Rubber Seed Oil Quality Assessment and Authentication

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ABSTRACT: Physicochemical and instrumental characterization of rubber (*Hevea brasiliensis* Müll. Arg.) seed oil (RSO) was carried out for the purposes of quality assessment, identification, and authentication. Properties such as color (Lovibond), specific gravity, percent FFA (as oleic acid), acid value, saponification value, iodine value, and viscosity were determined. FA composition and M.W. averages of RSO were determined using GLC and gel permeation chromatography (GPC), 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 ≈ 43.6 mg KOH/g). The saturated FA are palmitic (17.50%) and stearic (4.82%), and the main unsaturated FA are oleic (25.33%), linoleic (37.50%), and linolenic (14.21%). The oil can be classified as semidrying. GPC shows an unusual peak that is due to a very high M.W. (≈38,800) fraction that is not found in the chromatogram of known vegetable oils and is therefore unique to RSO. FTIR, ^{1}H NMR, and ^{13}C NMR analyses confirmed that RSO is composed mainly of TAG of saturated and unsaturated FA. Functional groups such as carbonyl, olefinic unsaturation, esters, glyceryl, methylene, and terminal methyl that are present in vegetable oils are also present in RSO.

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Vegetable oils are used not only for edible purposes but also for nonedible applications such as drying oils in paints. They are increasingly being used in the production of renewable industrial resources such as long-chain FA and oleochemicals for the chemical industry, hydroxyl acids, epoxy FA, conjugated unsaturated FA, plasticizers, surfactants, and adhesives (1–4). Positive industry trends indicate the continued development, use, and expansion of vegetable oils in fields such as environmentally friendly water-reducible alkyd resins. With the development of these new applications, determination of the quality of vegetable oils that is required in a particular field has become an important subject from both commercial/industrial and analytical perspectives. For instance, owing to price differentials between vegetable oils, a less expensive oil could be used to adulterate the more expensive one to the detriment of quality. A case in point was the adulteration of olive oil with rapeseed oil, the consumption of which led to the death of many persons (5). Characterization of vegetable oils and consequently their authentication help to control adulteration. Characterization also constitutes an integral part of determining their end uses, because the properties of the raw oil determine the quality of the derivable products (6). For example, the rate of drying of oil-modified alkyds is related not only to the level of unsaturation but also to the types and amounts of PUFA present in the oil.

Most current work on vegetable oil quality assessment is based on a number of instrumental techniques such as gel permeation chromatography (GPC), FTIR, MS, NMR, and HPLC (7–11). The technical literature is replete with information on the characteristics and authentication of vegetable oils with useful industrial applications (12–14).

This study concerns the characterization of rubber seed oil (RSO), which is assuming prominence as a binder in surface coatings and as a plasticizer/stabilizer for PVC and natural rubber (15–19). Characterization and determination of the quality features that would distinguish RSO from other vegetable oils were carried out using GPC, GLC, and FTIR and NMR spectroscopy as well as chemical analyses. The goals were to give better insight into the characteristic features of RSO in relation to its end uses and to establish a basis for studies of more complex products, e.g., alkyds, that are derivable.

EXPERIMENTAL PROCEDURES

Materials. Rubber seeds were collected from the estate of the Rubber Research Institute of Nigeria (RRIN). The 40-ha estate is planted with RRIN-developed high-latex-yielding clones (NIGERIA 800 series) that are about 17 yr old. The seeds were further dried, to a moisture content of about 7%, and their oil was extracted in the pilot mill of the institute. The yield was 23%.

Potassium hydroxide, hydrochloric acid, potassium iodide, potassium iodate, sodium thiosulfate, iodine trichloride, and other reagents used in the chemical characterization of RSO were all of analytical grade and were obtained from British Drug House (BDH), Poole, England.

Analysis of RSO. The physicochemical properties of RSO such as color, specific gravity, acid value, saponification value, iodine value, and viscosity were determined according to IUPAC standard methods (20).

GLC. The FA composition of RSO was determined using its methyl esters on a Hewlett-Packard 5890 Series II gas chromatograph with FID (Avondale, PA). The injection and detection temperatures were 250 and 300°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 20 mL/min.

GPC. M.W. averages of RSO were determined using GPC, performed on a Hewlett-Packard 1081 B high-performance liquid chromatograph with refractive index detector consisting of

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three Styragel HT3 columns (300 × 75 mm; Waters, Milford, MA) arranged in series. THF of HPLC grade was used as the mobile phase. Numerical values for the M.W. were obtained by comparison with calibration of propylene glycols as internal standards.

FTIR spectroscopy. A very thin film of RSO was spread on NaCl plates and the FTIR spectrum was recorded using a Nicolet 400D FTIR instrument (Madison, WI).

NMR spectroscopy. Proton and ^{13}C NMR of RSO was recorded on Bruker 300 MHz NMR spectrophotometer (Bruker Instruments Inc., Karlsruhe, Germany). About 20–30 mg of sample was dissolved in 1 mL of $CDCl_3$ and introduced into the NMR tube. Tetramethylsilane was used as internal standard.

RESULTS AND DISCUSSION

GLC analysis. The FA composition of RSO is about 22.3% saturated FA, comprising mainly palmitic acid (17.5%) and stearic acid (4.8%), and about 77.0% unsaturated FA, comprising essentially oleic acid (25.3%), linoleic acid (37.5%), and linolenic acid (14.2%) (Table 1). Other FA occur in minute quantities. 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. For instance, rapeseed oil contains about 50% oleic acid whereas soybean oil contains only 20% oleic acid. Conversely, the linoleic acid content in soybean oil is about 40% but about 20% in rapeseed oil. Palmitic acid content is used as an indicator of adulteration of cottonseed oil by palm oil because cottonseed contains only 26% palmitic acid and palm oil contains about 40% palmitic acid. The FA composition of RSO obtained in this study will be a useful tool for identifying RSO since the amount of individual FA in a vegetable oil can be used as indicator of quality or purity in the industry (12,21).

Similarly, in the area of industrial application, the suitability of a vegetable oil as a binder in surface coatings is related to its FA composition, especially the quantities of diene and triene acid. On this premise, linseed oil, with a very high content (approximately 52%) of linolenic acid (a triene acid), is the premium drying oil in the surface coating industry (22). However, owing to its high content of linolenic acid, films of linseed oil have a high tendency to "yellow" (i.e., deteriorate) on aging (23). Therefore, the lower linolenic acid content of RSO means that a film of the oil has a lower tendency to yellow. This advantage can be exploited in its application in surface coatings.

TABLE 1
FA Composition of Rubber Seed Oil

FA		%
Saturated		
$C_{16:0}$	Palmitic acid	17.51
$C_{18:0}$	Stearic acid	4.82
	Total	22.33
Unsaturated		
$C_{18:1}$	Oleic acid	25.33
$C_{18:2}$	Linoleic acid	37.50
$C_{18:3}$	Linolenic acid	14.21
	Total	77.04
Others		0.63

Physicochemical analysis. The physicochemical properties determined are as follows. The Lovibond index (22R, 23.2Y; 1″ cell) shows that RSO is dark in color. This indicates that, in its natural form, RSO is only suitable in applications where bright color is not the major consideration, e.g., pigmented coatings. However, it is possible to enhance the color of the oil by bleaching as has been reported by some workers (24). The specific gravity (0.916 at 30°C) is comparable to those of known vegetable oils and seems to indicate that no heavy element is present in the oil. The percent FFA (21.40% as oleic acid) and acid value (43.62 mg KOH/g) show that RSO is highly acidic, implying that larger amounts of polyol will be required in the transesterification reaction before the polyesterification reaction leading to the formation of alkyds. Alternatively, alkaline refining of the oil can be carried out to reduce the level of acidity. The saponification value of about 203 mg KOH/g shows that the nature of alkali hydrolysis of RSO is similar to that of common vegetable oils such as coconut oil (25). From the iodine value of 136 g I_2/100g, one can infer that RSO is a semidrying oil. This is further supported by the low PUFA content (Table 1). The bulk viscosity of pure RSO is 0.42 P.

All these characteristic quality features of RSO are exploited in the production of environmentally friendly solvent-borne and water-reducible coatings (15–17) and in other industrial uses (18,19).

M.W. analysis. The GPC chromatogram of RSO is shown in Figure 1. This figure provides useful information on the unique characteristics of RSO in terms of M.W., M.W. distribution, peak separation, and concentration. Figure 1 has four peaks, **a–d**. Comparison of Figure 1 with GPC chromatograms of other vegetable oils shows that the presence of the fourth peak (**d**) in the chromatogram of RSO is unique and is in contrast to the three peaks typically observed in the gel permeation chromatogram of known vegetable oils (26).

The M.W. averages and the percent concentrations corresponding to the peaks are presented in Table 2. From the comparison of Figure 1 with the chromatograms of known vegetable oils, the observed peaks can be assigned as follows: peak **a** comprises a low M.W. fraction consisting of a mixture of FFA and MAG, peak **b** is due to DAG, and peak **c** is a mixture of TAG and probably oligomeric TAG. Peak **d,** representing a rather high M.W. fraction (≈38,800 daltons), is believed to consist of higher M.W. species. The origin of this component in RSO can be traced to the polyisoprene molecules present in natural rubber latex as well as the tocotrienols found in RSO (24). Polyisoprene (about 1% w/w) has reportedly been isolated from the sludge obtained from degumming of RSO. This high M.W. fraction is believed to be responsible for the difficulty in filtering RSO. From Table 2, the FFA content of 20% agrees well with the value of 21.40% obtained from chemical

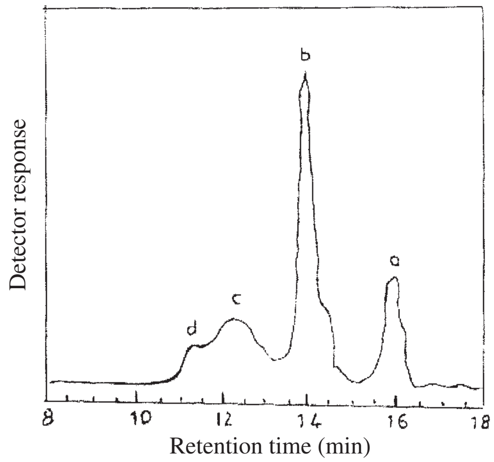

FIG. 1. Gel permeation chromatograph of rubber seed oil.

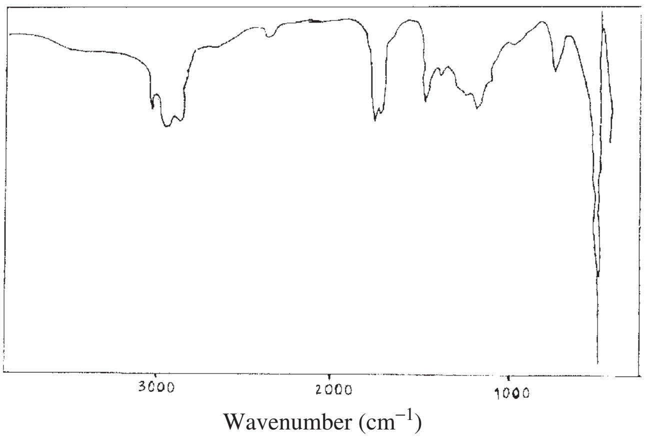

FIG. 2. FTIR spectrum of rubber seed oil.

analysis. Also, the equivalent weight of 276.53 estimated from the saponification value (23) compares favorably with the value of 249 obtained from GPC. The number average (M_n) and weight average (M_w) M.W. of RSO are, respectively, 7,393 and 13,076. The polydispersity or M.W. distribution (M_w/M_n) is 1.77.

FTIR analysis of RSO. Figure 2 shows the FTIR spectrum of RSO. The main peaks and their assignment to functional groups are given in Table 3. The results in Table 4 show that the functional groups present in RSO are similar to those in other vegetable oils (25). However, the carbonyl band occurs as a doublet, probably indicating two types of C=O. A similar observation has been reported for the IR spectrum of tung oil (27).

^{1}H NMR analysis of RSO. Figure 3 shows the ^{1}H NMR spectrum of RSO. The signal assignments are listed in Table 4. The distinguishable groups are the protons of the terminal methyl of the FA chain, the methylene group next to the terminal methyl (1.6 ppm), methylene groups adjacent to one double bond (2 ppm), and methylene groups adjacent to two double bonds (2.7 ppm). Others are methylene groups of glyceryl (α: 4.27–4.31 ppm and β: 5.29–5.41 ppm) and protons attached to C=C.

^{13}C NMR analysis of RSO. Figure 4 is the ^{13}C NMR spectrum of RSO. The signals, assigned in Table 5, can be grouped into four well-defined regions: 173–175 ppm due to the C-atom of the carbonyl group; 127–132 ppm due to unsaturated C-atoms; 62–69 ppm due glyceryl C-atoms; and 14–34 ppm due to methylene C-atoms in FA moieties. The methylene C-atoms are further distinguished into those adjacent to one double bond or single allylic (27 ppm) and those adjacent to two double bonds or double allylic (25.5 ppm). The adjacent double bond and carbonyl groups are believed to influence the methyl C-atoms and

TABLE 2
M.W. Averages and Percent Concentration of the Fractions Obtained from the Gel Permeation Chromatogram of Rubber Seed Oil

Peak	Ave. M.W. (daltons)	% Conc.
a	249	20
b	1,460	25
c	9,066	45
d	38,801	<1

TABLE 3
The Main Peaks in the FTIR Spectrum of Rubber Seed Oil and Their Assignment

Peak (cm^{-1})	Functional group
3009, 2925, 2854	C–H stretching vibration (aliphatic)
1745, 1711	C=O stretching vibration (ester)
1462	C=C bending vibration (aliphatic)
1239, 1165, 1099	C–O–C stretching vibration (ester)
722	C–H group vibration (aliphatic)

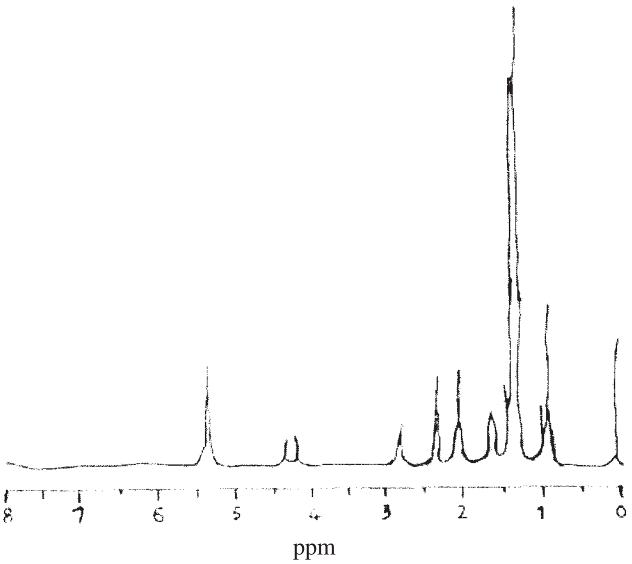

FIG. 3. ^{1}H NMR of rubber seed oil.

TABLE 4
Signals Present in ^{1}H NMR Spectrum of Rubber Seed Oil and Their Assignment

δ (ppm)	Assignment
0.85–0.97	–CH_3 terminal methyl
1.25–1.42	–CH_2 saturated aliphatic chain
1.61	–CH_2–C methylene α to terminal methyl
2.07	–CH_2–C=C allylic methylene
2.28–2.33	–CH_2–O–C=O acyl methylene
2.76–2.80	–C=C–CH_2–C=C– diallylic methylene
4.13–4.17	–CH_2–O–CO– in α position in glyceryl
4.27–4.31	–CH=CH– olefinic (FA chain)
5.29–5.41	–CH–O–CO– in β position in glyceryl

TABLE 5
Signals Present in ^{13}C NMR Spectrum of Rubber Seed Oil and Their Assignment

δ (ppm)	Assignment
14.01	ϖ^1
24.77	ϖ^2
26	Allylic carbon (double)
27	Allylic carbon (single)
31.46–33.94	Carbon α to carbonyl
62.03	C_1 or C_3 of glyceryl
68.86	C_2 of glyceryl
127.04–131.82	Olefinic carbon
172.73–173.79	Carbonyl carbon

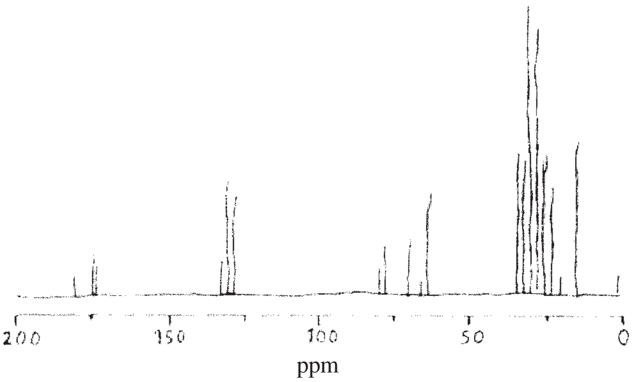

FIG. 4. ^{13}C NMR of rubber seed oil.

produce a slight difference in their chemical shifts compared with those of the standards. Both ^{1}H and ^{13}C NMR spectra of RSO are similar to those of known vegetable oils (28).

The results obtained in this study 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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