Research Article

Combined Effect of Sorghum and Barley Flours Incorporation on *in-vitro* Starch Digestibility, Estimated Glycemic Index and Consumer Acceptability of White Bread

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Abstract | Excess consumption of white bread has been linked towards the development of diabetes mellitus because of its high starch digestibility. Previous studies suggested that the addition of other grains, legumes and medicinal herbs to bread might reduce its starch digestibility. However, the incorporation of these non-wheat materials into bread is challenging from consumer acceptability perspective. Therefore, the current study investigated the effect of incorporating red sorghum and barley flours in white bread on *in-vitro* starch digestibility, estimated glycemic index (eGI) and consumer acceptability. Bread was formulated by incorporating blends (equal proportion) of sorghum and barely flours at 20%, 30%, 40% and 50% levels, each. Composite bread was compared with control bread (CB) made from 100% wheat flour only. *In-vitro* starch hydrolysis was determined enzymatically at 0, 20, 30, 45, 90 and 120 min. Rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and estimated eGI were calculated from starch digestion values. Consumer acceptability was determined using nine-point hedonic scale. The incorporation of sorghum and barley into wheat bread decreased *in-vitro* starch digestion at all incorporation levels than CB. RDS content significantly ($P < 0.05$) reduced in 40% and 50% sorghum and barley-containing bread (SBB), respectively, compared to CB. The eGI of SBB, at all incorporation levels, was significantly ($P < 0.05$) lowered compared to CB. Consumer sensory evaluation revealed that bread containing sorghum and barley flour, up to 40% level, was acceptable based on pre-set acceptability criteria. In conclusion, incorporation of sorghum and barley flour to white bread at 40% level is possible to decrease its digestibility and glycemic index, whilst maintaining consumer acceptability.

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Introduction

Reducing the global burden of diet-induced chronic diseases for instance diabetes mellitus (DM) and obesity is a major challenge in both industrialised and developing nations (de Waard *et al.*, 2019). Cereals are important targets in solving this problem since they are staple foods and substantial sources of energy across the world. Wheat is one of the most widely consumed cereal crop used to prepare various food products (Dhingra and Jood, 2002). Bread is the frequently consumed baked food which is mostly prepared form white wheat flour. As a result, the role of wheat in prevention of DM and other chronic diseases has been widely studied. Highly refined starchy foods are readily digested in the gut, resulting in rapid rise in blood glucose and therefore, are considered as risk factor for DM (Shewry *et al*., 2020). Therefore, reducing starch digestibility of wheat-based foods is essential, since it may result in delayed glucose release and a gradual rise in blood glucose levels (Prasad *et al*., 2015). Studies have shown that underutilized cereal grains have lower starch digestibility and hence, can be incorporated in white wheat bread to reduce its starch digestibility (Kaur *et al*., 2018).

Sorghum (*Sorghum bicolor* L.) ranks fifth in global cereal crop production but is still underutilized for human consumption. It is, nowadays, gaining interest as a source of several health promoting components including polyphenols, dietary fiber, slowly digestible starch (SDS) and resistant starch (RS) (Cardoso *et al*., 2015). Incorporation of sorghum flour into wheat-based foods has shown higher total phenolics, SDS and RS content and antioxidant activity *in-vitro* (Khan *et al*., 2013; Licata *et al*., 2014). Khan *et al*. (2013) reported that substitution of wheat semolina with sorghum flour, up to 40% level, reduced its digestibility without affecting consumer acceptability. Similarly, Yousif *et al*. (2012) revealed that wheat flatbread, incorporated with sorghum flour up to 40% level, reduced rapidly digestible starch (RDS) content compared to control wheat bread. Another study reported that tortillas, substituted with sorghum bran, had higher SDS content and decreased RDS content compared to brans from other cereals (Dunn *et al*., 2015). Moreover, sorghum-based foods had a lower glycemic index (GI) compared to wheat and rice-based foods (Prasad *et al*., 2015).

Barley (*Hordeum vulgare* L.) is another underutilized cereal which ranks fourth in cereal crop production. However, it is mainly used for feed and brewing purposes. The human consumption of barley is limited due to processing related issues. Barley is now gaining popularity as a component in functional foods due to high soluble dietary fiber content and antioxidant polyphenols (Thondre *et al*., 2012). The RDS content in barley flour is lower compared to rice, wheat, oat and corn flour (Soong *et al*., 2014). The higher content of polyphenols and lower digestible starch make barley an important functional food ingredient to regulate glycemic responses (Soong *et al*., 2014). In addition, the GI of barley is lower compared to wheat, rice, corn and oat, respectively (Soong *et al*., 2015). Besides, barley contained higher level of amylose compared to wheat and rice which may play a role in reducing glycemic responses (Soong *et al*., 2015). Various studies have shown that wheat products, supplemented with barley flour up to 15% and 30% level, were acceptable (Dhingra and Jood, 2002; Hussein *et al*., 2013). Hence, the incorporation of barley flour into bread would provide a product that may have potential health benefits.

Beside, providing health benefits, the human consumption of sorghum and barley is still limited. Their consumption can be improved by partially substituting these cereals in staple foods like white bread. Therefore, this study determined the effect of novel composite bread incorporated with different percentages of red sorghum and barley flours on *in-vitro* starch digestibility, eGI and consumer acceptability.

Materials and Methods

Raw materials

Refined wheat flour was obtained from local market. Red sorghum (var. JS-263) and barley (var. Jau-87) grains were obtained from Ayub Agriculture Research Institute (Faisalabad, Pakistan). Other ingredients (table salt, table sugar, oil (canola) and instant yeast) were procured from a local grocery store in Peshawar. Sorghum and barley (naked) grains were first cleaned manually to discard foreign materials such as pebbles, stones and seeds and then, washed. After washing, these grains were dried in sunlight to very low moisture content and ground in a flour mill (Warsak Road, Peshawar) to produce flour with 100% extraction rate and sieved via a 2 mm mash (Houssou and Ayernor, 2002). The resultant flours were packaged in black polyethylene bags until used.

Preparation of composite flours

For preparation of composite flour, wheat, barley and sorghum flours were weighed separately using a digital electronic weighing scale (Metra, model TL 600). Thereafter, red sorghum and barley flours were blended at equal proportion. Composite flour samples were, then, prepared by substituting refined wheat flour with the blend at 20%, 30%, 40% and 50% levels. The maximum incorporation level was determined by measuring dough strength by hand in a preliminary experiment. When more than 50% of flour blend was replaced for wheat flour, the dough strength was reduced, thus, limiting the use of flour blend by 50%. The replacement level above 50% was associated with increased dough mixing time, decreased bread volume, and denser crumb. The flours were thoroughly mixed to obtain a homogenous blend. The control sample was 100% refined wheat flour. The flour samples were saved in polyethylene bags (room temperature) until bread preparation and further analysis.

Bread preparation

Bread was prepared by mixing the flour blends at the rate of 100 g of composite flour with 5 g oil, 1 g salt, 3 g instant yeast, 6 g table sugar and water (65-70 ml) (Amendola and Nicole, 2003). The mixture was kneaded manually for 10 min to obtain soft and uniform dough. The dough was, then, kept in baking pans and fermented for 30 min at ambient temperature (28 °C). Afterwards, it was baked in electric oven (Panasonic Digital Oven) for 30 min at 230 °C. Control bread (CB) was made from refined wheat flour (100%) using similar procedure. After baking, bread were cooled and then, stored in polyethylene bags. Formulations were prepared in duplicate.

Samples preparation and storage

For chemical analysis of various parameters, the bread samples were dried in an air oven (50 °C, 24 hr). After that, dry bread samples were milled and passed via a 0.5 mm mesh. The milled samples were saved in airtight jars. The jars were covered using aluminium foil and stored in refrigerator (4 °C) until analyzed.

Chemicals

Sulfuric acid (H_2SO_4), boric acid (H_3BO_4), sodium hydroxide (NaOH), copper sulphate ($CuSO_4$), potassium sulphate (K_2SO_4), petroleum ether, acetone, methanol, α-amylase (AA), pepsin, amyloglucosidase (AMG), glucose-oxidase peroxidase reagent (GOPOD), potassium hydroxide (KOH) (2 M), sodium acetate (CH_3COONa) buffer (0.4 M, pH 3.8), sodium acetate buffer (0.2 M, pH 4.75), sodium phosphate (Na_3PO_4) buffer (0.1 M, pH 7), aqueous ethanol (80%), HCl-KCl buffer (0.01 M, pH 2), were all of analytical grade.

Proximate analyses

Proximate analyses were carried out on flour and bread samples. Moisture, crude fat, crude protein, crude fiber, ash and carbohydrate content of flour and bread samples were assessed using standard protocols (AOAC, 2002). Analyses were performed in quadruplicates and all values were calculated on dry basis.

Total starch determination

Total starch was determined by enzymatic colorimetric assay using the Megazyme Total Starch Assay kit (K-TSTA 06/2017; Megazyme Int. Ireland Ltd., Co. Wicklow, Ireland). Briefly, 100 mg defatted sample was mixed with 0.2 ml aqueous ethanol (80%) and stirred on vortex mixer. Next, 2 ml KOH (2 M) was added to each sample and stirred on ice water bath (20 min). Then, 8 ml sodium acetate buffer (1.2 M, pH 3.8) was put in each sample, and afterward 0.1 ml AA and 0.1 ml AMG were added in sample mixture. Sample mixtures were incubated (50 °C, 30 min) in a shaker water bath with alternating mixing using vortex. Next, the volume was made 100 ml using distilled water and aliquots of solution were taken and centrifuged at 3000 rpm for 10 min. GOPOD kit was used for determination of glucose content by colorimetric assay. For the assay, 0.1 ml of aliquots were taken in glass tubes and mixed with 3 ml GOPOD reagent followed by incubation (50 °C, 20 min). The color developed was, then, measured against a reagent blank (GOPOD reagent) at 510 nm. A conversion factor of 0.9 was used to calculate glucose as "mg of glucose x 0.9".

In-vitro starch digestibility and estimated glycemic index (eGI)

The method of Seczyk *et al*. (2017) was used to determine the *in-vitro* starch digestibility of bread samples. Briefly, 100 mg of defatted sample was mixed with 10 ml pepsin solution (0.2 g pepsin: 3500 U/mg per 100 ml of 0.01 M KCL-HCL buffer, pH 2) and incubated in a shaking water bath (37 °C, 80 rpm). Then, 15 ml of sodium phosphate buffer (0.1 M, pH 7) was added to the solution. Next, 5 ml sodium phosphate buffer (0.1 M, pH 7) containing AA (60 U/ml) was added to the sample mixture. Incubation was carried out for 120 min, during which 1 ml of aliquots from each sample were transferred to test tubes at time 0, 20, 30, 60, 90 and 120 min followed by inactivation of AA at 100 °C for 5 min. Next, 3 ml sodium acetate buffer (0.2 M, pH 4.75) and 60 µL AMG (196 U/ml) were added to aliquots to hydrolyze starch followed by incubation (60 °C, 60 min). The glucose content was determined using GOPOD assay. For each time point, starch digestibility was estimated as digested starch (DS) in g per 100 g dry starch using the following equation:

$$DS = 0.9 \times G_G \times 180 \times V/W \times S \times [100 - M]$$

Where; GG = glucose content (mmol/L); V= volume of digested starch (ml); 180 = glucose's molecular weight; W= weight of sample (g); S= sample's starch content (g/100 g dry sample); M= sample's moisture content (g/100 g sample); and 0.9 = stoichiometric constant for starch from glucose contents (Sopade and Gidley, 2009).

Rapidly digestible starch (RDS) (g/100 g dry starch) was measured by replacing G_G in the above equation with $(G_{20} - G_0)$ indicating the glucose content at 20 min minus the glucose content at time, 0 min. Likewise, slowly digested starch (SDS) (g/100 g dry starch) was measured replacing G_G for $(G_{120} - G_{20})$ (Rosin *et al*., 2002). Resistant starch (RS) (g/100 g dry starch) was determined as: 100 – RDS – SDS. Digestion curves of digested starch (g/100 g dry starch) compared with the time of digestion (min) for each bread sample corrected for baseline values (0 min) were constructed.

The hydrolysis index (HI) was computed as the ratio of the test bread sample's area under the 90-minute hydrolysis curve to the control bread sample's area under hydrolysis curve (reference).

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

The eGI was estimated using the 90-min HI using the formula of Goni *et al*. (1997):

$$eGI = 39.71 + 0.549HI_{90}$$

Consumer sensory evaluation

The sensory acceptance of control and treatment bread with varying percentages of sorghum and barley flours (20%, 30%, 40%, and 50%) was tested in the Department of Human Nutrition, University of Agriculture, Peshawar, by 50 untrained (25 male and 25 female) volunteers. The Department of Human Nutrition, Human Research Ethics Committee (HN-HREC/2017-0019) of The University of Agriculture, Peshawar approved the study protocol.

Sensory characteristics of breads such as texture, flavor, appearance and overall acceptance were determined using nine-point hedonic scale. The breads were served in disposable plates, coded with 3 digit numbers, in random order. Plain water was given for rinsing the mouth between the analyses of each bread. The subjects did not eat or drink for 3 hours pre-evaluation.

Two pre-set criteria were used to determine acceptability of composite bread in the present study (Clark and Johnson, 2002). The composite bread was deemed acceptable if (i) the mean sensory evaluation score for overall bread acceptance was ≥ 6, and (ii) the estimated population mean rating for overall bread acceptance was not less than 1 rating category lower than CB; i.e., if the 95% confidence interval (CI) for the mean difference (SBB – CB) was ≥ –1.0.

Statistical analysis

The SPSS software for Windows was used to examine the data. Quadruplicates were used in the analysis. Using one-way analysis of variance (ANOVA) with post-hoc LSD for multiple comparisons, the impact of sorghum and barley flours incorporation on proximate composition, *in-vitro* starch digestibility, eGI, and acceptability of bread was examined. A $P < 0.05$ was found to be significant.

Results and Discussion

Proximate composition

Results on proximate composition of flour and bread samples are given in Table 1. Sorghum and barley flours had significantly ($P < 0.05$) higher content of ash and fat compared to wheat flour. However, sorghum and barley flour had lower content of protein and carbohydrate compared to wheat flour. Furthermore, sorghum and barley flours had higher fiber content ($P < 0.05$) than wheat flour (control). The incorporation of sorghum and barley flour, at 20%, 30%, 40% and 50% levels, significantly ($P < 0.05$) improved the ash and dietary fiber content of composite bread. The protein and carbohydrate content significantly ($P < 0.05$) decreased when incorporated with sorghum and barley flours.

In-vitro starch digestibility

The incorporation of red sorghum and barley flours into wheat bread reduced its *in-vitro* starch digestibility at all degrees of incorporation, as shown by the lower response curve in the digestogram (Figure 1). The maximum incorporation, i.e., at 50%, resulted in the least digested starch. The CB, on the other hand, displayed the highest values at each time point.

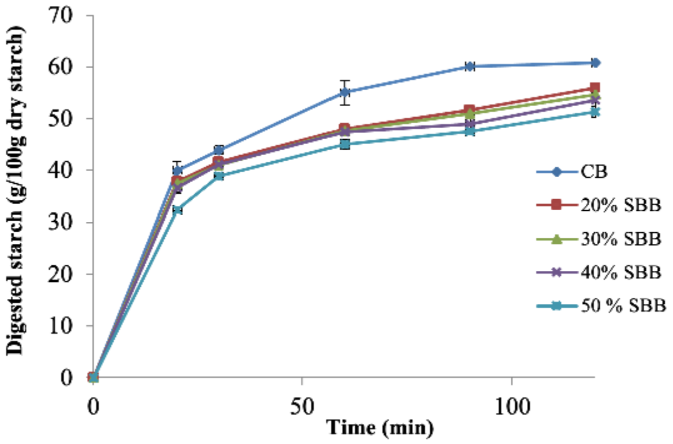

Figure 1: *Starch digestogram of control and bread incorporated with sorghum and barley flours at different levels. Values are presented as mean ± SD. CB = control bread; SBB = sorghum and barley-containing bread*

Starch fractions

The results on starch fractions of CB and SBB are shown in Table 2. Results exhibited significant ($P < 0.05$) effect of sorghum and barley flours incorporation on RDS and RS fractions but not on SDS fraction. The RDS content of bread, containing 40% and 50% sorghum and barley flours blends, was significantly lower ($P < 0.05$) relative to CB. The RS content was significantly greater ($P < 0.05$) in SBB compared to CB at all incorporation levels.

Hydrolysis index (HI) and estimated glycemic index (eGI)

Table 3 represents the HI and eGI of bread samples. The HI of SBB at all incorporation levels was significantly ($P < 0.05$) lower relative to CB. Similarly, the SBB, at all levels, had lower eGI compared to CB.

Table 1: *Proximate composition of flour and bread samples (% dry basis)*.*

Sample	Ash	Protein	Fat	Carbohydrate	Fiber
(A) Flour samples					
Wheat flour	1.10 ± 0.14^{h}	11.27 ± 0.04^{ab}	1.80 ± 0.03^{f}	84.00 ± 0.12^{a}	1.82 ± 0.04^{i}
Sorghum flour	2.02 ± 0.05^{ab}	10.20 ± 0.02^{e}	2.10 ± 0.02^{bcd}	79.50 ± 0.11^{g}	6.17 ± 0.04^{a}
Barley flour	2.06 ± 0.09^{a}	10.27± 0.08^{e}	2.22 ± 0.06^{bc}	79.86 ± 0.17^{f}	5.57 ± 0.06^{b}
20% SBF	1.31 ± 0.01^{fg}	10.73 ± 0.06^{cd}	1.82 ± 0.03^{ef}	83.07 ± 0.04^{b}	3.06 ± 0.03^{gh}
30% SBF	1.43 ± 0.04^{f}	10.74 ± 0.04^{cd}	2.00 ± 0.03^{de}	82.51 ± 0.07^{c}	3.31 ± 0.04^{f}
40% SBF	1.73 ± 0.04^{de}	10.48 ± 0.08^{de}	2.09 ± 0.03^{cd}	82.28 ± 0.12^{c}	3.41 ± 0.02^{e}
50% SBF	1.89 ± 0.02^{bc}	10.21 ± 0.02^{e}	2.30 ± 0.02^{b}	81.63 ± 0.02^{d}	3.96 ± 0.02^{c}
(B) Bread samples					
CB	1.06 ± 0.08^{h}	11.63 ± 0.06^{a}	4.26 ± 0.27^{a}	81.34 ± 0.26^{d}	1.70 ± 0.01^{j}
20% SBB	1.25 ± 0.02^{g}	10.94 ± 0.00^{bc}	4.24 ± 0.01^{a}	80.52 ± 0.07^{e}	3.04 ± 0.08^{h}
30% SBB	1.41 ± 0.04^{f}	10.50± 0.04^{de}	4.25 ± 0.07^{a}	80.67 ± 0.00^{e}	3.15 ± 0.06^{g}
40% SBB	1.65 ± 0.04^{e}	10.85 ± 0.05^{cd}	4.36 ± 0.02^{a}	79.76 ± 0.10^{fg}	3.36 ± 0.02^{ef}
50% SBB	1.81 ± 0.02^{cd}	10.83 ± 0.06^{cd}	4.37 ± 0.06^{a}	79.12 ± 0.04^{h}	3.85 ± 0.02^{d}

**Values are means ± SD of quadruplicates. Columns having means with different superscripts are significantly different ($p < 0.05$, LSD test). SBF: sorghum and barley-containing composite flour; CB: control bread; SBB: sorghum and barley-containing bread. Carbohydrate was calculated by difference using Atwater formula.*

Table 2: *Starch fractions of bread samples (% dry basis)**

Sample	TS	RDS	SDS	RS
CB	70.98 ± 0.46^{a}	39.97 ± 1.89^{a}	30.68 ± 1.75^{a}	29.35 ± 0.14^{c}
20% SBB	70.02 ± 0.68^{ab}	37.77 ± 0.15^{ab}	28.75 ± 0.15^{a}	33.48 ± 0.30^{b}
30% SBB	69.16 ± 0.73^{bc}	37.41 ± 1.23^{ab}	29.09 ± 1.23^{a}	33.50 ± 0.31^{b}
40% SBB	68.31 ± 0.29^{bc}	36.65 ± 0.08^{b}	28.56 ± 0.08^{a}	34.79 ± 1.23^{b}
50% SBB	67.97 ± 0.96^{c}	32.32 ± 0.63^{c}	28.57 ± 0.63^{a}	39.11 ± 1.08^{a}

**Values are expressed as mean ± SD of quadruplicates. Means in the same column with different letter are significantly different ($p < 0.05$). CB: control bread; SBB: sorghum and barley-containing bread; RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch; TS: total starch.*

Table 3: *Hydrolysis index (HI) and estimated glycemic index (eGI) of bread samples*.*

Sample	HI	eGI
CB	100.00 ± 0[a]	100.00 ± 0[a]
20% SBB	90.30 ± 0.44[b]	89.28 ± 0.24[b]
30% SBB	84.34 ± 0.83[c]	86.01 ± 0.46[c]
40% SBB	82.82 ± 0.25[d]	85.18 ± 0.14[d]
50% SBB	79.54 ± 1.05[e]	83.38 ± 0.58[e]

**Values are expressed as mean ± SD of quadruplicates. Values with different letters along the column are significantly different ($p < 0.05$). CB: control bread; SBB: sorghum and barley-containing bread; HI: hydrolysis index; eGI: estimated glycemic index.*

HI and eGI decreased with increasing the incorporation level of sorghum and barley flours in composite breads. The bread with the maximum incorporation (50%) had the lowest HI and eGI.

Consumer sensory evaluation

Results on consumer sensory evaluation are shown in Table 4. In comparison to CB, the consumer panel assigned SBB a lower ($P < 0.05$) score for color, flavor, texture and overall acceptance. The CB scored in the "like moderately" category for color acceptability, while the SBB with 20% and 30% incorporation levels scored in the "like slightly" range. SBB with 40% and 50% substitution levels scored in the "neither like nor dislike" category.

The CB bread received a "like moderately" rating in terms of flavor, texture (in the mouth), and overall acceptance, whereas bread with 20%, 30% and 40% incorporation scored in the "like slightly" range. Bread with 50% incorporation level rating was in the "neither like nor dislike" range.

The mean "overall acceptability" score for SBB containing 40% sorghum and barley flour blend was more than 6.0, stratifying the first pre-set acceptability criteria. Bread samples containing 50% sorghum and barley flours did not meet the first acceptability criteria, since they did not attain the minimal score of 6.0. For the bread comprising sorghum and barley flours at 20%, 30%, and 40% incorporation levels, the lower (95%) CI of the mean difference for overall acceptance between the SBB minus CB was ≥ minus one (–1.0). The mean differences for SBB containing 40% sorghum and barley flour was equal to –1.0, but not for bread containing 50% incorporation level. Hence, the bread sample containing sorghum and barley flour, blend up to 40% level, met the second pre-set acceptability criteria.

Table 4: *Mean score, mean difference and 95% confidence intervals of difference in consumer ratings of control and composite bread samples.*

Sensory attributes	Mean score	Mean difference (composite-control)	95% confidence interval of difference	
			Lower	Upper
Color				
CB	7.18[a]			
20% SBB	6.22[b]	-0.96	-1.47	-0.45
30% SBB	6.04[b]	-1.14	-1.65	-0.63
40% SBB	5.94[b]	-1.24	-1.75	-0.73
50% SBB	5.36[c]	-1.82	-2.33	-1.31
Flavor				
CB	7.12[a]			
20% SBB	6.34[b]	-0.78	-1.17	-0.40
30% SBB	6.12[b]	-1.00	-1.39	-0.62
40% SBB	6.06[b]	-1.06	-1.45	-0.68
50% SBB	5.32[c]	-1.80	-2.19	-1.42
Texture (in mouth)				
CB	7.02[a]			
20% SBB	6.30[b]	-0.72	-1.10	-0.34
30% SBB	6.10[b]	-0.92	-1.30	-0.54
40% SBB	6.04[b]	-0.98	-1.36	-0.60
50% SBB	5.40[c]	-1.62	-2.00	-1.24
Overall acceptability				
CB	7.14[a]			
20% SBB	6.42[b]	-0.72	-1.12	-0.32
30% SBB	6.26[b]	-0.88	-0.56	0.24
40% SBB	6.14[b]	-1.00	-1.40	-0.60
50% SBB	5.40[c]	-1.74	-2.14	-1.34

Mean scores for each section (A, B, C, or D) along the column having different letters are statistically different ($p < 0.05$; Post-hoc LSD test). CB: control bread; SBB: sorghum and barley-containing bread. Results of a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely).

The current study examined the effect of red sorghum and barley flours incorporation in wheat bread on *in-vitro* starch digestibility, levels of starch fractions, eGI and consumer acceptability. It was hypothesized that the incorporation of red sorghum and barley flours into white wheat bread would reduce its digestibility of starch and eGI without affecting consumer acceptability.

In compliance with the study of Collar and Angioloni (2014), incorporation of whole-grain red sorghum and barley flours at all levels enhanced ash, fiber and fat content of composite flours and bread samples relative to control samples. These results are also corroborated by Khan *et al*. (2013) and Yousif *et al*. (2012), who stated high ash and fiber content of pasta and flatbread incorporated with red sorghum flour. Previous studies have shown that the germ of sorghum and barley contain high level of fats, minerals and dietary fiber (Aboubacar *et al*., 2006; Collar and Angioloni, 2014). The reduction in the protein and carbohydrate content of composite breads after incorporation of sorghum and barley flours in the current study is corresponding with prior studies (Yousif *et al*., 2012; Khan *et al*., 2013). Previously, it has been shown that wheat flour contains high amount of protein than barley and sorghum flours (Al-Attabi *et al*., 2017). Therefore, the decreased protein content of composite bread might be related to the protein dilution after addition of sorghum and barley flours in the present study.

In the current study, the incorporation of sorghum and barley flour reduced the starch digestibility and eGI of composite bread samples relative to control bread. These findings are substantiated by previous *in-vitro* studies on the effect of sorghum and barley flours incorporation in food products such as flatbread, pasta and muffins (Yousif *et al*., 2012; Khan *et al*., 2014; Soong *et al*., 2014; Montalbano *et al*., 2016). Kaur *et al*. (2018) compared the *in-vitro* starch digestibility and eGI of various cereals. Their results indicated that barley and sorghum had lower starch digestibility than wheat and rice (Kaur *et al*., 2018). Thus, the decreased starch digestibility of the composite breads in the present study could be due to the addition of sorghum and barley flours.

The present study showed significant impact of sorghum and barley flour incorporation on RDS and RS content, with CB having substantially higher RDS content compared to composite breads. While the RS content was relatively higher in composite breads compared to CB. These values are comparable to those of Yousif *et al*. (2012), who studied the impact of incorporating red sorghum flour in wheat flatbread. These results are also supported by Kaur *et al*. (2018), who compared different cereals for starch fractions content and showed that barley exhibited the lowest RDS content, followed by sorghum. Moreover, barley and sorghum had higher level of SDS and RS compared to other cereals (Kaur *et al*., 2018). Thus, the significant differences in the starch fractions of composite breads could be attributed to incorporation of sorghum and barley flours.

In the current study, substituting wheat flour with red sorghum and barley flours significantly reduced the HI and eGI of composite breads compared to CB. In literature, the extent of starch hydrolysis has been shown to be correlated with the GI of foods (Englyst and Hudson, 1996). GI is a derivative of HI which is used to rate foods in terms of their ability to raise blood glucose levels (Kaur and Sandhu, 2010). The values of HI and eGI in the current study were comparable to those of Kaur *et al*. (2018), who showed HI and eGI of 80, 83.1 and 83.6, 85.3 for barley and sorghum, respectively. The eGI is also associated with the RDS content of foods as stated previously (Englyst *et al*., 1999). The RDS content was positively associated with the HI and GI of barley and sorghum whereas the SDS and RS content were inversely related (Kaur *et al*., 2018). The findings on the RDS level of the composite bread sample in the present study showed similar trend relative to HI and GI. The RDS and SDS values of food can, therefore, estimate the GI of starch-based foods (Englyst *et al*., 2003).

Starch digestibility and the resultant glycemic status are impacted by a number of elements such as resistant starch content, dietary fibers, amylose content, polyphenols, protein content and granule particle size (Rosin *et al*., 2002; Svihus *et al*., 2005; Absar *et al*., 2009). The granules of sorghum grain are embedded in a protein network linked through di-sulphide linkages that may limit the susceptibility to enzymatic hydrolysis and hence, reduce its digestibility (Taylor and Emmambux, 2010). Starch can interact with polyphenols forming resistant starch complexes, thus, reducing starch digestibility (Jakobek, 2015). In addition, polyphenols also inhibit the activity of pancreatic and salivary α-amylases in digesta thus reducing digestibility (Kim *et al*., 2011). Moreover, starch gelatinization during cooking forms complexes with protein that reduces digestibility of, both, starch and protein (Dunn *et al*., 2015).

The granule size may also affect starch digestibility. As the granule size increases, the surface area decreases and therefore, the possibility of enzyme action on substrate decreases (Svihus *et al*., 2005). Furthermore, the amylose contents of cereals also influence starch digestibility (Svihus *et al*., 2005). Cereals with high amylose level have reduced starch digestibility and greater RS levels (Sajilata *et al*., 2006). Dietary fiber in cereals are also implicated in lowering starch digestibility by raising viscosity of the gut content, thereby, reducing the mixing of digesta with enzymes

(Singh *et al*., 2010).

The present study has some potential limitations. In the current study, blend of whole-grain sorghum and barley flours were utilized to substitute refined wheat flour, hence, it is, unlikely, to isolate the influence of granule size on digestibility. In addition, the content of polyphenols, amylose and β-glucan were not determined to find any observed effect on starch digestibility and eGI. Therefore, future studies are warranted to address these limitations to find the effect of whole verses refine flour and also the interaction with various components reported in literature.

The decreased digestibility could be beneficial due to low caloric intake and glycemic responses from sorghum and barley-based foods and thus, may help in the prevention of obesity and other cardiometabolic health condition (Awika and Rooney, 2004). The future use of this study may involve the application of such products for their potential health benefits in subjects with cardiometabolic health condition such as DM and cardiovascular diseases.

Conclusions and Recommendations

In conclusion, incorporation of red sorghum and barley flours in wheat bread is a possible mean to lower its starch digestibility and eGI and enhance slowly digestible starch and resistant starch content without affecting consumer acceptability. The decreased RDS content in the composite bread shows that combining blend of whole-grain sorghum and barley flours with refined wheat flour bread might reduce the influence on postprandial glycemia. Human clinical trials are now suggested to see the effect of low GI bread with increased content of slowly digestible and resistant starch content on glycemic parameters in health and disease conditions. From consumer acceptability perspective, bread containing the higher acceptable level of sorghum and barley flours (i.e., 40%) can be used in future clinical trials for evaluation of health effects in various cardiometabolic health conditions.

Novelty Statement

The effect of combined incorporation of sorghum and barley flours in white bread on in-vitro starch digestibility, eGI and consumer acceptability is not investigated previously.

Author's Contribution

Imran Khan: Designed the study.
Bibi Hajira: Carried out the research study, analyzed the findings, and drafted the manuscript.
Imran Khan and Zia-ud-Din: Critically reviewed the article.
The final version of the manuscript was approved by all authors.

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

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