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Research Paper

Essential oils constituents of 'kuwing' oil from *Irvingia gabonensis* seeds ferment

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Abstract

Kuwing oil extracted from Irvingia gabonensis seed mash fermented over 6 days in Agoi Ibami 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 in the fermented product only. Five(5) organic acids constituents citric, glycolic, oxalic, malic and tartaric acids were identified in both the fresh seed and the ferment. while fifty one (51) chemicals were identified as volatiles or essential oils.The main constituents wereα -pinene, δ3-carene, trans-β-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, made up to 43% of the oil.

Keywords: Essential oils, *Irvingia gabonensis*, ferment, constituents, kuwing oil, fermentation.

In earlier times, *Irvingia gabonensis* (of *simaronbaceae* family) was sourced from the vast virgin forest. 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. Increased demand and market expansion for *Irvingia* cotyledon for culinary uses due to attractive revenue has led not only to harvest the fruits from tree trunks but also splitting fresh fruits to 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 *Irvingia* var *gabonensis*. Sun drying also enhances the quality of bush mango seeds which gives attractive prices to the product. Modernization has adversely affected the preparation and utilization of *'Itugha'* the age-long nutrient rich food (Ekpe *et al.*, 2007) appears to be gradually disappearing from the community dietary. Despite the high food value, is fast becoming extinct.

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).

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 *et al.*, 1994).

As such, identifying essential oil constituents of *itugha* can promote its other non-food utilization, expand its food uses and encourage its commercial production and industrialization.

The fresh seed is odourless, colourless and without flavour. Macerating/pounding, heating and fermenting mashed seeds in control conditions produce odour and flavor in the mash. This is known to increase its acceptance in the food industry and projects potential utilization prospects in the non-food industry. Essential oils, a class of highly volatile organic compounds found in plants, are extremely complex mixtures containing compounds of different functional-groups like terpenes, isoprenoids, alcohols, esters, aldehydes, ketones and phenols. These are designated and defined by the plant species and sometimes geographical location (McGraw-Hill Science & Technology Encyclopedia). No information on essential oil composition of 'kuwing oil' from *Irvingia gabonensis* is available. Kuwing oil produced from seeds of *Irvingia gabonensis* pulverized and fermented over 6 days and heated for 2 days, was investigated for it's essential oil composition.

These 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. (Britannica concised 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 and Jirovetz, 1994). Moreover, the bacteria do not acquire resistance to essential oils as they do with antibiotics, and plant essential oils are also known for their antimicrobial activity e.g. essential oils of *Dacryodis edulis*- African pear (Obame *et at* 2008). Today when so many illnesses causing 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.

Thus, this study would in addition to, exploring precursor compounds in fresh odourless *irvingia gabonensis* seed, highlight constituents of kuwing oil for its utilization prospects in industry especially for non-food purposes. In this paper, the chemical composition of essential oils of 'kuwing oil' extracted from *irvingia gabonensis* seed has been reported. 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 *irvingia gabonensis* seed mash has also been reported.

Materials and Methods

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

Extraction of oil

The fatty acid content of fresh *Irvingia* seed and Kuwing Oil sample were determined using the method of International laboratory (1993). The samples were first extracted with petroleum ether. The lipid extracts were then, methylated and the methyl esters of the respective fatty acids in the solvent fractions were analysed by gas-liquid chromatography.

Methylation of fat extract

The fat sample were heated for 2 hours under a current of nitrogen at 80-90^{0}C with 4% sulphuric acid in methanol.After cooling and addition of distilled water,the resulting methyl esters were extracted several times into hexane. The combined extracts were dried over sodium carbonate and anhydrous sodium sulphate (in a dessicator). 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 Megaseries, equipped with a Shimadzu data processor C-R3A, using the following experimental conditions: Glass capillary column 25m x 0.32mm i.d coated with SE 52,column temperature 60^{0}C, Injector and detector temperature 280^{0}C,carrier gas-hydrogen about 0.40 Kgcm^{-2}, Injection mode-split detector F ID (Field ion desorption), 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, Column temperature 60-240^{0}C at 3^{0}C/min and Ionizing voltage 70eV.

Organic acid determination

Organic acid content was determined in *Irvingia* seed and the ferment from which Kuwing oil was extracted, by Gas chromatography – Mass Spectrometry using 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 0.100 ml 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 oil/valatile oil

Essential/ volatile oils present in irvingia seeds and kuwing oil were identified using Giovanni Dugo and Anthonell Verzera (1993) method. In this method, fat extract was obtained from 10g of sample with petroleum ether (60-80^{0}C) by soxhlet extraction. Petroleum ether was distilled to afford an oily fraction prepared for gas-liquid chromatography analysis. 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 as detailed here.

GC-MC analysis of Volatiles

The volatile fraction were collected by steam distillation and the volatiles were extracted thoroughly into methylene dichloride and concentrated. The concentrated volatiles were separated by gas-liquid chromatographyon a Carlo Erba gas chromatograh 5160 Mega series, equipped with a shimadzu data processor C-R3A under the following experimental conditions: Glass capillary column 25m x 0.32mm i.d. coated with SE 52, Column temperature 60^{0}C to 100^{0}C at 3^{0}C/min, Injector and detector temperature 280^{0}C, Carrier gas, hydrogen about 0.40kgcm^{-2}, Injection mode-split detector field ion desorption (FID), 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,Column temperature 60-250^{0}C at 3^{0}C/min and Ionizing voltage 70eV.Relative amounts of detected compounds were calculated based on GC peaks.

Results and Discussion

Results of gas-liquid chromatography estimation of fatty acids in *Irvingia gabonensis* 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 *et al.* 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 *Irvingia gabonensis* seed, (Table 1). The high level of mystiric acid in the ferment could have been due to microbial enzyme hydrolysis of starches in *Irvingia gabonensis* 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 flavor (Maga, 1994). These acids could have been produced from the incomplete 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 bio-synthesis. The cell rupturing which took place during maceration of *Irvingia* seeds, 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 *itugha* production (Ekpe, 2009). Some quantity of organic acids identified in the fresh seeds were lost in the ferment e.g. malic acid in the seed estimated at 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, mystiric acid absent in the seed was detected in the ferment (Table 2).

Autolysis consisting of plasmolysis followed by proteolysis usually require of temperatures above 45^{O} C up to 24 hours. Plasmolysis can be initiated by different treatments including hot air drying (Saeki *et al*., 1989). This is 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 peaks of kuwing oil (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. Besides 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 *et al*.,2003; Solomons, 2006). α-pinene, camphene, δ3-carene, Trans-β-ocimene, Y-terpinene, octanol, cis-limonene oxide, neral and perillaldehyde constitute 75% of the oil

Odourless *Irvingia gabonensis* seed, macerated and fermented over six days produced Kuwing oil on exposure to temperatures above 45^{O} C . This oil was

obtained by compressing, extracting, or distilling off heat sensitive volatiles from crushed and fermented *irvingia gabonensis* seeds. Since 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 the oils found in health foods (Dolf De Rovira, 2008). When extracted for this purpose, extremely low pressure and low heat distillation is recommended (Baser and Buchbauer, 2010). On analyses for essential oils constituents, 75% of these constituents are established as essential oil constituents used in industries, and 45% of these find application in perfumery and aromatory industries. Others have been known to show antimicrobial and antifungal activity e.g.α-pinene, camphene, careen, octanol, limonene, neral, citronellal etc. Peaks one(1) to forty five(45) of the chromatograph in Fig 1 have been identified and the compounds listed while peaks forty six(46) to fifty one (51) are unknown.

Table 1. Composition of *Irvingia* seeds and ferment

Fatty acid	% Total lipid	
	Irvingia seeds	Ferment
Oleic acid	1.3	1.5
Linoleic	80	52
Stearic acid	1.3	21.3
Lauric acid	5	10.7
Behenic acids	4	37
Mystiric acid	-	29.3

Table 2 Organic acid composition of *Irvingia* seed and ferment

Organic Acid	samples (% dm)	
	Irvingia Seed	Ferment
Citric Acid	16.00+ 1.13	2.40+ 1.10
Glycolic Acid	1.26 + o.01	1.22 + 0.01
Oxalic Acid	6.59+ 1.20	2. 98+ 0.08
Malic Acid	6.28+ 1.40	0.11+ 0.00
Tartaric Acid	1.44+ 0.02	0.19+ 0.01

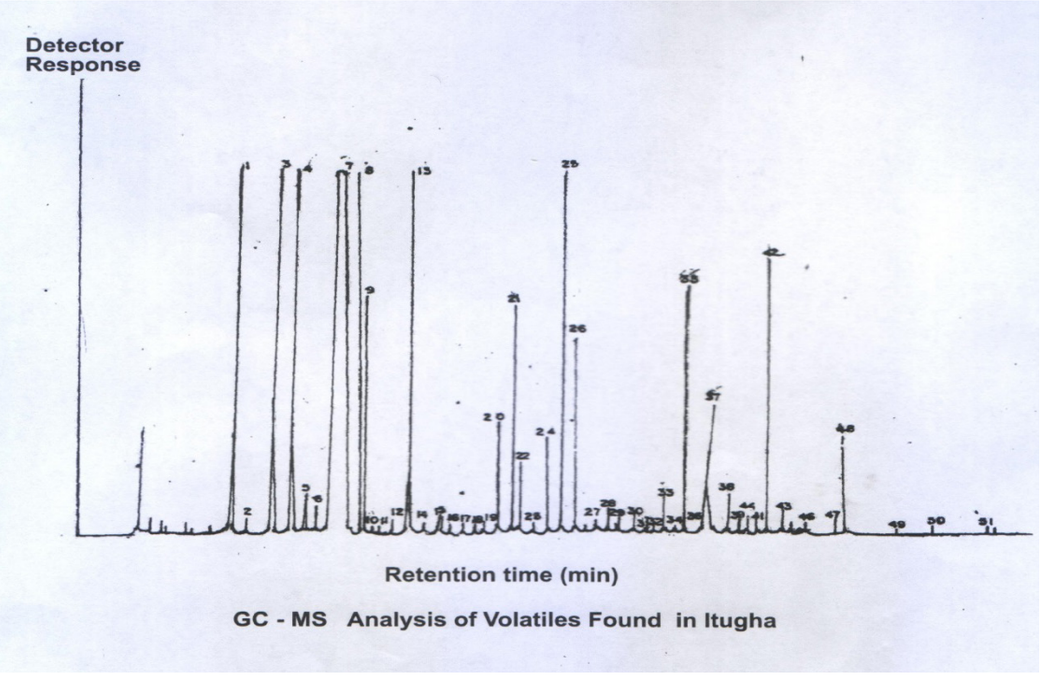

Figure 1. GC-MS Chromatogram for kuwing essential oil. Numbers refer to the listing in result.

Conclusion

Kuwing oil has been found to contain constituents of established essential oils in appropriate quantities and numbers. Nineteen (19) of the forty five (45)[in fifty one (51)] identified volatiles are known to be responsible for flavor and aroma. Therefore, this oil from fermented *irvingia gabonensis* seed paste/mash is recommended for listing as an essential oil. The identification of the remaining unnamed peaks is in progress.

Volatiles Identification in Kuwing Oil

Essential oils constituents of Kuwing oil identified against standards

1. α -Pinene , 2. β-Pinene, 3. Camphene, 4. δ3-Carene, 5. α-Terpinene 6. p-Cymene + Limonene, 7. Trans-β-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. β-Caryophyllene, 34. Trans-β-Bergamotene, 35. β-Humulene, 36. β-Sabtalene, 37. Aldehydic ester, 38. Germacrene-D, 39. Germacrane-B 40. Germacrane-D, 41. β-Bisobolene, 42. β-Sesquiphellandrene 43. Trans-α-Nerolidol, 44. Cis, trans-Fernesol, 45. Noot Katone

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