SciMedCentral
Journal of Human Nutrition & Food Science

Research Article

Technological Characterization of Hulless Barley (*Hordium vulgare*) Whole Grain Flour for Chapatti Making: Thermo-Mechanical, Physicochemical, Starch Digestibility and Retrogradation Traits

Hardeep Singh Gujral[1]*, Bharati Sharma[1], Harleen Kaur[1], Ashima Arora[1] and Vicky Solah[2]

[1]*Department of Food Science and Technology, Guru Nanak Dev University, India*
[2]*Food Science and Nutrition Program, College of Science, Health, Engineering and Education, Murdoch University, Australia*

***Corresponding author**
Hardeep Singh Gujral, Department of Food Science and Technology, Guru Nanak Dev University, Amritsar-143005, India, Tel: 91-183-2258802-3429, Fax: 91-183-2258820

Submitted: 02 October 2022
Accepted: 31 October 2022
Published: 30 October 2022
ISSN: 2333-6706

Keywords
- Hulless barley
- Chapatti
- Starch digestibility
- Retrogradation

Abstract

The study was aimed at investigating the technological performance of hulless barley whole grain flours for chapatti making in terms of thermo-mechanical characteristics, chapatti quality, and starch digestibility and retrogradation behavior. Vital gluten (7%) was added to improve the viscoelastic behavior of dough. The considerable influence of non-starchy polysaccharides in enhancing dough consistency after mixing (C1) and heating (C3, peak viscosity) is evident based on the results. Protein weakening and starch breakdown reduced significantly in barley chapattis in contrast to wheat chapatti. Water absorption, shrinkage and bake loss of chapatti increased as a function of non starchy polysaccharides. Whole barley flours demonstrated higher retrogradation as evident from the soluble starch and starch crystallinity analysis. Whole barley flour chapattis showed 76.74% more resistant starch and 20.48% more slowly digestible starch than wheat chapatti. Lower glycemic index (up to 62.04) was observed for whole barley flour chapattis demonstrating their potential benefit for diabetic patients. Thus, barley flour with added vital gluten may be effectively utilized in other bakery products to provide a slower digestibility.

INTRODUCTION

Barley plays a significant role in sustainable agriculture due to lower water uptake, high drought tolerance and adaptability in harsh and saline environment [1]. Barley is gaining greater recognition as a functional food due to the presence of bioactive components such as β-glucan, arabinoxylan and phenolic compounds that have been associated with health benefits such as control of diabetes, lowering of cholesterol and protective effects against certain types of cancer [2]. Dietary modification using products that contains resistant starch is a known strategy to assist in mitigation of risk factors associated with T2DM (Type 2 Diabetes Mellitus) [3].

Whole-grain cereals are widely promoted for various health benefits but very little of barley, especially hulless barley, is used in foods primarily due to lack of capability to form viscoelastic dough. However it could be used in many baked products [4] after addition of the vital wheat gluten. Most of the wheat in Indian subcontinent is stone milled and flour with higher extraction rate (up to 100%) is used to make chapattis. Hulless barley resembles wheat in morphology and can also be milled into whole flour possessing higher bioactive potential [1]. Popularity of non wheat flours is increasing as consumers are becoming aware of the health benefits and functional aspects of different cereal grains. Since wheat is mostly consumed in the form of chapatti in our region, the suitability of various healthier grains for chapatti making need to be explored. Whole barley flours can be made suitable for making chapatti by incorporating vital gluten to improve the viscoelastic behavior of dough. Vital gluten has earlier been used in the preparation of various food products like crackers, noodles, bread etc. [5,6].

An upsurge in number of working women and a rise in demand for convenience food in India has led to the large-scale

Cite this article: *Gujral HS, Sharma B, Kaur H, Arora A, Solah V (2022) Technological Characterization of Hulless Barley (Hordium vulgare) Whole Grain Flour for Chapatti Making: Thermo-Mechanical, Physicochemical, Starch Digestibility and Retrogradation Traits. J Hum Nutr Food Sci 10(2): 1149.*

manufacture and marketing of chapatti in packs but the consumer requires the product should retain the quality characteristics of fresh chapatti on consumption. Chapatti on storage has a tendency to become stale resulting in harder texture, loss of eating quality, decrease in moisture and water-soluble starch content and lower enzyme digestibility [7]. Hence monitoring the changes in quality characteristics of chapatti during storage has become of greater interest for researchers. Therefore, the present work attempts to study the feasibility of utilizing whole hulless barley flour for chapatti preparation after supplementing with vital gluten and evaluating thermo-mechanical characteristics, starch digestibility and retrogradation behavior.

MATERIALS AND METHODS

Materials

Hulless barley cultivars, Dolma and HBL-276 were obtained from Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya Bajaura; Geetanjali and Upasana (NDB-943) from Chandra Shekhar Azad University of Agriculture and Technology Kanpur and Narendra Dev University of Agriculture and Technology Faizabad, respectively; BHS-352 from Indian Agricultural Research Institute Shimla; Sindhu, Nurboo, SBL-8 and SBL-9 from High Mountain Arid Agriculture Research Institute Leh-Ladakh. HD 2967 cultivar of wheat variety was collected from Punjab Agricultural University Ludhiana, India. Vital wheat gluten (moisture content-7%, protein-75%, ash-1.5%) was procured from Pioneer Industries, Pathankot, India.

Preparation of flour

Wheat was milled in a stone mill (Trimurti, Amar Industries, India) by adjusting the feed rate to 5 Kg/hr and gap between the stones was so adjusted that a fine grind of flour obtained passed through 60 mesh sieve and this flour was labeled as whole wheat flour (WWF). Similarly barley was milled under same feed rate and fineness to obtain whole barley flour (WBF). The whole barley flour lacks enough gluten protein required for the development of viscoelastic network during dough formation hence to provide viscoelasticity vital wheat gluten was added. Initial trials of chapatti preparation were carried out by blending whole barley flour and vital gluten in various proportions and it was found that 7% vital gluten resulted in acceptable chapatti having good sheeting and puffing characteristics. The control whole wheat flour has been referred to as WWF and WBF supplemented with 7% vital gluten was labeled as GSWBF (gluten supplemented whole barley flour) throughout the manuscript.

Assay of β-glucan content

The total β-glucan was estimated using a 'β-glucan assay kit' (Megazyme International Ireland Ltd., Wicklow, Ireland) [1]. 500 mg of sample was mixed with 1 ml of ethanol (50% v/v) and sodium phosphate buffer followed by incubating in boiling water bath and further cooling to 40 °C. Lichenase enzyme (10 U) was added and tubes were kept at 40 °C for 1 h and centrifuged. Aliquot (0.1 ml) was transferred in three test tubes. To one tube (reaction blank), sodium acetate buffer was added and to other two tubes 0.2 U β-glucosidase was added. After keeping tubes at 40°C for 15 min glucose oxidase peroxidase reagent was added and stored at 40°C for 20 min. The absorbance was read spectrophotometrically at 510 nm (Shimadzu, UV-1800, Kyoto, Japan). The below mentioned formula was used to calculate β-glucan content:

$$\beta\text{-glucan } (\%) = \Delta A \times 100/\text{Absorbance of standard} \times 94 \times 0.001 \times 100/W \times 0.9$$

where, ΔA is (sample absorbance-blank absorbance); W is the weight of sample in mg.

Assay of arabinoxylan content

25 ml water was added to sample (125 mg) and vortexed. 1 ml of this suspension was taken to estimate total arabinoxylan according to the colorimetric method discussed by Douglas [8]. D-(+)-xylose (X-1500, Sigma) standard curve was used for calculating arabinoxylan content. The tests were repeated three times and result was expressed in percent (%).

Thermo-mechanical characteristics

Mixolab 2 (Chopin Technologies, France) was used to analyze the thermo-mechanical characteristics by subjecting dough to simultaneous mixing and heating conditions. The test was run using Chopin+ protocol at a hydration of 80 % because at lower hydration the dough wrapped around mixing blades for some varieties of hulless barley causing a sudden drop in torque to zero; hence a higher hydration level (80%) was selected. The sequence of test followed was mixing of dough at 30 °C for 8 min (C1 torque) which indicated optimum dough development. Further mixing caused the breakdown of dough matrix (C2 torque) which measured protein weakening. Then temperature increased to 90 °C (C3) depicting swelling and gelatinization of starch. There was continued mixing and holding duration of 7 min at this temperature (C4) indicating starch breakdown and finally, cooling of the dough to 50 °C indicating starch retrogradation (C5). The analysis was repeated thrice for each flour sample.

Chapatti characteristics

To obtain optimum water absorption, preliminary trials were performed subjectively by mixing flours with varying levels of water content. Dough preparation was done by adding WWF or GSWBF in a pin mixer (National Manufacturing Company, NE, USA) using the optimum water absorption (Table 2) obtained from preliminary trials according to the method discussed by Sharma et al. [9]. Chapattis were then prepared by following the method of Gujral and Gaur [4]. The amount of water lost from chapatti upon baking was reported as percent bake loss (%) and the reduction in diameter after baking as percent shrinkage (%). The sensory characteristics of chapatti were evaluated by semi-trained panel of twenty-five members (students and staff of the Department of Food Science & Technology, GNDU, Amritsar) using a 9-point Hedonic scale.

Retrogradation Analysis

Soluble starch and soluble amylose*:* Soluble starch and soluble amylose were determined as per the spectrophotometric method discussed by Sharma et al. [9] using soluble starch and soluble amylose (Sigma Aldrich) standard curve. The test was repeated three times.

Relative crystallinity*:* X-ray diffractometer (XRD-7000, Shimadzu, Japan) was used to obtain diffractograms of the fresh and retrograded chapattis. The diffractometer was fitted with a copper tube working at 40 kV and 30 mA irradiating the sample with monochromatic Cu kα radiation with a wave length of 0.154 nm. The spectra were scanned over a diffraction angle (2θ) range of 5-50° at scan speed of 2°/min. Relative crystallinity was obtained using Originpro 8.5.1 software by comparing the area under crystalline peaks to that of amorphous region. The analysis was performed three times.

In-vitro digestibility of starch and predicted glycemic index*:* In-vitro digestibility of starch was analyzed using glucose oxidase peroxidase (GOD-POD) kit (ERBA Diagnostics Mannheim, Germany) for measurement of free glucose. The digested starch was calculated using the following equation:

Digested starch (g/100g) = 0.9 × glucose concentration × Volume of digesta (ml)

Weight of sample (g) × Starch (%)

The digested starch obtained was then classified into slowly digestible starch (SDS), resistant starch (RS) and rapidly digestible starch (RDS). Hydrolysis Index (HI) was determined by comparing the area under curve for the chapatti sample with a reference food (white bread). The Hydrolysis index obtained was further used to predict glycemic index (pGI) as per the equation: pGI = 0.862 HI + 8.198 [5].

Statistical analysis

Each test was repeated three times on dry weight basis. Analysis of variance (ANOVA) was performed with the help of Microsoft Excel software. Fisher's least significant differences test was used to depict means with 95% ($p<0.05$) confidence. The Pearson correlation coefficient was calculated using Microsoft Excel software.

RESULTS AND DISCUSSION

Thermo-mechanical characteristics

Dough development: The initial mixing of dough caused hydration of proteins and development of a viscoelastic structure resulting in an increase in consistency (C1). WWF dough, which served as a control was characterized with low C1 (0.806 Nm) whereas for GSWBFs C1 ranged from 1.021-1.916 Nm (Table 1). This higher consistency in GSWBFs could be due to different nature of gluten as the vital gluten added to barley flours was slackly attached whereas the native gluten present in WWF was strongly bonded via covalent bonds. Our earlier study [10] revealed that the commercial vital gluten (obtained from Pioneer industries) had lower (254.4%) water absorption index while gluten prepared manually from WWF displayed a higher value (295.7%). Also, the vitality test for both the commercial vital gluten and wheat gluten (native) using Mixolab (Figure 1) indicated that the commercial vital gluten had lower consistency/strength and torque values as compared to fresh wheat gluten (extracted manually) [10].

Besides, higher levels of non starch polysaccharides (β-glucan and arabinoxylans) (Table 2) in the GSWBFs bind water [1] leaving less water available for gluten development leading to higher dough consistency (C1 torque). Significant correlation of C1 was observed with total β-glucan (r= 0.79) and total arabinoxylan content (r= 0.76). The highest C1 was observed for SBL 9 GSWBF that possessed higher levels of β-glucan (Table 2). The dough development time (DDT) for WWF was 0.9 min whereas GSWBFs took longer to develop; the DDT ranged between 1.0-2.52 min. The higher β-glucan and arabinoxylan content in barley flours also contributed to a rise in DDT attributed to interference from these fiber components in holding the gluten matrix, thus causing maximum resistance to extension at C1. This was also evident from positive association of DDT with total β-glucan (r= 0.54). It is also possible that vital gluten added to barley flours interact inferiorly with the intrinsic proteins present in flour and thus displaying more time for dough formation than the gluten present

Table 1: Thermo-mechanical characteristics of WWF and GSWBFs.

Cultivar	C1 (Nm)	DDT (min)	Stability (min)	Protein weakening (%)	C3 (Nm)	C4 (Nm)	C5 (Nm)	Breakdown (%)	Retrograda-tion (%)	β (Nm/min)
HD 2967	$0.81^{a}\pm0.07$	$0.90^{a}\pm0.07$	$1.95^{a}\pm0.07$	$27.92^{a}\pm1.49$	$1.36^{a}\pm0.02$	$0.89^{d}\pm0.08$	$1.20^{c}\pm0.07$	$34.42^{c}\pm0.53$	$26.13^{b}\pm0.99$	$0.256^{b}\pm0.03$
Upasna	$1.24^{c}\pm0.01$	$2.18^{b}\pm0.35$	$9.96^{e}\pm0.20$	$38.96^{d}\pm0.05$	$1.59^{b}\pm0.02$	$1.47^{h}\pm0.08$	$1.64^{e}\pm0.04$	$7.53^{a}\pm0.65$	$10.12^{a}\pm0.76$	$0.225^{a}\pm0.02$
Geetanjali	$1.02^{b}\pm0.01$	$1.38^{a}\pm0.07$	$10.78^{g}\pm0.35$	$36.41^{c}\pm0.43$	$1.66^{c}\pm0.02$	$1.27^{f}\pm0.03$	$1.84^{g}\pm0.04$	$23.62^{b}\pm0.87$	$31.19^{c}\pm0.13$	$0.222^{a}\pm0.03$
Dolma	$1.66^{g}\pm0.01$	$2.52^{b}\pm0.06$	$8.98^{d}\pm0.51$	$35.30^{c}\pm1.5$	$2.01^{g}\pm0.04$	$1.35^{g}\pm0.04$	$1.92^{h}\pm0.06$	$32.84^{c}\pm1.09$	$29.68^{c}\pm0.25$	$0.286^{b}\pm0.03$
HBL 276	$1.50^{e}\pm0.01$	$1.90^{b}\pm0.40$	$8.11^{d}\pm0.09$	$38.36^{d}\pm0.49$	$1.89^{f}\pm0.01$	$1.16^{e}\pm0.01$	$1.76^{f}\pm0.02$	$38.74^{d}\pm0.32$	$34.37^{c}\pm0.83$	$0.385^{c}\pm0.13$
BHS 352	$1.75^{i}\pm0.03$	$2.16^{b}\pm0.98$	$10.63^{f}\pm0.66$	$30.04^{b}\pm0.54$	$1.89^{f}\pm0.00$	$1.17^{e}\pm0.01$	$1.67^{e}\pm0.04$	$38.24^{d}\pm0.69$	$30.25^{c}\pm1.04$	$0.258^{b}\pm0.01$
Sindhu	$1.72^{h}\pm0.02$	$1.24^{a}\pm0.01$	$5.69^{b}\pm0.35$	$42.29^{f}\pm0.04$	$1.68^{c}\pm0.01$	$0.54^{a}\pm0.02$	$0.85^{a}\pm0.02$	$67.83^{h}\pm1.03$	$36.94^{c}\pm0.62$	$0.417^{d}\pm0.02$
Nurboo	$1.54^{f}\pm0.02$	$1.32^{a}\pm0.12$	$8.27^{d}\pm0.13$	$40.35^{e}\pm0.59$	$1.76^{d}\pm0.01$	$0.82^{c}\pm0.01$	$1.24^{c}\pm0.03$	$53.66^{f}\pm0.12$	$34.19^{c}\pm1.72$	$0.460^{d}\pm0.03$
SBL 8	$1.37^{d}\pm0.01$	$1.29^{a}\pm0.02$	$8.68^{d}\pm0.92$	$41.08^{e}\pm0.19$	$1.77^{d}\pm0.01$	$0.75^{b}\pm0.01$	$1.09^{b}\pm0.05$	$57.38^{g}\pm0.02$	$30.57^{c}\pm0.84$	$0.570^{e}\pm0.02$
SBL 9	$1.92^{j}\pm0.01$	$1.07^{a}\pm0.02$	$6.39^{c}\pm0.34$	$36.05^{c}\pm0.29$	$1.83^{e}\pm0.01$	$0.94^{d}\pm0.03$	$1.37^{d}\pm0.02$	$48.96^{e}\pm0.08$	$30.99^{c}\pm0.91$	$0.423^{d}\pm0.01$

Values represent mean of three replicates.
Superscripts a-i are significantly ($p \leq 0.05$) different column wise in different cultivars.
Protein weakening is (C1 at 8 min-C2)%; Breakdown is (C3-C4)%; Retrogradation is (C5-C4)%; β is starch gelatinization speed.
HD 2967 is WWF (control).

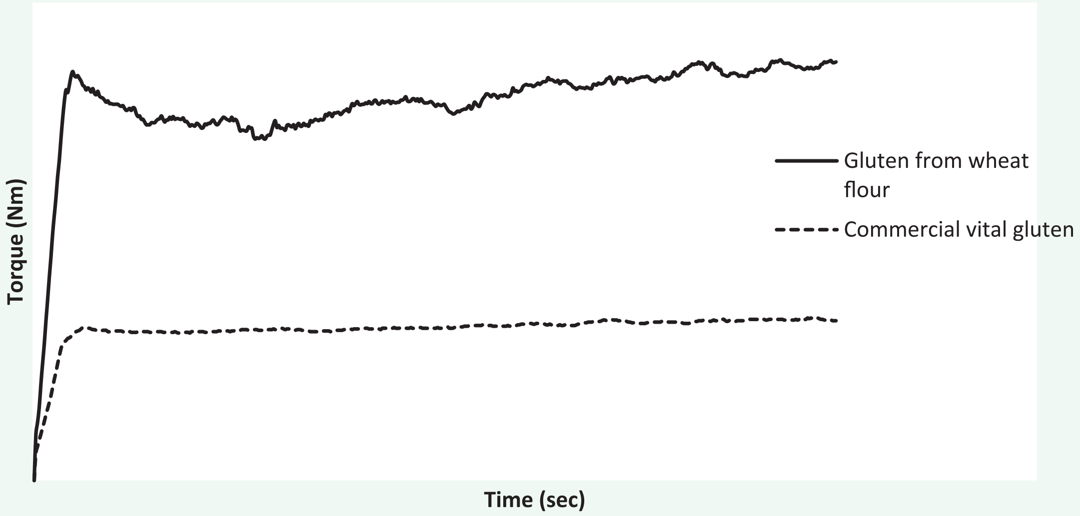

Figure 1 Mixolab curve showing the comparison of torque for gluten extracted from control wheat flour and vital gluten obtained commercially (Figure published in Sharma and Gujral [9]).

in WWF. Stability refers to the interval at which Mixolab curve persist within 11% of maximum consistency obtained through mixing period. WWF showed significantly lower stability (1.95 min) and it varied from 5.69-10.78 min for GSWBFs (Table 1). The higher stability of GSWBFs could be due to greater amount of β-glucan and arabinoxylans (Table 2) that formed a complex with added vital gluten and maintained the dough network [10]. This also implies that the GSWBFs were more resistant to mixing credited to the presence of fibrous compounds which was also evident from positive association of dough stability with total arabinoxylan (r= 0.40) and total β-glucan content (r= 0.71). Blandino et al. [11], and Izydorczyk et al. [12], highlighted the importance of fibrous components in amplifying the dough structure.

Protein weakening (%): The heating and mechanical shear led to destabilization of gluten matrix causing lowering of dough strength indicated by torque C2 [10]. Percent protein weakening (%) was found ranging between 30.04-42.29 % for GSWBFs and 27.92 % for WWF. The higher protein weakening in GSWBFs may be attributed to higher fiber content in barley flours that could compete with gluten protein for water, eventually producing internal turbulence and destabilization of protein matrix which could also be demonstrated by a positive correlation of protein weakening with the β-glucan content (r= 0.46). Another reason for higher protein weakening could be reduced vitality of the added vital gluten in GSWBFs as it has already undergone denaturation (as observed from Figure 1).

Starch gelatinization: Further heating resulted in swelling of starch granule that caused an increase in viscosity and rise in torque (C3) [9]. As heating proceeds, proteins have negligible effect and starch granules tend to have predominant role in increasing the torque. The C3 for WWF was 1.358 Nm whereas GSWBFs observed up to 32.34 % higher values indicating higher peak viscosity for GSWBFs. Significant positive correlations of C3 torque were noted with β-glucan (r= 0.91) and arabinoxylans (r= 0.91) content indicating the contribution of non starchy polysaccharides in increasing viscosity. Among GSWBFs, the highest torque at C3 was observed for Dolma and BHS 352 cultivar both of which possessed higher β-glucan and arabinoxylan content (Table 2). The speed of starch gelatinization (β) for the WWF was 0.256 (Table 1) whereas GSWBFs showed significantly higher values up to 0.57 indicating that the GSWBFs are being gelatinized at a higher speed than the WWF. A positive correlation (r= 0.50) between β (starch gelatinization speed) and β-glucan content was observed that indicated the positive role of fiber components in increasing the rate of gelatinization.

Starch paste breakdown: When dough was held at 90 °C for 7 min the torque continuously decreased till C4 was reached. The holding of flours at this temperature led to shear thinning and physical breakdown of hot starch granules causing a decrease in gel consistency. The percent breakdown (C3-C4) was calculated and was found to be 34.42 % in WWF while GSWBFs displayed values ranging between 7.53-67.83 % (Table 1). It was observed that all the GSWBFs showed more starch breakdown except Upasna, Geetanjali & Dolma indicating their greater gel stability. The reason for this higher breakdown could be the molecular interference caused by the non starchy polysaccharides and also the added vital gluten caused decreased resistance of the hot starch paste to shear thinning. Similar observation has been reported by Moza and Gujral [13]. Besides in WWF the native gluten protein may act as a protective layer around the gelatinized starch granules against shear thinning. Blandino et al. [11] reported that the replacement of refined wheat flour with the debranned inner kernel fraction of hulless barley led to significantly higher starch breakdown.

Starch retrogradation: During the cooling phase, an increase in torque was observed which might be due to the recrystallization of starch granules**.** Retrogradation that was calculated from the percent increase in torque from C4 to C5 was found to be 26.13% for WWF and 10.12%- 36.94% for GSWBFs, the least being demonstrated by Upasna and maximum by Sindhu cultivar (Table 1). The reason for higher retrogradation in GSWBFs could be that since vital gluten has been added, the quality of gluten is different and also mainly due to the different

Table 2: Non starchy polysaccharides (total β-glucan & total arabinoxylan) content, Water absorption, Shrinkage & Bake loss of WWF and GSWBF chapattis; Relative crystallinity of fresh and retrograded chapatti.

Cultivar	Total β-glucan content (%)	Total arabinoxylan content (%)	WA for chapatti making (%)	Shrinkage (%)	Bake loss (%)	Relative crystallinity (%)	
						Fresh	Retrograded*
HD 2967	0.60^{a}±0.05	0.81^{a}±0.08	80.0^{b}±0.71	2.54^{a}±1.20	22.35^{b}±1.85	8.2^{e}±0.35	22.4^{a}±0.09
Upasna	4.09^{b}±0.06	1.16^{a}±0.13	79.0^{a}±0.28	7.56^{c}±0.72	22.81^{b}±1.59	6.8^{c}±0.05	37.5^{i}±0.08
Geetanjali	4.18^{b}±0.041	2.05^{b}±0.13	80.0^{b}±0.71	5.29^{b}±0.49	19.96^{a}±0.73	9.4^{g}±0.06	36.0^{h}±0.07
Dolma	5.39^{c}±0.02	2.69^{b}±0.33	92.0^{e}±0.56	8.64^{c}±0.51	27.20^{c}±1.57	6.0^{a}±0.08	27.2^{c}±0.12
HBL 276	5.48^{c}±0.14	2.69^{b}±0.01	96.6^{g}±0.85	8.27^{c}±0.61	24.83^{b}±1.47	7.1^{d}±0.05	34.5^{g}±0.09
BHS 352	5.48^{c}±0.41	2.60^{b}±0.29	95.5^{f}±0.71	9.51^{c}±0.77	23.87^{b}±0.98	8.4^{f}±0.09	41.9^{j}±0.11
Sindhu	4.56^{b}±0.05	2.23^{b}±0.15	90.0^{d}±0.28	7.91^{c}±0.45	22.39^{b}±0.95	11.5^{i}±0.09	26.5^{b}±0.08
Nurboo	4.46^{b}±0.07	2.32^{b}±0.07	96.6^{g}±0.99	8.86^{c}±0.70	23.65^{b}±1.08	6.2^{b}±0.08	31.4^{e}±0.07
SBL 8	4.65^{b}±0.02	2.51^{b}±0.08	88.6^{c}±0.92	7.06^{c}±0.53	22.13^{b}±1.22	7.2^{d}±0.09	32.6^{f}±0.07
SBL 9	5.12^{c}±0.12	2.51^{b}±0.26	90.0^{d}±0.49	7.24^{c}±0.71	20.39^{a}±0.98	10.8^{h}±0.11	29.0^{d}±0.08

Values represent mean of three replicates.
Superscripts a-j are significantly ($p \leq 0.05$) different column wise in different cultivars and p, q superscripts are significantly ($p \leq 0.05$) different row wise within a cultivar.
WA is water absorption (subjective); HD 2967 is WWF (control).
Retrograded * - The relative crystallinity significantly increased after retrogradation for all chapatti samples.

Table 3: Soluble starch and soluble amylose content of raw flour & baked chapatti; Overall acceptability of fresh chapattis.

Cultivar	Soluble starch (g/100g)		Soluble amylose (g/100g)		Overall acceptability**
	Fresh	Retrograded*	Fresh	Retrograded*	
HD 2967	4.011^{g}±0.061	1.787^{g}±0.005	0.459^{g}±0.018	0.211^{e}±0.023	8.00^{a}±0.04
Upasna	0.865^{a}±0.092	0.094^{b}±0.007	0.090^{a}±0.005	0.017^{a}±0.002	7.88^{a}±0.25
Geetanjali	2.185^{f}±0.007	0.062^{a}±0.010	0.261^{e}±0.008	0.034^{b}±0.001	7.74^{a}±0.33
Dolma	1.990^{e}±0.001	0.745^{f}±0.007	0.285^{f}±0.007	0.115^{d}±0.007	7.25^{a}±0.09
HBL 276	1.947^{e}±0.005	0.060^{a}±0.002	0.265^{e}±0.004	0.022^{a}±0.001	7.00^{a}±0.44
BHS 352	1.225^{b}±0.021	0.096^{b}±0.024	0.183^{c}±0.011	0.049^{b}±0.002	6.00^{a}±0.37
Sindhu	1.570^{c}±0.113	0.162^{c}±0.060	0.209^{d}±0.004	0.037^{b}±0.003	7.85^{a}±0.08
Nurboo	2.113^{f}±0.108	0.469^{e}±0.118	0.258^{e}±0.009	0.070^{c}±0.005	7.00^{a}±0.35
SBL 8	1.734^{d}±0.014	0.442^{e}±0.005	0.219^{d}±0.002	0.073^{c}±0.007	8.45^{a}±0.06
SBL 9	1.205^{b}±0.007	0.330^{d}±0.028	0.142^{b}±0.008	0.043^{b}±0.008	7.00^{a}±0.49

Values represent mean of three replicates.
Superscripts a-g are significantly ($p \leq 0.05$) different column wise in different cultivars and p, q superscripts are significantly ($p \leq 0.05$) different row wise within a cultivar.
HD 2967 is WWF (control).
Retrograded*- The soluble starch and soluble amylose of chapatti decreased significantly after retrogradation.
Overall acceptability** (n=25).

nature of starch. Greater starch retrogradation has been reported earlier in pearling fractions of hulless barley [11].

Chapatti making behavior

Water absorption of dough for chapatti making*:* The water absorption (subjective, Table 2) for WWF dough was 80% whereas water absorption ranging from 79.0% to 96.6% was demonstrated by the GSWBFs (Table 2). Upasna and Geetanjali cultivars had lowest amount of non starchy polysaccharides (Table 2) which could be a reason for their less water absorption among GSWBFs. The highest content of total β-glucan in HBL-276 could be a probable reason for its higher water absorption. Water absorption correlated positively with arabinoxylan (r= 0.81) and β-glucan (r= 0.66) content displaying a linear association between the two parameters which was probably attributed to the hydrophilic hydroxyl groups of the non starchy polysaccharides that compete for water content. Both the barley flour and β-glucan significantly increased the water absorption for dough making and β-glucan and arabinoxylan have higher affinity to bind water which leads to increase in water absorption [14]. A greater increase in water absorption on replacement of wheat with a barley fraction containing higher levels of β-glucan has been reported [15].

Shrinkage & Bake loss*:* The shrinkage in chapatti is known to be caused due to contraction of gluten on baking. GSWBF chapattis displayed significantly ($p<0.05$) different shrinkage

Table 4: RDS (Rapidly digestible starch), SDS (Slowly digestible starch), RS (Resistant starch) and GI (Glycemic index) of fresh and retrograded chapatti.

Cultivar	**SDS (g/100g)**		**RDS (g/100g)**		**RS (g/100g)**		**GI**	
	Fresh	Retrograded	Fresh	Retrograded	Fresh	Retrograded	Fresh	Retrograded
HD 2967	33.61^{b}±0.83	35.95^{b*}±1.22	34.04^{d}±0.93	31.04^{d*}±1.06	0.30^{a}±0.01	0.96^{a*}±0.47	74.65^{d}±0.79	71.78^{e*}±0.39
Upasna	39.73^{d}±0.83	40.92^{c}±0.93	24.89^{a}±1.25	22.99^{b*}±1.07	0.18^{a}±0.16	0.89^{a}±0.93	63.91^{a}±0.94	61.84^{a*}±0.88
Geetanjali	30.92^{a}±0.73	31.54^{a}±0.91	28.35^{b}±0.74	27.53^{c}±1.84	1.03^{a}±0.07	1.20^{a}±0.06	66.13^{b}±0.69	63.46^{b*}±0.82
Dolma	35.11^{c}±1.00	37.81^{b*}±1.21	27.88^{b}±0.91	24.02^{b*}±0.65	0.30^{a}±0.17	1.46^{a*}±0.50	66.44^{b}±0.73	61.09^{a*}±0.87
HBL 276	38.68^{d}±1.98	41.02^{c}±0.74	26.41^{b}±1.26	24.04^{b*}±1.08	0.12^{a}±0.14	0.15^{a}±0.17	66.02^{b}±0.90	65.43^{c}±0.48
BHS 352	42.27^{e}±0.91	44.11^{d}±1.41	23.75^{a}±0.71	20.89^{a*}±0.64	1.29^{a}±0.28	2.31^{b*}±0.04	64.11^{a}±0.64	63.62^{b}±0.98
Sindhu	33.20^{b}±1.00	35.62^{b*}±1.26	24.38^{a}±1.25	21.29^{a*}±1.41	0.74^{a}±0.98	1.41^{a}±1.10	62.99^{a}±1.57	60.57^{a}±0.82
Nurboo	35.61^{c}±1.26	36.15^{b}±1.20	23.93^{a}±1.25	23.02^{b}±0.91	1.01^{a}±0.01	1.38^{a}±0.34	62.04^{a}±0.55	60.5^{a*}±0.47
SBL 8	30.72^{a}±1.84	32.04^{a}±1.41	26.56^{b}±1.70	25.38^{b*}±1.84	0.62^{a}±0.78	0.67^{a}±0.39	62.96^{a}±1.08	61.34^{a*}±0.93
SBL 9	36.50^{c}±0.60	37.44^{b}±1.09	30.09^{c}±0.91	28.33^{c*}±1.09	0.22^{a}±0.26	1.04^{a}±0.08	70.41^{c}±0.54	67.58^{d*}±0.51

Values represent mean of three replicates.
Superscripts a-d are significantly ($p \leq 0.05$) different column wise in different cultivars and p, q superscripts are significantly ($p \leq 0.05$) different row wise within a cultivar. HD 2967 is WWF (control).
* The values showed a significant difference after retrogradation.

values ranging between 5.29% to 9.51% while lower percent shrinkage (2.54%) was displayed by WWF chapatti (Table 2). A significant increase in shrinkage of chapatti on adding barley flour to wheat flour has been reported [14]. The percent shrinkage correlated positively with total β-glucan (r= 0.89) and total arabinoxylan content (r= 0.73) indicating the intermolecular interference produced due to these polysaccharides in the protein-starch matrix of the barley flours [14,15]. The fiber components like arabinoxylans and β-glucan might affect the dough characteristics by interfering the gluten formation process in dough. Also in GSWBFs the added vital gluten is not bound to the starch matrix the way the native gluten and starch are bound in WWF, yet another reason for higher shrinkage. Moreover, more shrinkage could also be attributed to the higher protein weakening observed in GSWBFs; which was also supported by a weak positive correlation (r= 0.42) between the two. Bake loss gives an indication of the quantity of water lost from chapatti upon baking and it was found to be 22.35 % for WWF chapatti whereas GSWBF chapattis showed a bake loss ranging from 19.96-27.2 %. Sharma and Gujral [14] reported similar findings. Higher the moisture in baked goods, lower the rate of staling [4]. The higher bake loss could also be related to higher hydration capacity (water absorption) of GSWBFs.

Sensory characteristics of chapatti*:* The quality characteristics of a good chapatti are softness, pliability, folding of chapatti in spoon shape and easy to tear, without being excessively brittle or leathery [16]. However, slight chewiness in mouth feel is desirable. When compared to traditional WWF chapattis, GSWBF chapattis received lower sensory scores and the difference was statistically insignificant (p<0.05). The barley flours have non-wheatish taste, color and typical barley flavor for which phenolic acids and proanthocyanidins are responsible that impart bitter and astringent taste [17]. The scores for color of chapattis prepared from GSWBFs decreased due to lighter color of the chapattis. The incorporation of barley flour to wheat flour at levels of 84 g barley flour/100 g wheat flour decreased the sensory score drastically and chapattis were unacceptable due to irregular shape of chapatti, dilution of gluten, gummy mouth feel, typical barley characteristic flavor/aroma and lighter color [14]. The chapattis prepared from Geetanjali cultivar received higher scores for texture which could be due to lower bake loss that could have resulted in more water retention thus making the chapattis softer in texture. Lower bake loss led to retention of higher moisture content and hence resulted in softer and pliable chapattis [9]. Among all the GSWBFs, chapattis prepared from SBL-8 were found to have greater acceptability due to its good taste. Knuckles et al. [18] reported that 20% incorporation of barley flour in wheat flour produced bread with acceptable sensory attributes however increasing the level of barley flour up to 40% decreased the sensory quality of bread.

Retrogradation analysis

Soluble starch & soluble amylose analysis*:* Starchy foods undergo retrogradation as a result of the recrystallization of gelatinized/cooked starch molecules and the process is affected by factors like moisture, amylose content, time of storage, and presence of non-starch components [19]. The soluble starch content varied significantly (p<0.05) for WWF and GSWBF chapattis (Table 3). WWF fresh chapatti showed soluble starch content of 4.011g/100g while it ranged between 0.86-2.18g/100g for GSWBF chapattis (fresh) (Table 3). The percent decrease in soluble starch content of control WWF chapattis after 48 hours storage at room temperature was 55.43% whereas this decrease ranged from 62.56-97.16% for chapattis prepared from GSWBFs indicating more retrogradation in GSWBFs. Less decrease in soluble starch on storage indicated higher retrogradation as their tendency to solubilize retards due to recrystallization of the starch molecules. Significant negative correlations of soluble starch contents were observed with the β-glucan (r= -0.81) and arabinoxylan (r= -0.5), indicating an inhibitory effect of non starchy polysaccharides on starch solubility. Moza and Gujral [13] also reported that non starchy polysaccharides compete with starch molecules for water uptake restricting their solubilization and swelling ultimately leading to inhibition of starch retrogradation. The decline in soluble amylose upon storage was due to aggregation of the linear amylose fraction into insoluble complexes. Upon storage for 48 hr decrease in content of soluble amylose was 54.08% for WWF chapatti whilst for GSWBF chapattis, the decrease was 59.64-91.58% (Table 3). The decrease in soluble amylose could be due to starch retrogradation that lowers the tendency of amylose molecules to leach out [20]. The decline in leached amylose content might be responsible for the inhibition of short-term retrogradation of starch. Interestingly, β-glucan correlated positively with percent decrease in soluble amylose (r= 0.46) and soluble starch (r= 0.51) content (more the content of soluble amylose in retrograded chapatti, lesser is the rate of staling) which portrays a significant role of non starchy polysaccharides in modulating the retrogradation behavior of chapatti. Also, in WWF, the gluten is present in bound form with starch hence less leaching of soluble starch occurred whereas in WBF the vital gluten is separately added and hence the barley starch is free leading to more leaching of soluble starch on storage. Shaikh et al. [7] reported that on storage of wheat flour chapattis for 2 days the decrease in content of soluble starch was 61.7%. Lesser the decrease in soluble starch and amylose content upon storage lower the rate of retrogradation which ultimately improves baking quality and restrict staling of chapattis [20].

Relative crystallinity (%): Relative crystallinity provides an indication of the crystalline nature of starch due to ordered arrays of double helices formed by the amylopectin side chains [21]. The relative crystallinity of fresh WWF chapatti was 8.2% and for fresh chapattis prepared from GSWBFs it ranged between 6-11.5% (Table 2). A lower degree of crystallinity was displayed by WWF and GSWBF chapatti with only one peak at 20° which is an indicative of V type polymorphism. Complexation of amylose with flour components at an early stage (in fresh chapatti) indicates an early amylose retrogradation. The amylose molecules tend to retrograde faster as compared to amylopectin molecules [22]. After 48 h storage, additional peaks at 15° and 17° were observed representing the presence of A-type and B-type crystallinity in retrograded chapatti. A significant increase in relative crystallinity was observed on storage for both the WWF and GSWBF chapattis that ranged between 22.4% and 26.5-41.9%, respectively which could be due to reassociation of amylose and amylopectin fractions on retrogradation as B-type crystallinity (ie. peaks at 17 and 23° 2θ) is usually a characteristic of retrograded starches. Overall, GSWBF chapattis had less increase in their crystallinity values than WWF chapattis indicating lower retrogradation. Positive association (r= 0.53) between non starchy polysaccharides and relative crystallinity could be observed indicating the positive effect of these in preventing retrogradataion. Sharma and Gujral [14] highlighted the anti-staling properties of b-glucans on addition into wheat chapatti. Primo-Martin et al. [23] reported that the relative crystallinity of the bread crust of crispy roll significantly increased during storage from 36% to 52% and form 41% to 68% for rusk rolls. Also, crystallinity of starch in the crumb after storage increased from 0 to 34% and 26% for rusk rolls and crispy rolls, respectively. The relative crystallinity of retrograded chapatti correlated positively with percent decrease in soluble starch (r= 0.75) indicating a linear relationship between increase in crystallinity on storage and retrogradation.

In-vitro starch digestibility and predicted glycemic index

The fresh WWF chapatti (fresh) had 34.04g/100g flour RDS content while chapattis prepared from GSWBFs showed values ranging between 23.75-30.09 g/100 g flour (Table 4), the lowest being shown by BHS 352 followed by Nurboo. It may be noted that this cultivar had higher content of β-glucan (Table 2) thus showed lower RDS values. Collar and Angioloni [24] reported that bread prepared by replacing 40% wheat flour with barley flour had lower RDS which was attributed to the reduced starch hydrolysis due to higher levels of β-glucan. RDS content decreased significantly ($p<0.05$) on storage. The RDS content of fresh chapatti positively correlated with soluble amylose content signifying a direct relationship between solubility (retrogradation) and digestibility of starch. Retrogradation enhanced resistance to digestive enzymes consequently lowering digestibility of starch. The reduction in RDS content after 48 hr storage was 8.81% for WWF chapattis and 2.91-13.84% for WBF vital gluten chapattis. The barley flours exhibited the property of increasing viscosity in the gut due to non starchy polysaccharides [25] which could also be a reason for the lowering of digestibility. Moreover, β-glucan and arabinoxylan exhibited a significant negative (r= -0.67) correlation with RDS of fresh chapatti indicating that greater levels of non starchy polysaccharides may show starch digestibility lowering effects [13].

The quantity of glucose released during 20 and 120 min of starch digestion (in-vitro) is slowly digestible starch (SDS) and it was found to be 33.61g/100g flour for WWF chapattis (fresh) and ranged from 30.72-42.27g/100g flour for GSWBFs chapatti. As per Thondre et al. [25], lowering of RDS and increase in SDS contents of chapatti on mixing β-glucan at 4 and 8% levels was reported. It was observed that SDS was more in BHS 352 that contained highest levels of non starchy polysaccharides (Table 2). The viscosity in gut is increased by β-glucan and this slows down the rate of starch digestion [13]. After 48 hr storage, SDS level improved by 6.51% for WWF chapattis and up to 7.14% for chapattis prepared from GSWBFs. The creation of aligned & ordered structures such as double helices of amylose and amylopectin crystallites as a result of retrogradation increases the resistance of starch to enzymatic hydrolysis. The rise in SDS of chapatti on storage could be a result of presence of partially intact starch granules and production of resistant starch which are in turn affected by the type and extent of processing conditions (baking time and temperature) and botanical source of starch. Also, total β-glucan content of refined flours correlated positively with SDS content of retrograded chapatti (r= 0.61, $p<0.05$) representing that presence of β-glucans may promote formation of slowly digestible starch upon retrogradation. Moza and Gujral [1] and Moza and Gujral [13] reported significant positive correlations of β-glucans and arabinoxylan with slowly digestible starch contents of flours.

Resistant starch is not absorbed in the small intestine of healthy individuals but is fermented in the large intestine and is important for human health to maintain the level of blood glucose. RS for fresh WWF chapatti was 0.3g/100g flour whereas for GSWBF chapattis it ranged from 0.12-1.29g/100g flour. The highest RS was observed for BHS 352 chapattis. On storage for 48 hours RS increased which was 68.75% for WWF chapatti and 14.17-79.78% for GSWBF chapattis. The retrogradation process of starch may affect the bioavailability of starch in gastro-intestinal tract, and recrystallized starch constitutes a starch fraction which was delivered to the large bowel. After storage, starch granules were less prone to enzymatic hydrolysis and therefore displayed less RDS but higher SDS and RS.

Glycemic Index (GI) was also predicted and a range of 62.04–70.41 was observed for chapattis (fresh) prepared from GSWBFs whereas the WWF chapattis showed a pGI of 74.65 (Table 4). Higher pGI was displayed by WWF and it was lower for all of the GSWBF chapattis; the lowest being observed for Nurboo cultivar. Lower GI could be a result of higher non starchy polysaccharides which was evident from a negative correlation between GI of fresh chapatti and total β-glucan ($r = -0.64$). RDS of fresh chapatti correlated positively with the GI of fresh ($r=0.94$) and retrograded chapatti ($r=0.83$). A positive correlation of RDS with the GI has been suggested earlier [9-13].

Correlating retrogradation by soluble starch/amylose, RDS, Mixolab & relative crystallinity

It was observed that the GSWBF chapattis exhibited higher retrogradation as compared to WWF chapatti. The percent decrease in soluble starch ($r= 0.75$, $p<0.05$) and soluble amylose ($r= 0.57$, $p<0.05$) correlated positively with relative crystallinity of retrograded chapatti indicating a significant relationship between the two parameters. Also, a negative correlation ($r= -0.54$, $p<0.05$) was observed between relative crystallinity of retrograded chapatti and RDS of retrograded chapatti indicating that the increased retrogradation leads to decrease in digestibility of starch. The torque during retrogradation phase (C5) correlated positively ($r= 0.50$, $p<0.05$) with the relative crystallinity of retrograded chapatti indicating that the mixolab retrogradation correlated significantly with the retrogradation obtained from the relative crystallinity data. The rapidly digestible starch of retrograded chapatti showed a negative correlation with the percent decrease in soluble starch ($r= -0.542$, $p<0.05$) and soluble amylose ($r= -0.43$, $p<0.05$) indicating lower susceptibility of enzymes to digest starch on retrogradation. It is possible that the addition of vital gluten can influence the extent of retrogradation of starch. Otherwise it was expected that the higher levels of β-glucan and arabinoxylan would have antistaling effects [14] however vital gluten changed this behavior.

CONCLUSION

The results of the present study reveal that addition of vital gluten to WBF resulted in higher water absorption because of the tendency of the non starchy polysaccharides and vital gluten to bind water. The chapatti parameters like shrinkage and bake loss altered significantly due to addition of vital gluten. Mixolab data showed higher protein weakening, viscosity and retrogradation in vital gluten incorporated blends (GSWBFs). Higher staling was observed in GSWBFs as evident from the soluble starch/amylose and crystallinity data. Chapattis prepared from whole barley flour vital gluten blends demonstrated higher levels of SDS, lower RDS and GI thus, making it suitable for diabetic patients and promoting various health benefits. Since chapattis form an essential component of the daily Indian diet therefore barley flour can be promoted as a functional food for chapatti making by incorporating vital gluten to provide the viscoelasticity required for dough formation as well as sheeting of chapattis and it would serve as an ideal medium for improving the nutritional quality of Indian diets.

Compliance with ethical standards

Conflict of interest: The authors hold no conflicts of interest regarding the work presented herein and foresee no conflicts in future associated with the publication of this work.

Compliance with ethics requirements: This article contains studies with human subjects as a part of sensory evaluation of the chapatti.

REFERENCES

1. Moza J, Gujral HS. Starch digestibility and bioactivity of high altitude hulless barley. Food Chem. 2016; 194: 561-568.
2. MS Izydorczyk, JE Dexter. Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products–a Review. Food Res. 2008; 41: 850-868.
3. Ang K, Bourgy C, Fenton H, Regina A, Newberry M, Diepeveen D, et al. Noodles Made from High Amylose Wheat Flour Attenuate Postprandial Glycaemia in Healthy Adults. Nutrients. 2020; 12: 2171.
4. Gujral HS, Gaur S. Instrumental texture of chapati as affected by barley flour, glycerol monostearate and sodium chloride. Int J Food Prop. 2005; 8: 1-9.
5. Wang F, Huang W, Kim Y, Liu R, Tilley M. Effects of transglutaminase on the rheological and noodle-making characteristics of oat dough containing vital wheat gluten or egg albumin. J Cereal Sci. 2011; 54: 53-59.
6. Li J, Hou GG, Chen Z, Gehring K. Effects of endoxylanases, vital wheat gluten, and gum Arabic on the rheological properties, water mobility, and baking quality of whole-wheat saltine cracker dough. J Cereal Sci. 2013; 58: 437-445.
7. Shaikh IM, Ghodke Sk, Ananthanarayan L. Inhibition of staling in chapati (Indian unleavened flat bread). J Food Process Preserv. 2008; 32: 378-403.
8. Douglas SG. A rapid method for the determination of pentosans in wheat flour. Food Chem. 1981; 7: 139-145.
9. Sharma B, Gujral HS, Solah V. Effect of incorporating finger millet in wheat flour on mixolab behavior, chapatti quality and starch digestibility. Food Chem. 2017; 231: 156–164.
10. Sharma B, Gujral HS. Characterization of thermo-mechanical behavior of dough and starch digestibility profile of minor millet flat breads. J Cereal Sci. 2019; 90: 102842.
11. Blandino M, Locatellib M, Gazzolaa A, Coisson JD, Giacosaa S, Travagliab F, et al. Hull-less barley pearling fractions: Nutritional properties and their effect on the functional and technological quality in bread-making. J Cereal Sci. 2015; 65: 48-56.
12. Izydorczyk MS, Hussain A, MacGregor AW. Effect of Barley and Barley Components on Rheological Properties of Wheat Dough. J Cereal Sci. 2001; 34: 251-260.
13. Moza J, Gujral HS. Mixolab, retrogradation and digestibility behavior

of chapatti made from hulless barley flours. J Cereal Sci. 2018; 79: 383-389.

14. Sharma P, Gujral HS. Anti-staling effects of β-glucan and barley flour in wheat flour chapatti. Food Chem. 2014; 145: 102-108.
15. Skendi A, Biliaderis Cg, Papageorgiou M, Izydorczyk MS. Effects of two barley β-glucan isolates on wheat flour dough and bread properties. Food Chem. 2010; 119: 1159–1167.
16. Banerji A, Ananthanarayan L, Lele SS. Dough browning inhibition of multigrain Indian flatbread (chapatti) using a combination of chemical and microwave treatment. J Food Meas Charact. 2019; 13: 807-820.
17. Holtekjolen AK, Kinitz C, Knutsen SH. Flavanol and Bound Phenolic Acid Contents in Different Barley Varieties. J Agric Food Chem. 2006; 54: 2253-2260.
18. Knuckles BE, Hudson CA, Chiu MM, Sayre RN. Effect of beta-glucan barley fractions in high-fiber bread and pasta. Cereal Foods World. 1997; 42: 94-99.
19. Chung HJ, Lim HS, Lim ST. Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch. J Cereal Sci. 2006; 43: 353-359.
20. Karim AA, Norziah MH, Seow CC. Methods for the Study of Starch Retrogradation. Food Chem. 2000; 71: 9-36.
21. Perez S, Bertoft E. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. Starch/Starke. 2010; 62: 389-420.
22. Indrani D, Rao SJ, Sankar KU, Rao GV. Changes in the physical-chemical and organoleptic characteristics of parotta during storage. Food Res Int. 2000; 33: 323-329.
23. Primo-Martina C, Van Nieuwenhuijzena NH, Hamera RJ, Van Vlieta T. Crystallinity changes in wheat starch during the bread-making process: Starch crystallinity in the bread crust. J Cereal Sci. 2007; 45: 219-226.
24. Collar C, Angioloni A. Nutritional and functional performance of high β-glucan barley flours in breadmaking: mixed breads versus wheat breads. Eur Food Res Technol. 2014; 238: 459-469.
25. Thondre PS, Monro J, Mishra S, Henry J. High molecular weight barley β-glucan decreases particle breakdown in chapattis (Indian flat breads) during in vitro digestion. Food Res Int. 2010; 43: 1476-1481.