

REFINING, MODIFICATION AND CHARACTERIZATION OF TOBACCO SEED (*Nicotiana tobacum*) OIL FOR ITS IMPROVED POTENTIALS FOR INDUSTRIAL USE.

V. G. Awolola*, D. S. Ogunniyi and O. O. Oluwaniyi

Department of Chemistry, University of Ilorin, Ilorin, Kwara State, Nigeria.

*Corresponding author: e-mail: vickyfani@yahoo.com, 08033557021

ABSTRACT

Tobacco seed oil (TSO) was extracted, refined and modified via epoxidation and hydroxylation. The physico-chemical properties of the unmodified oil samples and modified oil samples were carried out using standard methods. They were further characterized by infra-red (IR) spectroscopy and gas chromatography (GC). The gas chromatogram of the unmodified and modified oil samples showed a high content of polyunsaturated fatty acids. The IR spectrum of the modified products showed the presence of epoxy ring in the epoxidized oil with stretching vibration at 1239.83cm^{-1} and the opening of the oxirane in the hydroxylated oil was also confirmed by IR spectroscopy with the introduction of OH stretching signal at 3466.21cm^{-1} . The percentage oxirane, which is a measure of the degree of epoxidation, confirmed the success of the modification via epoxidation reaction. The epoxidized tobacco seed oil (ETSO) can be used as plasticizers-stabilizers, adhesives, ingredients for coating formulations, industrial toolings and composites. The hydroxylated tobacco seed oil (HTSO) can be used to produce polyhydroxyl compounds which are useful raw materials for polyurethane production.

Keywords: Epoxidation, hydroxylation, gas chromatography (GC), infra-red (IR) Spectroscopy, oxirane

INTRODUCTION

The application of modified natural fats and oils in the chemical industries is becoming more and more interesting because of their availability from renewable resources [Ikhuoria & Dadson, 2007]. Epoxidation is one of the chemical methods of transforming natural fats and oils which are also known as 'plants oils' into polymerizable monomers [Ikhuoria & Dadson, 2007]. Many plants oils such as soybean oil, rubber seed oil, Karanja seed oil, linseed oil, castor seed oil, African oil bean seed oil etc [Ikhuoria & Dadson, 2007; Okieimen *et al.*, 2005; Vaibhav *et al.*, 2007; Akpan *et al.*, 2006] have been modified in various forms to improve their industrial potentials. Epoxidation is the formation of an oxirane group (epoxides) by the reaction of peroxyacids (peracids) and olefinic and aromatic double bonds [Okieimen *et al.*, 2002]. Epoxides are of extreme commercial value. Epoxidised vegetable oils and esters derived from long chain olefins have found important applications as plasticizers and additives for polyvinyl chloride (PVC) [Gan *et al.*, 1995]. The plasticizers are used for polyvinyl chloride resins to improve its quality in terms of flexibility, elasticity and toughness and to impart stability to the polymer towards heat and UV radiation [Vaibhav *et al.*, 2007]. Due to the high reactivity of the oxirane ring, epoxides can also be used as starting materials for a variety of chemicals such as alcohols, glycols, alkanolamines, carbonyl compounds, olefinic compounds and polymers like polyesters, polyurethanes and epoxy resins [Carlson & Chang, 1985]. Today, one of the most important epoxidized vegetable oils is epoxidized soybean oil (ESO) and its worldwide production is about 200,000 tonnes/ year [Rusch *et al.*, 1999].

Tobacco plant from which the oil (TSO) is extracted belongs to the night shade family or *solanaceae* [Sheen & Calvert, 1969]. The plant is an annual and a potential woody plant. The leaves are usually used after aging and processed in various ways for smoking, chewing and snuffing [Sheen & Calvert, 1969]. The main food reserve of the seed is oil, while starch is negligible or absent. The composition of the fatty acids contained in oils generally (fatty acid spectrum) determines the use to which the oil is put [Hill, 2000]. Tobacco seed oil (TSO) contains linoleic acid [Bekele, 2000] as the main fatty acid and it is classified as a linoleic acid, which falls in the category of semi-drying oil and has a high degree of unsaturation. The properties of TSO suggest the potential use of the oil in food and coating industries [Mukhtar *et al.*, 2007]. Although, the refined oil of a certain tobacco variety has been reported to be edible [Talaquani *et al.*, 1986], tobacco seed oil (TSO) is not commonly used as an edible oil in most parts of Africa. This is because the tobacco plant is cultivated mainly for its leaves. TSO has been used for the production of soap, shoe polish and varnishes [Purseglove, 1991] and it has also been explored as an alternative to diesel fuel [Giannelos *et al.*, 2002]. There is a need to focus on the seeds of tobacco plant rather than the leaves since a lot of campaign is going on to reduce the number of tobacco smokers worldwide and the economic value of the leaves may likely decline with the continued campaign. Finding use for the seeds therefore will increase the utilization of the TSO and add economic value to the tobacco plantations [Ogunniyi & Odetoye, 2008]. Therefore, the aim of this article is to report the modification of TSO via epoxidation and hydroxylation, and to analyze the different oil samples for their physico-chemical properties and characterize them by infra-red (IR) spectroscopy and gas chromatography (GC).

MATERIAL AND METHODS

The tobacco seed that had been dehulled and dried was obtained from a local farmer in Ilorin, Kwara State, Nigeria. The oil was extracted from the grinded seed by solvent extraction using n-hexane. Analytical grade reagents (BDH Ltd, Poole, England) were used in all cases.

Refining of extracted oil

The methods described by Haas and Mittelbach [Haas & Mittelbach, 2000] were adopted for the refining of tobacco seed oil (RTSO). The extracted tobacco seed oil was degummed at 80°C with orthophosphoric acid while the degummed oil was neutralized with NaOH at 70°C. The neutralized tobacco seed oil was finally bleached with activated charcoal at 80°C to complete the refining process.

Modification of Tobacco seed oil

Epoxidation

Epoxidation of TSO was carried out at 70°C using peroxyacetic acid prepared *in-situ* by reacting hydrogen peroxide with acetic acid in the presence of small quantity of concentrated sulphuric acid [Okieimen *et al.*, 2002]. A known amount of TSO was placed in a 250ml flask containing the required amount of acetic acid and sulphuric acid, condenser, thermometer and stirrer. The flask was allowed to attain the reaction temperature in a thermostated bath (controlled to better than $\pm 2^\circ\text{C}$) and the hydrogen peroxide previously equilibrated at the reaction temperature was added dropwise with continuous stirring for about 1 hr. The stirring was controlled to ensure that the oil in the mixture was finely dispersed. Aliquot of the reaction mixture was withdrawn into a large excess of cold water in a separating funnel. The aqueous layer was drawn off and the oil layer was washed successively with warm water until it was acid free. The resulting epoxidized TSO (ETSO) was characterized using IUPAC standard methods [Paquot, 1979].

Hydroxylation

The hydroxylation reaction was carried out as described for epoxidation, with a small modification. At the end of the epoxidation reaction, the temperature of the reaction was raised to 80°C and the mixture held at this temperature for another 14 h. At the end of the reaction, the modified product (HTSO) was characterized using IUPAC standard methods [Paquot, 1979].

Characterization

Physico-chemical properties

The physical and chemical properties of the crude oil, refined and modified oil samples: density, specific gravity, refractive-index, iodine value (IV), acid value (AV), peroxide value (P.V), saponification value (SV), ester value (E.V.) and the oxirane content of the modified products were determined according to standard methods [Paquot, 1979].

Gas Chromatography

The fatty acid composition of RTSO and modified products (ETSO and HTSO) was determined using its methyl esters on a gas chromatograph (Hewlett Packard 6890 Powered with HP Chemstation Rev. A 09.01 [1206]) with a 30m long HP-INNOWAX (cross-linked PEG) type column while a flame ionization detector was used. The injection and detection temperatures were 230 and 275°C, respectively. Nitrogen was used as the carrier gas. The inside diameter of the column was 0.32mm and film thickness was 0.50µm.

Infrared spectroscopy

Infrared spectra of RTSO, ETSO and HTSO were recorded using a Model M 500 IR spectrophotometer. The samples were spread over NaCl cells. IR spectra were recorded in the range of 4000-500cm⁻¹.

RESULTS AND DISCUSSION

Physico-chemical properties of tobacco seed oil and modified products

The physical and chemical properties of the tobacco seed oil and modified products (ETSO, HTSO) are presented in Table 1. The values obtained for the four oil samples are close to and compare favorably well with those of conventional oils such as African oil bean seed oil [Ikhuoria & Dadson, 2007], rubber seed oil [Okieimen *et al.*, 2002], linseed oil, soybean oil and used in epoxidation studies and other industrial applications.

The saponification values of ETSO and HTSO are higher than crude tobacco seed oil (CTSO) and refined tobacco seed oil (RTSO). This increase is attributed to the introduction of oxygen and hydroxyl groups during the epoxidation and hydroxylation reactions respectively [Ikhuoria & Dadson, 2007]. The CTSO and RTSO have high I.V because of the high degree of unsaturation of the oils. As the oil was being modified via epoxidation and hydroxylation, however, the degree of unsaturation reduces, which therefore led to the decrease in the I.V of ETSO and HTSO respectively. This decrease in the iodine value is one of the indications of the successful modification of TSO. The percentage oxirane, which is a measure of the degree of epoxidation, also confirmed the success of the epoxidation reaction. ETSO has an oxirane content of 4.00% which reduced to 1.56% after hydroxylation. This is similar to observations from previous studies with other oils [Ikhuoria & Dadson, 2007; Okieimen *et al.*, 2005].

Table 1: Physico-chemical properties of CTSO, RTSO, ETSO, and HTSO

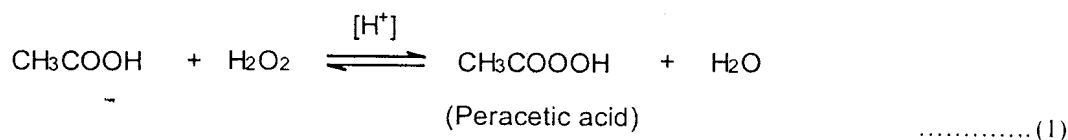
PROPERTIES	CTSO	RTSO	ETSO	HTSO
Density (g/cm ³)	0.897	0.906	0.911	0.917
Specific gravity (30°C)	0.897	0.906	0.911	0.917
Refractive index	1.445	1.443	1.446	1.451
Iodine value (gI/100g)	156.69	179.2	60.41	39.47
Acid value (mgKOH/g)	26.07	13.04	14.07	12.06
Saponification value (mgKOH/g)	212.2	212.2	215.4	234.7
Peroxide value (µg/g)	5.0	10.0	0.3	4.0
Ester value (mgKOH/g)	186.13	199.16	201.33	222.64
Oxirane %	NR	NR	4.00	1.56

NR= Not recorded

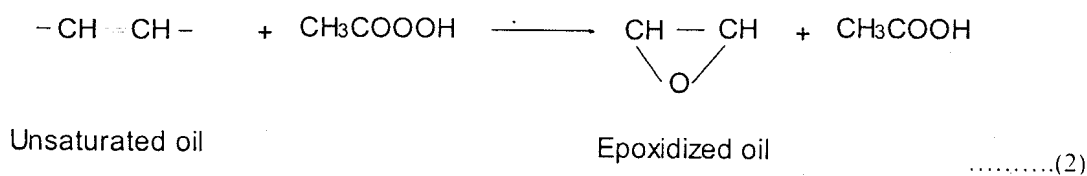
Mechanism of Modification (epoxidation and hydroxylation) of tobacco seed oil

The chemistry of the epoxidation and hydroxylation may be explained in terms of the following reaction processes [Okieimén *et al*, 2002].

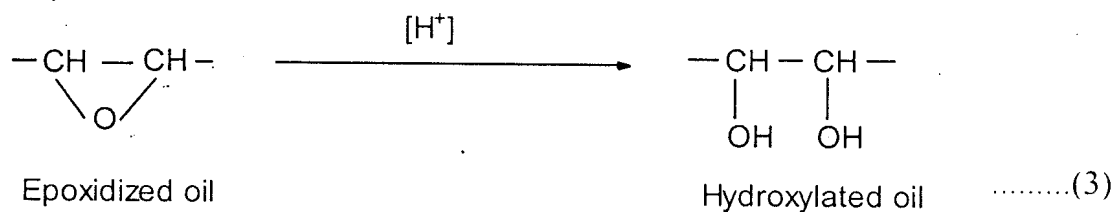
Formation of Peracetic acid /peròxyacid



Epoxidation



Hydroxylation



The formation of peracetic (peroxyacetic) acid from acetic acid and hydrogen peroxide is a reversible reaction (Equation 1). The peracetic acid generated *in-situ* in the aqueous phase is transferred and used up in the oil phase during epoxidation (Equation 2). The epoxidized tobacco seed was obtained at a reaction temperature of 70°C. At a slightly higher temperature of 80°C, the epoxy rings open up to yield hydroxyl compound (Equation 3). Thus, the epoxidized and the hydroxylated oil are the desired modified products which are also indications of the success of the modification processes.

IR analysis of the tobacco seed oil (CTSO, RTSO, ETSO and HTSO)

IR spectra of CTSO, RTSO, ETSO and HTSO are presented in Fig. 2 while the characteristic wavenumbers (ν) of the observed bands are recorded in Table 2. All the spectra have similar characteristic feature except the spectra for ETSO and HTSO. The spectra obtained for the two modified products are similar to CTSO and RTSO, except for the absorption due to the epoxy and hydroxyl group in ETSO and HTSO, respectively. Spectra of ETSO in Fig. 2C showed a characteristic oxirane ring band at about 1239.83 cm^{-1} which is attributed to the presence of an epoxide and also the confirmation of the epoxidation reaction. This band is completely absent in the IR spectrum of HTSO; this must have been due to epoxy ring opening to form hydroxylated products. Consequently, an O-H stretching signal at about 3466.21 cm^{-1} was obtained in the IR spectrum of hydroxylated oil in Fig. 2D. Also, the absence of the oxirane stretching signal at about 1239.83 cm^{-1} was probable due to the reduction in the oxirane content and the oxirane ring opening of HTSO in Fig. 2D. Again, the disappearance of the C=C band at about 1651.15 cm^{-1} in Fig. 2C and 2D, which is present in CTSO spectra (Fig. 2A) clearly shows that C=C in the starting material has reduced considerably. All other predominant bands in all the spectra showed characteristic -CH_2 bending at a range of 1377.68–1377.62 cm^{-1} , -CH_3 bending at a range of 1464–1463 cm^{-1} . There was also a characteristic C=O stretching band for all the spectra at a range of 1746–1744 cm^{-1} .

Table 2: The main IR peaks and their assigned functional groups of CTSO, RTSO, ETSO and HTSO

CTSO FREQUENCY (CM^{-1})	CTSO ASSIGNED FUNCTIONAL GROUP	RTSO FREQUENCY (CM^{-1})	RTSO ASSIGNED FUNCTIONAL GROUP	ETSO FREQUENCY (CM^{-1})	ETSO ASSIGNED FUNCTIONAL GROUP	HTSO FREQUENCY (CM^{-1})	HTSO ASSIGNED FUNCTIONAL GROUP
3008.96	CH stretching stretching	3008.70	CH stretching	3008.80	CH stretching	3466.21	-OH
2926.64	Olefinic C-H	2926.26	Olefinic C-H	2926.20	Olefinic C-H	1746.70	C=O
2854.51	Olefinic C-H	2854.59	Olefinic C-H	2854.68	Olefinic C-H	1463.83	CH ₂ bending
1746.68	C=O	1746.51	C=O	1744.37	C=O	1377.632	CH bending
1651.15	C=C	1463.97	CH ₂ bending	1464.04	CH ₂ Bending	-	-
1464.01	CH ₃ bending	1377.68	CH ₃ bending	1377.66	CH ₃ bending	-	-
1377.67	CH ₃	1164.84	C-O	1239.83	$\begin{array}{c} \text{---C---C---} \\ \\ \text{O} \end{array}$ oxirane ring	-	-
1164.30	C-O stretching	-	-	-	C-O stretching	-	-

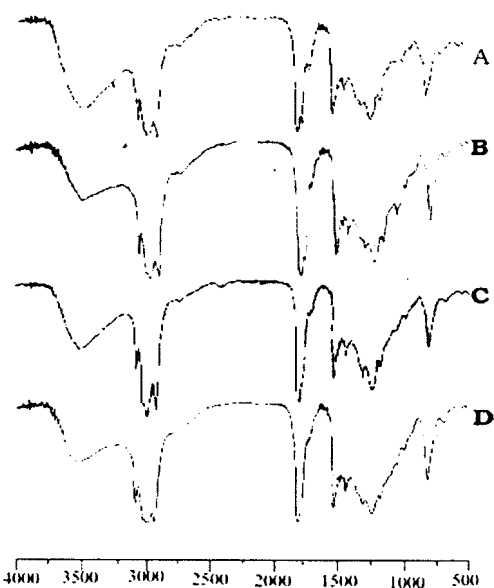


Fig. 1: IR spectra of CTSO (a), RTSO (b), ETSO (c) and HTSO (d)

Table 3: Fatty acid composition of CTSO, RTSO, ETSO and HTSO

OILSAMPLE	FATTYACID	STRUCTURE	(WT%)
CTSO	Linoleic	C18:2	60.51
	Oleic	C18:1	12.33
	Stearic	C18:0	15.36
	Palmitic	C16:0	11.68
RTSO	Linoleic	C18:2	65.26
	Oleic	C18:1	10.85
	Stearic	C18:0	14.03
	Palmitic	C16:0	9.82
ETSO	Linoleic	C18:2	15.40
	Oleic	C18:1	7.92
	Stearic	C18:0	64.36
	Palmitic	C16:0	11.99
HTSO	Linoleic	C18:2	12.80
	Oleic	C18:1	11.67
	Stearic	C18:0	63.61
	Palmitic	C16:0	10.92

Fatty acid composition of CTSO, RTSO, ETSO and HTSO

The main saturated and unsaturated fatty acids in CTSO were found to be 27.04% and 72.84% respectively. The unsaturated fatty acids were linoleic acid [C18:2] and oleic acid [C18:1]. Linoleic acid was the most abundant unsaturated fatty acid followed by oleic acid, with values as follows, CTSO (60.51% and 12.33%), RTSO (65.26% and 10.85%) while for HTSO (12.80% and 11.67%) respectively in each case. Palmitic acid and stearic acid were the saturated fatty acids (Table 3). However, the value of linoleic acid reduced (15.40%) for ETSO and HTSO (12.80%) which is due to the epoxidation reaction while the stearic acid [C18:0] which is a saturated fatty acid increased ETSO (64.36%) and HTSO (63.61%). This means that the degree of unsaturation was reduced as a result of epoxidation.

Mukhtar *et al.*, 2007 reported the total saturated and unsaturated fatty acids in crude tobacco seed oil to be 12.64% and 87.36% respectively which is closely related to the RTSO of this study. Linoleic acid (71.63%) and oleic acid (13.46%) were the most abundant among the unsaturated fatty acids while palmitic acid (8.72%) was the most abundant among the saturated fatty acids.

However, the results reported in this present work for CTSO and RTSO are close to those reported by Mukhtar *et al.*, 2007. The difference in the amounts of the different fatty acids may be due to crude nature of the oil and possibility of deterioration by peroxidation besides the different species of tobacco seed used in the present study or due to the different environmental or geographical conditions.

It is a general agreement that only acids with even number of carbons occur naturally, but it was found that tobacco seed oil contained little amounts of heptadecanoic acid and heptadecenoic acid containing odd number (17) of carbon atoms [Mukhtar *et al.*, 2007].

CONCLUSION

ETSO and HTSO were obtained from tobacco seed oil using peroxyacetic acid prepared in-situ by reacting hydrogen peroxide with acetic acid in the presence of small quantities of concentrated sulphuric acid. The CTSO and RTSO have high I.V because of the high degree of unsaturation of the oils but as the oil was being epoxidized and hydroxylated the degree of unsaturation reduces, which therefore led to the decrease in the I.V of ETSO and HTSO respectively. The decrease in the iodine value is one of the indications that epoxidation and hydroxylation actually took place with TSO. The high oxirane content of ETSO indicates that it can be used as plasticizer-stabilizers for PVC, adhesives, in coating formulations, industrial toolings and composites etc. Also the epoxidized oil can be hydroxylated; therefore hydroxylated product can produce polyhydroxyl compounds which may be useful as raw materials for urethane-type reactions.

REFERENCES

- Akpan, U.G., Jimoh, A. and Mohammed, A.D. (2006). Extraction, Characterization and Modification of Castor seed oil. *Leonardo Journal of Sciences*. **8**, 43-52. ISSN 1583-0233.
- Bekele, E. (2000). *Nicotiana tabacum L.* seed oil. Seminararbeit im Rahmen der Vorlesung Olpflanze-Zuchtung, Anbau. 5.
- Carlson, K.D and Chang, S.P. (1985). Chemical epoxidation of natural unsaturated epoxy seed oil from *Vernonia galamensis* and a look at epoxy oil markets. *J. Am. Oil. Chem. Soc.* **62**, 934-939.
- Gan, L.H., Ooi, K.S. Gan, L.M., Goh, S.H. (1995). Effect of epoxidation on the thermal oxidative stabilities of esters of fatty acids derived from palm olein. *J. Am. Oil. Chem. Soc.* **72** (4), 439-442.
- Carlson and S.P. Chang, (1985). Chemical epoxidation of natural unsaturated epoxy seed oil from *Vernonia galamensis* and a look at epoxy oil markets. *J. Am. Oil. Chem. Soc.* **62**, 934-939.
- Giannelos, P.N., Zannikos, F. Stournas, S. Lois, E and Anastopoulos, G (2002). Tobacco seed oil as an alternative diesel fuel: Physical and Chemical Properties. *Industrial Crops and Products*. **16**, 1-9.
- Haas, W and Mittalbach, W. (2000). Detoxification experiments with the seed oil from *Jatropha curcas L.*. *Industrial Crops and Products*. **12**, 111-118.
- Hill, K. (2000). Fats and oils as oleochemical raw materials. *Pure Appl. Chem.*, **72**, (7), 1255-1264.
- Ikhuoria, E.U and Dadson, G. E. (2007). Epoxidation and Hydroxylation of African Oil Bean (*Pentaclethra macrophylla*) seed oil. *Journal of Chemical Society of Nigeria*. **32**, (2), 264-268.

- Mukhtar, A. Habib, U. and Mukhtar, H. (2007). Fatty acid composition of tobacco seed oil and Synthesis of Alkyd Resin. *Chinese Journal of Chemistry*. **25**, 705-708.
- Ogunniyi, D.S. and Odetoeye, T.E. (2008). Preparation and evaluation of tobacco seed oil-modified alkyd resins. *Bioresource Technology*. **99**, 1300-1304.
- Okieimen, F.E.; Bakare, O.I. and Okieimen, C.O. (2002). Studies on the epoxidation of rubber seed oil. *Industrial Crops and Products*. **15**, 139-144.
- Okieimen, F.E., Pavithran, C. and Bakare, I.O. (2005). Epoxidation and Hydroxylation of Rubber Seed Oil: One pot multi-step reactions. *European Journal of Lipid Science and Technology*. **(107)**, 330-336.
- Paquot, C. (1979). IUPAC standard methods for analysis of oils, fats and derivatives. 6th Ed. Pergamon Press, Oxford, NY Toronto. 20-128.
- Purseglove, J.W. (1991). Tropical Crops. Longman, England. 539-555.
- Rusch, G, Klaas, M. and Warwel, S. (1999). Complete and Partial epoxidation of plants oils by lipase-catalysed perhydrolysis. *Ind. Crops Product*. **9**, 125-132.
- Sheen, S.J. and Calvert, J. (1969). Chemistry of Tobacco. *Tobacco Science*. **13**, 10-15.
- Talaquani, T.E., Shafik, J. and Mustafa, F.K. (1986). Fatty acid composition of the seed oil of certain tobacco varieties cultivated in Northern Iraq. *Ind. J. Agric. Chem*. **19**, 147-154.
- Vaibhav, V. G., Patwardhan, A. V., Dinda, S. and Pradhan, N. C. (2007). Epoxidation of Karanja (*Pongamia glabra*) oil catalysed by acidic ion exchange resin. *Eur. J. Lipid Sci. Technol*. **109**, 575-584. DOI 10.1002/cjH.200600298.