

## Effect of Beta-Glucan and Resistant Starch on Prebiotic Dough and Bread Properties

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### Abstract

White wheat flour is a poor source of dietary fiber. A demand therefore exists for the enrichment of bread with prebiotic non-digestible ingredients that exert a health-promoting effect. In this study, the effect of beta-glucan (BG) and resistant starch (RS) on the dough properties and bread-making characteristics has been investigated. The water absorption of doughs increased with increasing BG and RS amounts. The development time and farinograph quality number of BG-enriched doughs remained similar to the control while the stability of doughs decreased, and all of these values decreased when the RS was added. BG was more effective in increasing the softening of doughs than RS. The addition of RS or BG increased; the resistance to deformation, energy, maximum resistance and ratio number values, but their extensibility values had decreased compared to the control. Formulation containing BG/RS combination showed the best farinograph (development time, stability) and extensograph (resistance and extensibility) parameters. The application of BG and RS had similar or slightly smaller effect on specific volume and same or higher effect on the moisture content while it caused a decrease in firmness after five days of storage.

**Keywords:** Prebiotic; Bread quality; Beta glucan; Resistant starch; Dough rheology

### Introduction

White bread is a staple food in the human diet in many countries and a popular and convenient cereal product; it is also a poor source of dietary fiber, containing typically less than 2.5% fiber content. In fact, white wheat bread is commonly used as a high glycemic index reference in glycemic response [1]. A demand therefore exists for the development of bread with substances that are non-digestible polysaccharides or partially resistant to the digestive process.

Functional foods either contain (or add) a component with a positive health effect or eliminate a component with a negative one, as they must be safe, healthy and tasty [2]. Addition of healthy components such as prebiotics to food products is a common approach to development of these kinds of foods. According to the definition by [3], prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or more bacteria (probiotics) in the gastrointestinal tract and thus improve host health.

Oat BG is a soluble dietary fiber that mainly consists of the unbranched polysaccharides composed of (1→3), (1→4) linked β-D-glucopyranosyl units. BGs are main components of starchy endosperm and aleurone cell walls of commercially cereals such as oat, barley and wheat grains. The cereals consistency ranges of BG are from 1% in wheat grains, to 3–7% in oats, and 5–11% in barley [4]. Barley is the richest source for BG but has not been used in bakery products because it lacks gluten proteins and the end-products have poor sensory characteristic. Cheickna have demonstrated that BG-enriched wheat flour can produce dough with acceptable properties. BG has several physiological effects including the regulation of blood sugar and the lowering of the glycemic response, therefore assisting in the control of diabetes, the reduction of serum cholesterol levels and the prevention of cancer [5].

Starch is classified into three general types based on its rate of digestion: rapidly digestible (RDS), slowly digestible (SDS) and

RS. RS is resistant to digestion in the stomach and small intestine, and is a natural component that is present in many foods, having some nutritional benefits. When it reaches the large intestine, RS is fermented by large bowel microbiota, which increases the production of short-chain fatty acids (SCFAs) and fecal volume but reduces the pH of feces, lowering blood glucose levels and therefore assisting in the control of diabetes and plasma cholesterol levels, and therefore positively influences the function of the digestive tract and microbiota flora [6]. Many studies have shown that RS is a linear polysaccharide of (1→4) α-D-glucan, essentially derived from the retrograded amylose fraction, and has a relatively low molecular weight ( $1.2 \times 10^5$  Da) [7]. Therefore, RS levels are slightly increased by certain food processing methods, such as retorting, baking or drying at high temperatures.

### Prebiotic dosage of BG and RS

In fact, the U.S. Food and Drug Administration has claimed that the consumption of at least 3 g of BG per day, or 4 servings of a food product that supplies at least 0.75 g of BG per serving, has cholesterol lowering properties, with an effect on reducing the risk of heart disease [8]. Although the minimum healthy dose of RS is about 20 g/day [9], a low dosage in the range of 2.5 to 5 g/day has showed prebiotic effects as well [10]. The research has indicated that the amount of RS and BG used in flour fortification depends on the particular starch being used,

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extraction procedures, the application, factors such as dosage and molecular weight.

To our knowledge, no systematic studies have yet been performed on the effect of the supplementation of wheat flour with purified BG and RS in prebiotic dosage on dough rheology and bread characteristics. Therefore the present investigation was undertaken to study the dough rheological and bread-making properties when supplemented with BG and RS in prebiotic dosages, in order to improve the nutritional quality of bread.

## Materials and Methods

### Materials

White wheat flour, salt and yeast were purchased from local super markets. RS (high amylose corn starch) was obtained from Hi-maize 260, National starch, USA. BG was supplied by promOat™, Biovelop international AB Company, Sweden.

### Methods

**Flour quality assessment:** All the quality tests for white wheat flour were performed in accordance with established official procedures used to characterize wheat flour properties: protein, moisture, ash, wet gluten, gluten index, sedimentation value and falling number were determined by using standard AACC methods [11].

**Farinograph tests:** Separate doughs were fortified with BGs at levels of 0.8%, 1%, 1.2% w/w, RS at levels of 5.5%, 8%, 10.5% w/w, and one sample with the combination of 0.5% BG and 4% RS (w/w) was prepared. All of the samples were calculated on a flour dry weight basis and were tested according to the AACC Method 2000 (method 54-21). The BG or the RS in a dry powder form were first blended well with the wheat flour into the mixing bowl (300 g) of the farinograph (Brabender, Duisburg, Germany) that was connected with a circulating water pump and a thermostat which operated at  $30 \pm 0.2^\circ\text{C}$ . Farinograph water absorption, dough development time, dough stability, softening degree and farinograph quality number were then determined.

**Extensograph tests:** The control (unfortified), the BG, and the RS-enriched dough, as well as the BG and RS enriched dough, at first were each prepared in the 300 g mixing bowl of the farinograph (Brabender, Duisburg, Germany). The wheat flour was first blended well with the BG and/or RS at each concentration level previously detailed. Salt and water were then added to produce the dough samples with a consistency of 500 BU (Brabender Units), followed by 5 minutes of mixing. A test piece (150 g) was shaped into a ball, shaped into a cylinder and clamped into the fermenting cabinet. After 45, 90, and 135 minute reaction times in the fermenting cabinet at  $30\text{-}32^\circ\text{C}$ , each dough piece was stretched in the Brabender extensograph by a hook until rupture, as described in the AACC Method 2000 (method 54-10). The stretching force was thus recorded as a function of time, and the resistance to constant deformation (resistance to extension), the extensibility and energy in 45, 90, 135 minutes. Only the maximum resistance at 135 minutes and ratio number values were discussed.

**Bread-making process:** A uniform dough bread-making process was employed. The basic (control dough) formula on 100 g flour (at 14% moisture basis) consisted of salt (2 g), compressed yeast (2 g) and the amount of water required reaching 500 BU of consistency by the farinograph. Wheat flour was blended well with each of BGs at levels of 0.8%, 1%, 1.2% w/w (wheat flour basis), RS at levels of 5.5%, 8%, 10.5% w/w, and one sample with the combination of 0.5% BG and 4% RS (w/w). Bread doughs were prepared by mixing all ingredients and

fermenting in two-steps. After the first step, the fermented doughs were divided into four 500 g pieces, hand-moulded and put into tin pans for 45 minutes of proofing at  $30^\circ\text{C}$  and then baked at  $230^\circ\text{C}$  for 25 minutes. Following baking, the bread loaves were cooled at ambient conditions (for 2 hours). Subsequently, for the aging effect on some quality parameters, breads were packed in sealed plastic bags at room temperature for 1, 3 or 5 days.

**Specific volume:** Weight and volume were measured two hours after removal of bread loaves from the oven. The loaf volume was determined by the rapeseed displacement method and specific volume was calculated by dividing the volume by loaf weight ( $\text{cm}^3/\text{g}$ ).

**Moisture content determination:** The moisture content of breads stored for 1, 3 and 5 days (at  $25^\circ\text{C}$ ) in plastic bags was determined according to the AACC method 44-16 (2000).

**Bread firmness:** Breads were stored at room temperature in plastic bags in order to determine the bread staling at 1, 3, and 5 days. This was performed with the Instron M350-10CT (500 N load cell, Rochdale, England) texture analyzer, using a probe of 36 mm diameter, according to the AACC method 74-09 (2000). Compression tests were recorded on two slices from the center of each loaf, with an average thickness of 25 mm, cut with a knife. Samples were compressed to 40% at 100 mm/min speed. Measurements from 3 bread loaves were taken for each formulation and sampling time.

**Sensory analysis:** Breads were subjected to sensory evaluation by a panel of eight trained individuals (ranging in age from 25 to 35, non-smokers) at room temperature. The parameters were evaluated using the scoring system recommended by [12]. The maximum scores for each parameter were: chewiness 15, crust 15, texture 15, color of crumb 10, appearance 10, aroma 15 and taste/ flavor 20.

**Statistical analysis:** All experiments were carried out in three replicates. Data were shown as mean  $\pm$  SD or Median (Minimum to Maximum). Differences among means were identified by Analysis of Variance, followed by the Duncan's multiple range test and considering significant  $P$  values  $< 0.05$ . Kruskal Wallis and post hoc tests were used to analyze the sensory data. These calculations were performed by the SPSS version 13 (SPSS INC, IL, Chicago, USA) at 0.05 significance level.

## Results and Discussion

The physicochemical properties of wheat flour were: 10.43% protein, 0.629% ash, 13.15% moisture, 24.90% wet gluten, 72 gluten index, 22 (ml) sedimentation value and 557 (sec) falling number.

### Farinograph measurements

The water absorption results of prebiotics dough were compared with the control dough (without prebiotics addition) in Figure 1. Both BG and RS increased the water absorption capacity but BG produced a greater increase in the water absorption value than RS. As seen in Figure 1 all existing treatments, result in a significant increase ( $P < 0.05$ ) in water absorption compared to control samples and this increasing is enhanced by increasing the percentage of the substance which has been replaced. RS increased moisture content of dough because of high amylose content of RS in which amylose has higher binding capacity than native starch [13]. Also, increased water absorption has been reported in other studies where BG from different sources was used to fortify wheat flours [14].

According to the findings of [15,16], different hydrocolloids and dietary fiber increased water absorption due to the great number of

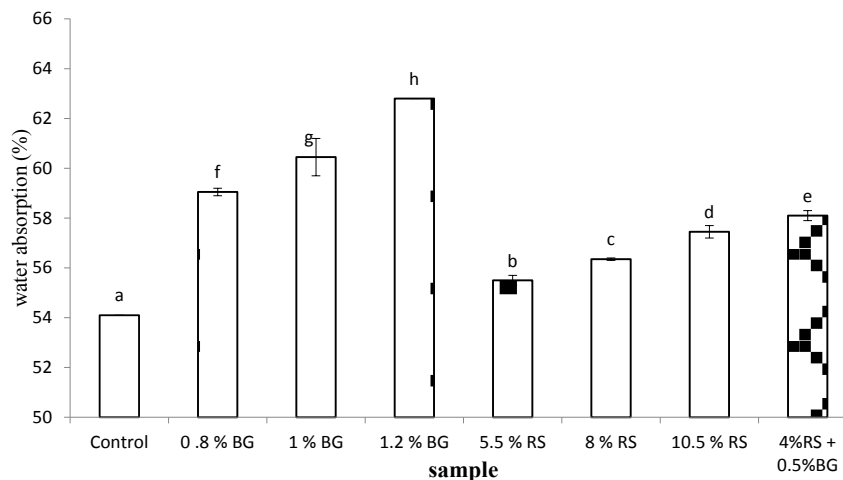


Figure 1: Comparison of water absorption values in dough supplemented with prebiotic components.

sample	Water absorption (%)	Development time (min)	Stability (min)	Degree of softening (BU)	Farinograph Quality Number
Control	54.10 ± 0.00 <sup>A</sup>	1.95 ± 0.25 <sup>C</sup>	4.65 ± 0.15 <sup>E</sup>	97.50 ± 0.50 <sup>A</sup>	49.00 ± 1.00 <sup>C</sup>
0.8 % BG	59.05 ± 0.15 <sup>F</sup>	1.60 ± 0.20 <sup>ABC</sup>	2.95 ± 0.15 <sup>C</sup>	122.00 ± 2.00 <sup>C</sup>	38.50 ± 1.50 <sup>BC</sup>
1 % BG	60.45 ± 0.75 <sup>G</sup>	1.55 ± 0.35 <sup>AB</sup>	2.20 ± 0.90 <sup>AB</sup>	133.00 ± 15.00 <sup>D</sup>	31.00 ± 16.00 <sup>AB</sup>
1.2 % BG	62.80 ± 0.00 <sup>H</sup>	1.75 ± 0.05 <sup>BC</sup>	2.70 ± 0.20 <sup>BC</sup>	125.00 ± 1.00 <sup>CD</sup>	43.50 ± 2.50 <sup>C</sup>
5.5 % RS	55.50 ± 0.20 <sup>B</sup>	1.50 ± 0.00 <sup>AB</sup>	3.25 ± 0.15 <sup>CD</sup>	105.00 ± 4.00 <sup>AB</sup>	27.50 ± 0.50 <sup>A</sup>
8 % RS	56.35 ± 0.05 <sup>C</sup>	1.60 ± 0.10 <sup>ABC</sup>	3.10 ± 0.40 <sup>C</sup>	103.50 ± 2.50 <sup>AB</sup>	28.00 ± 2.00 <sup>A</sup>
10.5 % RS	57.45 ± 0.25 <sup>D</sup>	1.30 ± 0.10 <sup>A</sup>	2.00 ± 0.20 <sup>A</sup>	111.00 ± 0.00 <sup>B</sup>	23.50 ± 1.50 <sup>A</sup>
4%RS+0.5%BG	58.10 ± 0.20 <sup>E</sup>	1.70 ± 0.20 <sup>BC</sup>	3.80 ± 0.10 <sup>D</sup>	102.50 ± 3.50 <sup>AB</sup>	46.50 ± 3.50 <sup>C</sup>

Presented data are mean value of three replication ± standard Deviation. Means in columns followed by a different letter are significantly different (P<0.05).

Table 1: Farinograph characteristics of dough supplemented with beta glucan (BG) and resistant starch (RS).

hydroxyl groups existing in the fiber structure, which allow more water interactions through hydrogen bonding. Therefore, in this study, increasing dough water absorption is expected due to the high water absorption capacity of BG and RS. Such effects have been related to the high water absorbing capacity of these polysaccharides and their ability to compete for water with other components in dough system. Ref. [17] showed that there is the relationship between the water absorption and bread properties in baking. The amount of water added to the flour is usually adjusted to reach 500 BU at optimum development time; at this hydration level, high quality bread is produced. Therefore, the addition of these substances to the flour showed beneficial impact on bread-making properties.

Table 1 shows farinograph results of BG and RS supplemented dough and the control one. Addition of RS to the dough formula decreased the development time significantly compared to control (except for 8% RS). But the doughs with combination of BG and RS or only BG were similar to the control dough (except for 1% BG). Overall, the magnitude of the changes in development time of doughs fortified with RS was greater than control dough. The farinograph properties of wheat flours are strongly dependent on protein content and quality except for 8%RS. [18] showed that there is a direct relationship between the protein content and development time of different kinds of flour. Thus this reduction can be the result of decreasing protein content that leads to spend rapidly development time and hydration time. There was a progressive (P<0.05) decrease in dough's stability value when BG, RS and both were added. So that the least resistance was related to the treatment which has 10.5% resistant starch.

The closest dough stability to the control dough was observed

in the dough with the combination of BG and RS. In contrast [19] found that, with increased level of BG, there was no large reduction in dough stability. It was noticed that the softening degree of dough has significantly increased at a level of 5% by adding more BG, however, RS had less effect on dough softening than BG, except the dough containing 10.5% RS which increased the softening significantly (Table 1). The softening degree of doughs containing both of BG and RS was similar to the control treatment. Generally it can be concluded that BG has less effect on dough softening than RS.

Dilution of gluten and interaction between fiber and gluten may result in an increase to the degree of dough softening. This conclusion confirmed the findings of [20] which investigated the impact of adding fiber and oat whole meal to bread. In general, in comparison with the control dough, RS has affected softening less than BG. Although there was no significant difference between them in control and all sample, but addition of RS and 1% BG to the doughs formula decreased the Farinograph Quality Number (FQN) that seems to be the result of making complex of BG and starch wheat flour.

According to the Ref. [21], gluten makes the original dough network structure and plays an important role in dough and bread-making properties. Therefore, the dilution of gluten by bran leads to dough deterioration, breaking of the starch-gluten network structure and a decrease of consistency [20]. Thus the substitution of some part of wheat flour with RS and BG resulted in protein content reduction, which was more significant in greater substitution levels. In contrast, some studies showed that, a few kinds of hydrocolloids and dietary fiber increased rheological properties of wheat flour such as water absorption, dough development time and dough stability and resistance [14,16].

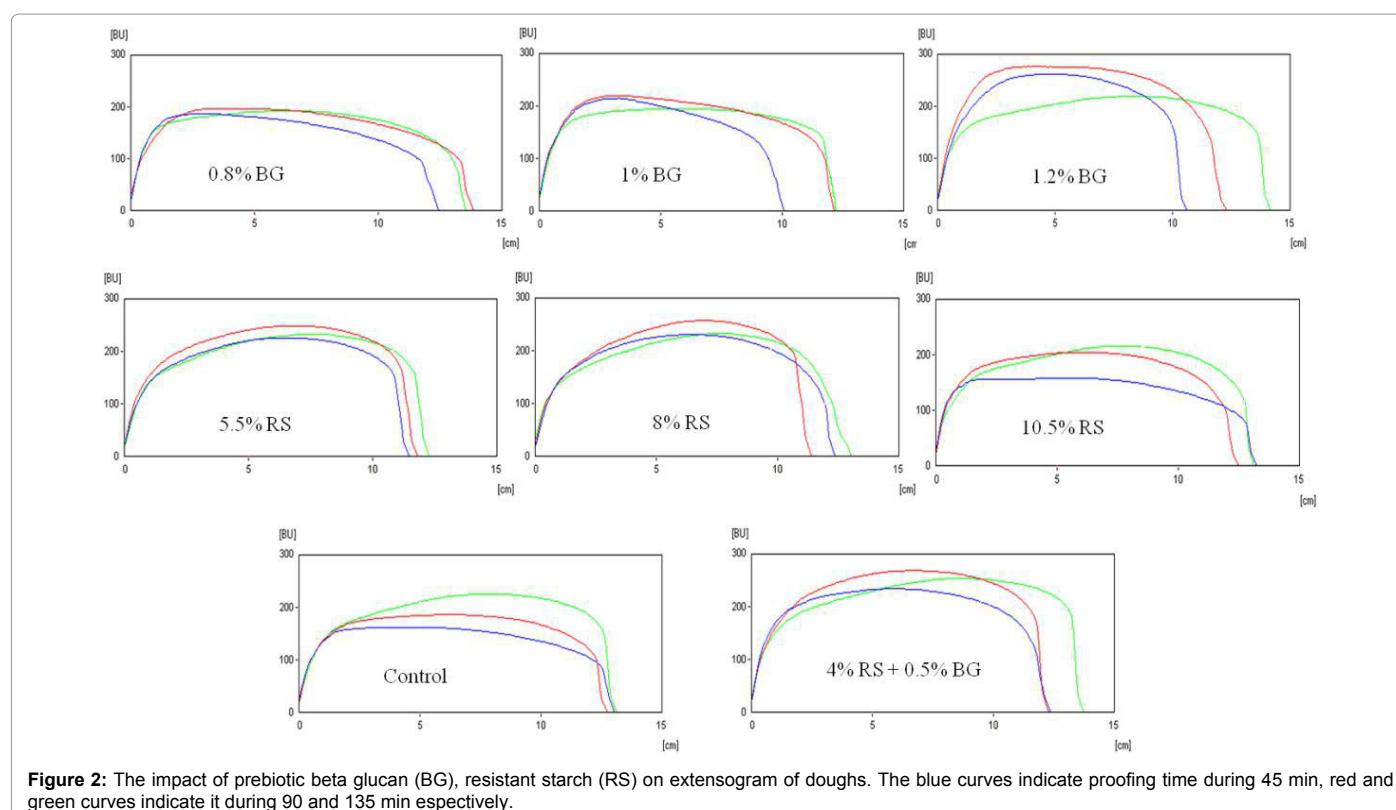
Sample	Resistance to extension (BU)			Extensibility (mm)			Energy (cm <sup>2</sup> )			Maximum Resistance (BU)	Ratio number
	45 min	90 min	135 min	45 min	90 min	135 min	45 min	90 min	135 min	135 min	135 min
Control	211.5 <sup>CD</sup>	180.5 <sup>A</sup>	155.5 <sup>A</sup>	133.5 <sup>BC</sup>	135.0 <sup>D</sup>	130.0 <sup>C</sup>	45.0 <sup>A</sup>	37.0 <sup>A</sup>	30.0 <sup>A</sup>	156.0 <sup>A</sup>	1.20 <sup>A</sup>
0.8 % BG	192.0 <sup>A</sup>	192.0 <sup>A</sup>	179.5 <sup>B</sup>	140.5 <sup>CD</sup>	137.5 <sup>D</sup>	120.0 <sup>B</sup>	42.5 <sup>A</sup>	39.5 <sup>AB</sup>	33.0 <sup>B</sup>	192.0 <sup>B</sup>	1.45 <sup>B</sup>
1 % BG	199.0 <sup>AB</sup>	212.0 <sup>B</sup>	180.0 <sup>B</sup>	150.5 <sup>CD</sup>	121.5 <sup>BC</sup>	109.5 <sup>A</sup>	43.0 <sup>A</sup>	39.5 <sup>AB</sup>	29.5 <sup>A</sup>	194.0 <sup>B</sup>	1.70 <sup>C</sup>
1.2 % BG	205.5 <sup>BC</sup>	275.5 <sup>D</sup>	252.5 <sup>E</sup>	139.0 <sup>C</sup>	124.5 <sup>C</sup>	107.0 <sup>A</sup>	46.0 <sup>A</sup>	51.5 <sup>E</sup>	40.0 <sup>DE</sup>	253.0 <sup>F</sup>	2.35 <sup>E</sup>
5.5 % RS	218.5 <sup>DE</sup>	240.5 <sup>C</sup>	225.0 <sup>D</sup>	133.5 <sup>BC</sup>	118.5 <sup>AB</sup>	112.5 <sup>A</sup>	46.0 <sup>A</sup>	43.5 <sup>DC</sup>	38.0 <sup>CD</sup>	228.5 <sup>DE</sup>	2.00 <sup>D</sup>
8 % RS	232.0 <sup>F</sup>	238.5 <sup>C</sup>	240.5 <sup>DE</sup>	121.0 <sup>A</sup>	114.0 <sup>A</sup>	123.0 <sup>B</sup>	42.5 <sup>A</sup>	41.0 <sup>BC</sup>	43.5 <sup>F</sup>	244.0 <sup>EF</sup>	1.95 <sup>CD</sup>
10.5 % RS	218.5 <sup>DE</sup>	221.5 <sup>B</sup>	203.5 <sup>C</sup>	126.0 <sup>AB</sup>	118.0 <sup>AB</sup>	122.0 <sup>B</sup>	44.0 <sup>A</sup>	40.0 <sup>B</sup>	36.5 <sup>C</sup>	205.5 <sup>BC</sup>	1.70 <sup>C</sup>
4%RS+0.5%BG	227.0 <sup>EF</sup>	242.5 <sup>C</sup>	221.0 <sup>CD</sup>	131.5 <sup>ABC</sup>	122.0 <sup>BC</sup>	125.5 <sup>BC</sup>	48.5 <sup>A</sup>	45.5 <sup>D</sup>	41.5 <sup>EF</sup>	222.0 <sup>CD</sup>	1.80 <sup>CD</sup>

Presented data are mean value of triplicates.

Mean values signed with the same letters in particular columns are non-significant at 0.05% level of confidence.

Values are medians (minimums to maximums) of sensory evaluation; Different letters in the same column are significantly different (P<0.05).

**Table 2:** Extensograph characteristics of dough supplemented with beta glucan (BG), resistant starch (RS) (at different level concentration) and the dough with both BG 0.5% and RS 4%.



**Figure 2:** The impact of prebiotic beta glucan (BG), resistant starch (RS) on extensogram of doughs. The blue curves indicate proofing time during 45 min, red and green curves indicate it during 90 and 135 min respectively.

On the other hand, [22] showed that the dilution of gluten with fiber solely cannot explain all of the observed changes in the addition of fiber to wheat flour. The different results from the impact of various kinds of fibers on dough properties can be explained by reaction between fibers and wheat flour gluten protein. Also, discrepancies related to the influence of BG and RS on the dough and the bread quality properties may arise from differences in the molecular size, solubility and the concentration range of the polysaccharides, as well as the flour types used for supplementation among the various studies [23].

### Effect of BG and RS on extensograph parameters

The effects of BG, RS and combination of RS and BG addition on the extensograph parameters throughout 135 min of proofing time are shown in Table 2 and Figure 2. At all resting times, all enriched samples exhibited higher resistance to extension values than did the control

(except for 45 min). On the other hand, the lowest resistance values were obtained in the control dough. Overall, the resistance to extension values at all resting times for RS-enriched doughs were higher than BG-enriched doughs (except for BG 1.2% dough). The dough supplemented with both BG and RS seemed to result in resistance to extension values similar to the doughs with RS. This confirms the finding of [23] that showed the BG addition to poor bread-making quality flour increased extensibility and resistance to extension values up to the good bread-making quality flour. Thus, the addition of BG and RS resulted in an enhancement of the gas retention properties of dough and possible improvement of the gluten network structure during proofing.

The extensibility values of BG-enriched doughs at 45 min proofing time remained similar but decreased at 90 (except 0.8% BG) and 135 minutes proofing times compared to the control (Table 2). However, they were lower for doughs supplemented with RS at 135



Sample	Moisture content (%)			Crumb Firmness (N)			Specific volume (cm <sup>3</sup> /g)
	1 day	3 day	5 day	1 day	3 day	5 day	
Control	35.08 ± 0.26 <sup>1A</sup>	34.19 ± 1.48 <sup>1A</sup>	26.18 ± 0.30 <sup>1A</sup>	1.03 ± 0.00 <sup>1D</sup>	1.10 ± 0.13 <sup>1BC</sup>	6.95 ± 2.22 <sup>2E</sup>	3.68 ± 0.12 <sup>C</sup>
0.8 % BG	35.32 ± 0.48 <sup>1AB</sup>	33.90 ± 0.19 <sup>2A</sup>	32.67 ± 0.90 <sup>3D</sup>	0.72 ± 0.12 <sup>1B</sup>	0.73 ± 0.11 <sup>1.2A</sup>	1.33 ± 0.32 <sup>2A</sup>	3.61 ± 0.01 <sup>BC</sup>
1 % BG	37.01 ± 0.44 <sup>1BC</sup>	32.11 ± 1.50 <sup>1A</sup>	28.11 ± 0.20 <sup>1AB</sup>	0.92 ± 0.04 <sup>1CD</sup>	0.91 ± 0.09 <sup>1AB</sup>	4.05 ± 0.86 <sup>2CD</sup>	3.62 ± 0.08 <sup>BC</sup>
1.2 % BG	37.77 ± 1.17 <sup>1C</sup>	34.86 ± 1.88 <sup>1A</sup>	28.77 ± 0.79 <sup>1AB</sup>	0.71 ± 0.07 <sup>1B</sup>	0.89 ± 0.06 <sup>2AB</sup>	2.40 ± 0.23 <sup>3ABC</sup>	3.44 ± 0.20 <sup>BC</sup>
5.5 % RS	36.26 ± 0.95 <sup>1ABC</sup>	35.33 ± 1.26 <sup>1A</sup>	29.24 ± 0.77 <sup>1BC</sup>	0.51 ± 0.00 <sup>1A</sup>	0.69 ± 0.01 <sup>2A</sup>	1.76 ± 0.22 <sup>3AB</sup>	2.95 ± 0.32 <sup>A</sup>
8 % RS	34.99 ± 1.56 <sup>1A</sup>	34.52 ± 1.64 <sup>1A</sup>	29.27 ± 3.36 <sup>1BC</sup>	0.62 ± 0.08 <sup>1AB</sup>	0.79 ± 0.01 <sup>1A</sup>	3.65 ± 0.13 <sup>2CD</sup>	3.62 ± 0.05 <sup>BC</sup>
10.5 %RS	34.89 ± 1.28 <sup>1A</sup>	33.32 ± 1.15 <sup>1A</sup>	32.04 ± 2.36 <sup>1CD</sup>	0.84 ± 0.09 <sup>1C</sup>	1.23 ± 0.07 <sup>1C</sup>	3.03 ± 0.56 <sup>2BC</sup>	3.36 ± 0.06 <sup>B</sup>
4%RS+0.5%B	34.68 ± 1.06 <sup>1A</sup>	31.79 ± 3.64 <sup>1A</sup>	30.27 ± 1.48 <sup>1BCD</sup>	1.26 ± 0.03 <sup>1E</sup>	2.94 ± 0.30 <sup>1D</sup>	5.27 ± 0.40 <sup>1D</sup>	2.97 ± 0.06 <sup>A</sup>

Presented data are mean values ± standard Deviation of triplicates.

Values followed by the same small letter within the same column or same numbers in the same line are not significantly different (P>0.05)

**Table 3:** Moisture content and crumb firmness of bread loaves stored for 1, 3 and 5 days at room temperature and specific volumes of breads.

Sample	Appearance	Crust	Aroma	Texture	Taste	Color	Chewiness	Total score
	Med	Med	Med	Med	Med	Med	Med	Med
Control	7.5 <sup>ABC</sup>	13.0 <sup>AB</sup>	13.5 <sup>ABC</sup>	13.0 <sup>AB</sup>	17.0 <sup>AB</sup>	9.0 <sup>A</sup>	12.0 <sup>A</sup>	84.5 <sup>AB</sup>
0.8 % BG	6.0 <sup>AB</sup>	12.5 <sup>AB</sup>	14.0 <sup>BC</sup>	10.5 <sup>AB</sup>	17.5 <sup>B</sup>	10.0 <sup>A</sup>	14.0 <sup>A</sup>	82.5 <sup>AB</sup>
1 % BG	7.5 <sup>ABC</sup>	13.0 <sup>AB</sup>	13.0 <sup>ABC</sup>	13.5 <sup>AB</sup>	15.0 <sup>AB</sup>	9.5 <sup>A</sup>	13.5 <sup>A</sup>	83.0 <sup>AB</sup>
1.2 % BG	8.0 <sup>ABC</sup>	12.0 <sup>AB</sup>	12.5 <sup>ABC</sup>	13.0 <sup>AB</sup>	16.5 <sup>AB</sup>	9.5 <sup>A</sup>	14.0 <sup>A</sup>	82.0 <sup>AB</sup>
5.5 % RS	8.0 <sup>BC</sup>	12.5 <sup>AB</sup>	11.5 <sup>ABC</sup>	12.0 <sup>AB</sup>	17.5 <sup>AB</sup>	10.0 <sup>A</sup>	14.0 <sup>A</sup>	82.5 <sup>AB</sup>
8 % RS	10.0 <sup>D</sup>	15.0 <sup>B</sup>	15.0 <sup>C</sup>	12.5 <sup>B</sup>	16.5 <sup>AB</sup>	10.0 <sup>A</sup>	14.5 <sup>A</sup>	94.5 <sup>B</sup>
10.5 % RS	9.0 <sup>C</sup>	12.5 <sup>AB</sup>	11.0 <sup>AB</sup>	12.0 <sup>AB</sup>	17.5 <sup>AB</sup>	10.0 <sup>A</sup>	11.5 <sup>A</sup>	82.0 <sup>AB</sup>
4%RS+0.5%B	5.5 <sup>A</sup>	8.0 <sup>A</sup>	10.0 <sup>A</sup>	9.5 <sup>A</sup>	11.5 <sup>A</sup>	9.0 <sup>A</sup>	10.5 <sup>A</sup>	68.0 <sup>A</sup>
<i>P value</i>	0.000	0.011	0.020	0.039	0.045	0.365	0.310	0.019

Values are medians (minimums to maximums) of sensory evaluation; Different letters in the same column are significantly different (P<0.05).

**Table 4:** Sensory scores of breads supplemented with beta glucan (BG), resistant starch (RS) (at different level concentration) and the dough with both BG 0.5% and RS.

minutes proofing time. RS produced a greater decreasing effect on the extensibility than BG (except for at 135 minutes) and the dough sample containing both BG and RS was similar to the control dough in extensibility values (except for 90 min). Desirable dough properties are usually associated with good dough resistance and extensibility values [23]. Therefore, desirable extensograph parameters (resistance and extensibility) were observed in the dough containing both BG and RS (Figure 2).

At all resting times, the lowest energy values were obtained in the control dough, whereas the dough supplemented with RS and BG had increased energy values. Generally, the results showed that increasing supplementation level increased the energy value of dough (except 10.5%). In addition, energy values of dough with both BG and RS were generally higher than doughs with either BG or RS. The energy values of all doughs after 135 minutes of proofing time had decreased. After 45 minutes of fermentation, there was no significant difference between under the curve area of treatments in the level of 5%, but at times 90 and 135 minutes the area under the curves of all treatments increased compared to control and only 1% BG treatment at 90 and 135 minutes and 0.8% BG treatment at 90 minutes were similar to control sample. The maximum resistance and ratio number values of doughs with BG, RS (at all substitution levels) and those with both of them were significantly higher than the control dough (only 135<sup>th</sup> minute values were discussed). It has been reported that maximum resistance and energy under extensograph can be used as indicators of dough strength [24], thus the results of maximum resistance and energy at extensograph indicated that BG addition increases dough strength.

### Specific volume

Specific volume values for loaves of bread are presented in Table 3. The volume of breads with addition of BGs was similar to

the control whereas the addition of RS to flour reduced the specific volume of the bread and was only similar to the control at 8% RS supplementation. Besides, lowest specific volume was observed when using the combination of BG and 5.5% RS. Similar behavior was observed by [25] for gluten-free flour substituted with RS. [2] reported a reduction in loaf volume of wheat breads fortified with BG. Ref. [23] reported that this depended on the molecular size, concentration of BGs and quality of base wheat flour. It is generally agreed that addition of some of the hydrocolloids to wheat flour reduces loaf volume. This result can be explained by several phenomena: I) Dilution of gluten [22]. II) Disruption of the gluten network structure by interactions between gluten and hydrocolloid material [22]. III) The strong water absorbing properties of hydrocolloids could suppress the amount of steam generated, resulting in reduced loaf volume [26].

Another possible explanation for the decrease in bread volume when adding RS to wheat flour is that during baking RS undergoes gelatinization [21], meaning that starch remains in granular form and is not decomposed by the amylolytic enzymes of yeast. Yeast is therefore not able to utilize RS for fermentation, slowing down the overall fermentation of the bread. The presence of undamaged starch granules could induce instability in cell walls [27]. These factors can negatively impact bread volume and cause uneven distribution of gas cells. The results of this study demonstrated that the BG-enriched breads had greater specific volumes than those supplemented with RS. Therefore, the type and level of prebiotic supplementation seems to affect the rheological properties of the dough and consequently influence the specific loaf volume of the fortified bread loaves.

### Influence of the BG and RS addition on moisture content of stored breads

By adding more BG, a continuous increase in the moisture content

of bread has been seen on the first day of storage (Table 3). After five days of storage, all samples showed higher moisture content than respective control breads. Whereas, at the first day storage, only those breads made from BG had higher moisture content than their control samples. This demonstrated that the water retention capacity of the fortified breads with RS was higher than those breads containing BG. On the other hand, the decreasing rate of moisture content in breads fortified with RS was lower than breads with BG.

The moisture content of each of the breads did not decrease significantly ( $P < 0.05$ ) during storage, except for 0.8% BG (Table 3). The results showed no significant difference among moisture content of breads at the third day of storage ( $P < 0.05$ ).

### Bread firmness

Generally crumb firmness of the breads decreased with adding BG and RS. In previous studies, crumb softening was reported by the addition of hydrocolloids [23,25]. Hardness increased with increasing BG and RS levels (on the first and fifth day of storage), but the values were generally smaller than the control (Table 3). The texture results showed that the firmness values of all BG and RS-supplemented bread crumbs, after three days of storage, were similar to or significantly lower than the control (except for breads with 10.5% RS and both BG and RS) (Table 3). The increase in hardness of the bread crumb fortified with RS, at the high level of addition (10.5%), may be a consequence of the thickening of the wall surrounding gas cells, as proposed by Ref. [15]. The bread fortified with both BG and RS exhibited higher firmness than the control after one and three days of storage. Crumb firmness of all breads was smaller than the control, after five days of storage. However, the hardening of each sample increased significantly during storage (except for breads with the combination of BG and RS).

The decrease in the crumb firmness observed when BG and RS is added to the bread formula could be attributed to their higher water retention capacity, and a possible inhibition of the amylopectin retrogradation [28], or it may be a consequence of an increase in the total area of gas cells [23]. The effects of the hydrocolloids on starch gel structure and mechanical properties results from two opposite phenomena; the first is an increase in the hardening as a consequence of the decrease in the swelling of the starch granules. Hydrocolloid added to wheat flour would compete for water with native wheat starch granules in the dough. This, in turn, might limit swelling and solubilisation of the starch during baking [26] and reduces the amylose leaking from the granules. The second is a weakening effect on the composite starch network structure due to the inhibition of the formation of cross-links among swollen granules [28]. When there are no changes in the moisture content, the formation of a cross-linked network rather than the development of amylopectin crystallites in the ageing gluten-starch composite matrix causes bread firming [29]. In the present study, no significant losses of moisture in breads after 5 days of storage were noted, whereas the firmness of the bread crumbs increased significantly. Ref. [30] noticed that the rate of crumb hardening is influenced by water content and it is considered as one of the most important factors for bread staling. In this study the water content of BG and RS enriched breads was significantly higher than the control bread, so all breads exhibited softer breadcrumbs than the control measured after five days of storage. Bread firmness is usually used as a tool to measure bread staling. However, staling is a very complicated process that cannot be explained by a single variant. It is maybe a combination of these factors that determine the overall effect on the mechanical properties of the bread structure, an effect that is dependent on each specific hydrocolloid used for fortification.

### Sensory

The sensory evaluation of the fresh bread was performed by eight trained panelists. The median, minimum and maximum ranks of the characteristics of breads supplemented with BG and RS, such as appearance, crust, texture, aroma, and taste/ flavor are presented in Table 4. Only color and chewiness scores of breads were not significantly influenced by the addition of BG, RS and combination of RS/BG compared to the control breads.

Results from sensory evaluation indicated that neither adding BG nor RS had any significant effects on appearance, crust, texture and total scores. Breads containing both of BG/RS were less appealing than breads containing 8% RS. According to Douglas and Sanders (2008), RS has less effect on sensory properties than the other dietary fibers. Panelists preferred the appearance properties of breads containing RS more than the bread enriched with both of BG/RS. Bread containing 8% RS was significantly better than all the other samples. Breads containing 0.8% BG had higher taste/ flavor scores than breads containing both of BG/RS, whereas all sample breads were similar to the control. Increasing RS level from 8% to 10.5% caused a decrease in aroma scores. Results from sensory evaluation indicated that the use of these prebiotic compounds has no bad effects on bread quality.

### Conclusion

Results showed that addition of BG and RS has significant effects on dough rheological properties. A continuous increase in the farinograph water absorption of the dough was observed with increasing fortification level; the BG showed a greater impact than did RS, presumably due to a higher water binding capacity of the former. RS showed a more significant effect on decreasing development time than BG. Overall, the effect of RS on increase of dough softening was lower than BG. Generally, the desirable dough in the farinograph parameters (water absorption, development time, stability) was observed in the dough that contained both of BG and RS. Therefore, flour fortified with both BG and RS produces the strongest dough.

The BG and RS addition increased the resistance to extension value, strengthened the gluten matrix, and increased the gas retention capacity of the dough, but decreased the extensibility value. The dough with the combination of BG and RS showed the best extensograph parameters (Resistance and Extensibility). Addition of BG and RS or both of them to the dough formula increased energy, maximum resistance and ratio values. The use of BGs had no significant effect on bread loaf volume, whereas, the addition of RS caused a decrease in specific volume of breads compare to the control (except for 8% RS). Bread enriched with BGs and RS had similar or higher moisture content than the control breads during storage time.

The texture results showed that the hardness values of BG and RS supplemented breads were smaller than the control after five days. In general, the sensory properties of all bread samples were similar to the control breads. BGs and RS did not undesirably or significantly affect the quality characteristics of the pan breads. These prebiotic compounds have the potential to be used in bread-making at prebiotic levels. The results of this work indicate that the type of prebiotic compounds used and their supplementation level influence the dough rheological properties and bread quality.

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