Research Article

Polyphenolic Content, Antioxidant Activity and *In Vitro* Starch Digestibility of Bread incorporated with Chamomile and Wild Thyme

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Abstract | This study was conducted to develop functional wheat bread by incorporating chamomile and wild thyme to enhance overall nutritional composition, phenolic contents and antioxidant activity. In this study, chamomile and wild thyme powders were first mixed at equal proportions and wheat flour was then replaced with the mixture at 1 ,2 and 3 % incorporation levels, to prepare composite test bread (CWB). Total polyphenols, total flavonoids content and antioxidant potential of the wheat flour, chamomile powder, wild thyme powder, mixture of chamomile and wild thyme at the ratio of 1:1, control bread (CB) and composite test bread samples were performed using standard methods. Folin-Ciocalteu, Aluminium chloride, and Colorimetric assays (ABTS and DPPH) were followed to determine total phenolic content, flavonoid contents and antioxidant status respectively along with"i*n-vitro* starch digestibility". The ash, fiber and protein content increased significantly while the carbohydrate content had significantly lower values (p, for all trends < 0.05) as the ratio of incorporation increased when compared with the control sample (data not shown here). The CWB also showed significantly (P<0.05) higher phenolic and total flavonoids content. Furthermore,CWB showed significantly (P<0.05) higher antioxidant activity, low starch digestibility and low hydrolysis index (HI) as evidenced by a lower response curve at all levels of incorporation, thus producing significantly low glycemic bread compared with CB. The CWB significantly improved the nutritional properties of the bread and was consumers acceptable.

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Introduction

Many phytochemicals like dietary polyphenols have unique medicinal properties. They are principally recognized for their extremely high antioxidant properties, perform several cellular activities in eliminating unhealthy cells, proving anticarcinogenic and anti-atherogenic associations (Duthie *et al.*, 2003), prevent neurodegenerative disorders, diabetes mellitus, provide anti-microbial, anti-inflammatory and cardio-protective characteristics (Scalbert *et al.*, 2005). Herbal and medicinal phytochemicals, which include dietary spices, dietary supplements, and functional foods, are used in little amounts but have a big impact on human health. This is due to the fact that they contain significant levels of different antioxi-

dants (Kochhar, 2008). Plants with medicinal properties are used to cure a variety of ailments (Bhardwaj and Gakhar, 2005). Herbal medications are mostly non-toxic (Nimbekar *et al.*, 2012), extremely safe, free of chemical reagents, more natural, less expensive and more effective with no or minimal adverse effects (Ramadan and Emam, 2012).

Chamomile and wild thyme are medicinal herbs mostly used in folk medicine to manage diseases especially diabetes.Wild Chamomile is also known as *Matricaria Recutita* L. (Linnaeus), chamomilla, Matricaria flowers, pinheads (Singh *et al.*, 2011) and Babuna (WHO, 1999). Chamomile extract has hypocholesterolemic, hypoglycemic and protective effects against diabetic complications in experimental studies on animal (Eddouks *et al.*, 2005; Emam, 2012; Kato *et al.*, 2008). Chamomile treated numerous inflammations, ulcers, wounds, skin diseases (eczema), neuralgia, and pains such as gout and rheumatic pains. Chamomile extract contains more than 120 bioactive compounds. The composition and phenolic content of chamomile was investigated by various researchers (Ayoughi *et al.*, 2011; Farhoudi, 2013; Roby *et al.*, 2013; Stanojevic *et al.*, 2016). Göger *et al.* (2018) also identified main components and chemical composition of the study material chamomile as "α-bisabolol oxide A (47.7 %), (E)-B-farnesene (21.5 %), α-bisabolol oxide B (6.2 %), α-bisabolene oxide A (5.7 %), chamazulene (4.1 %) and α-bisabolol (2.1 %)".

Another common medicinal plant is known as wild thyme (Thymus serpyllum L.) belongs to the genus Thymus (Labiatae), consists of about 350 species worldwide (Demissew, 1993). It grows naturally in northern areas (temperate zones) of Pakistan *e.g.* Skardu, Gilgit-Baltistan and Swat *etc.* It's known as Ben ajvain and Tumuro in the local dialect.Wild thyme is used for various purposes such as expectorant, anthelmintic, antiseptic, carminative, sedative, and tonic (Karnick, 1994). Wild thyme is also a rich source of polyphenolic compounds that decreased blood pressure, plasma lipid peroxidation significantly and induced vascular resistance in hypertensive rats. These results promoted this herb as a beneficial supplement in cardiac patients (Stanojevic *et al.*, 2016).

Previous investigations looked into the chemical composition and therapeutic effects of chamomile and wild thyme separately. Because optimal consumption of phytochemicals is not provided by a single food, this study aimed to improve the nutritional composition, polyphenols, total flavonoids, and antioxidant potential of bread by integrating medicinal plants/herbs rich in phenolic compounds, such as chamomile and wild thyme. As a result, research data suggests that efficient combinations of several foods may be more beneficial than a single food source (Das *et al.*, 2012a).

Materials and Methods

Procurement of rawmaterials

Wheat flour (Waqas general flour mills, district Gujrat, Punjab-Pakistan) and other baking materials including salt, sugar, butter and yeast were procured from the local market of Peshawar. Wild thyme was procured from authorized dealers in Skardu, Gilgit-Baltistan while chamomile was purchased from authorized dealers in Karachi (Pansari, Pakistan's first Herbal Store, Karachi) in sealed plastic containers. The plant samples (chamomile and wild thyme) were powdered using a commercial grinder and were stored in plastic jars until use.

Table 1: *Formulation of wheat bread incorporated with chamomile and wild thyme.*

Sample	Wheat Flour (%)	Chamomile Flour (%)	Wild Thyme Flour (%)
CB	100	0	0
1% CWB	99	0.5	0.5
2% CWB	98	1	1
3% CWB	97	1.5	1.5

CB: *Control bread;* **CWB:** *Chamomile and wild thyme incorporated bread.*

Bread preparation

The formulations of different bread types are shown in Table 1. Briefly, a basic dough recipe on a 100 g flour basis for control bread (CB) was followed by mixing the flour with weighted ingredients. The dough was prepared by adding ingredients including 6gm sugar, 1gm salt, 2gm yeast, 5gm oil and 60 ml water to 100 g wheat flour. For the preparation of treatment bread samples, the chamomile and wild thyme powders were first mixed at equal proportions. Wheat flour was then replaced with the mixture at 1, 2 and 3 % incorporation levels. All other ingredients were kept the same for the preparation of the treatment bread. The chamomile and wild thyme incorporated bread (CWB) sample was prepared by the straight dough method (Amendola and Rees, 2003). The dough was

kneaded with hands for 40 minutes at room temperature and transferred into baking pans for fermentation for 30 minutes. The bread was baked for 20 minutes at 220 °C in an electric baking oven (Panasonic Digital Oven). Phenolic, flavonoid and antioxidant content of the wheat flour, chamomile powder, wild thyme powder, mixture of chamomile and wild thyme at the ratio of 1:1, control bread and composite test bread samples were performed using standard methods.

Sample storage

After preparation of CB and CWB, the samples were desiccated in an oven for 24 h at 50°C, milled and passed through a "0.5 mm" sieve and were stored in "airtight" jars, covered with aluminium paper and kept at 4 °C in a refrigerator until use.

Reagents/Chemicals

The reagents used in this trail were bought from "Sigma-Aldrich" chemical suppliers, Pakistan and were of analytical grade. These were: methanol, sodium carbonate, gallic acid, aluminium chloride,, folin-ciocalteu reagent, potassium acetate, ABTS (2,2-azinobis (3-ethylben zothiazoline-6-sulfonate), DPPH (2,2-diphenyl-1- picrylhydrazyl), radical cation, trolox, KOH, sodium acetate buffer, amyloglucosidase, glucose oxidase/peroxidase (GOD/POD) reagent, α-amylase, pepsin and NaOH.

Sample extraction

Extraction of samples for the determination of phonelic contents and antioxidant levels were done by the method of Awika and Rooney (2004). Briefly, to 1g of sample, 10 ml methanol (acidified with 1% conc. HCL) was added and shaked for 2 hours at 120 rpm. Centrifugation was done at 3500 rpm for 20 minutes at 25 °C. The supernatant was retreated again as earlier. The final product was stored in a glass bottle covered with aluminium foil and refrigerated at 4 °C for later analysis.

Total phenolic content(TPC) determination

Folin–Ciocalteu (FC) method was used to find out TPC of the samples. FC is the combination of phosphomolybdic and phosphotungstic acid. FC reagent is changed to tungsten oxide (blue) and molybdenum oxide during the oxidation of phenolic content in the sample in the presence of sodium carbonate. A spectrophotometer was used to quantify the polyphenolic compounds in the sample through the intensity of the blue color. Briefly, a 0.8 ml of Folin-Ciocalteu reagent (FCR) (1 mol) was properly mixed with 0.20 ml of extracts. The product was neutralized by adding 2 ml saturated sodium carbonate (15 % w/v) to it, distilled water was mixed and the volume was increased to 5 ml and retained the mixture at room temperature for 1 h. Gallic acid was used in this method as a standard (Supplementary Figure 1) (Singleton *et al.*, 1999). For this purpose, exactly measured 0.5 g gallic acid was mixed in 10 ml absolute methanol (80 %) and the solution was made up to 100 ml. To obtain standard solutions, 0 - 5 ml of the resultant solution was added into a 100 ml flask and diluted to known concentration at the rate of 0-0.5 mg/ml of gallic acid. Then, 0.5 ml sample, 2.5 ml of ten-fold diluted FCR and 2 ml of sodium carbonate (7.5 %) were mixed. The tubes were protected with aluminium foil and kept at room temp. for 30 minutes. The absorbance was noted at 760 nm using a UV/V spectrophotometer (Model No. T80, China). TPC was expressed as milligram gallic acid equivalents (GAE) per 100 gm of the sample on dry basis (mg GAE/100g dry weight), measuring absorbance at 760 nm through a spectrophotometer.

Determination of total flavonoids

In the colorimetric determination of total flavonoids, quercetin was used as standard (Iqbal *et al.*, 2015). Quercetin's calibration curve with a range of 0-10 mg/100 ml was prepared (Supplementary Figure 2). Briefly, 1 M potassium acetate (0.1 ml), 10% aluminum chloride (0.1 ml), 1.5 ml of 80% methanol and distilled water (2.8 ml) were mixed to each test tube having 0.5 ml extract and 0.5 ml standard. The absorbance was noted against distilled water (blank) at 415 nm. The flavonoid content was expressed as mg quercetin equivalent (mgQE)/100 grams of sample (dry).

Determination of antioxidant activity

Antioxidant activity was determined by **DPPH** and **ABTS** assays.

DPPH (2,2-diphenyl-1- picrylhydrazyl) assay: Radical scavenging activity was measured by the DPPH method (Cheung *et al.*, 2003). For this purpose, 150 µl of sample extract was added to 2.85 ml of DPPH methanol (0.1 M). The mixture was thoroughly mixed with the help of a shaker and allowed to sit for 30 minutes under subdued light. Absorbance was noted at 517 nm against a blank (distilled water) on "UV–Vis spectrophotometer, T80, China". Trolox (0-1mM) was used as standard (Supplementary Fig-

ure 4). Results were shown in µl TE/g dry sample.

The %age of inhibition was measured by:

$$\% Inhibition = (Abs_{blank} - Abs_{sample}) \times 100/Abs_{bla}$$

ABTS (2,2-aazinobis (3-ethylben zothiazoline-6-sulfonate) radical cation: ABTS assay was used to estimate the scavenging activity of samples (Re *et al*., 1999). 7mM ABTS was added to 2.45 mM potassium persulphate solution to obtain ABTS radical cation. The mixture was kept overnight in the dark at 25 °C to stand, then it was further diluted by adding distilled water to get an absorbance of 1.4-1.5 at 414 nm (Forni *et al*., 1986). This diluted ABTS solution (2.85 ml) was mixed with 150 µl sample extract, trolox standard (0-1mM) (Supplementary Figure 3) solution was also added to it. The results were obtained by measuring absorbance 734 nm on Gynesis 10 (Spectrophotometer) and expressed as "µmol TE/g. (Leong and Shui, 2002).

In vitro starch digestibility and predicted glycemic index

Sopade and Gidley (2009) method was followed for *in vitro* starch digestion. A 100 mg sample was treated with 10ml pepsin suspension (0.2 gm pepsin: 3500 micrograms dissolved in 100 ml of HCL&KCl (0.01M) with pH of 7 and incubated in a reticulating water bath at 85 rpm for 30 minutes (37 °C). This mixture was neutralized with 0.01 M sodium phosphate (15 ml), adjusting the pH to 7. Then 60U/ml α-amalyse and 5 ml sodium phosphate (0.01M, pH 7) mixture was added to it and incubated for 120 min. Next, sample aliquots (1ml each) were shifted to test tubes at time intervals of 0, 20, 30, 45, 60, 90 and 120 min and inactivated by α-amalyse at 100 °C for 5 min. This procedure was followed by incubation (60 min at 60°C) and the addition of 3 mililiter "sodium acetate buffer(pH 7)" of 0.02M and 60 µL (196 U/ml) amyloglucosidase to the aliquots to hydrolyze the starch.

Starch digestibility was expressed in terms of digested starch (DS) (in gms/100 g dry starch) determined for each time point by;

$$DS = 180 \times V/W \times 0.9 \times S \times G_G \ldots\ldots (1)$$

Where;

V = Volume (in milliliters) of digesta, G_G = Glucose concentration, 180 = mol. weight of glucose, W= weight in gms of sample, 0.9 = stoichiometric constant, M = moisture (gms per 100 gms of sample) and S = starch of given sample (gms/100 gms dry sample), (Sopade and Gidley, 2009).

Digestogram of digested starch (g/100 g dry basis) for all bread samples were constructed in response to time and digestion after (0 min) baseline correction.

The hydrolysis index represented by HI is defined as "the ratio between the areas under the hydrolysis curve (0-90 min) of the test bread sample and the area under the curve of CB"and was calculated as follow:

$$HI = (AUC_{sample}/AUC_{reference}) \times 100$$

To find out the estimated/predicted glycemic index the 90 min hydrolysis index was used in the following formula (Goñi *et al*., 1997).

Predicted glycemic index (pGI) or (eGI) = $0.549HI_{90}$ + 39.71.

Statistical analysis

Statistical analysis was performed by SPSS, version 20.0 Armon, NY: IBM Corp. Values were analyzed in triplicate and reported in mean (standard deviation). One-way analysis of variance with LSD post hoc test for multiple comparisons was used to determine the effect of chamomile and wild thyme incorporation on outcome parameters. The significance level of 0.05 ($p<0.05$) was considered in all the analyses.

Results and Discussion

Polyphenolic and flavonoid content

Total phenolic contents (TPC) of CWB and CB are analyzed and results are presented in Table 2. TPC of CB significantly decreased as compared with wheat flour. Whereas TPC increased significantly ($p<0.05$) in CWB by 34.20%, 91.99 and 133.20 % respectively, as the supplementation increased from 1-3 %, when compared with CB.

The total flavonoid contents (TFC) of both bread samples (CB and CWB) are shown in Table 2. The TFC of CB decreased significantly ($p<0.005$) when compared with the wheat flour. However, 1% CWB incorporation increased flavonoid content by 30.33 %. Similarly, 2 and 3% CWB incorporation increased TFC significantly ($p<0.05$), by 72.97 and 113.16 %, respectively.

Antioxidant activity

Antioxidant status of the study constituents is shown

in Table 3. DPPH radical scavenging activity of CB reduced significantly compared with wheat flour. When these two medicinal plants were mixed at the ratio of 1:1 an intermediate value (204.24 ± 13.40) was observed. DPPH activity was concentration-dependent and significantly ($p<0.05$) improved as the incorporation increased from 1-3 %.

Table 2: *Total polyphenolic and flavonoid content.*

Material	Total Phenolic Content (mg GAE per 100gms)	Total Flavonoid Content (mg QE per 100 gms)
Wheat flour	54.32 ± 9.36^{f}	34.43 ± 3.84^{f}
CP	304.12 ± 8.12^{a}	136.72 ± 4.14^{a}
WP	260.31 ± 7.49^{c}	98.56 ± 3.45^{c}
CP+WP (1:1)	278.42 ± 8.19^{b}	115.16 ± 4.23^{b}
CB	36.08 ± 4.65^{h}	27.89 ± 3.86^{g}
1% CWB	48.42 ± 6.40^{g}	36.35 ± 4.37^{f}
2% CWB	69.27 ± 4.15^{e}	48.24 ± 4.16^{e}
3% CWB	84.14 ± 5.35^{d}	59.45 ± 3.83^{d}

Presented data are the mean value of three replications ± standard deviation. Means having different superscripts within the same column are significantly different. One way "ANOVA" with LSD test ($p<0.05$).

Table 3: *Antioxidant level of study materials and test breads (dry basis)*.*

Material	ABTS (µmol TE per 100gms)	DPPH (µmol TE per 100gms)
Wheat flour	45.06 ± 5.691^{g}	179.49 ± 15.78^{g}
CP	287.67 ± 8.61^{a}	227.83 ± 12.18^{a}
WP	192.43 ± 7.62^{c}	163.49 ± 11.21^{c}
CP+WP (1:1)	232. 93 ± 6.83^{b}	204.24 ± 13.40^{b}
CB	23.61 ± 2.67^{h}	140.30 ± 15.84^{h}
1% CWB	81.51 ± 8.71^{f}	191.43 ± 14.09^{f}
2% CWB	143.44 ± 5.32^{e}	217.71 ± 13.83^{e}
3% CWB	195.67 ± 8.14^{d}	246.58 ± 14.03^{d}

Presented data are the mean value of three replications ± standard deviation. Means having different superscripts within the same column are significantly different.One way ANOVA with LSD test ($p<0.05$).

Total antioxidant capacity (TAC) investigated by ABTS is presented in Table 3. The incorporation of chamomile and wild thyme powder at the proportion of 1, 2 and 3 % significantly ($p<0.05$) increased ABTS activity. At 1% incorporation, ABTS activity was 3.5 times greater, and at 2 and 3% incorporation, it was increased significantly ($p<0.05$) by 6 and 8 times, respectively.

In vitro starch hydrolysis and predicted glycemic index

In vitro starch digestibility of the control and incorporated breads (CWB) are shown in Figure 1. The hydrolysis rate increased rapidly in the first half an hour of reaction in all samples. Afterwards, hydrolysis proceeded and reached its peak in 120 min. The AUC (area under the curve) of incorporated bread (CWB) was lower than the CB. This specifies that the CB has a faster digestion rate. As shown in Fig. an increased digestion value of about 20% was noted for the CB during the first 30 min of reaction, at 120 min it increased to about 32%, which was greater (by 19–22%) than incorporated composite bread at 30 min of reaction. It was concluded that the CWB reduced *in vitro* starch digestibility at each time point and all levels of incorporation, as shown by the response curve in the digestogram (Figure 1). It means that the inverse relationship was found between incorporation and starch digestibility of the studied bread.

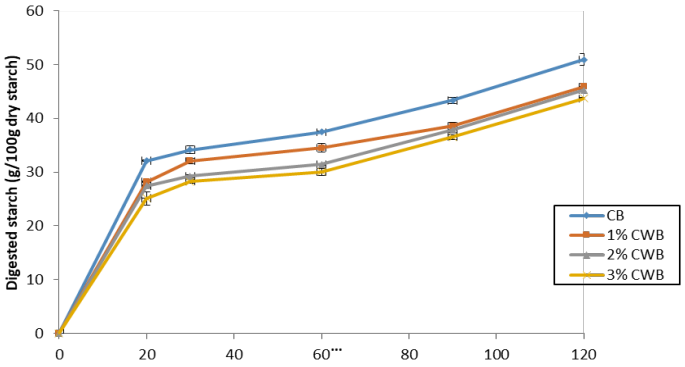

Figure 1: *Starch digestogram of CB and CWB. Values are means ± SD of triplicate samples at each time point. CB: control bread; CWB: chamomile and wild thyme incorporated bread.*

Table 4: *HI ande GI of CB and CWB.*

Material	HI	eGI
CB	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
1% CWB	90.51 ± 0.42^{b}	88.24 ± 0.33^{b}
2% CWB	86.94 ± 0.49^{c}	85.14 ± 0.36^{c}
3% CWB	85.72 ± 0.54^{d}	84.32 ± 0.30^{d}

Presented data are the mean value of three replications ± standard deviation. Means having different superscripts within the same column are significantly different. One way "ANOVA" with LSD test ($p<0.05$).

Hydrolysis index and predicted glycemic index (pGI) of CB and CWB

Hydrolysis index (HI) and predicted or estimated glycemic index (eGI) are shown in Table 4. HI decreased significantly ($p<0.05$) with the increasing rate of incorporation (chamomile and wild thyme) in the CWB as compared with the CB. The same signifi-

cantly decreasing trend was observed for the estimated glycemic index (eGI) in CWB as the incorporation increased from 1 to 3 %.

The main goal of this study was to prepare wheat bread by incorporating herbs that have known nutritional value. Chamomile and wild thyme are promising herbs with abundant bioactive compounds such as pholyphenols, flavonoids, and good antioxidant activity, which are common in the local population of the study area. Based on their chemical composition reported in the literature, it was hypothesised that incorporating them into wheat flour would improve overall wheat flour quality. Results of this study can be summarized as an overall increase in the ash, fiber, and protein with no change in fat content of incorporated wheat bread compared to control wheat bread (shown in our previous study). More importantly, when the carbohydrates content of both breads were compared, the CWB had significantly ($P<0.05$) lower carbohydrate content. In general, variation in carbohydrate contents of the test breads might be attributed to either individual food materials and/or hydrolysis of microflora enzyme that led to the production of other complex carbohydrates from other carbon-containing nutrients as the result of fermentation and combined effect of food fortification (Onoja *et al*., 2014). This condition may also be true for other nutrients (Hotz and Gibson, 2007; Odunfa, 1985). Similarly, the ash content of CWB improved in the current study. This increase in ash content of the test bread was a good sign of mineral availability in the product (Reebe *et al*., 2000). The CWB showed high pholyphenolic, flavonoid content and good antioxidant activity (Table 3). Furthermore, the CWB showed significantly low starch digestibility and low hydrolysis index (HI), thus producing significantly low glycemic bread (low estimated glycemic index) compared with CB with no change in consumers' acceptability.

These findings were consistent with several research trials conducted by Raba *et al*. (2007) and Balestra *et al*. (2011). They incorporated turmeric, garlic and ginger to enhance the phenolic content of wheat breads, respectively. In general, polyphenols are relatively heat resistant compounds and the baking process may lose some phenolic compounds (Cheynier, 2005). This trend was also observed in this trail and total pholyphenols, flavonoids and antioxidandsactivity of CB and CWB reduced significantly, compared with the wheat flour. However, regardless of this loss in these compounds during baking, incorporated bread (having turmeric) exhibited significantly greater phenolic compounds and antioxidant level (Lim *et al*., 2011). These findings were not surprising, and the additional rise in chemical compounds in the treated bread could be attributed to increased compound outflow from food sources (Vitali *et al*., 2009). Baking also requires heat processing, which may include various amounts of "Maillard reaction products (MRPs)," which contain antiradicals and antioxidants that react with DPPH and hydroxyl radicals, as well as oxygen peroxyl and Fe+2 & copper chelators (Dittrich *et al*., 2003). In addition, Gawlik-Dziki *et al*. (2009) suggested that products of Maillard reaction, phenolic acids of bread and flavonoids present in the incorporated plants/herbs prove a synergistic effect on the DPPH radicals, both as reducers and chelators. Awika *et al*. (2004) proved that phenolic and non-phenolic compounds (β-D-glucan) are the predominant source of antioxidant activity in bakery products like breads. Similar results were found by Gawlik-Dziki *et al*. (2009) for incorporation of tartary buckwheat flavones (TBF) to bread; Sikkhamondhol *et al*. (2009) used turmeric and turmeric essential oil residue; Gawlik-Dziki *et al*. (2013) used Onionskin powder; Das *et al*. (2012b) added coriander leaf powder; El-Megeid *et al*. (2009) used green tea powder, tomato paste & oregano mixture while Glei *et al*. (2006) supplemented bread with 1% green coffee. They all concluded that the improved antioxidant potential of bread was related to the high phenolic contents of the incorporated food materials into bread.

When the TFC and TAC of CWB were taken into account, the findings of this study were generally in line with those of Gawlik-Dziki *et al*. (2009), who used onion skin powder in bread. Flavonoids containing quercetin and its derivates have adequate antioxidant activity to trap free radicals and chelate metals, which inhibits lipid peroxidation and so reduces oxidative stress, which has been linked to a lower risk of major chronic diseases.Chamomile and wild thyme are the major sources of dietary flavonoids (quercetin) in many countries (Kulisic *et al*., 2005), respectively. So, these herbs may be incorporated in bread for bio-accessibility of quercetin content and to improve the TAC of bread without altering its sensory characteristics.

Regarding starch hydrolysis and *in vitro* starch digestibility, the results of this trial were parallel with

the results of Świeca *et al*. (2014). They evaluated the *in vitro* starch digestibility by the incorporation of bread with other materials like quinoa leaves (QL). These results were also considerably consistent with those reported for "cereal-based products" (Englyst and Englyst, 2005; Zhang *et al*., 2006).The incorporation of herbs into bread alters molecular interactions between dietary fiber, starch, lipids, and protein, causing fiber depolymerization and, as a result, enzyme assault. Similarly, indigestible fiber in the diet, as well as other related substances (non-fibrous), may reduce starch hydrolysis rate, resulting in suboptimal metabolic responses (Granfeldt *et al*., 1992). In the present study, CWB showed a significant drop in the HI and pGI values as compared to the control bread (CB) (Table 4). Reduction in HI and eGI values gave better structural characteristics like small particle size, high solvent retention capacity and viscosity to the incorporated bread. These characteristics are considered important in producing high-quality bread (Cleary and Brennan, 2006; Ho *et al*., 2015). Food products i.e. breads containing high amounts of fiber noticeably reduce its GI and could be a beneficial method to stabilize high spikes in blood glucose. Similarly, Rosin *et al*. (2002) demonstrated that HI and pGI was reduced by high polyphenols contents of these herbs (Rosin *et al*., 2002).

Conclusion and Recommendations

In conclusion, the CWB significantly (P<0.05) enhanced the TPC and TAC with a significant reduction in HI and eGI. The CWB significantly improved the nutritional properties of the bread and was consumers acceptable. On the whole, incorporation of chamomile and wild thyme had a positive effect on the bread quality in terms of polyphenols, flavonoids and total antioxidant activity. Furthermore, this modification improved the bread's glycemic index, making it excellent for preventing and treating hyperglycemia. In this study; only the total phenolic content, total flavonoid content and total antioxidant activity of all the study materials were determined, the free and bound phenolic/flavonoid contents and their antioxidant activity were not investigated. Therefore, future studies are proposed to investigate these parameters in bread samples incorporated with chamomile and wild thyme.

Novelty Statement

The present study is of its type done among fewer globally and the first one in Pakistan investigating the effect of bread incorporated with chamomile and wild thyme (herbal supplementation) on the nutritional composition and functional properties of wheat bread. This study suggested to see the combined effect of chamomile and wild thyme incorporated bread on blood glucose, plasma insulin, plasma total polyphenols, plasma total antioxidant capacity, biomarkers of oxidative stress and inflammation in diabetes. This is a notable contribution to the existing literature on this topic.

Author's Contribution

Imtiaz Ahmed: Principal author and PhD Scholar, who performed lab. work, collected and analyzed the data, interpreted the results. Finally wrote draft of the manuscript.
Imran Khan: Major supervisor, who proposed and designed the study and methodology. Helped in quality of the manuscript.
Zia-ud-Din: Helped in proofreading and improved quality of the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

Supplementary Material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.sja/2022/38.3.918.927

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