H NMR and ^{13}C NMR analysis confirmed that RSO is composed mainly of TAG of saturated and unsaturated FA.

Keywords: Triacylglycerol; Rubber seed oil; physicochemical properties

1. Introduction

The rubber tree (*Hevea brasiliensis*) is a perennial plantation crop, and it's indigenous to South America. Since its introduction to the orient around 1876, it has been cultivated as an industrial crop. The rubber tree grows in hot, humid climates [1], native of the tropical rain forests of the Amazon valley, is tall tree attaining a height of about 20m [2].

Rubber seeds yield from rubber plantations varies from 100 to 150 Kg/ha, depending on soil fertility and crop density. Rubber seeds are not available all the year round. There are two harvesting seasons in a year. The main season which is most prolific is from august to September and the second less prolific season is from January to February [3].

RSO is a semi-dried substance. The RSO has many applications for industrial purposes [4], possible uses for the manufacture of fatty acids [5], paint, alkyd resin, soap making [6], surface coatings [7], water-reducible alkyds [8], production biodiesel and use as fuel compression ignition engines [9].

Polyunsaturated fatty acids (PUFAs) consist of omega-6 (linoleic acid, γ-linolenic acid) and omega-3 (α-linolenic acid, eicosapentaenoic acid, docosahexaenoic) [10]. The importance of PUFAs came under highlight when these fatty acids proved to be essential to human health such as reduce cholesterol in body; reduce heart attack and stroke risk [11].

2. Materials and Methods

2.1 Materials

Rubber seeds were collected from (RRI) 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 (chloroform: methanol) as solvent at 60°C for 6 hours. RSO was subjected to degumming process using 20 ml water, 20 ml (0.2 %) H_3PO_4 and washed using 50 ml 95 % (v/v) ethanol, and 50 ml water.

2.2 Analysis of RSO

The physicochemical properties of RSO such as color, acid value, FFA%, iodine value, saponification value, unsaponifiable matter were determined [3].

2.3 GC

The FA composition of RSO was determined using its fatty acid methyl esters

which it's injected into GC for analysis. GC analysis was performed on shimadzu, GC equipped with flame ionization detector and capillary column (30 m × 0.25 mm × 0.25 µm 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. The identification of the peaks was carried out by retention times by means of comparing them with genuine standards analyzed under the same conditions [3].

2.4 HPLC

TAG of RSO was determined by using HPLC from waters model 1515 equipped with refractive index detector, and spherisorb C18 column, (150 mm × 4.8 µm ×3 mm). 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 into HPLC with total running time of 50 min [3].

2.5 FTIR spectroscopy

Nicollet 400D (Nicollet Instrument INC. Madison, WI) FTIR was used to measure FA content of the TAG. A very thin film of RSO was covered on NaCl cells (25mmi.d × 4mm thickness) and was used for analysis [3].

2.6 NMR spectroscopy

^{1}H and ^{13}C NMR of RSO was recorded on Bruker 300 NMR spectrophotometer (Bruker instruments Inc., korlsruhe, Germany). 20 mg of sample was dissolved in 1ml of $CDCl_3$ and introduced into the NMR tube [3].

3. Results and Discussion

The FA composition of the RSO is about 21.64±0.21% saturated FA, comprising mainly palmitic acid (9.10±0.06%) and stearic acid (12.63±0.01%), and about 77.40±0.26% unsaturated FA, comprising essentially oleic acid (25.31±0.13%), linoleic acid (36.31±0.09%), and linolenic acid (15.78 ± 0.18%) (Table (1)). The FA composition of vegetable oils is a main feature of their description and identification. It is also used as an indicator of the purity and quality of the oil because the type and quantity of each FA vary from one vegetable oil to another [12].

Table (1)
FAs composition of Rubber Seed Oil.

FA		*%*
Saturated		
C16:0	Palmitic acid	9.10±0.06
C18:0	Stearic acid	12.63±0.01
	Total	21.64±0.21
Unsaturated		
C18:1	Oleic acid	25.31±0.13
C18:2	Linoleic acid	36.31±0.09
C18:3	Linolenic acid	15.78±0.18
	Total	77.40±0.26
Others		0.96±0.06

The physicochemical properties determined as follows. The color of RSO (a * 0.60±0.01, b* 2.91±0.05, L* 30.91±0.6) shows that RSO is dark in color. This indicates that, in natural form, RSO is only suitable in applications where bright color is not the major consideration, e.g. pigmented coatings. However, it is possible by some workers. The percent FFA (8.76±0.03% as oleic acid) and acid value (17.43±0.06 mg KOH/g) show that RSO is highly acidic. Alkaline refining of the oil can carried out to reduce the level of acidity. The RSO shows high iodine value (134.44±0.31) comparing with Iodine value of

palm oil (52) [13] due to the high content of unsaturated fatty acids such as oleic acid (22.9%) which mean the RSO is semi-drying oil, and can use it in the paint industry.

The Saponification value of RSO (183.32±0.29) similar to the other typical seed oil such as sunflower, and corn oil [14] 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 [13], and *perah* seed oil [15], with average range saponification number of (175-250) [3]. 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 is 2.19±0.03%. The TAG level of RSO was 89.0%, indicating that the TAG percentage depend on the percent of both FFA and unsaponifiable matter.

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 [15]. The comparison of TAG was determined as the percentage of the peak area. The mechanism in separating the TAG involves the chain length and degree of instauration of the fatty acids [3]. Table (2) 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, and saturated TAG of PPP.

Table (2)
TAGs composition of Rubber seed oil.

TAGs	*ECNs*	*Relative composition (%)*
Polyunsaturated		
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
Monounsaturated		
PPL	46	6.4
POP	48	4.4
POS	50	1.0
Saturated		
PPP	48	3.9

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

FTIR analysis. The main peaks and their assignment to functional groups of the RSO are given in Table (3). The results showed characteristic strong absorption bands at 1744 cm^{-1} for the ester carbonyl (C=O) functional groups, at 1714 cm^{-1} for the carboxylic acid (C=O), and at 1463 cm^{-1} for a double bond, respectively. The functional groups present in RSO are similar to those in other vegetable oil such as *Jatropha curcas* seed oil [16].

Table (3)
The main peaks in the FTIR spectrum of rubber seed oil and their assignment.

Peak (cm^{-1})	*Functional group*
3466	O-H stretching vibration (alcohol)
3009, 2924, 2854	C-H stretching vibration (aliphatic)
1744	C=O stretching vibration (ester)
1714	C=O stretching vibration (carboxylic acid)
1463	C=C bending vibration (aliphatic)
1239, 1165, 1098	C-O-C stretching vibration (ester)
722	C-H group vibration (aliphatic)

The ^{1}H NMR analysis of RSO. The main signals assignments of RSO are listed in Table (4). The results shows the ^{1}H NMR spectrum of RSO methylene protons ($-CH_2$), terminal ($-CH_3$), protons α to the carbonyl groups ($-CH_2-C$), diallylic methylene ($-C=C-CH_2-C=C-$), and protons in α and β position in glyceryl.

The ^{13}C NMR analysis of RSO. The main signals assignments of the RSO are listed in Table (5). The results showed the ^{13}C NMR spectrum of RSO contain the allylic carbon atoms at 27 ppm, methylene carbon atoms at (27-30 ppm), glyceryl carbon atoms at (61.8-68.8 ppm), olefinic carbon atoms at (127.7-129.6 ppm), and carbonyl carbon atoms at (172.23-177.03 ppm).

Table (4)
The main signals present in ^{1}H NMR spectrum of RSO, and their assignment.

δ (ppm)	*Assignment*
0.77-0.86	$-CH_3$ terminal methyl
1.16-1.20	$-CH_2$ saturated aliphatic chain
1.93-1.95	$-CH_2-C$ methylene α to terminal methyl
2.19	$-CH_2-O-C=O$ acyl methylene
2.66-2.69	$-C=C-CH_2-C=C-$ diallylic methylene
4.02-4.19	$-CH_2-O-CO-$ in α position in glyceryl
5.20-5.23	-CH-O-CO- in β position in glyceryl

Table (5)
The main signals present in ^{13}C NMR spectrum of RSO and their assignment.

δ (ppm)	*Assignment*
27.04	allylic carbons (single)
28.9-29.6	Carbon α to carbonyl
61.9-64.6	C_1 or C_3 of glyceryl
67.5-68.8	C_2 of glyceryl
127.7-129.7	Olefinic carbon
172.4-177.8	Carbonyl carbon

Conclusion

The results obtained in this study by using (chloroform: methanol) as solvent not high difference comparing with using hexane as solvent and the results obtained are useful for identification purposes and for authentication of the quality of RSO. They also constitute baseline information for the study and quality control of products such as alkyds that may derived from RSO.

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الخلاصة

الخواص الكيميائية الفيزيائية لزيت بذرة المطاط (*Hevea brasiliensis* (kunth. Muell.)) قد انجزت لغرض تقييم الجودة، تحديد الهوية، والتوثيق. الخواص مثل الحوامض الدهنية الحرة (FFA%) ، اللون، قيمة التصوبن، قيمة اليود والمواد الغير متصوبنة قد تم تحديدها. تركيب الحوامض الدهنية (FA) وتركيب (TAG) قم تم تحديدها باستخدام (GC) و(HPLC) على التوالي. تركيب RSO قد تم تحديده باستخدام FTIR، ^{1}H NMR و ^{13}C NMR. الشكل الطبيعي للRSO عالي الحامضية (قيمة حامض= ١٧,٤٣±٠,٠٦ ملغم KOH/غم). الحوامض الدهنية المشبعة هي palmitic (9.10±0.06) و stearic (12.63±0.01) والحوامض الدهنية الرئيسية الغير مشبعة هي oleic (25.31±0.13)، linoleic (36.31±0.09) و linolenic (15.78±0.18). يمكن تصنيف الRSO كجزئي الجفاف. TAGs الغير مشبعة الرئيسية الموجودة في RSO هي LnLnL و LnOO. التحليل باستخدام FTIR، ^{1}H NMR و ^{13}C NMR أكد بان RSO يتكون بصورة رئيسية من الحوامض الدهنية المشبعة والغير مشبعة.