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Extraction and characterization of capsaicin from *Capsicum frutescens* and *Capsicum annuum*

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Abstract
The aim of this study was to determine the characteristics of capsaicin extracted from pepper fruit samples (fresh green hot dried green hot, dry red hot and dried sweet). The extraction of capsaicin was performed by maceration using 95% ethanol as a solvent. Determination of capsaicin content was performed by Gas chromatography-mass spectrometry (GC-MS) techniques. The results showed a high concentration of capsaicin in dry green pepper followed by the red pepper then fresh green pepper and then sweet pepper. The analysis shown considerable concentrations of some other constituents of pepper samples as ethanol extractable components.

Keywords: Capsicum, GC-MS, Capsaicin, Pepper, Chili, sweet pepper

1. Introduction

Rashid and Singh (2000) [1] reported that, Chili or Pepper (*Capsicum species*) origin in South America and spread into the new world tropics before subsequent introduction to Asia and Africa. Chilies are now widely grown throughout the tropics, sub-tropics and warmer temperature regions of the world. The actual cultivation of peppers by Indians was between 5200- 3400BC (Hui, 2010). From America, Peru and Mexico pepper spread to the world such as African, Caribbean and Pacific countries via Europe (Ashilenje, 2013) [2]. Liljana *et al* (2013) [3] reported that capsicum fruits or hot peppers have been used for a long time ago in the food industry, in traditional medicine, in agricultural industry and for many other aims. Pepper is used in fresh form or in cooking as popular food additive; also it has medicinal uses. Some chilies are used to manufacture teargas. Extracts from hot chilies are used as botanical pesticides against crop pests (Ashilenje, 2013) [2]. Pepper extracts also used to protect crops from insects and animals (Francis, 2004) [4]. β- Carotene is one of the important components of red chilies (Pawar, 2011) [5]. Capsanthin, capsorubin, zeaxanthin and cryptoxanthin are the main pigment found in capsicum (Zachariah and Gobinath, 2008) [6]. Chili pepper belongs to the crops that are cultivated throughout the world for their nutritional, medicinal and economic values. Member of the solanaceaes family, chili pepper is reported to be rich in proteins, lipids, fibers, mineral salts (Ca, P, Fe, and K), vitamins (A, D3, E, C, K, B2 and B12) and in capsaicin. Fresh green chili pepper contains more vitamin C than citrus fruits and fresh red chili pepper has more vitamin A than carrots (Orobiyi, *et al*, 2015) [7].

Capsaicinoids are phenolic compounds isolated from the genus capsicum, of which capsaicin is the most abundant constituent. By several studies it was noted the increase of capsaicinoids in capsicum fruits with regard to the fruit age, size, and different stages of development and nutrient stresses. The capsaicinoids accumulate in the early stages of fruit development and they reach a maximum rate as the fruit matures (Amruthraj. *et al*, 2014) [8]. Concentrations of capsaicin, not only, vary among cultivars but also among the fruits of the same cultivar (Singhal, Kulkarni and Rege, 1997) [9]. Capsaicin and dihydrocapsaicin are the most abundant of capsaicinoids which are the pungent metabolites in the fruits of capsicum species. Capsaicin and dihydrocapsaicin are responsible for about 90% of total pungency in the fruit. There are many less abundant capsaicinoids have been detected in capsicum extracts, including nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin (Stoica *et al*, 2015) [10].

Properties of capsaicin

Capsaicin ($C_{18}H_{27}NO_3$) is an odorless, colorless, hydrophilic, crystalline- waxy compound with molecular mass 305.4g/mol, melting point 62-65ºC boiling point at 0.01mmHg 210-220ºC and sublimate at 115ºC. Capsaicin is highly soluble in alcohol, ether, benzene and chloroform, slightly soluble in carbon disulfide and hot water. It is fairly resistant to acids and alkali solutions at room temperatures (Arora *et al*, 2011, Mortensen, 2009) [11, 12]. The chemical properties of capsaicin is denoted by its molecular structure which is consists of a hexagonal ring of bonded carbon atoms with a tail that contains a long hydrocarbon portion. This aromatic ring and its companion functional group form a basic vanillyl group (Mortensen, 2009) [12]. Capsaicin enhances digestion because it increases stomach acid secretion and stimulates the mucous membrane (Nwankwo, 2013) [13]. Both Khare (2007) [14] and El-Sakka (2010) [15] mentioned that capsaicin is used medicinally as analgesics relieve the pain, when it causes the brain to release endorphins as explained by Francis (2004) [4].

2. Materials and Methods

Fresh pepper sample was obtained from Khartoum state (Bahri local market). One part was treated as it in (fresh). A second portion was dried at room temperature and treated dried green pepper. The red pepper sample was obtained in a dry form. The sweet pepper was collected from the farm Abu Halema (village) and dried at room temperature. The last three samples were grinded separately using electrical grinder.

For extraction, (300) grams of fresh pepper sample and 60 grams from each of the other three samples were macerated by 95 % ethanol for three days, the extracts were filtered through

Whatman No.1 filter paper, the maceration process was repeated until pal color was obtained. In all extracts solvent was evaporated using rotatory evaporator at 60°C, the crudes of samples were weighed. Then the four samples were then analyzed using GC-MS and UV-VIS analysis.

Chemicals

Chemicals and solvents of analytical grade were used.

Instruments

Rotatory evaporator (Buchi R- 114 Water bath B– 480) - GC-MS instrument (Shimadzu 2010).

3. Results and discussion

According to the results obtained analysis showed that green hot pepper has a high concentration of capsaicin than the other samples. The sample of dried sweet pepper showed no capsaicin content, this is in line with the results obtained by Musfiroh *et al*. (2013) [16] which indicated that no peak is given by the sample of red sweet pepper (paprika). The lower content of capsaicin in fresh hot pepper may be attributed to the influence of extraction by the presence of water content in the sample. The peaks of capsaicin obtained by GC-MS appeared at retention time close to that observed by Nwokem, *et al* (2010) [17]. Table (1) show the weight of sample extracts; fresh green have highest weight that may because moisture contents.

Table 1: Weights of samples extracts

Sample	Fresh green	Dried green	Dry red	Dried sweet
Weight	9.503	7.860	7.596	7.247

The result of GC-MS was supported by sweet un-pungent taste of this fruit.

Table 2: Components of fresh green pepper

Peak	Component	R. time (min)	Area %
1	Lactic acid	5.698	13.58
2	L-Serine -2-d	7.811	0.9
3	Anisole, p-allyl	10.093	6.17
4	Anethole	11.454	5.45
5	Anethole II	11.732	0.80
6	1-phenyl-1-heptyne	14.270	1.07
7	Beta-lactose	14.409	10.23
8	Pentadecanoic acid	15.106	1.03
9	l-(+)-Ascorbic acid vanicol2,6-dihexadecanoate	15.728	8.90
10	Trans-Phytol	16.392	4.39
11	Linoleic acid	16.518	10.57
12	Octadecanoic acid	16.607	3.64
13	N-Vanillylnonanoamide	18.524	2.77
14	Diisooctyl phthalate	18.792	1.95
15	Capsaicin	19.113	14.52
16	N-Vanillylnonanoamide II	19.298	14.03

In the three samples; fresh green dried green and dry red pepper capsaicin appeared almost at the same retention time 19.113, 19.070 and19.078 minutes respectively. Three samples showed different number of components, 16 Components of fresh green pepper, 12 for dried green pepper; and 12for dry red pepper. That may be evidence of loss of volatile contents by drying.

Table 3: Components of dried green pepper

Peak	Component	R. time (min)	Area %
1	Ethyl hexopyranoside	14.295	1.24
2	Beta-lactose	14.370	6.42
3	Palmitic acid	15.703	13.64
4	Linoleic acid	16.500	42.22
5	Oleic acid	16.582	2.32
6	9-Octadecenoic acid,1,2,3-propanetriyl ester	16.967	1.22
7	Dipalmitoyl phosphatidyl ether	17.210	0.42
8	Alpha-Glyceryl linoleate	17.916	4.56
9	R-(-)-14-methyl-8-hexadecyn-1-ol	18.226	4.01
10	N-Vanillylnonanoamide	18.486	1.77
11	Capsaicin	19.070	9.90
12	N-Vanillylnonanoamide II	19.252	12.28

The sample of fresh green pepper showed low concentration of linoleic acid, whereas in the other samples it appeared in high concentrations. Some components appeared only in one sample, for instance lactic acid and anisole; p-allyl appeared in the fresh green sample only. Percentage of capsaicin in fresh pepper obtained by GC-MS was close to that noted by Stoica.*et al* (2015) [10] in (Habanero rouge) fruit.

Table 4: Components of dry red pepper

Peak	Component	R. time (min)	Area %
1	Palmitic acid	15.705	17.72
2	Palmitic acid ethyl ester	15.805	2.47
3	Lenoleic acid	16.503	46.03
4	Lenoleic acid, ethyl ester	16.571	6.47
5	9-Octadecenoic acid,1,2,3-propanetriyl ester	16.966	1.14
6	Palmitic acid diglycerin ester	17.206	0.50
7	Rhamnol	17.738	4.59
8	Alpha-Glyceryl linoleate	17.915	4.29
9	R-(-)-14-methyl-8-hexadecyn-1-ol	18.224	3.80
10	N-Vanillylnonanoamide	18.499	1.53
11	Capsaicin	19.078	5.70
12	N-Vanillylnonanoamide II	19.258	5.77

Table 5: Components of dried sweet pepper

Peak	Component	R. time (min)	Area %
1	6,10,14,18,22-tetracosapentaen-2-ol,3-bromo-2.6.10,15,19.23-hexamethyl	15.508	1.19
2	n-Pentadecanoic acid	15.710	18.47
3	Palmitic acid, ethyl ester	15.805	0.73
4	(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl-methanol	16.168	0.60
5	3-Chloropropionic acid, heptadecyl ester	16.230	0.30
6	14,17-Octadecadienoicacid,methyl ester	16.294	1.19
7	Linoleic acid	16.499	58.05
8	Linoleic acid	16.575	2.01
9	9-Octadecenoic acid,1,2,3-propanetriyl ester	16.969	2.13
10	Palmitic acid diglycerin ester	17.209	0.89
11	E,Z-1,3,12-Nonadecatriene	17.920	8.45
12	R-(-)-14-methyl-8-hexadecyn-1-ol	18.229	6.00

According to Scoville heat values the samples can be classified in levels of pungency as the hot pepper is very highly pungent whereas the sweet pepper is non-pungent.
Quality of pepper is affected by pungency level which must be determined by reliable methods.

Table 6: Contents of capsaicin expressed in (SHU)

Sample	Scoville Heat Unit (GC-MS)
Fresh green	73600
Dried green	208000
Dry red	112000
Dried sweet	0.0

4. Conclusion

The study concluded that the content of capsaicin in capsicum was influenced by the difference of species, maturity stage and the drying of sample. Also study shows the importance of pepper fruits which have nutritional components and health benefits with preferable flavor.

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