ISSN: 2347-467X, Vol. 07, No. (2) 2019, Pg. 532-546

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Extraction, Purification, and Characterization of Lycopene from Jordanian Vine Tomato Cultivar, and Study of its Potential Natural Antioxidant Effect on *Samen Baladi*

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Abstract

Lycopene is one of the carotenoids, a fat-soluble pigment that has antioxidant properties. Lycopene was extracted from vine tomato wastes, characterized and then introduced to Jordanian traditional sheep ghee (*Samen Baladi*). The quality attributes in term of peroxide value PV, acidity (%FFA), refractive index (RI) and iodine value (IV) were analyzed according to reference methods. CIE C*.H*. L* colour is measured using Konika Minolta CR-400 Chroma Meter handheld. All parameters were analyzed after storage for one month at room temperature (RT) and at 4°C.

Results showed that lycopene content in vine tomato wastes was 218.74 ppm, it has twice antioxidant power compared with that of ascorbic acid using DPPH scavenging method. The stability of lycopene was affected significantly after storage as its UV/ Vis spectral profile was changed dramatically.

Comparing the *Samen Baladi* with lycopene, the PV, and FFA% were significantly $p<0.05$ lower than those of *Samen Baladi* without lycopene while the IV was significantly $p<0.05$ higher. Upon storage for one month, at RT and 4°C, there were no significant differences in colour during storage, but those with lycopene were significantly $p<0.05$ different than

Article History

Received: 19 November 2018
Accepted: 20 April 2019

Keywords

Antioxidant;
DPPH;
Samen Baladi;
Lycopene;
Oxidative Instability.

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Doi: http://dx.doi.org/10.12944/CRNFSJ.7.2.22

those without lycopene in term of C*.H*. L* colour scale indicating that those with lycopene were duller and darker and the hue shifted to red.

We can conclude that lycopene may be a promised natural product to act as natural antioxidant but concerning the changes in colour, it may be a challenge in marketing as it negatively affects customer preference toward this sensory attribute.

Introduction

Lycopene is one of the carotenoids that naturally occur in many fruits and vegetables and found to be measured in the blood serum.[1] It is found mainly in tomatoes and other red fruits and vegetables.[2] Shi *et al.*,[3] reported that higher amounts of lycopene is found in fruits such as of watermelon, guava and pink grapefruit.[2-4]

Lycopene, is a fat-soluble pigment, and has antioxidant and antitumor properties, which proved to reduce the risk of cancer[5,6] and cardiovascular diseases,[6-8] as it was proven to lower the oxidative stress by either trapping the reactive oxygen species thus increasing the antioxidant potential9, or reducing the oxidative damage to lipids, proteins, and deoxyribonucleic acid.[6] From chemical point of view, lycopene is an acyclic carotenoid with a long chain of hydrocarbon. It is an isomer of beta-carotene, known with different names; 2,6,10,14,19,23,27,31-Octamethyl-dotriaconta 2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene, 4,4-carotene 9 ψ,ψ-carotene Lycopin, (all-E) lycopene, and all-trans lycopene as well.[9,11] Its molecular weight is 536. 89 and molecular formula is $C_{40}H_{56}$ (Figure 1).[12,13] It is a lipophilic compound with hydrophobic characteristics due to eleven linear conjugated double bonds and two unconjugated double bonds (Figure 1), which make it more soluble in organic solvents such as chloroform, hexane, benzene, methylene chloride, acetone and petroleum ether.[14] The chemical structure of lycopene is proposed to have increased affinity for singlet oxygen and radical scavenging capacity.[6,12,15,16]

The stable predominant thermodynamically form of lycopene in tomato is the all-trans lycopene form.[2] Heat, light, and chemical agents cause unstable isomer of lycopene which convert it into its cis- form,[2,14] meaning that during processing and storage of tomatoes, the lycopene may change into its cis- isomer, resulting in an unstable, energy-rich station.[3,14]

Currently, lycopene as a natural antioxidant, has attracted attentions because of its biological and physiochemical properties,[17] accordingly; its extraction and purification are highly requested.[13,18]

Ghee, the anhydrous butter fat is produced at home and small scale levels in many of the Middle East areas from sheep and goat milk[19] and it is well-known with different names, *samin, samna, samuli*...etc.[20] In Jordan, the traditional ghee is well known as *Samen Baladi*. It is one of dairy products produced from bovine, goat or sheep milk or mix.[20] The *Samen Baladi* produced from sheep milk is mostly preferred by the Jordanian consumers, and it's more expensive than those produced from bovine or goat (Personal communications). Ghee is manufactured in different methods based on (1) the raw material used: milk, cream, butter, (2) any required pre-treatment step for the raw materials, and (3) the handling of the semi-finished or fully formed ghee.[20] In Jordan, *Samen Baladi* has no controlled unified recipe, but generally it is produced at a small scale home-made industry, based on heat clarification of the butter, followed by addition of spices and herbs of producer specifications.[19] Among the herbs used are *Ruta graveolens, Melilotus officinalis*, thyme,

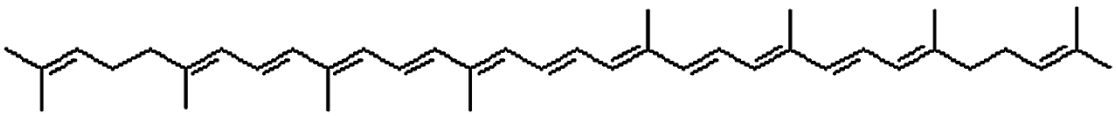

Fig. 1: Chemical Structure of Lycopene; Source, Molecular Weight= 536.89, Exact Mass= 536, Molecular Formula= $C_{40}H_{56}$, Molecular Composition= C: 89.49%, H: 10.51% [12]

and mint. Some producers use pieces of fruits such as apple to induce a special flavor. The Jordanian Organization of Standards and Metrology, has issued a technical rule (JS 201:2009) that stated the quality and health criteria of the Ghee: Peroxide Value (PV) less than 0.6m Eq O_2/ Kg, acidity <0.4% expressed as oleic acid, Refractive index (RI) 1.4524- 1.4561 at 40°C, and unfortunately the shelf life was not well specified.[21]

It is well known that Ghee develops oxidative and hydrolytic rancidity[19,22] and the extent of rancidity depends on storage conditions like light, air, and temperature or other bad practices.[23] The physicochemical changes that alter the quality of ghee or oils may be due to the high temperature of the frying or cooking process,[24] the compounds formed in deteriorated oil as a result of elevated temperature of cooking tend to increase foaming, colour intensity, viscosity, density, amount of polymeric and polar compounds as well as the free fatty acid content.[25] The reactions during the frying/ cooking process and storage conditions depend on factors such as the original quality of the oil, type of oil, concentration of antioxidants and oxygen.[25-27] Excessive lipid oxidation leads to formation of off-flavors and accumulation of oxidized chemical compounds such as aldehydes, ketones and many other organic acids[28,29] leading to many nutritional and health problems in addition to dramatical effect on product quality and its shelf life.[30] Incorporation of antioxidants can play a significant role in retarding lipid oxidation reactions in fat-based food products.[29,30] Among synthetic antioxidants the BHA and BHT, which are widely used as food additives, but currently their application has been questioned due of its possible carcinogenic components formed during their degradation.[28] Hence, there is a need for natural antioxidants that can be used for elongation of the shelf life and oxidative stability of stored products, among these natural antioxidants, the lycopene.

Table 1: Rf values for Purified Lycopene from Jordanian Cultivar Vine Tomato Wastes

Elution Development System	Rf
Petroleum ether: Dichloromethane 95: 5	0.962
5% methanol in Toluene	1.00
Toluene: Hexane 1:19	0.986

Despite its use standalone or in food preparations, the quality and stability of the traditional *Samen Baladi* have not been systematically investigated in Jordan, accordingly, this research is aimed to incorporate the lycopene extracted and well characterized from one of well-known cultivars of tomato in Jordan as a potential natural antioxidant, that may have a role in stabilizing the *Samen Baladi* and take the advantage of addition of a functional nutrient into such product.

Materials and Methods

Raw Materials, Chemicals and Reagents

Jordanian vine tomato cultivar (*Solanum lycopersicum, Var. cerasiforme*) was purchased from the local market. A freshly prepared sheep butter was purchased from a local market and kept refrigerated for further processing into *Samen Baladi*. Ruta commonly known as rue (*Ruta graveolens*) herb (known traditionally as Fagin), mint, Turmeric, bulgur, thyme leaves and salt were purchased from local market. Chemicals and reagent were purchased locally.

Tomato Sample Preparation

The fresh tomato was washed with tap water, followed by washing with distilled water (DW), then blended using home blender, the mixture was washed with running distilled water, the homogenate of seeds and peels were strained, then spurned to remove the free water, then dried by air forced oven at 50°C for 2 days. The dried seeds and peels were ground by local grinder and kept sealed at 4°C for further work.

Extraction of Crude Lycopene

The extraction of crude lycopene was done following Ranganna (1986)[31] with slight modifications. Thirty grams of the dried powdered tomato waste were transferred into a beaker containing 150 ml acetone, and agitated with magnetic stirrer for 30 min. The filtrate was taken, and re-extracted with extra 150 ml acetone for another 30 min. Filtrate was collected, and the extraction was repeated until the colour disappeared. Sixty (60) ml of petroleum ether was added into a separatory funnel and a small portion of the acetone extract was added as well. DW was

slowly added along the walls of the funnel. Two phases were separated, and the lower aqueous-acetone phase was discarded. Another portion of the acetone extract was added and the partitioning with petroleum ether was repeated until all of the extract was transferred into petroleum ether, then successive washings with DW were used to remove the residual acetone. The petroleum ether phase was collected and dried with anhydrous sodium sulfate, then evaporated by rotary evaporator at 35°C until the final volume (5 ml) resulted.

For quantification of lycopene, method of Rodriguez-Amaya and Kimura was followed.[32] fifty (50) µl aliquot of petroleum ether phase containing lycopene was taken into 10 ml volumetric flask and diluted to mark with petroleum ether. Absorption was measured using a spectrophotometer (Labomed UVD 2950 USA, spectrophotometer), in a 1 cm cell at 470 nm. Petroleum ether was used as a blank. Lycopene content was calculated using the equation below.[32]

Total Lycopene Content (µg/g)= A x volume (ml) x 10^4 / $A^{1\%}_{1cm}$ x sample weight (g)

Where A = absorbance; volume = total volume of extract, $A^{1\%}_{1cm}$: absorption coefficient of lycopene in petroleum ether = 3450. Multiply by 1000 to give the lycopene content in ppm.

Crude lycopene was tested for its refractive index and its DPPH scavenging power.

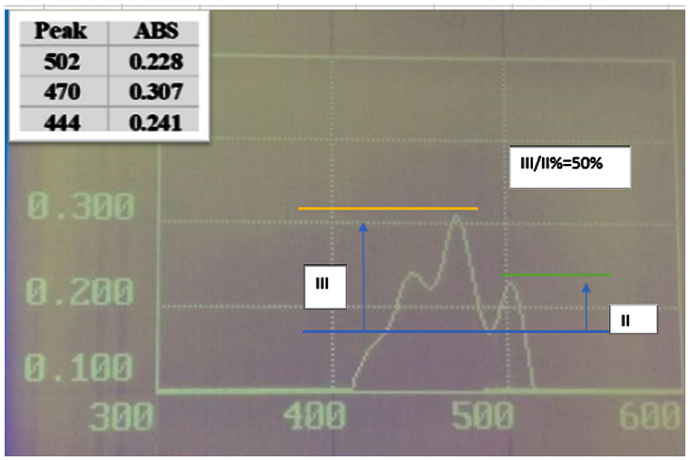

Fig. 2 (a): Lycopene UV/ VIS Spectra of Jordanian Vine Tomato Cultivar

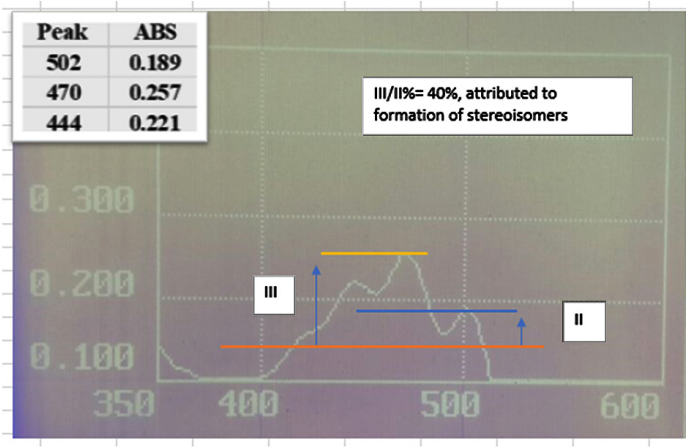

Fig. 2 (b): Lycopene UV/ VIS Spectra of Jordanian Vine Tomato Cultivar after Incubation for one month at RT, in Dark

The crude lycopene extracted and partitioned as mentioned above, was purified using column chromatography.[33] Purification was carried out to remove impurities and other carotenoids,[32] according to Lehman,[33] where, neutral activated alumina (activation in oven at 105°C/ 4h) was used as adsorbent. Acetone: hexane 1:9 mixture was used to elute carotene and lycopene from the column,[33] the purified lycopene was tested for its Vis-spectrum profile and TLC.

Thin Layer Chromatography (TLC)

The purified lycopene was subjected to TLC screening method.[32] The TLC was carried out using aluminum sheets (20 × 20 cm) pre-coated TLC Silica gel 60F254 sheets (Merck KGa A, Germany). Three elution solvent systems were selected; (1) petroleum ether: dichloromethane 95: 5, (2) 5% methanol in toluene and (3) toluene: hexane 1:19. The solvent was used and eluted in covered TLC developing tank. Visualization was performed using UV lamp.[32] The Rf value was measured; Rf = distance from origin to component spot (cm)/ distance from origin to solvent front (cm).

Detection of Vis-Spectroscopy Profile

For confirmation of lycopene and obtaining a fingerprint for lycopene extract the Visible- Ultraviolet spectra was conducted.[32,34] The purified lycopene was dissolved in ether and scanned from 300-600 nm wavelength. The %III/II is measured for the extracted spectrum, after purification and after storage for one month at RT. The %III/II is the ratio of the height of the longest-wavelength absorption peak, designated III, to that of the middle absorption peak, designated II, taking the minimum between the two peaks as the baseline, multiplied by 100.[32,34]

Measuring DPPH Radical Scavenging Capacity

Measurement of the scavenging ability of antioxidants was conducted towards a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as per Brand-Williams *et al.*,[35] with slight modification. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors.[36,37] A 0.1 ml of the crude lycopene in acetone at different concentrations was added to 3.9 ml of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (281689 Aldrich,) solution (20 mg/L in methanol). Methanol is conducted as control. The mixture was shaken well, incubated at room temperature for 30 min in dark, and then the absorbance at 517 nm was measured using (Labomed UVD_2950 USA, spectrophotometer). L-Ascorbic Acid (Sigma Aldrich 95210) was used for comparison. DPPH% inhibition = [(A1-A2)/A1] X 100, where A1 = the absorbance of the control reaction; A2 = the absorbance in the presence of the sample. The $IC_{50\%}$ value is the concentration (ppm) at which the scavenging activity was 50%.[38] Analysis was carried out in duplicate and the mean values with ± SEM are presented.

Preparation of *Samen Baladi*, with Crude Lycopene

Samen Baladi was made in the laboratory under controlled hygienic conditions following one of traditional methods used in Jordanian market. Sheep butter was washed with distilled water, transferred to heating pan, with distilled water, and heated to melt all the butter. Then melted mixture was transferred to refrigerator until assuring solidification. A hole was made to drain the water, and the fat now is ready for heating and skimming. Bulgur with salt was added to remove water traces, after finishing the skimming, the spices and herbs were added (Fagin, thyme leaves, mint leaves, and turmeric powder),

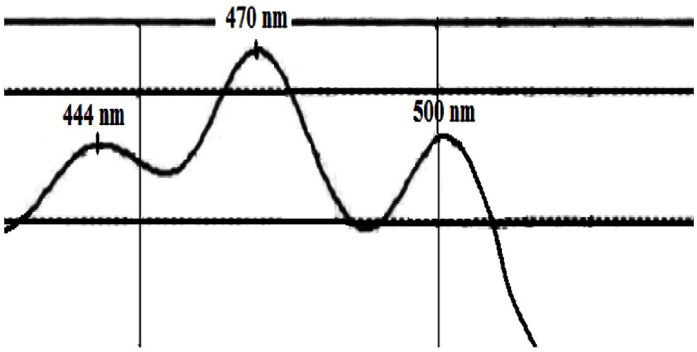

Fig. 3: Lycopene UV/ VIS p (Source[50])

heating continued until temperature reached 125°C. two batches were prepared one serve as control, the other is fortified with 250ppm crude lycopene. Samples were divided into:

- Zero-time samples (initial).
- One-month incubation at 4°C.
- One-month incubation at Room Temperature RT (26-32°C).

All samples were stored out of direct sunlight. All samples were analyzed for: PV, acidity, IV, RI, and colour.

Determination of Peroxide Value "PV"

Peroxide value was analyzed at zero-time and after one month stored at RT, and at 4°C according to AOAC[39]. Readings are represented as average of duplicate readings ± SEM. In brief, 5g of well homogenate *Samen Baladi* samples, was weighted into 250 ml stoppered conical flask then 30 ml of peroxide value solvent (3glacial acid:2 chloroform) was added to the sample then 0.5 ml saturated KI was added then the solution was shaken well and kept incubated at dark for exactly one min. Sample then was titrated with sodium thiosulfate standardized 0.01N using 0.5 ml starch solution as indicator until the endpoint "colourless". Blank was conducted.

PV expressed as mEqO2/ Kg= (V * N * 1000)/weight of sample. Where, V = ml of Sodium Thiosulphate consumed for sample - ml's consumed for blank. N = Exact normality of sodium thiosulphate solution.[39]

Table 2: The Inhibitory Percentage of Crude Lycopene Extracted from Jordanian Vine Tomato Wastes

Concentration ppm	DPPH% inhibition
0	0
21.87	24.69
2.19	12.84
0.22	7.41
0.02	7.16
Y = 0.7550*X + 8.437, correlation coefficient value ® = 0.9469, The $IC_{50\%}$=55.05ppm	
The $IC_{50\%}$ of Ascorbic acid: 110.85ppm	

Determination of Acidity (FFA %)

Acidity expressed as % of oleic acid as per AOAC[39] for samples at zero-time and after one month stored at RT, and at 4°C. Readings are represented as average of duplicate readings ± SEM. Briefly, 5gm is homogenate samples was weighted in Erlenmeyer flask. 50 ml of freshly neutralized ethyl alcohol then is added and boiled to insure well dissolving. Drops of phenolphthalein indicator solution was added. Titration is performed using 0.01N NaOH until the end point (pink colour stable for at least 15sec).

Acidity is expressed as % of oleic acid = (28.2 x V x N)/ Wt.; where: V = Volume in ml of 0.1N sodium hydroxide used, N = Normality of the potassium hydroxide solution or Sodium hydroxide solution, and Wt. = Weight in g of the sample.

Determination of Iodine Value "IV"

Iodine value expressed as mg I2/ 100g oil were analyzed for samples at zero-time and after one month stored at RT, and at 4°C, were determined as per AOAC.[39] Readings are represented as average of duplicate readings ± SEM. In a 500-mL glass-stoppered flask place 0.5gm of the sample. 25 ml of CCL_4 is added to dissolve the sample, then exactly 25 ml of Wij's solution was added and mix well. The flask is closed with stopper, and allow to stand in dark, for exactly 30 minutes with occasional shaking. Add 20 mL of potassium iodide solution (10%) of distilled water, and shake. Then, titration is performed until the endpoint using 0.1N standardized sodium thiosulfate (1 mL of starch 1% is added as indicator). A blank is conducted for back titration.

Iodine value = (a–b)*12.69*N / Weight (g) of sample, where; a: Volume (ml) of standardized 0.1N sodium thiosulfate consumed by the blank, b: Volume (ml) of standardized 0.1N sodium thiosulfate consumed by the sample, N: Exact normality of sodium thiosulfate.

Detection of Refractive Index "RI"

Refractive Index RI of the crude Lycopene was determined for characterization of the extracted tomato lycopene. Moreover, the RI is determined for samples of the prepared *Samen Baladi* at zero time and those incubated for one month at different conditions as well. Refractive Index is determined using digital refractometer (Reichert, 1310499, USA). All samples were heated to 40°C on water

bath, then the readings were obtained automatically from the device. Correction was made as per the following equation: RI $^{@40°C}$=RI instrument reading $^{@20°C}$ +(0.000385 *20). Readings were taken in duplicates, and presented as average± SEM.

Determination of Colour

Konika Minolta CR-400 Chroma Meter handheld, portable instrument was used to evaluate the colour of *Samen Baladi*. The identifying of Colour Differences was performed using CIE L*C*H*, Where, The L*C*h colour space using cylindrical coordinates. In this colour space, L* indicates lightness, C* represents chroma, and H* is the hue angle.

Statistical Analysis

Analysis was carried out in duplicate and the mean values with ± SEM are presented. Data obtained were analyzed using SAS statistical program.[40] Results were performed by ANOVA, and values were given as means ± SEM, while means were separated using LSD range test. Differences between treatment groups were tested by student's t test and paired t test. Levels of significance will be tested at P<0.05.

Results and Discussion

Lycopene Content and Characterization

The result revealed that lycopene content in dried Jordanian cultivar vine tomato waste was 218.74 measured at 470 nm and where ether was used as solvent, and molecular coefficient of 3450 with RI reading equals 1.4847 ± 0.0001. It was found that the vine tomato waste has a good source of natural lycopene that may exert possible promise for utilization as a functional nutrient in food industry additionally to its lipophilic antioxidant properties.

A study in Egypt, conducted on fresh waste of tomato released from tomato processing line, Basuny *et al.*,[41] reported that lycopene content was 145.5 ppm. In their study on the antioxidant activity of lycopene in different fractions of tomatoes; skin, pulp, and seed; Toor and Savage[42] revealed that lycopene content was 87, 28 and 16 ppm respectively.[42] Another study of Alda *et al.*,[43] on tomato and tomato products showed that lycopene content of fresh tomatoes was approximately 120 ppm, while the lycopene content in tomato paste approximately was 160 ppm, and in tomato boiled sauce was around 40 ppm, tomato Ketchup 170 ppm, and for those lycopene in spaghetti sauce was 160 ppm.[43] A study of Wawrzyniak *et al.*,[44] on polish market for tomato showed that lycopene content of fresh tomatoes ranged from 12.1 to 64.3 ppm and the average content of tomato pastes was 388.8 ppm, of ketchups was 111.2 ppm, and of tomato juices was 70.5 ppm.[44] A study on eight cultivars of tomatoes, conducted to evaluate the effect of thermal heat on lycopene contents, Temitope, and his colleagues[45]

Table 3: Data of PV, Acidity, IV, and RI of Semen Baladi with Lycopene compared with *Samen Baladi* without Lycopene at Zero time, and after incubation for One Month at RT (26 -32°C) and at 4°C

	Without Lycopene				With Lycopene			
	PV	FFA	IV	RI	PV	FFA	IV	RI
Zero time	$^{a}0.94_{c}$± 0.03	$^{a}0.09_{b}$± 0	$^{a}36.28_{a}$± 0.57	$^{a}1.4611_{a}$± 0	$^{a}0.90_{c}$± 0.02	$^{a}0.09_{c}$± 0	$^{a}36.79_{a}$± 0.79	$^{a}1.4613_{a}$± 0.0001
Ref temp	$^{a}2.56_{b}$± 0.02	$^{a}0.32_{a}$± 0.02	$^{a}34.74_{b}$± 0.024	$^{a}1.4610_{a}$± 0.0001	$^{b}1.80_{b}$± 0.06	$^{b}0.26_{b}$± 0.01	$^{a}34.73_{b}$± 0.07	$^{a}1.4609_{a}$± 0.0001
RT (26-32°C)	$^{a}5.00_{a}$± 0.24	$^{a}0.36_{a}$± 0	$^{b}32.76_{c}$± 0.1	$^{a}1.4612_{a}$± 0	$^{b}3.60_{a}$± 2.55	$^{b}0.28_{a}$± 0	$^{a}34.50_{b}$± 0.07	$^{a}1.4612_{a}$± 0.0001

PV: peroxide value expressed as $mEqO_2/kg$, FFA: free fatty acids expressed as %oleic acid, IV: iodine value expressed as mgI_2/g. Values represented the mean of duplicates ±SEM.

Different letter on the subscript at the same column indicate significant difference p<0.05.

Different letter on the superscript at the same raw between the blocks for the same test parameter indicate significant difference p<0.05.

reported that the lycopene content ranged from 70- 147 ppm. It has been published that lycopene content in the skin of tomato in Iraqi cultivar used for processing was 12 mg/100 g, and 54 mg/100 g in skin of tomatoes from Canada, while it ranged from 5- 14 mg/100 g in the skin of tomatoes grown outdoors in India as stated by Toor and Savage.[42]

Tomato waste as no commercial value and is currently disposed as a solid waste or used for animal feeding.[46-48] Currently, the management of these wastes is considered to be a worldwide problem for environmental and economic aspects.[48] These wastes are rich source of phytochemicals that could provide a potential source of natural lycopene.[16,48] The compositional variation in lycopene content in tomato is affected by consequence of varietal differences, climatic conditions, agricultural variables, stage of maturity, harvesting and post-harvest handling, conditions during storage, and transportation.[16] Generally speaking, the skin of tomatoes has been found to be richer sources of lycopene than the water insoluble fraction and the fibrous fraction[17,42,49] and processed tomato has higher amount compared to fresh tomato.[48] Differences in this study and those in the literatures may be attributed to many variables; among these the extraction methodology, the quantification parameters, cultivar type, and raw material analyzed in addition to differences in growing conditions.

Extracted and purified lycopene from the Jordanian vine tomato cultivar wastes were characterized through its TLC, and UV/ VIS spectral profile.

TLC silica gel chromatogram result of lycopene, developed with two development system: the 5% methanol in toluene and petroleum ether: dichloromethane 95: 5 is shown in the Table (1). The extracted, purified lycopene spots have special behavior on TLC, where the spots continue migration until almost the edge of the solvent front indicating the absence of functional groups in lycopene. It has been well documented that the influence of the double bonds is demonstrated by the adsorption affinities of the acyclic lycopene.[32,34]

The UV/visible absorption spectrum can be used as a first clue for the identification of lycopene.[32,34] The λ_{max} values of the lycopene in petroleum ether extracted and purified from Jordanian vine tomato cultivar wastes was shown in Figure (2a) as well the shape of spectrum. Lycopene absorb maximally at three wavelengths, resulting in a three-peak spectrum as documented in the literatures[32,34] and this agrees with the result of this study. The %III/II was measured. The %III/II is defined as the ratio of the height of the longest-wavelength absorption peak, designated III, to that of the middle absorption peak, designated II, taking the minimum between the two peaks as the baseline, multiplied by 10032, 34. The %III/II is a good indicator for successful purification and reflects any degradation that may take its place with time of storage.

A scanning of purified lycopene in ether (Figure 2b) after incubation period of one month in dark at room temperature RT (26-32°C), revealed a significant decrement in III/II% ($p<0.05$) and a peak in the UV-range (λ_{max} 360). This is can be explained by possible formation of different stereoisomers as a result of isomerization of all-*trans* lycopene into *cis*- lycopene, evidenced that lycopene biostability is affected by time of storage. These results are compatible with

Table 4: The L*C*H of Samen Baladi without lycopene and with lycopene

	Samen Baladi without Lycopene			Samen Baladi with Lycopene		
	L	C*	H	L	C*	H
Starting point	a55.62a	b20.01a	a121.75a	b53.77a	a23.01a	b100.09a
At 4°C	a55.50a	b21.34a	a121.66a	b53.50a	a22.91a	b101.10a
At RT (26-32°C)	a56.01a	b20.04a	a120.60a	b52.50a	a23.21a	b99.96a

Different letter on the subscript at the same column indicate significant difference $p<0.05$.

Different letter on the superscript at the same raw between the blocks for the same parameter indicate significant difference $p<0.05$.

the literatures that describing the degradation forms of lycopene and the formation of cis- form as storage time increased.[2,3,50,51]

The results of this study showed that the %III/II was 50% for purified lycopene (Figure 2a) compared with 40% of purified lycopene stored for one month, at RT and dark conditions (Figure 2b). As shown in Figure (2b), a new peak encountered at 360 nm in lycopene stored for one month, indicating the formation of stereoisomers during storage time.

Rodriguez-Amaya and Kimura,[32] indicated that the %III/ II of standard pure lycopene extracted was 65%. This was not our case as this significant difference may attributed to many reasons, the cultivar type and seasons effect, extraction method, endogenous enzymes effect during extraction, and environmental condition of the test.

The findings of Bunghez *et al.*,[50] (Figure 3), has been shown that the %III/ II is less than 60% which is also consistent to our findings.[50] Differences may be attributed to differences in methodology, raw material used and experimental design of the treatment.

Lycopene DPPH Scavenging Power

Antioxidants delay or prevent the oxidation of oxidizable materials by scavenging free radicals and minimizing the oxidative stress resulting from the reactive oxygen and/or nitrogen species.[52] These species can cause damage to the DNA as well as initiation of peroxidation of membrane lipids.[6,49] Many methods were proposed to measure the antioxidant activity.[6,37,49,53,54] These can be categorized in to two depending on the functionality: methods that depend on measuring the potential of donating an electron or α hydrogen atom to a specific ROS/ RNS, such as DPPH assay,[37] ABTS • assay: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), superoxide anion scavenging assay and Hydroxyl radical scavenging assay or measuring the ability to remove any source of oxidative initiation.[52,53]

Many authors showed that using DPPH method to evaluate the scavenging properties of lycopene could not be precise due to lipophilic tendency of lycopene toward non-polar solvents in addition to its insolubility in methanol.[9,55-57] In this current study, extracted lycopene was dissolved in acetone, then the aliquots were taken to be measured following DPPH method.

The inhibitory percentage of DPPH scavenging power of crude lycopene extracted and dissolved in acetone from Jordanian vine tomato wastes is shown in (Table 2). The DPPH scavenging power of lycopene extracted was compared with that of ascorbic acid. It has been found that the $IC_{50\%}$ DPPH inhibition percentage of lycopene was 55.05 ppm compared to 110.85 ppm for ascorbic acid, meaning that lycopene has twice antioxidant power of that of ascorbic acid. It may be suggested that lycopene extracted from vine tomato waste may exert a natural and available source of lipophilic antioxidants

These findings agree with those of Kaur *et al.*[54] who reported that lycopene $IC_{50\%}$ value of DPPH radical scavenging activity was 53.341ppm which was so close to our findings as shown in table (1). In their study, Pinela *et al.*[58] revealed that Portuguese tomato cultivars could contribute as sources of important antioxidants because of distinguished $IC_{50\%}$ of DPPH scavenging properties they may exhibit. Similar findings were reported by Borguini *et al.*,[57] when evaluating the antioxidant activity of the organic and conventional tomato cultivars but in different solvents extracts, results showed a high antioxidant activity when using DPPH scavenging method, nevertheless that antioxidant activity may attributed not only to lycopene, but to other polyphenols and ascorbic acid contents in tomatoes.[57]

Study of Chemical and Physical Analyses of *Samen Baladi* with Lycopene Kept for One month at Refrigeration and Room Temperature Condition (26 - 32°C)

Table (3) shows the PV, acidity, RI, and IV of *Samen Baladi* with lycopene compared with those without lycopene at zero point and after incubation for one month away from direct light at RT and at 4°C.

At zero time of preparing the Samen, PV, IV, acidity and RI were measured, it has been found that there were no significant differences ($p \geq 0.05$) between the blocks in the parameters of PV, acidity, IV and the RI.

Peroxide Value

It has been documented that oxidative rancidity could be major problem during ghee storage.[19] Peroxide

value is one of the most widely used chemical tests for the determination oxidative rancidity and the fats and oils quality[30,59] Determination of PV does not provide a complete evaluation of fats and oils flavor because of the intermediate transitory nature of peroxides and their breakdown to non-peroxide materials as increasing with time of storage.[30,59] Samples in this study were kept for one month, accordingly, the expected chance to reach the transitory nature of peroxide was neglected.

After storage for one month, the PV values increased significantly ($p<0.5$) in all treatments. The cumulative increment in samples with lycopene were significantly ($p<0.05$) lesser than those samples without lycopene. As shown in the Table3, the PV with time increase significantly ($p<0.05$) in treated samples and that of control, but at temperature of 4oC, the increment in PV was less than those at RT significantly ($p<0.5$). The PV of sample with lycopene was 1.80 mEqO2 which was significantly lower ($p<0.05$) than that without lycopene (2.56 $mEqO_2$) at refrigeration temperature of 4°C. While at RT, the PV values were 3.6 and 5.0 $mEqO_2$ for lycopene treated samples and the control respectively. Hence, the accumulation of peroxides in samples with lycopene were reduced with 40% compared with those samples without lycopene in both studied conditions. It can be suggested from these results that the addition of lycopene may play a positive role in controlling the PV development either in refrigerated conditions or at RT conditions.

Similar findings were reported by Siwach *et al.*,[22] who have conducted their study on lycopene as a natural antioxidant in extending the shelf-life of anhydrous cow milk fat; it has been revealed by their study that the lycopene can suppress the formation of peroxides,[22] as evidenced by significant reduction in peroxide values in samples with lycopene compared with samples without antioxidant addition. Moreover, Rohlik *et al.*,[60] reported that lycopene efficiently decreased lipid oxidation when added to paprika salami recipe and mention that lycopene has the same effect of protection in several meat products. [60] Accordingly, as evidenced in our results, and findings of the literatures, lycopene may add to suppress the oxidative rancidity in *Samen Baladi*.

Acidity

Results of acidity measured in term of FFA and expressed as % oleic acid had indicated that there are significant increases ($p<0.05$) in free fatty acid content between initial and after one-month storage at both conditions. This is consistent with findings of Divya and Vasudevan[23]), who reported that the FFA developed as storage time increased. Amr[19] reported also that as storage time increase, the hydrolytic rancidity represented by the FFA increased.

There were significant differences ($p<0.05$) between the FFA of *Samen Baladi* with lycopene and the FFA of those without lycopene after storing at different temperature conditions for one month. At refrigeration temperature FFA values were 0.26%, 0.32%, respectively with nearly 23% decrement in FFA values in those samples with lycopene. FFA for samples kept at RT for one month, the FFA% were 0.28, 0.36 for samples with lycopene and samples without lycopene respectively, with nearly 29% decrement in samples with lycopene. We can conclude that lycopene may has protective properties against rancidity, and the protective properties were noticed effectively at refrigeration temperature compared with RT (Table 3). The application of natural antioxidant significantly ($p<0.05$) reduced the development of free fatty acids, as it was proven by the results of Ullah *et al.*[61]

It has been documented that the amount of free fatty acids formed during lipid oxidation as a result of secondary oxidation of unsaturated aldehydes and other degradation products of hydroperoxides increased.[27] It was reported as well, that the hydrolytic rancidity (represented by the %FFA) can also be a problem in stored *Samen Baladi*,[19] because the excessive lipid oxidation lead to formation of off-flavors and undesirable oxidized chemical compounds such as the aldehydes, the ketones and many other organic acids.[28,29] The current study showed that the application of lycopene retarded the lipid oxidation this may be attributed to its antioxidant properties, accordingly the excessive degradation and productions of hydroperoxides will be reduced, causing significant decrements in the values of PV and FFA.

Our results were consistent with those of Siwach *et al.*,[22] who studied the lycopene as a natural antioxidant to extend the shelf-life of anhydrous cow milk fat in term of different quality attributes, among these was the effect of lycopene addition on the values of FFA. Moreover, findings of this study were similar to findings of Amr,[19] who studied the effective controlling effect of fennel extract as natural antioxidant on FFA in sheep ghee.

Iodine Value "IV"

There were no significant differences ($p \geq 0.05$) in Iodine values of samples treated with lycopene compared with those of control (without lycopene) at zero time (Table 3). The IV of samples without lycopene decreased significantly ($p < 0.05$) after storage, where the reduction is significantly lower ($p < 0.05$) at RT than those kept refrigerated.

Iodine value (IV) in lycopene treated samples at zero time and after one-month storage at 4°C and at RT were 36.79, 34.73 and 34.50 respectively. There were significant ($p < 0.05$) differences between those stored for one month at 4°C compared with those of control, and significant ($p < 0.05$) differences between those at zero time and those stored at RT, on the other hand, there were no significant differences between lycopene treated samples stored for one month at 4°C, and those samples stored at RT. Compared with results of control samples, there were significant decrement in IV between samples of zero time, those kept at 4°C, and those kept at RT, with values of 36.28, 34.74 and 32.76 respectively.

The decrements in IV with time may be achieved due to oxidation of unsaturated fatty acids of triglycerides.[59,62] Moreover self -antioxidant properties of oil may protect the oil from oxidation and the deduction of iodine value may reduce.[62] This may explain the higher values of IV in samples with lycopene when compared with those without lycopene, meaning that the lycopene as a natural antioxidant may protect the oxidation of unsaturated triglycerides.

In their study, Bukola *et al.*,[62] on the effects of different storage temperature on the iodine value of different cooking oils, it has been observed that iodine value decreases gradually during storage period, and the Iodine value for all oil samples stored in refrigerator after six weeks are higher than those stored at room temperature. Another study of Taghvaei and Jafari[63] conducted to assess the application and stability of natural antioxidant applied to edible oil has revealed that the Iodine values of sunflower oil treated with natural antioxidants of olive oil waste cake extract were higher significantly than those samples without treatment, and the effect on the Iodine value was dose-dependent. These results were consistent with ours in the current study, indicating that lycopene may has an effect on the protection the oxidation as evidenced by the Iodine values' results.

Refractive Index

Refractive index is used as identity indicator that provides useful information about the purity of oils which can be used to detect rancidity or deterioration in edible oil.[64] As shown in Table (3), there were no significant differences in the refractive indices of samples with and without lycopene upon storage at 4°C and RT (26- 32°C) for one month.

Alhibshi *et al.*,[65] reported that RI of oils will increase nonlinearly as the oil is exposed to heat or light.[65] Their study showed significant differences in refractive indices of selected studied oils exposed to light and heat.[65] During one month, all samples were kept away from any direct sunlight, and was not exposed to heat after processing, or air as they were kept tight., On other hand, the products of oxidation process that has taken place within storing time affect the PV readings, acidity, and the IV, but does not accumulate to a level that may affect the RI significantly. The results of insignificant differences in RI in current study were inconsistent to Alhibshi *et al.*,[65] findings, this may be attributed to differences in treatment parameters.

Colour Reading

Table (4) shows the results of the parameters of ΔL^*, ΔC^*, ΔH^* of both *Samen Baladi* with lycopene compared with those of *Samen Baladi* without lycopene.

There were no significant differences ($p \geq 0.05$) between readings of those kept refrigerated and those kept at RT (26 - 32°C) for one month in both blocks of *Samen Baladi* with lycopene and *Samen Baladi* without lycopene. Comparing L*C*H of *Samen Baladi* without lycopene and *Samen Baladi* with

lycopene, shows ΔL* was lighter in the *Samen Baladi* compared with those with lycopene. Visual sensation is compatible with these findings, the same for ΔC* which indicated that the *Samen Baladi* with lycopene is duller compared with *Samen Baladi* without lycopene. The ΔH* was clearly and significantly differ in *Samen Baladi* with lycopene as the colour is shifting to red more than that of *Samen Baladi* without lycopene (shift to yellow scale). However, this is not a surprise and it's well expected that lycopene addition will make *Samen Baladi* darker, duller, and its ΔH* is shifted from yellow scale into red scale comparing with *Samen Baladi* without lycopene addition. It is well reported that application of natural antioxidants will contribute to change in colour, and this current findings aligned with these findings.[22,63] The same effect was seen when lycopene is added to improve the sensory attributes of paprika salamis.[60] On other hand, Patange *et al.,*[66] has reported that as the storage period progressed, the scores for colour decreased significantly after five weeks of storage, and continued to decrease as storing period increased to eleventh week. These findings were inconsistent to current study, due to many factors, among these the storage period, methods of detection and the experiment design.

Conclusion

The Jordanian vine tomato dried waste has a substantial amount of lycopene with twice oxidative scavenging power of that of ascorbic acid. The addition of lycopene as natural antioxidant in to *Samen Baladi* has proven to control both types of rancidity expressed in PV, FFA and IV. The stability of extracted purified lycopene is a time- function, meaning that one should be careful while dealing with lycopene. Lycopene is proven to be GRAS, accordingly it can be added safely as there is no adverse-effect data for lycopene in animals or healthy humans6. The addition of lycopene into *Samen Baladi* produces a duller, darker product and the hue shifted to red, this may be a challenge in marketing due to t customer colour preference. Consequently, we can recommend the utilization of lycopene as natural antioxidant and colorant into *Samen Baladi* as there is a feasible need for functional nutrient and can be used as a promise antioxidant.

Acknowledgment

We would like to thank Dr. Radwan Ajo for his help in the statistical analysis.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest

Authors were cleared that no conflict of interest.

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