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## Studies on physico-chemical properties, GC-Mass and ED-XRF analysis of fatty oil of *Capsicum annum* Linn (dry chili) in Bangladesh

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### Abstract

*Capsicum Annum* linn. is locally known as dry chili in Bangladesh. It is used as spices all over the world. In this study fatty oil of the two varieties of dry chilly from two different places of Bangladesh were analyzed by GC-MS. Dry chili (*Capsicum Annum* linn.) Fatty oil has been widely used<sup>1</sup> industrially as an ingredient of many products, e.g. hot sauces, canned fish, ginger beer, as well as for some pharmaceutical products. Fatty oil of two varieties of dry chili was analysis by GC-MS and their composition were established. The GC-MS analysis showed that, Dinajpur's dry chili contained total nine fatty oils. These were Tetradecanoic acid, methyl ester (2.72%); 9-Hexadecanoic acid ,methyl ester (1.55%); Hexadecanoic acid ,methyl ester (17.40%); 9,12-Octadecadienoic acid(Z,Z)- methyl ester (44.64%); 10-Octadecanoic acid ,methyl ester (24.33%); 9-octadecenoic acid(z)-methyl ester (2.39%); Octadecanoic acid ,methyl ester (3.76%); Octadecanoic acid,9,10,12-trimethoxy-,methyl ester (1.43%); 4-tert-butyl-2-methoxyquinoline- 3-phenyl-2-piperidino-2-propen-1-a 4-butyl-2,2-dimethyl-2H-1-benzopyran (1.80%). On the other hand, Chittagong's dry chili contained total ten fatty oils. These were Dodecanoic acid,methyl ester (1.99%); Tetradecanoic acid,methyl ester(5.69%); 9-Hexadecanoic acid ,methyl ester (1.14%); Hexadecanoic acid ,methyl ester (30.15%); 9,12-Octadecadienoic acid(Z,Z)- methyl ester (29.30%); 9-octadecenoic acid(z)-methyl ester(17.82%); 8-octadecenoic acid,methyl ester(3.08%); Octadecanoic acid ,methyl ester (5.48%); Octadecanoic acid,9,10,12-trimethoxy-,methyl ester (2.02%); Methoxylate ester of methyl oleate (3.34%). Elemental analysis was also performed by ED-XRF Analyzer and was observed the mineral contents in fatty oil of the samples. In the minerals, % of K was major in both the samples (85.16% for Dinajpur and 82.54% for Chittagong).

## 1. INTRODUCTION

*Capsicum annum* Linn. is an important part of human diet since 7500 BC<sup>2</sup>. Chili peppers *originated in America*<sup>3</sup>. Chilies holds all the best properties for which it is considered as a food. It has been used as a food for many of years. Chilies are high in vitamin C (about twice that of citrus fruits) Even after cooking it only loses 30 percent of its vitamin C. Dried chilies are very high in vitamin A. Red chilies are a great source of  $\beta$ -carotene<sup>4-8</sup>. Chilies have antibacterial qualities, and contain bioflavonoid, anti-oxidants most common in apple juice. It is effective in protecting against cancer. Capsaicin cream is used to lower the sensation of pain in such conditions as arthritis, and other painful chronic condition<sup>9-11</sup>. Pungency in chili is due to the alkaloid "capsaicin" contained in the pericarp and placenta of fruits, it produces mild to intense spice when eaten. Numerous reviews have summarized the conditions under which capsaicin-containing pharmaceutical agents can best be used<sup>12-14</sup>. Chili is considered as one of the most important commercial spice crops and is widely used universal spice, named as wonder spice. Different varieties are cultivated for varied uses like vegetable, pickles, spice and condiments. Chili (botanically known as *Capsicum annum* L.; *Capsicum frutescens* L.), also called red pepper belongs to the genus capsicum, under the solanaceae family. Chilies are referred to as chillies, chile, hot peppers, bell peppers, red peppers, pod peppers, cayenne peppers, paprika, pimento, and capsicum in different parts of the world. Capsicum Annum (chili) plant oils & extracts have been used for a wide variety of purposes of many thousand of years. Chili grows best at 20–30°C. Growth and yields suffer when temperatures exceed 30°C or drops below 15°C for extended periods. The crop can be grown over a wide range of altitudes from sea level up to nearly 2100 meter<sup>15-16</sup>. However many researches have been carried out on dry chili (*Capsicum annum* Linn.), but no systematic research on comparative studies has been reported on the fatty oil of dry chili in Bangladesh. Some disagreement about the presence of its constituents was observed. Therefore, present work was undertaken to carry out a complete investigation of the fatty oil of nutmeg of two varieties including its physico-chemical properties along with GC analysis.

## 2. MATERIALS AND METHODS

The fresh Bangladeshi dry chili is available in the local markets. The dry chili was collected from two different parts of Bangladesh (Dinajpur and Chittagong). The collected samples were washed clearly by water to remove dust materials. Then they were dried. Finally the dried chili was ground by Fritsch mortar grinder, Germany for one hour. Then the powder was sieved prior to the extraction process. The mean particle diameters obtained were 0.25 and 0.50mm.

### 2.1 Physico-chemical studies<sup>17</sup>

Physico-chemical characteristics of seed such iodine value (pet-ether extract), moisture, ash, color, Solubility, crude fiber, protein, carbohydrate, food energy, specific gravity, refractive index were determined by following the standard procedures<sup>18-19</sup>, and the results were shown in Table-1.

#### 2.1.1 Isolation of fatty acids and preparation of methyl ester:

The neutral lipids-were extracted from the air-dry powdered fruit successively with light petroleum ether (40-60°C), n-hexane and chloroform in a Soxhlet apparatus each for 22h, respectively. Pet-ether, hexane and chloroform extracts were mixed to equal weight (2mg) to analyze the fatty acid in the fruit. The sample was kept in a nitrogen atmosphere in a refrigerator. Fruit sample was analyzed according to the method reported by griffin<sup>20</sup> for esterification and fatty acid methyl ester (FAME) extraction.

#### 2.1.2 Preparation of Standard fatty acid methyl ester (FAME)

Eleven standard free fatty acids (Caprylic acid ; Lauric acid; myristic acid ; palmitic acid; palmitoleic acid; linoleic acid , oleic acid; stearic; arachiaonic acid; behenic acid; and lignoceric acid) were individually weighed . About 5mg of each was taken in a reaction tube and BF<sub>3</sub>CH<sub>3</sub>OH reagent (5ml) was added to it. The mixture was boiled for 5 min. Hexane (5 ml) was added to it and boiled for further 1 min. After cooling the tube a solution of saturated salt was added and vortexes. Then the upper layer containing methyl esters was transferred to a vial with anhydrous sodium sulfate at the bottom. Then the ester was filtered through syringe filter and transferred to a small vial (2ml). The solvent was concentrated by blowing nitrogen gas and stored in a refrigerator before analysis by GC.

### 2.2 GC-MS Analysis

The essential oil of *Capsicum annum* Linn. (Dry chili) of two varieties were analyzed by Electron Impact Ionization (EI) method on GC-17A gas chromatograph, coupled to a GC-MS 2010 plus mass spectrometer; fused silica capillary column temperature of 40° C ( was held 2 min) was maintained with carrier gas helium at a constant pressure of 90kPa. Samples were injected by splitting with the split ratio 10. Essential oil sample was dissolved in chloroform. The operating condition were as follows: name of column- RTS-5MS, diameter 30 cm, length 0.25mm, temperature of the column- initial temperature 40°C ( was held 2 min) , injector temperature- 220 °C, holding time 5 min, column packing- column packing was done with 10% diethylene glycol succinate on 100-120 mesh diatomic CAW, splitting- samples were injected by splitting

with the split ratio 10, carrier gas- helium gas at constant pressure 90 kPa, sample dissolved- in chloroform, range of linear temperature increase- 10°C per min.

### 2.3 Preparation of fatty oil samples for GC-MS analysis

Fatty oil was diluted to 7% by chloroform. An inert gas (i.e. nitrogen) was introduced, from a large gas cylinder through the injection part, the column and the detector. The flow rate of the carrier gas was adjusted to ensure reproducible retention time and to minimize detector dirt. The sample was then injected by a micro syringe through a heated injection part when it was vaporized and carried into the column. The long tube of the column was tightly packed with solid particles. The solid support was uniformly covered with a thin film of a high boiling liquid (the stationary phase). The mobile and stationary phases were then partitioned by the samples and it was separated into the individual components. The carrier gas and sample component was then emerging from the column and passed through a detector. The amount of each component as concentration by the device and generates a signal which was registered electrically. The signal passed to a detector.

### 2.4 Identification of the components

The physicochemical properties of the fatty oil of *Capsicum annuum* Linn. (Dry chili) of two varieties from two different places of Bangladesh are presented in Table-1. Interpretation of mass spectroscopy (GC-MS) was conducted using data base of National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. The retention time, molecular weight, molecular formula and composition percentage of the sample material was recorded and presented in Table -2 and Table-3 (the fatty oil of *Capsicum annuum* Linn. (Dry chili) of two varieties from two different places of Bangladesh.)

Elemental analysis:

The mineral content was determined by EDXRF ANALYZER UNIQUNT (TM) STANDARDLESS METHOD in g/100g dry weight basis of the sample. The element analysis of *Capsicum Annuum* linn (dry chili) can be compared in different origins of Bangladesh.

*Capsicum Annuum* linn (dry chili) Mineral contents:

Mineral content such as K, Ca, Cl, S, Cu, Mn, Fe, Zn, Pb etc. were determined in its fresh *Capsicum Annuum* linn (dry chilli). Two varieties of *Capsicum Annuum* linn (dry chili) of different origin of Bangladesh like Dinajpur & Chittagong were collected from local area. The result of element analysis of its seed was determined by EDXRF ANALYZER UNIQUNT (TM) STANDARDLESS METHOD in g/100g dry weight basis of the sample. The result of minerals content of *Capsicum Annuum* linn (dry chili) from different origin of Bangladesh (Dinajpur and Chittagong) appeared as element basis in Table-4.

## 3. RESULTS AND DISCUSSION

The result of the Physico-chemical properties of *Capsicum annuum* Linn. (Dry chili) fatty oil of Dinajpur and Chittagong appeared in Table -1.

The slight variation of this oil content and the composition of the fatty oil depend on several factors such genotype, stage of maturity, cultivation peculiarities, soil composition and climate differences in various geographical locations. Fluctuation of the oil composition can import change in the organoleptic properties of the plant belonging to the botanical spices and variety. So far we aware till now no systemic investigation on the *Capsicum annuum* Linn. (Dry chili) have not been investigated in Bangladesh by using modern analytical techniques.

GC analyzed results which include the active principles with their retention time; molecular formula; molecular weight and composition of the fatty oil of *Capsicum annuum* Linn. (Dry chili) of two varieties were presented in Table-2 and Table-3.

Total nine fatty acids were identified from the fatty oil of Dinajpur dry chili. The major constituents were 9, 12-Octadecadienoic acid (Z, Z)-methyl ester, (44.64%), 10-Octadecanoic acid ,methyl ester (24.33%), Hexadecanoic acid ,methyl ester (17.40%).

On the other hand total ten fatty acids were identified from the fatty oil of Chittagong dry chili. The major constituents were Hexadecanoic acid ,methyl ester (30.15%), 9,12-Octadecadienoic acid(Z,Z)- methyl ester, (29.30%), 9-octadecenoic acid(z)-methyl ester(17.82%).

Results show that fatty oil from both of two areas oils are a complex mixture of numerous, many of which are found in trace amounts. It is worth monitoring that there is a great variation in the chemical composition of these two region oil of *Capsicum annuum* Linn. (Dry chili). This confirms that the reported variation in oil is due to geographic divergence and ecological conditions.

**Table 1:** Comparative studies on Physico-chemical properties of fatty oil of dry chili of Bangladesh (Dinajpur and Chittagong).

Physical properties		Dinajpur dry chili	Chittagong dry chili
Oil yield (%)		9.36%	7.06%
Organoleptic properties	Taste	Spicy pungent taste	Spicy pungent taste
	Odor	Spicy	Spicy
	Color	Deep red	Deep red
	Appearance at room temperature (30 <sup>0</sup> c)	Homogeneous, opaque liquid, lighter than water	Homogeneous, opaque liquid, lighter than water
Ash		9.74%	6.27%
Moisture		16.33%	16.67%
Crude fiber		25.44%	24.99%
Protein		3.37%	3.11%
Carbohydrate		8.35%	7.75%
Food energy		377.85cal/g	419.40cal/g
Specific gravity at 30 <sup>0</sup> c		0.92	0.91
Refractive index [ $n_D^{25}$ ]		1.47	1.49
Solubility in	Alcohol	Soluble	Soluble
	Distilled water	Insoluble	Insoluble
	Chloroform	Soluble	Soluble
	CCl <sub>4</sub>	Soluble	Soluble
	Pet-ether	Soluble	Soluble
	Diethyle ether	Soluble	Soluble
	n-Hexane	Insoluble	Insoluble
<b>Chemical properties</b>			
Acid value		10.17	8.19
Saponification value		188.46	197.09
Iodine value		133.83	139.59

**Table-2: Chemical constituents of the fatty oil of Dry chilly (from Dinajpur)**

Peak No:	Retention Time	Area (%)	Name of the Compound	Molecular weight	Molecular formula
1	17.634	2.72	Tetradecanoic acid,methyl ester	420.62	C <sub>26</sub> H <sub>44</sub> O <sub>4</sub>
2	20.943	1.55	9-Hexadecanoic acid ,methyl ester	268.4348	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>
3	21.440	17.40	Hexadecanoic acid ,methyl ester	270.4507	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
4	26.739	44.64	9,12-Octadecadienoic acid(Z,Z)- methyl ester,	294.47206	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
5	26.953	24.33	10-Octadecanoic acid ,methyl ester	296.4879	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
6	27.084	2.39	9-octadecenoic acid(z)-methyl ester	296.4879	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
7	27.874	3.76	Octadecanoic acid ,methyl ester	294.4721	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
8	33.346	1.43	Octadecanoic acid,9,10,12-trimethoxy-,methyl ester		C <sub>19</sub> H <sub>32</sub> O <sub>5</sub>
9	34.125	1.80	4-tert-butyl-2-methoxyquinoline 3-phenyl-2-piperidino-2-propen-1-a 4-butyl-2,2-dimethyl-2H-1-benzopyran		

Table-3: Chemical constituents of the fatty oil of Dry chilly (from Chittagong)

Peak No.	Retention Time	Area (%)	Name of the Compound	Molecular weight	Molecular formula
1	13.960	1.99	Dodecanoic acid,methyl ester		C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>
2	17.638	5.69	Tetradecanoic acid,methyl ester	420.62	C <sub>26</sub> H <sub>44</sub> O <sub>4</sub>
3	20.943	1.14	9-Hexadecanoic acid ,methyl ester	268.4348	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>
4	21.464	30.15	Hexadecanoic acid ,methyl ester	270.4507	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
5	26.673	29.30	9,12-Octadecadienoic acid(Z,Z)- methyl ester,	294.47206	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
6	26.898	17.82	9-octadecenoic acid(z)-methyl ester	296.4879	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
7	27.056	3.08	8-octadecenoic acid,methyl ester		
8	27.867	5.48	Octadecanoic acid ,methyl ester	294.4721	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>
9	33.335	2.02	Octadecanoic acid,9,10,12-trimethoxy-,methyl ester		
10	34.139	3.34	Methoxylate ester of methyl oleate		

Table-4: Elemental analysis in percent comparatively (on dry matter basis) of dry chili from two different parts of Bangladesh (Dinajpur and Chittagong).

S. No.	Component as Element	Dry chili of Dinajpur	Dry chili of Chittagong
1	K	85.16	82.54
2	Ca	6.44	5.26
3	Cl	5.31	8.89
4	S	1.62	1.79
5	Fe	0.713	0.500
6	Pb	0.322	0.646
7	Zn	0.258	0.137
8	Ti	0.0261	0.0609
9	Cu	0.0547	0.0476
10	Mn	0.0368	0.0709
11	Sr	0.0191	0.0257
12	Br	0.0081	0.0569
13	Ni	-	0.0099
14	Se	-	0.0040

Results of elemental analysis showed that percentage of minerals contained in fatty oils was varied in two different areas (Dinajpur and Chittagong). K 85.16%, Ca 6.44%, S 1.62%, Fe 0.71% in Dinajpur fatty oil. Whereas, K 82.54%, Ca 5.26%, S 1.79%, Fe 0.500% in Chittagong fatty oil.

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