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Characterization of total capsaicinoids, colour and volatile compounds of Habanero chilli pepper (*Capsicum chinense* Jack.) cultivars grown in Yucatan

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Abstract

Total capsaicinoids, colour and volatile compounds of 10 Habanero chilli pepper (*Capsicum chinense* Jack.) cultivars grown in Yucatan, grouped by their colours: four red, five orange and one brown, were determined. The content of capsaicinoids, responsible for the pungency of chilli peppers, varied between 41.8 and 65.9 mg g^{-1} dry fruit. Mean concentration of orange cultivars was 55.0 mg g^{-1}, while red cultivars had 45.0 mg g^{-1} dry fruit, indicating that the first ones are more pungent. The composition of volatile compounds of the Habanero chilli peppers differs clearly for the different cultivars. Orange and brown cultivars have in general higher amounts of esters, with their fruity odour notes, than red cultivars. These differences are reflected in the amount of total volatiles, which is higher in orange and brown cultivars in comparison with red cultivars. From these results it can be concluded that orange and brown cultivars are better in terms of their flavour-relevant chemical composition than red cultivars.
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1. Introduction

Chilli peppers are popular spices in many parts of the world, valued for their sensory attributes of colour, pungency and flavour. In Mexico, peppers represent a culinary tradition and a cultural symbol. Peppers are economically important because of the vast consumption of the diverse varieties. The food industry is the largest user of capsicums, where they are used as colouring and flavouring agents in sauces, soups, processed meats, snacks, candies, soft drinks and alcoholic beverages.

The genus *Capsicum* comprises more than 200 varieties (Pruthi, 1980). The fruits vary widely in size, shape, flavour and sensory heat. The genus *Capsicum* comprises five main species: *Capsicum annuum* (comprising the NuMex, Jalapeño and Bell varieties), *Capsicum frutescens* (Tabasco variety), *Capsicum chinense* (Habanero and Scotch Bonnet varieties), *Capsicum baccatum* (Aji varieties) and *Capsicum pubescens* (Rocoto and Manzano varieties) (Pruthi, 1980). The Habanero chilli pepper is very aromatic and is the hottest chilli pepper in the world. In Mexico, almost all Habanero pepper is harvested in Yucatan.

The pungency of capsicum fruit is due to a group of compounds called capsaicinoids which are present in hot pepper varieties in different amounts (Govindarajan, 1986). The concentration of capsaicinoids in fresh red pep-

per varieties, especially in paprika, ranges from 0.001% to 0.01%, and in strong chilli varieties the concentration ranges from 0.1% to <1% (Govindarajan, Rajalaksmi, & Chand, 1987).

Although more than 125 volatile compounds have been identified (Nijssen, Visscher, Maarse, Willemsens, & Boelens, 1996) in fresh and processed Capsicum fruits, the flavour significance of these compounds is not yet well-known. Recently, we studied the changes of volatile constituents in Habanero chilli peppers during maturation (Pino, Sauri, & Marbot, 2006).

This paper deals with the characterization of total capsaicinoids, colour and volatile compounds of 10 Habanero chilli pepper (C. chinense Jack.) cultivars grown in Yucatan, Mexico.

2. Materials and methods

2.1. Samples and chemicals

Ten cultivars of Habanero chilli peppers (C. chinense Jack.) were grown in the experimental fields of Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias and Instituto Tecnológico Agropecuario No. 2 in Yucatan, Mexico. They were named for their external colours: Red 1 to Red 4, Orange 1 to Orange 5 and Brown. Mature chilli pepper fruits were harvested from October to December 2003.

Standards of analysed compounds, as well as dichloromethane, were purchased from Aldrich (Milwaukee, USA) with a purity of >98%.

2.2. Physical and chemical analysis

The soluble solids of the pepper fruit were determined with an ATAGO refractometer, and the resulting data were reported as °Brix at 20 °C. Ash was determined as reported by A.O.A.C (1997). The pH was measured by homogenizing 40 g of the fruit in 100 ml distilled water in a Waring blender and then filtering.

2.3. Colour analysis

Colour was measured directly on three zones of the chilli pepper skin and the 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 hue angle ($\tan^{-1} b^*/a^*$) was calculated (Lee & Coates, 1999).

2.4. Determination of total capsaicinoid content

At harvest, 150 g fruits were divided longitudinally and dried in a single-wall transit oven at 60 °C until constant weight (~3 days) for capsaicinoids analysis. Dried tissue was ground, and samples of 1 g each were mixed with 10 ml of acetonitrile and kept for 4 h at 80 °C with shaking in a capped Erlenmeyer flask before the analysis acetonitrile containing capsaicinoid compounds was filtered (Collins, Mayer Wasmund, & Bosland, 1995). Capasaicinoids were quantified at 280 nm against a calibration curve with pure capsaicin.

2.5. Isolation of volatile compounds

The chilli peppers were cut open, the seeds removed, and the chilli peppers (100 g) macerated in a Waring blender for 1 min with 500 ml distilled water. Methyl nonanoate (2 mg) was added as internal standard and the resultant puree was immediately treated in an apparatus for simultaneous steam distillation–solvent extraction (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.

2.6. GC and GC/MS analyses

A Konik 4000A GC (Konik Instrument, Spain) equipped with a 30 m × 0.25 mm × 0.25 μm HP-5 fused sil-

Table 1
Physicochemical analyses of Habanero chilli pepper cultivars

Cultivars	Soluble solids (°Brix)	pH	Ash (%)	Colour				Total capsaicinoids (mg g^{-1} dry fruit)
				L^*	a^*	b^*	Hue angle	
Red 1	7.5	5.2	0.9	34.6	39.0	26.7	34.7	46.4
Red 2	6.4	5.4	0.9	28.7	39.1	23.4	31.1	41.8
Red 3	7.7	5.3	0.9	43.0	40.1	23.6	30.3	47.5
Red 4	7.4	5.4	1.2	41.6	42.5	28.5	33.7	44.4
Orange 1	7.3	4.9	0.9	58.9	18.5	55.8	71.6	47.8
Orange 2	7.8	5.2	1.0	63.9	20.9	60.8	70.6	64.9
Orange 3	7.9	5.2	1.0	69.8	22.1	41.6	62.3	52.7
Orange 4	9.0	5.4	1.0	60.8	18.9	49.0	70.5	53.5
Orange 5	4.6	5.4	0.5	61.6	13.4	48.3	72.8	54.8
Brown	9.1	5.2	1.0	28.1	4.7	4.0	40.5	65.8
Mean	7.5	5.3	0.9	49.1	25.9	36.2	51.8	51.9
RSD (%)	17.1	3.0	19.0	31.8	50.9	49.1	36.9	15.7

Table 2
Volatile compounds in Habanero chilli pepper cultivars (mg kg^{-1}dry fruit)

Compound	KI	Red 1	Red 2	Red 3	Red 4	Orange 1	Orange 2	Orange 3	Orange 4	Orange 5	Brown
Hexanal	800	t	t	t	t	t	0.01	0.01	t	t	0.01
(E)-2-Hexenal	854	–	0.01	0.01	t	0.02	0.02	0.03	0.01	0.02	0.03
(Z)-3-Hexenol	857	t	t	t	t	t	t	0.01	t	t	t
Hexanol	867	t	t	t	–	t	t	t	–	t	t
α-Pinene	939	–	t	–	–	t	0.01	t	–	–	t
Hexyl acetate	1009	–	–	–	t	t	t	–	–	–	0.02
Isobutyl isopentanoate	1018	–	–	–	–	–	t	t	–	–	t
Limonene	1031	t	t	t	t	t	t	t	–	t	t
Linalool	1098	t	t	t	–	t	0.01	0.01	t	0.01	t
Isopentyl 2-methylbutanoate	1100	–	–	–	–	–	t	t	–	t	0.01
Isopentyl isopentanoate	1103	0.01	t	–	–	0.02	0.06	0.06	0.02	0.04	0.06
2-Methylbutyl isopentanoate	1105	–	–	–	–	–	0.02	t	–	–	0.01
Hexyl isobutanoate	1149	0.02	0.04	–	0.01	0.05	0.07	0.06	0.02	0.02	0.09
Pentyl isopentanoate	1151	0.02	t	–	–	0.01	0.06	0.23	0.02	0.04	0.10
Isoprenyl pentanoate	1152	t	–	–	–	0.01	0.10	0.29	0.03	t	0.07
2-Isobutyl-3-methoxypyrazine	1186	–	–	–	–	0.01	0.01	0.01	0.01	0.01	0.01
α-Terpineol	1189	t	t	t	t	0.01	t	t	0.01	0.01	0.02
Methyl salicylate	1192	–	–	–	–	t	t	t	t	–	–
Hexyl 2-methylbutanoate	1236	0.08	0.25	0.09	0.07	0.24	0.37	t	0.18	0.40	0.39
Hexyl isopentanoate	1244	0.80	1.95	1.62	0.67	2.05	2.50	2.50	2.42	3.04	1.69
Heptyl butanoate	1291	0.10	0.11	0.10	0.11	0.12	0.10	0.10	0.11	0.11	0.13
(Z)-3-Hexenyl 2-methylbutanoate	1293	0.06	–	–	–	0.05	0.12	0.10	0.09	0.17	0.37
(Z)-3-Hexenyl isopentanoate	1295	0.31	0.77	0.24	0.16	0.18	0.63	0.72	0.54	1.34	1.40
Hexyl pentanoate	1298	0.21	0.66	0.14	0.12	0.09	0.55	0.84	0.52	1.49	0.91
(E)-2-Hexenyl pentanoate	1299	0.06	0.07	–	–	0.01	0.13	0.21	0.10	0.21	0.14
Heptyl isobutanoate	1300	0.05	0.06	0.08	0.07	0.06	0.08	0.06	0.05	0.09	0.07
Pentyl isohexanoate	1303	–	–	–	–	0.01	t	t	0.01	t	t
9-Decanolide	1308	–	–	–	0.01	–	0.01	t	–	–	–
Hexyl tiglate	1328	–	–	–	–	t	–	–	–	–	t
Heptyl 2-methylbutanoate	1333	-	t	–	–	–	0.03	0.06	0.01	0.01	0.04
Heptyl isopentanoate	1338	0.03	0.04	–	–	0.02	0.13	0.21	0.02	0.07	0.04
Hexyl isohexanoate	1342	0.02	0.05	–	–	0.05	0.09	0.16	0.10	0.29	0.04
Heptyl pentanoate	1376	0.02	0.16	0.11	t	0.42	0.49	0.48	0.15	0.69	0.45
(Z)-3-Hexenyl hexanoate	1382	t	0.01	–	–	t	–	0.01	0.01	0.05	t
Hexyl hexanoate	1384	t	t	–	–	0.02	0.02	0.05	0.04	0.11	0.03
Decanoic acid	1385	t	t	–	–	t	t	0.01	0.01	0.02	0.01
β-Cubebene	1388	t	0.01	–	–	0.01	0.03	0.01	0.01	0.03	0.02
3,3-Dimethylcyclohexanol	1392	0.07	0.60	1.98	0.07	2.05	1.84	1.59	1.36	1.52	1.90
Benzyl pentanoate	1396	0.02	0.04	–	–	0.01	0.04	0.07	0.05	0.09	0.13
Octyl 2-methylbutanoate	1418	t	0.05	0.07	–	t	0.18	0.16	0.05	0.15	t
β-Caryophylene	1419	–	–	–	–	t	0.01	–	t	0.02	t
(E)-α-Ionone	1430	t	t	–	–	0.01	0.01	0.02	0.02	0.08	0.02
Octyl isopentanoate	1440	t	0.06	–	–	t	0.11	0.15	0.06	0.19	0.19
2-Methyl-1-tetradecene	1445	0.01	0.07	t	–	0.05	0.42	0.13	0.05	0.39	0.11
α-Himachalene	1447	–	0.01	–	–	t	0.02	0.01	0.05	t	t
Heptyl hexanoate	1449	–	t	t	–	t	0.01	0.01	0.02	0.01	0.01
α-Humulene	1455	–	–	–	–	t	–	t	t	t	–
(E)-β-Farnesene	1457	–	–	–	–	t	0.08	t	0.01	0.04	t
2-Methyltetradecane	1462	0.02	0.13	–	–	0.03	0.47	–	0.06	0.26	0.06
γ-Himachalene	1483	0.03	0.13	0.09	0.03	0.11	0.38	0.22	0.28	0.27	–
Germacrene D	1485	0.01	0.02	t	–	0.04	0.14	0.09	0.02	0.09	t
(E)-β-Ionone	1489	0.01	0.02	0.01	t	0.05	0.11	0.06	0.05	0.18	0.05
γ-Cadinene	1514	–	–	–	–	t	–	–	–	–	–
δ-Cadinene	1523	t	t	–	–	0.01	0.05	0.02	0.02	0.10	0.01
trans-Cadina-1(2),4-diene	1535	–	–	–	–	–	t	–	0.01	0.02	–
Hexyl benzoate	1575	–	–	t	t	0.01	t	t	t	t	t
Oxacyclotetradecan-2-one	1577	0.01	0.02	–	–	–	0.19	0.04	0.01	0.03	0.01
Tetradecanal	1610	0.01	–	–	–	0.01	0.09	0.12	–	0.11	0.01
Oxacyclopentadecan-2-one	1650	0.01	t	–	–	t	0.01	t	t	0.01	0.01
Pentadecanal	1707	t	t	t	–	0.01	0.02	t	0.01	t	t
Benzyl benzoate	1762	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.02	t	0.01
Tetradecanoic acid	1782	0.01	0.01	0.01	0.02	0.01	t	t	0.02	t	t
Hexadecanal	1811	0.01	0.02	0.02	0.02	0.02	0.01	t	0.02	0.01	0.01
Total volatiles		2.02	5.38	4.59	1.37	5.90	9.85	8.93	6.68	11.84	8.69

– Not detected; t means lower than 0.01 mg kg^{-1}dry fruit.

ica 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) with two extractions for each sample with the use of the internal standard method, neglecting FID response factors.

GC/MS analyses were performed 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 FLAVORLIB data base and most identifications were confirmed by their relative retention indices.

3. Results and discussion

Table 1 summarizes some physical and compositional characteristics of 10 mature cultivars of chilli pepper fruits. The pH value was the only evaluated characteristic that showed little variation (RSD 3.0%). Soluble solids varied from 4.6 to 9.1 °Brix, while the ash content varied from 0.5% to 1.2%, showing great variability.

As three different colours of cultivars were studied, the a^* and b^* values, and the tone (hue angle) were different between the cultivars. In the red cultivars the mean value of a^* (contribution to red) was 40.19, while in the orange and brown cultivars it was 18.71 and 4.70, respectively. The b^* parameter (contribution to yellow) had a mean of 51.61 in the orange cultivars, 25.55 in the red cultivars and 4.00 in the brown cultivar. Due to the different values of a^* and b^*, the tone significantly differed between the cultivars. The L^* values varied in a wide interval, which indicates that colour brightness also differed between cultivars.

The content of capsaicinoids, responsible for the pungency of chilli peppers, varied between 41.8 and 65.9 mg g^{-1} dry fruit (15.7% RSD). Its mean concentration in orange cultivars was 55.0 mg g^{-1} dry fruit, while in red cultivars it was 45.0 mg g^{-1} dry fruit, indicating that the orange cultivars were more pungent. These results show the different physical and chemical characteristics of the evaluated cultivars.

The volatile constituents of Habanero chilli pepper were obtained by simultaneous steam distillation–solvent extraction and analysed by GC and GC–MS using fused silica capillary columns. No thermal degradation products of paprika (*C. annuum* L.) volatile compounds were found

using this isolation method (Guadayol, Caixach, Ribé, Cabañas, & Rivera, 1997).

The identified compounds are listed in Table 2 together with their semi-quantitative amounts. The yield of total volatiles, estimated by the addition of a measured amount of internal standard to the chilli peppers, was about 1.37 to 11.8 mg kg^{-1} for all cultivars. In total, 63 major volatile compounds were quantified. All of them have been reported earlier by Pino et al. (2006).

The presence of several aliphatic esters in Habanero chilli peppers has been reported (Pino et al., 2006). This abundance of aliphatic esters has not been found in other Capsicum species (Nijssen et al., 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). Major volatile compounds in all cultivars were hexyl isopentanoate, (*Z*)-3-hexenyl isopentanoate, hexyl pentanoate and 3,3-dimethylcyclohexanol.

The composition of volatile compounds of the Habanero chilli peppers differs clearly in the different cultivars, in particular between red and orange cultivars. Table 2 shows that orange and brown cultivars have in general higher amounts of esters with their fruity odour notes than red cultivars. Also, 2-isobutyl-3-methoxypyrazine was found in orange and brown cultivars. This pyrazine was found to possess an extremely potent odour similar to that of fresh green bell peppers (Luning, de Rijk, Wichers, & Roozen, 1994). All these differences are reflected in the total volatile amount, which is higher in orange and brown cultivars in comparison with red cultivars.

From these results it can be concluded that orange and brown cultivars are enhanced in terms of their flavour-relevant chemical composition than red cultivars.

Acknowledgements

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