Determination of the Chemical Volatile Compounds of a Chili Pepper Cultivar (*Capsicum Annuum*) Grown in Eastern Côte d'Ivoire

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ABSTRACT - ***Chilli is widely used throughout the world and, in Côte d'Ivoire in particular, for his nutritional and medicinal properties. Indeed, peppers are good sources of vitamins, polyphenols, minerals and volatile compounds. This work allowed to determine the volatile compounds of a pepper cultivar (Capsicum annuum) grown in eastern Côte d'Ivoire. The analysis of the volatile compounds was made by gas chromatography coupled to the mass spectrophotometer (GC-MS). The results showed that the fresh fruits of the grown pepper cultivar contained a total of 37 compounds. Majority compounds identified in green fruits are thymol (24.60%), d-limonene (21.80%) and hexyl isovalerate (09.90%) while those identified in fresh red fruits are thymol (45.90%), hexyl isovalerate (14.90%) and linalool (05.50%). The identified compounds belong to different families of compounds: terpenes (monoterpenes and sesquiterpenes), alcohols, aldehydes, fatty acids, esters, ketones and hydrocarbons. The fresh red fruits are rich in thymol and green fruits in D-limonene. This study shows that this chili cultivar has very interesting properties that can contribute to human health. As a result, this pepper could be a good pharmacological source.***

Keywords - Cultivar, chilli, *Capsicum annuum*, volatile compounds, extraction, essential oil, GC/MS

1. INTRODUCTION

Chili is the fruit of a set of variable species of the genus Capsicum belonging to the Solanaceae family **[1]**. To date, 35 species of Capsicum have been identified **[2]**, of which five, namely *C. annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens*, domesticated, are widespread. However, about 22 species are wild and endemic, especially in the Americas **[3]**. *Capsicum annuum* is the most widespread and cultivated species in temperate and subtropical countries **[4]**. The chilli cultivar (Capsicum annuum) in our study is a cherry pepper with a slightly elongated tip. This fruit, grown in eastern Côte d'Ivoire, is highly prized by the people of the region. In fact, pepper is widely used in many parts of the world for its sensory properties, including color, aroma, pungent taste and flavor **[5]**.

In Africa, chilli is used as a spice or as an ingredient in the preparation of dishes such as sauces and grills. It is consumed fresh or as a dry powder, and contains many chemical compounds that give it important antioxidant properties **[6] [7] [8]**. Numerous studies have shown that pepper contributes to the fight against certain diseases such as constipation, gastroenteritis, angina, obesity and diabetes **[9]**. In addition to their use in kitchens, peppers have been explored for their antimicrobial and antifungal properties **[10]**. Thus, studies **[11]** have reported that spice oils have an inhibitory effect on meat-damaging bacteria such as *Bacillus subtilis*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*, *Aspergillus flavus*, *Penicillium italicum* and *Cinnamomum zeylanicum*.

These studies have shown that a differentiation of the quality of essential oils is related to climatic conditions, geographical location of collection sites and other therapeutic and genetic factors. Like other tropical countries, Côte d'Ivoire has an abundance of chilli varieties that grow all year round. Chili is an important part of the diet of Ivorians. The pepper that is the subject of our study is used for its very fragrant properties in food and its therapeutic virtues, as the majority of peppers. We carried out a bibliographical research which revealed a lack of work relating to the essential oils of the cultivar which is the object of this work. Hence the interest of conducting research on volatile compounds of interest and their importance in human health.

2. MATERIAL AND EXPERIMENTAL METHODOLOGY

2.1. Plant material

The plant material consists of pepper fruits (*Capsicum annuum*) harvested in November 2018 on an experimental plot of Nangui Abrogoua University (Abidjan, Côte d'Ivoire), (Photo 1).

Photo 1 : Chilli cultivar plant (*Capsicum annuum*)

2.2. Experimental methodology

Harvests take place in the morning between 6 am and 9 am. Once in the laboratory, the fresh fruits were separated according to the uniformity of the grains and the coloring (green and red). Essential oils were obtained by hydrodistillation. Using a Clevenger-type distiller, one kilogram of fresh fruit of each color was distilled for three hours. Hexane was used to trap the essential oil. After extraction of essential oils, hexane is recovered by decantation. 10 mg of essential oil from each sample were then dissolved in 100 mL of hexane and analyzed by GC / MS. Each analysis is repeated three times. GC / MS was performed using an Agilent GC 7890B system equipped with a split-splitless injector and coupled to an Agilent MSD 5977B detector. 1 µl of 0.1% essential oil solution was injected and the analysis conditions were fixed as follows: injection mode without division (300 ° C.), capillary column HP-5MS (30 m × 0.25 mm, 0.25 µm), oven programming from 50 ° C (1 min) to 300 ° C for 5 min, at a rate of 5 ° C / min. The carrier gas is helium (1.2 mL / min). Mass spectra were recorded in electronic impact mode at 70 eV (swept mass range: 40 to 400 m / z). Source and quadrupole temperatures were set at 230 ° C and 150 ° C, respectively. The identification of the compounds was performed on the basis of the Chromatographic Retention Index (RI) measured on an HP-5MS column, and by comparison of the recorded spectra with a data library (Pal 600K®). IR calculations were performed in temperature program mode. A mixture of n-alkane (C7 to C30) homologues was used under the same chromatographic conditions. GC / MS was performed using an Agilent GC 7890B system equiped with a split-splitless injector and coupled to an Agilent MSD 5977B detector. 1 µl of 0.1% essential oil solution was injected and the analysis conditions were fixed as follows: injection mode without division (300 ° C.), capillary column HP-5MS (30 m × 0, 25 mm, 0.25 µm), oven programming from 50 ° C (1 min) to 300 ° C for 5 min, at a rate of 5 ° C / min. The carrier gas is helium (1.2 mL / min). Mass spectra were recorded in electronic impact mode at 70 eV (swept mass range: 40 to 400 m / z). Source and quadrupole temperatures were set at 230 ° C and 150 ° C, respectively. The identification of the compounds was performed on the basis of the Chromatographic Retention Index (RI) measured on an HP-5MS column, and by comparison of the recorded spectra with a data library (Pal 600K®). IR calculations were performed in temperature program mode. A mixture of n-alkane (C7 to C30) homologues was used under the same chromatographic conditions. The main components were confirmed by comparing their retention data with pure co-injected references (commercially available). The chromatograms of the volatile components are illustrated in figure 1. The yield (y) was determined by the following mathematical formula :

$$y(\%) = \frac{\text{Mass of the extract}}{\text{Mass of fresh fruits}} \text{x } 100$$

3. RESULTS AND DISCUSSION

The yield (y) was 0.027% for fresh green fruit and 0.045% for fresh red fruit. A total of 37 chemical compounds were identified, representing 100% of the compounds identified for green fruits and 99.90% of the compounds identified for red fruits (Table 1). In addition, the following four main compounds were observed: thymol, d-limonene, linalool and hexyl isovalerate (Figure 2). The identified compounds have been divided into different classes such as terpenes (monoterpenes and sesquiterpenes), alcohols, phenol, aldehydes, fatty acids, esters, ketones and hydrocarbons (Table 2). Two groups of sesquiterpenes have been identified.

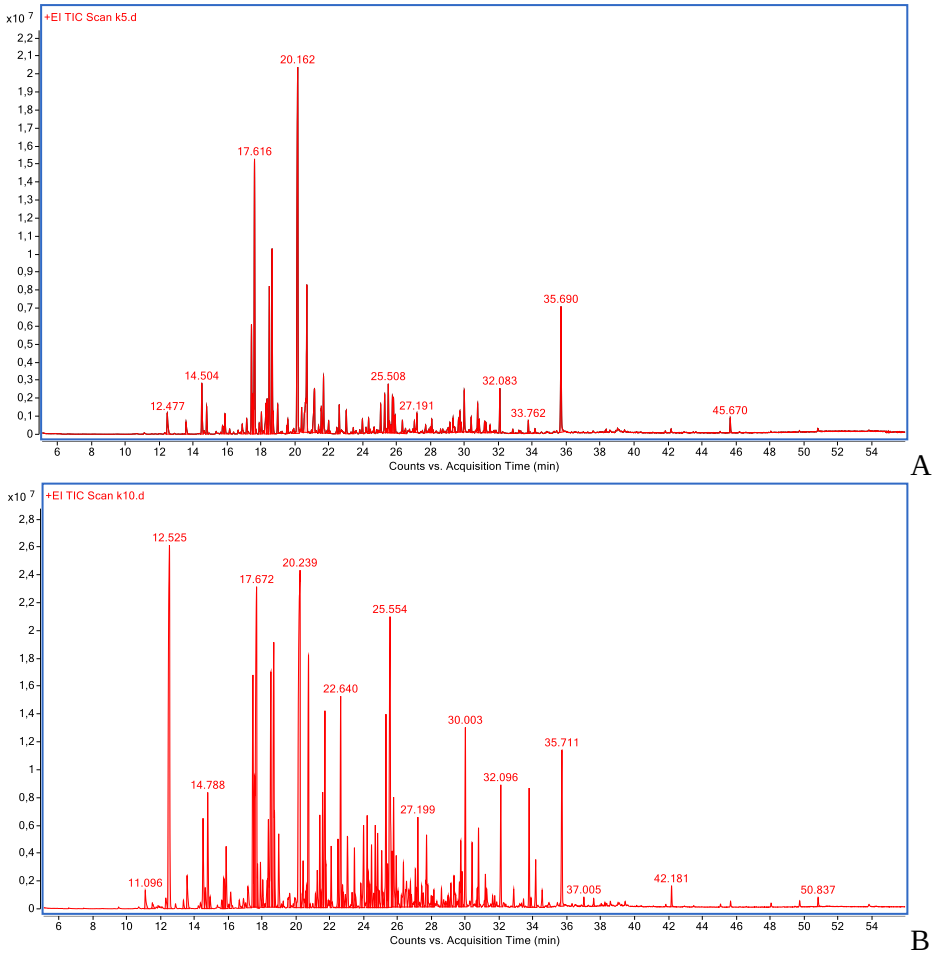

Figure 1 : Chromatographic profiles of the chilli cultivar (Capsicum annuum) at green (A) and red (B) stages of ripening.

The hydrocarbon sesquiterpenes represent the class of the most abundant compounds, numbering eight in the two extracts studied. However, they represent a small percentage ranging from 12.20% to 9.20%. B-elemene is only present in red fruits with a percentage of 1.90%. This compound has anticancer properties and its presence in fresh red fruits is beneficial for humans **[12] [13].** Indeed, β-elemene inhibits the proliferation of prostate cancer cells **[14]**. For other compounds of the same class, such as caryophyllene, beta-selinene and delta-cadinene, the percentage decreases during ripening, from green to red. The percentage of caryophyllene oxide increases in extracts by 0.20% for fresh green fruits, and by 1.00% for red fruits, and alpha-cadinol by 0.90% for fresh green fruits. and 1.20% for red fruits. The percentage of delta-cadinol remains constant at different stages of ripening. For oxygenated sesquiterpenes, the major compound is elemol; it is identified in both extracts with a percentage ranging from 0.90% to 0.80% respectively in fresh green and red fruits. Spathulenol is specific to fresh red fruits with a low rate of 0.30%. The class of oxygenated monoterpenes, 6 for green fruits represents 12.30%, and 5 for red fruits with a percentage of 9.60%. The most abundant compound is linalool. It is present in both extracts with a percentage that goes from 3.60% for green fruits to 5.50% for red fruits. This result is in agreement with that of **[15]** which showed that the percentage of linalool increases in the fruits of *Capsicum annuum* during the ripening stages. The presence of this compound in these extracts shows that these extracts contribute to anti-infectious actions with a broad spectrum of action. It is also an immune stimulant **[16]**. The hydrocarbon monoterpenes, 4 in number, represent 24.70% of the compounds identified in the fruits. The majority compound identified, d-limonene is the most important compound with a percentage of 21.80% for green fruits and 2.40% for red fruits. It was isolated in the red peppers at 0.43% **[17]**. This compound is present at all stages of ripening of the studied cultivar. He was identified in the *Capsicum annuum L. var. glabriusculum* at green and red stages of ripening of fruits **[18]**. The strong presence of d-limonene in this extract should be considered because some authors have shown that this compound has a broad spectrum of biological activity including chemo-therapeutic and chemopreventive effects. They have also demonstrated that oral administration of d-limonene in humans is well tolerated, even in high doses. According to **[19]**, it would be wise to consider this compound as a potential bioactive compound for cancer prevention. Thus, these various identified classes belonging to the terpenoid family have been demonstrated as compounds with great promise in terms of health promotion, including cancer prevention, protection against cardiovascular disease and antioxidant effects. However, a broad review covering the mechanisms behind these effects for each subclass of terpenoid was written by **[20]**.

Table 1 : Chemical composition of the essential oils of the chilli cultivar (*Capsicum annuum*) at the green and red ripening stages.

N°	Identified compounds	Ri[a]	Ri[b]	rc (%) gp	rc (%) gr
1	B-myrcene	979	1097	0.90	-
2	Octanal	982	1112	0.30	-
3	**D-Limonene**	1020	1148	**21.80**	**2.40**
4	Y-Terpinene	1031	1177	0.30	-
5	1-Octanol	1052	1185	1.40	1.40
6	**Linalool**	1081	1219	**3.60**	**5.50**
7	**Hexyl isobutyrate**	1127	1229	**4.00**	**2.70**
8	1-Nonanol	1149	1257	0.30	-
9	4-Carvomenthenol	1161	1317	0.90	1.50
10	Decanal	1183	1337	0.90	0.40
11	Terpineol	1198	1332	3.60	2.90
12	β-Citronnellol	1208	1350	0.70	1.70
13	**Hexyl isovalerate**	1222	1374	**9.90**	**14.90**
14	Geranial	1241	1408	-	0.50
15	1-Decanol	1256	1411	0.60	1.30
16	**Thymol**	1266	1436	**24.60**	**45.90**
17	Perilla alcohol	1274	1440	-	0.40
18	Decanoic acid	1344	1507	-	0.90
19	Copaene	1356	1539	0.70	0.40
20	β-elemene	1382	1551	-	1.90
21	2,5-Dimethoxy-α-cimene	1399	1569	1.70	-
22	α-ionone	1417	1585	0.90	0.30
23	Caryophyllene	1424	1592	3.10	1.10
24	β-Farnesene	1442	1613	1.70	-
25	α-Humulene	1456	1632	0.60	0.20
26	Myristicin	1482	1667	1.50	0.50
27	β-Selinene	1488	1671	3.70	2.40
28	δ-Cadinene	1519	1697	1.60	1.00
29	Elemol	1536	1730	0.90	0.80
30	Elemicin	1548	1737	2.30	1.70
31	Spathulenol	1576	1769	-	0.30
32	Oxyde de caryophyllene	1578	1778	0.20	1.00
33	δ-Cadinol	1618	1844	0.40	0.40
34	α-Cadinol	1650	1855	0.90	1.20
35	Asarone	1678	1859	2.30	-
36	Myristic acid	1748	1949	-	0.70
37	benzyl benzoate	1758	1978	3.70	3.60
	Total			100	99.90

Ri: retention index.
Ria: Identified by GC - MS software; names based on the NIST mass spectrum library, and comparing their Kovats retention indices.
Rib: Kovats retention indices of each component collected in the literature.
rc: relative concentration
-, absence; PV, green pepper; PR, red pepper

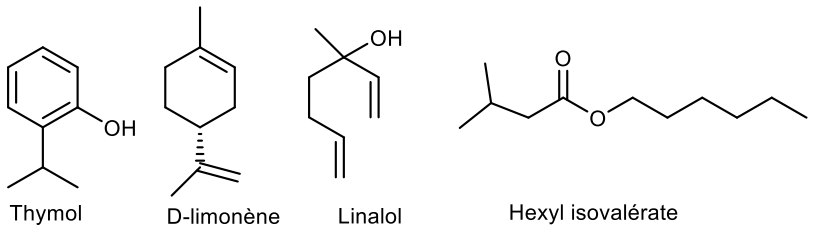

Figure 2 : Structures of major compounds identified

Table 2 : Classes of the identified chemical compounds of the chilli cultivar (*Capsicum annuum*) at the green and red ripening stages.

		gp		rp	
N°	**Classes**	tn	% tc	tn	% tc
1	Oxygen monoterpenes	4	8.80	5	12
2	Hydrocarbon monoterpenes	4	24.70	1	2.40
3	Oxygenated sesquiterpenes	1	0.90	2	1.10
4	Hydrocarbonated sesquiterpenes	8	12.20	8	9.20
5	Alcohols	3	2.30	2	2.70
6	Phenols	1	24.60	1	45.90
7	Aldehydes	2	1.20	2	0.90
8	Fatty acid	-	-	2	1.60
9	Esters	2	13.90	2	17.60
10	Aromatic hydrocarbons	4	8.20	4	6.20
11	Ketones	2	3.20	1	0.30
	Total	31	100	30	99.90

Tn: Total nomber; tc: total concentration; gp: green pepper; rp: red pepper

For phenols, the important compound identified is thymol with a percentage of 45.90% for fresh red fruits and 24.60% for fresh green fruits. The percentage of thymol increases from green to red. The strong presence of thymol in this cultivar, precisely at the red stage, gives it antioxidant properties **[21]**. In addition, this compound is nontoxic **[22]**. The same number of esters were identified in the two extracts analyzed with a percentage of 17.60% for fresh red fruits and 13.90% for fresh green fruits. The most abundant compound is hexyl isovalerate which is present at 14.90% in the extracts of the red fruits and at 9.90% in those of the green fruits. The percentage of Hexyl isovalerate increases from the green stage to the red stage of ripening. The presence of these compounds mainly explains the antispasmodic, anti-inflammatory and neurotonic properties (main properties of the esters) of the cultivar **[16] [23]**. This cultivar has a high level of esters in red fruits which gives it high odorous properties compared to fresh green fruits, unlike most *Capsicum annuum* according to **[15]**. Two fatty acids have been identified in the fresh red fruit extracts. These acids consist of a small percentage of decanoic acid (0.90%) and myristic acid (0.70%), represent 1.60% of the extracts of these fruits. Other classes such as alcohols, hydrocarbons, aldehydes and ketones are the minority classes identified with low percentages ranging from 0.30% to 8.20% for the various extracts analyzed. This result differs from those of **[24]**, which reports that aliphatic aldehydes, alcohols and branched hydrocarbons are the most representative chemical classes of the volatile fraction of Capsicum annuum L fresh peppers. Most of the compounds identified in this work are declining. During the ripening stage, or, disappear or appear at the red stage of ripening. The percentage of certain compounds such as thymol, hexyl isovalerate and linalool increases considerably. In addition, **[25]** has shown that the percentage of volatile aromatic compounds, mostly Capsicum annuum, varies from green to red. However, this observation takes into account the variety and method of analysis. As indicated **[26]**, the chemical composition of peppers varies during the ripening stage.

4. CONCLUSION

This study revealed the volatile chemical composition of a chili cultivar grown in eastern Côte d'Ivoire. Thus, the red fruits of the chilli cultivar (Capsicum annuum) have a high content of thymol, Hexyl isovalerate and Linalool while fresh green fruits are mainly composed of thymol, D-Limonene and Hexyl isovalerate. In addition, fresh red fruits have interesting biological properties that can contribute to human health. In addition, no valuation method for exploiting its active compounds exists. It would be wise to turn to this cultivar that could meet the needs of man. Also, this pepper could be used in the food industry or source of pharmacological product.

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