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Volatile patterns of different papaya (*Carica papaya* L.) varieties

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Summary

The aim of this research was to study the diversity of papaya volatiles in different cultivars and new breeding lines. Sensory evaluations have been conducted by hedonic, rank and profile analysis. The volatiles were extracted using different sample preparation methods (liquid-liquid-extraction and SBSE). Character impact compounds which contribute to the aroma impression were characterized by GC-O. For identification and semi-quantification GC-MS was used. The plant volatile patterns differ significantly between the genotypes resulting in very different sensory qualities. Especially the existence and content of terpenes show a high variability. Therefore the investigated genotypes have different potential regarding aroma quality in further steps of plant breeding programs.

Introduction

The popularity of papaya (*Carica papaya* L.) will be boosting as a fresh fruit crop which offering functional properties. The fruit is excellent in vitamin C content and pro-vitamin A as well as rich in dietary fiber. Indonesia is one of the leading countries on papaya production after Brazil, Nigeria, Mexico and India (SIDHU, 2006). In accordance to elevate the quality of local papayas, the Centre of Tropical Fruits Study (PKBT) at Bogor Agricultural University (IPB), Indonesia, has started a long term breeding program since 2003. This program targeted outstanding new cultivars having specifications and characteristics suitable for international market demands, high yield and resistant toward biotic and abiotic stress (ANONYMOUS, 2003). Until 2005, the program has been able to explore 75 genotypes of papaya collected from several areas in Indonesia and from abroad. This papaya gene bank collection has been divided based on size: small papaya and medium-big papaya, and its specific utilization purpose as vegetable/fruit papaya or papain producing papaya (ANONYMOUS, 2005).

'IPB-3' and 'IPB-6C' are two lines which were selected as outstanding varieties recently by the PKBT (WULAN, 2008; SRIANI, 2008, personal communication). Papaya 'IPB-3' is a selected line purified by selfing (self fertilization) with fruits of green to yellowish peel and orange to reddish pulp. The size is relative small with fruit weight of about 0.5 kg/fruit. 'IPB-6C' is an open pollinated big-size papaya originated from Cibodas village, Cicurug-Sukabumi area in West Java Indonesia. The fruit has green-yellowish peel and orange to reddish pulp. The weight of a single fruit is up to 2.8 kilogramms (ANONYMOUS, 2005; WULAN, 2008).

Plant volatiles possess different bioactive properties. In addition to its roles in signalling, attracting, defense and anti-oxidative potential, fruit aroma is important for their organoleptic quality and, therewith also for the consumers' decision to buy. During the process of domestication and plant breeding, plant volatiles patterns underlie a dramatic change. In the majority of the cases, wild types contain a higher diversity and higher concentration levels of volatiles than the cultivated ones (ULRICH et al., 2007; GOFF and KLEE, 2006).

The aim of this research is to study the differences and the diversity of papaya volatiles in two newly introduced IPB lines comparing to the commercially known cultivars. Besides identification of the volatiles by gas chromatography-mass spectrometry (GC-MS), odorants were characterized by gas chromatography-olfactometry (GC-O). In parallel to instrumental analysis human sensory was performed (profile analysis, hedonic and rank test).

Experimental

Materials: 'IPB-3' and 'IPB-6C' papaya fruits have been obtained from the PKBT of the Bogor Agricultural University. The cultivars 'Bangkok' and 'Burung' have been purchased in Bogor market (Indonesia), while the 'Brazil' variety was delivered by a Quedlinburg supermarket (Germany).

Profile analysis: The profile analysis has been conducted by using selected and trained panelists (n = 11) to evaluate the sensory profile of aroma (sour-putrid, fruity, flowery) and taste (sweetness, acidity, bitterness) using the scale 0 to 80 (0 = no value low and 80 = most intensive).

Hedonic test: The hedonic test has been conducted by 35 untrained panelists to evaluate the acceptability of color, taste, aroma, texture and overall. The hedonic scales used in this experiment are 1 for 'dislike very much' and up to 7 for 'like very much'.

Rank sensory test: The rank test has been conducted by 35 panelists to evaluate the overall acceptability of the samples by rank them from 1 as the most acceptable sample.

Sample preparation for instrumental analyses: For GC-O the volatiles of a proportionate mix of all genotypes were isolated using liquid-liquid extraction with the organic solvent methylene chloride. The flesh (200 g) of each cultivar was homogenized for 1 min in 200 ml of NaCl solution (18.6 % w/v). The homogenate was centrifuged at 4 °C for 30 min at 3000 rpm to give a supernatant. To obtain a clear juice, the supernatant was filtered through filter paper. A portion (250 ml) was extracted twice with 60 ml solvent in a separation funnel. 2,6-Dimethyl-5-hepten-2-ol (0.1 ppm v/v) was added as internal standard before extraction. The obtained extract was dried with sodium sulphate. Immediately before analysis the extract was concentrated to 100 µl by distilling off the solvent on a Vigreux column (20 cm length, 1 cm ID). An aliquot of 1 µl was injected manually into the GC using a cooled vial and syringe.

Additionally, an extraction by stir bar sorptive extraction (SBSE) in fruit homogenates of each genotype was used as rapid method. The homogenates were enzyme inhibited by saturation with NaCl. Details of the methods were published in KOMES, 2005 and WIJAYA et al., 2005.

GC-MS: A Hewlett-Packard GC-MS system (GC 5890 plus and MSD 5973) equipped with a split-splitless injector (manual injection) or a thermodesorption unit for SBSE (TDU by Gerstel, Mühlheim, Germany) was used at 250 °C. The MS detector temperature was 280 °C. A polar column (HP INNOWax, 0.25 mm ID 30 m length 0.5 µm film thickness) was used with the following temperature program: 45 °C held for 5 min, then raised to 200 °C at a rate of 2 K/min and held for 30 min. The flow rate of the carrier gas (He) was 1.0 ml/min. A volume of 1 µl of each sample was injected with the split ratio of 1:3 and 1:20, respectively. For compound identification the Wiley and NIST libraries were used. The analyses were performed in triplicate.

GC-O: The so called nasal impact frequency method (NIF) was used. The measurements were performed according to ULRICH et al. (2007).

Results and discussion

Human sensory. Based on the sensory evaluation, 'IPB-6C' was characterized by the highest acceptability in terms of overall attributes, whereas papaya 'Burung' was the least accepted. This result has also been supported by the results of the rank test. 'IPB-6C' has the highest rank, followed by 'IPB-3', 'Bangkok' and 'Burung', respectively. However, in case of aroma and taste attributes, 'IPB-3' showed better acceptance than 'IPB-6C' which is characterized by a acceptability in texture and color attributes.

The distinct flavour character of 'IPB-3' comparing to the other lines or cultivars can be seen as well from its flavour profile based on profile analysis (Fig. 1). It has been shown clearly that 'IPB-3' is more intense in sweet and flowery aroma attributes as well as in the sweet taste. Therefore, it is interesting to figure out the composition of volatile compounds in each line or cultivar.

Gas chromatography-olfactometry (GC-O). For the GC-O experiments a traditional liquid-liquid-extraction of a papaya blend of all available genotypes were used. This approach assures on the one hand the covering of the genotype diversity of the volatile patterns and on the other hand the extraction of the multiplicity and a broad polarity range of aroma compounds in comparison to rapid methods (KOMES, 2005). For the GC-O measurements a statistical non-dilution method (nasal impact frequency – NIF) was used. The advantage of the NIF method is the smoothing effect of inter-individual differences in the odour perception occurring in different individuals. Up to 32 different odour impressions per tester were detected in a single run. The so called NIF profile which was created by summation of the GC-O runs of all of the seven testers resulted in 12 important odour impressions for the genotype mix (Fig. 3). The odourants which contribute to the typical papaya aroma bouquet are comprised in Tab. 1. The compilation contains especially green, herbaceous and flowery odour notes. But also butanoic acid with its unpleasant odour contribute, albeit in distinct amounts, to the overall sensory sensation of papaya. The peak at a rentention time of 8.7 minutes with a potato-like smell belongs to an unknown substance. At the mass spectrometric chromatogram (TIC) no clear peak occurred above the baseline. The typical potato-like smell in plant extracts frequently are caused by (alkoxy-alkyl-) pyrazines. Because of the extremely low odour threshold of most of the compounds belonging to the pyrazin group an odour impression

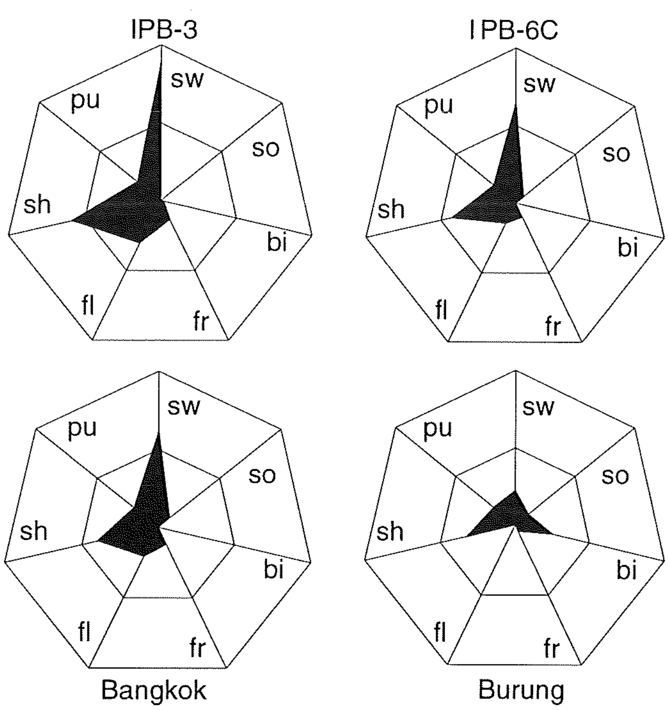

Fig. 1: Flavour profiles estimated by a profile analysis. All figures are given in the full intensity scale used. Nomenclature of the sensory impressions: taste (sw – sweet, so – sour, bi – bitter), aroma (fr – fruity, fl – flowery, sh – sweetish, pu – sour-putrit).

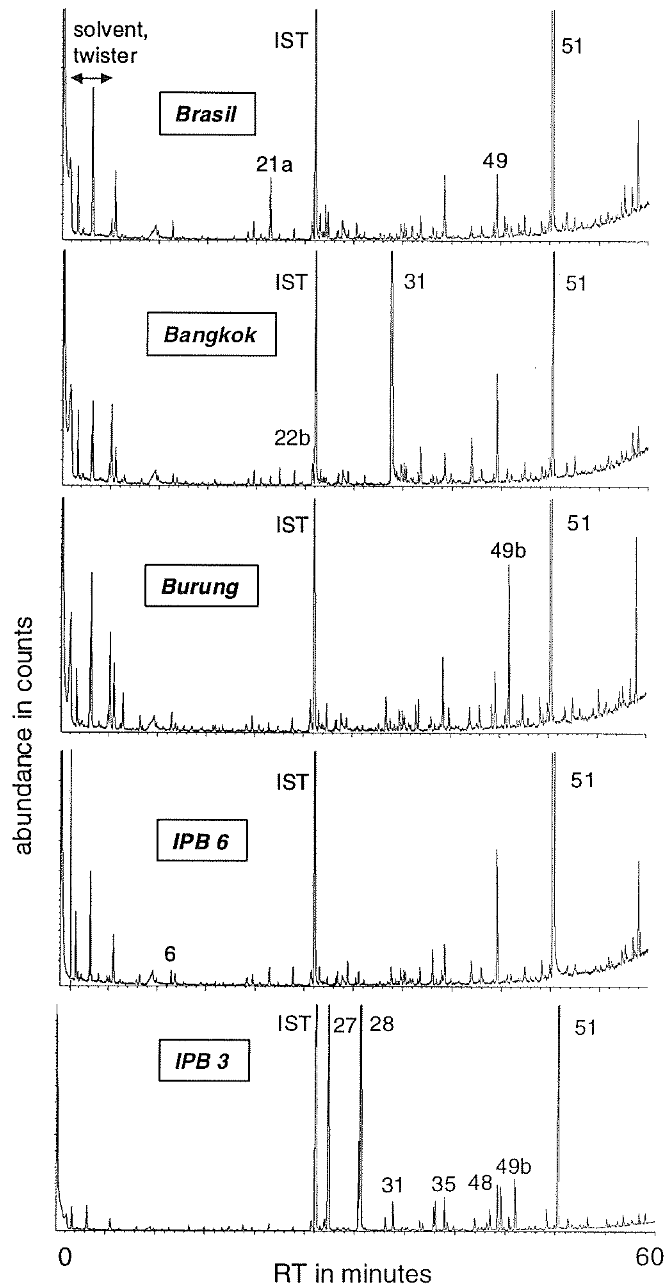

Fig. 2: Total Ion Chromatogrammes (TIC) of 2 lines and 3 varie-ties of papaya after SBSE of papaya homogenates and thermodesorption.

Tab. 1: GC-O analysis of an extract from a genotype mix using the NIF method and a panel of 7 trained testers.

no	substance	odour	NIF	RT GCO in min	RT MS in min
4	hexanal	herbaceous	4	3.4	9.90
4a	(Z)-2-pentenol	chemical	4	7.0	20.00
13	nonanal	herbaceous	5	7.9	23.00
13a	unknown	Potato-like	3	8.7	-
14	(Z)-linalool oxide	flowery	5	9.0	26.18
22	linalool	flowery	2	10.1	29.49
22a	DMSO	sweetish	2	10.4	30.60
24	butanoic acid	stinky	5	11.1	32.76
29	verbenone	floral	4	12.6	35.67
36	butanoic acid, phenyl methyl ester	sweetish, floral	3	14.8	41.68
43	δ-octalactone	flowery	3	16.5	45.00
45	Isothiocyanato benzene	smokey	3	18.9	49.35

is received in GCO experiments but the MS identification fails because of the very low concentration level (ULRICH et al., 1998).

Stirbar-sorptive extraction (SBSE). Using SBSE as a more rapid isolation method than liquid-liquid-extraction detailed chromatograms with more than 100 separated peaks each were obtained. As expected (KOMES, 2005) clear differences in volatile extraction ability (recovery rates) exist between the two used methods. With SBSE as sample preparation altogether 49 peaks were (tentatively) identified but two of the character impact compounds which were detected in GC-O with a liquid extract ((Z)-2-pentenol and dimethyl sulfoxid – DMSO) were not found. Strong substance discrimination of adsorption methods like SBSE or SPME are a known fact and have to be taken into account if used in aroma research.

As shown at Fig. 2, marked differences in volatiles patterns between the lines and cultivars were found using SBSE for determination of the diversity of volatile patterns. 'IPB-3' was richer in volatiles comparing to the others. It was an unexpected result that 'IPB-6C' has a more similar pattern to 'Brazil' than to 'Bangkok'. On the other hand, the 'IPB-3' line deriving from the solo type papaya which is phenotypically more similar to 'Brazil' showed a volatile pattern more similar to 'Bangkok'. Based on its characteristic, it is possible that this line is deriving from the Bangkok one generated by an open pollination on the local fields before being selected as an outstanding line.

The major volatile compounds in all papaya fruits detected by the SBSE method were dominated by isothiocyanatomethyl benzene (45) and benzyl nitrile (40) (Tab. 1). The presence of isothiocyanatomethyl benzene in papaya has been reported previously (FLATH and FORREY, 1977 (6); MACLEOD and PIERIS, 1983; FLATH et al., 1990). This compound has been confirmed by FLATH and FORREY (1977) as enzymatic derivative of benzyl glucosinolate which can be synthesized in bruised or injured papaya (PATIL and TANG, 1974). According to TANG (1971), it is a genuine metabolite emanating from intact green papaya which having protective function in the papaya fruit (PATIL and TANG, 1974). The presence of a significant amount of benzyl nitril (40) also was reported by MACLEOD and PIERIS (1983). The occurence of this volatile compound can be explained as another derivative product of benzyl glucosinolate in papaya (FLATH and FORREY, 1977).

The differences of detected volatile compounds and their relative concentrations in the 2 lines and 3 cultivars are presented in Tab. 2.

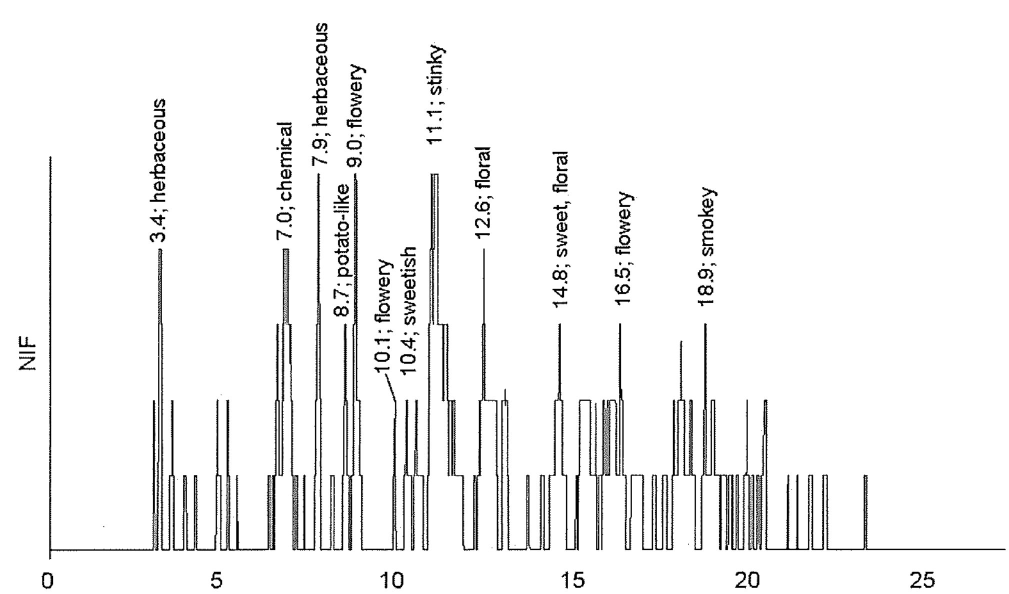

Fig. 3: GC-O analysis of an extract from a genotype mix using the NIF method and a panel of 7 trained testers.

Tab. 2: Relative concentration of major volatiles of 2 lines and 3 cultivars of papaya fruit measured by SBSE-GC. Genotypes: IPB-6C (I-6C), IPB 3,(I-3), Bangkok (BK), Brazil(BZ) and Burung (BR). The raw data (peak areas) are normalized by a internal standard (0.1 ppm v/v in the fruit homogenate). Missing values: the concentration of the compound is below the detection limit. Tentative identification of compounds was done by a library search (WILEY, NIST). The values are given as means of three repetitions. The standard errors for the individual substances are in the range from 5 to 20 %.

no	compound name	RT in min	BZ	BR	BK	IPB3	IPB6C
1	butanoic acid, methylester	6.46		0.769	0.009	0.015	
2	α-pinene	7.54		1.765	0.056		
3	butanoic acid, ethylester	8.22				0.027	
4	hexanal	9.92		1.838	0.066		
5	β-pinene	10.72		0.303	0.013		
6	3-carene	12.64		0.424	0.013		
7	β-myrcene	13.44				0.036	
8	D-limonene	14.97		0.083		0.018	
9	(E)-ocimene	16.76				0.021	
10	(Z)-ocimene	17.50				0.023	
11	o-cymene	17.60				0.010	
12	6-methyl-5-hepten-2-one	21.48	0.065	0.142		0.033	0.018
13	nonanal	23.91	0.010	0.378	0.011	0.022	0.021
14	(E)-linalooloxide (fur)	25.73			0.029	0.081	0.041
15	internal standard	26.09	1.000	1.000	1.000	1.000	1.000
16	unknown (linalooloxide derivative)	26.19				1.780	
17	acetic acid	26.58	0.028	0.512	0.012	0.035	0.026
18	unknown (linalooloxide derivate)	27.15				0.090	
19	(Z)-linalooloxide (fur)	27.41	0.026	0.564		2.945	0.014
20	decanal	28.42				0.016	0.016
21	benzaldehyde	29.42	0.009	0.700		0.016	0.031
22	linalool	29.49				15.820	0.013
23	benzoic acid, methyl ester	33.45		0.697		0.007	
24	butanonic acid	33.78		0.867	2.089	0.143	0.014
25	acetophenone	34.55				0.007	
26	furanmethanol	35.06	0.140	0.337			
27	isothiocyanato-cyclohexane	35.32				0.018	0.015
28	4-hexanolide	36.50				0.047	
29	verbenone	36.97	0.025	0.829	0.038	0.033	0.031
30	naphthalene	37.90		0.316		0.106	
31	(E)-linalooloxide (pyr)	38.04				0.120	0.069
32	(Z)-linalooloxide (pyr)	38.95				0.137	0.023
33	N.N-dibutyl-formamide	39.25	0.073	1.203	0.034	0.042	0.045
34	γ-hexalactone	39.86		0.493		0.026	
35	geraniol	41.98			0.050	0.071	0.035
36	butanoic acid, phenyl methyl ester	42.66				0.71	
37	benzyl alcohol	42.99	0.004	0.697	0.014	0.020	0.041
38	epoxy-linalool	43.19				0.035	
39	γ-octalactone	44.22	0.015			0.190	
40	benzyl nitrile	44.59	0.061	1.610	0.263	0.171	0.206
41	3,7-dimethyl-1,5,7-octatrien-3-ol	44.60				0.042	
42	benzothiazol	45.61	0.017	0.614		0.020	
43	δ-octalactone	45.98		3.536		0.211	
44	octanoic acid	49.15		0.780		0.089	0.015
45	isothiocyanatomethyl benzene	50.28	2.064	13.268	0.871	3.829	15.084
46	2-phenoxy ethanol	52.00			0.012		0.016
47	δ-decalactone	53.23				0.048	
48	4-methylthiazol	55.14		0.567			
49	unknown	55.30				0.050	

All substances were identified by MS library search and co-elution of authentic substances.
Reference compounds were not available for the substances 6, 10, 12, 16, 18, 27, 28, 30, 33, 36, 38, 41, 45, 46, and 48.

'IPB-3' is characterized by extremely high concentration of linalool (22), and also compound number 16 which was not detected in other genotypes. These compounds might be responsible for the more intense flowery aroma in this papaya line as shown in the profile analysis (Fig. 3). 'IPB-3' was rich in various linalooloxide isomers as well. δ-Decalacton (47) has been detected only in 'IPB-3' and was so far rarely reported as papaya odourant.

The 'Bangkok' variety was high in butanoic acid, which is known as typical stinky and unpleasant odour of papaya (Tab. 1). The presence of abundant amounts of this compound in Bangkok varieties might be also an indicator of overripeness of the fruits. Commonly, the unpleasant odour is detected in Burung. Moreover, in this experiment 'Burung' and 'IPB-3' were high in δ-octalacton which was reported to have odour descriptors like sweet, creamy and fatty with tropical and dairy nuances.

Conclusions

The 'IPB-6C' line was the most accepted followed by 'IPB-3', whereas Burung cultivar was the least accepted based on overall sensory attributes acceptability. 'IPB-3' showed richer flavour profile and received higher acceptability in term of taste and odour.

The typical aroma bouquet of papaya fruit is the result of numerous odour impressions arising from a huge number of aroma compounds. Character impact odourants were hexanal, (*Z*)-2-pentenol; nonanal, (*Z*)-linalool oxide, linalool, DMSO, butanoic acid, verbenone, phenyl methyl ester of butanoic acid, δ-octalactone and isothiocyanatomethyl benzene.

There are remarkable differences of relative concentrations and detected volatiles among the new papaya lines and cultivars. The differences in the sensory profile and the concentration pattern in the breeding lines in comparison to those of the used cultivars demonstrate the possibility to create sensory diversity by crossing and selection. In breeding programs which include the sensory quality as one of the targets, a control by human sensory and instrumental methods is essential. The methods used in this research like the effective sample preparation by SBSE are usable for this kind of topic but do not cover all character impact compounds responsible for the unique papaya aroma. The obtained knowledge will contribute to the breeders' ability in developing new papaya varieties with acceptable flavour properties.

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References

ANONYMOUS, 2003: Riset Unggulan Buah Tropis Indonesia. Kompas Edisi Jum'at, 19 Desember 2003. http://64.203.71.11/kompas cetak/0312/19/ispirasi/756234.htm. (accessed on 20th October 2007).

ANONYMOUS 2005: Pengembangan Varietas Pepaya. Pusat Kajian Buah-buahan Tropika. Laporan Akhir Rusnas 2005 (annual report). Pusat Kajian Buah-buahan Tropika, Bogor.

FLATH, A.R., FORREY, R.R., 1977: Volatile components of papaya (*Carica papaya* L. Solo Variety), J. Agric. Food Chem. 25, 103-109.

FLATH, R.A., LIGHT, D.M., JANG, E.B., MON, T.R., JOHN, J.O., 1990: Headspace examination of volatile emissions from ripening papaya (*Carica papaya* L., solo variety). J. Agric. Food Chem. 38, 1060-1063.

GOFF, S.A., KLEE, H.J., 2006: Plant Volatile Compounds: Sensory Cues for Health and Nutritional Value? Science 311 (5762), 815-819.

KOMES, D., ULRICH, D., LOVRIĆ, T., SCHIPPEL, K., 2005: Isolation of white wine volatiles using different sample preparation methods. Vitis 44, 187-193.

MACLEOD, A.J., PIERIS, N.M., 1983: Volatile components of papaya (*Carica papaya* L.) with particular reference to glucosinolate products. J. Agric. Food Chem. 31, 1005-1008.

PATIL S.S., TANG, C.S., 1974: Inhibition of ethylene evolution in papaya pulp tissue by benzyl isothiocyanates. Plant Physiol. 53, 585-588.

SIDHU, N.K., 2006: Tropical Fruits: Guava, Lychee, Papaya. In: Hui, Y.H., Jozsef, B. (eds.), Handbook of fruits and fruit processing, 597-634. Blackwell Publishing, Ames, Iowa.

TANG, C.S., 1971: Benzyl isothiocyanate of papaya fruit, Phytochemistry, 10, 117-121.

ULRICH, D., KOMES, D., OLBRICHT, K., HOBERG, E., 2007: Diversity of aroma patterns in wild and cultivated strawberry accessions. Genet Resour Crop Evol. 54, 1185-1196.

ULRICH, D., KRUMBEIN, A., SCHONHOF, I., HOBERG, E., 1998: Comparison of two sample preparation techniques for sniffing experiments with broccoli (*Brassica oleracea* var. *italica* Plenck). Food 42, 392-394.

WIJAYA, C.H., ULRICH, D., LESTARI, R., SCHIPPEL, K., EBERT, G., 2005: Identification of potent odorants in different cultivars of snake fruit [Salacca zalacca (Gaert.) Voss] using Gas Chromatography-Olfactometry. J. Agric. Food Chem. 53, 1637-1641.

WULAN, N.A., 2008: Californian van Java. http://www.trubusonline.com/mod/php?mod=publisher&op=allmedia&artid=227 (accessed on 29th September 2008).

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