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Evaluation of Zimbabwean tobacco varieties for potential as a source of tobacco seed oil (TSO)

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The impending curtailment of tobacco (*Nicotiana tabacum* L.) use in its traditional smoking form demands that economies previously dependent on tobacco as a cash crop find alternative sources of income. Alternatively, to enable the continued use of existing infrastructure and experience by growers, these economies could continue to grow the crop for other uses. The objective of this trial was, therefore, to explore alternative uses of tobacco, namely as a source of tobacco seed oil (TSO). Laboratory trials were conducted to optimize for TSO extraction, and to quantify and qualify the oil content of tobacco seed varieties from the Tobacco Research Board's tobacco germplasm collection. The quality of the extracted oil was assessed for acid, iodine and saponification values, free fatty acid content and specific gravity. The solvent extraction method, optimized for the local conditions was used for the extraction process. Results showed that the tested tobacco varieties contained 29-40% oil. Average values of density of 0.8937 g/mL, viscocity of 126.6 centipoise at 24.6°C, saponification value of 206.36 mg KOH/g oil, acid value 2.76 mg KOH/ g oil and iodine # of 145.75gl^2 were observed.

Key words: Parentals, hybrids, tobacco seed oil, fatty acids.

INTRODUCTION

The World Health Organization (WHO)'s Framework Convention on Tobacco Control (FCTC) established in 2003, to which Zimbabwe is a party to, calls on member states to implement measures to reduce tobacco leaf supply with the objective of enabling a reduction in global tobacco use. This is due to the perceived health hazards associated with the traditional form of tobacco consumption and will no doubt, lead to a massive reduction in the demand for tobacco leaf for use in traditional combustible cigarettes.

This scenario is potentially devastating for countries such as Zimbabwe, China, Brazil and India whose economy depends heavily on proceeds from tobacco leaf. Zimbabwe is well known for a vibrant tobacco industry anchored on its enabling climate, suitable soils, experienced growers and science-based tobacco production systems emanating from sustained research efforts. In terms of leaf production, in 2019 the country was reported as the largest producer of tobacco leaf in Africa and was ranked the fourth largest

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in the world, after China, India and Brazil (Shahbandeh, 2021).

The country exports more than 95% of its flavour-style tobacco leaf, which is in great demand for use in cigarette brands in over 60 countries across all five continents (TIMB Annual Statistics Report 2019). As a result of this, tobacco production makes an important contribution to GDP and to export revenue, and plays a major role in the national economy. The crop normally accounts for more than 50% of agricultural exports, 30% of total exports and nearly 10%of GDP. The uncertainty over the future availability of markets for the tobacco leaf in its traditional form has led to numerous initiatives aimed at identifying viable alternative crops that tobacco growers can adopt to decrease dependency on tobacco and improve the diversity of the farm economy (Clark et al., 2020). However, total diversification into new crops would lead to the abandonment of the tobacco related infrastructure in place and would require considerable investment in new facilities, training of farmers, extensive research and marketing efforts.

To enable the use of tobacco production related infrastructure, skills and technical know-how already in place, research efforts have been intensified towards exploiting tobacco for its non-conventional and economically viable alternative such as non-smoking related uses. Among the widely considered alternative uses of tobacco is the extraction of many valuable constituent phytochemicals. This is because tobacco is an excellent source of phytochemicals that include seed oil, nicotine, solanesol, edible proteins (green leaf) and organic acids (malic and citric) having pharmaceutical, agricultural and industrial uses (Sarala et al., 2013; Chiririwa et al., 2014). Oil in tobacco seed constitutes 33-40% of seed weight and according to literature could be used as an appropriate feedstock for biodiesel production (Tian et al., 2020) as well as raw material for the manufacture of soaps, paints, lubricants and fuels. The oil is also edible once refined (Sarala et al., 2013). The objective of this study was, therefore, is to explore the potential of Zimbabwean tobacco seed material for tobacco seed oil (TSO) production.

MATERIALS AND METHODS

Study site

The study was carried out at the Tobacco Research Board (Kutsaga Research Station) located 15 km East of Harare at latitude 17°55´S and longitude 31°08´E with an altitude of 1479 m above sea level. The average temperature is 32°C and 18°C in summer and winter, respectively, with annual rainfall between 800 to 1000 mm (FAO, 2006) and sand loam soil.

Tobacco Seed (TS)

Seed from the Tobacco Research Board's seed bank and the breeding lines (m/s BAZR 1-3-14, m/s ONCR 3-4-6, BAZR 3-6-18, XM 2 GR 1-1-26, m/s K326R 1-1-23, BAZ R 5-17-9, XSR 4-7-10, XZR 2-2-4, AWR 3R, MG, RWR K200B2, and K326) and seed hybrids (K RK26R, K RK66, K RK70, K RK71, K RK72, KR K73, K RK75 and K RK76) were used in this research. The parentals were selected for their popularity as the most suitable parents for our most popular hybrids, with good performance in terms of yield, disease resistance and quality. The most popular hybrids were chosen, and all seed samples were cleaned and air dried to reduce their moisture content to less than 8% before commencing the trial.

Instruments, apparatus, chemicals and reagents

An analytical balance for weighing the samples, electric mill for grinding the tobacco seed , soxhlet extractor (extracting the oil extraction from the seed), Liquid Chromatography Tandem Mass Spectrometer (LC-MS/MS) (LC/MS data is used to provide information about the molecular weight, structure, identity and quantity in this case of the fatty acids), Fourier Transform Infra-red Spectrometer (FTIR) (a technique used to identify the characteristic functional groups from the spectral bands that allow us to know the spectral characteristics of fatty acids), Viscometer (establishing the viscocity of the oil), rotary evaporator (solvent removal from the samples) and general laboratory glassware were used during the experiment.

Analytical grade and LC-MS/MS grade reagents were used in the extraction procedures and instrument analysis procedures. These included Ammonium formate, n-Hexane, sodium hydroxide, hydrochloric acid, potassium hydroxide, Methanol, Iodine, starch, glacial acetic acid, wiji's reagent, cyclohexane, sodium thiosulphate and deionised water. Fatty acid analytical standards of myristic, palmitoleic, stearic, oleic, linoleic, linolenic, palmitic acids were also used during the fatty acids' characterization and profiling.

Extraction Procedures

Quantifying oil content from tobacco varieties and breeding lines

Kutsaga breeding lines and hybrids were screened for TSO content using the standard method ISO 659:2009 for all seed regardless of size.

Optimizing oil extraction variables

Two of the highest TSO yielding hybrids were used to optimise the solvent extraction procedure for the following operational conditions;

1) Extraction time
2) Seed: solvent ratio using the established optimum extraction time of 5 h

TSO characterization and fatty acid profiling

Characterization of Fatty acid functional group properties of the parentals and hybrids were done using FTIR. These were analyzed using supplier instrument user manual method. The method entails passing infra-red radiation through the sample. Resulting in some radiation being absorbed and some passing through the sample. The absorbed radiation was converted into rotational and vibrational energy by the sample molecules giving FTIR bands at varying wave lengths. However, fatty acid profiling was then carried out by extracting the TSO using a previously used method (Omeje et al., 2019). Instrument analysis of the extract was carried out on

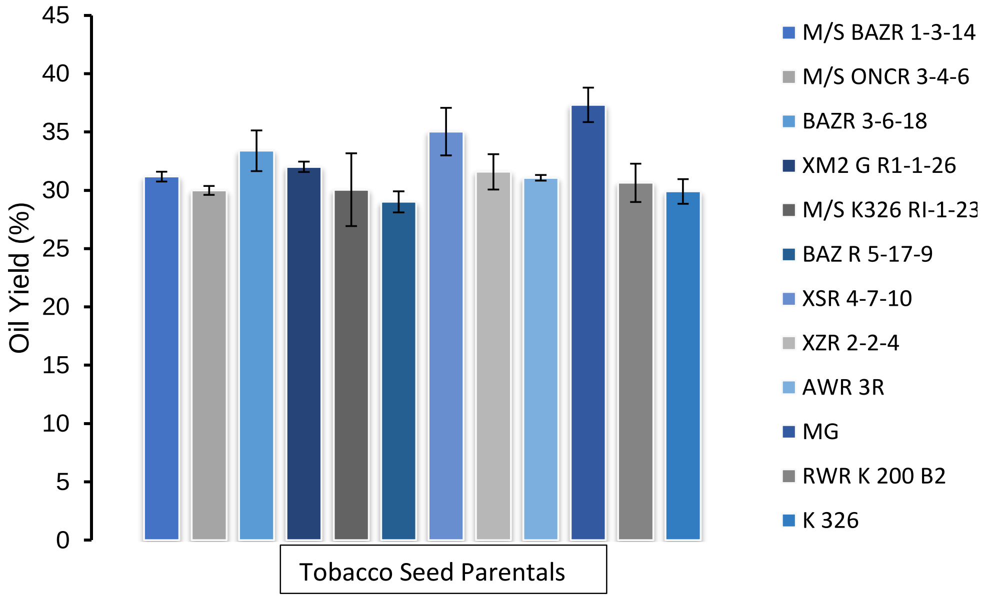

Figure 1. Oil yield for tobacco seed parental lines.
Source: Study

LC-MS/MS instrument using manufacturer supplied methods (after individually maximising the product and parent ions for each analyte) instead of the Gas Chromatography Flame Ionisation Detector (GC-FID) in the aforementioned method. Density was established by introducing the sample into a tight temperature controlled oscillating u tube and the change in oscillating frequency caused by the change in mass was used to determine the density. Acid value was determined through acid base titration using KOH. Saponification value was obtained by heating a weighed sample in KOH in a water bath and titration using HCl. Viscosity was established using a standard viscocity test tube. Iodine number was established using wiji's reagent and adding KI and back titrated with sodium thiosulphate. While peroxide value was obtained by titrating the Iodine liberated with sodium thiosulphate solution. All the research work was carried out at the Tobacco Research Board's Analytical Chemistry Services Laboratory.

RESULTS AND DISCUSSION

Extraction and quantification of tobacco seed oil from TRB hybrid seed varieties and parental lines

The results for tobacco seed oil quantification are shown in Figures 1 and 2. The mean range for both parentals and hybrids was between 29 and 40%, with the standard deviation being between 0.0781 and 0.9355 respectively.

The results show that MG gave the highest oil yield of 37.33 ± 0.5524 g followed by XSR 4-7-10 (35.04 ± 0.7136 g), with BAZ R 5-17-9 (29.01 ± 0.2615 g) giving the lowest oil yield.

Results show that KR K71 gave the highest oil yield followed by KR K76. The hybrid with the least oil yield is KR K26R.

The tobacco seed oil content of between 30 and 40% for both the parentals and hybrids is comparable to rice, sesame, tobacco seed, mustard, sunflower and safflower oil and also TSO extracted elsewhere which ranged from 30 to 43% (Irnawati et al., 2020; Chinweuba, 2013; Sarala et al., 2013; Faugno et al., 2016)). This high yield of tobacco oil makes tobacco seed a lucrative source of oil which has already found use in other countries as refined oil and as edible oil (Sarala et al., 2013) with India using it as a raw material in soap, varnishes, and lubricants. The extraction process can be upscaled commercially, making use of pressing machines that do not require the use of organic solvents which are expensive.

Optimization of the solvent extraction procedure

The TSO samples were optimized for sample extraction time and for sample weight to extraction solvent on the two highest yielding varieties (K RK71 and K RK76). The results are shown in Figures 3 and 4.

The TSO yield generally rose with increasing extraction time for both varieties and then decreased after five hours. The optimum extraction time was therefore, established to be five hours, which is less than the 8 hours recommended by the ISO 659:2009 standard method. Thus, for TSO production, the optimised n-hexane and Soxhlet extraction method proved to be more efficient for oil extraction from tobacco seed.

As the seed quantity increased, the volume of TSO obtained decreased with a reduction in the solvent: seed weight ratio and optimum solvent to seed weight ratio was determined to be 100 mL:1 g. This however, means

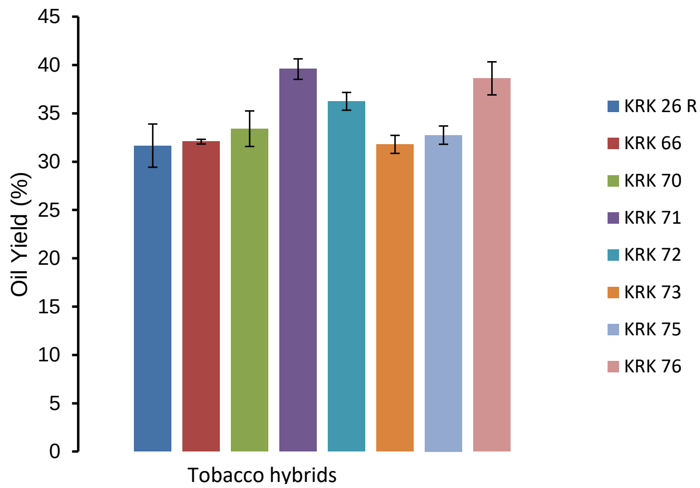

Figure 2. Oil yield for tobacco seed hybrids.
Source: Study

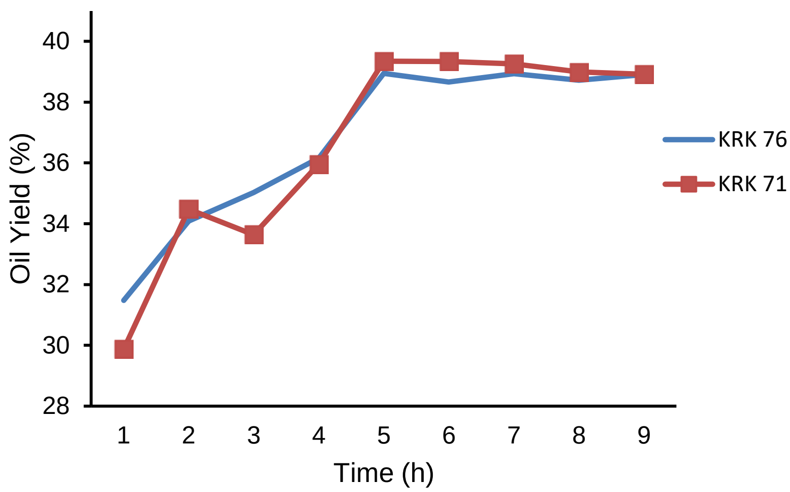

Figure 3. Effect of extraction time on oil yield.
Source: Study

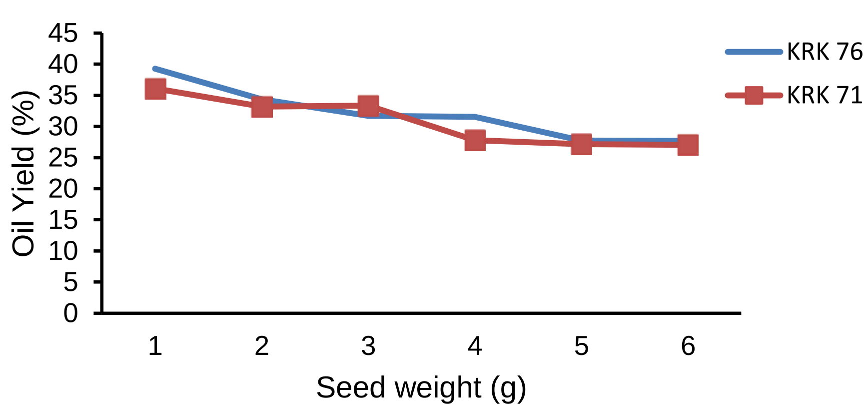

Figure 4. Effect of solvent to seed weight ratio on oil yield.
Source: Study

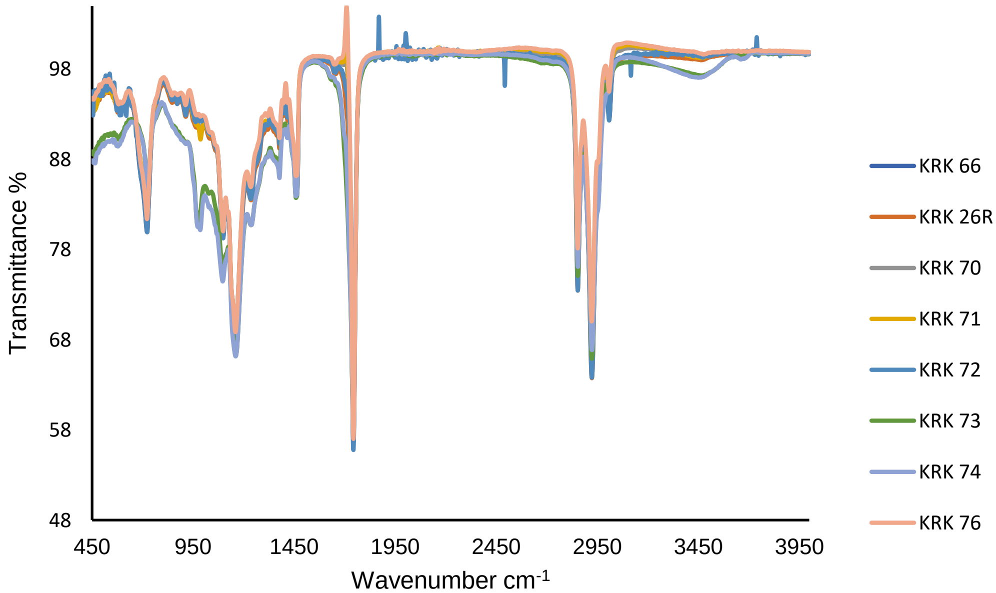

Figure 5. Combined FTIR plot for tobacco hybrids.
Source: Study

the extraction process is uneconomical on a large scale because one would need 100 L of organic solvent to extract I kg of seed for an oil yield of about 300-400 ml making this oil very expensive. Upscaling to get meaningful oil yields would require huge quantities of organic solvent. This experiment showed that tobacco seed can yield between 29-40% oil using solvent extraction. However, meaningful oil yields (79.47%) (Faugno et al., 2016) would require use of commercial techniques such as mechanical oil pressing which do not use expensive organic solvents but can give higher oil yields.

Characterization of the extracted tobacco seed oil

TSO FTIR spectra

Fourier Transform Infra-red Spectroscopy (FTIR) results were established for both the parentals and the hybrids for the TSO. The results are shown in Figures 5 and 6 for TSO hybrids and parentals respectively.

Observed wave lengths (λ) of 3009 cm^{-1} correspond to cis =CH, while λ of 2953 cm^{-1}, 2923 $cm^{-1}$and 2854 cm^{-1} arise from vibrations of C-H, namely asymmetric stretching of $–CH(CH_3)$, $-CH(CH_2)-$ and $–CH(CH_3)$ resp. Strong band at λ of 1743 cm^{-1} emanates from stretch vibration of C=O in triglycerides. The weak band at 1657 cm^{-1} is due to vibrations of the alkene group (C=C) from unsaturated fatty acids such as oleic and linoleic acids. Bands with strong and medium intensities at 1459 cm^{-1} and 1378 cm^{-1} arise from scissoring vibrations of $–CH_2^-$ and $–CH_3$ resp. The band at 1237 cm^{-1} originates from stretching vibration of C-C bonds of the hydrocarbon skeleton. These results are similar to those from Chiririwa et al. *(*2014). The FTIR plots for tobacco parentals and hybrids TSO show that all the extracts have similar absorption patterns.

The FTIR plots for both the parentals and the hybrids TSO extracts compared favourably with Linoleic, Linolenic, Palmitoleic and Oleic acid standards. The fatty acids compositions were established and confirmed using LC-MS/MS in Table 1.

TSO fatty acid characterisation by LC-MS/MS instrument

The LC-MS/MS results for two hybrids and three parentals indicated that TSO has an abundance of fatty acids (Table 1) such as Linoleic acid (45.95-58.27%), followed by Linolenic acid (18.62-24.62%), Palmitic (7.42-11.18%), Oleic (6.79-9.47%), Myristic (5.29-9.12%), Stearic (23%) and lastly Palmitoleic (0.28-0.87%) acids. These results agree with the FTIR spectra results (Figures 5 and 6) and are similar to those reported in other studies (Faugno et al., 2016; Warra, 2015; Francakova et al., 2015; Majdi et al., 2012). Studies have reported fatty acid compositions for linoleic (65-75%), oleic (7-16%), palmitic (8-15%), linolenic (0.48-1.16%), myristic (0.00-1.04%), stearic (2%-4.6%) and palmitoleic (0.09-0.29%) acids. Observed results from this research make tobacco seed a suitable raw material for the manufacture of soaps, paints, lubricants and fuels. The oil is also edible once refined (Sarala et al., 2013). Table1 shows the fatty acid contents of these two hybrids and three parentals.

Table 1. Percentage of fat acids in selected TSO varieties.

Varieties	Linoleic	Linolenic	Myristic	Palmitic	Palmitoleic	Stearic	Oleic
MS K326R (%)	45.95	24.46	9.12	11.18	0.87	ND	8.35
BZ RWR (%)	57.79	18.62	6.37	9.09	0.36	4.68	9.47
KR K76 (%)	52.23	23.91	5.29	9.24	0.41	1.31	7.61
KR K 71 (%)	51.25	24.61	6.8	7.81	0.28	1.88	7.37
KR K72 (%)	58.27	19.76	6.41	7.42	0.47	3.85	6.79

Source: Study

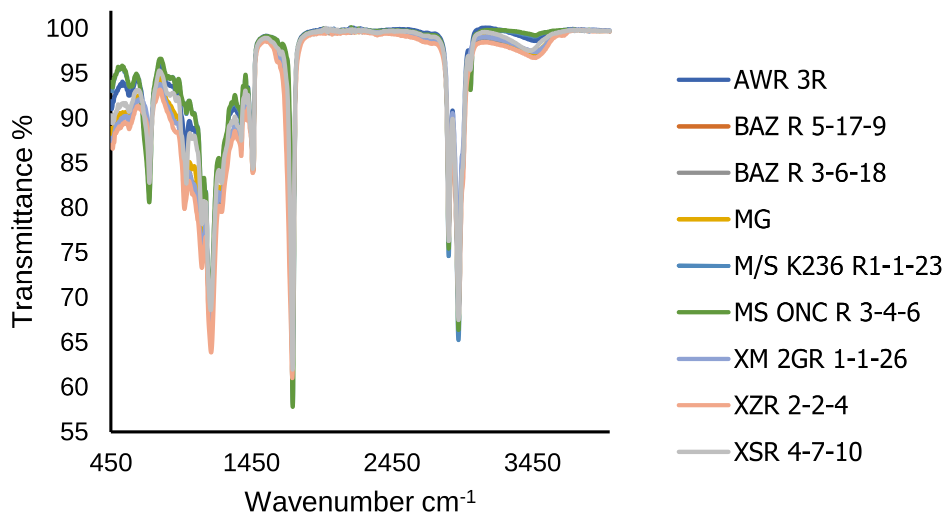

Figure 6. Combined FTIR plot for parental lines.
Source: Study

Table 2. Density, Acid value, Saponification value, Iodine number and Viscosity of TSO from two tobacco seed varieties.

Parameter	TSO Result		Methods
	K RK71	K RK76	
Density (g/**mL**)	0.8931	0.8943	ASTM D 4052-91 (1995C)
Acid value (mg KOH/g oil)	2.77	2.75	ASTM D664
Saponification value (mg KOH/g oil)	201.96	210.75	ASTM D1962
Iodine # (gI_2/100 g oil)	142	147	AOAC 993.330
Peroxide value (meq/Kg)	2.5	3.5	AOAC 965.20
Viscosity (Centipoise) at 24.6°C	126.8	126.4	ASTM D1545

Source: Study

Physico-chemical characterization of TSO

Density, acid value, saponification value, iodine # and viscosity: The extracted TSO for the two highest yielding varieties (K RK71 and K RK 76) was also further characterized for physicochemical properties. The results for density, acid value, saponification value, Iodine number and peroxide value obtained are shown in Table 2. Observed physicochemical results are generally in the range reported in other research (Gafar at al., 2012; Hariram and Rajan, 2016; Sarala et al., 2013; Paunovic at al., 2020).

Oil density relates to the calorific values and power generated per unit volume. The bigger the density value the greater the heating value and the generated power. However, larger density values result in increased viscosity making it difficult for the oil to burn should it be used as raw material for diesel manufacturing. The density value observed in this research was 0.8931 g/mL and 0.8943 g/mL for the two varieties (K RK71 and K RK 76) and these are on the border line of the recommended maximum value for oils for diesel production which is 0.890 g/mL. However, viscosity results observed were 126.4 centipoise and 126.8 centipoise which compare to that reported elsewhere by Sarala et al*.* (2013).

Saponification value indicates the average molecular weight of triglycerides in the oil. The high saponification values (201.96 and 210.75 mg KOH/g oil) for the oil obtained from the two varieties (K RK71 and K RK76) indicated that available triglycerides have shorter carbon chains. The shorter the carbon chain the more the fatty acid the oil has. These values were higher than reported for linseed seed oil (116.88 mg OH/g oil) and recommended for cosmetic application, but were in the same range as reported for tuber oil (193.33 and 188.33 mg KOH/g oil), for cotton seed oil (199.42 mg KOH/g oil) and for neem seed oil (213 mg KOH/g oil) (Suwari and Buang, 2017). Therefore, the oil can be used as raw material for manufacturing of soaps, paints and lubricants and can also be used as edible oil following refining.

Acid value indicates the number of mg KOH needed to neutralise free fatty acids in one gram of oil. The acid values observed in this research are 2.75 and 2.77 mg KOH/g oil (K RK71 and K RK 76) and these are in the range of 2.34 mg KOH/ g oil for linseed and 10.3 mg KOH/ g oil from shea nut butter recommended for soap making but way higher than 0.421 mg KOH/ g oil recommended for toothpaste production.

iodine number measures the degree of unsaturation or number of double bonds contained in the molecular state of oil expressed in gI_2/100g of oil. It influences the long-term stability of the properties of the oil which is important for storage and indicates susceptibility to rancidity with saturated lipids more susceptible. Observed Iodine values of 142 gI_2/100 g of oil and 147 gI_2/100g (K RK71 and K RK76 respectively) of the TSO indicate a higher content of unsaturated fatty acids compared to 119.78 gI_2/100g of oil reported from cotton seed and 30.33 gI_2/ g of oil from sponge Gourd recommended for soap and other cosmetics. This implies that TSO is slightly more prone to rancidity when compared with cotton and sponge gourd oil (Warra; 2015) upon storage.

Peroxide value is an indicator of the extent of oxidation of oils. Oxidation of lipids results in some undesirable off-flavours and loss of fat-soluble vitamins. TSO peroxide values obtained are 2.5 meq/Kg and 3.5 meq/Kg for K RK71 and K RK76. These results are within the 2.00-3.5 meq reported for sunflower oil and 2.25 – 6.5 meq reported for palm olein (Paunovic et al., 2020). These low peroxide values indicate that TSO is stable and may not be susceptible to oxidative rancidity.

Conclusion

Oil was successfully produced from tobacco seed. The seed yielded 29-40% of TSO. The TSO research results indicated that tobacco seed can yield oil comparable in terms of quantity and quality of seed oils produced elsewhere. The optimum extraction time was five hours with optimum solvent to seed weight ratio of 100 ml:1 g seed. The seed oil qualities exhibited desirable physicochemical characteristics and fatty acids composition that makes it suitable for value addition. The oil is abundant in fatty acids such as Linoleic acid (45.95-58.27%), followed by Linolenic (18.62-24.62%), Palmitic (7.42-11.18%), Oleic (6.79-9.47%), Myristic (5.29-9.12%), Stearic (23%) and lastly Palmitoleic (0.28-0.87%) acids. These results agree with those observed for the FTIR spectral analysis. The density value observed in this research was 0.8931 g/mL and 0.8943 g/mL for the two high yielding varieties (K RK71 and K RK76). The density results are on the border line of the recommended maximum value for oils for diesel production which is 0.890 g/ml. Viscosity results observed were 126.4 centipoise and 126.8 centipoise. Saponification value was 201.96 and 210.75 mg KOH/g oil, Acid value was 2.75 and 2.77 mg KOH/g oil, Iodine was 142 gI_2/100g oil and 147 gI_2/100g oil and Peroxide value was 2.5 mmol/Kg and 3.5 mmol/Kg for K RK71 and K RK76 respectively. These results indicate that TSO can be used as raw material in manufacturing of soap, paints, lubricants and fuel and it can be further refined for use as edible oil. It is recommended that partnerships be created with oil manufacturers to extract meaningful quantities which can be used as edible oil or as raw materials for further processing into soap and cosmetics.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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