Phytochemicals in Tomatoes- A Case for Optimum Thermal Processing

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ABSTRACT

Despite the recent emphasis on phytochemical contents of fruits and vegetables, evidence regarding their bioavailability remains inconclusive. In current study, a before and after 3 X 3 factorial design was adopted to compare phytochemistry of tomatoes under following variants of processing; temperature (raw vs.75°C vs.88°C) and time (5vs.15vs.30 minutes). Thermal processing was found to improve antioxidant, flavonoid and phenolic contents of tomatoes. Maximum values were noted after 30 minutes of heating at 88°C. Significant ($p<0.05$) improvements were observed in heat treated puree (30 minutes, 88°C) compared with raw in its antioxidant activity (8.05mg/100g to 46.87mg/100g), in phenolic content (20.02mg/100g to 26.99mg/100g) and in flavonoid content (1.71mg/100g to 4.61mg/100g). The potential influence of heat on antioxidant, flavonoid and phenolic content availability in tomatoes and products would be of interest to food technologists in all parts of the world as well as to the public health professionals in selecting and guiding about optimal processing temperatures.

Keywords: *Antioxidants, Flavonoids, Phytochemicals, Retention, Thermal processing.*

1. INTRODUCTION

With increasing trend of non-communicable diseases, there has been a recent surge in studying antioxidant and functional properties of food products. Phytochemicals are believed to be a part of healthy dietary pattern for their role in disease treatment as well as prevention (Zhang et al., 2015). In this fast paced world, demand for convenience and processed foods is on a rise because of which there is a recent concern about the effect of processing on these health enhancing components of food (Capanoglu, Beekwilder, Boyacioglu, De Vos, & Hall, 2010). It is a common belief that processed food items have lower nutritional values than their fresh counterparts. However, evidence has been inconclusive regarding non nutritional components and functional capacity of processed foods. This has been an area of contemporary research in addition to developing alternates to harmful products for reducing post-harvest changes in perishable food items (Masood et al., 2018). Various studies have demonstrated that bioavailability, antioxidant activity, and other nutritional properties of fruit products are enhanced by heat treatment (Nayak, Liu, & Tang, 2015). Research evidence reveals that thermal processing elevates the total antioxidant activity with no significant changes in total phenolic content and total flavonoids content (Nayak et al., 2015). Thermal treatments such as steaming, microwaving, frying, and drying has been found to result in higher antioxidant activity of fruits (Capanoglu et al., 2010). Heat processing results in breakdown of cell wall structures, thereby disrupting the chloroplast membranes and reducing cellular integrity, thus rendering several phytochemicals, more accessible to extraction (Kaur & Singh, 2008). These findings contrast the common belief that processed fruits and vegetables have lower health properties than fresh produce. Determination of optimum cooking time and temperature combination that can retain maximum phytochemical contents of fruits and vegetables is important. Tomatoes (Lycopersicum Esculentum), belonging to the Solanaceae family, are widely used plant which is consumed in both raw as well as cooked form all around the world (Alda et al., 2009; Perveen et al., 2015) . This study aimed to determine the effects of different cooking times, temperature and their combined effects on the phytochemistry of tomatoes.

2. METHODS

2.1. Study Design

A before and after 3 X 3 factorial design was adopted to compare phytochemistry of tomatoes under various temperature and time conditions.

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2.2. Sample Preparation

Ten kilogram of fresh, fully grown, mature and ripe tomatoes of ROMA variety (Solanum Lycopersicum) were purchased from a local farm house. Tomatoes were thoroughly washed and blanched for five minutes. The peels were removed and tomatoes were grinded and deseeded. Tomato puree was divided into seven equal portions. One portion of tomato puree was kept as raw in a sterilized jar. The other six portions were subjected to heat treatments. Three portions were heated at 75 °C for 5, 15, and 30 minutes whereas other three portions of tomato puree were heated at 85° C for 5, 15 and 30 minutes respectively. All seven samples were labeled and placed in sterilized jars.

2.3. Phytochemical Analysis

Estimation of antioxidant activity in each sample was done by the following methods:

2.4. Preparation of Extract

Tomato puree extracts were prepared according to Manzocco, Anese, and Nicoli (1998) with some modifications. Fresh samples of tomato puree (5g) were added to 20 ml water in a stoppered flask and left for shaking in an orbit shaker for 24 hours. Sample suspension was filtered through filter paper. Filtrate was evaporated in pre weighed china dish. Sample extracts were reconstituted by dissolving in 2-3 ml alcohol/ DMSO4 as required and stored in brown colored bottles. These extracts were used to estimate antioxidant activity in samples.

2.5. Determination of Antioxidant Activity

The antioxidant activity of extracts were determined with the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The bleaching rate of DPPH at a characteristic wavelength in the presence of the sample was monitored. DPPH scavenging assay of the prepared extracts was done according to Manzocco et al. (1998). The absorption of DPPH disappears from initial 517nm upon reduction by an antioxidant or a radical species.

Sample extracts were diluted with ethanol and DPPH solution. The bleaching of DPPH was followed at 517nm for 30 minutes. The percentage of the DPPH radical scavenging was calculated from the measured absorbance data. Ascorbic acid was used as a reference in this assay method.

$$\% \text{ of inhibition DPPH} = \frac{(\text{Abs.blank} - \text{Abs.sample}) \times 100}{\text{Abs.blank}}$$

2.6. Determination of Phenolic Content

Total phenolic content were determined by Folin Ciocalteu reagent according to Folin and Ciocalteu, (1927) as described by McDonald, Prenzler, Antolovich, and Robards (2001). Each sample (0.5g) was mixed with Folin - Ciocalteu reagent (1.5 ml, 1:10 diluted with distilled water) and 3.75 ml of 7% Na2CO3 and volume made up to 25 ml. The mixtures were allowed to stand for 15minutes and total phenolic content was determined at 760 nm using UV- spectrophotometer against blank. Gallic acid concentration curve was taken as a standard and results were expressed as milligram of Gallic acid equivalents (GAE) of fresh tomato puree.

2.7. Determination of Flavonoid Content

Aluminum chloride colorimetric method was employed for flavonoids according to Chang, Yang, Wen, and Chern (2002). Each sample of 1.5 ml was separately mixed with 3.25 ml of methanol, 0.25 ml of 5% aluminum chloride, and 0.25 ml of 1M potassium acetate. Volume was made up to 25 ml in the volumetric flask. It was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm using UV-spectrophotometer against blank. The calibration curve was prepared by preparing Quercetin solutions at concentrations 53 ug/ml in methanol. Quercetin was used as a reference standard and the total flavonoid content was express as milligram of Quercetin equivalents (mg QE/100g).

2.8. Statistical Analysis

All the data was analyzed using SPSS computer software program (version 21.0). For detecting significant differences between samples one-way and two-way analysis of variance (ANOVA) and MANOVA was applied followed by post hoc tests. p- values less than 0.05 were considered significant.

All laboratory work was performed in Pakistan Council of Scientific and Industrial Research (PCSIR), Plant Biotechnology and Organic Food Laboratory, Lahore, Pakistan

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3. RESULTS

This study was conducted to find out the effect of cooking time and temperature on the phytochemistry of tomatoes. Results in Table 1 indicate that there was significant difference F (2, 14), p<.001 in phenolic content, flavonoids and antioxidant activity of tomatoes cooked for varying time durations when analyzed by one-way ANOVA. Raw sample had the minimum phytochemical contents (phenolic content = 20.02±0.01; flavonoid content = 1.71±0.01; antioxidant content = 8.05±0.01) while tomatoes cooked for of 30 minutes had the highest phytochemical contents (phenolic content = 26.74±0.27; flavonoid content = 3.48±1.23; antioxidant content = 30.47±17.97). Results in Table 2 indicate that there was significant difference in phytochemical content F (1, 14), p<.001 for various temperature ranges. Unheated sample had the minimum phytochemical contents (phenolic content = 20.02±0.01; flavonoid content = 1.71±0.01; antioxidant content = 8.05±0.01) which gradually increased with increasing cooking temperatures. Tomatoes cooked at 88° C had the highest phytochemical contents (phenolic content = 24.94±2.32; flavonoid content = 4.24±0.38; antioxidant content = 27.83±14.41). Tables 3 and 4 show pair wise comparison of differing time and temperature conditions on phytochemical contents of tomatoes (post hoc Tukey's test). A significant difference in phenol, flavonoid and antioxidant content was found with different cooking times and temperature ranges (p<0.01). As shown in Table 5, the interaction of time x temperature also improved the phytochemical content significantly (Phenolic content = F (2, 14) =564, p<0.001; Flavonoid content = F (2, 14) =263, p<0.001; antioxidant content = F (2, 14) =3315366, p<0.001). The effect size of cooking time, cooking temperature and their interaction was found to be large (partial eta squared=1 and 0.99).

Figures 1 and 2 illustrate the effect of time and temperature respectively phytochemicals present in tomatoes. Though the effect of time and temperature was statistically significant for phenolic content, flavonoids and antioxidants, there was variation among these as well. Increase in phenolic and flavonoid contents was marginal, whereas the antioxidant content increased remarkably with both time and temperature. It can therefore be concluded that the antioxidants were affected the most by heat as compared to other phytochemicals.

Table 1. Effect of Thermal Processing Time on Phenolic Content of Tomatoes.

	Time (Minute)				ANOVA	
	Zero	5	15	30	F	*p*
	M±SD	M±SD	M±SD	M±SD	(2,14)	
Phenolic content	20.02±0.01^{a}	21.83±0.09^{b}	25.76±0.16^{c}	26.74±0.27^{d}	532125	<0.001
Flavonoids	1.71±0.01^{a}	2.74±1.11^{b}	3.34±1.11^{c}	3.48±1.23^{d}	9163	<0.001
Antioxidants	8.05±0.01^{a}	12.51±3.90^{b}	17.05±3.83^{c}	30.47±17.97^{d}	5229558	<0.001

Note: Means with different letters within row are significantly different $p<0.05$.

Table 2. Effect of Temperature of processing on Phytochemistry of tomato puree.

	Temperature (Degree C)			ANOVA	
	Zero	75	88	F	*P*
	M±SD	M±SD	M±SD	(1,14)	
Phenolic content	20.02±0.01^{a}	24.62±2.19^{b}	24.94±2.32^{c}	6090	<0.001
Flavonoids	1.71±0.01^{a}	2.14±0.31^{b}	4.24±0.38^{c}	193929	<0.001
Antioxidants	8.05±0.01^{a}	12.19±2.44^{b}	27.83±14.41^{c}	11012124	<0.001

Note: Means with different letters within row are significantly different $p<0.05$.

Table 3. Pairwise comparison of time range of thermal processing on Phytochemical Contents of tomato puree.

Group (I)	Group (J)	Mean diff.	SE	*P*
Phenolic Content				
Zero minute	5 minutes	-1.808*	0.006	<0.001
Zero minute	15 minutes	-5.740*	0.006	<0.001
Zero minute	30 minutes	-6.720*	0.006	<0.001
5 minutes	15 minutes	-3.932*	0.005	<0.001
5 minutes	30 minutes	-4.912*	0.005	<0.001
15 minutes	30 minutes	0.980*	0.005	<0.001

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Flavonoid content				
Zero minute	5 minutes	-1.029*	0.007	<0.001
Zero minute	15 minutes	-1.629*	0.007	<0.001
Zero minute	30 minutes	-1.773*	0.007	<0.001
5 minutes	15 minutes	-.600*	0.006	<0.001
5 minutes	30 minutes	-.744*	0.006	<0.001
15 minutes	30 minutes	-.144*	0.006	<0.001
Antioxidant content				
Zero minute	5 minutes	-4.460*	0.007	<0.001
Zero minute	15 minutes	-9.000*	0.007	<0.001
Zero minute	30 minutes	-22.415*	0.007	<0.001
5 minutes	15 minutes	-4.540*	0.006	<0.001
5 minutes	30 minutes	-17.955*	0.006	<0.001
15 minutes	30 minutes	-13.415*	0.006	<0.001

Note: *The mean difference is significant at 0.05 level.

Table 4. Pairwise comparison of temperature range of thermal processing on Phenolic content of tomato puree.

Group (I)	Group (J)	Mean diff.	SE	*P*
Phenolic Content				
Normal	75°C	-4.596*	0.006	<0.001
Normal	88°C	-4.917*	0.006	<0.001
75°C	88°C	.321*	0.004	<0.001
Flavonoid content				
Normal	75°C	-.429*	0.007	<0.001
Normal	88°C	-2.526*	0.007	<0.001
75°C	88°C	2.096*	0.005	<0.001
Antioxidant content				
Normal	75°C	-4.137*	0.007	<0.001
Normal	88°C	-19.780*	0.007	<0.001
75°C	88°C	15.643*	0.005	<0.001

Note: *The mean difference is significant at 0.05 level.

Table 5. Effect of Time and Temperature of thermal processing on Phenolic Content of tomato puree.

	SS	Df	MS	F	*P*	Partial η^2
Phenolic Content						
Intercept	10681.930	1	10681.930	140200326	<0.001	1.000
Time	81.086	2	40.543	532125	<0.001	1.000
Temperature	0.464	1	0.464	6090	<0.001	0.998
Timex Temp	0.086	2	0.043	564	<0.001	0.988
Flavonoid content						
Intercept	152.541	1	152.541	1495749	<0.001	1.000
Time	1.869	2	0.935	9163	<0.001	0.999
Temperature	19.777	1	19.777	193929	<0.001	1.000
TimexTemp	0.054	2	0.027	263	<0.001	0.974
Antioxidant content						
Intercept	5561.047	1	5561.047	55610467	<0.001	1.000
Time	1045.915	2	522.956	5229558	<0.001	1.000
Temperature	1101.212	1	1101.212	11012124	<0.001	1.000
Time x Temp	663.073	2	331.537	3315366	<0.001	1.000

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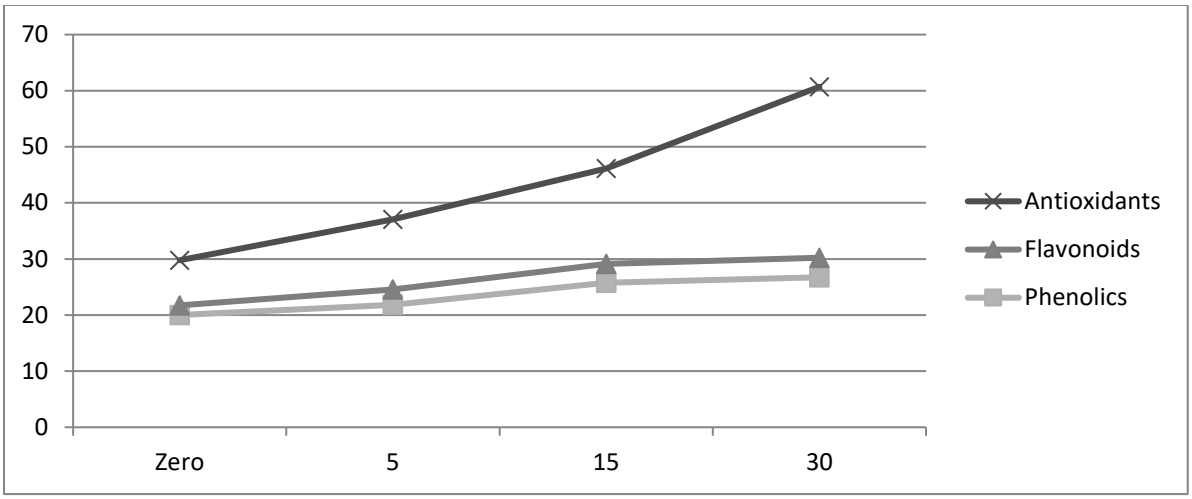

Figure 1. Effect of time of thermal processing on phenolic content, flavonoids and antioxidants of tomato puree.

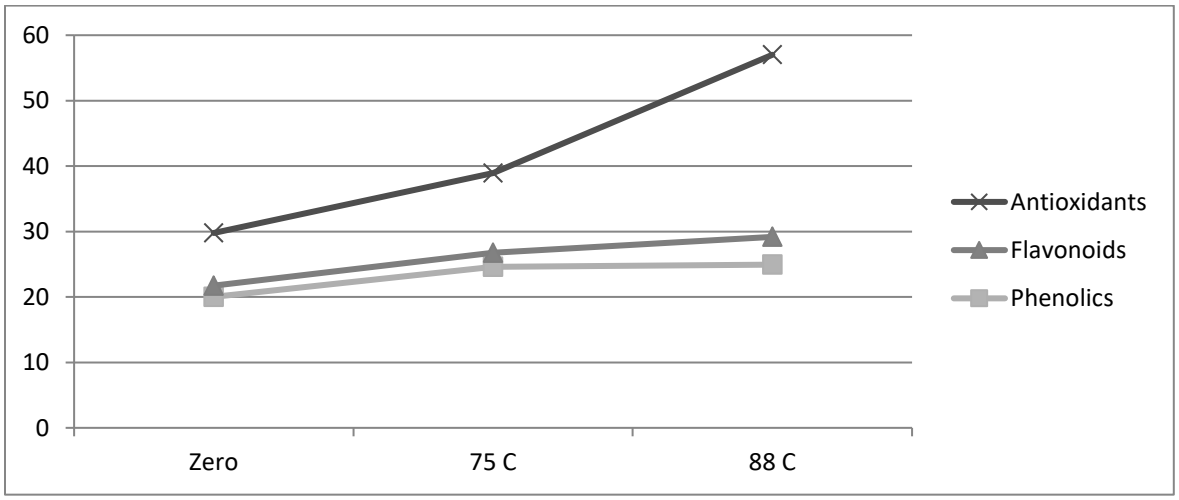

Figure 2. Effect of temperature of thermal processing on phenolic content, flavonoids and antioxidants of tomato puree.

4. DISCUSSION

Tomatoes are one of the most commonly used fruits consumed in raw as well as cooked form. . Role of tomatoes as functional food product is a topic of interest to many consumers. However, evidence for the effect of processing treatments on nutritional and non-nutritional composition of tomatoes is indecisive. The current study intended to evaluate the effect of thermal processing conditions on the phytochemistry of tomatoes. Antioxidant, phenolic and flavonoid contents of raw as well as cooked tomato puree were assessed using the standard methods of the Association of Official Analytical Chemist as described by Manzocco et al. (1998); McDonald et al. (2001) and Chang et al. (2002) respectively

The antioxidant activity of tomatoes increased with cooking time and temperature (Table 2). Capanoglu et al. (2010) and Kaur and Singh (2008) have reported higher antioxidant activity of thermally treated tomatoes. Enzymatic oxidation reactions in raw plant materials can cause loss of antioxidants. These enzymes are deactivated through heat treatment, therefore helping to retain the antioxidant capacity of cooked foods (Nayak et al., 2015). Udomkun et al. (2015) also confirmed that at high temperatures antioxidant active substances are formed which balance the loss of vitamin C and leads to higher antioxidant capacity. Tomas et al. (2017) concluded that industrial processing may lead to enhanced bioaccessibility of antioxidants. In the current study, maximum antioxidant activity of tomatoes was noted at 30 minutes of heating at 88°C. This may be regarded as an optimal temperature for maximizing the antioxidant capacity of tomato puree. Antioxidant capacity of foods has been reported to be dependent on phenolic contents (Udomkun et al., 2015). Nayak et al. (2015) reported non-significant changes in the

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total phenolic and total flavonoid content of tomatoes despite significant increase of antioxidant content due to thermal processing. The study at hand showed significant improvement in the total phenolic and flavonoid content of cooked tomato puree samples (Tables 1-4). These finding are supported by experiments conducted by Chang., Lin, Chang, and Liu (2006) and cited by Karam, Petit, Zimmer, Djantou, and Scher (2016) drying resulted in an increase in total flavonoid and total phenolic content as compared to the corresponding levels in fresh tomatoes. In our study, the flavonoid and phenolic contents were found to reach their maximum value at 88°C when heated for 30 minutes. The findings of current study can be valuable for the food manufacturers for developing efficient procedures aimed at maximum retention of phytochemicals in their products. Processed foods, for instance tomato puree, offering the convenience of usage as well as maximal functional values would be of interest to the consumers. The findings of this research can also be of help in planning nutrition education sessions for general public to maximize the retention of functional benefits of cooked tomatoes.

5. CONCLUSION

In the light of foregoing discussion, it is concluded that heat processed tomatoes have enhanced total antioxidant activity, phenolic and flavonoid content. Heating tomato puree at 80° C for 30 minutes can result in maximum rise of phytochemical contents. The potential influence of heat on antioxidant, flavonoid and phenolic content availability in tomatoes and tomato-based products would be of interest to food industries as well as to the consumers.

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CONFLICT OF INTEREST
The authors declare that they have no competing interests.

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