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Changes in volatile compounds of Habanero chile pepper (*Capsicum chinense* Jack. cv. Habanero) at two ripening stages

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Abstract

The steam volatile components of Yucatan Habanero chile pepper (*Capsicum chinense* Jack. cv. Habanero) at two ripening stages (green and orange) were analyzed using GC and GC/MS. Both samples had several compounds in common. One hundred and two compounds were identified, from which (*E*)-2-hexenal, hexyl 3-methylbutanoate, (*Z*)-3-hexenyl 3-methylbutanoate, hexyl pentanoate, 3,3-dimethylcyclohexanol, and hexadecanoic acid were found to be the major constituents. During Habanero chile pepper maturation, the majority of volatile compounds decreased or even disappeared, some of them with green odour notes while esters, which have fruity odour notes, increased at the same time.

Keywords: Habanero chile pepper; *Capsicum chinense*; Volatile compounds; Ripening

1. Introduction

Chile peppers are popular spices in many parts in the world, valued for their sensory attributes of colour, pungency, and aroma. This "hot" taste has long been known to be due to the presence of non-volatile capsaicinoids, particularly capsaicin (Reineccius, 1994). In Mexico, this spice represents a traditional and culture identity. Hot chile peppers are the main elements that have characterized Mexican cuisine for at least the past eight centuries. Chile peppers are economically important because of the vast quantity and the diverse varieties used. The genus Capsicum comprises five big species: *Capsicum annuum* (containing the NuMex, Jalapeño and Bell varieties), *Capsicum frutescens* (containing the Tabasco variety), *Capsicum chinense* (containing the Habanero and Scotch Bonnet varieties), *Capsicum baccatum*, (containing the Aji varieties) and *Capsicum pubescens*, (containing the Rocoto and Manzano varieties) (Pruthi, 1980). The Habanero chile pepper is very aromatic and is the hottest chile pepper in the world. In Mexico, almost all Habanero pepper is harvested in Yucatan. This chile pepper is dark green changing to orange, orange-red, or red when fully ripe. It is lantern-shaped, 1–2 in. long and about 11/2 in. in diameter, and it can be 50 times hotter than jalapeños and is used mainly in salsas, chutneys and marinades for seafoods or pickled (Bosland, 1992).

The main quality parameters for Capsicum varieties are colour and pungency (Govindarajan, 1986). However, current research is also focussing on the aroma as an important parameter for the quality of fresh fruits and vegetables (Cremer & Eichner, 2000; Guadayol, Caixach, Ribé, Cabañas, & Rivera, 1997; Luning, de Rijk, Wichers, & Roozen, 1994). Also, more than 125 volatile compounds have been identified (Nijssen, Visscher, Maarse, Willemsens, & Boelens, 1996) in fresh

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and processed Capsicum fruits, the significance of these compounds for the aroma is not yet well known. In all, 60 volatiles were identified in bell peppers, among which 2-isobutyl-3-methoxypyrazine, (*E*,*Z*)-2,6-nonadienal, and (*E*,*E*)-decadienal are important aroma compounds (Buttery, Seifert, Guadagni, & Ling, 1969). This pyrazine and other alkyl-methoxypyrazines are the character impact compounds of the genus Capsicum (Whitfield & Last, 1991). A relationship between the volatiles 2-isobutyl-3-methoxypyrazine, 2-sec-butyl-3-methoxypyrazine, (*Z*)-3-hexenol and some sensory-perceived "green" notes of three Capsicum cultivars has been suggested (Chitwood, Pangborn, & Jennings, 1983). During bell pepper maturation, the majority of volatiles decreased or even disappeared (Luning et al., 1994). So far, the changes of volatile constituents in Habanero chile pepper during maturation have not been studied.

This paper deals with the isolation and identification of the volatile compounds, at two ripening stages, of Habanero chile pepper (*Capsicum chinense* Jack. cv Habanero).

2. Materials and methods

2.1. Samples and chemicals

Freshly harvested *C. chinense* cv. Habanero, grown at Yucatan, Mexico, were purchased from a local retail market. Fruits were selected at two maturity stages: green and orange (ripe). Standards of analyzed compounds, as well as dichloromethane were purchased from Aldrich (Milwaukee, USA) with a purity of >98%.

2.2. Colour analysis

Colour was measured directly on three zones of the skin of chile pepper and average was calculated. The CIE L^*, a^*, b^*, values were measured with a Minolta (CR-200 model) tristimulus colorimeter with illuminant C. From CIE L^*, a^*, b^* values, the chroma $(a^{*2} + b^{*2})^{1/2}$ and the hue angle $(\tan^{-1} b^*/a^*)$ were calculated (Lee & Coates, 1999).

2.3. Determination of capsaicinoid content

At harvest, fruits were divided longitudinally and dried in a single-wall transit oven at 60 °C until constant weight (3 days) for capsaicinoid analysis. Capasaicinoid were quantified at 280 nm against a calibration curve with pure capsaicin (Collins, Mayer Wasmund, & Bosland, 1995).

2.4. Isolation of volatile compounds

The Habanero chile peppers were cut open, the seeds removed, and the peppers (100 g) macerated in a Waring blender for 1 min with 500 ml of distilled water. Methyl decanoate (2 mg) was added as internal standard and immediately the resultant puree was treated in a simultaneous steam-distillation-solvent extraction apparatus (Likens & Nickerson, 1964) for 1 h. Dichloromethane (40 ml) was used as an extracting solvent and the condenser was cooled at 10 °C. The volatile concentrate was dried over anhydrous sulfate and concentrated to 0.6 ml on a Kuderna–Danish evaporator and then, to 0.2 ml with a gentle nitrogen stream. Extractions were done in duplicate.

2.5. GC and GC/MS analyses

A Konik 4000A GC (Konik Instrument, Spain) equipped with a 30 m × 0.25 mm × 0.25 μ m HP-5 fused silica capillary column and a flame ionization detector (FID) was used. The injector and detector temperatures were 250 °C. The oven temperature was held at 50 °C for 2 min and then increased to 280 °C at 4 °C min^{-1} and held for 10 min. The carrier gas (hydrogen) flow rate was 1 ml min^{-1}. Linear retention indices were calculated against those of *n*-paraffins. Quantitative data were obtained from the electronic integration of the GC peak areas (EZChrom v 6.7 software) for two extractions of each sample with the use of the internal standard method, neglecting FID response factors.

GC/MS analyses were done on a Hewlett–Packard model 6890N GC equipped with a 5973 mass-selective detector (Agilent Technologies, USA). It was fitted with a HP-5MS fused silica column (30 m × 0.25 mm × 0.25 μm). The chromatographic conditions were the same as those described for GC (FID). Carrier gas (helium) flow rate was 1 ml min^{-1}. The detector operated in electron impact mode (70 eV) at 230 °C. Detection was performed in the scan mode between 30 and 400 Da. Constituents were identified by comparison of their mass spectra with those in NBS, NIST or our IDENT data base, and confirmed, in many compounds, by their relative retention indices. Mass spectra from the literature were also compared (Adams, 1995; McLafferty & Stauffer, 1989).

3. Results and discussion

The composition of carbohydrates, volatile compounds, colour pigments and capsaicinoids changes during the maturation of peppers (Govindarajan, 1986). It has long been known that capsaicinoid production increases with maturity to a maximum and then decreases by rapid turnover and degradation of up to 60% (Iwai, Susuki, & Fujiwaki, 1979). Recently, it was demonstrated that capsaicinoids, particularly capsaicin, increased continuously and reached a maximum after 45–50 days from fruit set in Habanero chile pepper

Table 1
Volatile compounds (ppm) in Habanero chile pepper at the ripening stages green and orange

Compound	RI[a]	Identification[b]	Green	Orange
Hexanal	800	A	0.98	0.25
5-Methyl-2(5H)-furanone	815	B	<0.01	<0.01
(*E*)-2-Hexenal	854	A	8.87	4.37
(*Z*)-3-Hexenol	857	A	1.59	0.44
(*E*)-2-Hexenol	861	B	1.87	2.44
Hexanol	867	A	1.16	0.57
2-Heptanone	889	A	0.05	0.04
Tricyclene	926	B	nd[c]	<0.01
α-Pinene	939	A	0.07	0.07
3-Hepten-2-one	942	C	0.05	0.03
Hexyl acetate	1008	A	nd	<0.01
4-Methyl-3-pentenoic acid	1011	C	<0.01	<0.01
Isobutyl 2-methylbutanoate	1015	A	<0.01	<0.01
Isobutyl isopentanoate	1018	A	<0.01	0.04
Isopentyl isobutanoate	1021	A	nd	<0.01
p-Cymene	1026	A	nd	<0.01
Limonene	1031	A	<0.01	<0.01
(*E*)-β-Ocimene	1050	A	nd	<0.01
Isopentyl butanoate	1060	A	<0.01	<0.01
Linalool	1098	A	0.26	0.22
Isopentyl isopentanoate	1103	A	0.20	0.52
2-Methylbutyl isopentanoate	1105	A	<0.01	0.10
Methyl octanoate	1126	A	<0.01	0.05
Pentyl 2-methylbutanoate	1142	A	<0.01	0.08
(*Z*)-3-Hexenyl isobutanoate	1145	A	0.41	1.00
Pentyl isopentanoate	1148	A	0.24	<0.01
Hexyl isobutanoate	1150	A	0.19	0.72
Isoprenyl pentanoate	1152	A	<0.01	0.32
Hexyl butanoate	1184	A	nd	0.06
(*E*)-2-Nonenal	1185	C	<0.01	nd
2-Isobutyl-3-methoxypyrazine	1186	B	0.01	<0.01
(*Z*)-3-Hexenyl butanoate	1187	B	nd	<0.01
α-Terpineol	1189	A	0.06	<0.01
Methyl salicylate	1190	A	0.67	0.69
Hexyl 2-methylbutanoate	1234	A	1.43	3.13
Hexyl isopentanoate	1243	A	9.92	25.5
Heptyl isobutanoate	1248	A	nd	0.08
Isopentyl hexanoate	1260	A	nd	<0.01
Heptyl butanoate	1291	A	nd	0.08
β-Cyclocitral	1292	B	<0.01	0.03
(*Z*)-3-Hexenyl 2-methylbutanoate	1293	A	0.98	1.77
(*Z*)-3-Hexenyl isopentanoate	1295	A	7.78	14.6
Hexyl pentanoate	1298	A	9.10	18.5
(*E*)-2-Hexenyl pentanoate	1299	A	1.84	2.47
Heptyl isobutanoate	1300	A	0.21	0.50
Pentyl isohexanoate	1303	C	<0.01	0.14
9-Decanolide	1308	C	0.07	0.14
Octyl isobutanoate	1311	B	<0.01	<0.01
(*E*,*E*)-2,4-Decadienal	1313	B	0.46	<0.01
4-Vinylguaiacol	1315	A	<0.01	<0.01
Heptyl 2-methylbutanoate	1332	B	1.53	2.10
Heptyl isopentanoate	1338	B	5.30	11.91
Methyl anisate	1340	B	nd	<0.01
Octyl isobutanoate	1348	A	0.37	0.76
(*Z*)-3-Hexenyl hexanoate	1382	B	0.29	0.78
Hexyl hexanoate	1383	A	0.33	2.62
Decanoic acid	1385	A	<0.01	3.22
β-Cubebene	1390	B	nd	<0.01
3,3-Dimethylcyclohexanol	1392	C	14.3	35.7
Benzyl pentanoate	1396	A	1.22	2.47
Octyl 2-methylbutanoate	1418	C	1.56	4.43
β-Caryophyllene	1420	A	0.74	1.48
(*E*)-α-ionone	1426	A	0.22	0.40

Table 1 (*continued*)

Compound	RI[a]	Identification[b]	Green	Orange
Octyl 2-methylbutanoate	1430	B	0.55	0.78
Octyl isopentanoate	1440	B	1.57	5.15
2-Methyl-1-tetradecene	1445	C	0.64	2.64
α-Himachalene	1447	B	0.41	1.08
Heptyl hexanoate	1448	B	<0.01	<0.01
α-Humulene	1454	A	0.05	0.13
(*E*)-β-Farnesene	1458	A	0.18	0.55
2-Methyltetradecane	1462	C	1.43	3.86
β-Chamigrene	1475	B	3.95	10.38
Germacrene D	1480	B	0.65	1.83
(*E*)-β-Ionone	1485	A	0.85	1.40
Pentadecane	1500	A	0.14	0.48
α-Muurolene	1502	B	0.16	0.48
γ-Cadinene	1513	A	<0.01	0.09
Cubebol	1515	B	<0.01	0.22
δ-Cadinene	1524	A	0.32	0.78
Cadina-1,4-diene	1531	B	<0.01	0.20
2-Methylpentadecane	1533	C	0.13	0.40
(*Z*)-Nerolidol	1534	A	nd	0.16
Hexyl benzoate	1576	B	0.69	3.18
Phenylacetic acid	1579	B	nd	<0.01
Hexadecane	1600	A	0.12	0.35
Nonyl pentanoate	1610	C	0.07	0.52
Tetradecanal	1613	A	nd	0.31
Cubenol	1642	B	0.20	0.67
Oxacyclopentadecan-2-one	1650	C	0.29	1.04
α-Cadinol	1653	B	0.14	0.52
Pentadecanal	1707	A	0.46	3.50
(*E*)-11-Hexadecenal	1759	C	0.22	2.24
Benzyl benzoate	1762	A	0.32	1.12
Tetradecanoic acid	1782	A	0.18	3.08
Hexadecanal	1811	A	<0.01	0.65
Pentadecanoic acid	1883	A	1.95	2.35
(*Z*)-11-Hexadecenoic acid	1915	C	3.39	9.35
Ethyl (*Z*)-9-hexadecenoate	1975	A	1.48	2.53
Hexadecanoic acid	1983	A	8.35	11.1
Octadecanol	2079	A	nd	0.08
Pentacosane	2500	A	0.07	0.12
Hexacosane	2600	A	<0.01	0.11

[a] Calculated retention indices on HP-5 column.

[b] The reliability of the identification proposal is indicated by the following: A, mass spectrum and Kovats index agreed with standards; B, mass spectrum and Kovats index agreed with database or literature; C, mass spectrum agreed with mass spectral database.

[c] nd, not detected.

(Contreras-Padilla & Yahía, 1998). The fruit used, in our study at the green stage (L^* 39.78, Hue angle 126.03 and Chroma 24.61), had a capsaicinoid content of 42.6 mg g^{-1} of dry weight, while at the final ripe colour (orange) (L^* 59.54, Hue angle 75.18 and Chroma 51.18), it increased to 50.5 mg g^{-1} of dry weight.

The volatile constituents of Habanero chile pepper were obtained by simultaneous steam-distillation-solvent extraction and analyzed by GC and GC–MS using fused silica capillary columns. It has been pointed that disruption of cell structure favours lipid oxidation and the formation of related alcohols, aldehydes and ketones in fresh bell peppers (Luning, 1994), but this behaviour is not a limitation in our results because both samples were isolated by the same procedure. In addition, tissue disruption is always necessary for commercial processing of chile peppers. Furthermore, no thermal degradation products of paprika volatile compounds were found using this isolation method at atmospheric pressure (Guadayol et al., 1997).

The identified compounds are listed in Table 1, together with their semiquantitative amounts. The yields of total volatiles, estimated by the addition of a measured amount of internal standard to the chile peppers, were about 103 and 228 mg kg^{-1} for green and orange fruits, respectively. In total, one hundred and two volatile compounds were identified, all of them reported for the first time in Habanero chile pepper, but they are often found in other vegetables (Nijssen, 1996). The presence of so many aliphatic esters in both samples, particularly at the orange stage, is interesting. This abundance of aliphatic esters has been not reported in other Capsicum species (Nijssen, 1996). Alkyl esters of isobutanoic, 2-methylbutyric, isopentanoic and hexanoic acids were the major ones. All these esters have powerful fruity odour notes (Arctander, 1969). In general, although pyrazines are present in small quantities in natural samples, their contribution to the flavour of these samples is considerable, as in the case of 2-isobutyl-3-methoxypyrazine, which was found to possess an extremely potent odour, similar to that of fresh green bell peppers (Luning, 1994). Interestingly, δ-3-carene, a terpene reported in bell pepper as red/green bell pepper odour (Luning, 1994), was not detected in this study. Major volatile compounds, at both maturity stages, were (*E*)-2-hexenal, hexyl 3-methylbutanoate, (*Z*)-3-hexenyl isopentanoate, hexyl pentanoate, 3,3-dimethylcyclohexanol, and hexadecanoic acid.

The composition of volatile compounds of Habanero green pepper differs clearly between the maturation stages. Table 1 shows that the majority of compounds increased, including esters, with their fruity odour notes, while some compounds decreased or even disappeared during ripening. For example, (*E*)-2-hexenal and (*Z*)-3-hexenol, which have green odour notes, significantly decreased. The green chile peppers have high concentrations of (*E*)-2-hexenal, hexyl 3-methylbutanoate, (*Z*)-3-hexenyl 3-methylbutanoate, hexyl pentanoate, 3,3-dimethylcyclohexanol and hexadecanoic acid.

Table 1 shows that the levels of (*E*)-2-hexenal and (*Z*)-3-hexenol decreased during Habanero chile pepper maturation while the levels of (*E*)-2-hexenol increased. As reported for tomatoes (Gaillard, Matthew, Wright, & Fishwick, 1977), the activities of several enzymes seem to change during ripening of Habanero chile pepper, especially those involved in the formation of these lipid-degraded products.

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