Indonesian Journal of Biotechnology, June, 2011 Vol. 16, No. 1, pp.11-16

Microorganisms Associated with Volatile Organic Compound Production in Spoilt Mango Fruits

Aliyu D. Ibrahim[1*], Bankole S. Oyeleke[3], Ummul Khaltum Muhammad[1], Adamu Aliyu Aliero[2], Sabo E. Yakubu[4], and Hadiza M. Safiyanu[1].

[1]Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Department of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University, Sokoto-Nigeria
[3]Department of Microbiology, Federal University of Technology, Minna, Nigeria
[4]Department of Microbiology, Faculty of Science, Ahmadu Bello University Zaria, .Nigeria

Abstract

Microorganisms associated with the production of volatile compound in spoilt mango fruits sold in Sokoto town were isolated and identified. The organisms include seven species of bacteria and a species of yeast. These include *Bacillus pumilus, Bacillus firmus, Brevibacillus laterosporus, Morganella morganii, Paenibacillus alvei, Staphylococcus saccharolyticus, Listeria monocytogenes* and *Candida krusei* respectively. GC-MS analysis revealed the presence of eleven and sixteen volatile organic compound in the healthy and spoilt ripe mango fruits. Octadecanoic acid, oleic acid, 1 – Butanol, 3 – methyl-, carbonate (2:1) and 3,7 – Dimethyl nonane were common to both healthy and spoilt fruits with the first three having higher concentration in healthy fruits than spoilt while the later had higher concentration in the spoilt. One methyl group of 3,3- Dimethyl hexane in healthy fruit was shifted to position two to yield 2,3-Dimethyl hexane in the spoilt fruits. 2,2-Dimethylbutane, Methyl(methyl-4-deoxy-2,3-di-O-methyl.beta.1-threo-hex-4-enopyranosid) urinate, 3-(4-amino-phenyl)-2-(toluene-4-sulfonylamino)-propionic acid, 2-Methyl-3-heptanone, 3,5-Nonadien-7-yn-2-ol, (E,E), Butanoic acid, 1,1-dimethylethyl ester, 1-methyl-3-beta.phenylethyl-2,4,5-trioxoimidazolidine, Pentanoic acid, 2,2-dimethyl, ethyl ester (Vinyl 2,2-dimethylpentanoate), 4-Methyurazole, 1-Tridecyn- 4 – 9 – ol, 1-Hexyl-1-nitrocyclohexane were unique to spoilt fruits. This study suggests that these unique volatile metabolites could be exploited as biomarkers to discriminate pathogens even when more than one disease is present thereby curbing post harvest loss during storage after further validation and the volatile organic compound could form the basis for constructing a metabolomics database for Nigeria.

Keywords : GC-MS, spoilage organisms, metabolomics, post harvest loss, volatile organic compound

Introduction

Food intake is essential for the survival of every living organism (Lindemann, 2001). The failure to detect spoiled or toxic food can have lethal consequences. Therefore, it is not surprising that humans use all their five senses to analyse food quality. A first judgement about the value of a food source is made on its appearance and smell. Food that looks and smells attractive is taken into the oral cavity. Here, based on a complex sensory analysis that is not only restricted to the sense of taste but also includes smell, touch and hearing (Drewnowski, 1997), the final decision about ingestion or rejection of food is made. Frequently, these complex interactions between different senses is inappropriately referred to as 'taste' although it should be better called flavour perception (Linden, 1993), because it uses multiple senses.

***Corresponding Author:**
Aliyu D. Ibrahim
Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria, e-mail: aid4life@yahoo.com

Microbial spoilage is manifested by a variety of sensory cues such as off-colours, off-odours, off- flavours, softening of vegetables and fruits, and slime. However, even before it becomes obvious, microbes have begun the process of breaking down food molecules for their own metabolic needs. Sugars and easily digested carbohydrates are used first, plant pectins are degraded. Then proteins are attacked, producing volatile compounds with characteristic smells such as ammonia, amines, and sulfides. This may be accompanied by the production of a wide range of metabolites which includes: alcohols, sulphur compounds, ketones, hydrocarbons, fluorescent pigments, organic acids, esters, carbonyls, and diamines. Microbial product quality or shelf-life indicators are organisms and/ or their metabolic products whose presence in given foods at certain levels may be used to assess existing quality or, better, to predict shelf-life. These may be: the spoilage organisms themselves, or their metabolic products (Doyle, 2007).

Fruits are infected prior to harvest if wounded by insects or other means or the rot may progress from infected stems and branches into the fruit. Postharvest infection of fruit may occur through wounds made during harvesting, transit or storage periods or when warmer fruit are washed in cooler contaminated water (Kucharek and Bartz, 2000). Early detection of spoilage would be advantageous in reducing food loss because there may be interventions that could halt or delay deterioration, and on the other hand food that had reached the end of its designated shelf life but was not spoiled could still be used. Numerous methods for detection of spoilage have been devised with the goals of determining concentrations of spoilage microbes or volatile compounds produced by these microbes. However, many of these methods are considered inadequate because they are time-consuming, labour-intensive, and/or do not reliably give consistent results (Doyle, 2007). This study is aimed at isolating and identifying microorganisms associated with spoilt mango fruits in Sokoto metropolis; extract and identifying volatile organic compounds associated with spoilt sweet pepper using GC-MS.

Materials and Methods

Sample collection

Ten spoilt and healthy intact ripe mango fruits were purchased from Gawon Nama area in Sokoto metropolis. Samples were collected into polythene bags and immediately transported to the research laboratory of Usmanu Danfodiyo University, Sokoto for further analysis.

Isolation and count of microorganisms

Bacteria were isolated by transferring an aliquot of 0.1 ml of a serially diluted (10^4) sample of spoilt mango fruits onto molten nutrient agar plates and incubated at 37^{o}C for 24 hours. The colonies that emerge were subculture continuously until a pure culture is obtained. For fungi isolation an aliquot of the mango fruits was inoculated onto molten SDA plates and incubated at room temperature for 7 days and was subculture continuously to obtain pure culture.

Identification of bacteria and fungi

The bacteria isolate were identified following series of biochemical test as described by Holt *et al*. (1994). Yeast colonies were studied by using Lactophenol Cotton Blue Mount (LPCB) as described by Oyeleke and Manga (2008).

Extraction of volatile metabolites

Volatile compounds were extracted using general purpose solvent Parliment (1997) as described by Ibrahim *et al*. (2011). Extraction of volatile compounds was done by direct solvent extraction method. Two gram of spoilt mango fruits and healthy ripe mango fruits was weighed into a bottle and saturated with 20 ml of diethyl ether. It was allowed to stand at room temperature for 24 h, filtered using Whatman No. 1 filter Paper and the filtrate was collected in a sterile bottle, closed tightly before the GC-MS analysis.

Gas chromatography mass spectrometry (GC-MS) analysis

GC-MS analysis was performed using GC-MS-QP2010 plus (Shimadzu, Japan) equipped with flame ionization detector (FID). The injection was conducted in split less mode at 250 ^{0}C for 3min by using an inlet of 0.75 mm i.d to minimize peak broadening. Chromatographic separations were performed by using DB-WAX analytical column 30 m 0.25 mm, 0.25mm (J&W scientific, Folsom C.A) with helium as carrier gas at a constant flow rate of 0.8 ml/min. The oven temperature was programmed at 60 ^{o}C for 5min, followed by an increase (held for 5 min), and finally at 10^{0}C/min to 280 ^{o}C (held for 10 min). The temperature of the FID was set to 250 ^{o}C. MS operating conditions (electron impact ionization mode) were an ion source temperature of 200 ^{o}C, ionization voltage of 70 eV and mass scan range of m/z 23- 450 at 2.76 scans/s.

Identification and quantification of volatile metabolites

The chromatographic peak identification was carried out by comparing their mass spectra with those of the bibliography data of unknown compounds from the NIST library mass spectra database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000). According to the method of (Wanakhachornkrai and Lertsiri, 2003) approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA). The results are presented as the peak area normalized (%).

Results

The microbial flora associated with volatile organic compound during spoilage of mango fruits were isolated and identified as seven species of bacteria and a species of yeast. These include *Bacillus pumilus, Bacillus firmus, Brevibacillus laterosporus, Morganella morganii, Paenibacillus alvei, Staphylococcus*

Table 2: Result of GC-MS analysis of healthy and spoilt mango fruits

RT (mm)	Compounds	Peak Area (%)	
		Healthy	Spoilt
3.84	4 - Methyl octane	7.03	-
3.85	2,2-Dimethyl butane	-	4.16
4.64	Methyl(methyl-4-deoxy-2,3-di-O-methyl.beta.1-threo-hex-4-enopyranosid) urinate	-	1.51
5.13	3-(4-amino-phenyl)-2-(toluene-4-sulfonylamino)-propionic acid	-	1.82
6.36	3,3 – Dimethyl hexane	5.85	-
6.37	2-Methyl-3-heptanone	-	4.35
9.67	3,7-Dimethyl nonane	4.99	5.77
11.02	1-Butanol, 3-Methyl-, Carbonate (2:1)	5.32	4.16
12.25	3,5-Nonadien-7-yn-2-ol, (E,E)	-	1.93
12.98	Butanoic acid, 1,1-dimethylethyl ester	-	0.69
14.19	1-methyl-3-beta. phenylethyl-2,4,5-trioxoimidazolidine	-	0.84
14.92	Allyl heptanoate (Heptanoic acid, 2-propenyl ester)	2.70	-
14.93	Pentanoic acid, 2,2-dimethyl, ethyl ester (Vinyl 2,2-dimethylpentanoate)	-	1.39
18.02	Naphthalane, 1-methyl (1-Methyl Naphthalane)	2.29	-
18.04	2,3-Dimethyl hexane	-	3.24
18.46	4-Methyurazole	-	2.26
27.61	Octadecanoic acid	32.39	30.47
28.77	Oleic acid	29.13	27.18
29.66	1-Tridecyn- 4 – 9 – ol	-	3.67
29.66	Dodecanoyl chloride (Lauric acid, chloride)	2.65	-
30.66	1-Hexyl-1-nitrocyclohexane	-	5.07
30.82	Hexadecanoic acid, 1-[[[2-amino ethoxy) hydroxyp hoshinyl]oxy]methyl]-1,2-ethanediyl ester	2.86	-
31.20	1- Heptadecyne	3.12	-

[1] Retention time (RT) on DB-WBX column in GC-MS.

saccharolyticus, Listeria monocytogenes and *Candida krusei* respectively.

The result of GC-MS analysis of diethyl ether extract obtained from healthy and spoilt mango revealed the presence of 11 and 16 compounds (Table 2), dominated among them are octadecanoic acid (32.39 and 30.47%), Oleic acid (29.1 and 27.18%) and a decrease in concentration of these dominant compounds were noticed in spoilt mango fruits which Butanoic acid, 1,1-dimethylethyl ester (0.69%) as the compound with the least value.

Discussion

The microbial flora associated with volatile organic compound during spoilage of mango fruits include *Bacillus pumilus, Bacillus firmus, Brevibacillus laterosporus, Morganella morganii, Paenibacillus alvei, Staphylococcus saccharolyticus, Listeria monocytogenes* and *Candida krusei* respectively. The source of these organisms could probably from soil, the fruits themselves, the harvesting/ packaging containers, mango handlers, air and dust (Jay *et al*., 2005). Mango fruits from Tashar Illela may not be safe for consumption as they may cause gastroenteritis because of the presence of *Listeria monocytogenes.* This result is in contrary to most authors as no *Pseudomonas spp* was isolated and the prevailing genera in this work are gram positive organism of the *Bacillus* genus. This probably is explained by the fact that *Bacillus spp* are able to overcome some of the intrinsic and extrinsic parameters that could have check their population due to their ability to form spores.

Several compounds were unique to disease mango fruits, which could be qualitatively used to discriminate disease fruits. Butanoic acid, 1,1-dimethylethyl ester, 2,2-Dimethylbutane, Methyl(methyl-4-deoxy-2,3-di-O-methyl.beta.1-threo-hex-4-enopyranosid) urinate, 3-(4-amino-phenyl)-2-(toluene-4-sulfonylamino)-propionic acid, 2-Methyl-3-heptanone, 3,5-Nonadien-7-yn-2-ol, (E,E), 1-methyl-3-beta.phenylethyl-2,4,5-trioxoimidazolidine, Pentanoic acid, 2,2-dimethyl, ethyl ester (Vinyl 2,2-dimethylpentanoate), 4-Methyurazole, 1-Tridecyn- 4 – 9 – ol, 1-Hexyl-1-nitrocyclohexane were unique to spoilt fruits. These unique metabolites can be used as biomarkers to detect the presence of the pathogens detected in this study. 4 - Methyl octane, 3,3 – Dimethyl hexane, Allyl heptanoate (Heptanoic acid, 2- propenyl ester), Naphthalane, 1-methyl (1-Methyl Naphthalane), Dodecanoyl chloride (Lauric acid, chloride), Hexadecanoic acid, 1-[[[2-amino ethoxy) hydroxyphoshinyl]oxy]meth yl]-1,2-ethanediyl ester and 1- Heptadecyne were unique to healthy mango fruits. 4 - Methyl octane was detected in green coffee (Gonzalez-Rios *et al*., 2006). These unique metabolites can be used as biomarkers to detect the presence of the pathogens detected in this study.

Four volatile organic compound were unique to healthy and spoilt mango fruits which include 3, 7-Dimethyl Nonane (4.99; 5.77%), 1-Butanol, 3-Methyl-, Carbonate (2:1) 5.32; 4.16%, octadecanoic acid (32.39; 30.47%) and oleic acid (29.13; 27.18%). However, a reduction was observed in the concentration of 1-Butanol, 3-Methyl-, Carbonate (2:1), octadecanoic acid and oleic acid.. The presence and/or absence of the above volatile organic compounds and the differences in their relative abundance could be considered for qualitative discrimination of healthy and spoilt mango fruits especially when unique compounds are absent and mixed infections, especially in the same lesion, are present. Similar result has been observed by Moalemiyan *et al*. (2006), that certain volatile metabolites were common to stem-end rot and anthracnose diseases of mango fruits.

The fatty acids detected in the spoilt and healthy fruits are octadecanoic acid and oleic acid. The hydroxyl form of 9- Octadecenoic acid (Z) that is hydroxy fatty acids (HFA) have been described as multifunctional molecules that have a variety of applications (Bódalo *et al*., 2005), and they and their derivatives are used in cosmetics, paints and coatings, lubricants and in the food industry (Bódalo *et al*., 2005). They are useful chemical intermediates in the synthesis of fine chemicals and pharmaceuticals (Bódalo *et al*., 2005). Moreover, some of them may protect plants against microbial infection, although the mechanism of these antimicrobial effects is poorly understood (Suzuki *et al*., 2005). The reduction in the relative abundance of octadecanoic acid and oleic acid might be that the microorganisms present in the spoilt mango fruits have converted them to other volatile compounds (Bódalo *et al*., 2005; Rodríguez, 2001). The importance of these esters has been describe to contribute to food aroma with the fact that esters with low carbon atoms are highly volatile at precursors (Izco and Torre, 2000; Nogueira *et al*., 2005). In addition to imparting a fruity floral character, esters can diminish or mask the sharpness of unpleasant free amino acid-derived notes (Yanfang and Wenyi, 2009).

In conclusion, this studies on microorganisms associated with volatile organic compound production that spoilt mango fruits sold in Tashar Illela contain organisms such as *Bacillus pumilus, Bacillus firmus, Brevibacillus laterosporus, Morganella morganii, Paenibacillus alvei, Staphylococcus saccharolyticus, Listeria monocytogenes* and *Candida krusei* respectively. Hence spoilt mango fruits from this market may not safe for consumption**.** The study revealed the presence of eleven and sixteen volatile organic compound in the healthy and spoilt ripe mango fruits. Octadecanoic acid, oleic acid, 1 – Butanol, 3 – methyl-, carbonate (2:1) and 3,7 – Dimethyl nonane were common to both healthy and spoilt fruits with the first three having higher concentration in healthy fruits than spoilt while the later had higher concentration in the spoilt. One methyl group of 3,3- Dimethyl hexane in healthy fruit was shifted to position two to yield 2,3-Dimethyl hexane in the spoilt fruits. 2,2-Dimethylbutane, Methyl(methyl-4-deoxy-2,3-di-O-methyl.beta.1-threo-hex-4-enopyranosid) urinate, 3-(4-amino-phenyl)-2-(toluene-4-sulfonylamino)-propionic acid, 2-Methyl-3-heptanone, 3,5-Nonadien-7-yn-2-ol, (E,E), Butanoic acid, 1,1-dimethylethyl ester, 1-methyl-3-beta.phenylethyl-2,4,5-trioxoimidazolidine, Pentanoic acid, 2,2-dimethyl, ethyl ester (Vinyl 2,2-dimethylpentanoate), 4-Methyurazole, 1-Tridecyn- 4 – 9 – ol, 1-Hexyl-1-nitrocyclohexane were unique to spoilt fruits. This study suggests that these unique volatile metabolites could be exploited as biomarkers to discriminate pathogens even when more than one disease is present thereby curbing post harvest loss during storage after further validation and the volatile organic compound could form the basis for constructing a metabolomics database for Nigeria.

References

Bódalo, A.; Bastida, J.; Máximo, M.F.; Hidalgo A.M. and Murcia M.D. 2005. Production of (E) 10-hydroxy-8-octadecenoic acid with lyophilized microbial cells. *Amer. J. Biochem. Biotechnol.*, **1**(1), 1-4.

Doyle, M. E. 2007. Microbial Food Spoilage - Losses and Control Strategies: *A Brief Review of the Literature.* Food Research Institute Briefings. University of Wisconsin–Madison

Drewnowski, A. 1997. Taste preferences and food intake. *Annu Rev Nutr,* **17**:237-53.

Gonzalez-Rios, O., Suarez-Quiroz, M. L. Boulanger, R., Barel, M., Guyot, B., Guiraud, J-P., and Schorr-Galindo, S. 2006. Impact of ''ecological'' post-harvest processing on the volatile fraction of coffee beans: I. Green coffee

Holt, J. G.; Krieg, N. R.; Sneath, P. H. A.; Staley J. T. and Williams S. T. 1994. Bergy's Manual of Determinative Bacteriology.9th Ed. Williams and Wilkins. Pp. 478-529.

Ibrahim, A.D.; Sani, A., Manga, S.B.; Aliero, A.A.; R.U. Joseph, Yakubu, S.E. and Ibafidon, H. 2011. Microorganisms

Associated with Volatile Metabolites Production in Soft Rot Disease of Sweet Pepper Fruits (Tattase) *Int. J. Biotechnol. Biochem.* **7**(6), 25–35

Izco J. M and Torre, P. 2000. Characterization of volatile flavour compounds in Roncal cheese extracted by the 'purge and trap' method and analysed by GC-MS. *Food Chem*. **70**, 409–417.

Jay, M. J.; Loessner, J. M and Golden, A.D. 2005. Modern food microbiology, 7th ed. Springer science, New York, USA. Pp 17- 54

Kucharek, T., and Bartz, J. 2000. Bacterial soft rots of vegetables and agronomic crops. Univ. of Fla. Coop. Ext. Serv. Fact Sheet (Plant Pathology) No. PP-12.

Linden, RW. 1993. Taste, British Dental Journal 175: 243-253. doi:10.1038/sj.bdj.4808291Lindemann, B., 2001. Receptors and transduction in taste. *Nature*. **413**, 219-225.

Moalemiyan, M., Vikram A, Kushalappa A. C. Yaylayan V. 2006. Volatile metabolite profiling to detect and discriminate stem-end rot and anthracnose diseases of mango fruits.Plant Pathology, 55 (6) , 792–802

Nogueira, M.C.L., Lubachevsky, G., Rankin, S.A. 2005. A study of the volatile composition of Minas cheese. Lebensm.-Wiss. *Technol*, **38**, 555-563.

Oyeleke, S.B. and Manga, S.B. 2008. Essentials of laboratory practical in microbiology. Tobest publishers, Minna. Nigeria. Pp. 33-34.

Parliment, T.H. 1997. *Solvent extraction and distillation techniques* In: Marsili, R. (E). Techniques for Analyzing food Aroma. Marcel Dekker. New York. Pp. 1 – 27.

Rodríguez, E., M.J. Espuny, A. Manresa and A. Guerrero, 2001. Identification of (E)-11-hydroxy-9-octadecenoic acid and (E)-9-hydroxy-10-octadecenoic acid by biotransformation of oleic acid by *Pseudomonas* sp. 32T3. *J. Am. Oil Chem. Soc.*,**78**,593-597.

Suzuki, Y., O. Kurita, Y. Kono, H. Hyakutake and Sakurai, A. 2005. Structure of a new antifungal C11-hydroxy fatty acid isolated from leaves of wild rice (*Oryza officinalis*). *Biosci. Biotechnol. Biochem*., **59**, 2049-2051.

Wanakhachornkrai, P and Lirtsiri, S. 2005. Comparison of determination method for volatile compounds in Thai soy sauce. *Food Chem.,* **83**, 619-629.

Yanfang, Z and Wenyi, T. 2009. Flavour and taste compounds analysis in Chinese solid fermented soy sauce. *Afr. Jour. Biotechnol.* **8**(4), 673-681