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Fortification Of Whole Wheat Cereal Bun With Green Tea Polyphenols And Its Effect On Estimated Glycemic Index, In-Vitro Digestibility And Nutraceutical Properties

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ABSTRACT

In this study, functional buns with the addition of green tea extract (GTE) were prepared to evaluate the in-vitro digestibility, glycemic response, physicochemical, thermal, and antioxidant properties. Green tea extract was obtained using an ultrasound-assisted extraction method. The extracted GTE was incorporated in whole wheat flour at different concentrations of 1, 3, and 5% to make green tea-fortified buns (GTEB). ATR-FTIR displayed a decrease in transmittance with an increase in concentration of GTE. Results revealed that the texture profile of functional buns varies non-significantly ($P \leq 0.5$) with the addition of GTE. Color values for crumb and crust showed significant difference ($P \leq 0.5$) with the addition of GTE. DPPH scavenging activity, reducing power (Rp), total phenolic content (TPC), and lipid peroxidation inhibition (ILP) were accessed which revealed that antioxidant activity increased significantly ($P \leq 0.5$) as the concentration of GTE increased. The *in-vitro* digestibility assay of buns fortified with green tea extract displayed a slow rate of digestion of functional buns which suggests it inhibits starch digestion. This method is anticipated to develop low glycemic bakery products for the expanding diabetic population of the world. Additionally, the buns developed from GTE can be utilized to produce functional foods with improved nutraceutical and antioxidant potential.

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The sensorial analysis of the functional buns indicated that the addition of the GTE did not impact the acceptability of the buns, as perceived by consumers.

Keywords: whole wheat flour, green tea extract, in-vitro digestiblity, nutraceutical properties.

Abbreviations

GTE Green tea extract

GTEB Green tea-fortified buns.

GI Glycemic index

HI Hydrolysis index

ILP Inhibition of lipid peroxidation

DPPH 1, 1-diphenyl-2- picrylhydrazyl) radical scavenging assay

RP Reducing power

PGI In-vitro Glycemic Index

TPA Texture profile analysis

ATR-FTIR Confirmational study by using attenuated total reflectance fourier transform infra-spectroscopy

TPC Total phenolic content

RDS Rapidly digestible starch

SDS Slowly digestible starch

RS Resistant starch

GOPOD Glucose-oxidase-peroxidase-amino antipyrine

GAE Gallic acid equivalent

AAE Ascorbic acid equivalent

To Onset temperature

Tp Peak temperature

Tc Conclusion temperature

Bn Native bun

S1 Bun fortified with 1% green tea extract

S2 Bun fortified with 3% green tea extract

S3 Bun fortified with 5% green tea extract

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1. Introduction

Wheat bread is being increasingly consumed as a traditional and an important part of the daily diet since 1995 (Kihlberg et al., 2004). Consumers are conscious enough that they prefer to consume foods with health benefits over taking supplements, and the demand for value-added wheat-based products has increased rapidly in recent decades. (Bhattacharya et al., 2003). This necessitates the revitalization of wheat-based foods with positive health effects by producers and the research communities. Bread contains a variety of elements that are either naturally present or have been added to it, including minerals, fiber, vitamins, and essential oils that naturally contain antioxidants (Magan et al., 2003). Because of the numerous health advantages connected with whole wheat products, there is an added rise in demand for foods made from them. Consumption of whole wheat flour may assist to reduce the risk of developing chronic illnesses like diabetes, obesity, and cardiovascular disorders (Bhat et al., 2016). Furthermore, the development of bun products enhanced with bioactive substances is a significant contribution to a wide array of food items with multiple health advantages. In this situation, the addition of green tea extract in baked goods can be quite beneficial for human nutrition. The demand for items made from wheat that have additional value has been rising quickly over the last few decades. Green Tea Extract (GTE) is composed of polyphenolic compounds, in which the major component is catechin. Catechin, including epicatechin, epicatechin-3-gallate, epigallocatechin, gallocatechin gallate, epicatechin, and epicatechin gallate (Zaveri, 2006), have been found to reduce the risk of chronic diseases like neurodegenerative and cardiovascular (Higdon et al., 2003), type 2 diabetes (Wu et al., 2004), cancer, and its antioxidant activity (Lu et al., 2010). It has also been reported that catechins could lower the enzymatic activity of pancreatic α-amylase and glucosidase during digestion by indirectly suppressing the acrylamide production, in the analysis of in vitro digestibility of GTE-fortified bread (Goh et al., 2015). Studies conducted by Liu et al., (2011); Yilmazer-Musa et al., (2012), and Koh et al., (2010) reported that tea polyphenols especially tea catechins inhibited the activity of α-amylase and α-glucosidase that are important enzymes for digestion of starch in humans. Another study found that tea polyphenols were the most effective in inhibiting the activity of α-amylase among the 4 types of digestive enzymes, pepsin, trypsin, α-amylase, and lipase, (He et al., 2007). These results raised the possibility that tea catechins may have an impact on the easy digestion of starch in a bun

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supplemented with GTE. Delayed starch digestion is advantageous for creating foods with a low glycemic index (GI) (Robyt et al., 2009). Low GI food consumption causes blood glucose levels to fluctuate less frequently (Englyst & Hudson, 1996), which lowers the risk of type 2 diabetes mellitus over the long term (Simmons et al., 2010). Therefore, enhancing buns with green tea polyphenols may be able to lower the bun's prospective glycemic index.

2. Materials and Methods

2.1. Materials and methods

Dried leaves of green tea (Camellia sinensis O. Kuntze) and wheat flour were procured from the local market Hazratbal Srinagar Jammu and Kashmir, India. Amyloglucosidase, α-amylase, and pancreatin enzymes was purchased from Sigma Aldrich, St. Louis, USA. GOPOD kit was obtained from Megazyme International, Wicklow, Ireland. The rest of the chemicals used to conduct experiment were of analytical grade.

2.2 Polyphenol extraction from green tea.

The dried green tea leaves were subjected to ultrasonication to liberate polyphenols using the method of Das et.al, (2018). Briefly, 300 mL of distilled water and 20 g of green tea was mixed in a beaker. After that, the suspension was ultrasonicated using an ultrasonic probe (Cole-Parmer, 04711–35) at an amplitude of 60%, power of 150 W and frequency of 20 kHz for 20 min at 45°C. The green tea extract (GTE) was transfered through a Whatman filter paper No. 1 and brought to room temperature (25°C). The extract was stored under refrigeration in an air-tight sterilized bottle.

2.3 Preparation of native and GTE fortified bun (GTEB)

Native bun and fortified bun with GTE 1, 3, and 5% of flour, were prepared using a method described by Wang and Zhou (2004), with certain modifications. The ingredients that are used for bun-making included 0.5 kg flour, 310 mL water, 15g shortening, 20 g sugar, 10 g salt, and 5 g dry instant yeast. Native bun and GTEB was labeled as Bn, S1, S2, and S3. All ingredients were mixed thoroughly. After mixing, the dough was rested for 10 min at 22°C and then divided into spherical shapes. The dough pieces were then proofed at 40°C and 85% relative humidity for 60 minutes. Finally, bake at 185°C for 15 min. After cooling at room temperature, the crumb sample was taken from the center of the bun and the crust from the surface. Samples of the bun were used for further analysis and in vitro digestibility.

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3. Proximate composition

The native bun and GTEB were analyzed for moisture (925.10), protein (920.87), ash (923.03), and fat (920.85), according to the method AOAC, 1990.

3.1 Resistant starch (RS)

RS content was measured using the Megazyme Assay Kit (Megazyme International, Wicklow, Ireland), following the approved American Association for Clinical Chemistry (AACC), 2000 method. Briefly, 100 mg sample and 4 mL of enzyme mixture (pancreatic α-amylase and amyloglucosidase) were added to each test tube, to hydrolyze digestible starch, the mixture was vortexed and incubated in a shaking water bath for 16 h at 37 °C. After incubation, the mixture was mixed with 4 mL absolute ethanol and vortexed again to deactivate the enzymes, and RS was collected as a pellet by centrifugation (1500 × g 10 min). The pellet obtained was washed with 50% ethanol to remove the digested starch. The sediment was dissolved in 2 mL of 2 M KOH by vigorously stirring for 20 min in an ice bath and neutralized with 8 mL sodium acetate buffer (1.2 M). The solution was mixed with amyloglucosidase (0.1 mL, 3300 U/mL) and then incubated in a water bath at 50 °C for 30 min, then centrifuged at 3000 × g for 10 min. 3mL of glucose-oxidase-peroxidase-amino antipyrine (GOPOD) was added to aliquots (0.1 mL) of the supernatant, and the mixture was incubated at 50°C for 20 min. Absorbance was measured using a spectrophotometer at 510 nm. Resistant starch was calculated as the amount of glucose × 0.9. Each sample was analyzed in triplicate.

3.2 In-vitro Glycemic Index (PGI)

The in-vitro glycemic indices of the samples were calculated according to the method given by Noor et al., 2021. Briefly, the sample (100 mg) was incubated with 10 ml HCl–KCl buffer (pH 1.5) and 200 ll pepsin solution (100 mg/ml HCl-KCl buffer) at 40°C for 1 h with constant shaking. The pH was maintained by the addition of 200 µl pancreatic α-amylase solution (1.5 mg/10 ml phosphate buffer; pH 7.8) and incubated at 37°C for 45 min. The reaction of the enzyme was halted with 70 µl Na_2CO_3 solution and samples were diluted to 25 ml with tris-maleate buffer (pH 6.9) and 5 ml of pancreatic α-amylase solution (3 U/5 ml tris-maleate buffer) and incubated at 37°C with constant shaking. From each sample aliquots of 1 ml were taken at 30, 90, and 120 min, to inactivate the enzyme reaction. The samples were placed into boiling water with vigorous shaking for 5 min and kept in a refrigerator (4°C) till the end of

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incubation time (180 min). 3 ml of 0.4 M sodium acetate buffer (pH 4.75) and 60 µl of amyloglucosidase (3300 U/ml) were added to each aliquot and incubated at 60°C for 45 min with constant shaking. The volume was then adjusted to 10 ml with distilled water and centrifuged before transferring 0.1 ml aliquots of the solution into glass test tubes for glucose measurement. The glucose released was measured using a glucose oxidase peroxidase (GOPOD) kit (K-GLOX, Megazyme Bray, Co. Wicklow, Ireland). Absorbance was measured at 510 nm against the reagent blank using UV–a vis spectrophotometer. The values were plotted on a graph and the area under the concentration-over-time curve (AUC) was determined using Sigma plot 10.0 (Systat Software, San Jose, CA, U.S.A.). The hydrolysis index (HI) was calculated as the percentage of total glucose released from the samples as compared to that released from standard glucose (0–180 min) (Barine & Yorte, 2016). The predicted glycemic indices of the samples were estimated according to the equation of Goni et al. (1997):

$$pGI = 39.71 + 0.549HI$$

3.3 Color

The color of samples was determined using a color flex spectrocolorimeter (Hunter Lab Colorimeter D-25, Hunter Associates Laboratory, Ruston, USA) after standardizing using Hunter Lab color standards and the Hunter values L (lightness), a (redness to greenness) and b (yellowness to blueness) were measured.

3.4 Sensory evaluation

Sensory evaluation of samples was used to determine the overall acceptability after the addition of GTE extracts. The bun samples were evaluated by a total of 10 trained panelists from the Department of Food Science and Technology, University of Kashmir, India. The panelists were given seven differently coded samples. Sensory attributes like hardness, cohesiveness, springiness, adhesiveness, chewiness, and resilience were determined based on a 9-point hedonic scale.

3.5 Texture profile analysis (TPA)

The hardness of the samples was determined using TA.XT Plus, Texture Analyser (Stable MicroSystems, Vienna Court, UK) that was fitted with a load cell of 50 kg. Kramer shear cells (blades, 3mm thick, 64mm high, and 82mm wide.) were set to move at a speed of 1 mm/s with a

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trigger force equal to 100 g. The peak force obtained from the force-displacement curve was taken as the breaking force of the bun. Results were reported as an average of three replicates.

3.6 Thermal properties

The thermal properties of the samples were studied with a differential scanning calorimeter (DSC-1 STARe System, Mettler-Toledo). 3.5 mg of samples were weighed into aluminum pans and 8 µl of deionized water was added. The pans were hermetically sealed and kept overnight at room temperature before analysis. The samples were heated at 10 °C/min from 20 to 150°C. An empty aluminum pan was used as a reference.

3.7 Confirmational study by using attenuated total reflectance fourier transform infra-spectroscopy (ATR-FTIR)

The spectra of the samples were recorded using an FTIR spectrometer system (Cary 630 FTIR, Agilent Technologies, USA), coupled with an ATR accessory. Analysis was done at room temperature, and spectra were obtained in the range of 4000-650 cm^{-1} at a resolution of 4 cm^{-1}, using Resolution Pro software version 2.5.5 (Agilent Technologies USA).

3.8. Antioxidant properties

3.8.1 DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging assay)

DPPH radical scavenging activity of the samples was determined using the method of Bhat et al., 2022. Briefly, a 100 mg sample was extracted by using 1 mL of methanol for 2 h and centrifuged at 3000×g for 10 min. The supernatant 100 µL was collected and mixed with 3.9 mL of a 6×10^{-5} mol/L of DPPH solution. Absorbance (A) (UV–Vis spectrophotometer, Model, UV-2450, Shimadzu, Japan) of the samples was measured at 517 nm using methanol as blank. Antioxidant activity was calculated using the equation given below:

$$\text{DPPH radical scavenging activity (\%)} = 1 - \frac{\text{A (sample)}}{\text{A (control)}} \times 100$$

Where A (control) is the absorbance of the control (including all reagents except the sample), A (sample) is the absorbance of the sample.

3.8.2. Reducing power (RP)

RP of the samples was done using the method of Zhao et al., (2008). Briefly, samples (500 mg) and 0.5 mL of 80% methanol were taken in a polypropylene tube for extraction.

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Results were measured as ascorbic acid equivalents (AAE)/g of a sample by preparing a standard curve using different concentrations of ascorbic acid.

3.8.3. Lipid peroxidation

Lipid peroxidation of the samples was measured by using the method of Bhat et al., (2022) with certain modifications. Lipid peroxidation was determined as % inhibition using the formula:

$$\%\text{Inhibition} = [1 - (\text{Ao of sample}/\text{Ac of control})] \times 100 \qquad \text{Equ no. } 1$$

Where Ao is the absorbance of sample and Ac is the absorbance of control.

3.8.4. Total polyphenol Content (TPC)

TPC of the samples was measured following the method of Gao et al., (2002). Sample 0.25ml was mixed with 1.75 mL of freshly prepared Folin - Ciocalteu reagent and allowed to equilibrate for 5 min. The mixture was neutralized with 0.5 mL of 7.5 % sodium carbonate solution (60 g/L) and incubated for 90 min at room temperature (25 °C). The absorbance of the samples was measured at 725 nm (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan). Methanol was used as a blank. The results were expressed as µg of gallic acid equivalents (GAE) per gram of sample.

3.8.5. Metal chelating (Fe^{2+}) activity

The chelating ability of samples was determined according to the procedure of Shah et al., 2015. The ability of all samples to chelate ferrous ions was calculated using the following equation:

$$\text{Chelating effect } (\%) = 1 - \frac{\text{A(sample)}}{\text{A (control)}} \times 100 \qquad \text{Equ no. } 2$$

Where A (sample) is the absorbance of the sample and A (control) is the absorbance of the control.

3.9. Statistical analysis

The results obtained are averages of triplicate observations. Analysis of variance with a significance level of 5% was done and Duncan's test was done to determine differences between means using the commercial statistical package (SPSS version IBM 20.0).

4. Result and discussion

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4.1 Physico-chemical properties

4.1.1 Proximate composition of native bun and GTEB

The composition of native bun and GTEB are presented in table 1. The moisture content of the native bun and GTEB increased significantly ($P \leq 0.05$) and the highest value was displayed by S3 (12.39±0.2) and the lowest by the native bun (10.74±0.17). The increase in moisture content could be due to the presence of dietary fibers and polyphenols in green tea extract which may store more water. According to Sivam et al., (2011), the interactions between water and the hydroxyl, carboxyl, or amine groups of the fibers and phenolics as well as wheat proteins may be the ground of high water retention. Protein, fat, and ash displayed no significant differences from each other. Similar results were reported by Ujihara and Hayashi (2015); Bhat et al., (2016).

Table 1. Proximate composition of native bun and GTEB.

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Bn	10.74±0.17[c]	10.69±0.29[a]	2.34±0.05[a]	1.55±0.06[a]
S1	11.02±0.05[ab]	10.76±0.07[a]	2.41±0.07[a]	1.57±0.15[a]
S2	11.92±0.11[ab]	10.93±0.23[a]	2.65±0.1[a]	1.68±0.04[a]
S3	12.39±0.2[a]	11.43±0.08[a]	2.73±0.04[a]	1.73±0.11[a]

Values (n = 3) are expressed as mean ± standard deviation. In a column, values preceded by the same letter do not differ significantly $P \leq 0.05$. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

4.1.2 In-vitro digestibility

In-vitro enzymatic digestion was carried out under controlled conditions to measure the amount of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) in bun samples (table 2). RDS levels of a native bun and GTEB decreased significantly ($P \leq 0.5$) as the concentration of GTE was increased and the highest value was displayed by the native bun (58.18±1.21) and lowest by S3 (16.14±0.45). The decrease in RDS showed that the magnitude of the glycemic response after consuming the GTEB might be reduced, which was advantageous for preventing abrupt increases in postprandial blood glucose levels. (Englyst & Hudson, 1996). In addition, the amounts of SDS and RS in the native and GTEB increased significantly and varied from 2.26±1.10 to 29.92±0.78 and 39.26±0.56 to 52.76±0.85,

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respectively. In the current study, the digestibility of buns supplemented with GTE decreased during the first 90 minutes of pancreatic digestion as the GTE concentration was increased. At the 20th, 60th, and 90th minute sampling periods, a significant reduction in glucose release was seen for all bun samples. The increase in SDS and RS could be due to the tea polyphenols that have an inhibitory effect on α-amylase activity during these times and may be the cause of a decrease in glucose release (He et al., 2007). Additionally, the high pH conditions in the pancreatic digesting phase may have caused the gallate groups of tea polyphenols (catechins) to dissociate into their anionic form, increasing the ionic contacts between the catechin and enzyme (Koh et al., 2010). All possible interactions between proteins and catechins have the potential to significantly alter the arrangement of the active site, and impair enzyme performance.

Table 2.SDS, RDS, and RS content of native bun and GTEB.

Sample	SDS (%)	RDS (%)	RS (%)
Bn	2.26±1.10[a]	58.18±1.21[d]	39.26±0.56[a]
S1	20.11±1.30[b]	34.04±1.18[c]	45.77±0.78[b]
S2	24.20±0.47[c]	27.02±0.74[b]	48.17±0.57[c]
S3	29.92±0.78[d]	16.14±0.45[a]	52.76±0.85[d]

Values (n = 3) are expressed as mean ± standard deviation. In a column, values preceded by the same letter do not differ significantly $P \leq 0.05$. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

4.1.3 Glycemic index

Table 3 shows the Hydrolysis Indices (HI) derived from the rate of hydrolysis over time and the related predictable Glycemic Indices (PGI). The HI and PGI of a native bun and GTEB decreased significantly ($P \leq 0.5$) and the highest value of HI and PGI was displayed by a native bun (49.99±0.98 and 66.62 ±0.56) and lowest by S3 (18.73±0.81 and 49.99±0.89). The decrease in HI and PGI could be due to the higher concentration of RS content in the samples (Jenkins et al., 2008). In this study, the GTEB released glucose more gradually than the native bun, which coincides with the idea that naringin's bioactive components cooperate to inhibit the enzymes that raise blood sugar levels (Shen, Xu, & Lu, 2012). Studies have shown that low GI and high RS diets can reduce insulin resistance, control blood glucose levels, enhance lipid metabolism, and avoid cardiovascular diseases (Chung et al., 2010). Thus, GI is linked to food nutritional

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quality, and a product with a low GI is preferred not just by diabetics, but also by the general public (Björck & Asp, 1994).

Table 3. HI and GI (%) of native bun and GTEB.

Sample	HI (%)	GI (%)
Bn	49.01 ± 0.98^{d}	66.62 ± 0.56^{c}
S1	24.69 ± 0.88^{c}	53.26 ± 0.70^{b}
S2	22.76 ± 0.67^{b}	52.21 ± 0.76^{b}
S3	18.73 ± 0.81^{a}	49.99 ± 0.89^{a}

Values (n = 3) are expressed as mean ± standard deviation. In a column, values preceded by the same letter do not differ significantly $P \leq 0.05$. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

4.1.4. Color

Color is the most important visual feature in determining product quality. Consumers tend to associate color with taste, safety, shelf life, nutritional value, and level of satisfaction because it correlates well with the physical, chemical, and sensory attributes of food quality (Pedreschi et al., 2006). Table 4 shows the color values of the crumb and crust of the native bun and GTEB. For the crumb, the L (lightness) and b (yellowness) values for the native bun and the GTEB samples decreased significantly ($P < 0.001$) as the concentration of GTE increased, with the lowest value displayed by S3 47.87±0.94a and 15.19±0.66 and highest by native bun 67.43±0.66 and 20.86 ±1.04 respectively. Whereas the a (greenness) value increased significantly ($P < 0.001$) from -1.34±0.24 to -0.37±0.24. These findings revealed that adding GTE to the bun resulted in a dark pink crumb with a small yellow tint. Green tea polyphenols get oxidized after baking, as did tea polyphenols engaged in caramelization and oxidized by reducing sugars. As a result, the GTE turned a dark pink color, making the bun crumb redder. Wang and Zhou (2004) and Wang et al. (2007) found that bread containing GTE reduced brightness and increased redness. Consumers found these to be satisfactory when compared to the commercially available unfortified bun.

For the crust, the L (lightness) and a (greenness) values for the native bun and the GTEB samples increased significantly ($P < 0.001$) as the concentration of GTE increased, with the highest values displayed by S3 61.46±0.73 and 8.74±0.08 and lowest by native bun 39.44±0.91 and

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3.43±0.34 respectively. whereas the b (yellowness) value decreased significantly($P < 0.001$), with the highest value displayed by native bun 35.66±0.59 and lowest by S3 (29.91±0.84). The increase in lightness could be attributed to tea polyphenols, which function as antioxidants by scavenging free radicals. Tea catechins could react with wheat protein, forming a crosslinking structure in bun dough due to broken disulfide bonds in the dough (Wang and Zhou (2004). The alterations of these crust color values are due to the presence of reducing sugars in the bun, which is linked to the Maillard reaction and caramelization. Similar results were reported by Fu et al., (2018). Similar color alterations have also been documented by Jusoh et al., (2009). Tea polyphenols prevented the Maillard reaction and caramelization in comparison to the control, giving the tea polyphenol bun crust a lighter color and higher L and b values.

Table 4. Color values of native bun and GTEB.

sample	L (Crust)	a	b	L (Crumb)	a	b
Bn	39.44±0.91^{a}	3.43±0.34^{a}	35.66±0.59^{d}	67.43±0.66^{d}	-1.34±0.24^{a}	20.86±1.04^{b}
S1	45.47±0.86^{b}	4.36±0.23^{b}	33.97±0.97c	57.54±0.67c	-1.19±0.20^{ab}	19.60±0.39^{b}
S2	56.97±0.71^{c}	7.75±0.28^{c}	32.04±0.68^{b}	52.91±0.54^{b}	-0.78±0.32^{bc}	16.29±1.36^{a}
S3	61.46±0.73^{d}	8.74±0.08^{d}	29.91±0.84^{a}	47.87±0.94^{a}	-0.37±0.24^{c}	15.19±0.66^{a}

Values (n = 3) are expressed as mean ± standard deviation. In a column, values preceded by the same letter do not differ significantly $P \leq 0.05$. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

4.1.5. Sensory evaluation

Sensory evaluation is a quantitative method for establishing legally binding links between product attributes and human perception (Lawless & Heymann, 1999). Color, texture, flavor, aftertaste, and overall acceptance of native bun and GTEB were assessed organoleptically (Table 5). Sensory examination revealed that the color measurement of the control bun (6.11±0.5) and S1 (6.75±0.82) scored significantly higher ($P < 0.05$) values, indicating that the panelists preferred both the control bun and GTEB. The S3 bun became dark green, it received a lower score (5.75±1.47). The release of certain green tea polyphenols during baking affected the color of the bun to some extent. Except for the S3, which had a dark yellow look and scored the lowest

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rating. The results also revealed that there was no significant change in flavor, aftertaste, or texture among all samples, although the GTEB was claimed to be a little firmer than the control. As a result, the addition of GTE to the formulation does not affect the mouth feel of GTEB, except for the S3 bun, which the panelists regarded as having a somewhat astringent flavor, the flavor scores were not significantly different ($P < 0.05$) among the samples ranged from 6.36±1.50 to 7.20±0.86). Even though the control bun 6.83±0.70 and the S1 bun 7.27±0.71 scored higher overall acceptance than other samples and were not significantly different ($P < 0.05$). Due to the same mouth feel as well as the acceptable flavor and look, the addition of GTE did not affect the overall acceptability of buns. Similar findings were reported by (Li et al., 2012).

Table 5. Sensory evaluation of native bun and GTEB.

Sample	Texture	Color	Flavor	Aftertaste	Overall acceptance
Bn	5.83±0.83^{a}	6.11±0.50^{ab}	7.20±0.86^{b}	7.13±0.82^{bc}	6.83±0.70^{a}
S1	6.08±0.82^{a}	6.75±0.82^{b}	7.58±0.71^{b}	7.45±0.50^{c}	7.27±0.71^{a}
S2	6.25±1.29^{a}	6.08±1.11^{ab}	6.05±1.12^{a}	6.81±1.78^{ab}	6.52±1.08^{a}
S3	5.78±0.70^{a}	5.75±1.47^{a}	6.36±1.50^{a}	6.50±1.47^{a}	6.50±0.82^{a}

Values ($n = 3$) are expressed as mean ± standard deviation. In a column, values preceded by the same letter do not differ significantly $P \leq 0.05$. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

4.1.6. Texture profile analysis

Hardness is a widely used criterion to determine bun quality since it is linked to bite force (Bourne, 2002). The hardness of samples increased as the concentration of GTE was increased (Table 6). On comparing the native bun and GTEB significant changes ($P < 0.05$) were detected and hardness increased from 10.48±0.66 to 14.87±1.14. The increase in hardness is due to the intertwining of cellulose to form a more dense mesh structure (Ning et al., 2017). The buns with tea polyphenols were slightly harder than the control. Similar results were reported by Pasrija et al. (2015). The chewiness is a measure of the energy needed to break down food particles for swallowing and was shown to increase non-significantly ($P \leq 0.05$), varying from 1.87±0.77 to

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2.37±0.26 as the GTE content was increased. Resilience which indicates the energy gained after the first cycle of compression is achieved and the values between the native and the GTEB displayed a non-significant difference ($P < 0.05$), varying from 0.28±0.03 to 0.60±0.14. Zhang et al. (2009) reported that resilience was associated with starch gelatinization and retrogradation characteristics. While springiness displayed the recovery of a sample between the first and second compression cycles, on comparing with the native bun, texture profile analysis (TPA) of the samples showed a non-significant ($P < 0.05$) increase in springiness. Cohesiveness displays the internal resistance of food structure and internal bonds in the sample, which is measured by the TPA in terms of the ratio of the second compression to the first compression. The cohesiveness of samples significantly decreased ($P < 0.05$) with the addition of GTE and varied from 0.27±0.10 to 0.46±0.14. The decrease in cohesiveness could be due to the loss of binding by tea polyphenols. Similar results were observed by Lu et al., (2010). Adhesiveness is the negative force between the first and second bite by the TPA software. The adhesiveness of the samples was observed to increase significantly ($p \leq 0.05$) from -7.19±0.06 to -0.39±0.06, as the concentration GTE was increased, which indicates the GTEB is more adhesive than the native bun. Thus samples with high negative values are more adhesive (Bhat et al., 2022). As the bun texture profile was determined on the day of manufacture, starch retrogradation did not affect the texture. The effects of starch stalling may be more pronounced if the GTEB is held for a longer period, increasing the hardness and chewiness values while decreasing the resilience value. Cohesiveness is still disregarded in the assessment of the overall bun quality, with hardness seen as the most significant texture criterion. It is preferable for the bun with GTE to not differ significantly from the control, as this will not have an impact on consumer acceptance of the bun. It is desirable for the bun containing GTE to be virtually identical to the native, as this will ensure that it is accepted by consumers.

Table 6. Texture profile analysis of native bun and GTEB.

Sample	Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness	Resilience
Bn	10.48±0.66^{a}	-0.39±0.06^{d}	0.56±0.10^{b}	0.46±0.14^{c}	1.87±0.77^{b}	0.60±0.14^{b}
S1	12.40±0.93^{b}	-0.71±0.06^{c}	0.49±0.08^{a}	0.39±0.10^{b}	1.78±1.20^{a}	0.32±0.06^{a}
S2	13.37±0.81^{c}	-3.48±0.05^{b}	0.49±0.28^{a}	0.37±0.14^{b}	1.87±0.18^{b}	0.29±0.06^{a}

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S3	14.87±1.14[d]	-7.19±0.06[a]	0.64±0.28[c]	0.27±0.10[a]	2.37±0.26[c]	0.28±0.03[a]

Values (n = 3) are expressed as mean ± standard deviation. In a column, values preceded by the same letter do not differ significantly P ≤ 0.05. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

4.1.7. Thermal properties

The thermal properties of the native bun and GTEB are presented in Table 8. The gelatinization transition temperatures that include onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) varied significantly ($P < 0.05$) among native and GTEB samples. The gelatinization transition temperatures, T_o, T_p and T_c varied from 55.38±1.22 to 58.78±0.40, 61.04±0.72 to 69.87±0.39, 66.17±0.60 to 71.50±1.64 respectively. The DSC results showed that the GTEB exhibited lower values for thermal parameters (T_o, T_p, T_c, and gelatinization enthalpy) compared to that of the native bun. These parameters determine the crystalline stability and its difference within the granules (Shi et al., 2013). The reduction in gelatinization temperatures (onset, peak, and conclusion) is due to the polyhydric structure of GTEPs, in which the -OH groups of GTPS were probably expected to interact with the side chains of amylopectin and bound to the amorphous region of starch granules. It might disrupt the interaction between crystallites and amorphous matrix, enabling the hydration of starch granules and lowering the temperatures and required energy for gelatinization (Zhu et al., 2009). Green tea polyphenols, may alter the pH and ionic strength of the system as well as interact with other substances like starch to alter the thermal characteristics of wheat flour (Zhu et al., 2015; Zhu et al., 2009). The enthalpy of gelatinization also varied significantly ($p < 0.05$) and ranges from 9.79±0.13 to 7.21±0.13. According to Wu et al. (2009), the concentration of tea polyphenol led to a clear shift in endotherms toward a lower temperature and a reduction in enthalpy. A significant difference ($P < 0.05$) in the gelatinization temperature range (T_c-T_0) was observed among samples with S3 (10.54±1.79) showing the lowest value and native bun displaying the highest (12.74±2.02). Several variables, such as the amylose concentration, lipid-amylose complex chains, crystalline-to-amorphous ratio, and protein content, influence the gelatinization transition temperatures (Gunaratne & Hoover, 2002).

Table 8. Thermal properties of native bun and GTEB.

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Sample	Onset	Peak	Endset	ΔH	T_c-T_0
Bn	58.78±0.40[d]	69.87±0.39[d]	71.50±1.64[d]	9.79±0.13[d]	12.74±2.02[b]
S1	57.86±0.40[c]	67.92±0.44[c]	69.06±0.65[c]	8.36±0.14[c]	11.19±0.52[a]
S2	56.78±0.91[b]	64.54±0.59[b]	67.33±0.82[b]	7.68±0.14[b]	10.79±1.67[a]
S3	55.38±1.22[a]	61.04±0.72[a]	66.17±0.60[a]	7.21±0.13[a]	10.54±1.79[a]

Values (n = 3) are expressed as mean ± standard deviation. In a column, values preceded by the same letter do not differ significantly $P \leq 0.05$. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

4.1.8. Confirmational study by using attenuated total reflectance fourier transform infra-spectroscopy (ATR-FTIR).

FTIR spectra of samples are presented in Fig. 1. The spectral bands are differentiated into four different regions: the stretching regions which lie between 800- 1500 cm^{-1}, and 1600-1800 cm^{-1}, the C-H region between 2800 - 3000 cm^{-1} and the O-H region between 3000 - 3600 cm^{-1}(Noor et al., 2021). The functional -OH group of GTEP and the starch molecules found in wheat flour were linked to the broad peak corresponding to the wave number between 3200 and 3400 cm-1 (Ahmad et al.,2020). All of the fortified samples show a peak shift towards a higher wave number, which shows that the -OH groups of GTEP and starch overlap. The peak between 990 and 1030 cm-1 reflects the C-O stretching of glucose rings vibration, and the peaks at wave numbers around 1613 cm-1, 1513 cm-1, and 1384 cm-1 correspond to the functional groups of C-C related to aromatic and benzene ring double bond structuring of GTEP (Fan et al., 2018; Liang et al., 2016). The assessment of these bands in the native and fortified samples revealed that as GTE content increased, the functional groups' intensity sharply decreased. The intensities may alter as a result of peak overlapping or GTE fortification, which forms unstable species that eventually transform into stable products. The changes in the molecular pattern of fortified buns confirm the presence of GTEP in a composite food system. Similar results were reported by Ahmad et al., (2021).

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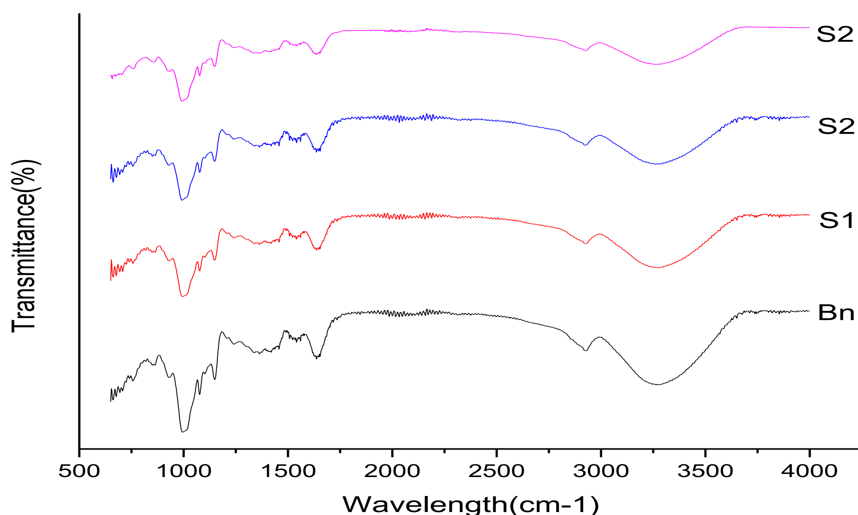

Figure 1. FTIR spectra of native bun (Bn), S1, S2, and S3. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

4.2 Antioxidant properties.

4.2.1 DPPH radical scavenging assay

DPPH radical scavenging activity of native bun and GTEB increased significantly ($p < 0.05$) as the content of GTE increased (Table 7). The sample S3 (43.94±0.59) had the highest DPPH scavenging activity, while the native bun had the lowest value (24.92±0.52). The increase in Dpph ability is attributed to GTP, which is a natural antioxidant; in addition, green tea contains other antioxidants such as vitamin C, carotenoids, and selenium (Ning et al., 2017). The inclusion of polyphenols, which can reduce reactive oxygen or scavenge free radicals, could explain the increase in antioxidant activity of compositions when compared to native (Wang et al., 2013).

4.2.2 Reducing power (RP)

The RP of native bun and GTEB increased considerably ($p< 0.05$) as GTE concentration was increased, and was found to vary from 0.14±0.04 to 0.86±0.02 mg AAE/g (Table 7). The increase in RP could be due to the presence of reductones, which are predominantly phenolic chemicals and flavonoids, which are thought to be responsible for cereals reducing power (Omwamba & Hu, 2010). During baking at higher temperatures, phenolic chemicals may be

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broken down into low molecular weight equivalents, increasing their reducing power (Kaur et al., 2012). The reducing power of low molecular weight reductones is greater than that of high molecular weight reductones. This could be the reason for the increased reducing power of buns. Green tea polysaccharides also showed a dose-dependent increase in reducing power, according to Chen et al., (2013).

4.2.3 Lipid peroxidation (ILP)

Table 7 shows the inhibition of lipid peroxidation of native bun and GTEB. When compared to native bun, GTEB had a considerably higher lipid peroxidation inhibitory capacity ($p < 0.05$). The percentage ILP of the samples ranged from 11.24±0.09 to 26.28±0.64. The highest ILP value was displayed by S3 and the lowest by Bn. The increased ILP in GTEB is owing to GTE's antioxidant action, which may prevent lipid oxidation and hence improve ILP levels (Wang et al., 2013). Green tea extract was found to have a strong antioxidant impact on lipid stability, inhibiting the generation of hydrogen peroxide and secondary oxidation products (Mildner-Szkudlarz et al., (2009). According to reports, blackcurrant extract, which is high in phenolics, has been shown to prevent lipid oxidation in beef patties (Jia et al., 2012). In addition, catechin-containing polyvinyl alcohol-starch films inhibited lipid oxidation in beef (Wu, Wang, and Chen 2010).

4.2.4 Chelating ability

Table 7 shows the chelating ability of native bun and GTEB to chelate Fe^{2+}. The ability of native and GTEB to chelate ferrous ions increases significantly ($P < 0.05$) and the S3 bun displayed the highest value of 6.72±0.50, whereas the native bun had the lowest value of 3.550.36. The increase in chelating ability could be due to catechol hydroxyl groups in ellagitannins (Sestili et al., 2007).

4.2.5 Total polyphenol content (TPC)

Table 7 shows the TPC of the native bun and GTEB. Green tea contains phenolic substances such as catechins, gallic acid, carotenoids, tocopherols, ascorbic acid, and a few phytochemical compounds (Stewart, et. al., 2005, Leung, et. al., 2001). GTE added into a wheat bun is projected to have more TPC than the native bun. The findings revealed that adding GTE to wheat bun increases TPC content significantly ($P < 0.05$) and increases from 542.54±0.13 to 1082.04±0.45 µgGAE/g flour. S3 had the highest value, while native bun had the lowest.

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According to Zhu et al., (2016), the increase in phenolic activity is dependent on the tea concentration.

Table 7. Antioxidant properties of native bun and GTEB.

Sample	TPC (µg GAE/g)	Dpph (%)	Metal chelating (%)	Reducing power (mg AAE/g)	Lipid peroxidation (%)
Bn	542.54±0.13[a]	24.92±0.52[a]	3.55±0.36[a]	0.141±0.04[a]	11.24±0.09[a]
S1	869.43±0.36[b]	27.70±0.74[ab]	4.62±0.12[b]	0.63±0.0.07[b]	16.82±0.57[b]
S2	969.13±0.66[c]	41.54±1.14[bc]	5.43±0.55[c]	0.77±0.03[c]	20.87±0.59[c]
S3	1082.04±0.45[d]	43.94±0.59[c]	6.72±0.50[d]	0.86±0.02[d]	26.28±0.64[d]

Values (n = 3) are expressed as mean ± standard deviation. In a column, values preceded by the same letter do not differ significantly $P \leq 0.05$. Where Bn, S1, S2, and S3 represent native bun and fortified bun with 1%, 3%, and 5 % green tea extract, respectively.

5. Conclusion

In this study, green tea extract was added to the wheat flour to develop functional buns using the ultrasonication method for extraction. The sensory analysis carried out in this study showed no significant differences in consumer preferences between the fortified and control buns, indicating that the addition did not affect acceptability. Buns supplemented with green tea extract (1% GTE) outperformed the control in all sensory metrics except texture, with a maximum score of 7.27. The results demonstrated an increase in anti-oxidant capacity, an increase in RS content, and a decrease in GI, demonstrating their beneficial effects for health-conscious people, particularly diabetes patients. Overall, the current study suggests that, in addition to being recommended for diabetes patients, GTE content in buns can be a rich source of health-promoting compounds and is therefore shown to be a potential nutraceutical to be exploited in the modern food industry.

Author Statement

Haamid Mujtaba: Investigation and writing the original draft. Banwar Lal Jatt: Supervision. Adil Gani: Supervision, resources & Conceptualization.

Declaration of competing interest

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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