Odor-contributing volatile compounds of a new Brazilian tabasco pepper cultivar analyzed by HS-SPME-GC-MS and HS-SPME-GC-O/FID

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Abstract
The cultivar BRS Avai was originated from recurrent phenotypic selection in the original American cultivar Tabasco Macllhenny™, used in the worldly famous Tabasco pepper (Capsicum frutescens L.) sauce. The determination of the aroma profile of this new cultivar could reveal important qualitative descriptors that will help in future crosses and in the improvement of the desirable characteristics. Headspace solid-phase microextraction combined with gas chromatography-mass spectrometry and gas chromatography-olfactometry techniques were used to analyze the volatile compounds from a new Brazilian cultivar, estimating the most powerful odor-active compounds. In the volatile fraction of BRS Avai pepper, esters were the predominant chemical class and major compounds were isohexyl and hexyl esters. Thirty-five odorants were detected and sixteen were considered as having moderate to high intensity, with hexyl 2-methylbutanoate, α-pinene, hexyl butanoate and hexyl 3-methylbutanoate contributing the most to the typical sweet, herbal and pepper-like aroma of this cultivar.

Keywords: Capsicum frutescens L.; chilli pepper; aroma; OCV; olfactometry.

Practical Application: This first GC-O study of a C. frutescens grown in Brazil identified main odor-contributing compounds that can be used as chemical markers in breeding studies for improvement of tabasco pepper quality.

1 Introduction
Peppers from the genus Capsicum are among the most consumed spices in the world, presenting a rich variation in size, color, flavor and pungency. They are also valued for their richness in bioactive compounds such as vitamins, polyphenols, flavonoids, carotenoids and other secondary metabolites with antioxidant properties (Di Cagno et al., 2009; Hamed et al., 2019). They comprise 38 species, six of which are domesticated: C. annuum, C. baccatum, C. assamicum, C. chinense, C. pubescens and C. frutescens (Ramchiary et al., 2013). Capsicum frutescens is one of the most used species in cooking, especially the varieties malagueta and tabasco, which are native to tropical regions of America (Carvalho et al., 2017).

The American cultivar Tabasco Macllhenny™, used in the worldly famous Tabasco pepper sauce, was introduced in Brazil in the 2000s, and originally grown in Ceará for exportation. However, considering the numerous difficulties arising from the periodic importation of seeds and production problems, such as the lack of plant uniformity, Embrapa’s researchers started a selection work to preserve and multiply the genetic base from the subpopulations of pepper used in the productive sector. The result was the cultivar BRS Avai, originated from recurrent phenotypic selection in the original American population (Pereira et al., 2014). According to these authors, the fruit has an elongated shape, red color, average length of 3 cm, capsacin content around 27,000 Scovilles and 24% fruit pulp. The productivity at the tested sites and the technological characteristics were superior to the Tabasco Macllhenny cultivar, showing that the selected genotype is adapted to the growing regions, but to obtain varieties of higher commercial interest, the breeding program must advance, seeking more productive and resistant genotypes, also taking into account the flavor they impart to foods.

A few studies have been conducted to identify volatile compounds present in Capsicum frutescens varieties. Bogusz et al. (2012, 2015), studying the volatile fractions of Brazilian Capsicum peppers by HS-SPME, identified isohexyl hexanoate, hexyl 2,2-dimethylpropanoate and hexyl isovalerate as major compounds in malagueta pepper, while Manikharda et al. (2018) identified mostly isohexyl esters as major compounds in the volatile profile of Shimatogarashi pepper, a typical C. frutescens domesticated in Japan. In its turn, the volatile composition determined in a native Chinese C. frutescens revealed only a small number of esters and a predominance of hydrocarbons, mostly alkanes (Liu et al., 2009).

However, only the knowledge of the odor-contributing volatiles (OCV) in plant materials is relevant for plant breeders, as improvement might be misleading if based only on quantitatively major compounds with low or no sensory contribution, while minor compounds, with much lower thresholds and higher odor impact, might be neglected.

The only studies in literature involving sensory determination of odor-contributing compounds in C. frutescens peppers were performed by Rodriguez-Burruezo et al. (2010) and Da...
Costa et al. (2012). In the first one, the authors evaluated 16 *Capsicum* accessions from the *annuum-chinense-frutescens* complex, from different geographic regions. The sniffing test revealed that the diversity of aromas among the cultivars is due to qualitative and quantitative differences of at least 23 OCV. *C. frutescens* accessions, from Laos, Spain and USA, with fruity/exotic aromas, were characterized by a high contribution of several esters and ionones (Rodríguez-Burruelo et al., 2010). On the second study, Da Costa et al. (2012) listed 34 OCV in 5 genotypes of chilly peppers. Among them, isohexyl esters were the major compounds, followed by γ-himachalene.

The aim of this study was to determine the odor-contributing volatile compounds of a Brazilian cultivar of *C. frutescens* pepper named BRS Avai, using a gas chromatography-olfactometry (GC-O) technique. The determination of the aroma profile of this new tabasco cultivar could reveal important qualitative descriptors that will help in future crosses and in the improvement of the desirable characteristics. This is the first GC-O study of a *C. frutescens* genotype grown in Brazil.

2 Materials and methods

2.1 Plant materials

The pepper cultivar BRS Avai (*C. frutescens*), originated from the population Tabasco Maclhenny™, was grown in Fortaleza, Ceará, Brazil (coordinates 3° 43' 46'' South; 38° 32' 36''; altitude 14 m), under greenhouse conditions during wet season, temperature range from 26 to 32 °C.

2.2 Volatile analysis

The volatile compound analysis was done according to method used by Garruti et al. (2013). The samples were washed in tap water, ground with NaCl (30% w/w) (Merck, Darmstadt, Germany) until a paste was formed, then weighed (2 g), packed in 10 mL vials, sealed and kept frozen (-18 °C) until analyses were made. The extraction of volatile compounds was carried out by manual headspace solid-phase microextraction (HS-SPME) using a 1-cm long fiber (Supelco Co., Bellefonte, USA) coated with 30 µm divinylbenzene/carboxen/ polydimethylsiloxane (DVB/Car/PDMS). The fiber was exposed to the sample's headspace at 45 °C for 60 min. Prior to the first extraction, the fiber was conditioned at 270 °C for 1 h in the injector port of the gas chromatograph. Between analyses, the fiber was reconditioned at 240 °C for 15 min (desorption time of 4 min).

A Shimadzu CG2010 gas chromatograph coupled to a QP2010 mass detector (Shimadzu Co., Kyoto, Japan) was used. The volatile compounds were separated with a DB-5 column (0.25 mm, 30 m, 0.25 µm, J&W Scientific, Folsum, USA) using the following oven temperature program: run at 50 °C, ramped up to 180 °C at a rate of 5 °C/min, with final hold of 5 min (total time 31 min). The injector was on the splitless mode for 1.0 min at 240 °C; carrier gas hydrogen at 1.5 mL/min; oven temperature initially at 50 °C, increasing to 180 °C at 5 °C/min and holding more 5 min (total time 31 min). The GC effluent was split, going to the flame ionization detector (FID) and to the olfactometer at the same time. Volatiles were carried to the judge's nose through the sniffing port by humidified nitrogen, to minimize the discomfort caused by excessive drying of the nasal mucosa.

Judges were selected by a series of triangular tests (ASTM E1885-04, 2011) using ethyl butanoate aqueous solutions in different concentrations (0.05, 0.025, 0.0125, 0.00625, 0.003125 M), always compared to water, and by the Odor Recognition Test (ISO Standards 5496 and 22935), with 16 different odoriferous substances. Initially, 17 participants with experience in sensory tests were recruited, however, only six judges (4 women, 2 men) were selected (75% of correct answers). The protocols of the sensory tests were previously approved by the Ethics Research Committee of the State University of Ceará (Approval no. 147,279).

Before olfactometry analysis, two preliminary runs were carried out in order the selected judges to be acquainted to the tasks. Their job was to verbally describe the quality of each perceived odor and, at the same time, rate its intensity using a 9-cm unstructured scale (0 = not perceived; 9 = extreme sensory impact) available at the time-intensity software named SCDTI (System of Time-Intensity Data Collection) (Cardello et al., 2003).

Analyses were performed in quadruplicate and the aromagram was built averaging 24 runs (6 x 4 replicates). Among all parameters provided by the software for each odorant substance in each run, only the maximum odor intensity (I_{max}) and the time it was perceived (T_{max}) were used to construct the aromagram, averaging 24 runs (6 judges x 4 replicates). Retention indices were calculated using T_{max} to ensure that the olfactometric data was related to the chromatographic data. The Odor Impact (OI) of each eluate was expressed as geometric mean percent (Equation 1), as proposed by Dravnieks (1992).

\[
\text{OI(\%)} = \sqrt[6]{F(\%)} \times \text{I}_{\text{max}}(\%)
\]  

where OI (\%) is odor impact; F(\%) is the detection frequency and I_{max}(\%) is the maximum intensity registered by the software in the time-intensity curve, all expressed in percentages.
3 Results and discussion

3.1 Volatile profile of C. frutescens cv. BRS Avai

Forty-four volatile compounds were detected in the headspace of tabasco pepper fruits (C. frutescens) cultivar BRS Avai. Among the 36 compounds identified (Table 1), 31 have previously been reported in the mentioned literature, indicating that the variation in the volatile profile of C. frutescens peppers is given more by quantitative rather than qualitative differences.

Esters were the predominant chemical class (23 compounds, 92% area), followed by alkanes (8 compounds, 7%). The volatile composition of BRS Avai pepper also included 2 alcohols (isooctanol, and tridecanol), 1 ketone (3,5-dimethyl-2-octanone), 1 lactone (oxacyclotetradecan-2-one) and 1 terpene (α-pinene). Among esters, half of compounds were branched, mainly 2-methylpropanoates, and 2- and 3-methylbutanoates. Major components (relative area > 2%) were hexyl and isohexyl esters: 4-methylpentyl hexanoate, 4-methylpentyl 3-methylbutanoate, 4-methylpentyl 2-methylbutanoate, 4-methylpentyl 2-methylpropanoate, hexyl hexanoate, hexyl pentanoate and hexyl 2-methylbutanoate.

The predominance of esters is in agreement with the results found in studies on the volatile fraction of other C. frutescens cultivars. The volatile profile of Brazilian malagueta pepper showed 33 esters, corresponding to 40% area, with compounds 2-methylpentyl hexanoate, hexyl 2,2-dimethylpropanoate and hexyl 3-methylbutanoate within the major compounds (Bogusz et al., 2012). Another study from the same research group analyzed Brazilian Capsicum peppers by comprehensive two-dimensional chromatography with a time-of-flight mass spectrometry detector (GC × GC -TOFMS) and confirmed that malagueta pepper is mainly composed by branched saturated esters (Bogusz et al., 2015). Different accessions of C. frutescens from Laos (Laotian), Spain (pebrera) and United States (tabasco) were characterized by high levels and diverse patterns of esters, mostly branched saturated esters corresponding to 2-methylpropanoate, 2-methylbutanoate, 3-methylbutanoate, and 4-methylpentanoate subgroups (Rodríguez-Burruezo et al., 2010). The ester group was also the largest, both in quantity and variety of compounds, in samples from three maturing stages of Shimatogarashi pepper (Manikharda et al., 2018). However, authors reported that during ripening, the ester content of this C. frutescens cultivar

Table 1. Active and non-active odor compounds identified in C. frutescens pepper cv. BRS Avai tabasco pepper.

<table>
<thead>
<tr>
<th>Peak</th>
<th>KI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Compound&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Area (%)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Odor description</th>
<th>Odor Impact (%)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Lit.&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>a</td>
<td>&lt; 700</td>
<td>Nd</td>
<td>-</td>
<td>sulphurous</td>
<td>31.2</td>
<td>4,7</td>
</tr>
<tr>
<td>b</td>
<td>&lt; 700</td>
<td>Nd</td>
<td>-</td>
<td>sweet, herbal</td>
<td>33.2</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>746</td>
<td>Nd</td>
<td>-</td>
<td>sweet, floral, cinnamon</td>
<td>31.0</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>811</td>
<td>Nd</td>
<td>-</td>
<td>green, herbal</td>
<td>40.2</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>853</td>
<td>Nd</td>
<td>-</td>
<td>green, leafy</td>
<td>29.9</td>
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</tr>
<tr>
<td>f</td>
<td>864</td>
<td>Nd</td>
<td>-</td>
<td>fruity, sweet</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>898</td>
<td>Nd</td>
<td>-</td>
<td>sweet, floral</td>
<td>45.2</td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>908</td>
<td>Nd</td>
<td>-</td>
<td>sweet, green</td>
<td>45.2</td>
<td></td>
</tr>
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<td>l</td>
<td>956</td>
<td>α-pinene</td>
<td>0.06</td>
<td>sweet, herbal</td>
<td>63.4</td>
<td>4,7</td>
</tr>
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<td>i</td>
<td>976</td>
<td>Nd</td>
<td>-</td>
<td>pepper; earth</td>
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</tr>
<tr>
<td>j</td>
<td>988</td>
<td>Nd</td>
<td>-</td>
<td>plastic; solvent</td>
<td>47.7</td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>999</td>
<td>Nd</td>
<td>-</td>
<td>solvent, rust</td>
<td>37.8</td>
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<tr>
<td>l</td>
<td>1009</td>
<td>Nd</td>
<td>-</td>
<td>malagueta pepper</td>
<td>19.2</td>
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</tr>
<tr>
<td>m</td>
<td>1017</td>
<td>Nd</td>
<td>-</td>
<td>plastic, solvent</td>
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</tr>
<tr>
<td>n</td>
<td>1046</td>
<td>Nd</td>
<td>-</td>
<td>sweet, floral</td>
<td>47.1</td>
<td></td>
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<tr>
<td>o</td>
<td>1054</td>
<td>Nd</td>
<td>-</td>
<td>green, pepper</td>
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<td>p</td>
<td>1057</td>
<td>Nd</td>
<td>-</td>
<td>Pentatomidae bug</td>
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</tr>
<tr>
<td>q</td>
<td>1064</td>
<td>Nd</td>
<td>-</td>
<td>pepper, green</td>
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<tr>
<td>r</td>
<td>1091</td>
<td>Nd</td>
<td>-</td>
<td>pepper, green</td>
<td>43.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1102</td>
<td>3-methylbutyl 2-methylbutanoate</td>
<td>0.10</td>
<td>pepper</td>
<td>46.4</td>
<td>1,47</td>
</tr>
<tr>
<td>3</td>
<td>1105</td>
<td>3,5-dimethyl-2-octanone</td>
<td>0.21</td>
<td></td>
<td>-</td>
<td></td>
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<td>4</td>
<td>1110</td>
<td>3-methylbutyl 3-methylbutanoate</td>
<td>0.15</td>
<td>pepper, oily, solvent</td>
<td>33.7</td>
<td>1,47</td>
</tr>
<tr>
<td>5</td>
<td>1115</td>
<td>4-methylpentyl 2-methylpropanoate</td>
<td>4.80</td>
<td>green, fruity</td>
<td>37.4</td>
<td>4,6,7</td>
</tr>
<tr>
<td>6</td>
<td>1153</td>
<td>hexyl 2-methylpropanoate</td>
<td>0.18</td>
<td>floral, grass</td>
<td>32.9</td>
<td>1,2,3,4,5,7</td>
</tr>
<tr>
<td>7</td>
<td>1162</td>
<td>hexyl butanoate</td>
<td>0.28</td>
<td>green, sweet, floral</td>
<td>55.8</td>
<td>1,2,3,4,5,7</td>
</tr>
<tr>
<td>s</td>
<td>1166</td>
<td>Nd</td>
<td>-</td>
<td>green, floral</td>
<td>25.4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1201</td>
<td>4-methylpentyl 2-methylbutanoate</td>
<td>14.85</td>
<td>green, herbal</td>
<td>76.3</td>
<td>4,6,7</td>
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<td>9</td>
<td>1208</td>
<td>4-methylpentyl 3-methylbutanoate</td>
<td>15.80</td>
<td>pepper, green, woody</td>
<td>51.1</td>
<td>4,6,7</td>
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<tr>
<td>10</td>
<td>1220</td>
<td>3-methylbutyl hexanoate</td>
<td>0.25</td>
<td>mint, pungent, sour</td>
<td>36.0</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Kovats retention index in DB-5-MS column; <sup>b</sup>Tentative identification (only by matching KIs and mass spectra from libraries); <sup>c</sup>Area percent from FID chromatogram; <sup>d</sup>Odor Impact percent, calculated as geometric mean of intensity and frequency for each perceived stimulus; <sup>e</sup>References in which the compound was reported for C. frutescens peppers: (1) Bogusz et al. (2015), (2) Bogusz et al. (2012), (3) Bogusz et al. (2011), (4) Rodríguez-Burruezo et al. (2010), (5) Liu et al. (2009), (6) Da Costa et al. (2012), (7) Manikharda et al. (2018); nd, not detected by GC-MS.
from Japan decreased (88.3% in green peppers to 81.8% in red peppers), while terpene content increased (from 2.3% to 7.3%). Major compounds in the red stage fruits were 4-methylpentyl 5-methylpentanoate, 4-methylpentyl 2-methylbutanoate, 4-methylpentyl 3-methylbutanoate, 4-methylpentyl-2-methylpropanoate, and 4-methylpentyl pentanoate.

The alkane group detected in cv. BRS Avai tabasco pepper comprised aliphatic and 2-methyl branched alkanes (C13 to C17). Most of these same compounds were also found in the *C. frutescens* domesticated in China and reported by Liu et al. (2009), in Brazilian malagueta pepper (Bogusz et al., 2015), and in Japanese Shimatogarashi pepper (Manikharda et al., 2018); nd, not detected by GC-MS.

Only three compounds were perceived with high odor impact: 4-methylpentyl 2-methylbutanoate (peak 8), described as herbal notes; a not-detected compound (peak q), described as herbal and pepper-like odors; and α-pinene (peak 1), described as sweet and herbal scents. Da Costa et al. (2012) also found 4-methylpentyl 2-methylbutanoate to be the most abundant odor-active volatile in red malagueta pepper, followed by other isohexyl esters, mainly 4-methylpentyl 3-methylbutanoate and 4-methylpentyl 2-methylpropanoate. However, compounds 4-methylpentyl 2-methylbutanoate and α-pinene have been detected in *C. annuum*, *C. chinense* and *C. frutescens* accessions without showing sensory importance in the CG-olfactometry study performed by Rodríguez-Burruezo et al. (2010). Instead, according to these authors, the highest sensory impressions corresponded to ethyl 4-methylpentanoate, followed by 3-isobutyl-
Aroma compounds of a new Brazilian tabasco pepper cultivar

According to the authors, the high content of esters in tabasco pepper lead to a different sweet-fruity impression from that of Laotian and Pekrera *C. frutescens* fruits, which presented floral-fruity smell along with paprika-like notes, given by ionones and the bell pepper pyrazine, respectively.

Another 10 odorants contribute to the typical aroma of *C. frutescens* pepper cv. BRS Avai with low impact (OI% < 30), mostly described as green, fruity, sweet, floral and pepper notes, indicating that their contribution may be sensorially less important. Among them are (Z)-3-hexenyl 3-methylbutanoate, heptyl 2-methylbutanoate, 3-methylbutyl 3-methylbutanoate and heptyl hexanoate (peak 26). Especially important were compounds 3-methylbutyl 2-methylbutanoate, 3-methylbutyl 3-methylbutanoate and heptyl 3-methylbutanoate that presented pepper-like aroma, as well as heptyl butanoate, scoring almost 60% for its odor impact. These compounds were also identified as OCV in the volatile fraction of *Capsicum chinense* peppers (Biquinho, BRS Seriema and CNPH 4080), described as fruity notes (Garruti et al., 2013).

Several esters, mainly ethyl 2-/3-methylbutanoate, hexyl 2-/3-methylbutanoate, and 4-methylpentyl 4-methylpentanoate were perceived with low intensity during sniffing analyses of the tabasco accession studied by Rodríguez-Burruezo et al. (2010), with odor impressions described as sweet and/or fruity. According to the authors, the high content of esters in tabasco pepper lead to a different sweet-fruity impression from that of Laotian and Pekrera *C. frutescens* fruits, which presented floral-fruity smell along with paprika-like notes, given by ionones and the bell pepper pyrazine, respectively.

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guiding breeding programs into developing new cultivars with better OCV patterns.

4 Conclusions

This study revealed the potent odorants responsible for the overall aroma of the Brazilian tabasco pepper cv. BRS Avai by the application of GC-O time-intensity technique. The volatile profile of this genotype is composed predominantly by esters (23), with isoxygen esters as very important compounds. Isohexyl 2-methylbutanoate, α-pinene, hexyl butanoate and isoxygen 3-methylbutanoate are the most powerful volatiles, contributing the most to the typical sweet, herbal and pepper-like aroma of this cultivar. Many compounds that were not detected by the instruments are also important and should be further investigated.

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References


