

Research Article

Characterization of the Chemical Composition of the Volatile Aroma Compounds of Egyptian Banana Belonging to the "Maghrabi" Cultivar by GC-MS

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Abstract: Banana is a favorite fruit for many people due to its distinctive taste and aroma. In the current investigation, the volatile aroma compounds of the Egyptian banana belonging to the "Maghrabi" cultivar were characterized, for the first time, using Gas Chromatography-Mass Spectrometry (GC-MS). Results indicate that 33 volatile compounds were identified, which represent 95.09% of the total eluted compounds from the GC column. Esters (21 compounds) were the major chemical group, representing 77.25% of the total identified volatiles, with isoamyl acetate being the most abundant ester (23.9%). Other volatile compounds belonging to different chemical groups, like alcohols (4.19%), ketones (3.2%), aldehydes (2.3%), and terpenes (6.75%), were also identified. The manuscript discusses the qualitative and quantitative differences between the aroma volatile compounds of the Egyptian "Maghrabi" cultivar and those from different banana plantation regions and genotypes.

Keywords: banana, "Maghrabi" cultivar, volatiles, aroma, GC-MS

1. Introduction

Banana (*Musa* spp.) is among the highly accepted and consumed fruits worldwide due to its delicious flavor, besides its nutritional value. It is a typical climacteric fruit that ripens after harvest due to increased respiration and ethylene production [1]. During the ripening process, different changes take place to bananas including pulp softening, starch-to-sugar conversion, color development, and volatile aroma compounds formation. Early studies indicated that the cultivar type of banana is an important factor that influences the profile of the volatile aroma compounds formation during ripening [2, 3]. In addition, the harvest season can also play a role in volatile formation [4].

Egypt is one of the banana-producing countries where the total production accounts for 1.2 million tons in 2022 [5]. This plantation is produced from an area of 68,481.9 acres with an average yield of 19.055 tons/acre. The most famous cultivar of Egyptian banana is called "Maghrabi", which belongs to the *Musa acuminata* cv. AAA. This cultivar is characterized by bigger plants and excellent bunches of good size and shape. In addition, the "Maghrabi cultivar is characterized by a distinctive aroma and flavor which makes it the consumer's first choice when shopping for bananas in

Egypt. The quality of the "Maghrabi" banana cultivar regarding the non-volatile compounds was previously investigated [6]. On the other hand, despite the flavor reputation of the "Maghrabi" cultivar, no attempts so far have been made to identify its volatile aroma compounds profile. Therefore, in the current investigation, the authors, for the first time, subjected banana from the "Maghrabi" cultivar to a basic gas chromatographic analysis including GC-Flame Ionization Detector (FID) and GC-MS. The first instrument is used for separation and quantification (as relative area %) of the eluted volatile aroma compounds. On the other hand, the second (GC-MS) is used for the characterization of these compounds based on their mass fragmentation patterns. The instrumental analysis used is thought to be sufficient to reveal the main difference in volatile aroma compounds between the "Maghrabi" cultivar and the other cultivars grown in different parts of the world, as will be revealed in detail in the results section.

2. Materials and methods

2.1 Chemicals

Ethrel® (2-dichlorophosphonic acid, also known as ethephon®) was obtained from Bayer Crop Science, Canada. Diethyl ether was purchased from Fisher Scientific (Leicestershire, U.K.). N-Alkane standard solution kit ranging from C_5 - C_{28} was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Calcium chloride (CaCl₂) was obtained from El-Naser Chemical Company, Cairo, Egypt.

2.2 Fruit materials

Mature green bunches of bananas belonging to the Egyptian "Maghrabi" cultivar were used in the current investigation. The bunches were selected using a combination of two criteria, as previously reported [7]. First, bunches age of 14 weeks after shooting (in the second half of September) and second, desirable finger angulation.

Banana bunches were picked from well-grown and uniform plants of *M. acuminata* cv. AAA. The plants were grown in a private orchard which specializes in growing this type of cultivar and is located in Oseim, Giza, Governorate, Egypt.

The bunches were transported on the same day to the Horticultural Crops Technology Department at the National Research Centre, Egypt, and stored after harvesting for one day as a wilting period. Bunches were dehanded then 2-3-4 hands were selected from one bunch and were cut from the top into individual fingers to avoid differences in physiological development. The characteristics indexes of the "Maghrabi" banana fingers, at that mature green stage, when the bunches were picked up from the suckers (zero time), were: peel color index (1), peeling condition (1), firmness "lb/inch²" (24.7), Soluble Solids Content (SSC) 3%, Titratable Acidity (TA) 0.261% and SSC/TA ratio was 11.49. These indexes as well as the experimental procedures used for their assessments were previously reported in detail [6].

2.2.1 Banana ripening after harvest

The mature green banana fingers were washed, drained, and exposed to submerging treatment for 3 minutes in water containing 500 ppm Ethrel® [6]. This compound is an ethylene-release chemical that is used to promote ripening. The treatments were replicated three times, and each replicate consisted of twenty banana fingers. Fruits were placed in a standard carton box (45-35-10 cm) and stored at room temperature of 15 ± 1 °C and relative humidity of 85-90% until they reached a suitable degree of ripening using a commercial peel color index [8].

2.3 Isolation of the volatile aroma compounds

Three batches of freshly peeled banana pulps at their ripened stage were mixed with a suitable amount of distilled water containing 1% CaCl₂ and homogenized in an electric blender until acquiring a liquid slurry consistency. Then, the slurry was poured into a 5-litre glass round bottom flask containing an oval-shaped magnetic bar and equipped with a Quickfit glass inlet-outlet adapter with a long stem, (having a "T" shape). The stem of the glass adapter was immersed inside the flask, 10 mm beneath the level of the banana slurry. Nitrogen gas at a rate of 30 ml/min was then

introduced from a tank to the flask through the T-shaped adaptor and allowed to bubble inside the banana slurry (which is magnetically stirred). That is to release and purge the volatile aroma compounds continuously into the headspace of the flask. The purged nitrogen gas, which carries the volatiles, is allowed to exit the flask and to enter, (through Teflon tubing), into a set of 3 traps connected in series via Teflon tubing. These traps are composed of 3 gas washing cylinders containing 150 ml diethyl ether each and immersed in an ice bath. The traps are used for receiving and capturing the purged volatile aroma compounds that were released from the headspace of the banana slurry by nitrogen gas.

After a time period of 1 h at 35 °C, the traps unit was disassembled, and diethyl ether from the 3 traps was collected in one conical flask, dried over anhydrous sodium sulphate, filtered, and concentrated using a flash rotary evaporator under gentle vacuum to about 20 ml. Then, the rest of the diethyl ether was evaporated using a slow stream of nitrogen to about 2 ml to give banana aroma concentrate, which was subjected to the GC and GC-MS analysis.

2.4 Analysis and identification of the volatile aroma compounds of banana 2.4.1 GC analysis

Three microliters of the banana aroma concentrate were diluted with 1 ml of pure diethyl ether, and then 2 μ l of this mixture were injected into Perkin-Elmer Autosystem XL Gas Chromatograph (USA) equipped with FID, at a split ratio of 1:10. A fused silica capillary column (60 m length \times 0.32 mm internal diameter \times 0.25 μ m film thickness) coated with DB-5 (5% phenyl, 95% methyl polysiloxane) was used to separate the different volatile aroma components of banana. The oven temperature was programmed at 40 °C with an initial time hold for 6 minutes to reach 150 °C at a rate of 2.5 °C/min, then to a final temperature of 230 °C at a rate of 10 °C/min. The injector and detector temperatures were 230 °C and 250 °C, respectively. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The abundance of each volatile constituent was expressed as a peak area percentage relative to the total peak areas of volatiles eluted from the GC column after detection using a Flame Ionization Detector (FID). All values of area percent reported in the study were the mean of three injections from three different extractions \pm SD. In addition, the retention indices (Kovats index) of each peak were calculated using a series of alkanes ranging from C₅ to C₂₈, and used in the identification table instead of retention time.

2.4.2 Identification using GC-MS analysis

GC-MS analysis was run for the identification of the volatile aroma compounds of the Egyptian "Maghrabi" banana cultivar. For that purpose, the same procedure, column, and conditions used for GC analysis were adopted and repeated in this section. The diluted banana concentrate was injected into a computerized Agilent 7890A gas chromatograph (Agilent Technologies Inc., USA), equipped with a 5975C quadrupole Mass Selective Detector (MSD). The ion source and the quadrupole were heated at 230 °C and 150 °C, respectively. The MSD operated in Electron Ionization (EI) mode at 70 eV with 2.05 scans/s. Identification of the volatile aroma components was carried out by matching their mass fragmentation pattern with that installed in a built-in electronic mass spectral library (National Institute of Standards and Technology (NIST) 98.1). A high matching percentage, not less than 96%, is considered a criterion for accepting the identity of the volatile compounds. This percentage limit shows perfect matching between the mass fragmentation pattern of the unknown banana volatile peak with its match that is suggested by the mass spectrum library (NIST).

3. Results and discussions

Banana volatile aroma compounds from the "Maghrabi" cultivar, which is native to Egypt, were isolated, quantified by GC-FID (as relative area %) and identified using GC-MS. Table 1 indicates that 33 volatile compounds are identified in the "Maghrabi" banana cultivar. These compounds represent 95.09% of the total compounds eluted from the GC column.

The formation of banana volatiles depends on the production of ethylene, which is the hormone associated with fruit ripening [3]. Therefore, mature green bananas were treated first with Ethrel®, which is a growth regulator used in major horticultural crops to stimulate ethylene release and promote ripening associated with the formation of volatile

aroma compounds [9]. The volatiles illustrated in Table 1 belong to five main chemical groups, namely esters, alcohols, ketones, aldehydes, and terpenes, with a minor 1 hydrocarbon member (toluene) (Figure 1).

Table 1. Aroma volatile components of ripened banana from the Egyptian "Maghrabi" cultivar

Identified volatile component*	IUPAC name	Chemical formula	Functional group	Retention index	Average area (%)**
Isobutanol	2-methylpropan-1-ol	$C_4H_{10}O$	Alcohol	620	4.02 ± 0.3
2-pentanone	methyl propyl ketone	$C_5H_{10}O$	Ketone	698	3.0 ± 0.17
Isoamyl alcohol	3-Methylbutan-1-ol	$C_5H_{12}O$	Alcohol	725	0.17 ± 0.05
Toluene	Methylbenzene	C_7H_8	Hydrocarbon	770	1.44 ± 0.2
Isobutyl acetate	2-methylpropyl ethanoate	$C_6H_{12}O_2$	Ester	781	10.1 ± 1.7
Ethyl butyrate	Ethyl butanoate	$C_6H_{12}O_2$	Ester	807	2.54 ± 0.1
Butyl acetate	Butyl ethanoate	$C_6H_{12}O_2$	Ester	821	4.9 ± 0.2
2-pentyl acetate	pentan-2-yl acetate	$C_7H_{14}O_2$	Ester	855	6.8 ± 0.2
2E-hexenal	(2E)-hex-2-enal	$C_6H_{10}O$	Aldehyde	863	1.9 ± 0.1
Isoamyl acetate	3-Methylbutyl ethanoate	$\mathrm{C_7H_{14}O_2}$	Ester	886	23.9 ± 2.7
2-heptanone	Heptan-2-one	$C_7H_{14}O$	Keton	918	0.2 ± 0.003
Isobutyl isobutyrate	2-methylpropyl 2-methylpropanoate	$C_8H_{16}O_2$	Ester	951	0.1 ± 0.001
Isobutyl butyrate	2-methylpropyl butanoate	$C_8H_{16}O_2$	Ester	961	2.5 ± 0.1
Sabinene	4-methylidene-1-propan-2-ylbicyclo[3.1.0]hexane	$C_{10}H_{16}$	Terpene	977	0.15 ± 0.001
Myrecene	7-Methyl-3-methylideneocta-1,6-diene	$C_{10}H_{16}$	Terpene	993	0.2 ± 0.001
Butyl butyrate	Butyl butanoate	$C_8H_{16}O_2$	Ester	1,001	1.5 ± 0.1
Butyl-2-methylbutyrate	Butyl 2-methylbutanoate	$C_9H_{18}O_2$	Ester	1,006	0.1 ± 0.001
Isobutyl isovalerate	2-methylpropyl 3-methylbutanoate	$C_9H_{18}O_2$	Ester	1,010	0.36 ± 0.05
Isopentyl butyrate	3-methylbutyl butanoate	$C_9H_{18}O_2$	Ester	1,016	0.8 ± 0.02
2-methylbutyl isobutyrate	2-methylbutyl 2-methylpropanoate	$\mathrm{C_9H_{18}O_2}$	Ester	1,030	2.0 ± 0.1
Limonene	1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	$C_{10}H_{16}$	Terpene	1,036	5.5 ± 0.6
2-heptyl acetate	Heptan-2-yl acetate	$C_9H_{18}O_2$	Ester	1,047	1.5 ± 0.1
Butyl isovalerate	Butyl 3-methylbutanoate	$C_9H_{18}O_2$	Ester	1,051	0.35 ± 0.01
Isoamyl isobutyrate	3-methylbutyl 2-methylpropanoate	$C_9H_{18}O_2$	Ester	1,065	12.0 ± 1.0
2-pentyl pentanoate	Pentanoic acid, 2-pentyl ester	$C_{10}H_{20}O_2$	Ester	1,079	0.1 ± 0.001
Isoamyl-2-methylbutyrate	3-methylbutyl 2-methylbutanoate	$C_{10}H_{20}O_2$	Ester	1,104	0.4 ± 0.02
Isoamyl-3-methylbutyrate	3-methylbutyl 3-methylbutanoate	$C_{10}H_{20}O_2$	Ester	1,112	5.5 ± 0.1
2-Methylhexyl butyrate	Hexyl 2-methylbutanote	$C_{11}H_{22}O_2$	Ester	1,195	0.3 ± 0.1
Neral	(2Z)-3,7-dimethylocta-2,6-dienal	$C_{10}H_{16}O$	Terpene	1,238	0.4 ± 0.007
Cumicaldehyde	4-propan-2-ylbenzaldehyde	$C_{10}H_{12}O$	Aldehyde	1,240	0.4 ± 0.03
Hexyl isovalerate	Hexyl-3-methylbutanoate	$C_{11}H_{22}O_2$	Ester	1,245	0.2 ± 0.007
Geranial	3,7-dimethylocta-2,6-dienal	$C_{10}H_{16}O$	Terpene	1,273	0.5 ± 0.06
Hexyl hexanoate	Hexyl hexanoate	$C_{12}H_{24}O_2$	Ester	1,352	1.3 ± 0.3
Total identified compounds (33)					95.09

^{*} Using GC-MS analysis ** After GC-FID

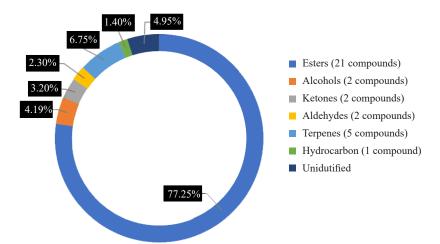


Figure 1. Main chemical groups and their percentages that constitute the volatile aroma compounds of the Egyptian banana "Maghrabi" cultivar

Esters (21 compounds) represent the major identified group in banana volatiles, accounting for 77.25% of the total identified compound (95.09%). This is much higher than the total esters found in other banana cultivars, such as cv. Nanicão (*M. acuminata*, AAA) and cv. Prata (*M. acuminata* × *M. balbisiana*, AAB), in which esters were just above 50% of the total volatile compounds [3]. The mechanism of ester formation in ripened bananas is based on the biosynthetic reaction between alcohols and Acetyl Coenzyme A (Acetyl-CoA), which is derived from amino acids and fatty acid metabolism [10].

Table 1 indicates that the ester, namely Isoamyl Acetate (IAA), represents the most abundant compound (23.9%) among all the total volatiles identified in banana and also represents 31% of the total 21 esters group (Figure 2). This ester is a character impact flavor compound due to its sharp characteristic ripened banana aroma [11]. The mechanism of formation of IAA is based on the metabolism of the amino acid leucine, which goes through a series of reactions during banana ripening, including deamination, decarboxylation, and esterification to end up with IAA [12]. Besides IAA, Table 1 indicates that the Egyptian "Maghrabi" cultivar also includes some other esters in lower abundance than IAA, like isoamyl isobutyrate (12%), isobutyl acetate (10.1%), 2-pentyl acetate (6.8%), and others, which are illustrated in detail in Figure 2.

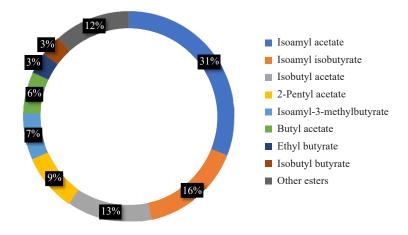


Figure 2. Distribution of 21 aroma esters identified in the Egyptian banana "Maghrabi" cultivar

Our current investigation, in addition to another [2] demonstrated the predominance of IAA as a major ester in banana volatiles. However, isobutyl butyrate with an odor described as "fruity", was reported to be the major constituent (29.6%), of the Brazilian *Musa* (AAA group) banana cultivar [4]. This ester was found in our study on the Egyptian "Maghrabi" cultivar as a minor ester accounting only for 2.5% (Table 1). Similarly, our results on the "Maghrabi" banana cultivar came in contradiction with other investigations [13, 14], which found that isoamyl butyrate (also called isopentyl butyrate) was the most abundant ester in banana cultivars grown in India and Madeira Islands, respectively. Interestingly, this ester was found as a trace (0.8%) in our study on the "Maghrabi" cultivar (Table 1). One should take into consideration that, besides the variation of cultivar type, the geographical origin of bananas can also affect the formation of IAA and isoamyl butyrate, as previously indicated [15]. From the aroma point of view, the odor impact of isoamyl butyrate is defined by gas chromatography-olfactometric analysis as fruity, floral, and acidic [16], but not a typical banana flavor as IAA.

Other ester like isoamyl-3-methylbutyrate (known as isoamyl isovalerate), was identified and ranked as the 2nd most abundant ester in some banana cultivars grown in India (up to 17.74% for grand naine, *Musa* AAA group) [13], and also in China (up to 14%, for *Musa* AAA Group, Brazilian) [2], depending on the season. In our study on the Egyptian "Maghrabi" cultivar, the same ester represents only 5.5% of the total identified volatile compounds (Table 1) and is ranked as 5th among the other esters (7% of the total esters group, Figure 2). It is worth indicating that the aroma of this ester in its pure state is more oriented toward a ripened red apple aroma rather than a banana aroma. Yet, it is commonly present in different cultivars of banana, where its aroma was described [17].

Besides esters, there are other minor volatile compounds that belong to different chemical groups, like alcohols, ketones, aldehydes, and terpenes, that are also identified in Table 1. Alcohols like isobutanol (4.02%) and isoamyl alcohol (0.17%) with a pungent and alcoholic aroma were reported in different banana cultivars [16]. Ketones, including 2-pentanone (3%) and 2-heptanone (0.2%), contribute to the fruity-banana aroma and herbaceous note, respectively [18]. These ketones are formed due to lipid oxidation during banana ripening and reach their maximum content (especially 2-pentanone) at the full ripening stage. Aliphatic aldehydes like 2*E*-hexenal (1.9%), which was also reported in approximate percentage compared to our study, are formed by the autoxidation of linolenic acid [18] and its aroma is described as a red apple. On the other hand, aromatic aldehyde like cuminaldehyde (0.4%, Table 1), which has a typical spicy-herbal aroma, was not reported before in previous publications on banana volatiles. Our group is still engaged in discussion about how this compound is formed during banana ripening. Terpenes (6.75%) represent another chemical group in the aroma volatiles of the Egyptian "Maghraby" cultivar banana. Limonene (5.5%) is the major terpene present in "Maghrabi" banana volatiles, followed by neral (0.4%) and geranial (0.5%) with much less amounts of sapinene (0.15%) and myrecene (0.2%) (Table 1).

4. Conclusion

The data presented in this investigation document, for the first time, the profile of volatile aroma compounds of the Egyptian banana, which belongs to the "Maghrabi" cultivar. These findings shed more light on the influence of cultivar on banana volatile compounds, which show a quantitative and qualitative volatile variation, especially isoamyl acetate and the other banana-characterizing esters. The investigation also presents insights that can be used in the flavor industry to develop more creative banana essences.

Conflict of interest

The authors declare no conflict of interest.

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