

Volatile Compounds and Capsaicinoid Content of Fresh Hot Peppers (*Capsicum Chinense*) Scotch Bonnet Variety at Red Stage

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Abstract: The aim of this study was to evaluate the capsaicinoids content and the volatile compounds of fresh hot pepper from Burundi at red stage. The Capsaicinoids were extracted in acetone and separated using column chromatography on silica gel, then evaluated quantitatively using a reverse phase High performance liquid chromatography/Photodiode array detection (RP-HPLC/PAD). The volatile compounds were extracted by hydrodistillation and then re-extracted and concentrated by SPME fiber at 55°C for 30 min and analyzed using Gas chromatography-mass spectrometry (GC-MS). Seventy volatile compounds were identified, of which aliphatic esters, alcohols, terpenoids and acids were the main classes. Hexyl pentanoate, hexyl isopentanoate, Pentyl 3- methylbutanoate, 10- undecenol, 3, 3- dimethyl cyclohexanol, β -chamigrene, Pentadecanoic acid, (E)- 9- tetradecenoic acid and Hexadecanoic acid were found to be the major volatile constituents. Capsaicin (CAPS) 47.632 mg/g and dihydrocapsaicin (DHCAPS) 23.096 mg/g were the major capsaicinoids and their contents converted in Scoville heat value (142931) show that the Scotch Bonnet variety is a high hot chili pepper according to the Scoville scale.

Key words: *Capsicum Chinense*, hydrodistillation, Scoville Heat Value (SHV), Scotch Bonnet pepper, Solid Phase Microextraction (SPME)

INTRODUCTION

The word pepper describes two different kinds of plants from two unlike species mainly used as spices and seasoning in foods. Thus the genus *Piper Nigrum* which is a flowering vine of the Piperaceae family and *Capsicum* which is a genus of plants of the Solanaceae family, native to Mexico but currently cultivated in Asia, Africa, and countries along the Mediterranean (Yao *et al.*, 1994; In-Kyung *et al.*, 2007). The genus *capsicum* includes five main species: *C. annum* (Containing Jalapeno, Serrano, Bell and *annuum* varieties), *C. Frutescens* (Containing the Tabasco variety), *C. chinense* (Containing the Habanero and Scotch Bonnet varieties). *C. baccatum* (Containing Aji variety) and *C. pubescens* (Containing the Rocoto and Manzano varieties) (Pino *et al.*, 2006). The Habanero chili peppers as well as the Scotch Bonnet varieties are renowned to be very aromatic and the hottest pepper in the world (Kurian and Starks, 2002). The aroma is provided by the volatile oil and the Solid Phase Microextraction (SPME) has been recommended for quantitative analysis of this aroma. SPME has the

advantage of being simple, fast, solvent-free, has high sensitivity and requires smaller sample volume (Stashenko and Martinez, 2007).

Hot taste is due to the presence of non-volatile capsaicinoids, particularly capsaicin {N-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methyl-non-6-enamide} and dihydrocapsaicin {N-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methyl-nonanamide} which are responsible for approximately 80-90% of the spiciness (Davis *et al.*, 2007; Higashiguchi *et al.*, 2006; Lopez Hernandez *et al.*, 1996). These capsaicinoids have been investigated by several methods. High- Performance Liquid Chromatography (HPLC) method is considered as the most reliable and accurate method for determining both capsaicin and dihydrocapsaicin contents (Kurian and Starks, 2002; Sanatombi and Sharma, 2008). Preliminary purification of the extract has been applied before HPLC analysis of capsaicinoids. Thin Layer Chromatography (TLC) and Column Chromatography (CC) methods are the most used. (Perucka and Oleszek, 2000; Romeo *et al.*, 2011).

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The Scotch Bonnet chili pepper, also known as Scot's Bonnet, Bonnie; originates in the Caribbean islands. Their fresh fruits ripe measure 2.5-4 cm long by 2.5-3.5 cm wide and change from green to colors ranging from pumpkin orange to scarlet red (Andrews, 1998).

In Burundi, this type of pepper is commonly called African Pili-Pili Mbuzi (Goat pepper). It is essentially cultivated and commercialized for culinary use and for preparing spicy sauces. However, there is no much or limited published information on Burundian *C. chinense* Scotch Bonnet variety regarding either their aroma (volatile) compounds or their capsaicinoids content.

Therefore, the purpose of this Study was to isolate and identify volatile constituents and to evaluate capsaicinoids content of fresh fruit (Pericarps) pepper (*C. Chinense* Scotch Bonnet variety) from Burundi at the red stage of maturity.

MATERIALS AND METHODS

Materials: Fresh peppers of the variety *capsicum chinense* var. Scotch Bonnet were purchased from the central market in Bujumbura the capital city of Burundi. Fruits were selected at the stage of full ripeness (red) and stored in the freezer (-20°C). Natural Capsaicin (65% capsaicin and 35% dihydrocapsaicin), HPLC-grade acetonitrile were purchased from Sigma-Aldrich chemical (China). All others chemicals reagents were obtained from National Chemical Reagent Co., Ltd. (Shanghai, China). Silica gel ZCX type 2 was from Qindao Haiyang chemical co. Ltd (Shandong, China).

Methods:

Extraction and isolation of capsaicinoids: The extraction of capsaicinoids was performed according to the method described by Thomas *et al.* (1998) with some modifications: Fresh fruit of *C. chinense* var. Scotch Bonnet (50.0 g) was grinded in mortar and extracted with 100 mL of acetone. The sample was further homogenized using a laboratory homogenizer for 5 min until all of the tissue was macerated in 50 mL of acetone. The organic extracts were centrifuged and solvents evaporated under reduced pressure at 40°C; producing 2.0 g of crude dried extract. The crude extract was dissolved in 5 mL of water and then subjected to column chromatography (CC) on silica gel (ZCX-type2) and subsequently eluted with an isocratic solvent system of Petroleum ether, Ethyl Acetate and Methanol (75:20:5). Five fractions were collected (F₁-F₅). All fractions were applied on TLC plate coated with silica gel 60 GF₂₅₄ and developed in the same solvent system as in column chromatography. Two plates were used. One of them was sampled with the five fractions

while the other one was sampled with the capsaicin standard solution (2 mg/mL). In order to identify the capsaicinoids; the plates were sprayed with the solution of 2, 6-dichlorochinonechloroimide (Perucka and Oleszek, 2000). The fractions containing capsaicinoids were mixed; evaporated under vacuum until dryness at 40°C; and dissolved in 2 mL of methanol and used for HPLC analysis.

Analysis of capsaicinoids: Chromatographic analyses were performed using an Agilent liquid chromatography 1100 (Waters, USA) with a UV detector (waters 2996). Separations were achieved by using an Atlantis TM 3.9 mm × 1.5 mm C-18 column (5 µm packing) with 1 mL/min as flow rate. The mobile phase consisted of 1% acetic acid in distilled water (eluent A) and acetonitrile (eluent B), using a gradient program as follow: 10-45% B (0-7min); 45% B (7-10min); 45-52% B (10-11min); 52%B (11-16min); 52-10%B (16-17min). The total run time was 20 min. The injection volume was 10 µL of the solution at 35°C. Capsaicin and dihydrocapsaicin were monitored at 280 nm.

The concentration of capsaicinoids was calculated by matching the peak areas for capsaicin and dihydrocapsaicin against their respective standard curve. The standard curves for capsaicin and dihydrocapsaicin were prepared by plotting HPLC peak area against concentration for analysis ranging from 10 to 60 ppm of a stock solution (250 ppm) of capsaicin and dihydrocapsaicin. This stock solution was prepared by dissolving 25 mg of natural capsaicin (65% capsaicin and 35% dihydrocapsaicin Aldrich label information) in 50 mL HPLC grade Methanol. Capsaicin and dihydrocapsaicin contents were expressed as mg/g dry weight pepper.

Isolation of volatile compounds: The essential oils were isolated by means of Hydrodistillation. 40 g of the Scotch Bonnet chili peppers were first frozen (-20°C) for 24 h and then thawed in distilled water. Afterward, they were cut open, the seeds removed, and macerated in a warring blender for 1 min with distilled water. The resultant puree was immediately subjected to Hydrodistillation using a clevenger-type apparatus for 3 h. Half milliliter of essential oil was collected and analyze with gas chromatography/mass spectrometry (GC-MS).

GC-MS analysis: The GC-MS analysis was performed as described by Song *et al.* (2010) with some modifications. As capsicum fruits are known to contain a little stream-volatile oil; a solid-phase micro-extraction (SPME) - fiber (75 µm, carboxen/poly-dimethylsiloxane) was used to

sample the volatile compounds and then assayed with a gas chromatograph-mass spectrometer (Finnigan Trace GC/MS, Finnigan, USA).

The chili pepper essential oil sample (0.5 mL) was placed into a 15 mL glass vial. The vial was sealed with PTFE/BYTL septum and left for 30 min at 55°C with the presence of SPME fiber to allow equilibration of the volatiles in the headspace. When the process was completed, the injection was conducted in a splitless mode for 3 min at 250°C. Separation of volatile compounds was carried out on a DB-WAX (30 m×0.25 mm×0.25 μm, J&W Scientific, Folsom, CA, USA) capillary column. The column flow rate was 1.8 mL/min, using helium as a carrier gas. The column temperature program was 40°C for 3 min, 40-80°C at 5°C/min, 80-160°C at 10°C/min for 0.5 min, 160-175°C at 2°C/min, 175-230°C at 10°C/min, and 230°C for 7 min. The GC was equipped with a mass spectrometric detector, operating in the electron impact mode with an energy voltage of 70 eV and emission current of 35 μA. The detector was set at a scanning range of 35 to 450 m/z at a rate of 4.45 scans/s.

Volatile compounds were identified by comparing mass spectra data of samples with those of the NIST 98 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the Wiley 6.0 (Wiley, New York, NY, USA) library. Mass spectra from the literature were also compared.

Scoville heat unit (SHU) or value (SHV) conversions:

The heat level of a pepper is measured in Scoville units or value named by Scoville (1912) who developed common Scoville scale in which a typical Bell pepper is rated at 0-100 SHV, the Habanero pepper 100.000-350.000 SHV while the pure capsaicin is rated at 16.000.000 SHV. (Sanatombi and Sharma, 2008).

Capsaicinoid contents were converted to Scoville Heat Value (SHV) by multiplying the pepper dry weight capsaicinoid concentration in parts per million (ppm) by the coefficient of the heat value for each compound, 9.3 for Nordihydrocapsaicin and 16.1 for both Capsaicin and Dihydrocapsaicin Todd *et al.* (1977).

$$\text{Total SHU} = [\text{CAPS (ppm)} + \text{DHCAPS (ppm)}] \times 16.1$$

Statistic analysis: All the experiments were run in triplicate. The statistical analyses of all experimental data were subjected to one way analysis of variance (ANOVA) using SPSS 16 (Chicago, USA) software and the significance differences (p<0.05) were identified by Duncan's multiple range test.

RESULTS AND DISCUSSION

Capsaicinoids content: In the present study, the extract obtained was composed of capsaicinoids and carotenoids pigments. Their separation was performed by column chromatography. Among five fractions collected and applied on TLC plates; fraction 3 and 4 showed the presence of capsaicinoids as blue dots appeared resulted from the reaction between capsaicinoids and 2, 6-dichlorochinonechloroimide. Additionally the *R_f* value corresponding to blue dots was 0.35 which agreed with the results reported by Perucka and Oleszek (2000). And their presence has proved to be responsible of the total pungency for the fresh fruit pepper as also published by Garcés-Claver *et al.* (2006).

In the previous reports, both Capsaicin and Dihydrocapsaicin gave strong, well-resolved peaks. (Thomas *et al.*, 1998; Perucka and Materska, 2003). Thus, the chromatogram A and B (Fig. 1) represent respectively a typical chromatogram of the pepper extract representing Capsaicin as the highest peak followed by that of dihydrocapsaicin, and the standard solution containing 65% capsaicin and 35% dihydrocapsaicin along with minor amounts of other capsaicinoids.

The capsaicin and dihydrocapsaicin content analyzed from the pepper extract were respectively 47.632 and 23.096 mg/g. These data represent 70% of the total capsaicinoids of pepper fruit which is below to 80-90% of the total capsaicinoids in Habanero pepper as reported by Davis *et al.* (2007).

The total Scoville value was determined by summing the concentrations of CAPS and DHCAPS expressed in SHV (142356 SHV). The capsaicin and dihydrocapsaicin contents and their corresponding pungency level measured in SHV (Table 1) are the main indicators for the hotness taste of the pepper and the same factors were evoked elsewhere by (Korel *et al.*, 2002; Topuz and

Table 1: Total SHV and Capsaicin CAPS, Dihydrocapsaicin DHCAPS content extracted from fresh pepper

	Extract concentration		SHV	Total calculated SHV
	mg/g	ppm		
Capsaicin (CAPS)	47.632±1.8	5954	16.1	142931
Dihydrocapsaicin (DHCAPS)	23.096±0.5	2924		

Values are mean of three samples, each sample in triplicate and were estimated on dry basis

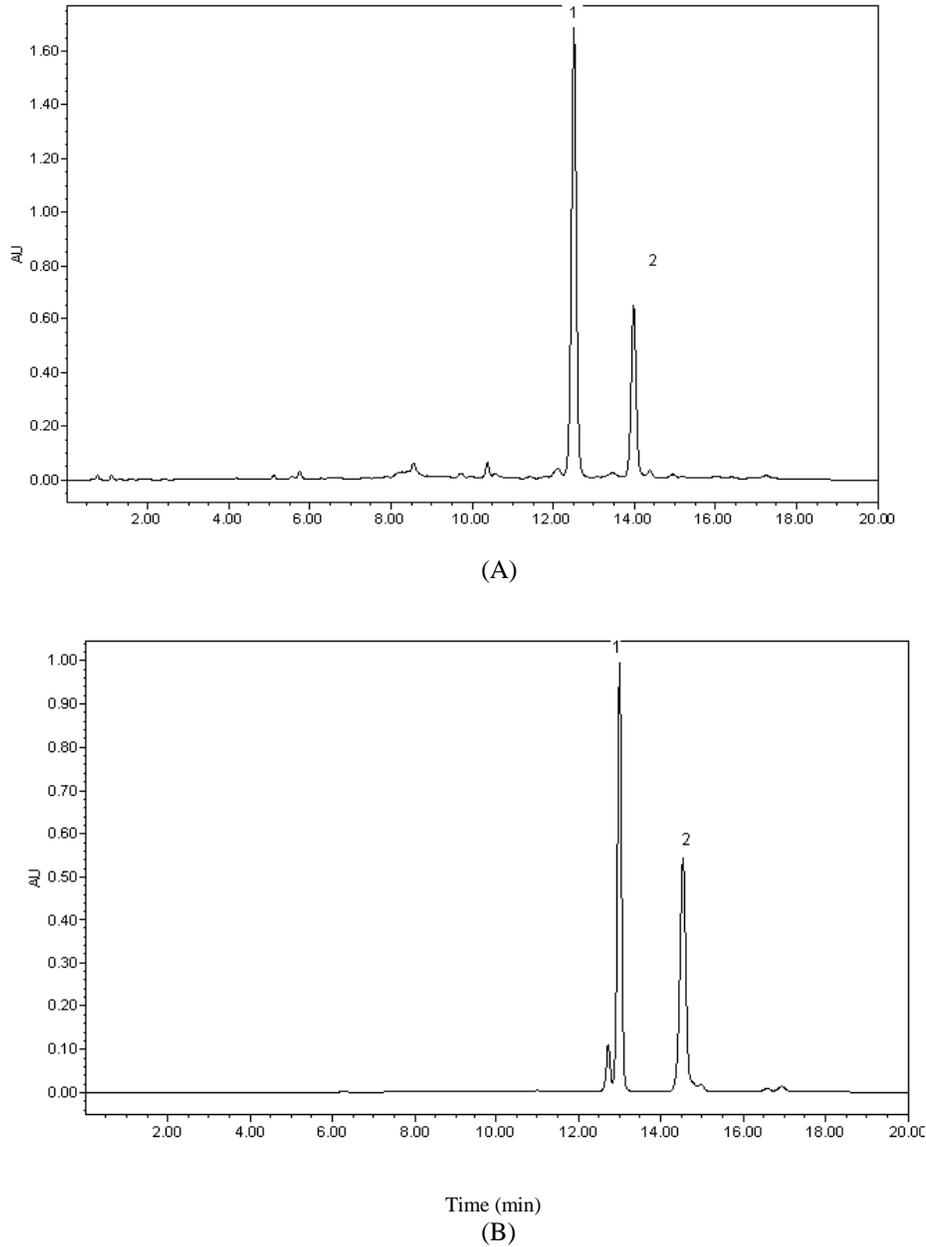


Fig. 1: HPLC Chromatogram A of extract of hot pepper fruits, B of standard solution, 1- Capsaicin 2- Dihydrocapsaicin

Ozdemir, 2007). Therefore, from the Total Scoville heat value (142356 SHV) obtained in this study; the chili pepper analyzed can be qualified as high hot pepper in accordance with the Scoville scale that ranks the high pungent peppers in the range of 100.000-350.000 SHV (Scoville, 1912). However, this pungency level of the Scotch Bonnet variety was found to be smaller than the

Habanero variety having the pungency ranging from 200.000 to 350.000 (Thomas *et al.*, 1998).

Volatile fraction composition: The aromas of chili pepper are the consequence of the characteristic components of their little stream essential oils (Gbalade and Soremekun, 1997; Forero *et al.*, 2009).

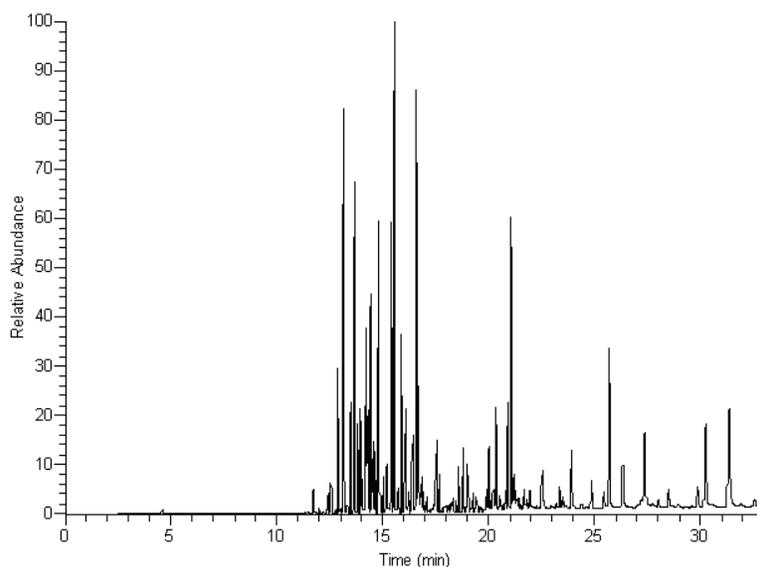


Fig. 2: Typical chromatogram of red chili pepper volatiles

Table 2: Volatile compounds identified in *Capsicum Chinense* scotch bonnet variety

No.	Volatile compounds	Retention time (min)	Relative content (%)
	Esters		34.16
			(25)
1	Hexyl butanoate	11.76	0.51
2	Hexyl isobutanoate	12.39	0.19
3	Pentyl isopentanoate	12.44	0.23
4	Hexyl 2-methylbutanoate	12.91	1.83
5	Hexyl isopentanoate	13.17	9.17
6	Hexyl pentanoate	13.68	5.62
7	[(Z)-3-hexenyl 2-methylbutanoate	14.03	0.48
8	[(E)-2-hexenyl pentanoate	14.11	0.31
9	[(Z)-3-Hexenyl isopentanoate	14.21	2.47
10	Hexyl hexanoate	14.54	0.84
11	Heptyl 2-methylbutanoate	14.63	0.67
12	Pentyl 3-methylbutanoate	14.84	4.21
13	Hexyl hexanoate	15.05	0.16
14	Octyl- 2-methylbutanoate	15.22	0.58
15	3,7-dimethyl-6-octenoic acid	15.50	0.16
16	Octyl-2-methylbutanoate	15.72	3.37
17	Menthyl pentanoate	17.54	1.11
18	Benzyl pentanoate	18.30	0.20
19	Z-7-tetradecenoic acid	19.28	0.25
20	hexadecanol	21.16	0.29
21	Ethyl hexadecanoate	21.20	0.54
22	Ethyl (Z)- 9-hexadecenoate	21.42	0.18
23	Ethyl (Z) -9- octadecenoate	23.34	0.45
24	Methyl octadecadienoate	23.53	0.20
25	Mandenol	23.93	1.29
	Hydrocarbons		4.4
			(4)
26	2-Methyltridecane	12.58	0.36
27	2-Methyl tetradecane	13.79	1.08
28	2-Methyl-1-tetradecene	13.96	1.35
29	Pentadecane	14.30	1.61

Table 2: (Continued)

No.	Volatile compounds	Retention time (min)	Relative content (%)
	Alcohols		19.68 (9)
30	2,3-Dimethyl-Cyclohexanol	14.45	2.59
31	3,3-Dimethyl-Cyclohexanol	15.41	4.61
32	10-Undecenol	15.59	9.20
33	(Z)-3-Octenol	16.23	0.40
34	(Z)-5-Octenol	16.42	0.99
35	2-cyclohexylethanol	20.24	0.26
36	(Z,Z)-9,12-octadecadienoic acid	20.36	1.19
37	1-Eicosanol	20.55	0.21
38	(Z)-11-Hexadecenol	21.27	0.23
	Terpenoids		15.63 (15)
39	α -Copaene	14.33	1.37
40	γ -Caryophyllene	16.09	1.29
41	B Chamigrene	16.60	5.64
42	β -Cubebene	16.67	0.54
43	Citronellol	16.71	1.70
44	Isocaryophyllene	16.84	0.41
45	δ -Cadinene	17.12	0.19
46	Citronellyl propionate	17.54	1.11
47	Limonene glycol	17.68	0.43
48	Geranyl isovalerate	18.61	0.51
49	(E)- α -Ionone	17.96	0.15
50	(E)- β -Ionone	18.79	0.81
51	Isoaromadendrene epoxide	19.43	0.25
52	Citronellyl formate	21.97	0.40
53	Pentacosane	23.47	0.15
	Ketone		5.92 (2)
54	Dihydrocarvone	21.05	3.85
55	Oxacycloheptadecan-2-one	30.25	2.96
	Aldehydes		2.17 (3)
56	Tetradecanal	19.00	0.63
57	Hexadecanal	19.90	0.34
58	Pentadecanal	20.02	1.20
	Acids		15.87 (11)
59	Decanoic acid	20.93	1.62
60	Undecanoic acid	21.71	0.30
61	(Z) 9-octadecenoic acid	21.80	0.19
62	Octadecanoic acid	25.46	0.46
63	(E)-9-Tetradecenoic acid	25.69	3.86
64	Tetradecanoic acid	26.34	1.57
65	(E)-9-Octadecenoic acid	27.05	0.39
66	Pentadecanoic acid	27.39	2.67
	Others		0.14 (1)
70	Dihydroactinidolide	22.38	
	Total (100)		96.79 (70)

Typical chromatogram of chili pepper at red stage is shown in Fig. 2 and seventy different volatile compounds were identified and listed in Table 2 with their relative content. They represent 96.79% of the whole essential oils including alcohols, aldehydes, esters, fatty acids, ketones, hydrocarbons, terpenoids and others.

However in the identified compounds aliphatic esters, alcohols, terpenoids and acids were most representative

chemical classes of the volatile fraction. The aliphatic esters with fruity odor type were most abundant with twenty-six compounds identified. Hexyl isopentanoate (9.17%), Pentyl 3-methylbutanoate (4.21%), Hexyl pentanoate (5.62%) were the major one. Hexyl isopentanoate and hexyl isobutanoate (0.19%) were reported to have a powerful fruity odor note by Forero *et al.* (2009). This abundance of aliphatic esters

has been reported in Habanero chili pepper at the ripening stages green and orange (Pino *et al.*, 2006) whereas in Calabrian *capsicum annum* varieties; aliphatic esters are less abundant as reported by Ziino *et al.* (2009). Nine alcohols; thirteen terpenoids and ten acids were identified, with 10-undecenol (9.20%), 3, 3-Dimethyl cyclohexanol (4.61%) as major alcohols; β -chamigrene (5.64%) as the most abundant terpene and (E)-9-Tetradecenoic acid (3.86%), Pentadecanoic acid (2.67%) and Hexadecanoic acid (4.69%) as the most important acids.

Aliphatic aldehydes, hydrocarbons and ketones were present in small quantities in contrast to the previous study on volatile compounds from different calabrian (Italian) varieties (*Capsicum annum* L) (Ziino *et al.*, 2009). Among aldehydes, Pentadecanal (1.20%) was quantitatively important and pentadecane (1.61%) was the major compound in Hydrocarbons. Dihydrocarvone (3.85%) was the major ketone. This ketone was for the first time found by Gildemeister (1913) in caraway oil and has a spicy odor type.

CONCLUSION

Scotch Bonnet variety commonly called African Pili-Pili from Burundi, used for the present study, was harvested at the red stage (ripe stage) of maturity. It has revealed to be mainly composed of capsaicin and dihydrocapsaicin as major capsaicinoids, thus responsible for its hotness/pungency taste. Their content converted in Scoville heat value revealed that this variety can be classified as a high hot pepper. The seventy volatile compounds were identified among them esters, alcohols, acid and terpenoids were the major classes. Further analyses of this variety at green and orange stages are suggested so that more volatile compounds can be identified. Furthermore the identification of the volatile compounds that are characteristic to their typical aroma and flavor will be more interesting.

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