Stability of Lycopene, β–Carotene and Vitamin A in Tomatoes and Some Tomato Products

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ABSTRACT

Tomatoes are among the most widely consumed vegetables worldwide and an important source of certain antioxidants including lycopene , β-carotene and vitamin A , and also have important anticarcinogenic properties. Consumption of tomato and tomato products for their content of antioxidants could be of a great benefit for human health. In the present study, lycopene, β-carotene and vitamin A contents of tomatoes and tomato products (including tomato juice, jam, paste, purée, sauce, soup, powder and ketchup) were determined by HPLC. Data showed that the concentrations of lycopene, β-carotene and vitamin A in tomato products ranged from 6596 to 214952 μg/100g, 170 to 5540 μg/100g and 283.63 to 9242.94 IU/100g, respectively. The highest concentrations of lycopene, β-carotene and vitamin A were traced in tomato paste with oil followed by tomato paste without oil. The stability of lycopene, β-carotene and vitamin A in tomato ketchup during storage for 3 months were influenced by environmental factors such as temperature and light. The stability of the aforementioned compounds A in tomato ketchup during storage at ambient temperature or at 4 C were affected by the light exposure as compared to samples which stored in the dark.

Key words: HPLC, processing, tomato products, lycopene, β-carotene , vitamin A .

INTRODUCTION

Carotenoids represent important biological compounds that are widely distributed in fruits and vegetables. Of the various carotenoids, lycopene has received considerable attention in recent years because of its possible role in the prevention chronic diseases such as prostate cancer (Rao & Agarwal, 1999). Epidemiological studies have also shown that the increased consumption of lycopene rich foods, such as tomatoes and tomato based products is associated with a low risk of cancer (Giovannucci, 1999). In addition to lycopene, both lutein and β-carotene are also present in tomatoes in a small amounts (Shim & Le Maguer, 2000). Some carotenoids possess vitamin A activity. Of the various vitamin A precursors, all-*trans* β -carotene is the most important because of its high (theoretically 100%) vitamin A activity (Tee, 1992). Some other carotenoids such as all-*trans* α- carotene have only half the biological value of all *trans* β-carotene. Both all *trans* α and all trans. β-carotenes are also present as positional isomers in foods such as carrots (Chen *et al.*, 1993).

Lycopene is a red colour pigment and in contrast to most other carotenoids that are widely distributed among great variety of fruits and vegetables, tomatoes and tomato products are main sources of lycopene in the diet. However, it is one of the major carotenoids in European countries and in the USA. Lycopene has unique structural and chemical features that may contribute to its specific biological properties (Clinton, 1998). It is also chemically quite resistant to heat and cooking. Because of its high number of conjugated double bonds, lycopene exhibits higher singlet oxygen quenching ability as compared to α-or- β-carotene (Di Mascio *et al.*, 1989).

In fresh and processed tomato juice, all– isomers of lycopene, lutein and β–carotene are present. It has been well documented that *cis* lycopene is more bioavailable than *trans* lycopene *in vitro* and *in vivo* probably because *cis*-isomers are more soluble in bile acid micelles and may be preferentially incorporated into chylomicrons (Boileau *et al.*, 1999). Thus, the amount and variety of lycopene isomers in tomato juice has to be determined.Lycopene bioavailability in processed tomato products is higher than in unprocessed fresh tomatoes. The composition and structure of the food also have an impact on the bioavailobility of lycopene and may affect the release of lycopene from the tomato tissue matrix. Food processing may improve lycopene bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and tissue matrix, thus making lycopene more accessible and enhancing the *cis* isomerization (Shim & Le-Maguer, 2000).

Lycopene in tomato paste is four times more boiavailable than in fresh tomatoes. Thus, processed tomato products such as pasteurized tomato juice, soup, sauce and ketchup contain the highest concentrations of bioavailable lycopene. Because lycopene is so insoluble in water and is so tightly bound to vegetable fiber, the bioavailability of lycopene increased by food processing. Cooking and crushing tomatoes (as in the canning process) and serving in oil rich dishes (such as spaghetti sauce or pizza) greatly increases assimilation from the digestive tract into the bloodstream. Lycopene is fat soluble so the oil is said to help absorption (Bowen *et al.*, 2002).

The purpose of the present work was to study the changes in lycopene, β-carotene and vitamin A contents in tomato products during processing and storage under different conditions (i.e., exposure to light and temperature).

MATERIALS AND METHODS

Preparation of Samples

Tomato fruits (*Lycopersicon esculentum*) of good quality were obtained from local market in Alexandria governorate during summer season and directly transported in ice box to laboratory of Faculty of Home Economics, AL-Azher Univ. The fruits were washed with tap water to remove dirt and each fruit was cut into quarter pieces, the pieces were comminuted in a Kitchen type blender, after removal of seeds by screening, the juice was divided into two portions. The first portion was analyzed as untreated tomato juice (unprocessed or as control), the second portion was utilized to produce processed tomato products such as jam, paste (with or without added sunflower oil), purée, sauce, soup and ketchup by concentrated tomato juice to different percentage of total solids according to type of the product.

Jam processing

The juice which prepared as mentioned before was blended with a tap water at ratio of 1:4 w/v. Sugar was added to the mixture at a level 1:1.5 w/w juice to sugar, then cooked for 40 minutes at 100°C with manual stirring. Citric acid was added at 0.1% level and cooking was continued for another 5-10 minutes at 100°C.The produced jam (67% TSS) was left to cool to 60-65°C. The product was packed in sterilized glass jars and stored at room temperature (Srivastava & Kumar, 2003).

Soup processing

Tomato soup was prepared using the following ingredients: tomato juice (1 kg), chicken soup cubs (4 g), salt (20 g), sugar (20 g), flour (10 g), chopped onion (20 g), chopped garlic (5g), headless clove (5 g), cumin, cardamom, black pepper, cinnamon (powdered) 1g each and water 350 ml).The finish tomato soup was packed in sterilized glass jars and stored at ambient temperature. The total soluble solids were not less than 7% (Srivastava & Kumar, 2003).

Ketchup processing

Ketchup was prepared using the following ingredients: tomato juice (1 kg), sugar (75g), salt (10g), chopped onion (50g), chopped ginger (10g), chopped garlic (5g), red chilli powder 5g, cinnamon, cardamom (Large), aniseed, cumin, black pepper (10g) each, clove (5g), vinegar (25ml). The ketchup was packed in crown cap bottles and stored at ambient temperature at 4°C, in the dark and light for 3 months (Srivastava & Kumar, 2003).

Tomato powder

To produce tomato powder, whole tomato fruits were dipped in hot water at 85-90°C for 1 min to remove the skin of tomatoes, and then tomatoes were cut into pieces. The tomato pieces were dried using the following two methods:-

1- Oven drying, where the tomato slices were placed on stainless steel trays in one layer and then placed on oven and dried at 70°C or 100°C, the trays were removed when the weight of tomato slices was being constant.

2- Sun drying under normal atmospheric conditions, where one layer of tomato slices was placed on stainless steel trays, exposed to the sun for 6 hours per day for 8 days at temperature of about 28-30°C and relative humidity of about 80-87% (Srivastava & Kumar, 2003). Two replicates for each sample were taken for lycopene, β-carotene and vitamin A analysis. These constituents were determined in ketchup at zero time and at the end of storage period.

Extraction and estimation of lycopene, β-carotene and vitamin A

A method developed by Lin & Chen (2003) was used for analysis of carotenoids (lycopene and β-carotene) and vitamin A. A sample of 10g tomato product (juice or processed or tomato powder) was mixed thoroughly with 2g of celite and then mixed with sufficient distilled water to produce a smooth paste containing 10% tomato solids and then poured into a 60 ml vial and mixed with acetone: ethanol: hexane (4:4:3 ml), and 0.2 g magnesium carbonate. The solution was shaken for 30 min, after which the upper layer was collected with 32 ml of acetone: ethanol: hexane mixture (4:4:3 ml) and shaken for 30 min. Again, the upper layer was collected and poured into the same flask. The lower layer was repeatedly extracted with 15 ml hexane and shaken for 20 min followed by addition of 5 ml hexane and the solution was homogenized at 12000 rpm for 5 min. The mixture was filtered through Whatman No. 1 filter paper and the filtrates were combined and poured into the same flask. Then, 150 ml distilled water and 100 ml Nacl (10%) were added to the filtrate, and the upper phase was also collected. The lower layer was extracted again with 20 ml hexane. All the filtrates were collected and evaporated to dryness in a flask under vacuum, the residue dissolved in 1 ml methylene chloride and filtered through a 0.2 um membrane filter for high performance liquid chromatography (HPLC) analysis.

Analysis by HPLC

The HPLC system model (Water 486) UV detector set at 254 nm and the data recorded by Millennium Chromatography Manger software 2010 (Waters, Milford MA 01757) was used. Reverse phase C18 Nova pack C18 column 3.9×150 m m, 10 µm (Waters U.S.A.) was used. An isocratic mobile phase system of acetonitrile: methanol: 2-propanol (44:54:2 by vol) was used (Stahl *et al.*, 1993). Lycopene, *β*-carotene and vitamin A standards were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). Peak identification was based on retention time and published absorbance spectral data. Lycopene and *β*-carotene content were calculated as µg per 100g sample, while vitamin A content of tomatoes and tomato products was calculated as IU per 100g sample.

RESULTS AND DISCUSSION

Effect of processing treatments on lycopene, *β*-carotene and vitamin A contents of tomato products

Lycopene a fat soluble carotenoid is a precursor of *β*-carotene (Sandmann, 1994) and has at least twice the antioxidant capacity of *β*-carotene (Di-Mascio *et al.*, 1989). Since lycopene has value as a phytonutrient, many breeders want to maximize lycopene content in their breeding lines and growers want to utilize production methods to increase lycopene content. Thus, simple and inexpensive assays to quantify lycopene are desirable prerequisites to developing produce with higher levels of this phytonutrient.

Data presented in Table (1) show that lycopene, *β*-carotene and vitamin A content were generally higher in tomato paste without oil (samples No.1, 2), ranging from 143560-195164 µg/100g, 3700-5030 µg/100g and 6173.08-8392.05 IU/100g,

Table 1: Lycopene, *β*-carotene and vitamin A contents of tomatoes and processed tomato products

Products	Lycopene (µg/100g)	*β*-carotene (µg/100g)	Vitamin A* (IU/100g)
Paste (with oil)	214952	5540	9242.94
Paste (without oil)1	195164	5030	8392.05
Paste (without oil) 2	143560	3700	6173.08
Powder (sun drying)	69840	1800	3003.10
Powder (oven 70°C)	126100	3250	5422.30
Powder (oven100°C)	142784	3680	6139.71
Puree	110192	2840	4738.26
Soup	51604	1330	2218.97
Sauce	55872	1440	2402.49
Juice	27936	720	1201.25
Jam	6596	170	283.63

* Retinol = Zero
1IU Vitamin A = 0.3 µg vitamin A alcohol
1IU Vitamin A = 0.6 µg β-carotene
1IU Vitamin A = 0.34 µg Retinol acetate

respectively as compared to tomato juice being 27936 µg/100g lycopene, 720 µg/100g β-carotene and 1201.25 Iu/100g of vitamin A. Panalaks & Murray (1970) reported that there was an increase in carotenoid levels as a result of cooking and processing. Most of these studies have focused on the changes in the levels of all-*trans* α- and β-carotene and subsequent losses in vitamin A activity due to thermal degradation and isomerization of these compounds. So our results agree with those reported by Simpson *et al.* (1976) which showed that lycopene is more stable in native tomato fruit tissues and matrices than in isolated or purified form. Heating of tomato juice was shown to result in an improvement in uptake of lycopene for humans (Stahl & Sies, 1992). Meanwhile Gartner *et al.* (1997) showed that tomato paste, has more bioavailable lycopene than fresh tomatoes when both are consumed in conjunction with corn oil.

Meanwhile, Khachik *et al.* (1992) reported that common heat treatments during food preparation such as microwaving, boiling, steaming and stewing did not significantly alter the carotenoid distribution in green vegetable and tomatoes. Thermal processing (bleaching, retorting and freezing processes) generally cause some loss of lycopene in tomato based foods (Shim & Le-Maguer, 2000). In processed tomato products, lycopene isomerization and autoxidation cause a decrease of total lycopene content, a decrease in the proportion of all *trans* lycopene, colour loss and development of grassy off flavours (Schierle *et al.*, 1997). Processing fruit makes the lycopene more bioavailabe by increasing the surface area available for digestion. More significantly, the chemical form of lycopene is altered by the temperature changes involved in processing to make it more easily absorbed by the body (Anonymous, 2007). Also, because lycopene is fat soluble (as are vitamins A, D, E and β-carotene), absorption into tissues is improved when oil is added to the diet. Although lycopene is available in supplement form, it is likely there is a synergistic effect when it is obtained from the whole fruit instead, where other components of the fruit enhance lycopene's effectiveness.

The results given in Table (1) indicate that there was a trend of higher lycopene, β-carotene and vitamin A contents for the tomato powder which was dried in oven at 100°C followed by oven at 70°C and finally sun dried. The highest concentrations of lycopene, β-carotene and vitamin A were noticed in tomatoes which was dried in oven at 100°C (142784 µg/100g, 3680 µg/100g and 6139.71 IU /100g, respectively) followed by samples dried in oven at 70°C being 126100 µg/100g, 3250 µg/100g and 5422.3 IU/100g, respectively. On the other hand, the concentrations of lycopene β-carotene and vitamin A in tomato powder (which was sun dried) were lower than those dried by the other methods. In low moisture products, like tomato powders, carotenoids are readily oxidized causing colour loss and off- flavours (Lovric *et al.*, 1970).

The results presented in Table (1) indicate that the lycopene, β-carotene and vitamin A contents of tomato puree were 110192 µg/100g, 2840 µg/100g and 4738.26 IU/100g, respectively. These results are in agreement with those reported by Rao *et al.* (1998) who stated that lycopene concentration was in the range of 42-365 ppm in tomato and the highest was in processed products such as tomato paste, tomato puree, chopped tomatoes and spaghetti sauce. However, cooked tomatoes generally had higher lycopene content than uncooked (Thompson *et al.*, 2000). The results given in Table (1) show that tomato jam had the lowest concentrations of lycopene, β-carotene and vitamin A among the studied tomato products in the present study.

Data regarding the changes in lycopene, β-carotene and vitamin A contents of ketchup samples which stored at ambient temperature and at 4°C, in dark and in light for a period of 3 months are shown in Table (2). The results show that after 3 months of light exposure of ketchup samples at ambient temperature (T2), or storage at 4°C in the light (T3), the lycopene, β-carotene and vitamin A were 87300, 59364 µg/100g, 2250, 1530 µg/100g and 3753.90, 2552.65 IU / 100g, respectively.

Processed ketchup samples which were stored for 3 months in the dark (either at ambient temperature or at 4°C) (T1 and T4) had lower amounts of lycopene, β-carotene and vitamin A contents as compared to those stored in the light. Sharma & Le-Maguer (1996b) demonstrated that the reaction rate of lycopene during storage of tomato puree at 25°C was 2.7 times greater than at 5°C. Also, Chen *et al.* (1994) reported that the dominant reaction isomerization or degradation may be dependent on many factors, such as temperature, illumination intensity and storage environment. In addition, it was found that a higher storage temperature could be destructive to all *trans* isomers.The β-carotene and its *cis* isomers and in most cases, the degradation of *cis* isomers proceeded faster than its formation.

Table 2: Effect of various storage conditions on lycopene, β-carotene and vitamin A contents of tomato ketchup

Treatment / constituents	Lycopene (μg/100g)		*β*-carotene (μg /100g)		Vitamin A (IU/100g)	
	Ziro time	After 3 months	Ziro time	After 3 months	Ziro time	After 3 months
T1	52669	53932	1375	1390	2276.11	2319.08
T2	86807	87300	2239	2250	3697.48	3753.90
T3	59267	59364	1524	1530	2546.92	2552.65
T4	53048	53156	1359	1370	2279.66	2285.71

T1 = Storage at ambient temperature and in dark
T2 = Storage at ambient temperature and light exposure
T3 = Storage at 4°C and light exposure
T4 = Storage at 4°C and in dark
Retinol = Zero
1 IU vitamin A = o.6 μg β-carotene
1 IU vitamin A = 0.3 μg vitamin A alcohol
1 IU vitamin A = 0.34 μg Retinol acetate

The increased formation of lycopene-5, 6-diol during storage at 45°C is consistent with the observed increased degradation of all- *trans* lycopene during storage at 45°C, as compared to storage at 6°C in the dark or light exposure at room temperature. This means that in the presence of oxygen, high temperature had the most unfavorable effect on lycopene stability because it increased autoxidation of lycopene (Anguelove & Warthesen, 2000). Extracted lycopene rapidly degraded when exposed to light, oxygen and high temperatures (Brumann & Grimme, 1981). Furthermore, Pesek & Warthesen (1987) reported that the degradation rate of lycopene was lower than *β*-carotene when a vegetable juice containing lycopene was exposed to light at 4°C for 8days. Obviously, the stability of lycopene may be variable in different food systems because of the complex nature of food components.

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ثبات الليكوبين والبيتا ـ كاروتين وفيتامين أ فى الطماطم وبعض منتجاتها

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تعتبر الطماطم من المنتجات واسعه الأنتشار والتى يتم استهلاكها بكميات كبيرة وذلك لما تحتويه من مواد مضادة للأكسدة تتمثل فى الليكوبين والبيتاكاروتين وفيتامين (أ) ولذا فإن للطماطم ومنتجاتها فوائد صحية.

أهتمت هذه الدراسة بتقدير وتقييم محتوى الطماطم ومنتجاتها من الليكوبين - البيتاكاروتين- وفيتامين (أ) وتم تصنيع بعض منتجات الطماطم معملياً (العصير، المربى، المعجون مضافا إليه زيت وغير مضاف إليه زيت، بيوريه، صلصة، شوربه، مسحوق، كاتشب) وتم أستخدام جهاز كروماتوجرافيا السائل عالية الإظهار (HPLC) فى تقدير تلك المكونات.

وقد أوضحت نتائج الدراسة أن تركيز كل من الليكوبين، بيتاكاروتين، فيتامين(أ) فى منتجات الطماطم المصنعة تراوحت بين ٢١٤٩٥٢ - ٦٥٩٦ ميكروجرام / ١٠٠جم و٥٥٤٠-١٧٠ ميكروجرام / ١٠٠جم و ٩٢٤.٩٤ -٦٣.٢٨٣ وحدة دولية / ١٠٠جم على الترتيب. وقد وجد أن أعلى تركيز لكل من الليكوبين، بيتا-كاروتين، فيتامين(أ) كان فى عينات معجون الطماطم المضاف إليها زيت يليها عينات معجون الطماطم غير المضاف اليها زيت. وقد وضحت النتائج أن المعاملة الحرارية للطماطم تؤدى إلى زيادة تركيز كل من الليكوبين، وبيتاكاروتين، فيتامين (أ).

وقد دلت النتائج أن ثبات كل من الليكوبين، بيتاكاروتين، فيتامين (أ) يتأثر بالعوامل البيئية مثل :- درجة الحرارة، الضوء. كذلك فقد تبين أن زيادة تكون الليكوبين، بيتاكاروتين، فيتامين (أ) فى عينات الكاتشب خلال عمليات التخزين سواء على درجة حرارة الغرفة أو فى الظلام تتأثر بوجود الضوء مقارنة بالعينات التى تم تخزينها فى الظلام وكانت الزيادة فى تكون تلك المركبات أكبر فى العينات التى تم تخزينها على درجة حرارة الغرفة مقارنة بتلك المخزنة على ٤°م.

مما تقدم ينصح باستهلاك الطماطم ومنتجاتها لما لها من فوائد صحية كبيرة فى الحماية من أنواع مختلفة من السرطانات مع الأخذ فى الأعتبار أن الفائدة تزيد بتصنيع الطماطم فى صورة منتجات نتيجة عملية التركيز.