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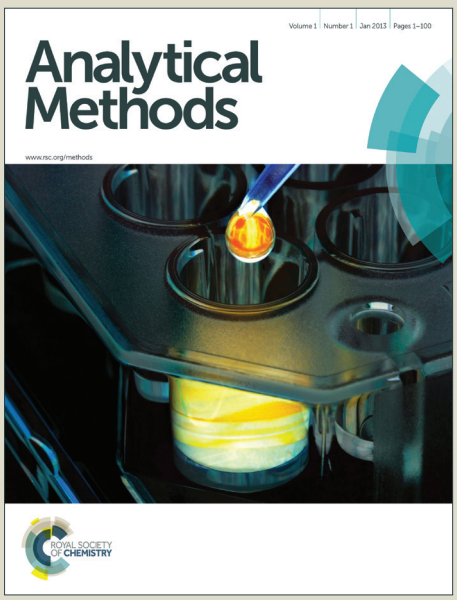

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Analysis of volatile compounds in *Capsicum* spp. by headspace solid-phase microextraction and GC×GC-TOFMS

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Abstract

A suitable method based on headspace solid-phase microextraction (HS-SPME), comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry detector (GC × GC-TOFMS) and chemometric approach was used aiming the investigation of the volatile fraction of Brazilian *Capsicum* peppers: malagueta (*C. frutescens)*, dedo-de-moça (*C. baccatum*) and murupi (*C. chinense)*. A total of 184 volatiles compounds were identified in the three pepper samples and 123 of these compounds were first described in Brazilian peppers. In addition, during Brazilian chili peppers maturation, as maturation time increases, it was noted that the majority of volatile compounds responsible for green odor notes disappeared being replaced to others whit fruity notes. The chemometric analysis (PCA) was able to separate samples according to their constituents, were malagueta was characterized by branched esters, murupi by terpenes and dedo-de-moça by the presence of aldehydes and terpenes.

Keywords: Comprehensive two-dimensional gas chromatography, *Capsicum frutescens* L.*, C. chinense* Jacq.*, C. baccatum* (Willd) Eshbaugh, volatile compounds, principal component analysis.

1. Introduction

Peppers from the genus *Capsicum* are very popular spices in various parts of the world, mainly due to their attributes of color, pungency and aroma. [1, 2] They can be consumed as fresh, dried, preserved or in pepper sauces. Industrially, they are used as coloring and flavor agents in a variety of types of food. [3] As a consequence of the biochemical processes that occur during maturation, differences in the flavor of *Capsicum* chili pepper can be perceived as a result of the degree of maturation. [4]

Although pungency is one of the most important attributes of *Capsicum* fruits, previous studies analyzed the pepper volatile fraction, since the chemical compounds present in this fraction directly affect the flavor. [5, 6] Moreover, the perception of the complex volatile mixture of the compounds is an important part of the consumers selection criteria for the acceptance of food. [12] Furthermore, the knowledge of volatile composition is an important tool for differentiating between the *Capsicum* types and also to establish measures for authenticity, quality control, guaranty of authenticity, fraud prevention and assurance of origin. [21] In addition, the food industry has an interest in obtaining concentrated aroma of peppers for flavored products without necessarily giving pungency to food. [7]

In the studies of peppers volatile fractions different extraction methods have been used, such as simultaneous steam distillation-solvent extraction (SDE), purge and trap and solid phase micro-extraction (SPME), but independent of the extraction method, gas chromatography (GC) coupled to mass spectrometry (MS) was the standard instrumental methodology used to investigate the volatile fraction. [7, 8, 9] However, even the most modern GC system is not able to separate all the compounds in the volatile fraction of complex samples as peppers. Then, it is common to have non-identified peaks on the chromatograms, those peaks commonly are constituted by two or three coeluted compounds. An alternative to the analysis of complex samples is the comprehensive two-dimensional gas chromatography (GC × GC), a powerful separation technique, whose resolving power is higher than that of conventional chromatography because the analytes in GC × GC are separated with the use of two sequential chromatographic columns with different separation characteristics. [22, 23] The entire effluent from the first column is reinjected in the second by the modulator which, by

focusing effect, decreases the peak width and increases the height of each peak, which increases analytical sensitivity.[10]

Following the former investigations on the chemistry of the volatiles in *Capsicum* [11, 12], this study aimed the development of a GC × GC methodology suitable for the characterization of the volatile fraction of malagueta (*C. frutescens*), dedo-de-moça (*C. bacccatum*) and murupi (*C. chinense*) Brazilian pepper varieties. Static headspace solid-phase microextraction (HS-SPME) and GC × GC-TOFMS were used to identify the characteristic compounds of each variety and assess the volatile compounds that could differentiate these samples. In order to extract the maximum information from data, Principal Component Analysis was employed to search for relationship among samples and variables.

2. Material and methods

2.1 Materials

The SPME fiber used was a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) from Supelco (Bellefonte, PA, USA). The gas chromatography columns tested in this study are: DB5 – (5%-phenyl)-methylpolysiloxane, DB1 – 100% dimethylpolysiloxane, DB-17 – (50%-phenyl)-methylpolysiloxane, DB-WAX – polyethylene glycol purchased from Agilent Technologies (Wilmington, DE, USA) and RT-LC50 (dimethyl [50% liquid crystal] polysiloxane) purchased from Restek Corporation, (Bellefonte, PA, USA).

2.2 Samples

Approximately 2 kg of samples, namely, malagueta, dedo-de-moça, and murupi pepper were botanically identified and supplied by the Campinas Agronomic Institute

(IAC, Campinas, SP, Brazil). The genotypes selected for the study were obtained from the germplasm bank of the Horticultural Center of the IAC. The plants were cultivated in 2009 from January to May, using similar fertilizer and irrigation treatments. They were harvested at two maturity stages: the physiological stage (maximum size development, but still immature) and the commercial stage (complete development of both size and color). The peppers were harvested in the morning and immediately transported to the laboratory in order to be analyzed. The period between harvest and analysis was no longer than 48 hours.

2.3 Samples Preparation

The volatiles were extracted by HS-SPME according to previously published procedure. [11, 12] Whole pepper fruits were grounded in a blender in 100 g batches, and 1.00 g aliquots of the ground material were weighed into 15.0 mL SPME flasks with screw tops and PTFE/silicon septa (Supelco - Bellefonte, PA, USA). The fiber was prepared before use according to the manufacturer instructions. The volatiles extraction conditions were: equilibration for 15 min followed by extraction during 80 minutes at 40 ºC. After extraction, the fiber was placed in the gas chromatograph injector and the analytes were desorbed in the split mode (1:20) at 250 ºC for 1.0 minute. After each extraction and desorption procedure, the fiber was reconditioned during 15 minutes at 250 ºC, this additional procedure was employed to eliminate analyte carry over between extractions. All of the samples were analyzed in triplicate and the results are mean values.

2.4 GC × GC analysis

The two-dimensional chromatographic analyses were carried out in the Comprehensive Two-dimensional Gas Chromatography Multi-User Nucleus of the Institute of Chemistry of the Federal University of Rio Grande do Sul (UFRGS), Brazil.

In the beginning, a comprehensive two-dimensional gas chromatography system coupled to a flame-ionization detector (GC × GC-FID) was used to analyze the pepper volatiles in order to find out the best chromatographic and injection conditions. The chromatograph used was the GC × GC-FID HP6890 (Agilent Technology, USA), equipped with a LECO cryogenic modulator (LECO Corporation, St. Joseph, MI, USA). The capillary column sets used to optimize the chromatographic separation are listed in Table 1.

The GC × GC-FID operational parameters were: injector at 250 ºC; temperature programming for the primary and secondary ovens: 40 ºC (0.2 min), with increments of 3 ºC min^{-1} to 230 ºC, remaining at this temperature for 4 min; carrier gas (hydrogen) at a constant flow rate of 1.0 mL min^{-1} and detector at 250 ºC. In order to optimize the chromatographic conditions it was tested: modulation periods of 4, 5, 6 and 8 seconds, and split ratios of 1:10; 1:20; 1:30 and 1:50.

The operational parameters used in the GC × GC-TOFMS were set as being injector temperature of 250 ºC and split ratio of 1:20; column set DB-5 (30 m x 0.25 mm i.d. x 0.25 µm stationary phase) and DB-WAX (2.60 m x 0.1 mm i.d. x 0.1 µm stationary phase); temperature gradient in the primary oven of 40 ºC (0.2 min), with increments of 3 ºC min^{-1} to 230 ºC, remaining at this temperature during 4 min; secondary oven 45 ºC (0.2 min), with increments of 3 ºC min^{-1} to 235 ºC, remaining at this temperature during 4 min; carrier gas (helium) at a constant flow rate of 1.3 mL min^{-1}; modulation period of 6 s; interface at 240 ºC; electron ionization source at 200 ºC electronic ionization at +70 eV; detector at 100 Hz, monitoring the mass range from 40-400 m/z and a multi-channel plate voltage of 1.7 kV. The identification criteria considered for the volatile compounds present in the *Capsicum* samples were minimum similarity search of 80% and LTPRI filtering (± 5 units). [13]

2.5 Statistical analysis

The Principal Components Analysis was carried out by using the Statistica v. 7 software (Statsoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1 Analysis of the Brazilian *Capsicum* pepper volatiles by GC × GC-TOFMS

After establishing the operational parameters for the GC × GC-FID system, the analysis was carried out in the GC × GC-TOFMS. Figure 1 demonstrates the GC × GC-TOFMS chromatogram for the immature malagueta pepper sample, highlighting the main chemical classes found in the sample. These classes were found grouped or structured in specific regions, since chromatographic structure is a result of the organization between compounds belonging to the same chemical class or group found in the same chromatographic separation space. The importance of such structuring is the possibility of applying the same chromatographic method to other similar samples, where compounds belonging to a specific chemical class trends to keep its positions in the same place at the structure. [10, 13, 14, 15]

Table 2 illustrates the results obtained in the identification of the volatile compounds from malagueta, murupi and dedo-de-moça peppers in ripe and unripe maturation states by HS-SPME and GC × GC-TOFMS. Considering all the samples analyzed, an amount of 184 volatiles were identified and grouped according to the following chemical classes: alkanes (26), alcohols (20), aldehydes (17), ketones (8), esters (68), ethers (3), terpenes (40) pyrazine (1) and sulfur compound (1).

A large number of esters and terpenes were detected in the chili peppers, which was expected since it has been previously reported in literature. [17, 18] Methyl and ethyl esters provide strong fruity notes in foods whereas terpenes provide wood, floral, fruity and spices notes. [12, 17, 18] Aldehydes are also especially important because of its low odor threshold described in sniffing analyzes of *Capsicum* as green, cucumber, pungent or herbaceous odor notes. [17, 18] On the other hand, alcohols have a higher odor threshold when compared to aldehydes and so their importance for the food aroma is relatively less to aldehydes.[12] Short-chain ketones, especially methyl ketones have powerful aroma, while pyrazines are considered strong odor compounds in *Capsicum* (odor notes of paprika and green). [17] Finally, the presence of aliphatic, aromatic and branched alkanes in *Capsicum* peppers has been reported in the literature related to capsaicins biosynthesis and degradation processes of carotenoids. [18]

For all peppers investigated in this research, the ripening process decreases the amount of aldehydes, which may explain the more pleasant and attractive aroma that exhibited the ripped *Capsicum* peppers. Additionally, the biosynthesis of *Capsicum* esters is described in literature as being directly related to the quantity and availability of the alcohols present in the sample. [18] In the maturation process of Brazilian peppers, the number of alcohols decreased in all of the samples and, in some cases, this reduction occurred by giving place to the appearance of new esters like heptyl hexanoate, (E)-3-hexenyl butyrate, 3-methyl butanoate, (Z)-3-hexenyl isopentanoate, methyl methacrylate and ethyl propionate, presents only in ripe peppers. Moreover, in this study pyrazines were found in all of the pepper samples and in all maturation stages.

The different analyzed peppers displayed characteristic compounds, identified by upper case letters in Table 2. For malagueta pepper, 104 compounds were identified in which 43 were only detected in this sample. These characteristic compounds consist

mainly of esters (total of 18) and alcohols (9). Whereas a total of 103 compounds were identified in murupi peppers, in which 40 were only detected in this sample. The characteristic compounds were referred as being mainly constituted of terpenes (14) and esters (13). Furthermore, in dedo-de-moça peppers it was detected and identified 68 compounds, in which 28 were exclusive to this sample. The characteristic compounds consisted mainly of terpenes (9) and aldehydes (9). In this way, it was identified 123 compounds, described for the first time in Brazilian peppers.

By comparing these GC × GC results with those obtained by using one-dimensional gas chromatography (GC-MS) for *Capsicum* peppers from Brazil, in the first case, a greater number of volatiles were identified probably due to the high resolving power of GC × GC. As an example, when were used one-dimensional chromatography only 83 volatiles were identified in malagueta, 77 in murupi and 49 in dedo-de-moça pepper. [13] To better understand, the similarities among the different pepper species, the standard recognizing method of Principal Component Analysis was employed.

3.2 Principal components analysis

Principal Component Analysis is a method mainly used to describe samples present in an n-dimensional space order for pattern recognition and is able to extract the relevant information from a given data set of a multivariate nature to aid in understanding the model. PCA has been reported in the literature to describe various problems involving food and agricultural matrices. [24] This is an unsupervised exploratory technique which reduces the dimensions of an initial multivariate dataset to a smaller number of uncorrelated variables with maximized variance, i.e., that permits the analysis of a dataset using the most important variables. Multivariate data analysis

methods, either supervised or unsupervised, used to reduce the dimensionality of multivariate dataset and provide aid to identify differences or similarities among the samples. PCA is a primary tool among the various multivariate data analysis methods. It is an unsupervised method and samples are clustered or separated due to similarities among profiles. [25]

In this research, thirty volatile compounds which were common to more than one pepper species and provides important odors notes to the peppers were evaluated by Principal Components Analysis. To this, peak area was used and the PCA method was made with mean center preprocess. The objective was determining the similarities and differences of these volatiles in those peppers. Figures 2 and 3 shows the sample scores and loadings plots, respectively, regarding on the two first principal components which captured 69.7% of the total variance explained.

The malagueta pepper samples were separated by the negative part of the Factor 2 in the scores plot. In accordance with the loadings plot, malagueta pepper were mainly characterized by compounds arising from the degradation of amino acids, such as the branched esters methyl hexanoate, iso-amyl isoisobutyrate and hexyl isovalerate (fruit, aromas) [7, 17] and by products formed from the degradation of fatty acids such as the aliphatic ester hexyl butyrate and hexyl hexanoate (with fruity notes). [17, 18] Compounds as alcohols, aldehydes, ketones and furan were also responsible for the separation of these samples e.g. 2-methylbutanal, 3-methylbutanal (green, almond, burnt, malty notes), 1-penten-3-one (pungent) and 2-pentylfuran (buttery, green bean-like).

The scores plot shows that murupi pepper was separated trough the positive part of Factors 1 and 2 and it was characterized, according to Figure 3, mainly by products from the terpenoid pathway, such sesquiterpenes copaene (woody) and δ-cadinene (green, sweet) [19], and also by products from amino acid degradation such as the

aromatic ester methyl salicylate (sweet) and the methyl esters as isopentyl isovalerate (with fruity aromas) [17].

On the other hand, the dedo-de-moça pepper was located at the positive part of Factor 2 and negative part of Factor 1 in the scores plot. The loadings plot shows that dedo-de-moça pepper was characterized by the presence of products derived from fatty acids, such as the aldehydes (E)-2-hexanal (apple-like, fruity, green), hexanal (grass, tallow, fat) and the aliphatic ester ethyl acetate (fruity) and compounds derived from amino acid degradation such as 2-isobutyl-3-methoxypyrazine (green peppers). [17, 19, 20]

4. CONCLUSIONS

The methodology developed for comprehensive two-dimensional gas chromatography and PCA was successfully applied to the analysis of the volatile fractions from malagueta, murupi and dedo-de-moça peppers in two stages of maturation. An amount of 184 volatile compounds was identified, being 123 of it described for the first time in Brazilian peppers. The Principal Components Analysis indicated that malagueta pepper is mainly described by compounds from branched ester group, while murupi peppers are considered sesquiterpenes abundant and the dedo-de-moça peppers characterized mainly by the presence of aldehydes and methoxypyrazine.

5. REFERENCES

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Figure captions

Figure 1: Chromatogram of the volatile compounds from immature malagueta peppers obtained by HS-SPME and GC × GC-TOFMS, with the main chemical classes respective structuring.

Figure 2: Scores plot regarding on the first two principal components obtained from PCA of the volatile compounds of malagueta, murupi and dedo-de-moça peppers.

Figure 3: Loadings plot regarding on the first two principal components obtained from PCA of the volatile compounds of malagueta, murupi and dedo-de-moça peppers.

Table 1: Column sets used in the GC x GC-FID system.

Set	Phase	1D – dimension	Phase	2D – dimension
1	DB5	30m x 0.25mm i.d. x 0.25µm	DB-WAX	2.60m x 0.1mm i.d. x 0.1µm
2	DB5	30m x 0.25mm i.d. x 0.25µm	DB-17	2.60m x 0.1mm i.d. x 0.1µm
3	DB5	30m x 0.25mm i.d. x 0.25µm	RT-LC50	2.60m x 0.1mm i.d. x 0.1µm
4	DB-WAX	30m x 0.25mm i.d. x 0.25µm	DB-17	2.60m x 0.1mm i.d. x 0.1µm
5	DB-WAX	30m x 0.25mm i.d. x 0.25µm	DB1	1.1m x 0.15mm i.d. x 0.1µm

DB5 – (5%-phenyl)-methylpolysiloxane
DB1 – 100% dimethylpolysiloxane
DB-17 – (50%-phenyl)-methylpolysiloxane
DB-WAX – polyethylene glycol
RT-LC50 (dimethyl [50% liquid crystal] polysiloxane)

Table 2: Volatile compounds from *Capsicum* peppers in two ripening stages and obtained by HS-SPME and GC × GC-TOFMS. Analytical conditions in the text. A: unripe malagueta pepper (*C. frutescens*); B: ripe malagueta pepper (*C. frutescens*); C: unripe murupi pepper (*C. chinense)*; D: ripe murupi pepper (*C. chinense)*; E: unripe dedo-de-moça pepper (*C. baccatum* var. *pendulum*); F: ripe dedo-de-moça pepper (*C. baccatum* var. *pendulum*).

Name	SI	LTPRI cal.	LTPRI lit.	Δ	A	B	C	D	E	F
Alkanes										
heptane [c]	909	701	700	1					x	
(E)-2-methyl-3-octene [b]	906	851					x	x		
ethylbenzene [a]	926	863	864	-1	x					
4-ethyl-2,3-dimethylhex-2-ene [b]	801	995						x		
2,7-dimethyl-1,7-octadiene [a]	807	1021			x					
3-hexyl-1,1-dimethylcyclopentane [b]	880	1247					x	x		
2-methyl-dodecane	870	1265	1266	-1	x	x	x			
tridecane	905	1301	1300	1	x	x	x	x	x	
(E)-3-methyldec-4-ene [b]	813	1345					x	x		
3,7-dimethylocta-1,6-diene [b]	833	1354					x	x		
2-methyl-1-tridecene [a]	885	1354			x					
(E)-6-tetradecene [b]	900	1354					x	x		
2-methyl-tridecane	923	1365	1365	0	x	x	x	x	x	x
3-methyl-tridecane	876	1373	1371	2			x	x	x	x
(E)-5-tetradecene [a]	923	1386	1387	-1	x	x				
(E)-4-tetradecene	904	1393					x			x
tetradecane	952	1401	1400	1	x	x	x	x	x	x
2-methyl-1-tetradecene	906	1447	1445	2	x	x	x	x	x	x
(E)-5-tridecene	892	1483						x	x	x
pentadecane	928	1501	1500	1	x	x	x	x	x	x
2-methyl-pentadecane	923	1565	1564	1	x	x	x	x		
3-methyl-pentadecane	871	1571	1570	1	x	x	x	x	x	x
hexadecane	941	1601	1600	1	x	x	x	x		
2-methyl-hexadecane [a]	895	1665	1666	-1	x	x				
8-heptadecene [a]	901	1681	1677	4	x					
heptadecane [a]	929	1701	1700	1	x	x				
Alcohols										
3-methyl-2-butanol	908	693	692	1	x	x	x			
1-penten-3-ol	858	694	694	0	x	x	x		x	
1-pentanol [c]	926	767	768	-1					x	
4-methyl-1-pentanol	951	837	838	-1	x	x	x	x		
(Z)-3-hexen-1-ol [a]	947	858	860	-2	x	x				
1-hexanol	877	870	865	5	x	x	x		x	
3-methyl-1-pentanol [b]	884	870	872	-2			x			

2-heptanol [a]	895	903	900	3	x	x				
6-methyl-2-heptanol [a]	855	968			x					
1-octen-3-ol [c]	844	981	983	-2					x	
(R)-2-octanol [a]	916	1003	998	5	x	x				
5-methyl-5-octen-2-ol [a]	816	1050			x	x				
3-ethyl-2-heptanol [a]	844	1081			x					
5,5-dimethyl-cyclohex-3-en-1-ol [b]	801	1195					x			
2-nonanol [a]	886	1103	1100	3	x	x				
2-decanol [b]	828	1182	1186	-4			x	x		
3-cyclohexene-1-ethanol [a]	813	1281			x					
3-cyclopentyl-1-propanol [b]	831	1343					x	x		
3,3-dimethyl-cyclohexanol [b]	845	1381					x	x		
(E)-2-hexadecacen-1-ol [a]	890	1483			x	x				
Aldehydes										
3-methyl-butanal	871	675	671	4	x				x	x
2-methyl-butanal	867	680	677	3	x		x		x	x
pentanal [c]	883	702	698	4					x	
hexanal	890	802	802	0	x	x		x	x	x
2-hexenal [c]	877	846	841	5					x	x
(E)-2-hexenal	939	854	853	1	x	x	x		x	
heptanal [c]	922	903	901	2					x	
(Z)-2-heptenal [c]	923	959	956	3					x	
(E,E)-2,4-heptadienal	811	1001	1000	1	x				x	
2-ethyl hexanal [a]	860	1041			x					
2-phenylacetaldehyde [a]	835	1053	1052	1	x					
(E)-2-octenal [c]	923	1061	1060	1					x	
(Z)-dec-7-enal [b]	822	1156					x	x		
(E,Z)-2,6-nonadienal [c]	875	1156	1155	1					x	
(E)-2-nonenal [c]	934	1163	1161	2					x	
2,4-decadienal [c]	898	1299	1297	2					x	
pentadecanal [c]	922	1718	1717	1						x
Ketones										
3-methyl-2-butanone [a]	801	677	673	4	x					
1-penten-3-one	896	696	691	5	x	x	x	x	x	x
2,3-pentanedione [b]	871	702	696	6			x			
2-heptanone [c]	837	891	887	4					x	
1-octen-3-one [c]	846	979	975	4					x	
2-nonanone	916	1092	1089	3	x	x			x	
2-decanone [a]	914	1193	1191	2	x	x				
2-undecanone [a]	843	1259	1257	2	x	x				
Esters										
ethyl acetate	941	614	610	4	x	x	x	x	x	x
methyl methacrylate [c]	884	712	710	2						x
ethyl propionate [c]	905	714	711	3						x
methyl isovalerate [a]	929	775	770	5	x	x				
methyl 2-methylbutanoate	884	775	771	4	x				x	

methyl pentanoate [a]	940	824	821	3	x	x			
methyl 3-methyl-2-butenoate [c]	951	844	842	2					x
ethyl 2-methylbutanoate [a]	914	848	846	2	x				
ethyl pentanoate [a]	908	901	898	3	x				
2-pentyl propionate [a]	820	918	916	2	x				
methyl hexanoate	911	924	922	2	x	x			x
α-methylbutyl isobutyrate [a]	837	962	958	4	x				
ethyl 4-methylpentanoate [a]	875	964	963	1	x	x			
hexyl acetate	817	977	972	5	x		x	x	
ethyl hexanoate [a]	892	998	996	2	x				
isobutyl 2-methylbutyrate [b]	915	1003	1002	1			x		
(Z)-3-hexenyl acetate [b]	898	1005	1001	4			x		
iso-butyl isovalerate [b]	940	1006	1003	3			x	x	
iso-amyl iso-butyrate	950	1011	1007	4	x	x	x	x	x
butyl 2-methylbutanoate [a]	894	1041	1039	2	x				
pentyl isobutyrate	942	1049	1047	2	x	x	x	x	
4-methylhexyl acetate [b]	821	1082					x	x	
methyl 6-methyl heptanoate [a]	838	1087			x				
pentyl butyrate	944	1093	1092	1	x		x	x	
isopentyl 2-methylbutanoate	895	1100	1099	1	x	x	x	x	
methyl benzoate [c]	891	1101	1096	5					x
isopentyl isovalerate	920	1106	1105	1	x	x	x	x	x
heptyl acetate [b]	879	1112	1110	2			x	x	
3-methyl butanoate [b]	856	1117	1116	1				x	
methyl octanoate [a]	884	1123	1120	3	x				
hexyl 2-methyl-2-propenoate [a]	839	1135			x				
(E)-3-hexenyl butyrate	883	1139				x	x	x	
pentyl 2-methylbutyrate	905	1139			x	x	x	x	
hexyl isobutyrate	926	1142	1139	3	x	x	x	x	
(Z)-3-hexenyl butyrate	899	1147	1142	5	x	x	x	x	
3-methyl-3-butenyl 3-methylbutanoate	877	1147			x	x	x		
3-methyl-2-butenyl pentanoate [b]	825	1149					x	x	
isopentyl pentanoate [a]	917	1154	1152	2	x				
hexyl butyrate	870	1191	1188	3	x	x	x	x	x
octanoic acid, ethyl ester [a]	853	1196	1194	2	x				
methyl salicylate	926	1202	1201	1	x	x	x	x	x
(Z)-3-hexenyl isopentanoate	879	1235	1235	0				x	x
hexyl 2-methylbutanoate	939	1237	1234	3	x	x	x	x	
hexyl isovalerate	871	1241	1240	1	x	x	x	x	x
(E)-2-hexenyl pentanoate [b]	838	1246	1243	3			x	x	
isopentyl hexanoate	921	1251	1250	1	x				x
hexyl pentanoate	819	1252	1247	5	x	x	x		
hexyl 3-methyl-2-butenoate	858	1287			x	x	x	x	
heptyl pivalate	846	1298			x	x	x	x	
benzyl isobutyrate	820	1301			x		x		
7-ethoxy-3-heptene [b]	822	1318					x	x	

heptyl 2-methylbutanoate	869	1335	1332	3	x	x	x	x		
heptyl pentanoate [a]	806	1341			x	x				
(Z)-3-hexenylpyruvate	851	1343			x	x	x	x		
(E)-4-hexenyl hexanoate [c]	839	1347							x	
hexyl hexanoate	827	1352	1348	4	x	x	x	x	x	x
(Z)-3-hexenyl hexanoate	890	1381	1379	2	x		x	x		
benzyl 3-methylbutanoate	892	1391	1387	4	x	x	x	x		
octyl pivalate [b]	814	1398					x			
octyl pentanoate [b]	819	1403					x	x		
octyl 2-methylbutanoate [b]	879	1433	1430	3			x	x		
octyl isopentanoate [b]	876	1440	1434	6			x	x		
n-heptyl hexanoate [a]	813	1450	1448	2		x				
(Z)-3-decenyl acetate [a]	826	1474			x					
phenylethyl pivalate [b]	835	1497					x	x		
benzyl hexanoate	810	1516			x		x			
benzoic acid n-hexyl ester [b]	873	1549	1545	4			x	x		
hexyl benzoate [a]	847	1550	1549	1	x	x				
Ether										
2-ethyl-furan	940	702	702		x	x			x	
2-pentyl-furan	880	991	990	1	x	x	x	x	x	x
(Z)-1-ethoxy-4-methylpent-2-ene	803	1244			x	x	x			
Pyrazine										
2-isobutyl-3-methoxypyrazine	874	1180	1180	0	x	x	x	x	x	x
Sulfur										
2-pentyl-thiophene	905	1166	1164	2	x	x			x	
Terpenes										
α-pinene	879	926	925	1				x	x	
α-tricyclene	894	926	923	3	x				x	x
(R)-α-pinene [c]	921	937	933	4					x	
camphene [c]	937	955	952	3					x	x
sabinene [c]	875	977	973	4					x	
β-myrcene [a]	843	990	988	2	x	x				
β-pinene [c]	878	990	985	5					x	x
α-phellanderene [c]	854	1009	1004	5					x	
o-cymene [c]	931	1029	1028	1					x	
limonene	911	1032	1031	1	x	x			x	x
eucalyptol [c]	920	1037	1035	2					x	x
(Z)-β-ocimene [a]	822	1047	1045	2	x	x				
γ-terpinolene [c]	882	1090	1089	1					x	
terpinen-4-ol [c]	804	1189	1184	5					x	
β-cyclocitral [a]	847	1227	1224	3	x	x				
α-cubenene [b]	908	1356	1352	4			x	x		
α-longipinene	866	1364	1360	4	x	x	x	x		
ylangene	869	1379	1375	4	x	x	x	x		
copaene	889	1386	1382	4	x	x	x	x	x	
β-cubebene [b]	904	1398	1394	4			x	x		

α-ionone [a]	851	1428	1427	1	x	x			
(E)-α-ionone [b]	860	1430	1428	2			x	x	
caryophyllene	911	1434	1432	2			x	x	x
germacrene D [b]	845	1443	1442	1			x		
aromadendrene [b]	800	1452	1451	1			x		
(E)-β-farnesene	871	1456	1454	2	x	x	x	x	
α-caryophyllene	922	1470	1467	3			x	x	x
(-)-germacrene D	866	1474	1470	4			x	x	x
9-epi-β-caryophyllene	879	1482	1477	5	x	x	x	x	
γ-muurolene [b]	914	1486	1481	5			x	x	
(E)-β-ionone	893	1486	1485	1	x	x	x	x	
longifolene-(v4) [b]	888	1492						x	
2-epi-(E)-β-caryophyllene [b]	862	1498					x	x	
α-selinene [b]	892	1502	1500	2			x	x	
6-epi-β-cubebene [b]	879	1506					x	x	
alloaromadedrene [b]	859	1515	1511	4			x		
δ-cadinene	897	1527	1524	3	x	x	x	x	x
L-calamenene [b]	840	1532	1530	2			x	x	
cadinadiene-1,4 [b]	883	1544	1539	5			x	x	
8,9-dehydro-neoisolongifolene [b]	819	1547					x	x	

$R.T_1$ = retention time in seconds for the first dimension.
$R.T_2$ = retention time in seconds for the second dimension.
SI = Similarity of the spectra obtained with those of the libraries.
$LTPRI_{Cal.}$ = Experimental linear temperature programmed retention index.
$LTPRI_{Lit.}$ = Literature linear temperature programmed retention index.
$\Delta = LTPRI_{Cal.} - LTPRI_{Lit.}$
[a] = compounds only characteristic of malagueta peppers.
[b] = compounds only characteristic of murupi peppers.
[c] = compounds only characteristic of dedo-de-moça peppers.

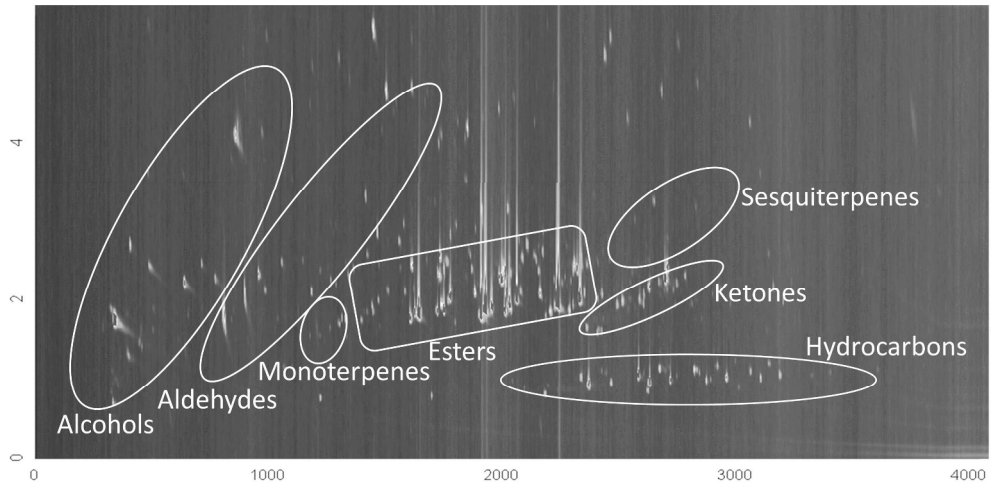

Figure 1: Chromatogram of the volatile compounds from immature malagueta peppers obtained by HS-SPME and GC × GC-TOFMS, with the main chemical classes respective structuring.
896x438mm (87 x 87 DPI)

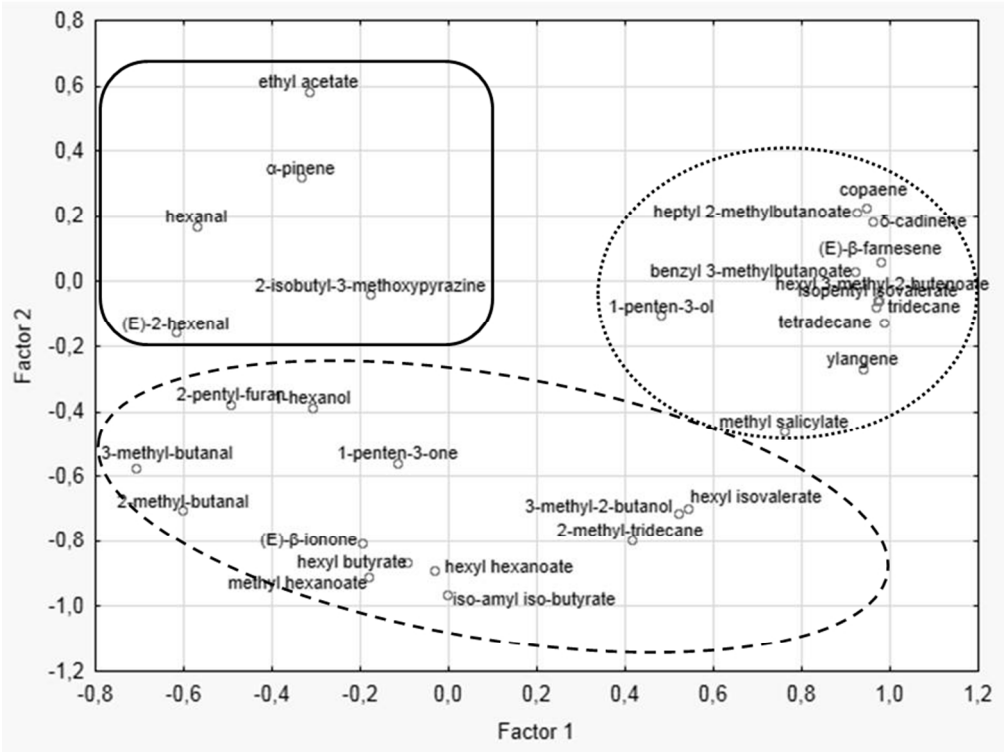

Figure 2: Scores plot regarding on the first two principal components obtained from PCA of the volatile compounds of malagueta, murupi and dedo-de-moça peppers.
254x190mm (96 x 96 DPI)

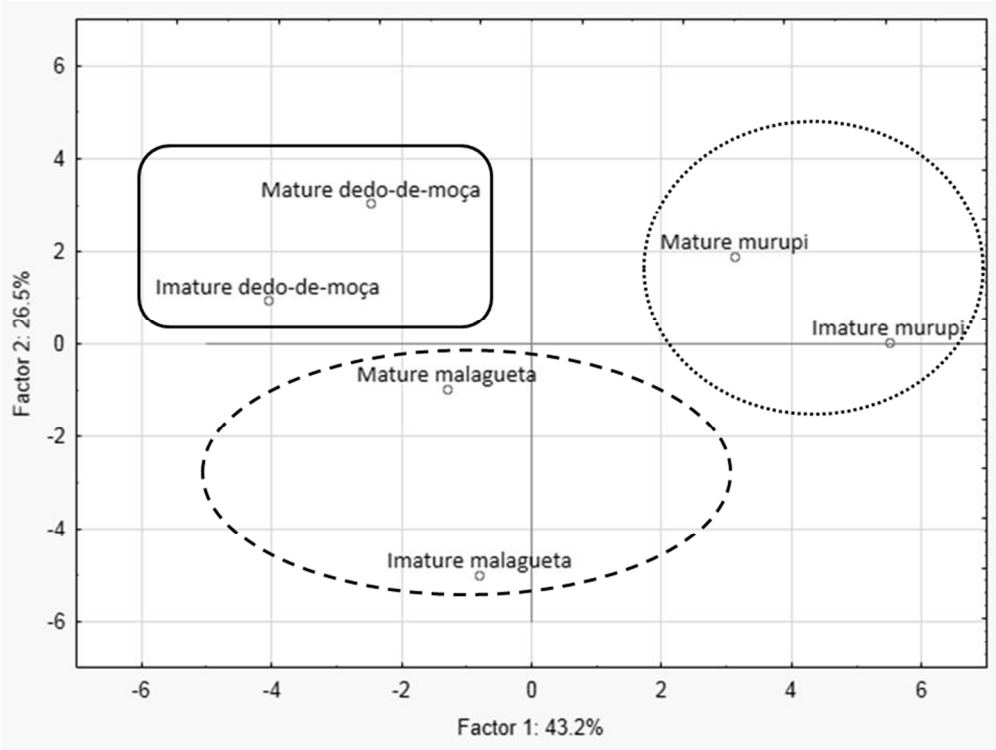

Figure 3: Loadings plot regarding on the first two principal components obtained from PCA of the volatile compounds of malagueta, murupi and dedo-de-moça peppers.
254x190mm (96 x 96 DPI)