# Constituents of ‘kuwing’ oil from irvingia gabonensis

ABSTRACT

Kuwing oil extracted from Irvingia gabonensis seed mash fermentated over 6 days in AgoiIbami community, Nigeria, was analysed for it’s essential oil constituents. Both the fresh seed and the ferment’s oil extracts were analysed for fatty acids, organic acids and essential oils, using GC and GC-MS methods of analysis. Six (6) fatty acids: Oleic, Linoleic, Stearic, Lauric, Behenic acids were found in both samples, while Mystiric was found only in the fermented product. Five(5) organic acids constituents Citric, Glycolic, Oxalic, Malic and Tartaric acids were identified in boththe fresh seed and the ferment. While fifty one (51) chemicals were identified as volatiles or essential oils , the main constituents are α -Pinene, Carene, Trans-Ocimene, α-Terpinene, Cis-Limonene Oxide, Perillaldehyde, Nootkatone, Germacrene-D, and Bornol, about 75% of the oil and nineteen (19) of the identified volatiles responsible for flavour and aroma, making up to 43% of the oil.

KEY WORDS: Essential oils, Irvingiagabonensis, Ferment, Constituents, Kuwingoil.

INTRODUCTION

In earliest times, Irvingiagabonensis (of simaronbaceae family) was sourced from the vast virgin forest. Then, fruits were allowed to ripen and drop from the tree top before they were hand-picked and usually, hunters gave information on the quantity of fruits on ground. Initial drops were regarded as the fruits “ testing the ground”. As the quantity of fruits on ground increased, collectors were alerted by hunters. Whoever found the fruits first, owned them.

No family lineage owns Irvingiagabonensis trees growing in virgin forests. However, with deforestation, some trees can now be found in secondary forests. The fruits were never harvested from the tree but, once they have dropped from the tree they are assumed matured. With increase promotion of non-timber forest products for agro-forestry, the number of irvingia trees’ plantations are on the rise.

Once collected, the fruits were heaped against trunks of big trees to rotten for de-pulping. After which, the seeds/nuts were cracked open to extract the edible cotyledons. Increase demand and market expansion for irvingia cotyledon for culinary uses due to attractive revenue has led not only to harvesting the fruits from tree trunks but also splitting fresh fruitsto obtain the cotyledons. The cotyledons are usually processed into a variety of products using different processing methods.

Traditionally, fermentation process is employed in the preparation of a number of products, one of which is ‘ itugha’ from irvingiavargabonensis (Ekpe, O. O, 2007). Sun drying also enhances the quality of bush mango seeds and this attribute give attractive prices for the sun-dried cotyledons. Modernization has adversely affected the preparation and utilization of ‘ Itugha’ and this age-long nutrient rich food (Ekpe et al, 2007) appears to be gradually disappearing from the community dietary. Distribution of ‘ Itugha’ is usually limited to the top family members and very close friends, which always resulted in disaffection among those not so favoured in its distribution. Despite the high food value usually placed on this food item, it is fast becoming extinct. To diversify its utilization, identification of secondary

products having other uses can expand and encourage itscommercial production and industrialization.

Even though the food value of any food product is a measure of its nutritional potentials, measured by it’s chemical composition. Safety indicators, the level of food toxicants as well as bioavailability of the nutrients are also important (Agube, 1991). Other applications of constituents in a product can be as important as it’s food value. As such, identifying essential oil constituents of itugha can promote it’sother non-food utilization as well as it’s food uses.

Essential oils are any class of highly volatile organic compounds found in plants. Chemically, essential oils are extremely complex mixtures containing compounds of very major functional-group class like terpenes, isoprenoids, alcohols, esters, aldehydes, ketones and phenols. Essential oils have three primary commercial uses; as odorants in perfumes, soaps, detergents and other products: as flavours in baked goods, candies, soft drinks and other foods: and as pharmaceutical in dental products and many medicines. ( Britannicaconcised encyclopedia, Aroma Web). Most people use essential oils for their therapeutic effects as they tend to leave beneficial bacteria intact while killing the pathogens or for their fragrance alone. Buchbauer&Jirovetz(1994) published an excellent survey on the uses of essential oils as medicament. Studies have shown that bacteria do not acquire resistance to essential oils as they do with antibiotics and plant essential oils are also known for their antimicrobial activitye. g. essential oils of Dacryodisedulis- African pear (Obameet at 2008).

Today when so many illnesses and bacteria are becoming resistant to antibiotics, the therapeutic effects of essential oils and their immune-boosting abilities may be just what we need to explore. Essential oils can be detected in all the cells of the body 21 minutes after application.

Essential oils are designated and defined by the plant species and sometimes geographical location( McGraw-Hill Science & Technology Encyclopedia). Kuwing oil produced from seeds of Irvingiagabonensis pulverized and fermented over 6 days and heat treated for 2 days, is investigated for it’s essential oil composition.

The value of non-timber forest products lies in their use as a supplementary food supply, as a source of vitamins, as snacks during hunting and gathering forays in the bush, as beverages, building materials, farm and kitchen tools and in the maintenance of traditional rites and pastimes (Alexandar etal , 1994). A key feature of the Irvingia study has been the gathering and documentation of indigenous knowledge on the species to expand the scope of available information on its possible utilization and application.. To the best of our knowledge, no literature information is available on essential oil composition of‘ kuwing oil’ fromIrvingiagabonensis.

Thus this study would in addition to, exploring precursor compounds in fresh odourlessirvingiagabonensis seed, highlight constituents of kuwing oil for its utilization prospects in industry especially for non-food purposes.

The fresh seed is odourless, colourless and without flavour. Macerating/pounding, heat treatment and fermenting mashed seeds in control conditions produces odour and flavourin the mash. This is known to

increases its acceptance in the food industry and projects potential utilization prospects in the non-food industry.

In this paper we are reporting the chemical composition of essential oils of ‘ kuwing oil’ extracted from irvingiagabonensis seed.

This study will cover identification of possible precursors of flavour compounds e. g. fatty acids and organic acids from fresh irvingia seeds and the volatiles or essential oils constituents, of kuwing oil, from heat treated fermented irvingiagabonensis seed mash.

MATERIALS AND METHOD

Fresh Irvingiagabonensis seeds was milled with the mill unit of a National blender, Model MX 495 for six (6) days under controlled condition. After each day’s milling, the mash was wrapped with *Piper umbellantum* leaves. This was to simulate the repeated milling under controlled conditions, that is necessary for the production of a fermented traditional spread from I. gabonensis called ‘ itugha’ . Oil drip from this ferment is the ‘ Kuwing’ Oil.

The fatty acid content of fresh Irvingia seed and Kuwing Oil sample were determined using the method of International laboratory (1993). In this method, the samples were first extracted with petiether to remove the oiliness in the samples. The lipid extracts were Methylated and the methyl esters of the respective fatty acids in the solvent fractions were analysed by gas-liquid chromatography. A 250ml flask was weighed( $w_o$ ), 5g of sample quantitatively weighed into a fat extraction thimble and 250ml petroleum ether poured into the previously weighed flask containing anti-bumping

chips. A soxhlet extractor into which the thimble with its contents had been introduced was then fitted into the round bottom flask and the extraction apparatus mounted on a heating mantle. The contents of the flask were heated and extraction process continued for about 15 hours. At the end of extraction, petroleum ether in the round bottom flask was distilled off the oily extract with the soxhlet and the little quantity finally evaporated off in a water bath at 50 $^{0}$ c. The flask and the fat extract were finally dried in a hot air circulating oven at 100 $^{0}$ C, cooled in a desiccator and weighed ($w_1$)

Methylation of Fat Extract

i. The fat sample were heated for 2 hours under a current of nitrogen at 80-90 $^{0}$ C with 4%sulphuric acid in methanol.

ii. After cooling and the addition of distilled water,

iii. the resulting methyl esters were extracted several times into hexane.

iv. The combined extracts were dried over sodium carbonate and anhydrous sodium sulphate (in a dessicator).

v. The solvent fraction was then reduced in volume by a stream of nitrogen.

Gas-Liquid Chromatography

Each methylated oil samples were analysed by gas-liquid chromatography on a Carlo Erba gas chromatograh 5160 Mega series, equipped with a shimadzu data processor C-R3A using the following experimental conditions:

(a)Glass capillary column 25m x 0. 32mm i. d coated with SE 52.

(b)Column temperature 60 $^{0}$ C

(c)Injector and detector temperature 280 $^{0}$ C.

(d)Carrier gas-hydrogen about 0. 40 Kgcm-2

(e)Injection mode-split detector F ID (Field ion desorption).

(f)Identification of compounds – retention time and by GC-MS using a Finnigan Mat ITD 800 with a 25m x 0. 32mm i. d. fused-silica capillary column coated with SE 52.

(g)Column temperature 60-240 $^{0}$ C at 3 $^{0}$ C/min.

(h)Ionizing voltage 70eV

Organic acid content was determined in Irvingia seed and the ferment from which Kuwing oil was extracted, by Gas chromatography – Mass Spectrometry, Bengtsson and Lehotay method (1996) with some modification. 1g of sample was pulverized with 1ml of distilled water, acidified with 1ml 1M HCl to a pH of about 1. 0, saturated with NaCl, then extracted with 3ml of ethyl acetate and 3ml of diethyl ether. The organic phases were combined and evaporated to dryness under nitrogen. The sample was derivatised with 0. 100ml of BSTFA-TMCS at 65 $^{0}$ C for 10 min, diluted with 0. 400ml of hexane/ethyl acetate (50% v/v) and 1 µ1 was injected into the GC-MS and analysed. Gas chromatographic, mass spectral and data analysis on Carlo Erba gas chromatograh 5160 Mega Series, equipped with a Shimadzu data Processor C-R3A: Sample was analysed by GC-MS by injecting 1 u1 of the sample in spliteless mode onto an open

tubular glass capillary column 25m x 0. 32mm i. d coated with SE 52, and the injector was kept at 250 $^0$ C. The carrier gas was hydrogen, with a flow-rate of 1ml/min. The GC oven was held at 90 $^0$ C for 4min, then raised at 8 $^0$ C/min. The peaks were identified by reference to a mass spectral library.

Essential/ volatile oils present in irvingia seeds and kuwing oil were identified using Giovanni Dugo and AnthonellVerzera (1993) method. In this method, fat extract was obtained from 10g of sample with petroleum ether. The sample of oil was prepared for gas-liquid chromatography on a Carlo Erba gas chromatograph 5160 mega series column 25m x 0. 32mm i-d, 60 $^0$ C at 3 $^0$ C/min and hydrogen carrier gas

1. 10g of sample was extracted with 100ml of petroleum ether (60-80 $^0$ C) by soxhlet extraction. Petroleum ether was distilled to afford an oily fraction prepared for gas-liquid chromatography analysis.
2. GC-MC analysis of Volatiles: The volatile fraction were collected by steam distillation and the volatiles were extracted thoroughly into methylene dichloride and concentrated.
3. The concentrated volatiles were separated by gas-liquid chromatography on a Carlo Erba gas chromatograh 5160 Mega series, equipped with a shimadzu data processor C-R3A under the following experimental conditions:

i. Glass capillary column 25m x 0. 32mm i. d. coated with SE 52.

ii. Column temperature 60 $^0$ C to 100 $^0$ C at 3 $^0$ C/min.

iii. Injector and detector temperature 280 $^0$ C

iv. Carrier gas0hydrogen about 0. 40kgcm $^{-2}$

v. Injection mode-split detector field ion desorption (FID).

vi. Identification compounds – retention time and by GC-MC using a Finnigam Mat ITD 800 with a 25mm x 0. 32mm i. d. fused-silica capillary column coated with SE 52.

vii. Column temperature 60-250 $^{0}$ C at 3 $^{0}$ C/min

viii. Ionizing voltage 70eV.

Relative amounts of detected compounds were calculated based on GC peaks.

Volatiles Identification in Kuwing Oil

Essential oils constituents of Kuwing oil identified against standards

1. α -Pinene

2. ï ¢-Pinene

3. Camphene

4. Carene

5. α-Terpinene

6. p-Cymene + Limonene

7. Trans-ï ¢-Ocimene

8. Y-terpinene

9. Octanol

10. Terpinolene

11. Trans-Sarbine hydrate

12. Nonanal

13. Cis-Limonene Oxide

14. Trans-limonene Oxide

15. Isopulegol

16. Citronellal

17. Borneol

18. α-Terpinol

19. Decanal

20. Nerola + Citronellol

21. Neral

22. Piperitone

23. Linalyl acetate

24. Geranial

25. Perillaldehyde

26. Undecanal

27. Nonyla acetate

28. α-ester

29. α-Terpernyl acetate

30. Citronellyl acetate

31. Neryl acetate

32. Heranyl acetate

33. ï ¢-Caryophyllene

34. Trans-ï ¢-Bergamotene

35. ï ¢-Humulene

36. ï ¢-Sabtalene

37. Aldehydic ester

38. Germacrene-D

39. Germacrane-B

40. Germacrane-D

41. ï ¢-Bisobolene

42. ï ¢-Sesquiphellandrene

43. Trans-α-Nerolidol

44. Cis, trans-Fernesol

45. NootKatone

DISCUSSION AND CONCLUSION

Gas-liquid chromatography estimation of fatty acids in Irvingiagabonensis seed and the ferment is shown in Table 1. Six fatty acid fractions were identified in the ferment and five in Irvingia seed. Oleic, linoleic, stearic, lauric and behenic acids were identified in the ferment. Linoleic acid was the most abundant fatty acid in Irvingia seed and ferment. The level of oleic acid was very low both in the ferment and Irvingia seed. Processing had little or no effect on its level. Stearic acid level in Irvingia seed was very low. However, processing increased its level significantly in the ferment. The levels of stearic, lauric and behenic acids were also increased in the ferment. The decreases in linoleic acid in the ferment is very revealing. Linoleic acid can be oxidatively degraded to $C_6$ aldehydes, alcohols and their esters. These $C_6$ compounds play significant roles in essential oils development (Kobayashi et al., 1994). This type of degradation might be the cause of decrease in level of linolenic acid from 80% total lipid in Irvingia seed to about 52% total lipid in the ferment.

There were good levels of stearic acid, behenic acid (a seed triglyceride) and mystiric acid in the ferment. Mystiric acid was not detected in Irvingia seed but was detected in good proportion in the ferment. McBurney etal. 1990)

reported that microbial fermentation of starch results in the production of some fatty acids, depending on the chemical composition of the starch. This could explain the appearance of mystiric acid in the ferment which was hitherto absent in Irvingiagabonensis seed. The high level of mystiric acid in the ferment could have been due to microbial enzyme hydrolysis of starches in Irvingiagabonensis seed, and subsequent degradation to aldehydes, alcohols etc.

Table 2 shows organic acid content of Irvingia seed and the ferment. Organic acids influenced pH that determines microbial growth and serve as preservatives. They influence the formation, type and rate of thermally produced flavour (Maga, 1994). These acids could have been produced from the non-total oxidation of sugars, as well as the deamination of amino acids, ascorbic acid and polyphenolic acids.

Formation of volatiles in food can also be attributed to enzymic biosynthesis. The cell rupturing which took place during maceration of irvingiaseeds, could have caused enzymes and precursors of essential oils to come in contact with one another. Bacterial growth suppression and primary metabolism can trigger biosynthesis of secondary metabolites in cell cultures ( Prahba et al., 1990). This agrees with bacterial growth suppression in controlled fermentation of the irvingia seed mash in itughaproduction( Ekpe, O. O. 2009). Some of the Organic acids identified in the fresh seeds were lost in the ferment e. g. Malic acid in the seed 6. 28% decreased to 0. 11% in the ferment, Citric acid in the seed 16. 0% to 2% in the ferment and Oxalic acid 6. 6% seed to 2% ferment while fatty acid like Linoleic acid decreased from 80% in the seed to 52% in the ferment.

Autolysis consisting of plasmolysis followed by proteolysis usually require up to 24 hours of temperatures above 45 $^{o}$ c. Plasmolysis can be initiated by different treatments including hot air drying( Saeki et al., 1989). This is in consonant with the observation that volatiles of kuwing oil were formed not less than 24 hours of hot air drying of the irvingia ferment. Each autolysate is known to have its own distinctive taste and odour(Lieske and Konrad, 1994).

Fig. 1 shows constituent of kuwingoil (essential oil) revealing the presence of Terpenes like Citronellal, Limonene, Terpinolene, α-Terpinene and isoprenoids among others. The extraction and synthesis of terpenes is the basis of the perfumery industry. They find a variety of uses in the food and pharmaceutical industry as flavor and odour improver. Citronellal is known to have insect repellant properties and research show its high repellant effectiveness against mosquitoes and strong antifungal qualities(Jeong-Kyu KIM et al., 2005; Kazuhiko NAKAHARA et al., 2003; Solomons, T. W. G 2006). α-Pinene, camphene, δ3-carene, Trans-β-ocimene, Y-terpinene, octanol, cis-Limonene oxide, Neral and Perillaldehyde constitute 75% of the oil

Odourlessirvingiagabonensis seed, macerated and fermentedover six days producedKuwing oil on exposure to temperatures above 45 degrees celcius. This oil was obtained by compressing, extracting, or distilling off heat sensitive volatiles from crushed and fermented irvingiagabonensis seedsSince essential oils often have odour and are therefore used in food flavouring/ perfumery, kuwing oil can qualify as of perfume quality oil which should be included among oils found in health foods(Wiley Dictionary of Flavours). When extracted for this purpose, extremely low pressure and low heat distillation is recommended( Wikipedia). On analyses for essential oils

constituents, 75% of these constituents are established essential oil constituents used in industries. And 45% of these find application in perfumery and aromatory industries. Others have been known to have antimicrobial and antifungal activity e. g. α-pinene, camphene, careen, octanol, Limonene, Neral, Citronellal etc. It is expedient then to list kuwing oil from irvingiagabonensis seed as an essential oil.