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USING OF TOMATO WASTES AS A SOURCE FOR LYCOPENE, NATURAL ANTIOXIDANTS AND ANTIMICROBIAL AGENTS IN MINCED MEAT

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ABSTRACT: ***This investigation was carried out to study the possibility of utilization of tomato processing wastes (peel and pulp residue) as a source for lycopene, natural antioxidants and antimicrobial agents in minced meat. In this respect, the optimum conditions for lycopene extraction were studied. A mixture of petroleum ether and acetone (1: 1, v/v) as a solvent at a ratio of (1: 25, w/v) sample to solvent for 5 min were the optimum extraction conditions; which gave 95.47 and 67.23 mg lycopene/100g dry peel and pulp residue; respectively. Lycopene stability was observed at 75 ^{o}C for 90 min when dissolved in sunflower oil. However, total polyphenols were as follows: 223.45 and 345.72 mg/100g dry peel and pulp residue; respectively. The antioxidant effects (as thiobarbituric acid value) of synthetic and natural additives mixed with minced meat and stored at -18 ^{o}C for 6 months were in the following order: pulp residue 250ppm > pulp residue 200ppm = peel 200ppm = peel 250ppm > pulp residue 150ppm > butylated hydroxyl toluene (BHT, 200ppm) = butylated hydroxyl anisole (BHA, 200ppm) = peel 150ppm > control. Disc- diffusion method was used for examination of the phenolic extracts at 200ppm diluted in 70% ethanol (1, 50 and 100%) of wastes and synthetic additives as antimicrobial agents in minced meat against some microbial strains namely, Bacillus subitils, Pseudomonas spp, Aspergillus niger and Candida guilerimondii FTI 20037. The results showed that no inhibitory effects of tomato wastes phenolic extract toward Pseudomonas spp at all used concentrations; while, it had a noticeable inhibitory effects at all applied concentrations toward Bacillus subitils, which highest effect at 1% level followed by 50%, then 100%. Peel and pulp residue phenolic extracts at levels 50 and 100% were effective against yeast and mold; the first level gave the highest. Generally, pulp residue was more effective than peel against the used microbial strains. Organoleptic evaluation of beef burger prepared from minced meat replaced with 6.73, 8.97 and 11.21% peel and 4.35, 5.80 and 7.25% pulp tomato wastes compared with control and synthetic antioxidants (200ppm) showed that, all ratios of tomato wastes gave an accepted burger from the judge's point of view, while both peel and pulp residue at percentage (6.73% peel and 4.35% pulp residue) were the best compared with other percentages.***

Keywords: ***Tomato peel, pulp residue, antioxidant, lycopene, antimicrobial, minced meat.***

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most widely used and versatile crops. It is consumed fresh and also used to manufacture a wide range of processed products (Madhavi and Salunkhe, 1998). Commercial processing of tomato produces a large amount of waste. Tomato paste manufacturing units generate 7–7.5% solid waste of raw material and 71-72% of this waste is pomace. The weight pomace contained 33% seed, 27% skin and 40% seed and 56% pulp and skin (Sogi and Bawa, 1998). Tomato industries yield a high amount of by-products mainly tomato peel and seeds. Since tomato peel is rich in lycopene, the direct addition of peel to food products could be a way to use this by-product to obtain a new product enriched in lycopene (Calvo *et al.,* 2008).

Tomatoes and tomato products are the major source of lycopene and are considered to be an important contributor of carotenoids to the human diet. Lycopene is one of the over 600 carotenoids found in nature. It accumulates in relatively few tissues, and can most easily be seen in ripe tomato, red pepper, watermelon and red grapefruit giving them a characteristic red pigmentation (Ben-Amotz and Fishler, 1998; Thompson *et al.,* 2000).

Tomato components like lycopene, phenolics, flavonoids and vitamins C and E are mainly responsible for the antioxidant capacity of raw tomatoes and processed tomato products. Due to their ability to quench singlet oxygen and trap peroxyl radicals, carotenoids have been described as excellent antioxidants (Leonardi *et al.,* 2000 and Beutner *et al.,* 2001).

Al-Wandawi *et al.* (1985) reported that tomato skin contains high levels of lycopene compared to the pulp and seeds. In addition, tomato skin and seeds were reported to contain essential amino acids, and the tomato seeds had particularly high amounts of minerals (Fe, Mn, Zn and Cu), and monounsaturated fatty acids (especially, oleic acid).

Stewart *et al.* (2000) reported that the majority of the flavonols in tomatoes are present in the skin. Similarly, Sharma and Le Maguer (1996) observed that most of the lycopene was associated with the skin and water insoluble fraction of the tomato pulp. Recently, George *et al.* (2004) studied antioxidant components in 12 field grown tomato genotypes, and reported that tomato skin had 2.5 times higher lycopene levels than the pulp. They also reported that the tomato skin had significant amounts of phenolics and ascorbic acid.

Toor and Savage, (2005) determined the amount of antioxidants in different fractions (skin, seeds and pulp) in three tomato cultivars, calculated on the basis of their actual fresh weights in whole tomato and found that the skin and seeds of the three cultivars on average contributed 53% of the total phenolics, 52% of the total flavonoids, 48% of the lycopene, 43% of the total ascorbic acid and 52% of the total antioxidant activity present in tomatoes.

The aim of the present study was to select the optimum conditions for lycopene extraction from tomato wastes, as well as to evaluate and utilize tomato wastes as a source for natural antioxidants and antimicrobial agents.

MATERIALS AND METHODS

MATERIALS:

Tomatoes (*Lycopersicon esculentum*) super strain B variety, were purchased from local market at Kafrelsheikh City. Fruits were washed and frozen at -18 ^{o}C. Fruits were put under tap water and peels were removed manually. Fruits without peels were passed from aluminum screen for juice extraction. Residual on the surface of screen after screening, which contained about seeds, fibers and other contents without peel or flesh are collected and called pulp residue. Beef meat (contained 20% fat) was purchased from local market in Kafrelsheikh City. Butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) were purchased from Al Gomhoria Company for Chemical and Drugs in Cairo, Egypt. Microbial strains, *Bacillus subitils, Pseudomonas spp, Aspergillus niger and Candida guilerimondii FTI 20037* were provided by Plant Pathology Department, Fac. of Agric., Kafrelsheikh University, Egypt.

METHODS:

Preparations of tomato peel and pulp residue:

Tomato peel and pulp residue were sun dried, then milled using blender mixer, then passed from 60 mesh screen, packed in polyethylene bags and stored at -18 ^{o}C until use.

Gross chemical composition of samples:

Moisture, ash, crude protein, ether extract and crude fibers of fresh tomato peel and pulp residue were determined according to the method described in A. O. A. C. (1990). Total carbohydrates were determined by phenol- sulphuric acid according to the method outlined by Dubois *et al.* (1956). The available carbohydrates were calculated by subtracting the percentage of crude fibers from the percentage of total carbohydrates content.

Solvents used for lycopene extraction from tomato wastes:

Acetone, hexane, ethanol 95%, mixture of hexane: acetone (6:4, v/v), mixture of hexane: ethanol (3:4, v/v), petroleum ether (40-60 ^{o}C) and mixture of petroleum ether: acetone (1:1, v/v) were used for lycopene extraction at different periods.

Lycopene stability test:

One gm of samples was used for lycopene extraction using the optimum conditions of extraction. Solvent was evaporated using rotary evaporator at 40 ^{o}C. Extracted lycopene was put into test tube and 10 ml of distilled water or sunflower oil was added and mixed thoroughly. Samples were put into

oven at 50, 75, 100, 125 and 150 °C for 30, 60, 90 and 120 min. Absorbance was measured at 445 nm (Chen *et al.,* 2009).

Lycopene content determination:

Lycopene content of sun dried tomato peel and pulp residue was colorimetrically determined using the standard method of lycopene estimation (Rao *et al.,* 1998). The absorbance of lycopene was recorded at 445 nm. The amount of lycopene (mg) was calculated by the following equation: Lycopene (mg) = خطـأ! الإشـارة المرجعيـة غيـر معرّفة. $\frac{A\ x\ dil\ x\ ml\ x\ 10}{E^{1\%}_{1cm}}$

Where A, absorbance of the solution in 1 cm cuvette, dil; dilution factor, ml; total ml of the sample and $E^{1\%}_{1cm}$ **: specific extinction coefficient for lycopene in petroleum ether which is (3450).**

Polyphenols content determination:

Total polyphenols contents were determined by the Folin- Ciocalteau colorimetric method (Singleton *et al.,* 1999). Extracted solution (0.5 ml) was mixed with 0.5 ml of the Folin- Ciocalteau reagent and 1 ml of saturated Na_2CO_3, then absorbance was measured at 760 nm after 1 h of incubation at room temperature. Total polyphenol contents were expressed as mg tannic acid equivalents/100g sample.

Preparation of beef burger:

Tomato peel and pulp residue powder (as a polyphenol content) were mixed with minced meat at ratios 6.73, 8.97 and 11.21% of peel as substituted of minced meat. Also, pulp residue was added at ratios 4.35, 5.80 and 7.25% as minced meat substitution. These ratios were equivalent to 150, 200 and 250ppm total polyphenols. Meat samples were blended with selected ratio of tomato wastes. BHA and BHT were also used at 200ppm (the recommended ratio) as synthetic antioxidants. Treated and untreated samples were packed in polyethylene bags and stored at -18 °C for 6 months. Beef burger was prepared using minced meat mixed with tomato wastes according to the method described by El-Akary (1986) as the following formulas in Table (A):

Table (A): Different recipes for burger making.

Ingredients (%) / No of recipes	Meat	Salt	Spices*	Onion	Water	Peel	Pulp residue
Control	85.0	2.0	2.0	1.0	10.0	0.0	0.0
BHA 200ppm	85.0	2.0	2.0	1.0	10.0	0.0	0.0
BHT 200ppm	85.0	2.0	2.0	1.0	10.0	0.0	0.0
Peel 150ppm	78.27	2.0	2.0	1.0	10.0	6.73	0.0
Peel 200ppm	76.03	2.0	2.0	1.0	10.0	8.97	0.0
Peel 250ppm	73.79	2.0	2.0	1.0	10.0	11.21	0.0
Pulp residue 150ppm	80.65	2.0	2.0	1.0	10.0	0.0	4.35
Pulp residue 200ppm	79.20	2.0	2.0	1.0	10.0	0.0	5.80
Pulp residue 250ppm	77.75	2.0	2.0	1.0	10.0	0.0	7.25

*** Spices mixture %(black pepper 50%, coriander 30%, cubeb 5%, cinnamon 5%, cumin 5% and red pepper 5%).**

Determination the antioxidant activity of tomato wastes:

It was determined by measuring the thiobarbituric acid (TBA) values every 2 months according to the method described by Tarladgis *et al.* (1960) and modified by Rhee, (1978).

Antimicrobial activity of tomato wastes test:

The disc-diffusion method was used for detection the antimicrobial activity of tomato wastes. Some bacterial strains represent gram negative bacteria: namely *Pseudomonas spp* and gram positive bacteria: *Bacillus subitils* were used. In addition, fungi isolate was also examined namely *Aspergillus niger.* Moreover, one strain of yeast namely, *Candida guilerimondii FTI 20037* was also tested. Phenolic compounds from 8.97g peel and 5.80g pulp residue were extracted using cold methanol then solvent was evaporated using rotary evaporator. Extracts contained 20 mg phenolic compounds (200ppm). Extracts were mixed with 2 ml twin 20 and diluted with 70% ethanol (v/v) to give solution 1% (0.1 ml of extract in 10 ml ethanol 70%), 50% (equivalent volume of ethanol 70%) and 100% (extract without ethanol 70%). Nutrient agar (NA) and potatoes dextrose agar (PDA) media were used to detect the total count of bacteria and yeast and mold; respectively, according to Difco Manual (1977). The appropriate media were poured into sterile plates (12 cm diameter), left to solidify at room temperature. The organisms were inoculated on the surface of the prepared media. A sterile

disc, 6 mm diameter of Whatman No. 1 filter paper were dipped in the appropriate extract phenolic solution, blotted, and then placed on the surface of inoculated plates. The inhibitory effects of the 70% ethanol and phenol solution 1% (w/v) were also tested by placing saturated disc with only 70% ethanol or 1% phenol solution on each inoculated plate (Conner and Beuchat, 1984).

Sensory analysis:

Treated and untreated beef burgers were subjected to organoleptic evaluation. Samples were served to panel of 14 judges. The panelists were asked to evaluate color, taste, odor, texture and overall acceptability on a 1 to 10 hedonic scale as described by (Watts *et al.,* 1989).

Statistical analysis:

The obtained data were statistically analyzed using General Linear Models Procedure Adapted by Statistical Package for the Social Sciences (SPSS, 1997).

RESULTS AND DISCUSSION

Chemical compositions of tomato peel and pulp residue:

Gross chemical composition of tomato processing wastes was determined and the data were recorded in Table (1). The results showed that pulp residue contain a high amount of crude protein and ether extract comparing with tomato peel. On contrary, available carbohydrates and crude fibers of tomato peel were higher than those of pulp residue. These results are mostly in harmony with those reported by Attia *et al.* (2000) they found that protein content was 27.9 and 16.8%, fat content was 24.1 and 7.2% and fiber content was 18.1 and 57.7% in tomato seeds and peels; respectively.

Table (1): Gross chemical composition of tomato peel and pulp residue on dry weight basis.

Constituents (%)	Tomato peel	Pulp residue
Moisture	73.29b	84.52a
Dry matter	26.71a	15.48b
Crude protein	11.30b	22.38a
Ether extract	11.60b	17.69a
Ash	3.05b	4.88a
Total carbohydrate	74.05a	55.05b
-Available carbohydrate	16.82a	6.39b
-Crude fibers	57.23a	48.66b

Values are means of three replicates.

Values having the same letter(s) within a row are not significantly different (P > 0.05).

The obtained results are also similar with those reported by Arafa *et al.* (2008). Some variation may be attributed to effect of the cultural practices in tomato cultivars or processing methods (Brodowski and Geisman, 1980).

Selecting the optimum conditions for lycopene extraction:

Extraction of lycopene from different tomato wastes (peel and pulp residue) was influenced by several factors including solvent type, sample to solvent ratio and extraction period.

A- Effect of solvents type on lycopene extraction:

To study the efficiency of organic solvents type on the extraction of lycopene from tomato peel and pulp residue, eight solvents were used [acetone, hexane, ethanol 95%, a mixture of hexane and acetone (6:4, v/v), a mixture of hexane and ethanol (3:4, v/v), a mixture of acetone and ethanol (3:4, v/v), petroleum ether (40-60^{o}C) and finally, a mixture of petroleum ether and acetone (1:1, v/v). The obtained results are shown in Table (2). From given results it should be noted that, petroleum ether and acetone mixture (1:1, v/v) was the most efficient solvent for lycopene extraction from tomato peel and pulp residue.

Table (2): Effect of using different solvents on lycopene extraction from tomato wastes.

Solvent type	Absorbance at 445 nm	
	Peel	Pulp residue
Acetone	1.553 c	1.281 c
Hexane	1.109 d	0.867 e
Ethanol 95%	1.525 c	1.218 c
Hexane : acetone (6 : 4, v/v)	1.137 d	1.137 d
Hexane : ethanol (3 : 4, v/v)	1.603 b	1.325 b
Acetone : ethanol (3 : 4, v/v)	1.519 c	1.277 c
Petroleum ether (40 – 60 ^{o}C)	1.641 b	1.379 b
Petroleum ether : acetone (1: 1, v/v)	3.458 a	3.152 a

Values are means of three replicates.
Values having the same letter(s) within a column are not significantly different (P > 0.05).
Samples were extracted at room temperature.

No significant difference was found between petroleum ether only and a mixture of hexane: ethanol (3:4, v/v), both of them were in the second order.

However, the efficiency of using hexane and the mixture of hexane and acetone (6:4, v/v) as extraction solvents were the lowest. So, the mixture of petroleum ether and acetone (1:1, v/v) will be used in the next experiment for lycopene extraction from tomato wastes. These results are in agreement with those reported by (Choi, 2002).

B- Effect of sample to solvent ratio on lycopene extraction:

Table (3) represents the influence of using different sample to solvent ratios on the efficiency of lycopene extraction from tested samples. It should be noted that, the percentage of extractable lycopene not significantly differed as a function of increasing solvent to sample ratio from 1:25 to 1:50. So, the optimum ratio to recover the highest yield of lycopene was 1 gm of sample to 25 ml of solvent (a mixture of petroleum ether and acetone 1:1, v/v) for 5 min at room temperature. These results may be due to the presence of enough amounts of solvent requires dissolving and separating the maximum amounts of lycopene that found inside the cells. These results are in line with those of Choi, (2002). Based on the aforementioned advantage, the previously mentioned ratio will be used in the next experiments.

Table (3): Effect of sample to solvent ratios (w/v) on the extraction* of tomato wastes lycopene.

Sample to solvent ratio	Absorbance at 445 nm	
	Peel	Pulp residue
1 : 15	3.27 b	3.03 b
1 : 25	3.46 a	3.15 a
1 : 35	3.47 a	3.16 a
1 : 50	3.47 a	3.16 a

***Extractable lycopene was carried out at room temperature for 5 min using a mixture of petroleum ether and acetone (1:1, v/v).**
Values having the same letter(s) within a column are not significantly different (P > 0.05).

C- Effect of extraction period on lycopene extraction:

Extractable lycopene was increased with prolonging the extraction period from 1 to 5 min; while, no significant increase was observed at 5 to 9 min (Table 4). From the recorded results, it should be concluded that, the optimum period for maximum lycopene extraction was 5 minutes. Similar results were found elsewhere (Choi, 2002) who reported that, 5 min was the optimum time for maximum extraction of carotenoids.

Table (4): Effect of using different periods on the extractability* of lycopene from tomato wastes.

Extraction period (min)	Absorbance at 445 nm	
	Peel	Pulp residue
1.0	2.95 c	2.80 c
3.0	3.11 b	2.94 b
5.0	3.46 a	3.15 a
7.0	3.50 a	3.17 a
9.0	3.51 a	3.19 a

Extraction of lycopene was carried out at room temperature using a mixture of petroleum ether and acetone (1:1, v/v) as a solvent, in 1:25 (w/v) sample to solvent ratio.
Values having the same letter(s) within a column are not significantly different (P > 0.05).

Optimum conditions for lycopene extraction:

From the previous results it should be concluded that, the highest yield of lycopene was recovered from different tomato wastes by using the following conditions; a mixture of petroleum ether and acetone (1:1, v/v) as a solvent in a ratio of 1 sample: 25 solvent (w/v), where the extraction was carried out for 5 min. The amounts of extractable lycopene at the mentioned recommended conditions are shown in Table (5). Tomato peel had higher value of total lycopene (95.47mg/100g dry weight) than pulp residue (67.23mg/100g dry weight). Similar results were reported by Markovic *et al.* (2006); while, the obtained results are higher than those obtained by Calvo *et al.* (2008), who reported that lycopene concentration of the dry tomato peel was 55.70 mg/100 g of peel.

Table (5): Optimum conditions of lycopene extraction.

Condition / Samples	Solvent type	Sample to solvent ratio (w/v)	Extraction period (min)	Total lycopene (mg/100g dry sample)
Peel	Petroleum ether and acetone (1:1, v/v)	1 : 25	5	95.47 a
Pulp residue	Petroleum ether and acetone (1:1, v/v)	1 : 25	5	67.23 b

Means of treatments having the same case letter(s) within a column are not significantly different at (p > 0.05).

Effect of thermal treatment on lycopene stability:

The stability of lycopene extracted from tomato wastes (dissolved in water or sunflower oil) at different temperatures and times were studied. The obtained results were presented in Table (6). The data cleared that lycopene contents decreased as a function of temperature increasing from 50 to 150 ^{o}C in water and oil media. Total lycopene decreased also, as a result of heating time from 30 to 120 min in both water and oil media at the same temperature. The data also, cleared that thermal stability of lycopene that dissolved in sunflower oil are higher than that dissolved in water. Lycopene stability was observed at 75 ^{o}C for 90 min when dissolved in sunflower oil. These results are in agreement with those reported by Shi *et al.* (2008), they found that the high heating temperatures (120 and 140 ^{o}C) increased isomerization of lycopene and resulting in degradation of total lycopene and cis-isomers in both water and oil based tomato products. They also, found that the levels of degradation of total lycopene contents and cis-isomers were greater in water based samples than in oil based model systems under different treatments. Also, isomerization converts all-trans isomers (in tomatoes, lycopene exists in the all-trans form). Cis-isomers of lycopene are better absorbed than the all-trans form because of the shorter length of the cis-isomers and the greater solubility in mixed micelles. Gartner *et al.* (1997) reported that lycopene is fat soluble, so absorption is improved when oil is added to the diet causing much of the ingested lycopene to pass through the body.

Table (6): Effect of thermal treatment at different periods on tomato waste lycopene stability dissolved in water and sunflower oil.

Sample	Time (min)	Temperature (^{o}C)									
		50		75		100		125		150	
		Water	Oil	Water	Oil	Water	Oil	Water	Oil	Water	Oil
		Absorbance at 445 nm									
Peel	30	a2.93b	a3.35a	a2.86b	a3.32a	b2.52c	a2.85b	b2.03d	a2.51c	b1.85e	a2.10d
	60	b2.80b	a3.31a	b2.65c	a3.27a	c2.36e	c2.54d	c1.87g	b2.14f	c1.11h	b1.85g
	90	d2.31b	b3.00a	c2.11c	b2.93a	d1.88d	d2.25b	d1.21e	d1.87d	d0.93f	d1.22e
	120	f1.83d	d2.64a	e1.71e	d2.45b	f1.51f	f2.00c	e1.01g	f1.64e	g0.40h	e1.07g
Pulp residue	30	a 3.02a	b3.07a	a2.85b	b3.01a	a2.63c	b2.70c	a2.13e	a2.45d	a1.96f	a2.13e
	60	b2.83b	b3.00a	b2.67c	b2.93a	c2.27d	d2.33d	c1.83f	c2.00e	e0.63h	c1.49g
	90	c2.45c	c2.90a	c2.17d	c2.63b	e1.75e	e2.15d	d1.25f	e1.76e	f0.51h	e1.13g
	120	e1.95c	d2.63a	d1.85d	e2.25b	g1.03f	g1.80d	f0.87g	g1.14e	g0.37h	f0.97f

Lycopene absorbance at 25 $\pm$ 2 ^{o}C were 3.46 in peel and 3.15 in pulp residue.
Absorbance as affected by temperatures having the same right case letter(s) within a row are not significantly different (p > 0.05).

Absorbance as affected by time having the same left case letter(s) within a column are not significantly different (p > 0.05).

Determination of total polyphenol content in tomato waste extract:

Total polyphenol contents in methanolic extract of tomato wastes are shown in Table (7). Methanolic extract of tomato peel had lower value of total polyphenol content (223.45mg/100g dry weight) than pulp residue (345.72mg/100g dry weight). Toor and Savage (2005) found that the skin and seeds of the three cultivars of tomato on average contributed 53% to the total phenolics, 52% to the total flavonoids, 48% to the total lycopene, 43% to the total ascorbic acid and 52% to the total antioxidant.

Table (7): Polyphenol contents in tomato wastes (calculated on dry weight basis).

Values / Samples	Total polyphenol (mg/100g dry weight)	Dry weight (g) equivalent to total polyphenol (ppm) content		
		150ppm	200ppm	250ppm
Peel	223.45 b	6.73	8.97	11.21
Pulp residue	345.72 a	4.35	5.80	7.25

Means of treatments having the same case letter(s) within a column are not significantly different at (p > 0.05).

Effect of adding tomato wastes to minced meat as natural phenolic antioxidants:

Changes in thiobarbituric (TBA) acid values (as indicator of antioxidant activity) of minced meat mixed with different levels of tomato wastes comparing with synthetic antioxidants and stored at -18 ^{o}C for 6 months are shown in Table (8). The data revealed that TBA values were increased as prolonging of storage time. On the other hand, significant differences were found between synthetic antioxidants (BHA and BHT) and natural additives (tomato wastes) comparing with control. Generally, the antioxidant effects of synthetic and natural additives after 6 months of storage were in order: pulp residue 250ppm > pulp residue 200ppm = peel 250ppm = peel 200ppm > pulp residue 150ppm > BHT = BHA = peel 150ppm > control. Shi and Le Maguer (2000) mentioned that although lycopene contains no provitamin A activity, lycopene does exhibit a physical quenching rate constant with singlet oxygen almost twice as high as that of β-carotene. Thus, the increase in lycopene content might probably enhance the antioxidant activity of tomato wastes.

These results show that removal of skin and seeds from tomato during home cooking and processing resulting in a significant loss of the major

content of antioxidants. Therefore, it is important to consume tomatoes along with their skin and seeds, in order to attain maximum health benefits.

Table (8): Change of thiobarbituric acid (TBA) values of minced meat mixed with different levels of tomato peel and pulp residues (calculated as total polyphenolic contents) during storage at -18 °C for 6 months.

Storage time (months)	0	2	4	6
Treatments	TBA values (mg of malonaldehyde/kg meat)			
Control	0.00 d	A 0.234 c	A 0.584 b	A 0.889 a
BHA (200 ppm)	0.00 c	E 0.00 c	E 0.062 b	B 0.335 a
BHT (200 ppm)	0.00 c	E 0.00 c	F 0.008 b	B 0.359 a
*Peel (150 ppm)	0.00 d	B 0.070 c	B 0.273 b	B 0.328 a
Peel (200 ppm)	0.00 d	B 0.070 c	D 0.086 b	D 0.228 a
Peel (250 ppm)	0.00 c	E 0.00 c	D 0.091 b	D 0.225 a
**Pulp residue (150 ppm)	0.00 d	C 0.062 c	C 0.132 b	C 0.294 a
Pulp residue (200 ppm)	0.00 d	D 0.039 c	D 0.101 b	D 0.239 a
Pulp residue (250 ppm)	0.00 d	E 0.008 c	F 0.023 b	E 0.120 a

Means of treatments having the same left case letter(s) within a column are not significantly different (p > 0.05).
Means of storage periods having the same right case letter(s) within a row are not significantly different (p > 0.05). *Peels (150ppm= 6.73g, 200ppm= 8.97g and 250ppm= 11.21g). **Pulp residue (150ppm= 4.35g, 200ppm= 5.80g and 250ppm= 7.25g).

Antimicrobial activity of tomato wastes extract:

The antimicrobial effect of methanolic extract of phenolic compounds (20mg in extract) in tomato wastes towards two strains of bacteria (*Bacillus subitils and Pseudomonas spp.*), one strain of yeast (*Candida guilerimondii FTI 20037*) and one strain of fungi (*Aspergillus niger*) was investigated. Three concentrations of methanolic extract for tomato peel and pulp residue (1, 50 and 100%) diluted in 70% ethanol were examined and compared with 1% phenol. The diameters of inhibition zones were taken as indicator of the antimicrobial activity degree of the extracts. The obtained results are given in Table (9). The results show that no inhibitory effects of methanolic extract of tomato peel and pulp residue toward *Pseudomonas spp.* at all used concentrations; but phenol 1% produced inhibition zone equal to 11.25 mm.

Table (9): Diameters of inhibition zones (mm) resulted from the effects of phenolic extracts of tomato peel and pulp residue toward some microorganisms.

Microorganisms / Sample extract	*Bacillus subitils*	*Pseudomonas Spp.*	*Aspergillus niger*	*Candida guilerimondii FTI 20037*
	Diameters of inhibition zones (mm)			
Ethanol 70% (blank)	0.00 g	0.00 b	0.00 e	0.00 e
Phenol 1%	14.00 b	11.25 a	0.00 e	0.00 e
1% Peel in ethanol 70%	13.45 c	0.00 b	0.00 e	0.00 e
50% Peel in ethanol 70%	10.50 d	0.00 b	16.50 b	20.00 b
100% Peel	7.66 f	0.00 b	6.80 d	7.50 d
1% Pulp residue in ethanol 70%	15.00 a	0.00 b	0.00 e	0.00 e
50% Pulp residue in ethanol 70%	10.00 d	0.00 b	19.50 a	22.00 a
100% Pulp residue	8.50 e	0.00 b	8.66 c	9.00 c

Means of treatments having the same letter(s) within a column are not significantly different (p > 0.05).

Methanolic extract of phenolic compounds of tomato wastes possessed noticeable inhibitory effects at all applied concentrations toward *Bacillus subitils*. Methanolic extracts of phenolic compounds of tomato peel and pulp residue at level 1% gave the most wide inhibition zones (13.45 and 15.00 mm) followed by level 50% (10.50 and 10.00 mm); respectively, while using of 100% concentration produced the most narrow zone (7.66 and 8.50 mm); respectively.

The results also, revealed that the effect of methanolic extracts of phenolic compounds of tomato wastes toward *Aspergillus niger* were detected upon using level 50% and 100% only. Using of 50% methanolic extracts of phenolic compounds of tomato peel and pulp residue produced more size of inhibition zone (16.50 and 19.50 mm) followed by level 100% (6.80 and 8.66 mm); respectively.

Apparent also from the same Table that the effects of 50% from methanolic extracts of phenolic compounds of tomato peel and pulp residue were much more than 100%. Methanolic extracts of phenolic compounds of tomato wastes at 1%, phenol 1% and ethanol 70% did not possess inhibitory effects on the used fungal strain.

The inhibitory effects of methanolic extracts of phenolic compounds of tomato peel and pulp residue toward yeast strain (*Candida guilerimondii FTI 20037*) were noticed upon using 50% or 100% only. The effect of 50% was much more than 100%, where the diameters of inhibition zones were 20.00 and 7.50 mm for methanolic extracts of phenolic compounds of tomato peel, and 22.00 and 9.00 mm for methanolic extracts of phenolic compounds of tomato pulp residue; respectively. Generally, pulp residue was more effective than peel against used microbial strains. These results are in agreement with Østerlie and Lerfall (2005), they mentioned that tomato products had acidic characters that led to decrease the pH of minced meat, thus the growth of microorganisms were reduced.

Effect of tomato wastes on the organoleptic properties of beef burger:

The organoleptic evaluation of the beef burger prepared using various percentages of tomato peel (6.73, 8.97 and 11.21%) and pulp residue (4.35, 5.80 and 7.25%) as replacement of beef meat was performed and the means of results were recorded in Table (10). The results indicated that as for storage time, no significant changes for color among all treatments during storage for six months, while samples mixed with BHT, pulp 5.80% and 7.25% gave the lowest scores for taste comparing with other treatments during storage period. As for odor, no significant differences among all samples treated with natural additives and synthetic antioxidants; while control was lowered significantly during storage. Texture of all treated and untreated samples was not differed significantly during storage with the exception of sample that mixed with 7.25% pulp residue which lowered during storage.

From overall acceptability, the results in Table (11) revealed that control and samples treated with BHA and BHT had the highest scores for all characteristics; while, samples mixed with 6.73% peel and 4.35%pulp residue come in the second order. The other treatments were in the following order: peel 8.97% > pulp residue 5.80% > peel 11.21% > pulp residue 7.25%. Generally, all ratios of tomato wastes used in this experiment as substitution for meat gave burger accepted from the judge's point of view. The obtained results are in line with those of Østerlie and Lerfall (2005), who reported that adding of lycopene from tomato products to minced meat could lead to a different taste, better color and with a well, documented health benefit. Calvo *et al.* (2008) used dry tomato peel in sausage manufacture at levels 0, 0.6, 0.9 and 1.2%. They found that the sensory and textural properties and overall acceptability of all sausages were good, indicating that tomato peel could be added to dry fermented sausages to produce a meat product enriched in lycopene.

Table 10

Table (11): Effect of tomato wastes on the organoleptic properties of beef burger.

Treatments	Storage time (months)			
	0	2	4	6
	Overall acceptability			
Control	A 8.75 ns	A 8.63	A 8.60	A 8.50
BHA 200ppm	A 8.55 ns	A 8.48	A 8.43	A 8.43
BHT 200ppm	A 8.55 ns	A 8.43	A 8.40	A 8.35
Peel 150ppm	B 7.73 ns	B 7.65	B 7.60	B 7.58
Peel 200ppm	BC 7.35 ns	BC 7.25	BC 7.20	B 7.13
Peel 250ppm	DE 6.65 ns	D 6.60	D 6.48	C 6.43
Pulp 150ppm	B 7.58 ns	B 7.48	B 7.48	B 7.38
Pulp 200ppm	CD 7.03 ns	CD 6.90	CD 6.78	BC 6.75
Pulp 250ppm	E 6.20 ns	D 6.48	D 6.00	D 5.88

Means of treatments having the same left case letter(s) within a column are not significantly different (p > 0.05).
Means of storage periods having the same right case letter(s) within a row are not significantly different (p > 0.05).
Peels (150ppm= 6.73g, 200ppm= 8.97g and 250ppm= 11.21g). Pulp residues (150ppm= 4.35g, 200ppm= 5.80g and 250ppm= 7.25g).

They also, reported that it is the first time that dry tomato peel, a by-product of the tomato industries, has been used as a source of lycopene in foods. The direct use of this by-product, avoiding lycopene extraction has an obvious health advantages.

Conclusion

Tomato wastes could be used as a good source of lycopene pigment. Lycopene pigment showed stability at 75 ^{o}C for 90 min in oil media, so that, it can be used as a natural colorants in some food products. Furthermore, these wastes are considered as a valuable added ingredient for preparing some food products specially, fatty foods such as minced meat and burger to prevent oxidative and microbial deterioration.

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استخدام مخلفات الطماطم كمصدر لصبغة الليكوبين ، مضادات للأكسدة والميكروبات في اللحم المفروم

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الملخص العربي

أجريت هذه الدراسة بهدف دراسة إمكانية الاستفادة من مخلفات تصنيع الطماطم (القشور ومتبقيات اللب بعد العصر) كمصدر لصبغة الليكوبين (اللون الاحمر) وكذلك كمضادات أكسدة طبيعية في اللحم المفروم. حيث تم دراسة الظروف المثلى لاستخلاص صبغة الليكوبين ووجد أن مخلوط من مذيبات الايثير البترولي والأسيتون بنسبة ١: ١ (بالحجم) ، ١ عينة : ٢٥ مذيب (وزن /حجم) لمدة ٥ دقائق كانت هي انسب الظروف للاستخلاص حيث أعطت ٩٥.٤٧ ، ٦٧.٢٣ مجم ليكوبين/١٠٠جم عينة جافة في كل من القشور ومتبقيات اللب على الترتيب.

- وبدراسة تأثير درجات الحرارة المختلفة (٥٠ ، ٧٥ ، ١٠٠ ، ١٢٥ ، ١٥٠) درجة مئوية لمدة ٣٠ ، ٦٠ ، ٩٠ ، ١٢٠ دقيقة على ثبات صبغة الليكوبين المذابة في الماء وكذلك المذابة فى زيت دوار الشمس وجد أن صبغة الليكوبين ثابتة على ٧٥ درجة مئوية لمدة ٩٠ دقيقة خاصة المذابة في الزيت والتى يقل ثباتها بارتفاع درجات الحرارة ولمدة زمنية أطول. لذلك يمكن استخدامها كملونات طبيعية في بعض المنتجات الغذائية.
- وكانت كمية الفينولات الكلية ٢٢٣.٤٥ ، ٣٤٥.٧٢ مجم/١٠٠جم عينة جافة لكل من القشور ومتبقيات اللب على الترتيب.
- كما تم دراسة التأثير المضاد للأكسدة (المقدر كحامض الثيوباربتيوريك) لمخلفات الطماطم كإضافات طبيعية بالمقارنة بمضادات الأكسدة الصناعية التي تم خلطها مع اللحم المفروم وخزنت على –١٨ درجة مئوية لمدة ٦ شهور وكان النشاط المضاد للأكسدة كالتالي: متبقيات اللب بنسبة ٢٥٠جزء/مليون > متبقيات اللب بنسبة ٢٠٠جزء/مليون= القشور بنسبة ٢٠٠ جزء/مليون = القشور بنسبة ٢٥٠ جزء/مليون > متبقيات اللب بنسبة ١٥٠ جزء/مليون > BHT (٢٠٠ جزء/مليون) = BHA (٢٠٠ جزء/مليون) = القشور بنسبة ١٥٠ جزء/مليون > الكنترول.

- تم أيضا دراسة التأثير المضاد للميكروبات للمستخلصات الفينولية لوزن ٨.٩٧جم قشور ، ٥.٨٠جم متبقيات اللب والتى تعطى ٢٠ مجم فينولات كلية ثم عمل تركيزات (١ ، ٥٠ ، ١٠٠% منهم فى الايثانول ٧٠%) وذلك بطريقة Disc-Diffusion method ضد سلالتين من البكتريا أحداهما موجبة لصبغة جرام والأخرى سالبة لنفس الصبغة وهما *Bacillus subitils* ˇ *Pseudomonas spp* على الترتيب، وأيضا سلاله من الفطريات هي *Aspergillus niger* وسلاله من الخمائر وهى *Candida guilerimondii FTI 20037* وأظهرت النتائج أنه: بالنسبة لبكتريا *Pseudomonas spp* لا يوجد تأثير تثبيطي لجميع التركيزات المستخدمة من المستخلصات الفينولية لمخلفات الطماطم المذابة في إيثانول ٧٠% (بتركيزات ١ ، ٥٠ ، ١٠٠%) على هذه البكتريا. بينما بالنسبة لبكتريا *Bacillus subitils* وجد أن مستخلصات مخلفات الطماطم لها تأثير تثبيطي عليها لكل التركيزات المستخدمة وأن تركيز ١% أعطى أعلى تأثير تثبيطي يليه تركيز ٥٠% ثم تركيز ١٠٠%. ومن ناحية أخرى وجد أن مستخلص القشور ومتبقيات اللب بنسبتي ٥٠ ، ١٠٠% كان لهما تأثير مضاد لنمو كل من الفطر والخميرة وان تركيز ٥٠% كان الأعلى. وعموما فأن مستخلصات متبقيات اللب كانت هي الأكثر تأثيرا ضد السلالات الميكروبية المستخدمة مقارنه بمستخلصات القشور.
- وبدراسة الخواص العضوية الحسية للهمبورجر المصنع من اللحم المفروم المضاف إليه قشور الطماطم بنسب استبدال ٦.٧٣ ، ٨.٩٧ ، ١١.٢١% ومتبقيات اللب بنسب ٤.٣٥ ، ٥.٨٠ ، ٧.٢٥% مقارنه بمضادات الأكسدة الصناعية BHA ، BHT (٢٠٠ جزء /المليون) أوضحت النتائج أن كل النسب المضافة أعطت همبورجر مقبول حسيا وكانت النسب ٦.٧٣% قشور ، ٤.٣٥% متبقيات اللب هي الأفضل مقارنة بباقي النسب المستخدمة.

ومما سبق يتضح انه يمكن استخدام مخلفات صناعة الطماطم (القشور ومتبقيات اللب) كمصادر لصبغة الليكوبين الحمراء لاستخدامها كملونات طبيعية في بعض الأغذية وكذلك يمكن استخدام هذه المخلفات كمصدر لمضادات الأكسدة الطبيعية والمضادات الميكروبية وذلك لما لها من فوائد صحية بدون آثار جانبية.

Table (10): Effect of tomato wastes on the organoleptic properties of beef burger.

Attributes	Color				Taste				Odor				Texture			
Treatments	Storage time (months)															
	0	2	4	6	0	2	4	6	0	2	4	6	0	2	4	6
Control	a 8.5ns	a 8.4	a 8.4	a8.3	a9.0ns	a9.0	a8.8	a8.7	a9.0a	a8.7ab	a8.7ab	a8.5b	a8.5ns	a8.4	a8.4	a8.4
BHA 200ppm	a 8.4ns	a 8.4	a 8.3	a8.3	a8.8ns	ab8.7	a8.6	a8.6	a8.7ns	a8.5	a8.5	a8.5	ab8.3ns	a8.3	a8.3	a8.3
BHT 200ppm	a 8.4ns	a 8.3	a 8.3	a8.2	a8.8a	b8.4ab	a8.4ab	a8.3b	a8.7ns	a8.7	a8.6	a8.6	ab8.3ns	a8.3	a8.3	a8.3
Peel 150ppm	b 7.9ns	b 7.8	a 7.8	b7.7	b7.5ns	c7.5	b7.3	b7.3	b7.6ns	b7.5	b7.5	b7.5	bc7.9ns	b7.8	b7.8	b7.8
Peel 200ppm	c 7.2ns	c 7.0	c 7.1	c7.0	cd7.0ns	d7.0	c6.8	cd6.6	b7.5n.s	b7.3	bc7.2	bc7.2	c7.7ns	b7.7	b7.7	b7.7
Peel 250ppm	d 6.4ns	d 6.3	d 6.3	d6.1	e6.2ns	f6.1	d6.0	e6.0	b7.2ns	bc7.2	c 6.9	c 6.9	e6.8ns	d6.8	d6.7	c6.7
Pulp 150ppm	b 7.8ns	b 7.6	b 7.6	b7.5	bc7.3ns	cd7.1	bc7.1	c6.8	b7.6ns	b7.6	b7.6	b7.6	cd7.6ns	b7.6	b7.6	b7.6
Pulp 200ppm	c 7.0ns	c 7.0	c 6.8	c6.8	d6.8a	e6.6ab	cd6.4ab	de6.3b	b7.1ns	cd 6.9	cd 6.8	cd 6.8	de7.2ns	cd7.1	cd7.1	c7.1
Pulp 250ppm	d 6.2ns	d 6.2	d 6.2	d6.2	e5.9a	f5.8ab	d5.9a	f5.4b	c6.5ns	d6.4	d6.3	d6.3	f6.2a	e5.7b	e5.6b	d5.6b

Means of treatments having the same left case letter(s) within a column are not significantly different (p > 0.05).
Means of storage periods having the same right case letter(s) within a row are not significantly different (p > 0.05).
Peels (150ppm= 6.73g, 200ppm= 8.97g and 250ppm= 11.21g). Pulp residues (150ppm= 4.35g, 200ppm= 5.80g and 250ppm= 7.25g).