Research Article

Extraction and Utilization of Tomato Carotenoids as Antioxidant and Natural Colorants in Sunflower Oil and Spaghetti

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Abstract | In the present study carotenoids from tomato peel was elucidated by HPLC to utilize it as natural colorants in sunflower oil and spaghetti. Tomato peel contained 720 mg/100g of carotenoids consisting phytoene (3.15%) followed by phytofluene (2.31%), β-carotene (2.11%), cis-lycopene (1.71%), lutein (1.51%), cis-ζ-carotene (0.61%), ζ-carotene (0.56%), and γ-carotene (0.52%). Lycopene was found in highest amount (87.52 %). Antioxidant activity of carotenoids from tomato peel was assessed by measuring Rancimat's test and diphenyl-1-picrylhydrazyl (DPPH) by using 50 to 200 mg/kg carotenoids in sunflower oil and spaghetti. The sunflower oil containing 50 to 200 mg/kg carotenoids showed higher antioxidant activity in comparison to 200 mg/kg Butylated hydroxytoluene (BHT). Optimum cooking time, its weight and swelling index were remained same at different level of oil carotenoids tomato peel powder when compared with control. On the other hand spaghetti cooking becomes decrease by increase of carotenoids level in spaghetti when compared with control. Organoleptic evaluation of spaghetti prepared from lycopene revealed maximum score of smell, taste, odors, color texture and overall acceptability when compared with other tested sample and control.

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Introduction

Common colors from plant items have drawn a fair amount of consideration around the world. These pigment show different colors and are comprised of distinctive colors, for example, orange (β-carotene), yellowish-green (lutein), green (chlorophyll), and blue-purple (anthocyanin) (Mortensen, 2006). Lycopene is the red color found in red colors products of the soil, for example, tomato, papaya, pink grapefruit, pink guava and watermelon. Lycopene is a carotenoid hydrocarbon (additionally called carotene). The amplified conjugated double bond arrangement of these compounds is a critical component in the carotenoids in charge of their alluring colors that it frames the light retaining chromospheres (Rodriguez and Kimura, 2004).

The presence of visible colors in these compounds has conjugated double bonds. The more noteworthy the quantity of conjugated double bonds, the higher a wavelength esteem for most extreme assimilation

(Rodriguez, 2001). Lycopene is one of the mainstream colors most important in nourishment industry as nutritional substance for its medical advantages (Rao and Rao, 2007). The compound has gives more importance because of their broad utilization in food, makeup and pharmaceuticals. In the interim, the costs of crude materials are expanding also their accessibility is diminishing (Amin and Mukhrizah, 2006).

The defensive impacts of lycopene have been indicated on oxidative anxiety, cardiovascular infection, hypertension, atherosclerosis, diabetes and others. Then again, there are still no decisive results reported because of the fact studies on the role of lycopene against these diseases is still ongoing. Oxidative anxiety is one of the significant danger elements of chronic disease (Rao and Rao, 2007).

Free radicals or oxidants are potential benefactors prompting oxidative anxiety. In vitro, ex vivo, and in vivo studies have been completed to show the impacts of lycopene against oxidative stress. In this connection, lipid, protein and DNA oxidation are firmly identified with oxidative stress. Lycopene is the red color that assumes an essential part in plant and animals. In human wellbeing, much proof demonstrates that utilization of lycopene rich nourishments can help in avoiding degenerative sicknesses. However, exceptionally restricted studies have discovered a gainful part of the utilization of lycopene alone. The association of lycopene with other dynamic compound is essential for acquiring its ideal capacity in human wellbeing. The peel part of tomato waste contains lycopene up to five times more than the peel (on wet basis), however its high dampness levels and vulnerability to microbial decay make the capacity and handling of this material very tricky (Kaur et al., 2008).

Lycopene utilization as coloring agent in the food, nourishment, nutraceuticals and pharmaceutical commercial ventures, in spite of the fact that its organic properties, as hostile to oxidant and against cancer-causing specialists, have been increasing expanded consideration in the decade ago (Stahl and Sies, 2005). Because of this, lycopene utilization is firmly prescribed for decreasing the danger of atherosclerosis, coronary heart maladies and a few sorts of tumor (Shi and Maguer, 2000). Lycopene, an individual from carotenoid family, and is an antioxidant produced by plants and soluble in lipid mostly it is not produced by human, animals and micro organism (Kaur et al., 2008). Where it serves as an adornment light-assembling colors and secures them against the lethal impacts of oxygen and light. It is a red pigment and good precursor of vitamin A that play important role in rhdophsin cycle. The purpose of the study was to concentrate on the investigation of carotenoids profile from tomato peel and make its utilization as common cancer prevention agent, food colorants in food commodities, for example, sunflower oil and spaghetti with different levels.

Materials and Methods

Roma tomatoes were purchased in Feb, 2016 from the local market of Dera Ismail Khan. They were peel down for further processing in the Laboratory. The solvents utilized for spectral and HPLC study were of ACS grade and acquired from Sigma compound. Refined sunflower free from antioxidant potential were collected from Agriculture Research Institute Ratta Kulachi. Manufactured antioxidant agent, specifically butylated hydroxyl toluene (BHT) and standard carotenoids were of commercial grade.

Extraction of carotenoids from tomato peel

Carotenoids were extracted by method described by (Hackett et al., 2004). Tomato peel was dried in broiler dryer at 40°C until the moisture content come to 6% then ground and passed through 0.15mm strainers. Hundred grams of tomato peel powder and 500 ml of ethanol were added in a beaker mixed for 20 min. The blend was then homogenized for 1 min. After that the blend was separated through Whatman filter papers. The filtrate was blended with 250 mL of acetone/hexane solution (50:50, v/v) and homogenized for 1 min. A separatory funnel was used to separate the non polar hexane layer containing lipid materials from the water-soluble fraction and solvents were evacuated. The crude of carotenoids extracted from tomato peel was kept in container to be solidified then further screening was carried out.

Determination of total carotenoids

Carotenoid was measured by spectrophotometer to (Hornero and Munguez, 2001). Carotenoid removed from tomato peel was dissolved in hexane and the absorbance was measured using spectrophotometer at 472 and 508 nm.

Determination of lycopene

The extracted solution of lycopene samples were analyzed in an HPLC system consisting of a Shimadzu HPLC pump LC-20AD×2 units, Shimadzu 996

photodiode array detector (PDA- UV/VIS detector) and Rheodyne 7725i manual sample injector with a 20-µL sample loop. The system was controlled with Chromatography software (Shimad zu HPLC shimadzu 996 photodiode array detector (PDA-UV-VIS) Rheodyne 7725i).

Determination of carotenoids

The carotenoids (lyco-red) separated from tomato peel were determined by Knauer HPLC pump 64 as indicated by the technique reported by (Gaylek et al., 1987) utilizing octadecyl silane C 18, 3.9 x 150mm. For both HPLC columns, two solvents were used for elution: (1) methanol (2) ethyl acetic acid derivation. The flow rate was 1.8ml/min and absorbance was measured at 475 nm. A mixture of methanol and ethyl acetic acid (54:46) as mobile phase, (sample amount: 20µL, flow, 1.8mL/ min) and identified at 475nm.

Assessment of antioxidant activity

Carotenoid separated from tomato peel was screened as antioxidant agent by Rancimat technique 50, 100, 150, and 200 ppm of the carotenoids removed from tomato peel were blended with 25g of sunflower oil in a conical flask, against a specimen 25g of sunflower oil blended with 200 ppm of manufactured BHT in a flask. The control was sunflower oil. The method was described by Mubarak, (2003).

Physical and chemical characteristics of spaghetti

Moisture, crude protein, crude fat, ash, fiber, minerals, and falling number were determined according to the procedure described in the AOAC (2007). Total carbohydrate was calculated by the Molish and Benedict test.

Sample Preparation

Spaghetti was prepared 72% extract wheat flour, water 35% and salt 1%. Every sample was mixed with different level of carotenoid from tomato peel (1.0, 2.0, 3.0, 4.0 and 5.0%. The blend of ingredient was put in a blending dish (Kitchen Aid Mixer) and blended at speed 1 for 1 min., water was poured and blending was proceeded at speed 1 for 2 min., trailed by blending at speed 2 until the batter solidified. Protected with plastic wrap, permitted to rest around 30 min. hand plied for around 1 min and sheeted with wooden moving pin to around 1.5 cm. thickness. Spaghetti was cut into strips 5mm wide, held tight bar, solidified on air dried for 24 h at 25°C± 2°C. The spaghetti was dried to 6.40% succulence in circulated air through broiler at70°C for 24 h, cooled at room temperature and the delivered spaghetti was pressed in polyethylene pack until dried. Cooking characteristics of spaghetti under scrutiny to be specific ideal cooking time, spaghetti cooking weight, were measured by the method of (Dexter et al., 1983).

Antioxidant capacity

Radical Scavenging Activity (RSA %) test and Free radical Scavenging Activity (RSA) of the sample was measured by using the procedure of (Brand-Williams et al.,1995) also, communicated as percent of inhibition of the DPPH radical and was calculated by the following equation:

$$RSA\% = Abs\,(test) - Abs\,(control) \times \frac{100}{Abs\,(control)}$$

Organoleptic Evaluation

Organoleptic evaluation was done by ten specialists at Food Tech. Research Organization (FTRI). The specialists were requested to evaluate color, taste, and texture and in general worthiness for cooked spaghetti as by the methodology described by (Dexter et al., 1983).

Statistical analysis

All the values from Organoleptic evaluation were taken in triplicates and were analyzed by using Duncan's numerous extent test to significant difference at the 0.05 probability ($p < 0.05$).

Table 1: *Represent the chemistry of peel of tomato.*

Ingredients	Tomato peel
Crude protein	11.01
Ether extract	3.01
Ash	3.99
Crude fiber	49.95
Carbohydrates content	32.04
Total carotenoids (dry weight)	690
Phenolic content mg/100	270.30
Lycopene	580

Results and Discussions

Proximate analysis of tomato peel

Tomato peel was analyzed for proximate chemical composition and data is presented in Table 1. The results demonstrated that, the tomato peel contain 11.01% crude protein (Dry Weight), 3.99% slag (DW), Ash, 3.01% (DW) ether extract 49.95% (DW) and 32.04% (DW) total carbohydrate. Then again, the aggregate carotenoid, absolute Polyphenols and

lycopene in tomato peel were 690 mg/100g, 270.30 mg/100g and 580 mg/100g dry weight respectively. These findings are in agreement with previous investigation (Rodriguez et al., 2004), expressed that, the measure of carotenoid from tomato peels range from 515 to 960 mg/100g (Aghel et al., 2011) found that the lycopene is the significant sources of carotenoid in tomato peel and this color indicated to more than 85% of all carotenoid, its focus can shift from 25 to 220 mg in the crisp items or from 440 to 2000 mg/100g dry weight basis.

Table 2: *Represent the Physical and chemical composition of Flour.*

Component	Wheat flour %
Crude Fiber	0.98
Ash	0.42
Fat	85.21
Crude Protein	12.87
Carbohydrates	85.21
Mg	215.121
Na	130.67
Zn	3.8
Mn	16.5
Fe	5.11
Ca	88.45
K	223.13
Cu	3.7
Falling no	298
Liquefaction no	24.1

Physical and Chemical composition of Spaghetti

Spaghetti were analyzed for the purpose to determine the moisture content, Protein, Fiber, Ash, Fat, Carbohydrates, minerals, Falling number and liquefaction number. The results values indicated that moisture, protein, fiber, ash, fat, and carbohydrates of 72% extract. were: 11.77, 12.87, 0.98, 0.522, 0.42 and 85.21% respectively. This data is in the same line with that obtained by (Farvilli et al., 1997). The mineral content of wheat flour was: 215.121, 130.663, 3.577, 16.443, 5.113, 88.449, 223.132, and 3.633 (mg 100g flour) for Mg, Na, Zn, Mn, Fe, Ca, K, and Cu, respectively. The results were in agreement with those obtained by Bedeir (2004) in minerals contents of Na, Zn, Mn, Fe, Ca, and Cu, but higher in K content and lower in Mg content. These results are agreement with that obtained by (Farvilli et al., 1997) (Table 2).

Carotenoid profile of tomato peel

The carotenoids extracted from tomato peel were run on HPLC and recognized as1) lutein, 2) lycopene, 3) cis-lycopene, 4) γ-carotene, 5) cis-ζ-carotene, 6) ζ-carotene, 7) β-carotene, 8) phytofluene, and 9) phytoene. The results presented in Table 3 and (Figure 1) showed that the lycopene was the real carotenoid which was 87.52 % of aggregate carotenoids from tomatoes followed by 3.15% phytoene, 2.31% phytofluene, 2.11% β-carotene, 1.71% cis-lycopene, 1.51% lutein, 0.61% cis-ζ-carotene, 56% ζ-carotene, and 52% γ-carotene individually. These results are concurrence with (Aghel et al., 2011). Results likewise demonstrated that the lycopene was the prevailed color in tomato peel, trailed by phytoene, phytofluene, β-carotene and cis-lycopene individually, while minor measures of 9-cis-lutein, 13-cis-β-carotene and 9-cis-β-carotene were additionally identified.

Table 3: *Represent the identification of carotenoid extracted from tomato peel.*

Peak	Identification of carotenoid	Area %	Retention time
1	Lutein	1.50	5.86
2	Lycopene	86.13	22.21
3	Cis-lycopene	1.71	25.15
4	ɣ-carotene	0.52	31.6
5	Cis- ζ carotene	0.61	35.8
6	ζ-carotene	0.50	39.1
7	Beta carotene	2.11	41.9
8	Phytofluene	2.31	43.9
9	Phytoene	3.1	54.9

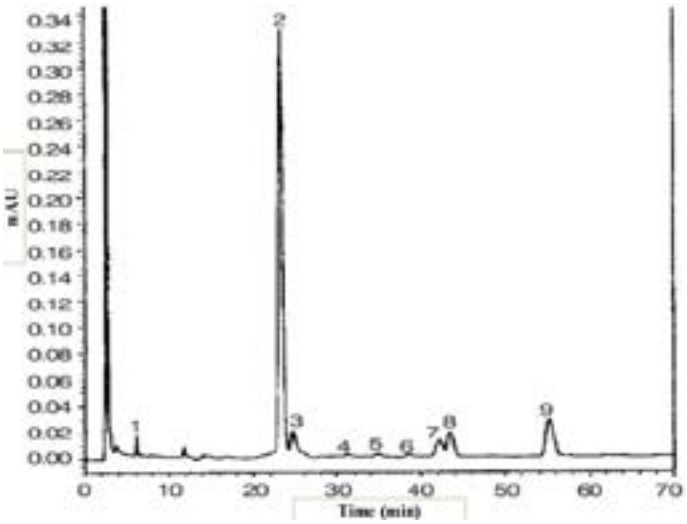

Figure 1: *Represent the HPLC Analysis of Carotenoid from Tomato Peel*

Antioxidant efficiency of carotenoid extracted from tomato peel

Antioxidant activities are normally added to fats, oils and nutrition substances containing fats keeping in

mind the end goal to repress the advancement of off-flavor emerging from oxidation of unsaturated fatty acid. Table 4 demonstrates the stimulation time of sunflower oil containing diverse convergences of carotenoids removed from tomato peel as characteristic antioxidant by the Rancimat test at 110°C. Results demonstrated that, stimulation time of sunflower oil was expanded by expanding the extra of carotenoids from tomato peel. The induction period was 3.25 and 7.20 h for sunflower oils without including antioxidant and that contained 200 mg/kg BHT, individually, in the interim, our finding demonstrated that the induction period was expanded step by step by including and expanding the grouping of carotenoids removed from tomato peel as normal cancer prevention agents. These estimations of prompting periods expanded to 9.40, 12.30, 16.22 and 20.70 h for sunflower oil that contained 50, 100, 150 and 200 mg/kg of carotenoids extracted from tomato peel, individually. These results are like those given by (Nilson et al., 1999) who demonstrated that, the carotenoids act as cancer prevention agents by wrecking the free radicals. Likewise (Mubarak, 2003) reported that, carotenoids lyco-red as regular cancer prevention agent concentrates of 0.1% fixation expanded the impelling time of sunflower oil measured by Rancimat system. Substitution of engineered cancer prevention agent by lycopene common antioxidant may have advantages because of wellbeing ramifications of functional parameter, for example, soundness in both oil and water. Lycopene, Carbon-40 poly isoprenoid compound containing 13 double bonds, is the abundant carotenoid, representing give or take 80–90% of the aggregate colors substance in tomatoes. With its 11 conjugated and two non-conjugated double bonds, it was observed to be more proficient antioxidant (singlet oxygen quencher) than β-carotene, α - carotene, and α –tocopherol (Chun et al., 2009). Lycopene as cancer prevention agent has a solitary oxygen extinguishing capacity twice as high that of β-carotene and 10 times higher that α tocopherol.

Cooking characteristics of spaghetti

Cooking qualities of spaghetti arranged with distinctive proportion of carotenoid from tomato peel are outlined in Table 5. Ideal cooking time was 10 min, cooking weight of 10g dry matter of spaghetti 32g, Swelling Index value was 6.0. 32 gm. These qualities were not contrasted with every level of carotenoids colors and control test. These finding are in concurrence with (Rizk and Tolba 2010).

Table 4: *Relation between induction periods of sunflower oil containing different ratios of carotenoids as natural antioxidant.*

Sunflower oil treatment	Induction Period
Free from Additives	3.25c
Having 200ppm BHT	7.20b
Having 50 ppm carotenoids	9.40a
Having 100 ppm carotenoids	12.30c
Having 150 ppm carotenoids	16.22d
Having 200 ppm carotenoids	20.70e

Values are significantly different at P<0.05

Table 5: *Characteristic of spaghetti prepared with different ratio of Carotenoids extracted from tomato peel.*

% adding of pigment	Dry matter loss at optimum cooking time of Spahagetti
Control	10
1% carotenoids	7.40
2% carotenoids	6.30
3% carotenoids	5.40
4% carotenoids	3.30
5% carotenoids	3.00

At the end of the day, as the level of carotenoid from tomato peel is higher in arranged spaghetti the lower is the cooking time. Case in point, cooking rate was7.40, 6.30, 5.40, 3.30 and 3.0% for spaghetti contained 1.0, 2.0, 3.0, 4.0 and 5.0% carotenoid from tomato peel individually. The steadiness of decrement of weight reduction may be because of the ability of carotenoid from tomato peel to be available as intricate with protein or lipoprotein. Their submicroscopic structure might likewise be an element in the extraordinary security of carotenoid from tomato peel amid assembling of spaghetti. (Dexter et al., 1983).

Antioxidant efficiency

Radical Scavenging Activity (RSA) of spaghetti arranged with diverse proportions of carotenoid from tomato peel amid capacity period is outlined in Table 6 explained that, supplementing spaghetti with carotenoid from tomato peel enhanced the Radical Scavenging Action (RSA) in the spaghetti. It was seen that, RSA% of control Vs supplemented with 1% and 5% concentrate expanded by 75.39% and 195.04% individually contrasted and control at zero time, while the relative rates after 6 months were 82.81% and 216.93% in the similar condition. On the other hand, during the storage period for all treatment the percent of radical Scavenging activity of spaghetti slowly decreased down.

Table 6: *Radical Scavenging Activity (RSA) of spaghetti prepared with different ratios of Carotenoid from tomato peel during storage period.*

Storage Period	Control	Level of Carotenoids				
		1%	2%	3%	4%	5%
Zero time	26.41	47.56	59.32	63.34	73.02	81.02
One month	24.03	42.86	52.12	59.90	70.13	76.94
Two month	21.10	39.29	50.01	56.04	66.29	73.01
Four month	20.78	38.03	42.90	53.43	63.04	68.03
Six month	18.36	34.90	39.05	46.67	57.03	59.76

Table 7: *Tangible parameters of spaghetti arranged with diverse proportion of carotenoids from tomato peel.*

Treatment	Quality characteristics of spaghetti			
	Color	Taste	Texture	Overall acceptability
Control	4.10 e	4.40 e	4.50 e	4.20 e
1% Carotenoids	5.10 d	5.20 d	5.40 d	6.30 d
2% Carotenoids	7.43 c	7.31 c	7.43 c	7.52 c
3% Carotenoids	9.27 a	9.37 a	9.60 a	9.47 a
4% Carotenoids	8.90 a	9.23 ab	9.12 ab	9.20 a
5% Carotenoids	8.45 b	8.66 b	8.49 b	8.64 b

Value in the same column with different letter are significant at <0.05.

Tactile parameters of spaghetti arranged with distinctive proportion of carotenoids from tomato peel

Method for the score given by the specialist for spaghetti made with Spaghetti 72% and treated with different proportion of carotenoids from tomato peel contrasted and the control (without carotenoids colors) were recorded with the after effects of measurable tests shown in Table 7. There were a huge distinction in coloring, taste, composition and general worthiness basis among diverse spaghetti either in the control or those arranged with including lycopene colors. While, there is no noteworthy distinction between spaghetti arranged with including 3.0 and 4.0% carotenoids from tomato peel however there were critical contrasts between the specimens and other checked spaghetti in all recorded tangible qualities. In the interim, spaghetti including 3.0% carotenoids colors got the higher coloring, taste, surface and general agreeableness score took after by 4.0%, 5.0%, 2.0%, and 1.0% and the control separately. Then again, control spaghetti test got the lower score in every single tactile rule. Results indicated additionally, that the coloring model was much related with the last item as both coloring and general adequacy had the same letter of centrality in all examples. Consequently, the best level carotenoids from tomato peel enhanced the tactile quality properties of arranged spaghetti were 3.0% to 4.0% however under 2.0% of carotenoids from tomato peel was less worthy for eating spaghetti qualities.

Conclusion

It was concluded that antioxidant potential of carotenoids extracted from tomato peel was good. Store sample showed strong antioxidant activity due to carotenoids. All conditions of cooking time, its weight and swelling index of spaghetti was kept uniform. While on the other hand, spaghetti cooking losses were decreased with increased level of synthetic carotenoids when compared with standard (control). Moreover, organoleptic evaluation of spaghetti cooked from carotenoid pigments from tomato peel revealed the highest score of color, taste, texture and overall acceptability when compared with control and other tested samples. Furthermore, utilization of extracted carotenoid from tomato and its utilization as food grade colorant in food industry were quite unique techniques for the nutritional as well as medicinal benefit.

Authors' Contribution

MW and AAS conceived and designed the experiments and analyzed the data; MW performed the experiment sand wrote the paper. MI and OB provided reagents/materials/analysis tools.

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