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A Comprehensive Study on the Identification of Natural Existence and its Gradual Development of Methanol Traces in the Raw Chilli (*Capsicum annuum*) and its Oleoresin

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ABSTRACT

The present research was aiming to understand the possibility of the natural existence of methanol in raw chilli, oleoresins and its gradual increment on long storage. The results of the researchers inferred that (1) the chilli raw material irrespective of its geographical origin, maturity or parts it contains methanol traces. (2) The traces of methanol ppm will increase gradually when we keep it for long period of time (3) when comparing the storage conditions the elevation of methanol ppm in oleoresin is less in the products which are keeping less than 10 º C. The results are supporting the findings of Frenkel et.al, in 1998 and fall et.al, 1996. The former find the reason for the development of methanol in tomato fruits at the time of ripening. It is due to the demethoxylation of pectins present in the cell wall of plants by the catalytic activity of the enzyme pectin methyl esterase (PME, EC 3.1.1.11). According to fall natural methanol emission is happening from the natural plant leaves due to the pectin metabolism. The cell wall of the chill is also contained the pectin compound as a building block. It might be the reason for the development of methanol traces in the raw chilli. During the time of extraction, it may accumulate in the concentrated products. From the results, it is confirming that there is a chance for natural existence of methanol in raw chilli and its value-added products in trace level.

Key words: *Chilli, Oleoresin, Gas Chromatography, Methanol*

1. INTRODUCTION

Chilies are known from the pre-historic times in Peru. It is considered as one of the most important spice crops comes under the genus capsicum and family solanaceae. It is an important part of the human diet since 7500 BC. Mac Neish, *et al.*, 1964 [1].The genus capsicum consists of about 22 species in which five being domesticated Bosland, *et al.*, 1996 [2] viz. C. annuum, C. chinense, C. frutescence, C. pubescence, and C. baccatum. Despite their vast trait differences, most chilli cultivars cultivated commercially in the world belong to the species C. annuum. It is an annual tropical plant growing all over the world. The top ten chilli producing countries are India, China, Ethiopia, Myanmar, Mexico, Vietnam, Peru, Pakistan, Ghana and Bangladesh, contributing 85% of the world production. Among this, the major share is holding by India. India is not only the largest producer but also the largest consumer in this world. It is cultivated in all the states and union territories of the country. In India, Andhra Pradesh is the major producer followed by Karnataka, Orissa, Bengal, Maharashtra, and Madhya Pradesh (Report -Ministry of Agriculture, Government of India 2009).

Green Chilli Plants

Green Chilies with Fruit

Chillies are integral and the most important ingredient in many different cuisines around the world as it is adds pungency, taste, flavor, and color to the dishes (Zhuang, *et al.*, 2012)[3].It is using in salads, baked dishes, stuffed dishes, stew, salsa, pizza, cheese and pickles in various forms.

Green Chilli Fruit

Green Chilli Oleoresin

Chilli contains many chemicals, including water, fixed oils, carotenoids, capsaicinoids, fibers, and minerals. Many of these are contributing the taste, aroma, and color. The two most important group of chemicals found in chilli are the carotenoids and capsaicinoids (Bosland and Votava, 2000).Carotenoids contributing the color and capsaicinoids providing the pungency to the food matrix. Green chilli is using as a vegetable in Indian as well as global cuisines. The presences of capsaicinoids in green chilli help them to find a suitable position in the pharmaceutical products also. Modern food industries are equipped with lots of new technologies to manufacture newly demanded products and it will force them to find new forms of raw materials.

Red Chilli

Chilli Oleoresin

The flavors of many spices are due to the presence of phenols, alkaloids, terpenes and other aromatic compounds. Plant tissues are the main source of R-tocopherol, ascorbic acid, carotenoids, and phenolic compounds. Spice oleoresins are the natural flavors which can meet the modern demand of the food industries. Oleoresins are essentially the concentrated liquid form obtained from spices. Its recovery from plant materials is generally carried out by solvent extraction, super critical fluid extraction (SCFE) , ultra sonication, membrane adsorption , molecular imprinting or by enzyme-assisted extraction. Enzyme-assisted extraction is the recent approach for extracting bio ingredients from the plant materials.

New food regulations are always a challenge to the food and ingredient manufactures .Recently evolved food regulation related to capsaicin in China, Thailand, and Philippine demanding methanol free capsaicin. Most of the industries are isolating the capsaicinoids from chilli, either by using methanol or ethanol. But in many circumstances it has been noticed that the existence of methanol ppm in chilli capsaicin oleoresin even if methanol was not used as an extraction medium.

In the present study the "Existence of Methanol peak in Chilli and its products" aiming to understand and establish the natural existence of methanol peak in chilli and its oleoresins. The periodical increment of the methanol PPM level in final products after long storage, even if methanol has not been using as a purification medium at any stage of production.

The research was carried out by different stages

- Identification of the purity of solvents used for the extraction and purification purpose.
- Identification of the existence of Methanol PPM in foreign origin capsicum oleoresin.
- Identification of the existence of Methanol PPM in Indian origin capsicum oleoresin.
- Identification of the existence of Methanol PPM in raw chilli powder and seeds

2. MATERIALS AND METHODS

The commercial solvents used for the extraction and purification purpose were Ethanol, Ethyl Acetate, Hexane, and Acetone. The purity of the solvents was identified by using Gas Chromatography (GC)

2.1 Method of Residual solvent Analysis Methanol (FCC method for 'Residual solvent in Oleoresin' using Gas Chromatography**) Equipment**

a. Gas Chromatograph (Agilent 6820GC) with a column: DB- 1.3meter, 0.25mm ID- 0.25 micrometer film thickness. Carrier: Hydrogen flow 0.8Ml/minutes, Detector: F.L.D, Detector gas flows- Air 350mL/minutes, Hydrogen- 38mL/minutes, Detector temperature: 200Deg.C, Injector temperature: 180Deg.C, Column 80Deg.C Isothermal and injection volume: 0.2 micro litter.

b. Pipettes

c. Distilling Head: Use of Clevenger trap designed for oils heavier than water.

d. Reagents

Potassium Carbonate: Anhydrous Sodium sulfate: Anhydrous. Detergent & Antifoam: Any such products that are free from volatile compounds should be used. Reference Solution: 0.63% v/w of acetone in water

Sample Preparation

Place 50.0g of sample .10g of anhydrous sodium sulfate, 50ml of water. And a small amount of detergent and antifoam in a 250ml round bottom flask with a 24/40 ground glass neck. Attach the distillation head, a 400mm water-cooled condenser, and a receiver and collect approximately 15ml of distillate. Add 15g of anhydrous potassium carbonate to the distillate, cool while shaking. Place this solution in a 50ml RB flask with a 24/40 ground glass neck. Add a few boiling chips and 1.00ml of the reference solution and collect 1ml of distillate, which will contain any methanol from the sample together with acetone as the internal standard. Inject 20µl to the GC.

e. Calibration of Gas Chromatograph

Reagents

- 0.63% solution of acetone in water
- Solvents LR grade

f. Procedure

- Check for gas leaks: All gas lines joints are checked using soap solution for leaks
- Measure gas flow rates.

2.2 Response factor for Methanol

- Take accurately 1ml of reagent B in a test tube
- Add 1µl of methanol using 2µl syringe
- Set the Chromatographic conditions as per Annexure* on the GC.
- Note the areas of Methanol and acetone from the chromatogram and calculate response as

$$\text{Constant } K = \frac{\text{Area of Acetone} * 18.2}{\text{Area of Methanol}}$$

Calculation of Methanol (ppm) =sample area X Constant (K)*50

Standard area X Sample weight

Reference: FCC 9th Edition page 1442-1443 Response factor Tolerance limit is 10%

2.3 Oleoresin Capsicum/Paprika Obtained from Moroccan and Spanish Origin

Sample – I (Capsicum and paprika) is obtained from Morocco, sample - II (Capsicum/ paprika) is obtained from Spain. The paprika sample is obtained from Spain.

2.4 Oleoresin Capsicum Obtained from Indian Origin

Samples collected from Indian origin material.one set of samples are collected from the crude extract done by ethyl acetate and hexane. The second set of samples are collected after methanol purification.

2.5 Chilli Raw Material Powder with and without Seeds from Different Origin

Different raw materials are collected from different origin with and without seeds for the trials. Byadagi chilli powder #1 (without seed), Paprika Powder (Spanish), Capsaicin chilli powder (Ugandan with seed), Byadagi chilli seed, Seed oil and Hubli Medium Colour powder.

2.6 Capsicum Storage Study under Different Conditions

Methanol purified capsicum oleoresin (CAP6021/1404) kept under different storage conditions and analyzed for methanol traces and its influences based on storage conditions. Samples kept in go down at the normal atmospheric condition, R&D at the normal atmospheric condition and one sample kept in lab freezer at 5 to 10 Deg. C.

3. RESULT AND DISCUSSION

After analyzing the solvents for purity it was found that there were no traces of methanol (Table-1). It will help us to make sure that there are no chances for the contamination from solvents. The same solvents were used for the further experiments.

Table -1 Methanol Peak is not present in any of these Solvents

Solvent	Date of analysis	Purity	Methanol traces
Ethanol	3/7/2015	99.92%	Not detected
Ethyl acetate	13/10/2015	99.73%	‘’
Hexane	13/10/2015	99.60%	‘’
Acetone	8/12/2015	99.97%	‘’

The oleoresin samples of paprika and capsaicin from different origin were analyzed for methanol PPM at different intervals (Table-II). Capsicum sample – I and II analyzed in the mid of September 2015 and was found 52ppm and 62ppm methanol respectively and the same materials were analyzed after one and a half month and have got a result of 78ppm and 95ppm in the sample –I and II. The result reviled that there was an increment of 50% more than the target on storage of capsicum samples. In the same way paprika sample number I, II, and II were analyzed on mid of October 2015 and has got a result of 6ppm, 8ppm, and 6ppm respectively. After one month the analysis was repeated with the same set of material and was found an increment of 16 to 130% (14ppm, 9ppm, 7ppm).

Table-2 Results of Oleoresin Capsicum/Paprika Obtained from Moroccan and Spanish Origin

Sample(origin)	Date of I st analysis and Methanol level	Date of II nd analysis and Methanol level
OR Capsicum Sample –I	26/09/2015 – 52 ppm	10/11/2015 – 78 ppm
OR Capsicum Sample – II	26/09/2015 – 62 ppm	10/11/2015 – 95 ppm
OR Paprika Sample- I(Moroccan)	29/09/2015 – 6 ppm	10/11/2015– 14 ppm
OR paprika Sample - II(Spanish)	11/10/2015 – 8 ppm	11/11/2015 – 9 ppm
OR paprika – sample III)	13/10/2015 – 6 ppm	10/11/2015 – 7 ppm

The results of the analysis inferred that inherent methanol ppm can be a notice in chilli extract and its percentage will increase on storage.

Trials conducted by using various Indian origin material extracted with ethyl acetate-hexane mix as well as ethyl acetate-hexane mix extract followed my methanol purified. All the samples without methanol purification were subjected to methanol ppm analysis

and were found that there were no traces of methanol ppm in all these products. 5 to 10 months intervals the same materials were checked for methanol ppm and was found methanol trace at 2 to 30ppm. Two capsicum samples after methanol purification were also analyzed for identifying the existence of methanol ppm and was found that 4ppm in both the samples. After 2 months the trial was repeated with one sample and was found an increment of 250% (14ppm) and the second sample was analysed after 7months and was found an increment of 575% (27ppm).

Table-3 Results of Oleoresin Capsicum Obtained from Indian Origin

Sample codes	Date of Ist analysis and Methanol level	Date of IInd analysis and Methanol level
CAP606/110	15/11/2014 – Not detected	23/09/2015 – 2 ppm
CAP606/116 (EA+Hex extract)	02/12/2014 – Not detected	09/10/2015– 6 ppm
CAP606/134 (EA+Hex extract)	04/07/2015– Not detected	26/10/2015 – 7 ppm
CAP609/011 (EA+Hex extract)	31/07/2013 – 9 ppm	23/09/2015 – 32 ppm
CAP614/029 (EA+Hex extract, Methanol washed)	15/07/2014 – 4 ppm	22/09/2015 – 14 ppm
CAp614/034 (EA+Hex, Methanol washed)	04/01/2015– 4 ppm	22/09/2015 – 27 ppm

From the above experiments, it was confirmed that even if we are not using methanol as a solvent at any stage of production there is a chance for methanol traces formation in chilli by the natural biochemical reactions. In the same time the second part of the trials proving that there are chances for the development of methanol in capsicum products after long storage. The trials with different period proving that the percentage of residual methanol increasing when the time of storage is increasing.

The trials for the identification of the natural existence of methanol traces in the raw chilli was conducted by using various raw chillies from different sources. The present trial used Byadagi chilli powder #1 (without seed), Paprika Powder (Spanish), Capsaicin chilli powder (Ugandan with seed), Byadagi chilli seed, Seed oil, Byadagi chilli seed (washed) and Hubli Medium Colour chilli powder. After analysis, it was noticed that methanol traces were existing in all variety of chilli irrespective of its origin and even in its seeds also. When it is quantifying the methanol traces is varying from 7 to 149ppm. The minimum result was observed in seed oil and the maximum was noticed in high colour Hubli chilli. From the results it was confirmed that methanol traces are naturally existing in chilli raw material.

Table-4 Results Chilli Raw Material Powder with and without Seeds from Different Origin

Sample Name	**Date of analysis**	**'Methanol' level**
Byadagi chilli powder #1(without seed)	1/12/1995	127 ppm
Byadagi chilli powder #2 (without seed)	2/12/1995	116 ppm
Paprika Powder (Spanish)	2/12/1995	111 ppm
Capsaicin chilli powder (Ugandan with seed)	2/12/1995	27 ppm
Paprika powder	2/12/1995	138 ppm
Byadagi chilli seed	8/12/1995	45 ppm
Seed oil	12/12/1995	7 ppm
Byadagi chilli seed (washed)	16/12/1995	51 ppm
Hubli Med. Colour powder	23/12/1995	149 ppm

To understand the influence of storage conditions and the development of methanol traces in methanol purified capsicum product was studied under different storage conditions analyzed the methanol traces and documented. The same sample was kept in going down, R&D room at normal atmospheric conditions and one sample was kept in R&D freezer at 5 to 10 ^{o}. C. Initial methanol traces was noticed and it was 24ppm. Then it was analyzed with the interval of one month and finally after one year each. The study demonstrated that there is a gradual increase of methanol ppm in sample irrespective of its storage conditions. Sample kept in go down started with 24ppm and the final result was 250ppm. The same was in R&D lab showed 24ppm and 182ppm and the same in R&D freezer showed 24ppm to 58ppm. The mark able observation from the study was that the increment of methanol traces in sample kept in go down was very high compared to R&D room and R&D freezer. The gradual development of methanol traces in the final product is very less if it can be kept in cold condition with less than 10Deg.C.

Table-5 Results of Methanol Traces in Capsicum Oleoresin Kept in Different Storage Conditions.

Date	Storage area	Methanol ppm
	P&F Go down	27
7/3/2014	R&D Lab	29
	R&D Freezer	24
	P&F Go down	38
17/4/2014	R&D Lab	39
	R&D Freezer	32
	P&F Go down	52
20/6/2014	R&D Lab	57
	R&D Freezer	37
	P&F Go down	52
21/7/2014	R&D Lab	57
	R&D Freezer	37
	P&F Go down	62
08/12/2014	R&D Lab	72
	R&D Freezer	32
	P&F Go down	230
04/11/2015	R&D Lab	171
	R&D Freezer	60
	P&F Go down	250
01/01/2016	R&D Lab	182
	R&D Freezer	58

The results inferred that methanol traces can be increase in chilli product on long storage irrespective of its storage conditions. It can be controlled in an extend by keeping in cold conditions with less than 10 º. C.

4. CONCLUSION

Chili is a natural vegetable growing in different climatic conditions of various geographical areas. It contains number of active components like carotenoids and capsaicinoids. Chilli oleoresin is developing by traditional solvent extraction, CO2 extraction and Enzyme-assisted technology. Among the three solvent extraction cheaper but the retention of the solvent residual balance is a big concern in food industries. Recently many regulatory bodies are raising the concern of the existence of methanol in chilli oleoresins and trying to avoid methanol usage in the production process. Some countries are trying to implement it as a rule.The fact which is well known by the scientific world is that capsaicinoids are the alkaloids present in chilli is giving hotness to the chilli . The coloring factor carotenoid is isolating from chilli oleoresin is either by ethanol or popularly using methanol as the purification solvent to remove the capsaicinoids. The product purified methanol will definitely retain a minimum ppm level of methanol in the final product. The suspect and regulatory requirement is a very big concern in many oleoresin manufacturing companies. Many scientists is working in many research institutes and industries to find an alternate method to isolate the capsaicinoids from chilli extract except methanol. Many of them are succeeded to remove the capsaicinoids from chilli extract without methanol. But even though methanol is not a purification medium in the production process of capsaicinoids. The final product is showing methanol traces after GC analysis.

The present research was aiming to understand the possibility of the natural existence of methanol in raw chilli, oleoresins and its gradual increment on long storage. The results of the research inferred that (1) the chilli raw material irrespective of its geographical origin, maturity or parts it contains methanol traces. (2) The traces of methanol ppm will increase gradually when we keep it for long period of time (3) when comparing the storage conditions the elevation of methanol ppm in oleoresin is less in the products which are keeping less than 10 º. C. The results are supporting the findings of Frenkel et.al, in 1998 [5], fall et.al, 1996 [4] and Nemecek et.al, 1995[6]. The former find the reason for the development of methanol in tomato fruits at the time of ripening. It is due to the demethoxylation of pectins present in the cell wall of plants by the catalytic activity of the enzyme pectin methyl esterase (PME, EC 3.1.1.11). According to fall natural methanol emission is happening from the natural plant leaves due to the pectin metabolism. The cell wall of the chill is also contained the pectin compound as a building block. It might be the reason for the development of methanol traces in the raw chilli. During the time of extraction, it may accumulate in the concentrated products. From the results, it is confirming that there is a chance for natural existence of methanol in raw chilli and its value-added products

in trace level. More intense research is required to understand the exact scientific reason for the increase of the methanol trace on long storage and its variations under different storage conditions.

5. REFERENCE

[1] Mac Neish, Richard S. Ancient Mesoamerican civilization. Peabody Foundation Bosland, Paul W. "Capsicums: Innovative uses of an ancient crop." Progress in new crops. ASHS Press, Arlington, VA 1996: 479-487.n, 1964.

[2] Bosland, P.W., Votava, E.J., Peppers: vegetable and spice capsicums. 2012; Vol. 22. CABI, UK.

[3] Zhuang, Y., Chen, L., Sun, L., Cao J., Bioactive characteristics and antioxidant activities of nine peppers. J. Funct. Foods. 20124; (1), 331-338.

[4] Fall, R. and Benson, A.A., Leaf methanol—the simplest natural product from plants. Trends in Plant Science, 1996; 1(9), pp.296-301.

[5] Frenkel, Chaim, Jeanne S. Peters, Denise M. Tieman, Martin E. Tiznado, and Avtar K. Handa. "Pectin methylesterase regulates methanol and ethanol accumulation in ripening tomato (Lycopersicon esculentum) fruit." Journal of Biological Chemistry 273, no. 8 1998; 4293-4295.

[6] Nemecek-Marshall, Michele, Robert C. MacDonald, Jennifer J. Franzen, Cheryl L. Wojciechowski, and Ray Fall. "Methanol emission from leaves (enzymatic detection of gas-phase methanol and relation of methanol fluxes to stomatal conductance and leaf development)." Plant Physiology 1995; 108, no. (4) 1359-1368.

