



Research article

Tomato powder and crude lycopene as a source of natural antioxidants in whole wheat flour cookies

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ABSTRACT

The nutritional quality of bakery products keeps on degrading from the process of baking, packaging, transportation and storage. The present study was carried out to evaluate the effect of addition of tomato powder (2 & 4/100 g of flour) and crude lycopene (50 & 100 mg/100 g of flour), which have potent antioxidant activity, on the nutritional quality and shelf life of cookies prepared from whole wheat flour. Color values i.e., a^* and b^* of freshly prepared cookies containing tomato powder (TP) were found in the range of 5.40–6.21 and 33.20–33.40 respectively, and that of crude lycopene (CL) in the range of 5.18–5.24 and 32.50–34.90 respectively, higher than the control (4.53 and 32.50, respectively). Significant ($p < 0.05$) and non-significant ($p > 0.05$) increase was observed in the total phenolic content of dough containing TP (0.54–0.72 mg GAE/g) and CL (0.46–0.59 mg GAE/g), when compared to control (0.38 mg GAE/g). Antioxidant properties like, DPPH scavenging activity, reducing power and inhibition of lipid peroxidation (ILP), and total carotenoid content (TCC) of dough and cookies increased significantly ($p < 0.05$) upon incorporation of TP and CL. Sensory properties of enriched cookies were comparable with that of control. Color values (a^* and b^*), hardness, TCC, ILP and TPC were reduced significantly ($p < 0.05$) with storage.

1. Introduction

The demand for foods that offer health benefits beyond basic nutrition has increased to a large extent among the consumers. It necessitates the formulation of food products that have health promoting effects like antioxidant, anti-cancerous, anti-inflammatory and anti-diabetic properties. Foods with such attributes may also be used as an alternative to dietary supplements in terms of safety, consumption, delivery and effectiveness of bioactive compounds in vivo (Škrbic and Cvejanov, 2011).

Cereal-based bakery products are widely accepted and consumed by almost all people around the world. They have long shelf-lives which make them convenient for use (Arshad et al., 2007). In addition, the nutritional quality of these products can be improved by incorporating a number of bioactive ingredients into their formulation. In this way, bakery products like cookies, cakes etc. can be used as a medium for sufficing health promoting dietary needs. However, an important aspect in preparing cookies with improved nutritional value is the preservation of their sensory attributes as consumer acceptability has the priority in determining the effective application of a newly developed product (Škrbic and Cvejanov, 2011).

Bakery products contain high amount of fats and oils which oxidize slowly during storage leading to rancidity and deterioration of sensory attributes of the product. However, the auto-oxidation of fats or oils in these products can be prevented by using antioxidants. The use of natural antioxidants derived from plants has received much attention in recent years (Dillard and German, 2000) due to the toxicity associated with the use of synthetic antioxidants such as butylated hydroxy anisole and butylated hydroxy toluene (Gazzani et al., 1998). In this context, tomato powder and its extract can be used as a source of natural antioxidants (Stajcic et al., 2015) in bakery products to prolong their shelf life as well as to increase their nutritional quality.

Tomato (*Lycopersicon esculentum*) is an important agricultural commodity containing high concentration of lycopene. It is regarded as an important contributor of carotenoids to human diet. Lycopene is an efficient radical scavenger with strong physical quenching rate for singlet oxygen. Additionally, it is known to induce cell to cell communication and modulate hormone system, immune system and other metabolic pathways (Navarro-González et al., 2011). Balasundram et al. (2006) reported that phenolic compounds present in tomatoes have several physiological properties such as antioxidant, anti-microbial,

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antithrombotic, anti-inflammatory, anti-allergenic, cardioprotective and vasodilating. Limited studies have been carried out on incorporation of tomato powder and its extract in cookies. The objective of the present study was to prepare whole wheat flour cookies with added tomato powder and its extract and investigate the properties of the same during the storage period of nine months.

2. Material methods

2.1. Sample collection and preparation

Wheat cultivar HS-240 (*Triticum aestivum*, L.) was procured from Sheri-Kashmir University of Agricultural Sciences & Technology, Kashmir (SKUAST-K), Srinagar, India. The grains were ground into flour in a laboratory mill (Amar Industries, Amritsar, India) and sieved (50 mesh) to obtain the whole wheat flour with uniform particle size. The moisture, protein, fat, crude fiber and ash contents of the flour sample were observed as 10.5, 9.00, 2.00, 4.60 and 1.53 g/100 g (dw), respectively.

The tomato lycopene was extracted using modified method of [Mayeaux et al. \(2006\)](#). 12 mL of each of petroleum ether and acetone were added to tomato powder (2 g) in the dark amber colored flask. The extraction of the sample was carried out at room temperature for 24 h, followed by addition of 8 mL of water. After extraction, the samples were centrifuged at 1500 g for 10 min to separate the sample extract into distinct polar and non-polar layers. The supernatant layer was transferred to round bottomed amber colored flask. This supernatant solvent was then evaporated using rotary vacuum evaporator (Equitron Roteva, India). The dried extract was kept in HPLC vial and stored under refrigeration (4 °C) till further analysis.

2.2. Preparation of cookies

Whole wheat flour cookies supplemented with crude lycopene (extracted from tomatoes) and tomato powder were prepared by mixing flour with the crude lycopene (50 & 100 mg/100 g of flour) and tomato powder (2 & 4/100 g of flour). The control cookies without any supplement were also prepared in order to check the contribution of supplements. Whole wheat flour (250 g) was mixed thoroughly with 100 g of shortening in a laboratory mixer. It was followed by the addition of 112.5 g of sugar, 30 g of skim milk powder, 1.5 g of salt, 1.5 g of baking powder, 25 g of egg white and water as per the requirement (approximately 10–12 %). The dough samples were represented as DC (control), DL₅₀ (50 mg of lycopene/100 g), DL₁₀₀ (100 mg lycopene/100 g), DT₁ (2 g of tomato powder/100 g), and DT₂ (4 g of tomato powder/100 g), sheeted to a uniform thickness of 6 mm and cut into round shapes using a round cutter of 5.00 cm diameter. After this, the cookies were placed on aluminum trays and baked at 160 °C for 10–15 min and then allowed to cool. The cookies were labeled as CC (control), CL₅₀, CL₁₀₀, CT₁ and CT₂, respectively. The cookies were packaged in air tight laminated pouches, stored at room temperature (18–25 °C) under natural relative humidity conditions for storage studies (0–9 months).

2.3. Proximate composition of cookies

The proximate composition of freshly prepared cookies including moisture (925.10), protein (984.13), crude fat (920.85) and ash (923.03) content of the cookies was determined using the standard methods of [AOAC \(1990\)](#).

2.4. Physical properties – Weight, diameter, thickness & spread ratio

Four cookie samples selected randomly were weighed using analytical balance and then were placed next to each other and the total diameter was measured using Vernier Caliper. The average of the two readings divided by four was taken as a diameter of a cookie. Thickness of

the cookies was measured by stacking four cookies one above the other and restacking four times. Further, the spread ratio of the cookies was measured and expressed as diameter/thickness.

2.5. Texture

Hardness of cookies was measured by using TA.XT Plus, texture analyser (Stable MicroSystems, Vienna Court, UK) that was set with pre-test speed = 1 mm/s, test speed = 3 mm/s, post-test speed = 10 mm/s and trigger force = auto. Each cookie was centrally placed on the base plate and the blade was lowered to break the cookie at a distance of 5 mm. The peak force was measured that represented the hardness of cookies. The analysis of each sample was performed in triplicates.

2.6. Color

Hunter color *L** (lightness), *a** (redness) and *b** (yellowness) values of the cookies were determined using Color Flex Spectrocolorimeter (Hunter Lab Colorimeter D-25, Hunter Associates Laboratory, Ruston, USA) after being standardized using Hunter Lab color standards.

2.7. Total phenolic content (TPC)

The TPC of the samples was determined using the Folin-Ciocalteu spectrophotometric method ([Gao et al., 2002](#)). Each sample (200 mg) was taken in a centrifuge tube and extracted by adding 4 mL of acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) to it and kept at room temperature (25 °C) for 2 h. After this, 1.5 mL of freshly prepared (10 fold diluted) Folin-Ciocalteu reagent was added to 200 µL of this sample extract. The reagents were mixed, allowed to equilibrate for 5 min and neutralized with 1.5 mL of sodium carbonate solution (60 g/L). This was followed by incubation at room temperature (25 °C) for 90 min. The absorbance of the samples was taken at 725 nm (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan). Results are expressed as mg of gallic acid equivalents (GAE) per gram of sample using the gallic acid standard curve.

2.8. Antioxidant properties

2.8.1. DPPH (2, 2-diphenyl-2-picrylhydrazyl) radical scavenging assay

DPPH radical scavenging activity was determined according to the procedure described by [Brand-Williams et al. \(1995\)](#) with minor modifications. 100 mg of sample was extracted with 1 mL of absolute methanol for 2 h and then centrifuged at 3000 × g for 10 min. The supernatant (100 µL) was collected and reacted with 3.9 mL of a 6×10^{-5} mol/L of DPPH solution. Absorbance (A) (UV-vis spectrophotometer, Model, UV-2450, Shimadzu, Japan) at 515 nm was read at 0 (control) and 30 min (samples) using a methanol blank. Antioxidant activity was measured as % scavenging activity of DPPH radical calculated as below:

$$\text{DPPH radical scavenging activity (\%)} = [1 - (\text{A of sample } t = 30 / \text{A of control } t = 0)] \times 100$$

2.8.2. Reducing power

The reducing power of samples was determined by using the method of [Zhao et al. \(2008\)](#). Samples were extracted by adding 0.5 mL of 80% methanol to 500 mg (db) sample in polypropylene tubes. A standard curve was prepared using different concentrations of ascorbic acid and results are expressed as ascorbic acid equivalents (AAE)/g of sample.

2.8.3. Lipid peroxidation

Lipid peroxidation values were determined by using the method described by Wright et al. (1981) with slight modification. Sample extraction was carried out using acidified methanol (HCl/methanol/water, 1:80:10, v/v/v). A sample of 200 mg was taken in a small beaker to which 4 mL of acidified methanol was added and placed on a magnetic stirrer for 2 h for extraction. This was followed by centrifugation at 3000 × g for 10 min. After centrifugation 250 µL of the extract was taken in a centrifuge tube to which was added 1 mL of linoleic acid (0.1% in ethanol), 0.2 mL of ferric nitrate (20 mM), 0.2 mL of ascorbic acid (200 mM) and 0.2 mL of hydrogen peroxide (30 mM). The mixture was then incubated at 37 °C in water bath for 1 h. After incubation, the reaction was terminated by the addition of 1 mL TCA (10%, w/v), followed by addition of TBA (1 mL, 1% w/v). The reaction mixture was placed in a boiling water bath for 20 min and then centrifuged at 5000 rpm for 10 min. Absorbance of the samples was measured at 535 nm against a blank. Lipid peroxidation was measured as % inhibition using the formula given below:

$$\% \text{ Inhibition} = [1 - (A \text{ of sample} / A \text{ of control})] \times 100$$

2.9. Total carotenoids

Total carotenoid content was estimated by using the method of de Carvalho et al. (2012). Sample (15 g) was taken in a dark amber colored flask (prevent photo-degradation), to which 25 mL of acetone was added. The mixture was then homogenized for 1 min at maximum speed (WiseTis Homogenizer, Wisd Laboratory Instruments, Seoul, Korea) to maximize the extraction of carotenoids and kept overnight on a magnetic stirrer in dark. This process was repeated several times until the sample became colourless. After extraction, the mixture was filtered using whatman filter paper No. 1. The filtered extract was then transferred to a separatory funnel (500 mL) already filled with 40 mL of petroleum ether, followed by steady addition of distilled water in order to remove acetone. The aqueous phase containing acetone was discarded. The procedure was repeated four times until no residual acetone remained in the mixture. After this, the extract was transferred to a volumetric flask (50 mL) to which 15 g of anhydrous sodium sulfate was added. Total volume was made to 50 mL by using petroleum ether and absorbance was taken at 450 nm. Total carotenoid content was calculated as:

$$\text{Carotenoids content (} \mu\text{g / g)} = \frac{A \times V(\text{mL}) \times 10^4}{A_{1\text{cm}}^{1\%} \times P(\text{g})}$$

where, A = Absorbance; V = Total extract volume; P = sample weight; $A_{1\text{cm}}^{1\%}$ = 2592 (β -carotene Extinction Coefficient in petroleum ether).

2.10. Sensory evaluation

Sensory evaluation of the freshly prepared and stored whole wheat flour cookies was done in order to check the overall acceptability of the product after addition of tomato powder and its extract. The samples were presented to a total of 20 semi-trained panelists from the Department of Food Science and Technology, University of Kashmir, India. Five differently coded samples were presented to panelists. The panelists rinsed their mouth thoroughly with portable water and tasted the cookies one by one. Sensory attributes like appearance, texture, mouth feel, flavor and overall acceptability were recorded on the basis of 9-point hedonic scale (Bhat et al., 2018).

2.11. Statistical analysis

Results were expressed as averages of triplicate observations and mean \pm standard deviation. An analysis of variance (ANOVA) with 5% level of significance was performed. Duncan's test was used to calculate

the differences between means using statistical package (SPSS, Inc, Chicago, IL, USA).

3. Results and discussion

3.1. Proximate composition

Proximate composition of freshly prepared cookies is presented in Table 1. Moisture, protein, crude fat and ash content of the cookies were observed in the range of 2.27–3.55 g/100 g, 6.11–6.72 g/100 g, 26.08–27.14 g/100 g and 1.71–2.03 g/100 g (dw), respectively. Protein content of the composite cookies i.e. cookies containing crude lycopene and tomato powder did not vary significantly ($p < 0.05$) compared to control. However, crude fat and ash content of the cookies were observed to increase significantly ($p < 0.05$) upon incorporation of crude lycopene and tomato powder. The highest and lowest fat content was found in CL₁₀₀ (27.14 %) and CC (26.08 %) cookies, respectively. Increased fat content of the former may be due the incorporation of crude lycopene which forms micro globules with fat (Tan et al., 2014), thereby preventing it from oxidative deterioration. Bhat and Ahsan (2015) reported that the ash content of the cookies increased after incorporation of tomato powder which is consistent with the results observed in the present study.

3.2. Physical characteristics

Physical properties of the cookies are shown in Table 2. Diameter of the cookies was observed in the range of 5.30–6.04 cm. Enriched cookies exhibited significantly ($p < 0.05$) lower values of diameter and higher values of thickness in comparison to control cookies. Consequently, spread ratio of the enriched cookies was observed lower than the control cookies. Such behavior could be attributed to the better rising ability of the cookies containing tomato powder and crude lycopene (Olapade and Adeyemo, 2014). Decrease in the spread ratio of cookies containing tomato powder may be attributed to the high water absorption capacity of the later. Bhat and Ahsan (2015) have reported a decrease in the spread ratio of wheat flour cookies following the addition of tomato powder at a level of 7.5% and 10%, which is in line with the results obtained in the present study.

Spread ratio of the cookies was observed to decrease significantly ($p < 0.05$) upon addition of crude lycopene. Since lycopene is fat soluble, it is likely to reduce the amount of free fat available for wheat flour, thereby decreasing the spread ratio of cookies. According to Ayo et al. (2014), the fat content of cookies has a direct relationship with their spread ratio.

3.3. Texture of cookies

Effect of incorporation of crude lycopene and tomato powder during storage period of 0–9 months on the texture of cookies is presented in

Table 1. Proximate composition (db) of cookies supplemented with tomato powder and crude lycopene (n=3).

Sample	Moisture (g/100 g)	Protein (g/100 g)	Crude fat (g/100 g)	Ash (g/100 g)
CC	2.36 \pm 0.04 ^a	6.13 \pm 0.02 ^a	26.08 \pm 0.06 ^a	1.71 \pm 0.03 ^a
CL ₅₀	2.39 \pm 0.12 ^a	6.14 \pm 0.04 ^a	26.63 \pm 0.12 ^c	1.81 \pm 0.05 ^b
CL ₁₀₀	2.27 \pm 0.06 ^a	6.11 \pm 0.02 ^a	27.14 \pm 0.06 ^d	1.86 \pm 0.03 ^{bc}
CT ₁	3.45 \pm 0.05 ^d	6.16 \pm 0.08 ^a	26.30 \pm 0.14 ^b	1.91 \pm 0.02 ^c
CT ₂	3.55 \pm 0.05 ^d	6.11 \pm 0.14 ^a	26.62 \pm 0.03 ^c	2.03 \pm 0.07 ^d

Results are expressed as means (n = 3) \pm standard deviation.

Values followed by same letter in a column do not differ significantly ($p < 0.05$). CC: Control cookie without any supplement;; CL₅₀: Cookie with added crude lycopene (50 mg/100g of flour); CL₁₀₀: Cookie with added crude lycopene (100 mg/100 g of flour); CT₁: Cookie with added tomato powder (2 g/100 g); CT₂: Cookie with added tomato powder (4 g/100 g).

Table 2. Physical characteristics and hardness of cookies supplemented with tomato powder and crude lycopene (n = 3).

Parameters	Hardness (N)			Storage (Months)				
	Weight (g)	Diameter (cm)	Thickness (cm)	Spread ratio	0	3	6	9
CC	12.42 ± 0.05 ^a	6.04 ± 0.03 ^d	0.59 ± 0.00 ^a	10.24 ± 0.17 ^e	17.16 ± 1.00 ^{cp}	13.65 ± 0.43 ^{bp}	12.72 ± 0.23 ^{bp}	9.33 ± 0.41 ^{ap}
CL ₅₀	12.14 ± 0.20 ^a	5.50 ± 0.02 ^c	0.66 ± 0.01 ^c	8.48 ± 0.14 ^c	33.65 ± 0.29 ^{cq}	31.54 ± 1.20 ^{cr}	24.96 ± 0.23 ^{bq}	20.4 ± 0.10 ^{aq}
CL ₁₀₀	11.93 ± 0.54 ^a	5.55 ± 0.02 ^{bc}	0.61 ± 0.02 ^b	9.09 ± 0.16 ^d	36.01 ± 0.15 ^{cr}	31.95 ± 1.40 ^{br}	26.67 ± 0.29 ^{ar}	23.5 ± 0.32 ^{ar}
CT ₁	12.45 ± 0.02 ^a	5.49 ± 0.03 ^b	0.69 ± 0.01 ^d	7.96 ± 0.13 ^b	36.11 ± 0.80 ^{br}	26.88 ± 0.17 ^{aq}	26.06 ± 0.90 ^{ar}	24.1 ± 0.07 ^{as}
CT ₂	12.51 ± 0.14 ^a	5.30 ± 0.03 ^a	0.71 ± 0.01 ^a	7.47 ± 0.09 ^a	43.78 ± 0.29 ^{cs}	32.83 ± 0.95 ^{br}	32.66 ± 0.65 ^{bs}	27.6 ± 0.41 ^{at}

Results are expressed as means (n = 3) ± standard deviation.

Values followed by same letter in a row & in the column do not differ significantly (p < 0.05).

CC: Control cookie without any supplement;; CL₅₀: Cookie with added crude lycopene (50 mg/100g of flour); CL₁₀₀: Cookie with added crude lycopene (100 mg/100 g of flour); CT₁: Cookie with added tomato powder (2 g/100 g); CT₂: Cookie with added tomato powder (4 g/100 g).

Table 2. Hardness of the freshly prepared cookies was observed in the range of 17.16–43.78 N. It increased significantly (p < 0.05) after the addition of tomato powder and crude lycopene. The highest and lowest values for hardness were observed in CT₂ and CC, respectively. The increase in the hardness of CT₁ and CT₂ could be due the increase in the amount of fiber upon addition of tomato powder when compared to control. It has been reported by Bhat and Ahsan (2015) that the hardness of cookies increased after the incorporation of tomato powder (5%), which is in line with the results of the present study. The hardness of cookies was reported as 49.69 N by Zouari et al. (2016) which is in agreement with the results obtained in the present study. Kaur et al. (2014) reported the hardness of cookies prepared from different wheat cultivars between 30.25 and 78.87 N. Incorporation of crude lycopene also increased the hardness of cookies. This might be due to the formation of micro globules between lycopene and fat as reported by Tan et al. (2014) in a study related to incorporation of lycopene in liposomes. As fat hinders the formation of gluten network, its limited availability presumably increases the hardness of cookies.

Hardness of cookies decreased significantly (p < 0.05) during the storage period of 0–9 months. Decrease in the hardness of cookies during storage may be attributed to the increase in water activity (Chieh, 2006).

3.4. Color

Hunter color values L* (lightness), a* (redness) and b* (yellowness) of cookies are presented in Table 3. Results revealed that freshly prepared enriched cookies had lower L* value (45.4–54.8) which implies their darker appearance as compared to control. The highest L* value was found in CC (55.9) and lowest in CT₂ cookie (45.4). Hunter color values i.e. a* and b* of the freshly prepared cookies were observed in the range of 4.53–6.21 and 32.5–35.4, respectively. However, the values of redness and yellowness were found higher in cookies containing tomato powder and crude lycopene when compared to control. Kaur et al. (2017) reported the L*, a* and b* values for whole wheat flour cookies as 61.66,

9.96 and 26.33, respectively, which is slightly more than the values observed in the present study. The values of the same parameters reported by Bhat and Ahsan (2015) for cookies containing tomato powder are in agreement with the results obtained in the current study.

Storage period of 0–9 months resulted in a significant (p < 0.05) increase in L* value while, a significant (p < 0.05) decrease in a* and b* values was observed in all the cookies. The decrease in a* and b* values may be attributed to the degradation of carotenoids upon storage. It has been reported by Chen et al. (1996) that decrease in the amount of α-carotene occurred from 25.4 to 20.7, 19.7 and 19.3 μg/mL respectively, at the temperatures of 4, 25 and 35 °C during the storage period of 3 months which might also be the case in our study.

3.5. Total phenolic content (TPC)

TPC of dough and cookies is shown in Table 4. TPC of the control dough varied non-significantly (p > 0.05) from DL₅₀ and DL₁₀₀. This might be due to the lesser amount of phenolic compounds in crude lycopene. However, TPC of DT₁ and DT₂ increased significantly (p < 0.05) due to the presence of tomato powder as compared to control and were observed in the range of 0.38–0.72 mg GAE/g. The increase in TPC might be due to the presence of phenolic compounds such as rutin, naringenin, chlorogenic acid, protocatechuic acid, caffeic acid, gentistic acid, p-coumaric acid, ferulic acid etc. in tomato powder (Georgé et al., 2011). TPC of all cookies in general increased significantly (p < 0.05) upon baking. It may be a result of generation of Maillard reaction products during baking (Lindenmeier and Hofmann, 2004).

Storage time of 0–9 months resulted in a non significant (p > 0.05) decrease in TPC content of all the cookies. Decrease in TPC content may be due to degradation of polyphenols during storage (Şaponjac et al., 2016). TPC of enriched cookies was however, observed to remain higher compared to control throughout the storage which indicates their higher antioxidant potential.

Table 3. Hunter color values of cookies supplemented with tomato powder and crude lycopene (n = 3).

Sample	Storage (Months)			Storage (Months)			Storage (Months)					
	0	3	6	0	3	6	0	3	6	9		
	L			a			b					
CC	55.9 ± 0.4 ^{at}	55.9 ± 0.2 ^{at}	56.2 ± 0.5 ^{bt}	56.5 ± 0.2 ^{bs}	4.5 ± 0.4 ^{bp}	4.5 ± 0.3 ^{bp}	3.5 ± 0.2 ^{ap}	3.1 ± 0.1 ^{ap}	32.5 ± 0.6 ^{cp}	30.5 ± 0.1 ^{bp}	30.2 ± 0.4 ^{bp}	29.1 ± 0.1 ^{ap}
CL ₅₀	54.8 ± 0.5 ^{as}	54.9 ± 0.2 ^{as}	55.8 ± 0.8 ^{abs}	55.9 ± 0.2 ^{br}	5.2 ± 1.7 ^{cq}	4.7 ± 0.1 ^{bpq}	4.1 ± 0.7 ^{apq}	4.0 ± 0.3 ^{aq}	33.7 ± 0.3 ^{cq}	32.5 ± 0.3 ^{bq}	32.3 ± 0.2 ^{br}	31.4 ± 0.0 ^{ar}
CL ₁₀₀	51.7 ± 1.4 ^{aqr}	51.9 ± 0.4 ^{ar}	52.6 ± 0.0 ^{abr}	53.6 ± 0.1 ^{cq}	5.2 ± 0.5 ^{cq}	4.9 ± 0.0 ^{bq}	3.4 ± 0.0 ^{ap}	3.3 ± 0.0 ^{ap}	35.4 ± 0.5 ^{dr}	34.9 ± 0.6 ^{cr}	32.5 ± 0.1 ^{br}	31.6 ± 0.1 ^{ar}
CT ₁	50.7 ± 0.9 ^{aq}	50.8 ± 0.1 ^{aq}	50.9 ± 1.4 ^{ap}	51.9 ± 0.3 ^{abp}	5.4 ± 0.2 ^{dq}	4.5 ± 0.1 ^{cp}	4.4 ± 0.1 ^{bq}	4.1 ± 0.2 ^{aq}	33.2 ± 1.4 ^{dq}	32.8 ± 0.0 ^{cq}	31.5 ± 0.3 ^{bq}	30.0 ± 0.2 ^{aq}
CT ₂	45.4 ± 1.1 ^{ap}	45.9 ± 2.1 ^{ap}	51.2 ± 0.3 ^{bq}	51.5 ± 0.0 ^{bp}	6.2 ± 0.4 ^{cr}	5.3 ± 0.1 ^{br}	4.9 ± 1.0 ^{ar}	4.8 ± 0.4 ^{ars}	33.4 ± 0.5 ^{cq}	32.9 ± 1.1 ^{bq}	32.2 ± 0.1 ^{br}	31.2 ± 0.4 ^{ar}

Results are expressed as means (n = 3) ± standard deviation.

Values followed by same letter in a row & in the column do not differ significantly (p < 0.05).

CC: Control cookie without any supplement;; CL₅₀: Cookie with added crude lycopene (50 mg/100g of flour); CL₁₀₀: Cookie with added crude lycopene (100 mg/100 g of flour); CT₁: Cookie with added tomato powder (2 g/100 g); CT₂: Cookie with added tomato powder (4 g/100 g).

Table 4. Antioxidant properties of cookies supplemented with tomato powder and crude lycopene (n = 3).

Sample	DC	CC	DL ₅₀	DL ₁₀₀	CL ₅₀	CL ₁₀₀	DT ₁	DT ₂	CT ₁	CT ₂
Storage (Months)	TPC (mg GAE/g)									
0	0.38 ± 0.0 ^P	0.80 ± 0.03 ^{brs}	0.46 ± 0.07 ^{Pq}	0.59 ± 0.04 ^{Pq}	0.84 ± 0.01 ^{crs}	0.86 ± 0.01 ^{bs}	0.54 ± 0.12 ^q	0.72 ± 0.12 ^r	1.22 ± 0.05 ^{bt}	1.27 ± 0.09 ^{ct}
3	-	0.77 ± 0.07 ^{abp}	-	-	0.82 ± 0.01 ^{bp}	0.84 ± 0.10 ^{abp}	-	-	1.17 ± 0.10 ^{abq}	1.25 ± 0.08 ^{cq}
6	-	0.73 ± 0.02 ^{abp}	-	-	0.77 ± 0.04 ^{bcpq}	0.83 ± 0.04 ^{abp}	-	-	1.10 ± 0.07 ^{abr}	1.08 ± 0.05 ^{br}
9	-	0.71 ± 0.01 ^{ap}	-	-	0.74 ± 0.07 ^{apq}	0.77 ± 0.03 ^{aq}	-	-	1.03 ± 0.02 ^{ar}	0.91 ± 0.04 ^{as}
	DPPH scavenging assay (%)									
0	15.3 ± 0.1 ^P	20.7 ± 0.51 ^{cq}	22.9 ± 1.14 ^{qf}	26.1 ± 2.25 ^s	32.6 ± 2.81 ^{ct}	34.5 ± 0.60 ^{ct}	21.1 ± 1.70 ^q	24.9 ± 2.40 ^{rs}	29.5 ± 1.90 ^{ct}	32.8 ± 1.80 ^{ct}
3	-	18.3 ± 0.90 ^{bp}	-	-	31.3 ± 0.51 ^{ct}	32.9 ± 0.90 ^{ct}	-	-	27.7 ± 1.30 ^{cq}	31.2 ± 1.60 ^{ct}
6	-	17.4 ± 0.82 ^{bp}	-	-	23.6 ± 0.20 ^{br}	23.9 ± 0.81 ^{br}	-	-	21.9 ± 0.50 ^{bq}	24.5 ± 1.00 ^{br}
9	-	12.2 ± 0.80 ^{ap}	-	-	15.9 ± 0.60 ^{aq}	14.8 ± 0.10 ^{aq}	-	-	16.1 ± 0.30 ^{aq}	17.8 ± 0.44 ^{ar}
	Reducing power (mg AAE/g)									
0	0.23 ± 0.1 ^P	0.34 ± 0.04 ^{bq}	0.31 ± 0.04 ^q	0.38 ± 0.01 ^q	0.57 ± 0.01 ^{ds}	0.63 ± 0.05 ^{cst}	0.30 ± 0.07 ^q	0.50 ± 0.04 ^r	0.56 ± 0.00 ^{crs}	0.68 ± 0.02 ^{ct}
3	-	0.30 ± 0.05 ^{abp}	-	-	0.53 ± 0.01 ^{cqr}	0.59 ± 0.08 ^{crs}	-	-	0.52 ± 0.02 ^{cq}	0.63 ± 0.05 ^{cs}
6	-	0.31 ± 0.00 ^{abp}	-	-	0.48 ± 0.03 ^{br}	0.37 ± 0.04 ^{bq}	-	-	0.47 ± 0.01 ^{br}	0.50 ± 0.51 ^{br}
9	-	0.22 ± 0.01 ^{ap}	-	-	0.44 ± 0.02 ^{ar}	0.25 ± 0.02 ^{ap}	-	-	0.42 ± 0.02 ^{ar}	0.36 ± 0.03 ^{aq}
	Lipid peroxidation (% inhibition)									
0	10.9 ± 0.3 ^{Pq}	6.4 ± 0.36 ^{bp}	12.2 ± 0.70 ^{Pq}	22.9 ± 2.25 ^r	10.7 ± 0.72 ^{cpq}	7.70 ± 0.72 ^{bpq}	30.3 ± 1.70 ^s	37.0 ± 0.40 ^t	9.27 ± 0.30 ^{dq}	13.4 ± 0.80 ^{cq}
3	-	5.1 ± 0.24 ^{pp}	-	-	9.34 ± 0.83 ^{cqr}	5.32 ± 0.90 ^{abp}	-	-	7.02 ± 0.50 ^{cpq}	10.2 ± 0.20 ^{brct}
6	-	3.2 ± 0.62 ^{ap}	-	-	6.54 ± 0.23 ^{bpq}	4.50 ± 0.80 ^{abpq}	-	-	3.54 ± 1.50 ^{bp}	8.18 ± 0.05 ^{bq}
9	-	1.7 ± 0.31 ^{ap}	-	-	4.36 ± 0.51 ^{aq}	3.00 ± 0.85 ^{apq}	-	-	1.43 ± 0.20 ^{ap}	2.25 ± 0.23 ^{ap}

Results are expressed as means (n = 3) ± standard deviation.

Values followed by same letter in a row & the column do not differ significantly (p ≤ 0.05). The letters 'a, b, c, d' denote difference within a row and 'p, q, r, s' within a column.

DC: Control dough without any supplement; CC: Control cookie without any supplement; DL₅₀: Dough with added crude lycopene (50 mg/100 g of flour); DL₁₀₀: Dough with added crude lycopene (100 mg/100 g of flour); CL₅₀: Cookie with added crude lycopene (50 mg/100 g of flour); CL₁₀₀: Cookie with added crude lycopene (100 mg/100 g of flour); DT₁: Dough with added tomato powder (2 g/100 g); DT₂: Dough with added tomato powder (4g/100 g); CT₁: Cookie with added tomato powder (2 g/100 g); CT₂: Cookie with added tomato powder (4 g/100 g).

3.6. Antioxidant properties

3.6.1. DPPH scavenging assay

DPPH radical scavenging activity of dough containing crude lycopene and tomato powder was significantly (p < 0.05) higher than control (Table 4). The possible reason for the increased DPPH radical scavenging activity could be the presence of phenolic antioxidants i.e. caffeic and chlorogenic acid in tomatoes (Takeoka et al., 2001).

Baking resulted in an increase in the DPPH radical scavenging activity of cookies. It may be due to the formation of malanoidins generated in Maillard reaction at elevated temperature (Xu and Chang, 2008). In addition, it may also be due to the *trans-cis*-isomerization of lycopene that takes place at higher temperature increasing its ability to scavenge the DPPH radicals (Phan-Thi and Waché, 2014).

A significant (p < 0.05) decrease in DPPH radical scavenging activity of control cookies and the cookies with added crude lycopene and tomato powder was observed during storage, except the cookies stored for three months where the decrease was non-significant (p > 0.05). The decrease in DPPH radical scavenging activity may be attributed to degradation of phenolic compounds upon storage.

3.6.2. Reducing power

Reducing power of dough was observed to increase significantly (p < 0.05) from 0.23 to 0.50 mg AAE/g upon addition of crude lycopene and tomato powder (Table 4). The reducing power of dry tomato powder has been previously reported by Kim and Chin (2016).

In freshly prepared cookies, reducing power increased significantly (p < 0.05) upon baking and was observed in the range of 0.34–0.68 mg AAE/g. The highest and the lowest reducing power were observed in CT₂ and CC, cookies respectively. The formation of Maillard reaction products upon baking might have increased the reducing power of cookies. A similar increase in reducing power of wheat flour cookies on baking has been reported by Sharma and Gujral (2014).

Reducing power decreased during the storage period of 0–9 months. A similar trend was observed in other antioxidant assays like TPC and DPPH radical scavenging activity. However, reducing power of enriched cookies was observed to remain high throughout the storage period when compared to control.

3.7. Lipid peroxidation

Lipid peroxide formation is accompanied with the formation of a secondary end-product called malondialdehyde (MDA), the presence of which can be used as an indication for the occurrence of lipid peroxidation. The percent inhibition of control dough and dough containing crude lycopene and tomato powder is presented in Table 4. Inhibition of lipid peroxidation (ILP) by dough was observed to increase significantly (p < 0.05) in the range of 10.88–37.02% after addition of crude lycopene and tomato powder. These results may be attributed to the presence of antioxidants in crude lycopene and in tomato powder that prevent lipid peroxide formation. Highest value of ILP was observed in case of DT₂ (37.02%) and lowest was observed in DC (10.88%). Alshatwia et al. (2010) reported the tomato powder to be more protective against lipid peroxidation than lycopene. It may be a reason of higher ILP values of dough containing tomato powder.

Baking of cookies in general resulted in a decrease in ILP values. However, it was observed to decrease non-significantly (p > 0.05) in CC and CL₅₀, while as in case of CL₁₀₀, CT₁ and CT₂ it decreased significantly (p > 0.05) upon baking. The decrease in ILP might be due to increase in oxidation of fats upon heating (Kumar and Singhal, 1992).

ILP of cookies was also found to decrease significantly (p < 0.05) during storage period of 0–9 months. The decrease in ILP may again be attributed to oxidation of lipids during storage. Mesías et al. (2015) reported the formation of lipid oxidation products in cookies after 60 days of storage which is also presumed to occur in our study.

3.8. Total carotenoids content (TCC)

TCC of dough and cookies is shown in Figure 1. Incorporation of crude lycopene and tomato powder significantly ($p < 0.05$) increased the TCC of the dough. It increased in the range of 1.71–7.06 $\mu\text{g/g}$, being highest in DL₁₀₀ and lowest in DC. Ndolo and Beta (2013) reported the TCC of whole wheat grain as 2.57 $\mu\text{g/g}$, which is close to the results obtained for control dough in the current study.

Baking of cookies significantly ($p < 0.05$) reduced the levels of TCC. Maximum reduction in TCC was observed in cookies containing tomato powder (38–38.41% reduction). However, in case of control cookies and cookies with added crude lycopene the reduction in TCC upon baking was around 28.65% and 20–27.19%, respectively. This might be because of higher stability of lycopene compared to tomato powder. It has been reported by Ranhotra et al. (1995) that upon baking of whole wheat bread carotene losses are 4–5%. In a similar study conducted by Leehardt et al. (2006), a loss of 40% in TCC of bread was observed during baking which is in agreement with the results of the present study.

Storage time (0–9 months) had an adverse effect on TCC. Results revealed a significant ($p < 0.05$) decrease in TCC of cookies, presumably due to degradation of carotenoids during storage. Mellado-Ortega and Hornero-Méndez (2016) have also found a significant decrease in TCC of durum wheat during storage.

3.9. Sensory analysis

The results of sensory analysis of cookies are presented in Table 5. Analysis was made in terms of appearance, mouth feel, flavour and overall acceptability of control and composite cookies. The results of sensory analysis showed highest rating for texture of freshly prepared CC as compared to those containing tomato powder and crude lycopene. Appearance of the cookies containing lycopene gained maximum score as compared to control and cookies with added tomato powder. The appearance score was based on the number of cracks on the surface of cookies. In general, the overall acceptability of enriched cookies was comparable to control except for CT₂ cookie. The later rated lowest for all the sensory attributes. It depicts that addition of tomato powder (2g/100 g) and crude lycopene (50 and 100 mg/100 g) to cookies have the

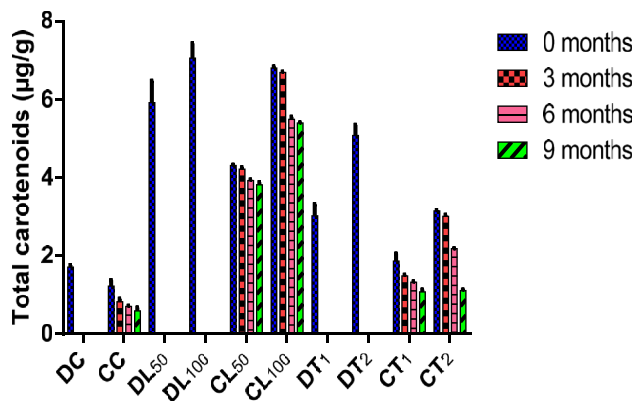


Figure 1. Total carotenoid content of cookies supplemented with tomato powder and crude lycopene ($n = 3$). Results are expressed as means ($n = 3$) \pm standard deviation. Values followed by same letter in a row & the column do not differ significantly ($p \leq 0.05$). The letters 'a, b, c, d' denote difference within a row and 'p, q, r, s' within a column. DC: Control dough without any supplement; CC: Control cookie without any supplement; DL₅₀: Dough with added crude lycopene (50 mg/100 g of flour); DL₁₀₀: Dough with added crude lycopene (100 mg/100 g of flour); CL₅₀: Cookie with added crude lycopene (50 mg/100 g of flour); CL₁₀₀: Cookie with added crude lycopene (100 mg/100 g of flour); DT₁: Dough with added tomato powder (2 g/100 g); DT₂: Dough with added tomato powder (4g/100 g); CT₁: Cookie with added tomato powder (2 g/100 g); CT₂: Cookie with added tomato powder (4 g/100 g).

Table 5. Sensory scores of cookies supplemented with tomato powder and crude lycopene.

Storage (Months)	CC	CL ₅₀	CL ₁₀₀	CT ₁	CT ₂
Texture					
0	8.25 \pm 0.41 ^{ds}	7.00 \pm 0.21 ^{bs}	7.50 \pm 0.43 ^{cr}	7.00 \pm 0.11 ^{bs}	5.62 \pm 0.63 ^{as}
3	7.80 \pm 0.32 ^{dr}	6.83 \pm 0.66 ^{br}	7.25 \pm 0.22 ^{cq}	6.87 \pm 0.09 ^{br}	5.41 \pm 0.10 ^{ar}
6	7.00 \pm 0.10 ^{cq}	6.64 \pm 0.13 ^{bq}	7.22 \pm 0.11 ^{cq}	6.55 \pm 0.11 ^{bq}	5.20 \pm 0.76 ^{aq}
9	6.32 \pm 0.21 ^{cp}	6.30 \pm 0.13 ^{cp}	6.45 \pm 0.28 ^{cp}	5.54 \pm 0.33 ^{bp}	5.00 \pm 0.81 ^{ap}
Appearance					
0	7.75 \pm 0.78 ^{cr}	7.75 \pm 0.32 ^{cr}	8.00 \pm 0.42 ^{dr}	7.50 \pm 0.87 ^{br}	6.80 \pm 0.54 ^{aq}
3	7.50 \pm 0.21 ^{bq}	7.56 \pm 0.23 ^{bq}	7.81 \pm 0.20 ^{cq}	6.89 \pm 0.13 ^{aq}	6.81 \pm 0.30 ^{aq}
6	7.00 \pm 0.66 ^{bp}	7.50 \pm 0.67 ^{cq}	7.85 \pm 0.33 ^{dqr}	6.70 \pm 0.15 ^{ap}	6.76 \pm 0.31 ^{ap}
9	6.90 \pm 0.10 ^{bp}	7.33 \pm 0.77 ^{cp}	7.45 \pm 0.34 ^{cp}	6.67 \pm 0.52 ^{ap}	6.71 \pm 0.76 ^{ap}
Mouth feel					
0	7.25 \pm 0.41 ^{cr}	7.68 \pm 0.02 ^{cq}	7.50 \pm 0.12 ^{dr}	6.75 \pm 0.30 ^{br}	5.81 \pm 0.55 ^{ar}
3	7.25 \pm 0.31 ^{cq}	7.66 \pm 0.19 ^{dq}	7.25 \pm 0.51 ^{cq}	6.71 \pm 0.66 ^{br}	5.78 \pm 0.72 ^{ar}
6	7.15 \pm 0.32 ^{cq}	7.77 \pm 0.81 ^{dq}	7.66 \pm 0.45 ^{ds}	6.00 \pm 0.44 ^{bq}	4.99 \pm 0.49 ^{aq}
9	6.82 \pm 0.21 ^{cp}	7.00 \pm 0.32 ^{dp}	6.97 \pm 0.55 ^{cdp}	5.21 \pm 0.43 ^{bp}	4.01 \pm 0.90 ^{ap}
Flavour					
0	7.50 \pm 1.01 ^{ds}	7.51 \pm 0.22 ^{dr}	7.00 \pm 0.27 ^{cr}	6.75 \pm 0.60 ^{bs}	6.00 \pm 0.15 ^{ar}
3	7.00 \pm 0.32 ^{dq}	6.75 \pm 0.71 ^{dq}	6.51 \pm 0.56 ^{cq}	6.00 \pm 0.33 ^{aq}	6.23 \pm 0.89 ^{bs}
6	7.21 \pm 0.33 ^{er}	6.80 \pm 0.47 ^{dq}	6.55 \pm 0.82 ^{cq}	6.21 \pm 0.10 ^{br}	5.01 \pm 0.97 ^{aq}
9	6.66 \pm 0.71 ^{ep}	5.55 \pm 0.35 ^{dp}	5.09 \pm 0.88 ^{cp}	4.10 \pm 0.19 ^{bp}	3.04 \pm 1.14 ^{ap}
Overall acceptability					
0	7.62 \pm 0.11 ^{cr}	7.59 \pm 0.43 ^{cr}	7.62 \pm 0.19 ^{cs}	6.87 \pm 0.34 ^{br}	6.30 \pm 0.18 ^{ar}
3	7.59 \pm 0.68 ^{cr}	7.60 \pm 0.45 ^{cr}	7.45 \pm 0.31 ^{cr}	6.88 \pm 0.10 ^{br}	6.03 \pm 0.23 ^{aq}
6	7.21 \pm 0.23 ^{cq}	7.43 \pm 0.15 ^{dq}	7.21 \pm 0.78 ^{cq}	6.52 \pm 0.21 ^{bq}	5.89 \pm 0.43 ^{aq}
9	5.23 \pm 0.32 ^{cp}	5.88 \pm 0.32 ^{dp}	5.02 \pm 0.99 ^{bp}	4.88 \pm 0.32 ^{bp}	3.32 \pm 0.27 ^{ap}

Results are expressed as means ($n = 3$) \pm standard deviation. Values followed by the same letter in a row & the column do not differ significantly ($p \leq 0.05$). The letters 'a, b, c, d...' denote difference within a row and 'p, q, r, s...' within a column. CC: Control cookie without any supplement; CL₅₀: Cookie with added crude lycopene (50 mg/100g of flour); CL₁₀₀: Cookie with added crude lycopene (100 mg/100 g of flour); CT₁: Cookie with added tomato powder (2 g/100 g); CT₂: Cookie with added tomato powder (4 g/100 g).

potential to provide desirable sensory properties to the final product, in addition to improving its antioxidant profile. The sensory attributes reported by Bhat and Ahsan (2015) for cookies prepared by incorporating tomato powder were lower as compared to that obtained in the present study. It may be because of the reduced particle size of tomato powder used in present study unlike the ones used in the previous study.

The sensory score of cookies decreased significantly ($p < 0.05$) with the increase in storage period. However, the product was moderately liked by panelists as per the 9-point hedonic scale ratings following six months of storage.

4. Conclusion

The present study demonstrated that considerable improvement in the physical characteristics and antioxidant properties of whole wheat flour cookies could be attained by the addition of tomato powder and crude lycopene. Results revealed that spread ratio of the cookies decreased after incorporation of tomato powder and crude lycopene thereby indicating a better rising ability of the enriched cookies. Texture analysis of the cookies showed that the cookies supplemented with tomato powder and crude lycopene were hard as compared to control cookies. The Hunter color values a^* and b^* of the enriched cookies were also comparable to control cookies. The results of this study suggest that novel cookies with added tomato powder and crude lycopene can be produced with improved antioxidant properties without having any adverse effect on their physical and organoleptic properties.

Declarations

Author contribution statement

Naseer Ahmad Bhat: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Idrees Ahmed Wani: Conceived and designed the experiments.

Afshan Mumtaz Hamdani: Contributed reagents, materials, analysis tools or data.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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