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Increase in percent purity of β carotene content in organically cultivated tomatoes estimated by SPE and HPLC

Nitika Thakur

ABSTRACT

Nutrition and health are the two most important related areas which always need to be refreshed. Tomato has become an important vegetable of the world in view of the increasing demand for the fresh consumption as well as for processing industries. As a fresh commodity and as a processed product, tomato represents a major source of essential nutrients. The present studies were carried out to evaluate the beta-carotene content of organic and conventionally grown tomatoes by combining SPE and HPLC techniques. The analysis of the sample revealed that β-carotene percentage recorded in organic samples were higher than that the control. Sample T_3 organic was recorded with 13.41 per cent purity, followed by T_4 (8.3%). Minimum percentage purity (4.81%) was recorded in the conventional treatment (control). The results clearly indicated a higher percentage of β-carotene in organic treatments, as compared to farmer's practice (control).

Keywords: Tomato; HPLC; SPE; Organic; Conventional.

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INTRODUCTION

Tomato fruits are a rich source of vitamin C, and the antioxidant properties of this fruit helps in prevailing cancer and occurrence of heart diseases. The abundance of anthocyanin has made it a more valuable vegetable crop. Increased interest in organic tomato production is imposed by the need to evaluate the quality and nutritional value of organic tomato. From the nutritional point of view, tomato is popularly known as "The Poor Man's Apple", which is one of the chief vegetable crops in India. The French people called it as 'The Apple of Love' whereas the Germans referred to it as 'The Apple of Paradise" .Besides their rich nutritional composition, the important and valuable phytochemical components make them highly favored by the consumer worldwide (Tonucci et al., 1995).Tomatoes are not only seasonal but highly perishable after harvesting due to various chemical and physical processes. The fruit lasses its desired quality, nutritional attributes and some are likely to turn out to be total waste (Idha et al., 2007). Tomato is climacteric in nature, having a respiratory peak during ripening due to release of ethylene (Sammi et al., 2007);(Wills et al., 2002). To extend the shelf life of tomato, the respiratory metabolism must be hindered or slowed down either by low temperature storage or storage in a high carbon dioxide atmosphere (Kalt et al., 1999). Various chemicals generally considered as safe (GRAS) are widely used to improve the shelf life of perishable commodities.

Concern about the possible consequences of using increasing amount of chemical fertilizers has led to a strong interest in alternative strategies to ensure competitive yields and protection of crops. Indiscriminate use of pesticides and herbicides could cause diverse changes in biological balance and could lead to an increase in the incidence of cancer and other diseases, through the toxic residues present in the edible produce. The new approach to farming often referred to as sustainable agriculture, seeks to introduce agricultural practices that are ecofriendly and maintain the long term ecological balance of the soil ecosystem. Organic Farming, Food Quality and Human Health complement the strong environmental arguments for going organic. Thus emphasize the use of management practices in preference to use of off farm inputs, taking into account the fact that regional condition requires locally adapted system.

Keeping in view the above facts, the present studies were carried out with an open pollinated and indeterminate tomato variety (cv. Solan Lalima), which has been recently released by University of Horticulture and Forestry (UHF-Nauni) for commercial cultivation of tomato, having superiority over the present tomato hybrids available in the markets in terms of fruit quality and productivity. Being an open pollinated variety, it's a suitable option for organic cultivation. Therefore, farmers can produce its seeds at their own farm. The studies were therefore, conducted to see the influence of different organic and inorganic nutrient sources on the beta-carotene content in tomato.

MATERIALS AND METHODS

Experimental location

The experimental trial was set up in the farmer's field, located at village Basal, 5Km away from Solan town, under Solan block of Solan district, Himachal Pradesh at an elevation of 1270m above mean sea level located 30-52' North and latitude 77-11' east. The experimental area lies under the sub-temperate, sub- humid mid-hill agro-climatic zone of Himachal Pradesh, where summers are moderately hot during May-June, while winters are severe during December-January. The average rainfall in this area ranges from100 to300cm, most of which was received during monsoon months of July and August.

Experimental design of the field

The experiment was laid out in an RBD (Randomized Block Design) with eight treatments replicated five times. The design consisted of 40 plots in which tomato seedlings were planted at a distance of 90 cm x 30 cm having 24 plants per plot. The six (T_1-T_6) organic treatments were applied in different consolidated blocks separated at a distance of 7m from the farmer's and chemical treatments (T_7 and T_8) which were laid out separately. Package of practices were followed separately for organic, conventional and chemical cultivation during the entire course of studies. Different farm inputs were used during field preparation. The doses of the manures and biofertilizers have been formulated by carrying out the soil and manure analysis and doses recommendations prescribed in organic package of tomato crop.A random selection of ten plants was considered from each bed.

Sources of organic amendments and inputs

Manures used

FYM (Farm yard manure) and VC (Vermicompost) were procured from the farmer's field having compost pits and vermi-bed.

Biofertilizers and Biocontrol agents

AZO (Azotobacter), PSB (phosphate solubilizing bacteria), Neem cakes, *Trichoderma viridae*, *Pseudomonas fluorescence* and Asafotida were procured from Poabs Green Pvt. Limited- Kerala.

Chemicals used

All the chemicals used were of analytic grade. All media component silica as sorbent was tested. The SPE (Solid Phase Extraction) column (500mg/6ml; Stepbio, Bologna, Italy) was obtained from Samprep (italy).

Seed source: Procured from Department of Vegetable Crops Dr. Y.S. Parmar -UHF Solan.

Tomato variety used: SolanLalima (open pollinated).SolanLalima is an open pollinated and indeterminate variety of tomato having superiority over the present tomato hybrids available in the markets in terms of fruit quality and productivity. Being open pollinated variety, it's a suitable option for organic cultivation.

Seed rate: 400 g/ha (40 gm/biga)

Seed treatment: The seeds were treated with Beejamrut (6g/40g seed) and *Trichoderma viridi* (0.32g). The seeds were dried in the shade and again treated with a mixture of Azotobacter and PSB (0.8g each).Finally the seeds are dried in shade and sown within 8 hrs of treatment.

Quantitative analysis of Beta -Carotene

Sample preparation

Acetone: Hexane extraction method (Shahzad et al., 2014)

The tomato samples of different designed treatments were collected from the field at the last harvest stage for beta content estimation. The tomato samples were chopped and dehydrated in a cabinet dryer (70^{0}C) with circulating hot air and ground in a laboratory grinder. The samples (0.6g) were weighed in diferent beakers to which 5ml BHT-acetone solution (0.05% w/v) was added. The beaker was placed in a bowl of ice on a magnetic stirring plate for 15 minutes and 3ml distilled water was added. It was finally shaken for 5 minutes on ice and incubated for 5minutes at room temperature to allow the two layers to separate. The upper layer was transferred to a clean vial and finally subjected to SPE (Solid phase extraction).

The SPE column (Solid phase extraction, Flow rate, 1g/6ml; octadecyl C18) used for the separation of pigments was first equilibrated with 10ml hexane.

After this, loading of sample (2ml) was done. After the loading process was over, the first elution process was carried out with 6 ml hexane and remaining fractions were eluted with 6ml acetone. The elutions gave orange (beta-carotene) and yellow fractions (lycopene). Each sample was eluted with four fractions and these fractions were subjected to spectrophotometric analysis from a range of 360nm, 443nm, 476nm and 503nm respectively. The sample fractions with maximum absorbance (503nm for lycopene; 452 for beta carotene) were selected for further analysis in comparison to control. The samples were subjected to HPLC analysis and different peaks were detected.

RESULTS

Quantitative analysis of β-carotene

The eluted fractions obtained into before the lycopene fractions were dark-orange in colour (Table 1).

Table 1. SPE Fractionation for β –carotene.

Wavelengths	**Fractions**			
T_1 sample	**1**	**2**	**3**	**4**
360	0.417	0.420	0.049	0..417
443	0.415	0.421	0.045	0.419
476	0.250	0.254	0.062	0.245
503	0.248	0.254	0.062	0.243
T_2 Sample				
360	0.652	0.466	0.090	0.623
443	0.652	0.467	0.090	0.620
476	0.259	0.244	0.108	0.024
503	0.259	0.244	0.107	0.024
T_3 Sample				
360	1.066	0.297	0.767	0.513
443	1.067	0.297	0.764	0.513
476	0.210	0.222	0.688	0.251
503	0.209	0.222	0.653	0.249
T_4 Sample				
360	0.937	1.185	1.148	0.230
443	0.938	1.185	1.048	0.228
476	0.235	0.205	0.730	0.206
503	0.232	0.202	0.725	0.203
T_5 Sample				
360	0.534	0.589	0.674	0.513
443	0.567	0.297	0.674	0.513
476	0.210	0.222	0.645	0.251
503	0.209	0.222	0.653	0.249
T_6 Sample				
360	0.327	0.297	0.417	0.397
443	0.411	0.297	0.409	0.345
476	0.254	0.062	0.401	0.297
503	0.209	0.063	0.254	0.295
Wavelengths	**T_7 Sample**			
360	0.420	0.049	0.039	
443	0.405	0.045	0.046	0.406
476	0.254	0.058	0.053	0.255
503	0.209	0.077	0.053	0.210

The eluted fractions obtained before the lycopene fractions were dark-orange in colour. The fractions were divided into a series of four fractions before subjecting them to spectrophotometric analysis (from 441 to 663nm), recording absorbance from 441nm, 453nm, 645nm and 663nm, respectively (Table 1).

HPLC analysis for quantification of β-carotene

The samples (T_3, T_4 and T_7) were subjected to HPLC analysis (Table 2) for the total quantification of beta-carotene.

Table 2: HPLC quantitative estimation of β-carotene.

Analyte	**Retention time**	**% Area**
Std. β- carotene	20.3	100%
Organic sample (T_3)	20.3	13.41%
Organic sample (T_4	20.3	8.3%
Farmer's practice (C)	20.3	4.81%

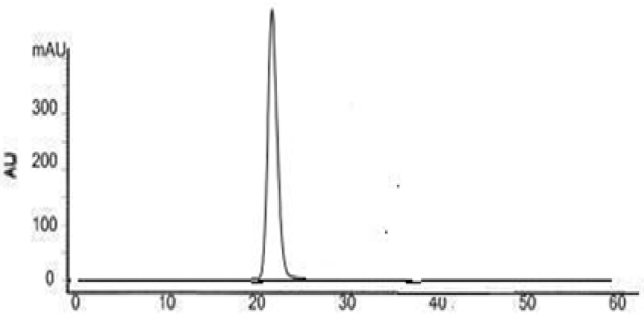
The retention time recorded for the analysis of beta-carotene was 20.3 minutes (Fig 1a).

Fig 1a: HPLC chromatogram (standard) of β-carotene (RT 20.3 min).

The analysis of the sample revealed that β-carotene percentage recorded in organic samples were higher than that in the control. Sample T_3 organic was recorded with 13.41 per cent purity (Fig 1 c), followed by T_4 (8.3%) (Fig 1b).

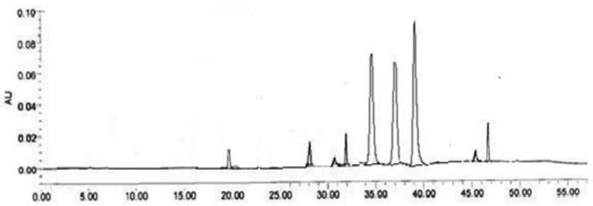
Fig 1c: HPLC chromatogram of purified organic tomato (T_3) extract showing peak of β-carotene (RT 20.3 min) with 13.41 per cent purity.

Fig 1b: HPLC chromatogram of purified organic tomato (T_4) extract showing peak of β-carotene (RT 20.3 min) with 8.3 per cent purity.

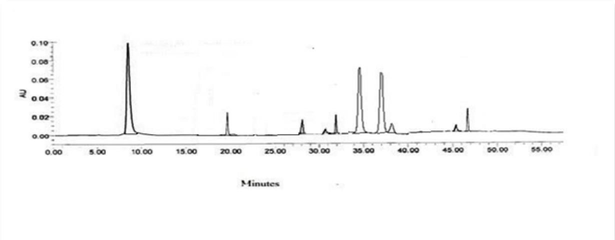


Minimum percentage purity (4.81%) (Fig 1 d) was recorded in the conventional treatment (control).

Fig. 1d. HPLC chromatogram of purified conventional tomato extract (T_7) showing peak (peak 10) of β-carotene (RT 20.3 min) with 4.81 per cent purity.



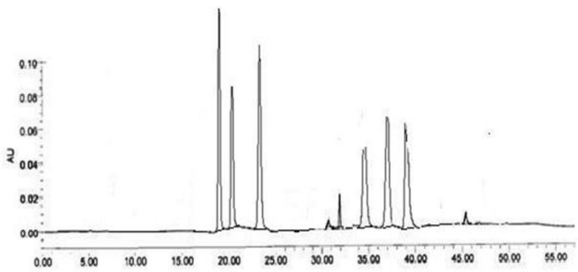
The results clearly indicated a higher percentage of β-carotene in organic treatments,



whereas a very low percentage of lycopene was observed in farmer's practice (control).

DISCUSSION

India has made spectacular breakthrough in the production and the consumption of fertilizers during the last four decades, but consumption of renewable form of energy (chemical fertilizers) will be quite a limiting



factor for increasing agriculture production in future. Because of the escalating energy cost, chemical fertilizers are not available at affordable prices to the farmers. Moreover, the imbalanced and continuous use of chemical fertilizers is leading to a reduction in the crop yields and results in imbalance of nutrients in the soil which has adverse effects on soil health. These organic materials with varying C:N ratios and biochemical composition release nutrients at different pace. Under dynamic multiple cropping systems, the choice of crops in sequence should be based on crop value as its quality and productivity as well as restoration of soil fertility and economics. In sub-tropical climate of eastern India, high value crops like tomato can be successfully grown as dry and wet season crop respectively, in a cropping sequence. The crop seems to be promising and gaining popularity with multiple advantages of meeting increasing demand of vegetables. Tomato requires nutrient elements such as N, P, K, Mg, Ca, Na and S for improved production. These nutrients are specific in function and must be supplied to the plant at the right time and in the right quantity (Shukla et al., 1993).With intensification of cropping and heavy use of chemical fertilizers, the supplementary and complementary roles of organic materials are being strongly felt for retaining soil productivity (Laudicina *et al*., 2013). Use of organic farming techniques to grow crops has gained popularity in recent years as a result of both an increase in consumer demand for organically grown produce and a genuine desire on the part of many growers to sustain or improve the soil health Moreover, higher price of organically produced food than conventional produce (Lester, 2006) prompting producers to grow crops organically. The increased consumer demand appears to be driven primarily by the perception that organically grown produce is safer and more nutritious to eat than produce grown conventionally (Lumpkin, 2005) Significantly higher lycopene content was recorded under vermicompost and farm yard manure integrated with various biofertilizers treatments compared to the control. HPLC analysis of organic sample showed maximum increase of 19.66 per cent and 11.06 per cent in lycopene content (T_3 and T_4) over control.. Significantly higher beta-carotene content was recorded under vermicompost and farm yard manure integrated with various biofertilizer treatments compared to the control. HPLC analysis of organic sample showing maximum beta-carotene was registered with an increase of 8.6 per cent and 3.49 per cent over the control. Similar increase in lycopene and β-carotene content in organically grown tomatoes and carrots was also reported by (Maronik *et al.*, 1964) in comparison to plants raised with inorganic fertilizers. Furthermore, VC and FYM are also known to contain vitamins (Vitamin B_{12} and other vitamins) with higher hormonal and enzymatic activity which has been reported to affect the vitamin synthesis.

The HPLC analysis revealed a higher % of beta-carotene under combination of organic manures and biofertilizers in comparison to individual application of each. The organic samples thus were recorded at a higher side in increasing the attributes in comparison to the control.

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Nitika Thakur*
Department of Biotechnology,
Shoolini University,
Bhajol, Solan-(HP),
India
E-mail: nitikathakur45@gmail.com