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Volatile compounds trigger the pleasant strong aroma of new cultivar Gama Melon Parfum during growth and maturation

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KEYWORDS

Cucumis melo
Development volatile
Flavor
Principal component analysis

ABSTRACT

Melon (*Cucumis melo L.)* cv. Gama Melon Parfum (GMP) is the new cultivar from cross-breeding of Natsuno Omoide (NO3) female parent and Miyamauri (MR5) male with phenotypic characteristics that is prominent such as very strong pleasant aroma when ripe. The physiological characteristics will be changed which have an impact on the formation of volatile compounds during fruit development. Therefore, the profile of strong volatile aroma compounds during fruit development to is critical to be identified. The volatile compounds analysis was performed on fruits harvested at 7, 14, 21, 28, and 35 days after pollination and storage at -20°C. Gas chromatography-mass spectrometry (GC-MS) was used for volatile identification. The results showed some volatile compounds changed during fruit development consisted of 8 esters, 2 alcohols, 1 acid, 3 terpenoids, and 3 hydrocarbons. The profile of volatile compounds was dominated by esters, followed by alcohols and acid respectively. Interestingly, the characteristics of the volatile compounds can differentiate between the ripe stage and unripe stage using principal component analysis. The findings of this study can be used to improve the quality aroma of GMP.

Introduction

Universitas Gadjah Mada has assembled two new cultivars of melon plants. One of those is a cultivar Gama Melon Parfum (GMP). It has a prominent characteristic phenotype that has very strong pleasant aromas like perfume but it is bitter so it is not suitable for consumption (Hasbullah et al., 2019). Therefore, GMP cultivar has the potential to be developed as the manufacture of perfumes and flavoring agents.

Melon aroma is composed of volatile compounds. Some researchers remain that the aroma of melon produce from volatile compounds from the group comprising aldehydes, alcohols (Shan et al., 2012; Oh et al., 2011), acetate and nonacetate esters (Obando-Ulloa et al., 2008, 2009, 2010; Fallik et al., 2001), isoprenoids (Kourkoutas et al., 2006), hydrocarbons (Beaulieu and Grimm, 2001) and sulfurs (Beaulieu, 2006). The volatile compounds and aroma of the melon will be formed and developed during fruit development. Melon cultivar variation will lead to differences in the profile of volatile compounds and aroma (Shan et al., 2012; Oh et al., 2011; Obando-Ulloa et al., 2010, 2009, 2008; Beaulieu, 2006; Kourkoutas et al., 2006; Fallik et al., 2001; Scalzo et al., 2001; Beaulieu and Grimm, 2001; Mattheis and Fellman, 1999). Therefore, the new GMP cultivar is thought to have a different profile of volatile compounds from other cultivars.

There has not been any research conducted to identify the profile of volatile compounds from GMP cultivar when ripe or during development. Hence, this study aimed to identify the profile of volatile compounds that trigger pleasant strong aroma during fruit development of GMP cultivar after pollination and differentiate the production

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of the volatile compounds between development stages using principal component analysis.

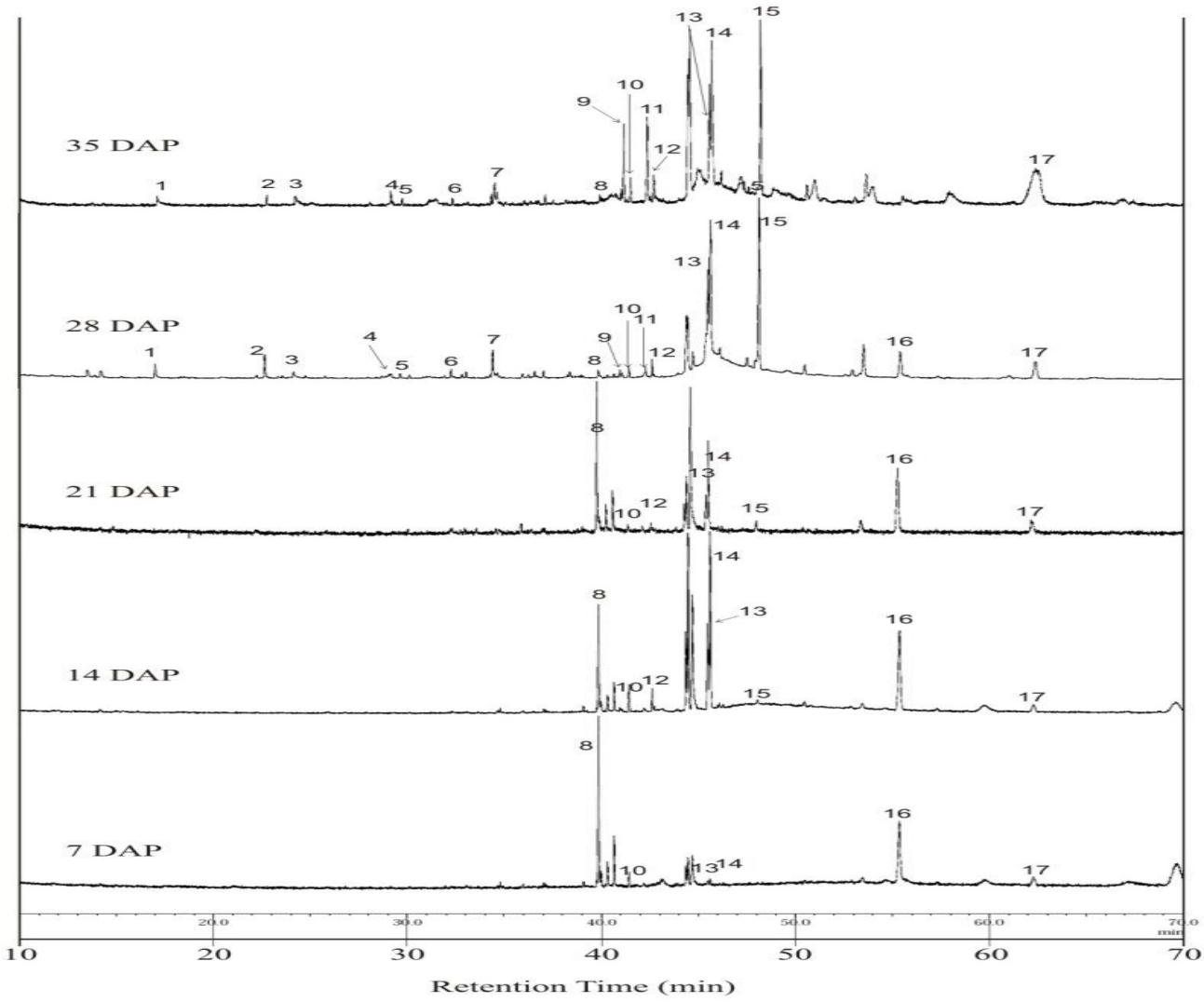

Figure 1. Chromatogram of volatile compounds development. The compounds name at numbered peaks can be seen in Table 1

Research Methods

Fruit materials

Cucumis melo L. cv. Gama Melon Parfum were grown in Yogyakarta, Indonesia. Fruits were harvested weekly for 5 weeks at 7, 14, 21, 28, and 35 days after pollination (DAP). The rind of fruit was removed from the flesh and frozen with liquid nitrogen (Samator) and stored at -20°C until analysis.

Volatile compounds extraction

Volatile compounds from samples were extracted by the cold maceration method. The frozen rind (50 g) of the fruit was homogenized by a dry blender. Following through homogenization, the sample was added with 120 mL pentane/dichloromethane mixture (2:1, v/v) (Ajax, Australia) and internal standard, 5 µL 1-octanol (4.12 mg/5 µL) (Sigma), respectively. The volatile extraction was performed for 24 h at -20°C. The extract was decantated and dried over anhydrous sodium sulfate (Merck, Germany) and concentrated to a final volume of 5 ml in Vigreux column. Following that, it was collected in the dark glass vial and stored at -20°C until analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

A GCMS-QP2010S Shimadzu with an electron impact mode (EI) generated at 70 eV was used. The capillary column Agilent HP-5MS (30 m x 0.25 mm; film thickness 0.25 µm) was used for separation.

Table 1. Volatile compounds detected during GMP development

Peak no.	Compounds	CAS	OT (ppb)	Concentration (ppm)					Odor Activity Value (OAV)					Odor description
				7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	
	Alcohols													
1.	1-Octanol	111-87-5	130	n.d.	n.d.	n.d.	2.16	0.23	n.d.	n.d.	n.d.	16.62	1.77	Waxy, green, citrus, aldehydic and floral with a sweet, fatty, coconut nuance (a)
14.	9,12,15-Octadecatrien-1-ol	2774-90-5	u.k.	0.13	6.52	1.12	25.27	2.13	u.k.	u.k.	u.k.	u.k.	u.k.	
	Esters													
2.	Octyl Acetate	112-14-1	12	n.d.	n.d.	n.d.	2.41	0.09	n.d.	n.d.	n.d.	200.83	7.50	Woody, tar, burnt plastic, fruity, slightly fatty, waxy-floral odor, green apple-like (b)
4.	(Z)-3-Octen-1-ol acetate	69668-83-3	u.k.	n.d.	n.d.	n.d.	0.45	0.18	u.k.	u.k.	u.k.	u.k.	u.k.	Green, earthy, fruity with oily nuances (c)
5.	Decyl Acetate	112-17-4	1000	n.d.	n.d.	n.d.	0.54	0.07	n.d.	n.d.	n.d.	0.54	0.07	Sweet fatty-fruity odor of pineapple, rosy waxy undertone (d)
9.	(Z)- Methyl 9-hexadecenoate	1120-25-8	u.k.	n.d.	n.d.	n.d.	0.52	0.63	u.k.	u.k.	u.k.	u.k.	u.k.	Old leather (c)
10	Methyl hexadecanoate	112-39-0	u.k.	0.28	0.81	0.06	1.09	0.21	u.k.	u.k.	u.k.	u.k.	u.k.	Oily waxy fatty (c)
12.	Ethyl hexadecanoate	628-97-7	1500	n.d.	0.82	0.07	1.76	0.21	n.d.	0.55	0.05	1.17	0.14	Fatty, rancid, fruity, sweet (e)
13.	Methyl 9,12-hexadecadienoate	2462-80-8	u.k.	0.09	3.04	0.42	16.46	1.25	u.k.	u.k.	u.k.	u.k.	u.k.	
16.	Dioctyl 1,2-benzenedicarboxylate	117-84-0	u.k.	2.42	6.09	1.56	5.74	n.d.	u.k.	u.k.	u.k.	u.k.	u.k.	
	Acid													
11.	(Z)-9-Octadecenoic acid	112-80-1	u.k.	n.d.	n.d.	n.d.	1.52	0.80	u.k.	u.k.	u.k.	u.k.	u.k.	Faint fatty waxy lard fried (c)
	Terpenoids													
3.	Chavicol	501-92-8	u.k.	n.d.	n.d.	n.d.	0.74	0.04	u.k.	u.k.	u.k.	u.k.	u.k.	Phenolic odor, penetrating, betel (d)
7.	Germacrene D-4-ol	74841-87-5	u.k.	n.d.	n.d.	n.d.	3.81	0.32	u.k.	u.k.	u.k.	u.k.	u.k.	
8.	Phytol	150-86-7	u.k.	2.82	3.88	1.59	0.72	n.d.	u.k.	u.k.	u.k.	u.k.	u.k.	Delicate floral balsam powdery waxy (c)
	Hydrocarbon													
6.	Hexadecane	544-76-3	u.k.	n.d.	n.d.	n.d.	0.88	0.05	u.k.	u.k.	u.k.	u.k.	u.k.	Faint mild waxy(c)
15.	Eicosane	112-95-8	u.k.	n.d.	0.22	0.12	24.81	2.18	u.k.	u.k.	u.k.	u.k.	u.k.	Waxy (c)
17.	Octacosane	630-02-4	u.k.	0.43	0.72	0.29	5.21	2.74	u.k.	u.k.	u.k.	u.k.	u.k.	

OT, Odor Threshold; K1-5, GMP Age 1-5 Week After Pollination; n.d., not detected; u.k., unknown; (a) (Casaburi et al., 2014); (b) (Narain et al., 2010); (c) thegoodscentscompany.com; (d) (Werkhoff et al., 1998); (e) (Welke et al., 2014)

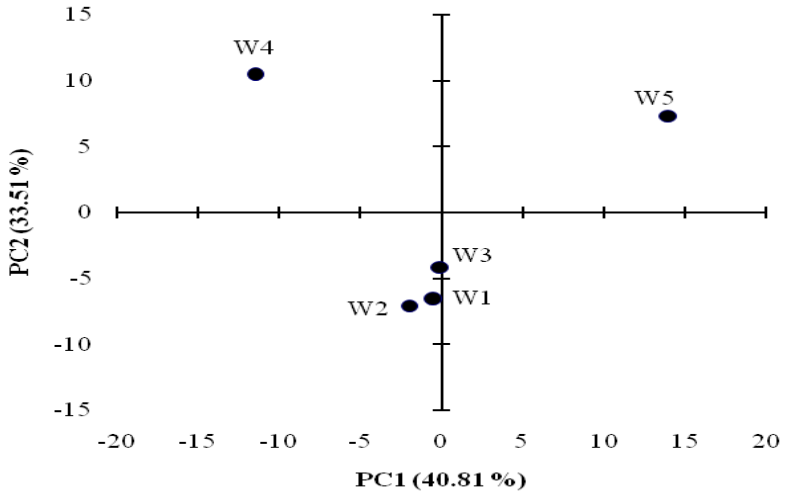

Figure. 2 Principal component plot of profile volatile compounds during GMP melon development after pollination

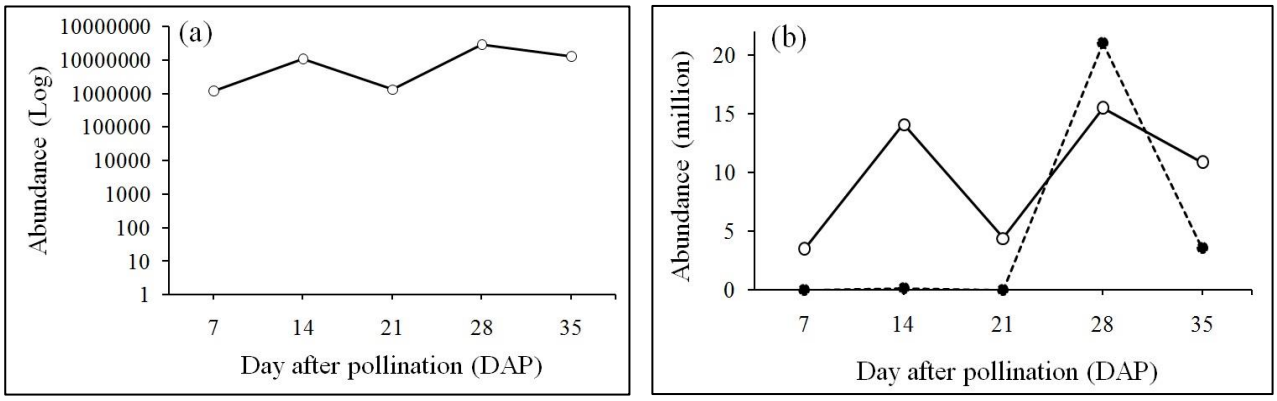

Figure 3. (a) Changes in esters during fruit development, (b) Changes in alcohols (—○—) and acids (- -● - -) during fruit development

Volatile concentrate (5 µL) was injected with a splitless mode. The injection temperature was maintained at 260°C and the column temperature was programmed from 40°C at a rate of 3°C/min to 80°C, held 1 min, and then to 130°C at a rate of 5°C/min, held 2 min, and then to 240°C at a rate of 6°C/min, held 30 min.The MS detector was set to 250°C and continuous scan from mass to charge ratio (m/z) 28 to 600. Helium was the carrier gas at the rate of 69.3 ml/min and pressure of 13.7 kPa. Identification of the compounds was based on a comparison of their mass spectra with those of authentic standards in the Wiley 229 Library, NIST 62 Library, dan NIST 12 Library.

Statistical analysis

The volatile data were subjected to Principal Component Analysis (PCA) using the XLSTAT beta version.

Results and Discussion

Volatile profile compounds during development of fruit

Volatile compounds changes grow up during fruit development (Figure 1). Detection of volatile compounds in the rind GMP uses the Agilent HP 5MS column which has a semi-polar characteristic. Seventeen main compounds were identified including 8 esters, 2 alcohols, 1 acid, 3 terpenoids, and 3 hydrocarbons (Table 1).

Fruit will be changed in the volatile compounds that contribute to fruit aromas during the development phase of pollination to ripening. Fruit contains hundreds of different volatile compounds in very small amounts but those can be detected by human noose (Hui, 2010). Alcohols are produced in 28 DAP and decreases in 35 DAP. The OAV of 1-octanol was more than one value so its important compounds form GMP

aroma. The contribution of these compounds is especially for the aroma description of green and fruity, sweet.

Esters showed a similar pattern with alcohols for octyl acetate; (Z) 3-octen-1-ol acetate and decyl acetate. (Z) Methyl 9-hexadecenoate was produced in 28 DAP and rode on 35 DAP. Methyl hexadecanoate; ethyl hexadecanoate; methyl 9,12-hexadecadienoate and dioctyl 1,2-benzenedicarboxylate were produced in 7 DAP and those experienced up and down in 14 and 21 DAP, in 28 DAP the compound concentration increased then decreased again in 35 DAP. Octyl acetate had OAV more than one value and contributed to the aroma description of fruity and floral. Acid was produced in 28 DAP and decreased in 35 DAP.

Terpenoids, including cavicol and germacrene D-4-ol was produced in 28 DAP and decreased in 35 DAP. While the phytol was produced in 7 DAP and its concentration decline until 28 DAP. Phytol is one component that plays a role in the biosynthesis of chlorophyll (Vavilin and Vermaas, 2007; Stenbaek and Jensen, 2010). Hydrocarbons, including heneicosane were produced in 28 DAP and decrease in 35 DAP, while eicosane and octacosane were produced in the 7 DAP and peaked at 28 DAP and decrease in 35 DAP.

Principal Component Analysis (PCA) of Volatiles Development

Principal component 1 (PC1) represented 40.81% of variables and principal component 2 (PC2) represented 33.51% of variables (Figure 2). PCA from PC1 and PC2 represented 74.32% of variables. GMP melon 35 DAP (W5) was separated with melon 28 DAP (W4) in PC1. W5 was in the right position (positive), while the W4 was in the left position (negative). GMP melon that was still in the growth phase or a raw state (W1, W2, and W3) was separated with a melon that entered the phase of maturation and ripening (W4 and W5) in PC2. W4 and W5 were in the top position (positive), while W1, W2, and W3 were in the down position (negative). PC1 represented 50% of the total esters, 30% of the total alcohols, aldehydes, ketones, terpenoids, and isoprenoids, 25% of hydrocarbons, lactones, and sulfur. PC2 represented 50% of the total ester, 30% of the total alcohol, 70% of the total acid, and 25% hydrocarbons.

Principal Component Analysis (PCA) was effective for classifying age based on the produced volatile compounds. It can be seen that the unripe GMP melon was been able to distinguish mature and ripe in PC2. Obando-Ulloa et al. (2010) also reported that PCA was able to group types of melons based on identified volatile compounds.

Changing of esters, alcohols, and acids

There are changes in ester compounds during fruit development (Figure 3a). Those were produced from the initial phase of fruit development and increase when entered the maturation phase (28 DAP). Alcohols were grown in the initial phase, then declined and increased when the maturation phase. Acids peaked production in 28 DAP (Figure 3b).

The amount of esters declined when entered the ripening phase (35 DAP). It may because most esters have much evaporated into the environment, so that extracted in the rind become less. Softening of the fruit texture makes permeability of cell wall changed that allows ester compound to be easier to get out and evaporate into the environment. It is also able to explain why the amount of ester in 28 DAP was more than 35 DAP. Cell walls of 28 DAP more tend to firm than 35 DAP, so esters produced are stuck in the cell. This is because the cell wall permeability is better so that the ester did not freely come out and evaporate into the environment. A decrease of alcohols and acids in the ripening phase is possibly used for esterification and respiration.

Conclusion

In conclusion, during the development stage of GMP occurred volatile compounds profile changes i.e. 8 esters, 2 alcohols, 1 acid, 3 terpenoids, and 2 hydrocarbons. Principal component analysis was also useful to group the GMP base on volatile production, showing which GMP is different in an unripe stage and ripe stage.

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