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*Full Length Research Paper*

# A comparative study of the oils from the seed arils of *Trichilia emetica* from Mozambique

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**The physicochemical properties and fatty acid composition of the edible aril oils from four variants of *Trichilia emetica* Vahl (Meliaceae), obtained by Soxhlet extraction, *were* determined. The oil yields ranged between 42.2 – 53.8% (*w/w*). Determination of the stability parameters, acid value (AV) and peroxide value (PV), indicated that all four oil samples were stable to hydrolytic and oxidative stress (AV = 0.88 – 1.02 mg KOH/g and PV = 2.02 - 2.62 mEq/kg). The refractive indices (RI = 1.466 – 1.467) and the iodine values (IV = 63.42 – 78.02 mEq/kg) indicated that the four oil samples were all moderately unsaturated, while the saponification values (SV = 183.71 – 189.03 mg KOH/g) indicated that the oil samples consisted mainly of medium length fatty acid chains. GC-MS determination of the fatty acid profiles (16:0 = 40.29 – 46.76%, 18:1 = 25.28 – 30.44%, 18:2 = 24.99 – 27.64%) confirmed the moderate unsaturation and showed that all four oil samples consisted mainly of C16 and C18 carbon chains. The GC-MS findings were closely corroborated by $^{1}$H-NMR determination of the fatty acid classes. The study showed that the characteristics of the oil from the seed arils from the four variants of *T. emetica* were all similar to each other and compared favourably with the properties of some well-known edible household oils such as palm oil and olive oil. Hence, the four variants are all equally suitable for further evaluation towards commercial exploitation of the fruit oil.**

**Key words:** *Trichilia emetica* Vahl, mafura, seed aril oil, fatty acid composition, physicochemical properties, stability parameters, oil yield.

## INTRODUCTION

*Trichilia emetica* Vahl (Meliaceae), commonly called ‘Mafura tree’, ‘Cape Mahogany’ or ‘Natal Mahogany’, has significant socio-economic importance in Mozambique, where it thrives very well particularly in the southern provinces, where it is cultivated for protection around home yards and farm lands. The tree grows wildly along coastal low lands and is also intensively cultivated by farming communities (Deveza, 1996; Matakala et al, 2005). The fruits of the mafura tree are pear-shaped containing 3-6 shiny black seeds with a bright coloured fleshy aril almost covering the seed. The fruits are usually ripe for picking from December/January to March (Grundy and Campbell, 1993).

The ripe mafura fruit yields two types of oil: the edible ‘mafura oil’ which is extracted from the fleshy seed envelope/aril and the bitter tasting, emetic and inedible “mafura butter” which is extracted from the seed kernel. In traditional extractions, the two oils are extracted separately. For the extraction of the edible mafura oil, the seeds are sun or smoke dried, soaked for several hours in hot water and then the flesh (aril) is separated from the kernel. The semi-solid emulsion is boiled and the mafura oil floats on the surface and is decanted from the aqueous phase. The oil thus obtained (locally called *munhantsi*) is highly appreciated for human consumption. The oil is added to meat or vegetables to prepare various dishes. Furthermore, mafura oil is used as a hair conditioner, a colorant, applied to the skin and

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also used for medicinal purposes. The mafura butter which is obtained from the seed kernel on the other hand is used in making soap, candle and wood polish. The butter is also used as a body ointment and for medicinal purposes (Mashungwa and Mmolotsi, 2007). Thus the mafura fruits are cultivated, harvested and processed in many rural households in Mozambique as a source of income and more importantly as part of the diet. Besides being a source of oil, the fruits are consumed fresh or soaked in water and mixed with sugar and then stirred into a creamy yogurt-type texture.

Rural communities have for generations extracted seed oils for local use as sources of food, medicine and for cosmetic applications. Thus there are many non-conventional seed oils in rural communities all over the world whose agro–economic potential has not been properly evaluated (Mitei et al., 2008; Kleiman et al., 2008; Choudhary et al., 2014), due to lack of modern scientific data. There are reports in the literature on the physicochemical properties and fatty acid composition of the seed kernel oil of *Trichilia emetica* (Khumalo et al., 2002; Vermaak et al., 2011; Adinew, 2014 and 2015). These studies confirmed its various uses such as in nourishing and revitalising the skin and as hair conditioner (Phyto Trade Africa). It has been reported that a 4-step consecutive processing (degumming, neutralization, bleaching and deodorization) of the mafura butter can produce an oil with an acceptable flavour (Fupi and Mork, 1982). However scientific reports about the oil from the fleshy envelope/ aril of *T. emetica* fruit are rather scanty in the literature. Thus the mafura aril oils have not been formally analyzed with a view to study their general characteristics and evaluate their potential for domestic and industrial applications. We here report on a comparative study of the *T. emetica* aril oils obtained from four different variants of *T. emetica* species. This study focused on the yields, physicochemical properties and the fatty acid compositions of the oils from the red-bitter, red-sweet, orange-sweet and white-sweet arils in order to obtain some definitive scientific information about the general characteristics of the *T. emetica* aril oil, to investigate any differences that may occur in the properties of the oils among the different variants and to evaluate their potential for possible commercial exploitation.

## Experimental Procedures

### Materials

*T. emetica* fruits (Mafura) were purchased from a market in Maputo, Mozambique, and sundried. Four different aril variants: red-bitter, red-sweet, orange-sweet and white-sweet, according to colour and taste were used for this research.

### Extraction: Solvents and Reagents

Solvents and reagents used in this work, unless otherwise stated, were all of analytical grade. The solvents were obtained from Rochelle Chemicals (South Africa) and BDH (Merck Chemicals, UK). The dried pulp/aril of the mafura fruits was separated from the seeds and macerated in a Warring commercial blender (Gateshead, UK). The powders were extracted with a 3:1 *(v/v)* mixture of *n*-hexane and 2-propanol in a Soxhlet apparatus for 6 h.

### Physicochemical Properties

The bulk physicochemical properties: refractive index (RI), relative density (RD), saponification value (SV), acid value (AV), iodine value (IV), peroxide value (PV), *p*-anisidine value (*p*-AV) and unsaponifiable matter (UM), (Table 1), were determined according to standard IUPAC methods for the analysis of fats and oils (Dieffenbacher and Pocklington, 1987). All experiments, unless otherwise stated, were conducted in triplicate.

### Lipid Class Composition

Analysis of the lipid classes, Table 2, in each oil sample was carried out by adsorption column chromatography using florisil (7% water w/w, Saarchem Pty Ltd, Muldersdrift, Republic of South Africa) and gradient elution as follows: *n*-hexane (100%), mixtures of *n*-hexane/diethyl ether, diethyl ether, methanol (100%) and acetone (100%), (Sempore and Berzard, 1996).

### Fatty Acid Composition:

### Preparation of FAME

The mafura oil samples (5 g each) were derivatised into the fatty acid methyl esters (FAME's) by refluxing in dry methanol that contained ethanoyl chloride (150:10 mL) for 2h. The resulting solution was neutralized with 2M NaOH and extracted with diethyl ether (3 x100 mL), dried under anhydrous magnesium sulphate and evaporated using rotary evaporator. The FAME's were stored under nitrogen and used for GC-MS and proton-NMR analyses.

### Instrumentation and Separation Conditions

The FAME samples in dichloromethane were analyzed in a Hewlett-Packard model 6890 gas chromatograph, equipped with a polyethylene glycol-TPA modified capillary column (ZB-FFAP, 30 m x 0.32 mm id x 0.25 µm: Chrompack: Middleburg, Netherlands) and interfaced with a micromass Autospec ETOF in the electron ionization mode with electron energy of 70eV. The initial temperature of 100 $^oC$ was held for 3 minutes and then ramped up to 210 $^oC$ at 10 $^oC$ per minute, then raised to 220 $^oC$ at 1 $^oC$ per minute, before finally raising the temperature to 250 $^oC$ at 20 $^oC$ per minute. Helium was used as a carrier gas at a rate of 0.8 mL/min. Peak identification was performed using AMDIS/NIST software while quantification of fatty acids was performed on WSEARCH software using peak

**Table 1.** Physicochemical properties of the seed aril oils from four variants of *Trichilia emetica*

| *T. emetica* | Red-bitter | Red-sweet | Orange-sweet | White-sweet |
|---|---|---|---|---|
| % Yield | 42.2 | 49.0 | 50.0 | 53.8 |
| RI | 1.467±0.002 | 1.466±0.000 | 1.466±0.008 | 1.467±0.002 |
| RD (g/mL, 30 ºC) | 0.911±0.007 | 0. 910±0.033 | 0.909±0.000 | 0.898±0.006 |
| SV (mg KOH/g) | 183.71±0.86 | 187.42±0.89 | 187.02±0.26 | 189.03±1.44 |
| IV (Wijs) | 78.02±1.09 | 69.93±1.60 | 71.54±1.03 | 63.42±0.75 |
| AV (mg KOH/g) | 1.02±0.229 | 0.89±0.011 | 0.89±0.005 | 0.88±0.036 |
| PV (mEq/kg) | 2.53±0.25 | 2.02±0.20 | 2.51±0.05 | 2.62±0.15 |
| *p*-AV | 2.12±0.36 | 2.22±0.99 | 2.12±0.80 | 2.90±0.44 |
| UM (%w/w) | 0.32±0.079 | 0.24±0.003 | 0.27±0.054 | 0.31±0.007 |
| Oxidation value[a] | 7.18±0.26 | 6.26±0.65 | 7.14±0.56 | 8.14±0.32 |
| Flavor score[b] | 5.19±0.23 | 5.51±0.54 | 5.20±0.45 | 4.85±0.29 |

Values represent the average of three replicate analyses ± SD;
[a] Calculated from Holm's equation (List et al., 1974); [b] Calculated from List's equation (List et al., 1974);
RI = Refractive index (40 ºC); RD = Relative density (30 ºC); SV = Saponification value; IV = Iodine value (Wijs method); AV = Acid Value; PV= Peroxide value; *p*-AV = *p*-Anisidine value; UM = Unsaponifiable matter.

**Table 2.** Percentage composition of lipid classes of the seed aril oils from four variants of *Trichilia emetica* - as estimated from adsorption column chromatography [a]

| *T. emetica* | Hydrocarbons *n*-Hexane 100% | Sterol esters *n*-H:DEE 95:5% | TAG + FFA *n*-H:DEE 85:15% | Free sterols *n*-H:DEE 75:25% | DAG *n*-H:DEE 50:50% | MAG *n*-H:DEE 10:90% | Glycolipids Acetone 100% | Phospholipids MeOH 100% |
|---|---|---|---|---|---|---|---|---|
| Red-bitter | 3..62 | 3.22 | 79.43 | 1.99 | 2.61 | 2.07 | 2.33 | 4.73 |
| Red-sweet | 4.54 | 1.49 | 78..83 | 4.27 | 1.20 | 1.93 | 1.40 | 6.34 |
| Orange-sweet | 4.15 | 3.43 | 79.44 | 4.00 | 1.31 | 1.25 | 1.22 | 4.85 |
| White-sweet | 3.83 | 2.47 | 77.58 | 4.84 | 3.65 | 1.23 | 2.00 | 4.40 |

[a] Values represent average of two replicate analysis; DEE = diethyl ether; FFA = free fatty acids; *n*-H = *n*-Hexane, DAG = diacylglycerols; MAG = monoacylglycerols.

areas. Sample spectra were compared with the spectra of fatty acid standards to confirm the identity of the components, reported in Table 3.

### NMR Analysis

Proton NMR spectra of the mixture of FAME's, dissolved in $CDCl_3$, were acquired at 300 MHz using a Bruker Avance DPX300 spectrometer. The relative composition of the saturated, monounsaturated, diunsaturated and α-linolenic fatty acids, together with their average chain lengths, shown in Table 4, were determined from the relative sizes of the integrals of the signals for the allylic, diallylic and methyl protons applying Holmback's equations (Holmback, 2000).

### Data Analysis

All experiments were conducted in triplicate unless otherwise stated and results are expressed as mean values ± SD. A statistical analysis was carried out using a one way ANOVA with a significance of $P$ <0.05.The software used for the statistical analysis was the SPSS for Windows statistical package (v.10.0.6; SPSS, Chicago, IL, USA).

## RESULTS AND DISCUSSION

The investigation started with the determination of certain important physicochemical properties of the mafura aril oil samples in order to learn about the general characteristics of the oils from the four *T. emetica* variants. These data are reported in Table 1, which also reports on the average yields of the four variants. The oil yields range from 42.2 – 53.8% (*w/w*) with red-bitter having the lowest yield and white-sweet having the highest yield. This range of oil yield compares very favourably with the oil yields of well-known commercial oil sources like sunflower (55%),

**Table 3.** Fatty acids composition (% w/w) of the seed aril oils from four variants of *Trichilia emetica* obtained by GC-MS analysis of the FAMEs from test oils.

| Fatty acids class | Red-bitter | Red-sweet | Orange-sweet | White-sweet |
|---|---|---|---|---|
| 16:0 | 46.76 | 46.73 | 43.42 | 40.29 |
| 18:0 | 1.41 | 1.90 | 1.79 | 1.36 |
| 18:1n-9 | 25.28 | 26.15 | 27.27 | 30.44 |
| 18:2n-6 | 25.48 | 24.99 | 27.14 | 27.64 |
| 18: n-3 | ND | ND | ND | ND |
| Total saturated | 48.17 | 48.63 | 45.21 | 41.65 |
| Total unsaturated | 50.76 | 51.14 | 54.41 | 58.08 |

Values represent the average of two replicate analyses; ND= not detected.

groundnut (46%), rapeseed (41%) sesame (51%) and others (Bockish, 1998). Thus the aril oils from the *T. emetica* variants are potentially good sources of commercial vegetable oil for both food and nonfood uses in southern Mozambique where they tend to grow very well.

The values of the refractive indices (RI) and the relative densities (1.467 – 1.466 and 0.911 – 0.909, respectively) are virtually the same for the four variants of *T. emetica*. RI values are a measure of chain length and unsaturation, especially polyunsaturation, and tend to increase with increase in these properties. Thus this indicates that the four oils contain similar levels of unsaturation and average chain length. The RI values for the test oils all fall within the range of RI values for olive oil (1.4677-1.4705), (Kirk and Sawyer, 1991), and thus allows the prediction that the oils of the *T. emetica* species would be only moderately unsaturated and that the average chain length of the fatty acids would be between C16 and C18, a prediction which is upheld by the range of iodine values determined (IV = 63.9 – 78.0 (*Wij's)*), as shown in Table 1 and the fatty acid composition given in Table 3. The saponification values of the mafura oils range between 183.71 to 189.03 mg KOH/g. These values also fall within the range of SV's for most of the common commercial seed oils like olive, sunflower and groundnut oils (Kirk and Sawyer, 1991), again indicating that medium chain length fatty acids dominate the aril oils of *T. emetica*. The range of acid values (AV = 0.88 – 1.02 mg KOH/g) for the aril oils of the *T. emetica* variants fall well within the Codex recommended standards for unrefined vegetable oils, as also is the range of peroxide values (PV = 2.02 – 2.62 mEq/kg), which are within the Codex standards for refined vegetable oils (Kirk and Sawyer, 1991). Indeed the *p*-anisidine values (*p*-AV = 2.12 – 2.90) shown in Table 1, indicate that not much secondary oxidation reactions have occurred in the test oils. This observation is borne out by the oxidation values (OV) and the corresponding flavor scores (*F*) shown in Table 1. The OV's were calculated using Holm' equation, OV = *p*-AV + 2(PV), which is used to describe the degree of oxidation of oils, and the flavor scores were calculated from List's equation, *F* = 7.7-0.35(OV), which is used for predicting theoretical flavor scores (List et al., 1974). Table 1 shows good oxidation values and flavor scores (all below 10 units) for all the oils, indicating that not much primary oxidation activity had taken place in the oils and consequently there was very little presence of secondary oxidation products in the four oil samples. These stability parameters indicate that the oils are stable to both hydrolytic and oxidative stress and should therefore have good keeping capacity. This fine quality of the oils can be attributed to the low levels of polyunsaturation in the mafura aril oils. The range of percent unsaponifiable matter (UM = 0.24 – 0.31 % (w/w)) given in Table 1, falls well within the Codex recommended maximum for all the common household cooking oils like sunflower, soybean and olive oils (Kirk and Sawyer, 1991), which again indicates the wholesomeness of the unrefined mafura oils. The physicochemical parameters given in Table 1 for the aril oils of the *T. emetica* variants seem to justify the use of the unrefined mafura oils for food preparations by the local communities, as the quality of the oils, especially regarding their stability parameter's, compares very favourably with virgin olive oil.

Table 2 shows the results of the determination of the percent composition of lipid classes in the oil samples by adsorption chromatography. As would be expected the predominant lipid classes in the oils were found to be triacylglycerols with a range of 77.58 to 79.83%. Thus by a good margin non-polar lipid classes dominate the aril oils of the *T. emetica* variants. The content of monoacylglycerols, MAG, and diacylglycerols, DAG, in the mafura oils was quite low which indicates that not much lipase catalyzed hydrolysis had taken place in the oils, an observation which supports the low AV's shown in Table 1. While the content of glycolipids in the oils of the *T. emetica* varieties (1.2 – 2.0%) was rather low, the phospholipid content (4.40 – 6.34%) on the other hand was quite substantial; indicating that in a commercial production of the mafura aril oil a degumming process will have to be applied. Overall, Table 2 shows that the oils of the *T. emetica* variants are predominantly made of nonpolar triacylglycerol lipids.

The fatty acid compositions of the oil samples obtained by GC-MS analysis of the fatty acid methyl esters,

**Table 4.** Composition of fatty acid classes of the seed aril oils from four variants of *Trichilia emetica* determined from proton NMR integrals of the FAMEs.

| *Trichilia emetica* | $CH_2$ (methylene envelope) | | $CH_2$ – diallylic | | $CH_2$ – allylic | | $CH_3$ | | $CH_3$ – ω3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | δ/ppm | Integral | δ/ppm | Integral | δ/ppm | Integral | δ/ppm | Integral | δ/ppm | Integral |
| Red-bitter | 1.29 | 20.59 | 2.79 | 0.50 | 2.07 | 2.01 | 0.92 | 3.00 | - | - |
| Red-sweet | 1.30 | 21.15 | 2.81 | 0.49 | 2.10 | 1.99 | 0.92 | 3.00 | - | - |
| Orange-sweet | 1.29 | 20.75 | 2.80 | 0.52 | 2.07 | 2.07 | 0.91 | 3.00 | - | - |
| White-sweet | 1.29 | 20.48 | 2.80 | 0.52 | 2.07 | 2.22 | 0.91 | 3.00 | - | - |

Estimated amounts of fatty acid classes

| *Trichilia emetica* | α-Linolenic | Diunsaturated | Monounsaturated | Total Unsaturated | Total Saturated |
|---|---|---|---|---|---|
| Red- bitter | ND | 25.0 | 25.0 | 50.0 | 50.0 |
| Red- sweet | ND | 24.5 | 25.0 | 49.5 | 50.5 |
| Orange-sweet | ND | 26.0 | 25.8 | 51.8 | 48.2 |
| White-sweet | ND | 26.0 | 29.5 | 55.5 | 44.5 |

% Composition of FA classes were estimated from one $^{1}$H NMR experiment for each oil sample;
CN = Average carbon Number; ND = Not detected.

FAMEs, are as shown in Table 3. These results are in full agreement with the saponification values reported in Table 1, which indicated that the bulk of each oil sample was composed of medium chain fatty acids such as C16 and C18 fatty acids. The single most abundant fatty acid in each oil sample was palmitic acid, 16:0, ranging from 40.29% for the white-sweet variant to 46.76% for the red-bitter variant. In contrast, the white-sweet variant oil contained the largest amount of oleic acid, 18:1n-9, at a level of 30.44%, whilst the red-bitter contained the lowest amount of oleic acid at 25.28%. The orange-sweet contained virtually equal amounts of oleic and linoleic, 18:2n-6, acids at 27.27% and 27.14%, respectively. This trend is also shown by the red-sweet variant which contained 25.28% of oleic acid and 25.28% of linoleic acid. The oil of the white-sweet variant contained the largest amount of linoleic acid, making its total unsaturation composition to be 58.08%, which was the highest among the four variants. The orange-sweet variant had the second highest unsaturation at 54.41%, followed by the red-sweet and red-bitter variants at 51.14% and 50.76 %, respectively. It should be recalled that this moderate level of unsaturation was predicted from the IVs reported in Table 1.

The fatty acid profiles of the four mafura variants as determined by GC-MS analysis of the FAMEs are in close agreement with the composition of the fatty acid classes estimated from proton NMR analysis of the FAMEs as shown in Tables 3 and 4. In the NMR analysis, the composition of the fatty acid classes was determined from the integrals of the proton signals in the olefinic region using Holmback's equations (Holmback, 2000). Both Tables 3 and 4 show that the mafura aril oils contained about equal amounts of linoleic (diunsaturated) and oleic (monounsaturated) fatty acids and that overall the ratio of total unsaturated to total saturated fatty acids was roughly 1:1. Thus the NMR analysis strongly complements the more accurate GC-MS technique. The ratio of the total saturation to total unsaturation in the mafura aril oils is rather similar to that of palm oil and indeed, at ambient temperatures both mafura and palm oils exist in two forms, a semi-solid lower fraction and a liquid fraction at the top. The oil extracted from the mafura aril therefore has the potential to be fractionated into two portions that can be used for different purposes, i.e., food and non-food uses, as is the case for palm oil.

The general fatty acid profile of the mafura oil reported in the literature is often that of the mafura seed oil, i.e., the mafura butter which is obtained from the mafura seed kernels, where Engelter and Wehmeyer (1970), reported that oleic acid was the dominant fatty acid [38.8% 16:0, 2.2% 18:0, 48.5% 18:1(n-9), 10.4% 18:2 (n-6) and 1.0% 18:3 (n-3)], whereas Khumalo et al. (2002), [0.5% 14:0, 52.7% 16:0, 1.9% 18:0, 27.8% 18:1 (n-9), 16.3% 18:2 (n-6) and 0.3 18:3 (n-3)] reported that palmitic acid was the dominant fatty acid. Both reports put the content of linoleic acid as being much lower than that of oleic acid. In this work the investigation focused on the mafura fruit aril oil and we here report that the content of linoleic acid in the pulp oil was about equal to that of oleic acid, Tables 3 and 4. It can therefore be inferred that the increased polyunsaturation in the aril oil allows a large portion of it to remain in the liquid state at room temperature. Thus the major difference between the mafura seed oils, i.e., the mafura butter and the mafura aril oil is the presence of higher levels of polyunsaturation in the mafura aril oil.

The comparative study of the aril oils from the four *T. emetica* variants has in fact shown that the oils from all the four variants have very similar properties: they all have good hydrolytic and oxidative stability, the average chain length of their fatty acids is very much the same (C16-C18) and above all, their fatty acid profiles are very similar with roughly equal amounts of total saturated and total unsaturated fatty acids. The high oil yields of the *T. emetica* arils, together with the favourable characteristics of the oils, make all the variants of the species attractive for commercial development for both food uses and non-food uses such as feedstock for the production of biodiesel. Indeed with its similar fatty acid composition as that of palm oil, the mafura fruit aril oil can be developed to become an important source of edible seed oil for the world market.

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