Research Article

Proximate Analysis, Starch Fractions and Sensory Evaluation of Bread Incorporated with Chamomile and Wild Thyme

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Abstract | The Nutritional quality of wheat bread can be enhanced by incorporating certain nutrient-rich ingredients in its formulation. The main objective of the present study was to investigate the feasibility of incorporating chamomile and wild thyme powder in wheat bread to further improve its nutritional profile. For this purpose, three composite test breads (CWB) were prepared by incorporating chamomile and wild thyme at an equal proportion of 1, 2 and 3%. Proximate analyses of wheat flour, chamomile powder, thyme powder, a 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. In-vitro starch digestibility and total starch were determined by Megazyme starch assay kit. A nine-point hedonic scale assessed bread samples for consumer acceptance. The findings of proximate composition showed a significant increase in ash at 2 and 3 % incorporation. The fiber content also significantly increased at the incorporation ratio of 1, 2 and 3%. In CWB, fat concentration at 2 and 3% incorporation remained almost equal to that of CB. The protein content increased "significantly in a dose-dependent manner with the increasing incorporation of these two herbs" when compared with the CB. When the carbohydrate content of both breads was compared, the functional bread had significantly lower values (p, for all trends < 0.05), respectively, as the ratio of incorporation increased. Total starch (TS) and DS reduced non-significantly at 1 and 2 % and significantly at 3% incorporation as compared with CB. Resistant starch (RS) content of CWB increased significantly ($p<0.05$) at 1, 2 and 3% incorporation of these herbs with high RS at the highest level of incorporation. Slowly digestible starch (SDS) content non-significantly decreased. Rapidly digestible starch (RDS) reduced significantly ($p<0.05$) at 1% compared with CB, with no significant change at 1 and 2 and 2 and 3 % incorporation. SDI decreased significantly ($p<0.05$) with the increasing rate of incorporation of chamomile and wild thyme in the CWB. The acceptability score of test bread was ≥6 on a 9-point hedonic scale. It was concluded that this incorporation significantly improved the bread's nutritional properties and starch digestibility and was acceptable for consumers.

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Introduction

Wheat bread has been a staple food for thousands of years (Weegles, 2019), including a wide range of nutrients such as carbohydrates (which provide energy), essential nutrients, dietary fiber, and phytochemicals (Kumar *et al*., 2011), all of which are necessary for growth and development (Kourkouta *et al*., 2017).

The nutritional value of bread is determined by the type of flour used in its production, as well as the inclusion of ingredients such as seeds or oil. Being staple, wheat bread can be used as a carrier for numerous nutrients that are deficient in diet (Akhtar *et al*., 2011; Balk *et al*., 2019). Addition of any such ingredient will further improve the nutritional quality of wheat bread. Fortification of wheat bread with iron and zinc is in general practice throughout the world (Balk *et al*., 2019). Other product developments, such as the addition of fiber, whole grains, seeds, and omega 3 fatty acids, have helped improve bread's nutritional characteristics, and these innovations are expected to continue (Engle-Stone *et al*., 2017).

Chamomile and wild thyme are two famous herbs and are potential fortificants (Adebo *et al*., 2020) that are expected to improve the nutritional quality of wheat bread. Chamomile has numerous ingredients important for health (Bhaskaran *et al*., 2010). Chamomile has been shown to improve iron absorption. Wild thyme is rich in nutrients that have antioxidant characteristics (Mihailovic-Stanojevic *et al*., 2013). Based on a number of other food formulations using chamomile and wild thyme, we are of the view that wheat bread incorporated with chamomile and wild thyme would rate high in terms of nutritional composition and higher proximate constituents. Although, Bread fortification is viewed as a way to improve human health and well-being; nevertheless, chamomile and wild thyme compatibility with other ingredients is critical from both a nutritional and acceptability standpoint; their functionality must be examined through wheat bread processing. While enhancing the nutritional quality of wheat bread, maintaining its technological and sensory attributes is also important.

In this regard, this study aims to investigate the suitability of wheat bread as the main foodstuff to deliver valuable nutritional ingredients in chamomile and wild thyme considering influence of these herbs on the nutritional and sensorial characteristics of wheat dough and bread. Chamomile and wild thyme inclusion in dough systems may affect the pasting characteristics of starch and proteins and, consequently, the baking process and bread structure. Therefore, this study was planned to add chamomile and wild thyme to wheat bread and to determine proximate composition and related characteristics (e.g., *in-vitro* starch digestibility, and consumer acceptability etc.) of such enriched wheat bread.

Materials and Methods

Procurement of raw materials

All-purpose wheat flour (Waqas general flour mills, district Gujrat, Punjab-Pakistan) and other baking materials including butter, sugar, salt, 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 samples were powdered using a commercial grinder and were stored in plastic jars until use.

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 measured ingredients. The dough was prepared by adding ingredients including 6g sugar, 1g salt, 2g yeast, 5g oil and 60 ml water to 100 g all-purpose 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 as well as the control bread 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).

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.

Samples 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 aluminum paper and kept at 4 °C in a refrigerator until use.

Reagents/ chemicals

The reagents used in this trial were bought from Sigma-Aldrich chemical suppliers, in Pakistan and were of analytical grade. These were: megazyme total starch assay kit (K-TSTA 06/2017; Megazyme Int. Ireland Ltd. Co. Wicklow, Ireland), sodium acetate buffer, α-amylase, amyloglucosidase, glucose oxidase/ peroxidase (GOD/POD) reagent, pepsin, HCL, KCl, sodium phosphate, pepsin and NaOH.

Proximate analysis

Proximate analyses of raw materials and bread were performed using standard AOAC methods (Feldsine *et al*., 2002). All the bread samples were analyzed for ash, crude fiber, moisture, crude protein and fat content. The moisture content was determined by drying the samples in an electric oven at 105°C for 4 hours. Ether extract percentage (% EE) was determined using Soxhlet system HT-extraction technique using AOAC method No 922.06. In the end, crude fat was determined by the difference in the weight of the sample (Min and Steenson, 1998). Crude protein percentage (% CP) was determined by Kjeldahlapparatus using AOAC method No 920.87. The percentage nitrogen obtained was used to calculate the % CP using the relationship: % CP = % N X 6.25. Crude fiber percentage (% CF) was determined by dilute acid and alkali hydrolysis using AOAC method 991.43. Percentage ash (%) was determined by incinerating the samples in a muffle furnace at 550°C for four hours. The ash was cooled in a desiccator and weighed. Carbohydrate was calculated by difference using the following equation:

$$Carbohydrate\ (\%) = 100 - (Ash + Moisture + Crude\ Fiber + Crude\ protein + Crude\ fat)$$

Total starch (TS) determination

Total starch was determined using the Goni *et al*. (1997) technique. In this method megazyme total starch assay kit (K-TSTA 06/2017; Megazyme Int. Ireland Ltd. Co. Wicklow, Ireland) was used. Concisely, 100 milligrams of sample was added into 0.2 ml ethanol (80 %) while shaking on a mixer. Added 2 ml KOH (2 M) to it, incubated for 20 minutes. After that, 8 ml of sodium acetate was added as a buffer (pH 3.8, 1.2 M). Next, α-amalyse and amyloglucosidase (0.1 ml each) were mixed. After that, the mixture was incubated in a shaking water bath for 30 minutes at 50 degrees Celsius. By adding distilled water, the amount increased to 100 ml and centrifuged at 3000 rpm for ten minutes. GODPOD reagent (3 ml) was added to 0.1 ml of aliquots to find glucose. After incubating for 20 min at 50 °C, color developed and was measured at 510 nm against a blank GODPOD reagent. TS was calculated by the following formula:

$$Total\ starch = glucose\ in\ mgs \times 0.9$$

Starch digestibility

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 per dissolved in 100 ml of HCL and 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 were 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 ml "sodium acetate buffer (pH 7)" of 0.02M and 60 µL (196 U/ ml) amyloglucosidase to the aliquots to hydrolyze the starch. GOPOD method was used to find out glucose content.

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

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

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

Rapidly digestible starch (RDS) (g/100 g dry starch) was determined by replacing G_G in Equation 1 with ($G_{20-}G_{0)}$: The difference between glucometer reading at 20 min and 0 min. RDS is the starch digested in 20 min. Similarly, slowly digested starch (SDS) expressed in g/100 g dry starch is the starch digested between 20-120 min, was calculated by substituting G_G for (G_{120}-G_{20}) in Equation 1 (Rosin *et al*., 2002). For calculation of Resistant starch (RS) expressed in g/100 g dry starch the following equation was used:

$$RS = 100 - (SDS+RDS)$$

Starch digestion Index (SDI) was calculated by the equation SDI = RDS ÷ TS x 100 (Rashmi and Urooj, 2003).

Sensory evaluation

Sensory evaluation of CB and CWB was carried out by 50 untrained subjects after approval of the experimental protocol by the Ethics Committee of the Department of Human Nutrition (HN-HREC/2018-0014), The University of Agricultural, Peshawar. The texture, flavor, color and overall consumer acceptability of CB and CBW was determined by a nine-point hedonic scale; showing 1 as "dis-like extremely", 5 as "neither like nor dislike", 9 as "like extremely". Breads were offered with mineral water in trays (disposable). The subjects did not take anything to eat and drink for 3 hours before evaluation.

The incorporated bread (CWB) was acceptable if the consumers rated the breads ≥ 6, showing; "like slightly" on the "nine-point" hedonic scale.

Statistical analysis

Statistical analysis was performed by SPSS, Version 20.0 Armon, and 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. To evaluate bread's acceptability one-way repeated ANOVA was used. The significance level of 0.05 ($p<0.05$) was considered in all the analyses.

Results and Discussion

Proximate composition

The proximate composition of the wheat flour, chamomile powder, thyme powder, mixture of chamomile and wild thyme at the ratio of 1:1, the incorporation of test herbs at 1%, 2% and 3% in the test bread and control bread are shown in Table 2. The ash content slightly decreased in control bread (CB) and 1 % incorporation compared with wheat flour. However, this level increased significantly ($p<0.05$) at 2 and 3 % incorporation. The fiber content of thyme powder was significantly ($p<0.05$) high followed by a 1:1 ratio of chamomile and wild thyme. Also, the fiber content of incorporated bread significantly ($p<0.05$) increased in the incorporation ratio of 1, 2 and 3%. The fats content at 1, 2 and 3%" incorporation of chamomile and wild thyme did not affect significantly and remained almost equal to that of CB. The protein content increased significantly in a dose-dependent manner with the increasing incorporation of these two herbs as compared with the CB. The CWB samples recorded higher protein values than the CB. When the CHO content of both breads was compared, the CWB at 1, 2 and 3% incorporation had significantly low values, compared with the CB.

Total starch (TS) determination

Starch fractions of CWB and CB are presented in Tables 3 and 4. Total starch (TS) and digestible starch (DS) reduced non-significantly at 1 and 2 % and significantly at 3% incorporation as compared with CB. However, resistant starch (RS) content of CWB increased significantly ($p<0.05$) at 1, 2 and 3% incorporation of these herbs with high RS at the highest level of incorporation. Slowly digestible starch (SDS) content decreased non-significantly ($p>0.05$). Rapidly digestible starch (RDS) reduced significantly ($p<0.05$) at 1% compared with CB, with no significant change at 1 and 2 and 2 and 3 % incorporation. SDI decreased significantly ($p<0.05$) with the increasing rate of incorporation (chamomile and wild thyme) in the CWB (Table 4).

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Table 2: *Proximate analysis of raw ingredients and bread samples (% dry basis)*.*

Ingredients	Ash	Fiber	Fat	Protein	CHO
All-purpose flour (Wheat)	1.75± 0.35[ef]	2.00 ± 0.05[fg]	1.25 ± 0.34[e]	11.59 ± 0.30[c]	83.40 ± 0.30[a]
CP	13.75±0.35[a]	16.30 ± 0.28[c]	3.75± 0.35[d]	13.12 ± 0.00[b]	53.07 ± 0.42[f]
WP	9.25 ± 0.38[c]	21.50 ± 0.70[a]	4.30 ±0.28[cd]	17.30 ± 0.28[a]	47.65 ± 0.21[g]
CP+WP (1:1)	11.75 ± .39[b]	18.50 ± 0.56[b]	4.25 ± 0.07[d]	17.45 ± 0.21[a]	48.05 ± 1.20[g]
CB	1.25 ± 0.35[f]	1.50 ± 0.70[g]	5.25 ± 0.35[ab]	10.50±0.61[db]	81.50 ± 0.08[b]
1% CWB	1.10 ± 0.13[f]	2.45 ± 0.07[ef]	4.85 ± 0.07[bc]	11.50 ± 0.42[c]	80.10± 0.70[c]
2% CWB	2.10 ± 0.14[de]	3.00 ± 0.14[de]	5.20 ± 0.14[ab]	12.11 ± 0.01[c]	77.59± 0.15[d]
3% CWB	2.75 ± 0.35[d]	3.35 ± 0.07[d]	5.45 ± 0.07[a]	12.95± 0.21[b]	75.50 ± 0.70[e]

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: *Digestible and resistant starch proportions of CB and CWB.*

Sample	TS	RS	DS
CB	72.92 ± 0.37[a]	21.98 ± 0.86[d]	50.94 ± 0.62[a]
1% CWB	71.87 ± 0.35[ab]	25.03 ± 0.73[c]	46.84 ± 0.54[ab]
2% CWB	71.06 ± 0.49[ab]	26.51 ± 0.91[b]	44.55 ± 0.71[ab]
3% CWB	70.31 ± 0.28[b]	27.91 ± 0.66[a]	42.4 ± 0.47[b]

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

Table 4: *Relevant starch fractions and starch digestion index (SDI) of bread samples.*

Sample	RDS	SDS	SDI (%)
CB	32.11 ± 0.24[a]	28.82 ± 0.81[a]	44.03 ± 0.44[a]
1% CWB	27.68 ± 0.25[b]	28.98 ± 0.88[a]	39.58 ± 0.59[b]
2% CWB	26.55 ± 0.21[bc]	27.09 ± 1.26[a]	37.83 ± 0.66[c]
3% CWB	25.12 ± 0.23[c]	27.18 ± 1.27[a]	35.18 ± 1.77[d]

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

RDS and RS are negatively correlated with one another, and RS also showed a negative relationship with SDI (Table 5), as shown by r values of _0.991 and _0.998 with p0.05, respectively. Because the RS fraction is not absorbed in the small intestine, it is unable to augment the postprandial glycaemic response. The relative rate of starch digestion as evaluated by SDI (r = 0.988, P<0.01) had a positive and strong connection with RDS. In research on starch digestibility, a similar connection exists (Rosin *et al*., 2002). Because the samples in this study primarily consist of DS, which triggers stronger glycaemic reactions, the substantially positive connection between SDI and RDS is unsurprising (Rosin *et al*., 2002). RDS induces quick digestion and absorption in the small intestine, resulting in a high glycemic response (Englyst and Englyst, 2005).

Table 5: *Pearson's correlation between starch fractions.*

Starch fractions	RS	SDI
RS	1	-0.998**
SDS	-0.8NS	0.779NS
RDS	-0.991**	0.988*

*RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; SDI, starch digestion index; NS, Non-significant; * Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).*

Table 6: *Sensory characteristics of the investigated bread samples.*

Bread samples	Appearance	Texture	Taste	Overall acceptance
CB	7.02±0.94[a]	6.92±1.14[a]	7.04±1.05[a]	7.06±1.13[a]
1% CWB	6.52±0.61[b]	6.46±1.47[b]	6.56±1.28[b]	6.68±1.43[ab]
2% CWB	6.32±1.75[b]	6.24±1.42[b]	6.28±1.23[c]	6.44±1.45[b]
3% CWB	6.16±1.58[b]	6.12±1.91[b]	5.96±2.01[c]	6.22±1.80[b]

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).

Sensory analysis

The sensorial qualities of the bread samples assessed by fifty untrained panelists are presented in Table 6.

The incorporation of herbs into bread to enrich food products and enhance food values is an emerging field of research. It is noteworthy that this approach is non-toxic, highly safe, free of chemical reagents, more natural, and appropriate physical method to alter molecular organization (Collar and Armero, 2018). Wheat is a staple food of the majority of the population and any least invasive treatment that incorporate sherbs may improve its nutritional quality and is expected to positively impact the nutrition of the local populace. Therefore, the primary objective of this study was to prepare wheat bread by incorporating herbs that have known nutritional value. Chamomile and wild thyme are promising herbs common in the local population of the study area and looking at their chemical composition reported in the literature, it was hypothesized that their incorporation in wheat flour would improve overall wheat flour quality. Results of this study can be summarized as an overall improvement in the composition of the supplemented wheat bread. These results have public health nutrition implications as any effort directed to improve the nutritional value of food has positive effects on the health outcomes of the community.

Briefly, there was an overall increase in the ash, fiber, and protein with no change in fat content of incorporated wheat bread compared to control wheat bread. More importantly, when the carbohydrate contents of both breads were compared, the CWB had significantly lower carbohydrate content. The findings of the present study showed significant improvement in the nutritional and structural properties of the CWB, with no change in consumers' acceptability as compared with CB. Changes in the chemical composition of treated bread in the present study were not unexpected.

To our knowledge, however, there is little to no information on the use of chamomile and/or wild thyme in wheat for the production and formulation of bread. The literature search indicated only one experimental study, conducted by El-Zainy *et al.* (2016). El-Zainy *et al.* (2016) added 3% chamomile to oat flour biscuits (OF biscuit) and found that the amount of moisture and ash rose significantly with improvement in shelf-life and the sensory properties as a result. Additionally, they noted that chamomile powder has the following compositions: 9.97±0.22 moisture, 18.34±0.33 protein, 8.57±0.09 ash, 3.72±0.03 fat, and 59.41±0.29% carbs (on a wet weight basis).

In the past, other researchers made efforts to incorporate various nutritionally rich ingredients in wheat flour. They (Odedeji *et al.*, 2014; Oyetayo and Oyedeji, 2017; Salazar *et al.*, 2017; Yu and Beta, 2015) reported that in general, the incorporation of herbs improved the nutritional quality of wheat flour by comparatively lowering the moisture content. It is noteworthy that the intermediate moisture content in treatment bread helps prevent the growth of microorganisms and a further reduction in moisture content reduced moulds production, increasing shelf life (Odedeji *et al.*, 2014).

The results of the present study showed a decrease in the carbohydrate content of CWB. 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 (Odedeji *et al.*, 2014). This condition may also be true for other nutrients (Hotz and Gibson, 2007; Odunfa, 1985). Similarly, the % age of ash of test breads 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). It was also observed that at 1 % CWB decreased fat concentration to 4.85 ± 0.07, but this effect was not significant statistically. The low lipid level of the CWB was expected because of the low lipid level of chamomile (CP) and wild thyme powder (WP) (3.75± 0.35 vs. 4.30 ±0.28%). Low lipid levels reduced chances for rancidity and guarantee longer shelf life for the breads (Beuchat and Worthington, 1974). Fat concentration at 2 and 3% incorporation remained almost equal to that of CB, without any significant changes and was found to be 5.20 ± 0.14 and 5.45 ± 0.07, respectively.

Regarding starch hydrolysis and *in vitro* starch digestibility, the results of this trial were parallel with the results of Świeca *et al.* (2014). They looked at *in vitro* starch digestibility based on TS and RS and found an 11.8 percent decrease in digestibility, a 42.36 percent increase in resistant starch at 5% supplementation, and a significant reduction in starch and protein digestibility when bread was combined 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). Starches are categorized into TS, RS, SDS and RDS. Their digestibility depends upon the type and concentration, its rate of digestion (reaction time) and absorption site, during *in vitro* starch digestion (Collar and Armero, 2018; Englyst *et al*., 2003). In this study, in general, the starch digestibility and RS content of CWB rose significantly ($p<0.05$) when compared to CB (Table 3). For dietary starch portions, high RS, SDS, and low RDS levels are considered to represent a favorable nutritional trend (Englyst *et al*., 2003), promoting the microbial flora in the intestinal, preventing diabetes, and hyperlipidemia and reducing chronic diseases. This additional increase and improvement in nutritional composition in the treated bread was related to the increased discharge of compounds from the food sources. The decrease in SDS in treated bread were caused by the availability of starch to acids, solvents and hydrolyzing enzymes, causing de-polymerization of fiber. These differences were the result of crystals breakage, improved molecular interactions, polymorphic translation and granule surfaces disruption. The incorporation of herbs into bread altered molecular organization especially the chemical structure of RDS into SDS and RS (Collar and Armero, 2018). Another reason for the change in starch digestibility and bread-making quality was the reduction in high-molecular-weight proteins (extractable protein levels) and increase in SDS due to the incorporation of phenolic acid into all-purpose wheat flour (Han and Koh, 2011). The incorporation of bread increased molecular interaction among dietary fiber, starch, lipids and/or protein resulting in a change in structures and hence enzyme attack. In "*in vitro* and *in vivo*" studies, indigestible dietary fiber and several related non-fibrous substances slowed starch breakdown and induced low metabolic responses (Granfeldt *et al*., 1992; Granfeldt *et al*., 1994). Thus, the attachment of starches decreased the chances of enzyme attack and declined the release of sugar molecules. Furthermore, combination of added fibers and indigenous natural polymers resulted in a closely packed bread crumb structure with entrapped starch granules and protein (Ho *et al*., 2015).

Sensory characteristics of foods are important attributes in human nutrition. The appearance and color of food promotes the initial response, though, the flavor regulates the final rejection or acceptance by the customer (Onoja *et al*., 2014; Teratanavat and Hooker, 2006). In the present study, different formulations of breads were assessed by the hedonic test to define the degree of liking. The CWB had acceptable flavor, taste and color and was much similar to CB at all levels of incorporation (Table 6) and no significant difference was found between CB and CWB. This means that the inclusion of these herbs had no effect on the color or flavor of CWB. In terms of overall acceptance of CWB, no significant differences were seen between CB, 1, 2, and 3% incorporation. When comparing the three incorporation levels, the 1% incorporation level had the highest score of 6.68 when compared to the control. The crumb of CWB was as solid as before, with the same level of bubbles, which had no negative impact on the look or overall quality of the bread. As a result, it's vital to emphasize that the addition had no discernible effect on the bread's sensory characteristics. The overall quality score of 3 percent CWB was 6.22 in this trial, compared to 7.06 in the control sample, but did not diminish the bread's overall quality. These results were comparable to the findings of Lim *et al*. (2011). They concluded that substituting other ingredients (turmeric powder) for wheat flour in the bread-making process had no effect on color, flavor, or other sensory attributes such as aroma and texture, and showed general customer approval.

Conclusions and Recommendations

In conclusion, the addition of chamomile and wild thyme to bread improved the ash, protein, and fiber levels substantially. Reduction in rapidly digestible starch (RDS), and starch digestion index (SDI) is positively correlated with the inclusion of chamomile and wild thyme. The overall acceptability score of CWB was ≥ 6 on a 9-point hedonic scale CWB, with no change in consumers' acceptability as compared with CB. In this study, shelf life and taste attributes related to staling of the CWB was not determined. Therefore, future studies are proposed to investigate these parameters in bread samples incorporated with chamomile and wild thyme.

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Novelty Statement

The innovative sort of bread that is generally referred to as herbal bread is identified in this study, which is the first of its kind in Pakistan. In this study, the nutritional value and usefulness of wheat bread are examined in relation to the effects of bread incorporated with chamomile and wild thyme (herbal supplements).

Author's Contribution

Imtiaz Ahmed: Principal author, who collected and analyzed the data, interpreted the results. Finally wrote draft of the manuscript.
Waseem Abbas: Performed laboratory work.
Imran Khan: Major supervisor, who proposed and designed the study and methodology.
Helped in proof reading and improved quality of the manuscript.

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

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