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RESEARCH ARTICLE

Effects of resistant starch type 4 supplementation of bread on in vitro glycemic index value, bile acid-binding capacity, and mineral bioavailability

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Abstract

Background and objectives: RS4 is an alternative dietary fiber source with some potential physiological benefits giving better quality end products. Common dietary fiber sources including bran usually have deteriorative effect on color, textural properties, and consumer acceptability of breads. This study was designed to investigate the effect of RS4 supplementation on the bread quality as well as mineral bioavailability, bile acid-binding capacity, in vitro glycemic index.

Findings: Loaf volumes of the breads supplemented with RS4 were higher than the bread supplemented with wheat bran (WB). Among the bread samples, the one supplemented with WB had the darkest color and highest firmness values. During storage, WB supplementation caused the highest increase in bread firmness. RS4 supplementation caused higher total dietary fiber (TDF) and mineral bioavailability values and lower in vitro glycemic index than bran supplementation.

Conclusions: RS4 supplementation of bread caused increases in TDF content, bile acid-binding capacity, and mineral bioavailability and has less deteriorative effect on the quality than WB supplementation. Breads supplemented with WB had a faster staling rate than the breads supplemented with RS4.

Significance and novelty: Besides the better appearance and texture of the RS4 supplemented bread samples, the improvement in nutritional properties proved that RS4 is a better dietary fiber source than WB.

KEYWORDS

in vitro bile acid-binding capacity, in vitro glycemic index, mineral bioavailability, resistant starch type 4

1 | INTRODUCTION

Resistant starch (RS) is defined as the fraction of starch, which escapes digestion in the small intestine but may be fermented in the colon. It has been classified into four categories (RS1, RS2, RS3, RS4; Englyst, Kingman, & Cummings, 1992). RS1 is a starch form, which is physically nondigestible. It has been trapped in the food matrix such as partly milled grains and seeds. RS2 is ungelatinized granular starch. It comprises of native, uncooked granules like raw potato or unripe banana starches (Sharma, Yadav, & Ritika, 2008). RS3 is retrograded starch and is produced by gelatinization followed by retrogradation of starch molecules (Sajilata, Singhal, & Kulkarni, 2006). RS4 is a chemically modified starch and include starches, which are etherized, esterified, or cross-linked (Nugent, 2005). Amylose-lipid complexes have also been proposed as resistant starch type 5 (RS5), because of its resistance to enzyme hydrolysis (Hasjim, Ai, & Jane, 2013).

Product developers and nutritionists are interested in resistant starch as a food ingredient for two reasons: the potential physiological benefits and better quality of end products. Many studies report that RS has physiological functions similar to dietary fiber (DF; Baixauli, Salvador, Martínez-Cervera, & Fiszman, 2008). Because of the bile-binding ability of fibers, bile acids are excreted from the body after the consumption of high fiber products. Thus, gall bladder signals the liver to increase bile acid synthesis from serum low-density lipoprotein (LDL) cholesterol. The LDL cholesterol removed from the bloodstream is used to synthesize new bile acids, causing a drop in the LDL serum cholesterol levels (Hinkle, 2013). Glycemic index (GI) is the value that shows the increase in blood glucose after the intake of carbohydrate-containing foods, compared to the increase in blood glucose following the digestion of white bread (Rizkalla, Bellisle, & Slama, 2002). RS has been associated with reduced digestibility, providing prolonged, and slow glucose release; therefore, a low GI compared to regular starch. Foods with low GI are known to reduce the risk of cardiovascular disease and type 2 diabetes. Besides, RS produces short-chain fatty acids (SCFAs) due to its fermentation by the colonic microorganisms and SCFAs promote normal colonic function (Nugent, 2005) and prevents colonic cancer (Hasjim et al., 2013). RS has a fine particle size, white color, mild flavor, lower water-holding capacity, and higher water-binding capacity. Moreover, it can be either water soluble or insoluble. Because of their functional properties, resistant starches can be added in a wide range of foods including dairy products, breads, cakes, muffins, pasta, and battered foods (Homayouni et al., 2014; Sanz, Salvador, & Fiszman, 2008).

Due to the increased interest in healthy foods, dietary fiber is currently accepted as an essential food ingredient. Popular food products, such as bread, are being used as vehicles for increasing DF consumption (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). However, high fiber breads have several negative attributes such as dark color, decreased loaf volume, poor mouthfeel, and undesirable flavor (Sajilata et al., 2006). In comparison with conventional fibers, such as bran, whole grains, or fruit fibers, RS has advantages of affecting the sensory properties of the end products less and it provides better appearance, texture, and mouthfeel than conventional fibers (Fuentes-Zaragoza et al., 2010). In a study evaluating the effects of RS on bread characteristics and comparing their performance with traditional fibers (cellulose, oat fiber, wheat fiber), two commercial RS samples (HylonVII and Novelose 240) were used. The breads supplemented with RS had greater loaf volume and better cell structure compared to the breads supplemented with traditional fibers (Baghurst, Baghurst, & Record, 1996; Sajilata et al., 2006).

RS4 is a distinctive class of RS due to the large variety of possible chemical modifications, which might decrease digestibility (Stewart & Zimmer, 2017) and can be produced from various starch sources such as maize, tapioca, and potato. Functional properties of RS4 can vary depending on the source of starch and type of chemical modification (Sajilata & Singhal, 2005; Stewart & Zimmer, 2017). These variations affect their functionality, digestibility, and fermentability as they are added into food formulations (Maningat & Seib, 2013; Stewart, Wilcox, Bell, Buggia, & Maki, 2018). RS4 has been useful in formulations needing pulpy texture, smoothness, flowability, low pH, and high temperature storage (Sajilata & Singhal, 2005). Since new types of RS4 have been emerging, the studies on RS4 as a food ingredient are intensified in the recent years (Stewart et al., 2018). There are some studies investigating the effect of RS4 supplementation on the volume, textural properties, color parameters, firmness, and total dietary fiber (TDF) values of the breads in the literature.

A study on the effect of RS4 (acetylated retrograded starch) on bread quality indicated that breads supplemented with RS4 had a lighter crust color than the control bread. Moreover, TDF contents of the bread samples increased significantly with increasing level (10%, 20%, 30%, and 40%) of RS4 supplementation (Wojciechowicz et al., 2015). In another study, bread samples supplemented with 25% RS4 (phosphorylated cross-linked) had significantly lower loaf volume compared to the control bread (Miller & Bianchi, 2017).

To the best of authors' knowledge, there are no studies investigating the mineral bioavailability, in vitro glycemic index, and bile acid-binding capacity in RS4 supplemented breads. The aim of this study was to investigate the effect of RS4 supplementation on the bread quality as well as mineral bioavailability, bile acid-binding capacity, in vitro glycemic index. For this purpose, RS4 samples were added into the bread formulations at different levels (15%, 20%, 25%; flour basis). Wheat bran was also used (at 15% level) in the bread formulation for comparison.

2 | MATERIALS AND METHODS

2.1 | Materials

Bread wheat flour was obtained from Bagislar Un. RS4 (phosphorylated cross-linked) sample was kindly donated by Demirpolat Inc. Wheat bran was obtained from Ankara Halk Ekmek Inc. and milled by using a Perten 3100 (Perten Ins.) to pass 500 µm. $CaCO_3$ as Ca and Fe–Zn mixture were obtained from UNO for mineral analysis. The chemicals used in this study were of analytical reagent grade, unless otherwise specified.

2.2 | Chemical and physicochemical analyses

Moisture, ash, and protein (Velp-NDA 701 Dumas Nitrogen Analyzer) contents of the samples (wheat flour, wheat bran, and RS4) were determined by using the AACC Method 44-15A, 08-01.01, and 46-30 (AACC International, 2009), respectively. The Zeleny sedimentation value of the flour sample was measured according to the AACC Method 56-60 (AACC International, 2009). Dry gluten content (Perten Glutomatic and Glutork 2020) of the flour sample was determined according to the AACC Method 38-12A (AACC International, 2009).

The TDF contents of RS4, bran, and bread samples were determined according to AACC Method No. 32-07.01 (AACC International, 2009) using TDF determination kit (Megazyme Int.). Sequential enzymatic digestion was applied to the samples using heat-stable α-amylase, protease, and amyloglucosidase to remove digestible starch and protein. Enzyme digestate was treated with alcohol before filtering through a preweighted crucible containing celite as a filter aid. TDF residue was washed with alcohol and acetone, dried, weighed, and expressed as % (g TDF/100 g dry sample).

2.3 | Bread making and storage experiment

Bread samples were prepared according to the AACC Method 10-10B (AACC International, 2009) as modified by Ozturk, Koksel, and Ng, (2009). The formula included 100 g of flour, 25 ml salt solution (noniodized, 6.0%), 25 ml yeast suspension (8.0%), and water according to the Farinograph water absorption value. Doughs were mixed by using a stand mixer (Kitchenaid). The dough samples were punched after 30 min of fermentation and left for fermentation for another 30 min. After the second fermentation, the dough was molded and panned. Final proof was 55 min. The loaves were baked in a laboratory rotary oven (Simsek Laborteknik) at 230°C for 25 min. RS4 and wheat bran samples were added into the respective bread formulations at different levels (15%, 20%, 25% for RS4, and 15% for wheat bran; flour basis). Wheat bran supplementation level was kept at 15%, due to its extreme deteriorative effects on bread quality. The bread making was performed in triplicate, and mean values were reported. The bread samples were cooled at room temperature for 2 hr. Afterward, they were packed in plastic bags and stored at room temperature for 1 and 3 days.

For determination of in vitro calcium, iron, and zinc bioavailability; calcium carbonate (Vitamiks) and Fe–Zn mixture (Vitamiks) were added to the bread formulation at the levels of daily intake values stated at the Turkish Food Codex Regulation on labeling and provision of food information to consumers (Table 1; Codex, 2017).

2.4 | Bread quality

The volume, textural properties, and color parameters of the bread samples were determined after cooling to room temperature. The volumes of bread samples were determined using the rapeseed displacement method in a loaf volumeter (National Mfg). Bread firmness was measured according to AACC Method 74-09 (AACC International, 2009). A texture analyzer (Stable Microsystems; TA-XT plus) equipped with 5 kg load cell, and a 30-mm cylinder probe was used for the texture analysis. The force (firmness, N) required to compress 40% of two slices (1.25 cm each) was determined at 1.7 mm/s test speed. The crust color of breads was assessed using a spectrophotometer (Minolta; CM-3600d).The color of each sample was measured from eight different points according to CIE *L**, *a**, *b** color space parameters.

TABLE 1 Daily reference intake values for vitamins and minerals indicated in the Turkish Food Codex (Codex, 2017)

Nutrient	Nutrition reference value (mg/100 g)[a]
Calcium	800
Iron	14
Zinc	10

[a] Acceptable for healthy individuals at 4 years and over.

2.5 | In vitro glycemic index value

The samples were digested according to the method of Englyst, Veenstra, and Hudson, (1996). For this purpose, 100 mg of sample was weighed into 50 ml tubes containing 10 glass beads (5 mm diameter). Two milliliters of HCl (0.05 M) containing pepsin (5 mg/ml, Sigma; P7000) was added to the tubes, and the tubes were incubated at 37°C in a shaking water bath for 30 min. Sodium acetate buffer (4 ml, 0.5 M, pH 5.2), 1 ml of enzyme solution containing 0.104 g pancreatin (Sigma; P7545), and 14.45 U amyloglucosidase (3,300 U/ml; Megazyme Int.) were added to each tube. The tubes were incubated horizontally at 37°C in a shaking water bath. Aliquots (100 µl) were taken into Eppendorf tubes at 0 and 90 min intervals and mixed with 1 ml of absolute ethanol. These solutions were centrifuged at 800 *g* for 10 min, and glucose content was measured with glucoseoxidase–peroxidase (GOPOD) reagent (Megazyme Int.) by using a spectrophotometer (Shimadzu 1601) at 510 nm wavelength. The hydrolysis index (HI) shows starch digestion rate, and in vitro glycemic index (GI) indicates the digestibility of the starch against white bread (reference, GI:100). The hydrolysis index (HI) is the ratio of the area under the hydrolysis curve of sample to the area under the hydrolysis curve of white bread as reference sample. The HI was calculated as follows:

$$\mathrm{HI} = \frac{\text{Area under the curve of the sample}}{\text{Area under the curve of white bread}}$$

The in vitro GI was determined by using the following equation of Goñi, Garcia-Alonso, and Saura-Calixto, (1997).

$$\mathrm{GI} = 39.71 + 0.549\mathrm{HI}$$

2.6 | In vitro bile acid-binding capacity

Bile acid (BA)-binding capacity of the samples was determined according to the method described previously (Zacherl, Eisner, & Engel, 2011) with some modifications. Sample (50 mg) was dissolved in water (ratio 1:2) and homogenized. After adding HCl (1 ml, 0.01 N), the samples were incubated in a 37°C water bath for 60 min. After the incubation, NaOH (0.1 ml, 0.1 N, pH 6.3) and pancreatin-bile acid mixture (*w*:*w*, 1:9.4, 1 ml) were added. Sodium taurodeoxycholate hydrate (TDC) was used as the bile acid. After the incubation, the sample was centrifuged at 21,734 *g* for 10 min; the supernatant was kept in boiling water for 5 min to inactivate enzymes. After cooling, methanol and KH_2PO_4were added to the solution. The sample was filtered through a 0.45 μm filter to be used for HPLC analysis. The mobile phase for HPLC was methanol–sodium phosphate–water (*v*:*v*:*v*, 70:20:10) solution and a flow of 0.8 ml/min at a temperature of 40°C. The results were measured using Diode Array Detector (DAD) at wavelength of 200 nm. A calibration curve prepared with bile acid standard solution was used for the quantification of the bile acid. Unconjugated bile acid content was calculated using the area under the curve. BA-binding value of the samples was calculated by subtracting the unconjugated bile acid from the total bile acid content and expressed as μmol/100 g and % bound relative to Cholestyramine.

2.7 | In vitro mineral bioavailability

In vitro mineral bioavailability is expressed as a ratio of the amount of the mineral released during enzymatic digestion to the total amount of the mineral contained in the sample. Enzymatic digestion was made according to Suliburska and Krejpcio, (2014). In vitro mineral bioavailability was determined for the samples supplemented with 15% RS and 15% WB. Control sample (100% wheat flour) was also analyzed.

The samples (10 gr) were mixed with deionized water (100 ml). The pH of the mixture was adjusted to pH 2.0 with 0.1 N HCl solution and treated with pepsin (0.5 ml). The samples were incubated in a 37°C shaking water bath for 2 hr. After the incubation, the pH was adjusted to 6.8–7.0, subjected to pancreatin (25 ml; 0.4 g/100 ml $NaHCO_3$) and incubated in a shaking water bath under the same conditions for 4 hr. After the digestion, the samples were centrifuged for 20 min at a speed of 15,093 *g*. The supernatant (25 ml) was transferred to Teflon vessels and ashed with HNO_3 (7 ml) by means of a closed pressurized system microwave oven (MARS-5 CEM). Then, it was filtered into a volumetric flask (100 ml). Lanthanum chloride solution (1 ml) was added (0.1%, *w*/*v*) for calcium determination. Lastly, it was diluted to 100 ml with deionize water and the minerals were determined by atomic absorption spectrophotometer (AAS; Thermo Scientific).

For the determination of total amount of the mineral contained in the sample, approximately 1 g sample was weighed and ashed with HNO_3 in Teflon vessels in a microwave oven. The concentration of Ca, Zn, and Fe were determined by AAS.

2.8 | Statistical analysis

All of the results are reported as means of at least duplicate analyses. Data were analyzed by using one-way analysis of variance (ANOVA). When significant ($p < .05$) differences were found, Duncan's test was used to determine the differences among means.

3 | RESULTS AND DISCUSSION

3.1 | Chemical and physicochemical properties of flour, wheat bran, and RS4 samples

Zeleny sedimentation value, protein (N × 5.7, dry basis), moisture, ash, TDF, and dry gluten contents of the flour sample were 61 ml, 13.6%, 13.9%, 0.50%, 4.3%, and 10.7%, respectively. A flour sample with relatively high gluten quality was selected to tolerate the potential negative effects of resistant starch and bran addition on the quality of bread. Moisture, ash, and TDF contents of wheat bran were 9.5%, 5.50%, and 60.0%, respectively, while the corresponding values of the RS4 sample were 5.4%, 0.05%, and 85.4%, respectively.

The TDF contents of bread samples are presented in Table 2. TDF content of control bread was 7.0%. TDF contents of RS4 supplemented breads increased significantly ($p < .05$) with RS4 supplementation and reached to 30.6% at the 25% supplementation level. While significant increase in TDF content of wheat bran supplemented bread was observed compared to the control bread, TDF content of this bread was significantly lower as compared to the bread supplemented with RS4 at the same level ($p < .05$). A study on RS4 supplemented bread indicated that RS4 supplementation resulted in significant increases in TDF content ($p < .05$; Stewart et al., 2018). These results are in line with our study.

3.2 | Physical properties of bread samples

Crust properties of breads supplemented with RS4 and wheat bran are shown in Figure 1, and loaf volume, crust color, and firmness values of the breads supplemented with different levels of RS4 and wheat bran are presented in Table 3. Significant decreases in loaf volumes of RS4 supplemented breads were observed as the supplementation level increased. The results of the present study are in line with various other studies (Ozturk et al., 2009; Rosell, Santos, & Collar, 2010) indicating that RS weakens the

TABLE 2 TDF contents of bread samples

Sample	Total dietary fiber (%) Day 0	Day 1	Day 3
CB	7.0 e	6.7 e	7.3 e
RS15	20.4 c	20.0 c	20.8 c
RS20	25.3 b	24.3 b	25.7 b
RS25	30.6 a	28.7 a	30.8 a
WB15	16.3 d	16.3 d	16.7 d

Note: Means with different small letters within each column are significantly different ($p < .05$).

Abbreviations: CB, control bread; RS15, breads supplemented with RS4 at 15% level; RS20, breads supplemented with RS4 at 20% level; RS25, bread supplemented with RS4 at 25% level; TDF, total dietary fiber; WB15, breads supplemented with wheat bran at 15% level.

gluten and decreases the specific volume of bread. While the difference in the loaf volumes of the breads supplemented with 15% and 20% RS4 was not significant, their loaf volumes were significantly higher than the bread supplemented with 25% RS4 ($p < .05$). The lowest loaf volume was observed for the bread supplemented with 15% wheat bran. The loaf volume of this bread was significantly lower as compared to the breads supplemented with RS4 at all levels ($p < .05$). Although loaf volumes of all breads supplemented with RS4 were significantly lower than the control bread, their loaf volumes were acceptable. Shyu, Hwang, Huang, and Sung, (2018) reported that the loaf volumes of breads made from flours substituted with 10% RS4 (Fibersym™70) were not significantly different from those of the control, whereas the substitution with 20% and 30% RS4 decreased the loaf volume.

Table 3 displays color values of crust for the bread samples. Crust L^* and b^* values of the breads increased significantly with increasing level of RS4 supplementation. On the other hand, supplementation of wheat bran caused decreases in L^* and b^* values as compared to the control bread. The a^* values of breads decreased with both RS4 and wheat bran supplementation. The results of the present study are in line with Barros, Telis, Taboga, and Franco, (2018) indicating that the higher the concentration of RS, the higher the L^*, and the lower the a^* values. Similarly, the crust L^* values of bread increased with resistant starch supplementation in another study. However, a^* and b^*values decreased. Since Maillard reaction caused the browning of the crust during baking, the dilution of the wheat proteins might have been resulted in a lighter colored crust (Altuna, Ribotta, & Tadini, 2015).

Firmness values of the bread samples are presented in Table 3. The firmness value of wheat bran supplemented bread was significantly higher as compared to the breads supplemented with RS4 at all levels. Significant increases in firmness values of RS4 supplemented breads were observed as the supplementation level increased ($p < .05$). Similar results were also observed in a study in which breads were supplemented with different levels (10%, 20%, 30%) of Novelose330 (RS3; Ozturk et al., 2009). They indicated that Novelose330 addition caused increases in firmness values above the 10% level. In another study, the textural properties of bread containing resistant starch at the 20% level were significantly different from those of the controls (0% RS) and 10% level (Shyu et al., 2018).

Within each storage day, the firmness values of the breads supplemented with RS4 increased and wheat bran supplementation caused the highest increase in firmness values. These increases were statistically significant ($p < .05$). The firmness values of the breads increased over days 1 and 3 during storage. These increases were statistically significant for RS4 and bran supplemented samples and control bread ($p < .05$). The results indicated that the breads supplemented with wheat bran had a faster staling rate than the breads supplemented with RS4. The RS4 sample used in the present study is highly cross-linked, and it is quite likely that starch molecules will not leach out of the granules within the bread loaf during baking (<100°C). In other words, due

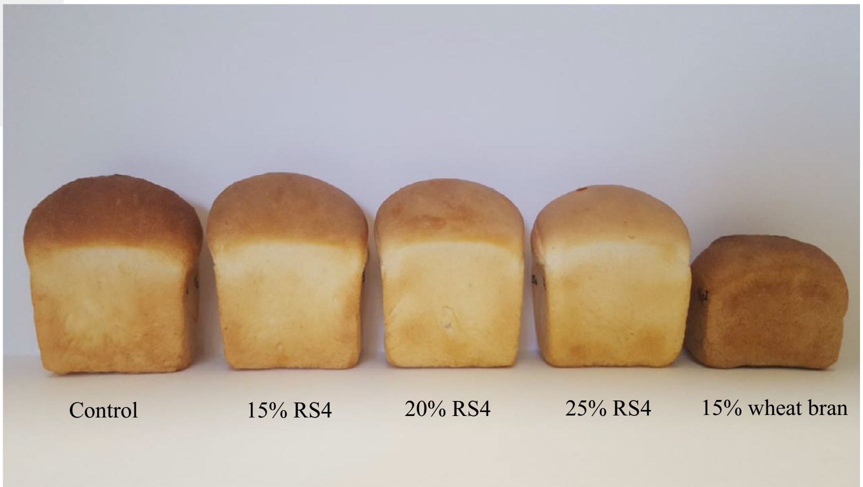

FIGURE 1 External properties of breads supplemented with RS4 (15%, 20%, and 25%) and wheat bran (15%) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Loaf volumes and quality characteristics of breads supplemented with different levels of RS4 and wheat bran samples

Sample	Addition level (%)	Loaf volume (cm^3)	Crust color			Firmness (g)		
			*L**	*a**	*b**	Day 0	Day 1	Day 3
CB	0	658 a	45.67 d	15.67 a	26.00 c	55.00 eC	116.00 eB	182.67 eA
RS15	15	638 b	54.33 c	14.00 b	27.67 b	66.67 dC	133.33 dB	227.00 dA
RS20	20	630 b	56.33 b	13.33 b	28.67 ab	94.00 cC	154.67 cB	243.00 cA
RS25	25	613 c	61.33 a	12.33 c	30.00 a	111.33 bC	179.33 bB	268.33 bA
WB15	15	478 d	43.67 e	12.33 c	22.33 d	146.33 aC	207.67 aB	326.00 aA

Note: Means with different small letters within each column are significantly different ($p < .05$).
Means with different capital letters within each row are significantly different ($p < .05$).
Abbreviations: CB, control bread; RS15, breads supplemented with RS4 at 15% level; RS20, breads supplemented with RS4 at 20% level; RS25, breads supplemented with RS4 at 25% level; WB15, breads supplemented with wheat bran at 15% level.

TABLE 4 Bile acid-binding and in vitro GI value of bread samples

Sample	Bile acid (BA)-binding capacity						In vitro GI		
	Bound BA (µmol/100 g)			Bound BA relative to cholestyramine (%)					
	Day 0	Day 1	Day 3	Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
CB	0.52 d	0.56 d	0.58 d	5.2 d	5.6 d	5.8 d	100.0 aA	91.3 aB	82.6 aC
RS15	0.82 c	0.84 c	0.84 c	8.2 c	8.4 c	8.4 c	82.2 cA	76.6 bB	70.6 bC
RS20	0.87 b	0.89 b	0.89 b	8.7 b	8.8 b	8.9 b	80.8 dA	71.2 cB	66.0 cC
RS25	0.89 a	0.92 a	0.93 a	8.9 a	9.2 a	9.3 a	75.0 eA	66.9 dB	56.8 dC
WB15	0.87 b	0.89 b	0.89 b	8.6 b	8.8 b	8.9 b	88.4 bA	87.4 aA	82.2 aB
Cholestyramine	10.04								

Note: Means with different small letters within each column are significantly different ($p < .05$).
Means with different capital letters within each row are significantly different ($p < .05$).
Abbreviations: CB, control bread; RS15, breads supplemented with RS4 at 15% level; RS20, breads supplemented with RS4 at 20% level; RS25, breads supplemented with RS4 at 25% level; WB15, breads supplemented with wheat bran at 15% level.

to high level of cross-linking, starch molecules do not have enough mobility to interact with each other and go through retrogradation. Therefore, the level of retrogradation (hence staling rate) is expected to be limited in the loaves supplemented with RS4. It is also reported that RS decreases amylopectin retrogradation rate (Sanz-Penella, Wronkowska, Soral-Śmietana, Collar, & Haros, 2010) or behave as an inert ingredient in the system (Almeida, Chang, & Steel, 2013). These are probably the main reasons for the slower staling effect in the samples supplemented with RS4 as compared to the one supplemented with bran. Therefore, RS4 seems to be a better dietary fiber source than wheat bran in terms of affecting the bread staling rate.

3.3 | Nutritional properties of bread samples

The in vitro GI values of bread samples are presented in Table 4. RS4 supplementation caused a great decrease in GI values. The breads supplemented with RS4 and wheat bran had lower in vitro GI values than the control sample (white bread). The in vitro GI values of the breads supplemented with RS4 ranged from 57.4 to 83.9, whereas the in vitro GI values of the breads supplemented with bran were relatively higher (90.0–92.1). RS4 supplementation of bread caused a lower GI compared to bran supplementation. Foods are classified as low (GI ≤ 55), medium (GI 56–69), and high (GI ≥ 70) glycemic index foods (Kumar et al., 2018). According to the results, the bread samples supplemented with 20% or higher RS4 levels can be categorized as medium or low GI food.

Within each storage day, GI values of the RS4 supplemented breads significantly decreased ($p < .05$) as the RS4 supplementation level increased. Furthermore, as the GI values of breads are compared at each RS4 supplementation level, significant decreases were observed in both 1st and 3rd storage days ($p < .05$). A similar trend was also observed in control and wheat bran supplemented breads during the storage. Bread staling is closely related to starch retrogradation, and it is known that RS3 is produced due to retrogradation of starch molecules. The decrease in GI can be associated with the formation of RS3 during the storage of the bread samples.

Bile acid binding is one of the most important characteristics of dietary fiber. Similar to other types of dietary fiber, RS binds to bile acids, prevents their reabsorption, and removes them from the body (Hinkle, 2013; Sharma et al., 2008). BA-binding capacity values of the bread samples are presented in Table 4. BA-binding capacity results are stated in two different ways: the amount of BA bound (μmol/100 g) and percent BA bound relative to Cholestyramine (%). BA-binding capacity of bread samples increased significantly with increasing level of RS4 supplementation. The highest BA-binding capacity was observed in the bread supplemented with 25% RS4. The BA-binding capacity of the bread supplemented with 15% wheat bran was equal to that of the bread supplemented with 20% RS4. Hence, both of the breads supplemented with 20% RS4 and 15% wheat bran may have same health-protecting potential due to their same BA-binding capacity. They are expected to have similar cholesterol-lowering effect. The in vitro binding of bile acids by taro starch and taro resistant starch relative to Cholestyramine reported by Simsek and El, (2012) were comparable to (5.2% and 7.6%, respectively) the results of the present study.

In vitro bioavailability of minerals of bread samples supplemented with 15% RS4 and wheat bran are presented in Table 5. Ca, Fe, and Zn were added to the bread formulation according to reference values (Table 1) 800 mg/100 g, 11.7 mg/100 g, and 10 mg/100 g, respectively. When the bread supplemented with RS4 was compared to the control bread, it was detected that supplementation of RS caused small but significant increases in Ca and Zn bioavailability. However, Ca and Zn bioavailability values of the bread sample supplemented with 15% RS4 were significantly greater than the sample supplemented with 15% wheat bran. While the difference in the Fe bioavailability of breads supplemented with RS4 and control bread was not significant, Fe bioavailability of breads supplemented with RS4 was significantly higher than the bread supplemented with wheat bran($p < .05$). To the best of authors' knowledge, there are no studies investigating the mineral bioavailability in RS4 supplemented breads. In a study about in vitro bioaccessibility of minerals from bread samples, the Ca and Zn bioaccessibility values of white bread were detected as 36.10% and 20.63%, respectively (Ting & Loh, 2016).These results are similar to the values obtained for the control bread in the present study.

TABLE 5 Bioavailability of calcium, iron, and zinc of bread samples

	Minerals		
Sample	Ca (%)	Fe (%)	Zn (%)
CM	40.0 b	11.3 a	21.9 b
RSM15	42.5 a	11.5 a	24.3 a
WBM15	22.5 c	6.1 b	15.0 c

Note: Means with different small letters within each column are significantly different ($p < .05$).

Abbreviations: CM, breads supplemented with $CaCO_3$ and Fe–Zn mixture; RSM15, breads supplemented with RS4 at 15% level and $CaCO_3$ and Fe–Zn mixture; WBM15, breads supplemented with wheat bran at 15% level and $CaCO_3$ and Fe–Zn mixture.

4 | CONCLUSIONS

RS4 has a good potential to be used as a dietary fiber source in bread formulations. RS4 provides better appearance and texture than wheat bran. The wheat bran had substantial deteriorative effect on the crust color, loaf volume, and firmness values of the breads. The RS4 resulted in a lighter crust color than the control bread and bread supplemented with wheat bran. Hence, the bread supplemented with RS4 is expected to be more desirable than the bread supplemented with wheat bran in terms of consumer acceptability. In addition to its lower negative effects on baking quality, RS4 supplementation caused increases in TDF, BA-binding capacity, and mineral bioavailability values and decrease in vitro glycemic index as compared to the wheat bran supplementation. The results of this study proved that RS4 is a better dietary fiber source than wheat bran in terms of mineral bioavailability and BA-binding capacity and RS supplementation level could be increased, without substantial adverse effects on bread quality.

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CONFLICT OF INTEREST

The authors have no conflict of interest regarding the content of this paper.

AUTHOR CONTRIBUTIONS

H. Koksel and M. Aribas: designed the study; M. Aribas: involved in chemical and physicochemical analysis, bread making and quality determination, in vitro bile acid-binding capacity, glycemic index value and mineral bioavailability, total dietary fiber content analysis, statistical analysis, and manuscript writing under H. Koksel supervising; K. Kahraman: involved in in vitro glycemic index value, total dietary fiber content analysis, statistical analysis, and manuscript writing.

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REFERENCES

AACC International (2009). *AACC International approved methods*. https://doi.org/10.1094/aaccintmethods

Almeida, E. L., Chang, Y. K., & Steel, C. J. (2013). Dietary fibre sources in bread: Influence on technological quality. *LWT - Food Science and Technology*, *50*(2), 545–553. https://doi.org/10.1016/j.lwt.2012.08.012

Altuna, L., Ribotta, P. D., & Tadini, C. C. (2015). Effect of a combination of enzymes on dough rheology and physical and sensory properties of bread enriched with resistant starch. *LWT - Food Science and Technology*, *64*(2), 867–873. https://doi.org/10.1016/J.LWT.2015.06.024

Baghurst, P. A., Baghurst, K. I., & Record, S. J. (1996). *Dietary fibre, non-starch polysaccharides and resistant starch: A review*. Retrieved from https://publications.csiro.au/rpr/pub?list=BRO&pxml:id=procite:1fb4f67a-47a3-4c8d-84a1-e3709102a1c2

Baixauli, R., Salvador, A., Martínez-Cervera, S., & Fiszman, S. M. (2008). Distinctive sensory features introduced by resistant starch in baked products. *LWT - Food Science and Technology*, *41*(10), 1927–1933. https://doi.org/10.1016/J.LWT.2008.01.012

Barros, J. H. T., Telis, V. R. N., Taboga, S., & Franco, C. M. L. (2018). Resistant starch: Effect on rheology, quality, and staling rate of white wheat bread. *Journal of Food Science and Technology*, *55*(11), 4578–4588. https://doi.org/10.1007/s13197-018-3393-6

Codex, T. F. (2017). *Turkish Food Codex Regulation on labeling and provision of food information to consumers*.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*(Suppl 2), S33–S50.

Englyst, H. N., Veenstra, J., & Hudson, G. J. (1996). Measurement of rapidly available glucose (RAG) in plant foods: A potential in vitro predictor of the glycaemic response. *The British Journal of Nutrition*, *75*(3), 327–337.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M. J., Sánchez-Zapata, E., & Pérez-Álvarez, J. A. (2010). Resistant starch as functional ingredient: A review. *Food Research International*, *43*(4), 931–942. https://doi.org/10.1016/J.FOODRES.2010.02.004

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, *17*(3), 427–437. https://doi.org/10.1016/S0271-5317(97)00010-9

Hasjim, J., Ai, Y., & Jane, J. (2013). Novel applications of amylose-lipid complex as resistant starch type 5. In Y. C. Shi & C. C. Maningat (Eds.), *Resistant starch* (pp. 79–94). Hoboken, NJ: John Wiley and Sons.

Hinkle, E. (2013). *A study of cooking and varietal effects on potato in vitro bile acid binding capacity*. Honors College. https://digitalcommons.library.umaine.edu/honor

Homayouni, A., Amini, A., Keshtiban, A. K., Mortazavian, A. M., Esazadeh, K., & Pourmoradian, S. (2014). Resistant starch in food industry: A changing outlook for consumer and producer. *Starch - Stärke*, *66*(1–2), 102–114. https://doi.org/10.1002/star.201300110

Kumar, A., Sahoo, U., Baisakha, B., Augustine Okpani, O., Ngangkham, U., Basak, N., … Sharma, S. (2018). Resistant starch could be decisive in determining the glycemic index of rice cultivars. *Journal of Cereal Science*, *79*, 348–353. https://doi.org/10.1016/j.jcs.2017.11.013

Maningat, C. C., & Seib, P. A. (2013). RS4-type resistant starch: Chemistry, functionality and health benefits. In Y. C. Shi & C. C. Maningat (Eds.), *Resistant starch* (pp. 43–77). Chichester, UK: John Wiley & Sons.

Miller, R. A., & Bianchi, E. (2017). Effect of RS4 resistant starch on dietary fiber content of white pan bread. *Cereal Chemistry Journal*, *94*(2), 185–189. https://doi.org/10.1094/CCHEM-03-16-0048-R

Nugent, A. P. (2005). Health properties of resistant starch. *Nutrition Bulletin*, *30*(1), 27–54. https://doi.org/10.1111/j.1467-3010.2005.00481.x

Ozturk, S., Koksel, H., & Ng, P. K. W. (2009). Farinograph properties and bread quality of flours supplemented with resistant starch. *International Journal of Food Sciences and Nutrition*, *60*(6), 449–457. https://doi.org/10.1080/09637480701822450

Rizkalla, S. W., Bellisle, F., & Slama, G. (2002). Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. *British Journal of Nutrition*, *88*(S3), 255. https://doi.org/10.1079/BJN2002715

Rosell, C. M., Santos, E., & Collar, C. (2010). Physical characterization of fiber-enriched bread doughs by dual mixing and temperature constraint using the Mixolab®. *European Food Research and Technology*, *231*(4), 535–544. https://doi.org/10.1007/s00217-010-1310-y

Sajilata, M. G., & Singhal, R. S. (2005). Specialty starches for snack foods. *Carbohydrate Polymers*, *59*(2), 131–151. https://doi.org/10.1016/J.CARBPOL.2004.08.012

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch? A review. *Comprehensive Reviews in Food Science and Food Safety*, *5*(1), 1–17. https://doi.org/10.1111/j.1541-4337.2006.tb00076.x

Sanz, T., Salvador, A., & Fiszman, S. M. (2008). Resistant starch (RS) in battered fried products: Functionality and high-fibre benefit. *Food Hydrocolloids*, *22*(4), 543–549. https://doi.org/10.1016/J.FOODHYD.2007.01.018

Sanz-Penella, J. M., Wronkowska, M., Soral-Śmietana, M., Collar, C., & Haros, M. (2010). Impact of the addition of resistant starch from modified pea starch on dough and bread performance. *European Food Research and Technology*, *231*(4), 499–508. https://doi.org/10.1007/s00217-010-1294-7

Sharma, A., Yadav, B. S., & Ritika, A., (2008). Resistant starch: Physiological roles and food applications. *Food Reviews International*, *24*(2), 193–234. https://doi.org/10.1080/87559120801926237

Shyu, Y.-S., Hwang, J.-Y., Huang, T.-C., & Sung, W.-C. (2018). Effect of resistant starch on physicochemical properties of wheat dough and bread. *Journal of Food and Nutrition Research*, *6*(5), 335–340. https://doi.org/10.12691/jfnr-6-5-9

Simsek, S., & El, S. N. (2012). Production of resistant starch from taro (*Colocasia esculenta* L. Schott) corm and determination of its effects on health by in vitro methods. *Carbohydrate Polymers*, *90*(3), 1204–1209. https://doi.org/10.1016/j.carbpol.2012.06.039

Stewart, M., Wilcox, M., Bell, M., Buggia, M., & Maki, K. (2018). Type-4 resistant starch in substitution for available carbohydrate reduces postprandial glycemic response and hunger in acute, randomized, double-blind, controlled study. *Nutrients*, *10*(2), 129. https://doi.org/10.3390/nu10020129

Stewart, M., & Zimmer, J. (2017). A high fiber cookie made with resistant starch type 4 reduces post-prandial glucose and insulin responses in healthy adults. *Nutrients*, *9*(3), 237. https://doi.org/10.3390/nu9030237

Suliburska, J., & Krejpcio, Z. (2014). Evaluation of the content and bioaccessibility of iron, zinc, calcium and magnesium from groats, rice, leguminous grains and nuts. *Journal of Food Science and Technology*, *51*(3), 589–594. https://doi.org/10.1007/s13197-011-0535-5

Ting, S. R., & Loh, S. P. (2016). In vitro bioaccessibility of calcium, iron and zinc from breads and bread spreads. *International Food Research Journal*, *23*(5), 2175–2180.

Wojciechowicz, A., Gil, Z., Spychaj, R., Czaja, A., Pejcz, E., Czubaszek, A., & Zmijewski, M. (2015). Effect of acetylated retrograded starch (resistant starch RS4) on the nutritional value and microstructure of the crumb (SEM) of wheat bread. *Journal of Food Processing & Technology*, *6*(6), 2. https://doi.org/10.4172/2157-7110.1000450

Zacherl, C., Eisner, P., & Engel, K.-H. (2011). In vitro model to correlate viscosity and bile acid-binding capacity of digested water-soluble and insoluble dietary fibres. *Food Chemistry*, *126*(2), 423–428. https://doi.org/10.1016/J.FOODCHEM.2010.10.113

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