academicJournals

Vol. 13(42), pp. 4119-4123, 15 October, 2014
DOI: 10.5897/AJB2013.13261
Article Number: BE111EC48143
ISSN 1684-5315
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Volatile compounds produced in two traditional fermented foods of the Congo: Nsamba (palm wine) and bikedi (retted cassava dough)

J. R. Dhellot, S. N. Mokemiabeka, R. Moyen, S. C. Kobawila* and D. Louembe

Equipe Pluridisciplinaire de Recherche en Alimentation et Nutrition (EPRAN-Congo), Faculté des Sciences et Techniques, Université Marien NGOUBI, Brazzaville-Congo.

Received 11 September, 2013; Accepted 2 September, 2014

The "nsamba" (palm wine) and "bikedi" (retted cassava dough) are respectively produced by fermentation from the sap of the oil palm (*Elaeis guineensis* Jack) and lactic acid fermentation from cassava root (*Manihot cassava* Crantz). The study is focused on the determination of volatile compounds present in these two traditional fermented foods of Congo at the end of fermentation. The characterization of these volatile compounds at the end of fermentation for "nsamba" and "bikedi" revealed as the main compounds esters, terpenes, fatty acids and long chain alcohols. Indeed, it was noted: for "nsamba" 86% esters (ethyl caprylate, ethyl decenoate, N-ethyl decanoic, ethyl laurate) and decanoic acid; for "bikedi" 43% terpenes and 37% alcohols: estragol, limonene, linalol, myrcene and menthol.

Key words: Palm wine, dough, cassava, aroma, fermentation.

INTRODUCTION

In Congo, people have developed, since immemorial times, at the level of family or operative units, traditional processes of fermentation to produce fermented foods and beverages. Within the identification and characterization of constituents responsible for flavor, two traditional fermented foods have been the subject of our study because of their economic, cultural and sociological impact to the population and the type of plant raw material, the root and the sap. It is about the retted cassava roots "bikedi" and palm wine "nsamba".

Food aromas are the main compounds responsible for the taste of foods, more particularly fermented foods. Several bacteria and yeasts synthesize aromas molecules during their metabolism (Belitz and Grosch, 1999; Monroy-Rivera et al., 1990; Bourgeois and Larpent-Gourgaud, 1990; Malonga et al., 1993).

Some specific aromas synthetised by microorganisms can be used as additives. It is the case for aromatic compounds produced by lactic acid bacteria, which can enhance the savour of many foods (Spinnler and Desmazeaud, 1996; Takeoka, 1998; Campbell-Platt, 1987). The cassava (*Manihot esculenta Crantz*) belongs to the botanical family of Euphorbiaceae. It is a perennial plant that can reach about 2-4 m height according to the variety. Leaves and tuberous roots are the main food products from this plant (Gomez and Valdivieso, 1985; Louembe et al., 2001; Louembe and Kobawila, 2003).

The cassava (*M. esculenta Crantz*) is an important

*Corresponding author. E-mail: kobawila.simon@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

source of calories because it covers 60% of daily caloric needs of populations in tropical Africa and Central America (Nartley, 1968). Cassava roots are a few inches of the soil surface and are 5 to 10 number. In Central Africa, particularly in Congo, the cassava root is consumed mainly as fufu (flour from fermented root) or chikwangue (cassava bread from fermented dough) (Louembe et al., 2001; Kobawila, 2003; Achi and Akomas, 2006). The fermentation of cassava roots is a lactic fermentation involving the lactic acid bacteria, mainly *Lactococcus lactis*, *Leuconostoc mesenteroides* and *Lactobacillus plantarum*; other bacteria, the *Bacillus* (*Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus polymyxa*), the Enterobacteria (*Actinomycetes*, *Streptococcus* spp.); the yeasts, the *Saccharomyces cerevisiae* species and the genus *Candida*; and the molds.

The main metabolites products are lactic acid (0.45%), butyric acid (0.40%), acetic acid (0.145%), propionic acid and ethanol (0.305%).

Among the local beverages consumed in Congo is palm wine. The sweet sap is obtained from the male inflorescences of oil palm-tree *Elaeis guineensis Jack*, which is left to rest for a few hours or days for fermentation to obtain the fermented palm wine (the type of fermentation is alcoholic) (Brassir, 1962; Faparusi and Brassir, 1972; Malonga et al., 1995; Lasekan and Abbas, 2010; Ho et al., 2007). Among the identified micro-organisms, we noted mainly *S. cerevisiae* and *L. lactis* (Amoa-Awua et al., 2007); others also present were *Bacillus*, *Corynebacterium*, *Pseudomonas* and *Hanseniaspora*. The main metabolites produced are ethanol (3.27%), acetic acid (3.95%), isobutyric acid (0.91%) and isovaleric acid (0.42%) (Malonga et al., 1995; Nur Aimi et al., 2013).

MATERIALS AND METHODS

Cassava roots aged 18 months were gotten from plantations around Brazzaville. The "bikedi" from a production workshop located in the south of Brazzaville. The fermented sap of the oil palm (*Elaeis guineensis Jack*) was collected in the vicinity of Brazzaville.

Preparation of retted cassava roots

The internal production process was made with cassava roots whole or peeled. Fresh roots were immersed in water (or peeled and immersed) for fermentation two to five days. The retted tubers were removed from the water, peeled, washed and drained (or washed and drained in the case of pre-peeled tubers). The fermented tubers were then defibrated by extracting the central fiber; they fall in clumps to give retted cassava dough.

Collection of the oil palm-tree sap

Oil palm-tree male inflorescence was cut and the sap which escapes was collected in the sterile bottles during several hours.

Preparation of samples

Case of "bikedi"

Samples of tubers were placed in tubes noted (T_0, T_1, T_2, T_3 and T_m) corresponding to tubers before soaking in water (T_0), after one, two and three days of soaking (T_1, T_2, T_3), 48 and 72 h after soaking and fermented cassava roots (T_f tube of accomplished product). For each sample of tubers (T_0, T_1, T_2, T_3 and T_f), grinding was done with Waring Blendor. 15 g obtained pulp were weighed and placed in a test tube. The tubes were then noted TT_0, TT_1, TT_2, TT_3 and TT_f. For each sample, three trials were prepared.

Case of "nsamba"

For each sample of "nsamba", V_0, V_1, V_2, V_3 and S correspond respectively to the beginning (V_0) after one, two and three days of fermentation (V_1, V_2 and V_3) and palm wine (S). The sample was stirred to mix well, then 15 g of the obtained liquid was placed in test tubes, respectively noted as TV_0, TV_1, TV_2, TV_3 and TS. For each sample three trials were prepared.

The analysis was realized with the fermented products accomplished (T_f and TS) which were appreciated by tasters panel.

Preparation and analysis of extracts

20 ml of ethyl ether (solvent 1) were added to the first tube and 20 ml of cyclohexane (solvent 2) was added in the last two tubes. Then all the tubes were placed for 3 min in vortex for sedimentation and finally, the supernatants were collected in screws tubes which were placed in the refrigerator. Analysis of samples was carried out by gas chromatography (GC) coupled with mass spectrometry (GC / MS). The apparatus used was a Hewlett Packard 5973/6890, equipped with an injector (280°C) and a HP-5 column (25 m x 2.25 mm, 0. 25 µm film thickness). The temperature programme was 50°C (5 min) the temperature was increased to 300°C at the rate of 5°C / min. The carrier gas was helium at a flow rate of 1.1 ml / min. The injected volume was 1 µl of the sample diluted at 10% (v/v) with acetone sample. Retention indices of all aroma compounds were determined according to Van Den Dool approach (Van Den Dool and Kratz, 1963). The identification of compounds was performed by comparing their mass spectra with those presented by Mc Lafferty (Mc Lafferty and Stauffer, 1989), Adams (Adams, 2001) and Joulain (Joulain and Konig, 1998), and their retention indices with those given in literature.

RESULTS AND DISCUSSION

The results of the identification and quantification indicate: a) for "nsamba" (palm wine) 15 compounds (Table 1), including: 86.05% esters; 6.48% of fatty acids, octanoic, decanoic, dodecanoic and hexadecanoic acids; 0.67% of terpene represented by (E)-beta-farnesene and dillapiole; 0.59% phenyl ethylic alcohol; 0.18% of aldehyde, tridecanal. b) For the "bikedi" (fermented cassava paste) 51 compounds (Table 2) including: 41.19% terpenes; 37.11% alcohols such as linalool, estragol, anethole and cravacol. The estragole is the most represented (33.82%); 6.18% methyl esters with angelate, citronnellyl formate, ethyl decanoate, bornyl acetate, geranyl acetate, and thymol methyl ester; 4.57% alkanes $nC_{12}H_{26}$, $nC_{15}H_{32}$, $nC_{16}H_{34}$; 1.51% fatty acids with nonanoic,

Table 1. Compounds identified in the extract of “nsamba"

Chemical constituent	Percentage (%)
Ethyl hexanoate	1.7135
Phenyl-ethylic alcohol	0.5946
Octanoic acid	1.8381
Ethyl caprylate	28.2519
Phenyl ethyl acetate	0.3402
Ethyl 3-phenyl propionate	0.2417
Decanoic acid	3.6444
Ethyl 9-decenoate	23.7702
N–Ethyl decanoate	22.5624
(E)-Beta-farnesene	0.3903
Tridecanal	0.1781
Dodecanoic acid	0.8117
Ethyl laurate	3.7615
Dill apiole	0.2777
Ethyl palmitate	0.3072
Hexadecanoic acid	0.1922
Ethyl 9-hexadecanoate	1.4718
Isomeric ethyl 9- hexadecanoate	0.2517
Ethyl palmitate	1.8893
Ethyl oleate	0.3251
Ethyl oleate	0.9766
Ethyl stearate	0.1981

Table 2. Compounds identified in the extract of "bikedi"

Chemical constituent	Percentage (%)
Myrcene	2.4749
Limonene + eucalyptol + para-cymene	23.9185
(Z) beta ocimene	0.7087
(E) beta ocimene	0.5456
Gamma terpinene	1.7114
2-Nonanone	1.0451
Linalol GIVAUDAN	2.4808
CIS-ROSE OXIDE	0.5274
Ocimene « Neo-allo »	0.7592
Camphor	0.5182
2-Methyl butyl angelate	0.2858
Menthone	2.4612
Iso-menthone	1.1662
Terpinene-4-ol	1.1721
Methyl salicylate	0.7165
Estragol	33.8179
Methyl thymol ether	0.7945
Methyl carvacrol ether	0.4405
L-Carvone	0.3358
Piperitone	0.4528
Citronnellyl formiate	1.8292
Nonanoic acid	0.6219
Bornyl acetate	1.6852
Anethol	0.6951
2-Undecanone	0.2734
Methyl nonylcetone	0.7623
Carvacrol	0.1182
Citronnellyl acetate	0.4974
Neryl acetate	0.1557
Dehydro acetic acid	0.5773

decanoic and dehydro acetic acids.

The identification and quantification of responsible compounds for aromas in the two fermented traditional foods "bikedi" and "nsamba" reveal a clear difference of compounds between the two extracts. They showed the presence of terpene hydrocarbons in larger amounts (41.19%) than alcohol (37.11%) in the aromatic extract of "bikedi." As for the aroma of "nsamba", they are mostly composed of fatty acid esters (86.05%) with mainly ethyl caprylate (28.25%) (Anli et al., 2007). This difference is due, in part, to the constituents of the sap of oil palm and of cassava root and, secondly, the microorganisms involved in the fermentation and the ability of these microorganisms to synthesize aroma compounds from the present products. The microorganisms produce intra and extra cellular enzymes which contribute to generative reactions for flavor and aromas. The aldehydes, ketones and carboxylic acids may result from a degradation reaction of oxidation catalysed by lipoxigenase and hyperoxidase enzymes (Leejeerajumnean et al., 2001; Zhao et al., 2006; Nzigamasabo, 2012).

In the "bikedi" limonene representing 23.92% of the aromas could come from the breakdown of sugars. It allows the production of mevalonic acid and leads to the formation of geranyl pyrophosphate (GPP), a precursor of limonene (Nyako, 1977; Mann and Davidson, 1994).

Limonene is used in food and pharmaceutical industry to flavor bitter alkaloids. It possesses also phytotherapeutic virtues because it is recognized as an anticancer agent (Daeschel et al., 1988; Tsuda et al., 2004). Estragole (33.82%) was also identified in these extracts. It is used as a food aroma in some liqueurs and in perfumery (Scheier, 1979). The estragole is also suspected to be carcinogenic and genotoxic (Annan et al., 2003).

Among the phenols, we noted the presence of carvacol and thymol. Phenols have the antioxidant power and the most interesting bactericidal or bacteriostatic activity (Richard, 1992).

Myrcene, monoterpene hydrocarbon, identified in "bikedi" and "nsamba" is a key molecule for the synthesis of various compounds, including vitamins A and E but also the geraniol and its derivatives. It allows the reduction of the sensitivity to pain by increasing endogenous morphinopeptides (Andah and Muller, 1973).

In "nsamba", the following compounds were identified: tridecanal, dillapiole, dodecanoic acid and ethyl stearate. This qualitative difference may be support of the difference in aromatic typical of these foods (Umoh et al.,

1985; Onyango et al., 2004; Lasekan and Abbas, 2010).

Conclusion

The characteristic aromatic notes of "nsamba" could be due to the presence of: a) constituents compounds of the oil palm-tree sap, rich in carbohydrates, fats and proteins, while the cassava root, raw material of "bikedi", is essentially rich in carbohydrates; b) mixed populations of yeasts and bacteria whose the action lead to alcoholic and lactic acid fermentation, respectively for the oil palm-tree sap and the cassava root as well as the particular synthesis of aromas molecules according to the micro-organisms species. The qualitative difference in the composition of these two fermented foods is at the basis of the difference in the aromatic notes between "bikedi" and "nsamba". The characterization of these volatile compounds is an important piece of information for understanding the perception of odours, flavor and aromas (Shittu and Adedokun, 2010; Biasioli et al., 2011; Fiches et al., 2013).

In perspective, to improve the taste and aromas of the products, it is possible to use the technique of starters which is more suitable to the use of pure cultures in traditional methods as it is indicated by the studies of Ko (1985), Nout et al. (1987, 1992, 1993), and Tuncel et al. (1989). The knowledge of the biochemical phenomenon occurring in these food fermentations could allow to improve the traditional processes of production for obtaining products which have better quality.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Achi OK, Akomas NS (2006). Comparative assessment of fermentation techniques in the processing of fufu, a traditional fermented cassava product. Pak. J. Nutr. 5 (3):224-229.

Adams RP (2001). Identification of Essential Oil components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corp. Carol Tream. IL.

Amoa-Awua WK, Sampson E, Tano-Debrah K (2007). Growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm (Elaeis guineensis) in Ghana. J. Appl. Microbiol. 102:599-606.

Andah A, Muller HG (1973). Studies on koko, a Ghanian fermented maize porridge. Ghana J. Agric. Sci. 6:93-102.

Annan NT, Poll L, Sefa-Dedeh S, Plahar WA, Jakoksen M (2003). Volatile compounds produced by *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough. J. Appl. Microbiol. 94:462-474.

Belitz HD, Grosch W (1999). *Food chemistry*. Second edition, Springer-Verlag, Berlin Heidelberg (Allemagne), 992 pp.

Biasioli F, Yeretzian C, Gasperi F, Mark TD (2011). PTR-MS monitoring of VOCs and BVOCs in food science and technology. Trends Anal. Chem. 30(7):968-977.

Bourgeois CM, Larpent-Gourgaud M (1990). Les fermentation salimentaires, Techniques et documentations, Lavoisier (Paris).

Brassir O (1962). Observations on the fermentation of palm-wine. W. Afr. J. Biol. Appl. Chem. 6:21-25.

Campbell-Platt G (1987). Fermented foods of the world - a dictionary and guide. London. Butterworths. ISBN: 0-407-00313-4.

Daeschel MA, Fleming HP, MCFEETERS RF (1988). Mixed culture fermentation of cucumber juice with *Lactobacillus plantarum* and yeasts. J. Food Sci. 53:862-864.

Faparusi SI, Brassir O (1972). Factors affecting the quality of palm-wine. W. Afr. J. Biol. Appl. Chem. 15:17-28.

Fiches G, Deleris I, Saint-Eve A, Brunerie P, Souchon I (2013). Modifying PTR-MS operating conditions for quantitative headspace analysis of hydro-alcoholic beverages. 2. Brandy characterization and discrimination by PTR-MS. Int. Food Res. J. 20(11):369-376.

Gomez G, Valdivieso M (1985). Cassava foliage: chemical composition, cyanide content and effect of drying on cyanide elimination. J. Sci. Food Agric. 36:33-441.

Ho CW, Wan Aida WM, Maskat MY, Osmann H (2007). Changes in volatile compounds of palm sap (Arenga pinnata) during the heating process for production of palm sugar. Food Chem. 102: 1156- 1162.

Joulain D, König WA (1988). The Atlas of Spectral data of Sequiterpene Hydrocarbons. E.B. Verla, Hamburg.

Ko SD (1985). Some microbial aspects of tempe starters. Proceedings, Asian Symposium on non-salted soybean fermentation, Tsukuba, Japan, July.101-109.

Kobawila SC (2003). Les Aliments Fermentés d'Afrique Centrale : cas du CONGO, Thèse de Doctorat D'état. 144.

Lasekan O, Abbas KA (2010). Flavour chemistry of palm toddy and palm juice : a review. Trends Food Sci. Technol. 21 (10):494-501.

leejeerajumnean A, Duckham SC, Owens ID, Ames IM (2001). Volatile compounds in bacillus-fermented soybeans. J. SC. Food Agricult. 81 : 525-529.

Louembe D, Kobawila SC (2003). Etude microbiologique des Feuilles fermentées de manioc : « Ntoba mbodi ». TROPICULTURA. 21(3) : 106-111.

Louembe D, Kobawila SC, Massamba JP, Malonga M, Mavoungou O (2001). Caractéristiques socio-démographiques dans la filière pâte rouie de manioc au Congo-Brazzaville. Tropicultura 19(4): 213-217.

Malonga M, Mavoungou O, Kobawila SC, Louembe D (1995). Etude microbiologique et biochimique du vin de palme (*Elais guineensis Jacq*) en République du Congo. Microbiol. Aliments Nutr. 13:195-200.

Malonga M, Mavoungou O, Kobawila SC, Louembe D, Brauman A (1993). Les bactéries lactiques au cours du rouissage : caractérisation et évolution. Microbiol. Aliments Nutr. 11:471-475.

Mann J, Davidson RS (1994). Natural Products. Addison Wesley Longman Ltd., Harlow, UK. 308-309.

Mc Lafferty FW, Stauffer DB (1989). The Wiley NBS registry of Mass Spectral Data. 2nd Edition. J. Wiley and Son. NY.

Monroy-Rivera JA, Leberteau A, Marty C, Muchnik J, Bimbenet JJ (1990). Determination of cyanogenic coumpounds in cassava during heating air drying. Science des aliments 10:647-648.

Nartley F (1968). Studies on cassava, Cyanogenesis: the biosynthesis of linamarin and lotaustralin in etiolated seedlings. Phytochemistry 7: 1307-1312.

Nout MJR (1993). Processed weaning foods for tropical climates. J. Food Sci. Nutr. 43:213-221.

Nout MJR, Martoyunwono TD, Bone PCJ, Odamtten GT (1992). Hibiscus leaves for the manufacture of usar, a traditional inoculum for tempe. J. Sci. Agric. 58:339-346.

Nout MJR, De Dreu MA, Zuurbier AM, Bonan TS, Van Laar Houen IAG (1987). Ecology of controlled soja beans acidification for Tempe manufacture. Food Microbiol. 4:165-172.

Nur Aimi R, Abu Bakar F, Dzulkifly MH (2013). Determination of volatile compounds in fresh and fermented Nipa sap (Nypa fructicans) using static headspace gaz chromatography-mass spectrometry (GC-MS). Int. Food Res. J. 20(1):369-376.

Nyako KO (1977). Palm wine, an alcoholic beverage of Ghana. Symposium of indigenous fermented foods (Bangkok, Thailand).

Nzigamasabo A (2012). Volatile compounds in Ikivunde and Inyange, two Burundian cassava products. Glob. Adv. Res. J. Food Sci. Technol. 1(1):001-007.

Onyango C, Bley T, Raddatz H (2004). Flavour compounds in backslop fermented Uji (an East African sour porridge). Eur. Food Res. Technol. 218:579-583.
Richard H (1992). Connaissance de la nature des arômes. Les arômes alimentaires. Coll. Sciences et techniques Agro-alimentaires. Ed. Lavoisier. 21-37.
Scheier P (1979). Flavor composition of wines: a review. CRC Crit. Rev. Food Sci. Nutr. 12:59-111.
Shittu TA, Adedokun II (2010). Comparative evaluation of the functional and sensory chracteristics of three traditional fermented cassava products. J. Nat. Sci. Eng. Technol. 9(2):106-16.
Spinnler HE, Desmazeaud M (1996). La maîtrise des flaveurs. In: C.M. Bourgeois , J.P. Larpent , Microbiologie alimentaire. 2 : aliments fermentés et fermentations alimentaires (408-429). Sciences et Techniques Agroalimentaires, 2. Paris, FRA : Lavoisier Tec et Doc.
Takeoka G (1998). Flavor chemistry of vegetables. In: Flavor chemistry. Thirty years of progress. Teranishi R. et al. (Ed.), Cluwer Academic/Plenum Publishers, New York. pp. 287-304.
Tsuda H, Ohshima Y, Nomoto H, Fujita K, Matsuda E, Ligo M, Takasuka N, Moore MA (2004). Cancer prevention by natural compounds. Drug Metab. Pharmacokinet. 19(4):245-63.
Tuncel G, Nout MJR, Rombouts FM (1989). Effect of acidification on the microbial composition and performance of tempe starter. Food Microbiol. 6:37-43.
Umoh I, Ongunkoya F, Oke O (1985). Effect of thiamin status on the metabolism of linamarin in rats. Ann. Nutr. Metal. 29:312-324.
Van Den Dool H, Kratz PD (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. 11:463-471.
Zhao DY, Tang J, Ding XL (2006). Analysis of volatile components during potherb mustard (Brassica juncea, coss) pickle fermentation using SPME-GC-MS. LWT Food Sci. Technol. 40(3):439-447.