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Quantitative analysis of lycopene content in two commercially available tomato sauces: HPTLC based quality check

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ABSTRACT

The demand for high nutritional quality food is increasing because of the commercial opportunities offered by such products due to their visual and functional properties, increasing consumer awareness for the relationship between food and health and the widespread industrial use for nutrient supplementation. An attempt has been made by author to develop a simple method for standardization and quantification such nutritional products. Two different brands (B1 and B2) of Tomato containing edible products (Tomato sauces) were quantitatively analyzed for the detection of lycopene, the major ingredient of tomato edible products. HPTLC profile was developed using Petroleum ether: toluene: water (5:5:0.5) as a mobile phase. The Rf values of lycopene in different brands of tomato edible products was found to be 0.94. The total peak areas of the lycopene and the corresponding peak areas of different formulations were compared and lycopene contents were estimated to be 5.43, 7.27 μ g mL-1 in B1 and B2 respectively. The present study rationalizes the use of HPTLC fingerprint profiles for ascertaining the identity, purity and quality of the different tomato edible products and also for generating data which may be useful in setting standards for these kinds of products.

Keywords: Lycopene, HPTLC, Tomato edible products.

INTRODUCTION

Tomatoes (Lycopersicon esculentum), belonging to Family Solanaceae commonly used in the Mediterranean diet, are a major source of antioxidants and contribute to the daily intake of a significant amount of these molecules ^[1]. They are consumed fresh or as processed products such as canned tomatoes, sauce, juice ketchup and soup^[2]. Among the carotenoids, lycopene is the compound that presents the largest number of unsaturations, having a total of 13 double bonds, with 11 of them conjugated in the structure. Fruits and vegetables, particularly tomatoes, contain high concentrations of lycopene. Various epidemiology studies have provided evidence to indicate that diets containing high concentrations of carotenoids may lower risks for the development of chronic diseases such as cancer and heart disease. Lycopene has received particular attention in recent years as a result of studies that have reported that it is a highly efficient antioxidant and has a high singlet-oxygen and free-radical scavenging capacity.^[3]. These conjugated double bonds are responsible for the red color and antioxidant activity of the lycopene [4]. Tomato-based foods rich in Z-lycopene are potentially more bioavailable and have greater bioefficacy compared to natural tomato products which mainly contain all-E-lycopene^[5]. Lycopene is a high value nutraceutical having wide use ^[6], lycopene can be considered to be a commercially important natural health-promoting ingredient for the market of nutraceutical products ^[7]. The health benefits that have been associated with presence of lycopene in the diet increase researcher's interest to perform innovative studies on it ^[8]. Lycopene is not merely a pigment but a powerful antioxidant, neutralizes free radicals especially those derived from oxygen, present under the lipid membrane and skin cover. Lycopene is produced by phytochemical synthesis in plants and microorganisms whereas animals do not produce it ^[9]. Although lycopene is present in many fruits and vegetables, the majority of studies addressing its biological activity or extraction are centered on tomatoextracted-lycopene. Tomatoes, commonly eaten in both raw and processed forms, contain high levels of lycopene, ranging from 39.2 μ g g⁻¹fresh fruit weight in fresh tomatoes to as high as 682 μ g g⁻¹ wet basis for processed tomato paste ^[10]. Lycopene concentration increases with the maturity of the tomato berries, causing the development of red color ^[11], processing of tomatoes into pastes or sauces increases the resulting lycopene concentration ^[12]. The most common source of lycopene in the diet is processed tomato products such as tinned tomatoes and tomato puree. Lycopene is transported around the body largely bound to the low density lipoprotein (LDL) in the plasma ^[13] Carotenoids are a group of C40 isoprenoid molecules that play diverse biological and ecological roles in plants. Tomato is an important vegetable in human diet and provides the vitamin a precursor of β -carotene ^[14]

Since cooked and processed foods derived from tomatoes were shown to provide optimal lycopene

boost, products such as paste, puree, juice, etc. are nowadays gaining popularity as dietary sources. Since the human body cannot manufacture lycopene, this nutrient must be supplied externally in a diet ^[15].

Thermal processing (bleaching, retorting, and freezing processes) generally cause some loss of lycopene in tomato-based foods. Heat induces isomerization of the all-trans to cis forms. The cis-isomers increase with temperature and processing time. In general, dehydrated and powdered tomatoes have poor lycopene stability unless carefully processed and promptly placed in a hermetically sealed and inert atmosphere for storage.

Recent epidemiological studies have suggested that the consumption of tomatoes and tomato-based food products reduce the risk of cancer (oral cavity, pharynx, esophagus, stomach, rectum, colon, urinary bladder, prostate and breast) in humans.^[16] Various experimental and epidemiologic studies have suggested that high lycopene intakes are associated with decreased risks for atherosclerosis ^[17]. Also lycopene has been proposed to protect against prostate cancer through various properties including decreased lipid oxidation, inhibition of cancer cell proliferation, and most notably potent antioxidant properties [18] An increase in serum lycopene after supplementation can reduce oxidative stress which may play a role in endothelial function ^[19].Endothelial function is considered one of the best indicators of vascular health, and its dysfunction is viewed as the common pathway between coronary risk factors and the development of atherosclerosis. Serum concentrations of lycopene may play an important role in the early stage of atherosclerosis ^[20]. Thus, diet rich in lycopene may protect humans from various kinds of diseases. Dietary lycopene is derived mainly from tomatoes and processed tomato products. Pink grapefruit, watermelon, rosehip, apricot, guava, and papaya contain minor amounts of lycopene. It is a potent antioxidant and the most efficient quencher of singlet oxygen protecting cell lipids, lipoproteins, proteins and DNA from the oxidative damage. The inhibitory effects of lycopene on carcinogenesis could involve free radical scavenging, up regulation of detoxification systems, interference with cell proliferation, induction of gap-junctional communication, inhibition of cell cycle progression, and modulation of signal transduction ^[21]. Also Lycopene's major commercial use is as a coloring agent in the feed, food, nutraceutical and pharmaceutical industries, although its biological properties, as anti-oxidant and anticarcinogenic agent, have been gaining increased attention in the last decade [22]. In addition to its antioxidant properties, lycopene has also been shown to induce cell to cell communication and modulate hormonal, immune systems and other metabolic pathways which may also be responsible for the beneficial effects ^[23].Lycopene may also stimulate the modulation of cell growth, inflammatory processes, immune function and others ^[24].

Lycopene has somewhat higher antioxidant activity than b-carotene. For example, lycopene is destroyed to a greater extent than b-carotene in human skin irradiated with UV. Other studies have shown that lycopene is more efficient than b-carotene in scavenging singlet oxygen and peroxyl radicals and in protecting lymphocytes against NO₂-induced membrane damage and cell death. It is important to note that lycopene is only structurally different from b-carotene in lacking the b-ionone ring (and thus devoid of vitamin A activity). Thus, the possibility that lycopene may have similar pro-oxidant activities as b-carotene^[25].

A significant increase in serum lycopene level is observed on ketchup and capsules intake by healthy humans. Lipid and protein oxidation were also reduced significantly, based on these result there should be intake of 5 to 10 mg lycopene per day ^[26].

Due to the anti-oxidant properties of lycopene, several attempts are being made to isolate lycopene from tomatoes in large quantities and formulate it in suitable dosage forms for human use in risk reduction of certain types of cancer. Lycopene can be extracted from several sources but tomato being one of the best source yields high quantity of lycopene^[16].

Lycopene has been determined in food or biological samples by many analytical methods, such as UV-Visible spectrophotometry, liquid chromatography connected to electro spry-ionization (LC/ESI-MS), reverse phase liquid chromatography (RP-LC), supercritical fluid chromatography(SFC), matrix assisted desorption ionization(MALDI) and especially LC with spectrophotometric detection ^[27]. HPLC is too expensive and too slow for the routine screening of fruit from the many plants produced in breeding programs designed to develop new cultivars ^[28].

High performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique based on the full capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, etc. enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic, and biomolecules. HPTLC allows fast, inexpensive method of analysis in the laboratory as well as in field. Modern quantitative HPTLC, when properly performed by welltrained analysts, can be advantageous compared to high-performance liquid-column chromatography in many analytical situations. The modern HPTLC technique, combined with automated sample application and densitometry scanning, is sensitive and completely reliable, suitable for use in qualitative and quantitative analysis. HPTLC is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images. To Special advantages of HPTLC include high sample throughput and low cost per analysis; multiple samples and standards can be separated simultaneously, and sample preparation requirements are often minimal because the stationary phase is disposable ^[29].

MATERIALS AND METHODS

Chemicals and reagents

HPTLC analyses were performed on Merck $10 \text{ cm} \times 10 \text{ cm}$ HPTLC silica gel 60F254 (0.25 mm) plates. All the chemical and reagents used in the experiment were of analytical grade and were supplied by Merck, Darmstadt, Germany.

Preparation of standard solution

1mg of Lycopene was dissolved in 10 ml of methanol to obtain the concentration of 100μ g/ml. Further dilutions were made with Methanol to obtain working standards 20, 40, 60, 80 and 100μ g mL⁻¹.

Preparation of sample solution

Commercially available tomato sauces of two different brands (B1 (Brand 1), B2 (Brand 2) containing lycopene as active ingredients were purchased from local market. 5 mg of each product were weighed accurately and extracted with 10 ml of methanol by the help of magnetic stirrer for 2 hours and filtered with the help of whatman filter paper.

Instrumentation and chromatographic conditions

The following were the instruments and chromatographic conditions used. Spotting device using a CamagLinomat V TLC applicator device, CAMAG (Muttenz, Switzerland), Syringe: 100 μ L Hamilton (Bonaduz, Switzerland).TLC chamber: glass twin trough chamber (20 \times 10 \times 4 cm); CAMAG. Densitometer: TLC Scanner 3 linked to winCATS software V.4.06; CAMAG. HPTLC plates: 10 \times 10 cm, 0.2 mm thickness precoated with silica gel 60 F₂₅₄; E. Merck (Darmstadt, Germany). Experimental conditions: temperature, 25±2°C; relative humidity, 40%. Solvent system: petroleum ether: benzene: water (5:5:0.5) Detection wavelength: 266 nm..Slit dimension: 5.00 \times 0.45 mm. Scanning speed: 10 mm s⁻¹ and source of radiation: deuterium lamp.

Detection and Quantification

Following sample application, plates were developed in a Camag twin through glass tank pre-saturated with the mobile phase, petroleum ether: Toluene: water (5:5:0.5) ^[30] for one hour, after drying quantitative analysis of the compounds was done by scanning the plates using Camag TLC scanner equipped with Wincats software (Camag) at wavelength (λ max) 266 nm, absorption-reflection scan mode.

RESULTS

Under the chromatographic conditions described above, the Rf values and Area under curve for different concentrations of working standards of lycopene are mentioned in table 1, while the Rf value of lycopene in both test samples (B1and B2) was found to be0.92 .The Chromatograms of standard lycopene are shown in Figure 1 (a-e) and that of lycopene in commercial samples are shown in figure 2 (a-b). 3-D display of all tracks at 266nm is given in figure 3 while the spectral comparison of all the tracks at 276 nm is given in figure 4. The calibration curve was linear in the range of 20 to100 μ g /ml as illustrated in Figure 5.

From the regression equation, y = 341.5x - 104.0, $R^2 = 0.94$, the concentrations of the test samples i.e. B1 (Track 6) and B2 (Track7) was estimated to be about 5.43, 7.27µg /ml respectively. The methods applied were simple, precise, specific, sensitive, and accurate and can also be used in routine quality control of the raw materials as well as the eatable products containing such kind of phytoconstituents.

Table 1:	Rf values and	Area under curv	e for different	concentrations o	f working s	tandards of Ly	copene for line	ar calibration curve

Tracks	Concentrations of working standard of	Rf value	Area under Curve(AUC)
	Lycopene(µg/ml)		
Track 1	20	0.89	119.0
Track 2	40	0.90	669.5
Track 3	60	0.92	1082.9
Track 4	80	0.92	1140.7
Track 5	100	0.93	1591.2

DISCUSSION

Nowadays in developing countries like ours, Adulteration and substitution is quite prevalent, particularly in eatables like commercially available mustard oil, milk, jaggery and sauces (tomato and chili available in road side fast food joints) etc. In order to make both ends meet, people do not hesitate in playing with the lives of fellow humans by adulterating the most common eatables, particularly being consumed by children's. In the present research work, authors had taken up commercially available tomato sauces to quantitatively estimate the lycopene content (or is it just a red/orange or green dye?). Indian Institute of Toxicological Research (formerly I.T.R.C.), Lucknow, had already published many studies regarding the deleterious effects of dyes in eatables. The growing interest in the authenticity of edible products requires reliable verification methods. Quality assurance of edible products may be ensured by proper quality control of the main ingredients present in these products. In recent years, there are many researches being conducted on lycopene. The increasing interests arise because of the many beneficial properties of lycopene. In addition, lycopene reduce arterial stiffness and the risk of CVD (Cardiovascular disease) ^[31]. Thus it is essential to review the analytical methods available for lycopene extraction, separation, detection and preparative isolation as high-purity lycopene is in demand for use in food, pharmaceutical, cosmetic and dye industries ^[20].



Figure 1(a): Densiometric chromatogram of working standard Lycopene ($20\mu g/ml$): Track 1



Figure 1(c): Densiometric chromatogram of working standard Lycopene ($60\mu g/ml$): Track 3



Figure 1(b): Densiometric chromatogram of working standard Lycopene (40µg/ml):Track 2



Figure 1(d): Densiometric chromatogram of working standard Lycopene (80µg/ml): Track 4



Figure 1(e): Densiometric chromatogram of working standard Lycopene (100µg/ml): Track 5



Figure 2(a): Densiometric chromatogram of tomato edible product B1 (20 μ g/ml): Track 6

Figure 2(b): Densiometric chromatogram of tomato edible product B2 (20µg/ml): Track 7



Figure 3: 3D Spectra of all Tracks at 266nm



Figure 4: Spectral comparison of all Tracks at 276nm.

Lycopene has been ranked as being most potent among the following antioxidants: lycopene > N α -tocopherol> N α -carotene> N β cryptoxanthin> N zeaxanthin = β -carotene >N lutein ^[32]. Because of the established beneficiary effects of lycopene (free-radical scavenger) on human health, lycopene is often used as a food supplement as well as a natural food colorant [33]. As the Quality of tomato products is greatly related to their lycopene content [34], thus TLC densitometric method was established for the simultaneous quantification of lycopene in two commercially available tomato sauce brands using HPTLC fingerprinting. After qualitative and quantitative studies it was found that brand B1 was having less quantity of lycopene, than brand B2 (conc. of lycopene: Brand B2 >Brand B1). Since Lycopene is emerging as valued antioxidant and nutritional supplement [35], thus use of such tomato sauces just not gives taste benefits but also it improves the health of consumer as lycopene is having high nutritional value, thus this method applied which is simple, precise, specific, sensitive, and accurate can be used in routine quality control of the raw materials as well as the food preparations containing such kind of phytoconstituents. By means of data generated it was possible to assess the quality assured by subjected brands of tomato sauces. The identity of the marketed brands has not been disclosed due to ethical reasons.

CONCLUSION

Commercial nutritional products are high in demand due to their nutritional value and ease of availability, but the high risk of adulteration remains their! Whether these products are meeting federal standards or not? If yes than they are safe but what if the products are not of that quality what they claim , the major issue lye here is that consumers are unknown of the fact that the product which they are consuming are not of that standard what it ought to be. Thus present work is an attempt to assess one of these products quantitatively; author quantified the amount of lycopene in tomato sauces of two different brands. Data generated from the study proclaimed that quantity of lycopene in tomato sauces was in accordance to its brand popularity and the quality assured, that is the renowned brand consisted of higher lycopene content than the less known brand. This is a benchmark outcome that the quality assured by the "Brand B2" was authentic and contained more amount of lycopene than the "Brand B1" which was a very less popular brand. By this study it can be concluded that HPTLC is highly précised indispensible quality assessment device for eatable products as well and can become a useful analytical tool for checking the quality of such nutritional products .Thus by figuring out necessity to generate scientific/technical standards for these food products will keep a check on intentional/unintentional adulterations and can assure the necessary quality

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