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In vitro glycemic index, bile acid binding capacity and mineral bioavailability of spaghetti supplemented with resistant starch type 4 and wheat bran

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ABSTRACT

In this study effects of resistant starch type 4 (RS4) on nutritional and quality properties of spaghetti were investigated. RS4 was added into spaghetti at different levels (15, 20 and 25%). Wheat bran was also added to spaghetti at 15% level. RS4 supplementation caused lower negative effects on quality properties (total organic matter value, color and textural properties) than bran supplementation. The results point out that RS4 supplementation of spaghetti caused higher total dietary fiber content and comparable glycemic index value at the same bran supplementation level. RS4 caused an increase in bile acid binding capacity and expected to have positive effects on cholesterol metabolism. Moreover, RS4 supplemented spaghetti had generally better mineral bioavailability values compared to bran supplemented and control spaghettis. Overall results indicated that RS4 supplementation provided improvement in nutritional properties of spaghetti and can be an alternative ingredient for fiber supplemented spaghetti compared to bran.

1. Introduction

Pasta is the most commonly consumed food made of wheat following bread. It has been consumed in the Mediterranean countries for centuries (Tazrart, Lamacchia, Zaidi, & Haros, 2016). A good quality pasta should have a bright yellow color and "*al dente*" textural characteristics, good surface integrity and not have sticky surface (Dhiraj & Prabhasankar, 2013). Unlike simple sugars which offer a quick and short-term boost of energy, pasta products are a good carbohydrate source providing a slow release of energy (Gull, Prasad, & Kumar, 2018). While starch content of pasta products is generally high, their dietary fiber, mineral, vitamin and phenolic compound contents are relatively low (D'Amico et al., 2015). Several studies have been focused on enhancing nutritional and functional value of pasta with addition of proteins, dietary fibers, resistant starches, legume flours and banana flour (Menon, Padmaja, Sajeev, & Sheriff, 2012). Supplementation of pasta with fiber generally cause detrimental effects on the quality parameters such as texture, flavor, color, cooking time and dry matter loss to cooking water (Vernaza et al., 2012).

Resistant starch is defined as a fraction of starch that cannot be digested in the small intestine and passes to the colon to be fermented by the microbiota. There are 5 types of resistant starch (Birt et al., 2013). Resistant starch type 1 is physically entrapped in cellular matrix such as whole grains or partly milled cereal grains (Joye, 2019). Resistant starch type 2 is ungelatinized starch granules such as high amylose corn starch, unripe banana and raw potatoes. Resistant starch type 3 is a retrograded starch. It is found in cooked and cooled potatoes, rice and pasta (Snelson et al., 2019). Resistant starch type 4 (RS4) is a chemically modified starch which is formed typically through esterification, cross-linking or transglycosylation (Mah, Garcia-Campayo, & Liska, 2018). These modifications can prevent its digestion by blocking enzyme access (Nissar, Ahad, Naik, & Hussain, 2017). Its resistance to digestion depends on the type and extent of the chemical modification. Although it is widely used in commercial applications, producers share limited information about nature of RS4 and its modification levels. Different forms of RS4 are produced from different starch sources such as potato, tapioca, wheat, and/or high-amylose maize and supplied by commercial companies (Roman & Martinez, 2019). Resistant starch

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type 5 is starch that the amylose component forms complexes with lipids (Hasjim, Ai, & Jane, 2013).

When resistant starches are compared to the conventional fibers such as whole grains, bran and fruit fibers, they have many advantages such as less color change, bland flavor, lower water absorption capacity, better sensory properties, higher gelatinization temperature, and good extrusion product quality when included in food formulations (Sajilata, Singhal, & Kulkarni, 2006; Sanz-Penella, Wronkowska, Soral-Śmietana, Collar, & Haros, 2010; Vernaza et al., 2012).

Resistant starches are valuable ingredients as fiber source (Erickson, Carlson, Stewart, & Slavin, 2018). All types of RS have a lot of health benefits such as increasing the number of probiotic microorganisms in the colon, releasing glucose slowly and lowering insulin response, reducing the activity of lipogenic enzymes such as fatty acid synthesis, reducing the risk of the development of type 2 diabetes, obesity, coronary heart diseases, inflammatory bowel diseases, and gastrointestinal disorders (Gelencsér et al., 2008; Ghodke & Ananthanarayan, 2008; Homayouni et al., 2014). On the other hand, RS is a good ingredient which does not negatively affect textural and taste properties and improves sensory properties compared with the traditional fibers (Buttriss & Stokes, 2008). Cooking time, total organic matter, water absorption, cooking loss and the textural properties are the most important characteristics influencing the quality of pasta products (Gelencsér et al., 2008). The supplementation of foods with functional fibers reported to cause undesirable changes in sensory properties of the final products and consumer acceptability can decrease due to these changes (Homayouni et al., 2014). There are several studies investigating the effect of RS4 supplementation on the cooking quality, color parameters and starch digestibility values of pasta in the literature. In a study investigating the cooking properties of RS2 and RS4 supplemented pasta samples, it was found that RS did not disrupt the starch-protein interaction and quality characteristics of pasta samples were not negatively affected during cooking. 10% and 20% RS4 supplementation caused a lower starch digestibility value [the total area under curve (AUC, ($mg_{glucose}/g_{sample}$) × min)] than control sample (Gelencsér et al., 2008). In another study, 10% RS4 supplementation caused a decrease in the L* and b*color values and caused an increase in the a* value. The cooking loss values of pasta containing RS4 at the 5% and 10% level were lower than the control sample (0% RS4). Additionally, RS2 and RS4 supplementation resulted in significant increases in water absorption values (Bustos, Pérez, & León, 2011). Makhlouf et al. (2019) indicated that the supplementation of spaghetti with 5% and 10% RS4 caused a decrease and 15% RS4 supplementation caused an increase in cooking loss values.

To the best of authors knowledge, there are no studies investigating textural properties (uncooked and cooked), TDF contents, *in vitro* mineral bioavailability and bile acid binding capacity in RS4 supplemented spaghetti. The difference of this study from the previous ones was to investigate the effect of RS4 supplementation on the nutritional properties of spaghetti such as total dietary fiber (TDF) content, *in vitro* mineral bioavailability, *in vitro* glycemic index (GI) and bile acid (BA) binding capacity. Common dietary fiber sources including wheat bran usually cause undesirable changes on quality and some of the nutritional properties of spaghetti resulting in decreased consumer acceptability and mineral bioavailability. This is a major problem in high fiber spaghetti production. The aim of this study was to develop high fiber spaghetti by RS4 supplementation with better consumer acceptability and improved nutritional properties. Therefore, RS4 was added into the spaghetti at different levels (15%, 20%, 25%; semolina basis). Wheat bran (WB) was also used (at 15% level) in the spaghetti formulation for comparison.

2. Materials and methods

2.1. Materials

Semolina, RS4 (phosphorylated cross-linked wheat starch with 85.4% TDF content) and commercial wheat bran were obtained from Nuh'un Ankara Makarna (Ankara, Turkey), Demirpolat Inc. (Konya, Turkey) and Ankara Halk Ekmek Inc. (Ankara, Turkey), respectively. Bran was milled by using Perten 3100 laboratory grinder (Perten Ins., Hudinge, Sweden) equipped with 500 μm sieve. Ca (in the form of $CaCO_3$) and Fe-Zn mixture were obtained from UNO (Istanbul, Turkey). The chemicals used in this study were of analytical reagent grade, unless otherwise specified.

2.2. Methods

2.2.1. Chemical and physicochemical analyses

Moisture and ash contents of the samples (semolina, bran and RS4) were determined using AACC Method 44-15A and 08-01.01, respectively (AACC International, 2009). Protein content of the semolina sample was determined using AACC Method 46-30 with Dumas Nitrogen Analyzer (Velp-NDA 701, Usmate, Italy). SDS sedimentation value and dry gluten content of the semolina sample were measured according to Williams, El-Haramein, Nakkoul, and Rihawi (1988) and AACC Method 38-12A, respectively (AACC International, 2009).

2.2.2. Spaghetti processing and drying

Semolina samples were processed into spaghetti using laboratory scale spaghetti processing equipment (Namad, Italy) according to the method of D'Egidio et al. (1982). RS4 and wheat bran samples were added into the respective spaghetti formulations at different levels (15%, 20%, 25% for RS4 and 15% for wheat bran; semolina basis, final mass was kept constant). 15% bran supplementation level is commonly used by commercial pasta processing industry. Furthermore, bran supplementation at 15% level and above could have more deteriorative effects on quality parameters of spaghetti especially cooking loss value. Therefore, in this study bran supplementation level was used as 15%. Wheat bran or RS4 was first blended with semolina in a 5-liter container and transferred to a pre-mixer (Namad, Italy) and was mixed for 15 min prior to water addition. The amount of water was in the range of 31–33% based on the level of RS4/bran. Water was added to the semolina or semolina-RS4/bran mixture and dough was mixed by using the pre-mixer (Namad, Italy) for 15 min. The spaghetti was extruded under vacuum at a pressure of 500 mmHg. Spaghetti samples (1.7 mm thick) were dried in a pilot-scale drier (Namad, Italy) at 40 °C to a final moisture content of 12%. Spaghetti production was performed in duplicate. The spaghetti samples were packed in plastic bags and stored at room temperature until the analyses.

For the determination of *in vitro* calcium, iron and zinc bioavailability; calcium carbonate (Vitamiks, UNO, Turkey) and Fe-Zn mixture (Vitamiks, UNO, Turkey) were added to the spaghetti formulation at the levels of daily intake values stated in the Turkish Food Codex Regulation on labeling and provision of food information to consumers (Anonymous, 2017). Daily Reference Intake Values are the amounts of minerals recommended per day for healthy individuals at four years and over and were indicated as 800, 14 and 10 mg/100 g for calcium iron and zinc, respectively.

2.2.3. Texture analysis of uncooked spaghetti

The textural properties of uncooked spaghetti (flexure) were determined using a TA.XT.plus texture analyzer (Stable Micro Systems, Godalming, Surrey, England) equipped with a load cell of 5 kg according to AACC Method No. 16.50 (AACC International, 2009). The spaghetti flexure (A/SFR) probe was used to measure the compression and flexure. The uncooked spaghetti sample of 200 mm length was located between upper and lower supports in centrally located holes of the probe for the flexure measurement. Then, the average force (g) and distance (mm) to break was measured. The test was done with a pre-test speed of 0.5 mm/s, test speed of 2.5 mm/s and post-test speed of 10 mm/s.

2.2.4. Spaghetti quality

The quality properties of spaghetti were evaluated by several physical, chemical, textural, and nutritional characteristics. Cooking time is

defined as the time required to obtain complete gelatinization of starch and determined according to AACC Method No. 66-50 (AACC International, 2009). Total organic matter (TOM) is the quantity of organic substances released from the surface of cooked pasta during exhaustive rinsing. It was determined by a chemical method according to D'Egidio et al. (1982). Cooking loss (CL) is the amount of solid substance lost to cooking water. The cooking water was collected in a tared beaker and evaporated in an air oven at 98 °C. The residue was weighed and expressed as percentage of the starting material.

The water absorption of the samples was evaluated by using 25 g dry spaghetti sample. The cooked spaghetti sample was weighed five minutes after draining. Water absorption was expressed as grams of water absorbed (during cooking) per gram of dry pasta (Ozderen, Olanca, Sanal, Ozay, & Koksel, 2008).

The sensory characteristics of the cooked spaghetti samples were evaluated by a panel of experts. It was determined according to the methods of D'Egidio, Mariani, Nardi, and Novaro (1993) under test conditions of the International Standard 7304 (ISO, 2016). Final judgment was obtained by averaging the values of all the experts. The sensory properties evaluated were hardness (or firmness), adhesiveness (or stickiness) and bulkiness (or clumpiness). Hardness was stated as the force required to bite through the cooked spaghetti strand with the incisors. Adhesiveness was defined as the material adhering to the surface of cooked spaghetti and bulkiness was defined as the adhesion degree of cooked spaghetti strands among each other. All of the sensory properties were evaluated by a score of 10–100. Judgment scores for bulkiness and adhesiveness were assigned as: < 20 = very high, 40 = high, 60 = average, 80 = almost absent, 100 = absent. Judgment scores for hardness were: < 20 = very low, 40 = low, 60 = sufficient, 80 = good, 100 = very good (Basman, Koksel, & Atli, 2006; Pestorić, Summary, & Pestoric, 2012).

The textural properties of cooked spaghetti (firmness and stickiness) were determined using a TA.XT.plus texture analyzer (Stable Micro Systems, Godalming, Surrey, England) equipped with a load cell of 5 kg according to AACC Method No. 16.50 (AACC International, 2009). The stickiness and firmness were determined after cooking the spaghetti for the optimum time. The pasta firmness/stickiness probe (HDP/PFS) was used to determine stickiness. The test was done with a hold time of 2 s and tracking speed of 0.5 mm/s. Stickiness (g) was stated as the maximum force to separate the probe from the sample's surface upon retraction of the probe. Cooked spaghetti quality firmness probe (A/LKB-F) was used to determine firmness. Firmness (g·cm) was determined as the force used to cut through the sample.

The color of spaghetti sample was measured using a spectrophotometer (Minolta, CM-3600d, Japan) according to CIE L*, a*, b* color space parameters.

The microstructure of surface of the uncooked spaghetti samples was determined by scanning electron microscopy (SEM). Samples were mounted on circular metal stubs coated with double-sided adhesive carbon tape. Each stub had 1 longitudinal piece for one sample selected at random from a larger sample. The mounted samples were coated with gold for 4 min using a Sputter Quorum Coater. Samples were viewed using a Zeiss Geminisem 300 SEM (Germany) at 250× magnification level.

2.2.5. 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). 2 mL 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 (3300 U/mL, Megazyme Int., Ireland) 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 x*g* for 10 min, and glucose content was measured with glucoseoxidase-peroxidase (GOPOD) reagent (Megazyme Int., Ireland) by using a spectrophotometer (Shimadzu 1601, Japan) at 510 nm wavelength.

Several researchers showed a high correlation between the rate of starch digestion and the glycemic response by various *in vitro* digestion methods that imitate the *in vivo* methods (Goñi, Garcia-Alonso, & Saura-Calixto, 1997). Goñi et al. (1997) stated that the kinetics of *in vitro* digestion is followed by a nonlinear model with a first order equation of $C = C_{\infty}(1 - e^{-kt})$, where C is the percentage of starch hydrolyzed at time t (min), C∞ is the equilibrium percentage of starch hydrolyzed after 180 min, and k is the kinetic constant. The hydrolysis index (HI) shows starch digestion rate and estimated glycemic index (GI) indicates the digestibility of the sample against white bread. The hydrolysis index (HI) is the ratio of the area under the hydrolysis curve of the sample to the area under the hydrolysis curve of white bread as reference sample. The HI was calculated as follows (Eq.1);

$$\mathrm{HI} = \frac{\text{Area under the curve of the sample}}{\text{Area under the curve of white bread}} \quad (1)$$

The *in vitro* GI was determined by using the following equation (Eq. (2)) of (Goñi et al., 1997).

$$\mathrm{GI} = 39.71 + 0.549\mathrm{HI} \quad (2)$$

2.2.6. Total dietary fiber

The total dietary fiber contents of RS4, bran and spaghetti samples were determined according to AACC Method No. 32–07.01 (AACC International, 2009) using total dietary fiber determination kit (Megazyme Int. Ireland). 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 pre-weighted crucible containing dietary fiber residue. Then it was washed with alcohol and acetone, filtered, dried, weighed and expressed as % (g TDF/100 g dry sample).

2.2.7. In vitro bile acid binding capacity

In vitro bile acid binding capacity was determined according to the method of Zacherl, Eisner, and Engel (2011) with some modifications. Sample (50 mg) was dispersed in water (ratio 1:2) by brief vortexing. HCl (1 mL, 0.01 N) was added and 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 21,700 x*g* for 10 min, the supernatant was kept in boiling water for 5 min to inactivate the enzymes. After cooling, methanol and KH_2PO_4 were added to the solution. The sample was filtered through a 0.45 μm filter for HPLC analysis. The mobile phase for HPLC was methanol–sodium phosphate-water (v:v:v, 70:20:10) solution with a flow rate 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. Bile acid binding values of the samples were calculated by subtracting the unconjugated bile acid content from the total bile acid content and expressed as μmol/100g and % bound relative to Cholestyramine.

2.2.8. 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 carried out according to Suliburska and Krejpcio (2014). *In vitro* mineral

bioavailability was determined for the samples supplemented with 15% RS4 and 15% WB. Control spaghetti sample (100% semolina) was also analyzed.

The samples (10 g) 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 h. 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 h. After the digestion, the samples were centrifuged for 20 min at a speed of 15,100 *xg*. The supernatant (25 mL) was transferred to Teflon vessels and ashed with HNO_3 (7 mL, 65%) by means of a closed pressurized system microwave oven (MARS-5 CEM, USA). 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 deionized water and the minerals were determined by atomic absorption spectrophotometer (AAS) (iCE 3000, Thermo Scientific, USA).

For the determination of total amount of the minerals 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.

2.2.9. Statistical analysis

All of the spaghetti samples were produced in two replicates. The results are reported as means of duplicate analyses on each replicate spaghetti sample (2 replicate spaghetti sample × 2 duplicate analyses). The data in this study are presented as mean ± SD. Data were analyzed by using one-way analysis of variance (ANOVA). When significant ($p < 0.05$) 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 semolina, wheat bran and RS4 samples

Moisture contents of semolina, bran and RS4 samples were 12.8%, 9.5% and 5.4% respectively and ash contents of semolina, bran and RS4 samples were 0.93%, 5.50% and 0.05%, respectively. SDS sedimentation value, protein (Nx5.7, dry basis), and dry gluten contents of the semolina sample were 24 mL, 13.3% and 12.2%, respectively. RS4 and bran supplementation have weakening effect on protein matrix of spaghetti, causing inferior quality. Therefore, a semolina sample with relatively high gluten content and good quality characteristics was used in the production of RS4 and bran supplemented spaghetti to eliminate their potential deteriorative effects on spaghetti quality.

3.2. Quality properties of spaghetti samples

Spaghetti was cooked in boiling water and samples were taken at certain time intervals. The cooking time was determined by squeezing the cooked spaghetti between two clear plastics. When white center core just disappeared the time was recorded as the "cooking time". The optimum cooking times of the spaghetti samples supplemented with different levels of RS4 (0, 15, 20 and 25%) and wheat bran were 13.0 min, 12.0 min, 12.5 min, 12.8 min and 14.0 min, respectively.

Total organic matter (TOM), cooking loss (CL) and water absorption values of the spaghetti samples supplemented with different levels of RS4 and wheat bran are presented in Table 1. Significant increases in TOM values of RS4 supplemented spaghetti samples were observed as the supplementation level increased. The highest TOM value was observed for the spaghetti sample supplemented with 25% RS4. TOM value of the sample supplemented with wheat bran was significantly higher as compared to the sample supplemented with RS4 at the same level. It was indicated that semolina proteins are linked together by disulfide, hydrogen, and hydrophobic bonds to form a matrix. The continuity and strength of this protein matrix depends on inter- and intra-molecular bonds. This matrix slowly breaks apart during the cooking and stickiness on the cooked pasta surface begins to increase (Tudorică, Kuri, & Brennan, 2002). Due to this increase, total organic matter on the pasta surface also increases. In this study, RS4 and bran supplementation caused weakening of these bonds. TOM values of the RS4 and bran supplemented samples increased because of weakening these bonds. D'Egidio et al. (1982) had stated that the TOM values can be used for the quality classification of spaghetti samples: TOM values lower than 1.4% indicate very good quality spaghetti, the values between 1.4% and 2.1% indicate good quality spaghetti and the values greater than 2.1% indicate poor quality spaghetti. TOM value of the control sample was 1.15%, indicating a very good quality. The spaghetti samples supplemented with 15% RS4, 20% RS4 and 15% bran had TOM values of 1.56%, 1.67% and 1.74%, respectively, indicating good quality. The supplementation of spaghetti with 25% RS4 caused a significant increase in TOM value (2.21%) indicating the poor quality of the sample.

Table 1
Total organic matter (TOM), cooking loss and water absorption values of spaghetti samples supplemented with different levels of RS4 and wheat bran.

Sample	Addition level (%)	TOM (%)	Cooking Loss (%)	Water Absorption (%)
CS	0	1.15 ± 0.03^{e}	5.95 ± 0.21^{e}	179.97 ± 3.34^{a}
RS15	15	1.56 ± 0.05^{d}	6.13 ± 0.01^{d}	154.97 ± 1.19^{b}
RS20	20	1.74 ± 0.21^{b}	6.39 ± 0.02^{c}	150.63 ± 2.51^{bc}
RS25	25	2.21 ± 0.10^{a}	6.55 ± 0.03^{b}	147.18 ± 1.12^{c}
WB15	15	1.67 ± 0.05^{c}	8.01 ± 0.22^{a}	151.70 ± 0.37^{bc}

CS; Control spaghetti, RS15; spaghetti supplemented with RS4 at 15% level, RS20; spaghetti supplemented with RS4 at 20% level, RS25; spaghetti supplemented with RS4 at 25% level, WB15; spaghetti supplemented with wheat bran at 15% level, S; Semolina.
[a,e]Means with different small letters within each column are significantly different ($p < 0.05$).

The cooking loss values increased significantly with RS4 and bran supplementation. The spaghetti sample supplemented with bran had the highest CL value (8.01%) among the fiber supplemented samples. Sato et al. (2019) stated that up to 6% CL, the pasta can be specified as high quality; CL values between 6% and 8% means medium quality pasta and CL higher than or equal to 10% means poor quality pasta. Grzybowski and Donnelly (1979) also indicated that 8% CL value is the maximum acceptable level for pasta. According to the CL values, the spaghetti samples supplemented with RS4 (at all levels) and bran can be classified as medium quality spaghetti. A study on quality characteristics of pasta supplemented with different types of RS rich ingredients (RS2; Himaize™260, Hi-maize™1043 and RS4; Fibersym™70) indicated that, the CL values of pasta supplemented with 10% and 20% RS ranged from 5.2 to 6.1% (Gelencsér et al., 2008). These results are in agreement with the values obtained for the samples supplemented with RS4 in the present study. It was stated that the weakening or disruption of the protein-starch matrix causes high cooking loss in pasta (Izydorczyk, Lagassé, Hatcher, Dexter, & Rossnagel, 2005). Due to disruptions in the protein matrix, the spaghetti samples supplemented with RS4 and bran had higher cooking loss values than the control. It is known that addition of nonendosperm components to dough promote physical disruption of the gluten protein matrix (Gan, Ellis, Vaughan, & Galliard, 1989). The decrease in the gluten concentration (dilution due to added material) is one of the responsible factors causing the weakening of the gluten network (Brennan & Cleary, 2007; Courtin & Delcour, 2002). On the other hand, some researchers showed that inclusion of fibers into a dough system weakens the gluten network by disrupting intermolecular associations of gluten proteins (Skendi, Biliaderis, Papageorgiou, & Izydorczyk, 2010; Sullivan, O'Flaherty, Brunton, Arendt, & Gallagher,

2011) and altering protein-matrix continuity (Bustos, Perez, & Leon, 2015). The most affected parameter of cooked pasta due to fiber addition is the cooking loss. The disruption in the gluten matrix allows more gelatinized starch leach from the pasta during cooking which results in an increase in the cooking loss (Brennan, Kuri, & Tudorica, 2004; Inglett, Peterson, & Carriere, 2005; Manno et al., 2009). Aravind, Sissons, Egan, and Fellows (2012) investigated the effect of fiber and resistant starch addition on the pasta structure. Based on the SEM images, bran addition appears to alter protein matrix continuity. On the other hand, resistant starch appears to be easily incorporated into pasta structure while maintaining the integrity of protein matrix (Bustos, Perez, & León, 2013; Manthey & Schorno, 2002). Since RS4 does not gelatinize at spaghetti cooking temperature, it is not expected to results in an increase in the cooking loss. Aravind et al. (2012) stated that the addition of bran with a particle size of 150–500 μm had negative effects on various sensory and technological properties of pasta. Resistant starch has a particle size of 10–15 μm (Sajilata et al., 2006). Particle size difference between wheat bran and resistant starch may be the other reason of their different effects on the cooking loss. The higher particle size causes more disruption in the gluten matrix, therefore higher cooking loss.

The water absorption values of the spaghetti samples supplemented with RS4 and wheat bran was significantly ($p < 0.05$) lower than that of the control sample (Table 1). While the sample supplemented with 25% RS had the lowest water absorption value, there were no significant differences between the samples supplemented with 20% RS4 and wheat bran. Gelencsér et al., 2008 stated that RS supplementation did not cause significant effect on water absorption compared to the control pasta produced from durum wheat. It was indicated by Bustos et al. (2015) that water absorption of pasta is associated with starch swelling and gelatinization. A disrupted gluten matrix will cause granules to absorb more water and gelatinize, so that an increase of water absorption will be observed. On the other hand, it has been stated that strong protein-starch matrix caused an increase in the water absorption of pasta (Nilusha, Jayasinghe, & Perera, 2019). Hence, there seems to be some conflicting conclusions in the literature on this matter and water absorption is not generally accepted as a major quality parameter in spaghetti.

Sensory properties (hardness, adhesiveness, bulkiness) of the spaghetti samples supplemented with different levels of RS4 and wheat bran are presented in Table 2. Hardness, adhesiveness and bulkiness values of the samples supplemented with RS4 and bran were lower than those of control sample, as they caused disruption of the protein matrix. The increase in the RS4 supplementation level caused significant decreases in the hardness, adhesiveness and bulkiness ($p < 0.05$). The lowest average hardness, adhesiveness and bulkiness score were obtained for the spaghetti sample supplemented with 25% RS4 and 15% bran ($p < 0.05$). The spaghetti supplemented with 15% RS4 had the best scores of hardness, bulkiness and adhesiveness among the RS4 and bran supplemented samples. It also had lower TOM values (Table 1), indicating better quality.

Table 3 displays the textural properties of spaghetti samples supplemented with different levels of RS4 and wheat bran. The flexure values were measured for the uncooked spaghetti samples, while stickiness and firmness values were measured for the cooked spaghetti samples. The breaking force values decreased significantly with increasing RS4 supplementation level. The lowest breaking force value (31.43 g) was observed for the spaghetti sample supplemented with 25% RS4. While supplementation of bran caused decrease in breaking force value of the sample, there was no significant difference between samples supplemented with 20% RS4 and bran. The breaking strength of dry spaghetti can be a good indicator of how well it has been processed. It also indicates spaghetti's gluten quality and protein matrix integrity, how well it will cope with the handling, storage and cooking process. A low breaking force indicates a weak spaghetti (Aranibar et al., 2019; Smweing, 1997). The loss in the integrity of the protein matrix will cause a decrease in the breaking strength of dried spaghetti. As the incorporation of RS and bran causes disruption in the protein matrix, a decrease in the breaking force was observed. It was stated that fiber supplemented noodle with small particle size had higher breaking force value than the noodle supplemented with large particle size fiber (Shiau, Wu, & Liu, 2012). In the present study, the bran supplemented spaghetti had lower breaking force value as compared to 15% RS4 supplemented one probably because of its larger particle size compared to RS4. Hence, a higher concentration of RS4 (20%) was necessary to result in a comparable breaking force value provided by bran supplementation. The breaking distance values had a similar trend with the breaking force values.

Table 2
Sensory characteristics (hardness, adhesiveness, bulkiness) of spaghetti samples supplemented with different levels of RS4 and wheat bran.

Sample	Sensory characteristics		
	Hardness	Adhesiveness	Bulkiness
CS	65.2 ± 2.2[a]	62.4 ± 1.6[a]	61.1 ± 1.2[a]
RS15	57.4 ± 1.3[b]	55.3 ± 1.5[b]	54.5 ± 2.8[b]
RS20	49.2 ± 1.3[c]	49.1 ± 0.9[c]	47.4 ± 2.1[c]
RS25	41.1 ± 1.1[d]	42.1 ± 2.1[d]	41.2 ± 1.2[d]
WB15	42.3 ± 1.4[d]	45.0 ± 1.3[d]	43.1 ± 1.3[d]

CS; Control spaghetti, RS15; spaghetti supplemented with RS4 at 15% level, RS20; spaghetti supplemented with RS4 at 20% level, RS25; spaghetti supplemented with RS4 at 25% level, WB15; spaghetti supplemented with wheat bran at 15% level, S; Semolina.
[a,d]Means with different small letters within each column are significantly different ($p < 0.05$).

The stickiness values of the spaghetti samples increased significantly with RS4 and bran supplementation. While the difference in the stickiness values of the spaghetti samples supplemented with 20% RS4 and bran was not significant. Their stickiness values were significantly lower than the spaghetti sample supplemented with 25% RS4 ($p < 0.05$) which had the highest stickiness value. RS4 and bran supplementation caused increases in the stickiness on the cooked pasta surface as they weaken protein matrix during the cooking process. Adhesiveness/stickiness is related to the quantity of amylose leached to the cooking water (Bustos et al., 2015) and as stated above, the disruption in the gluten matrix allows more gelatinized starch to leach from the pasta during cooking, causing an increase in the stickiness. The difference in the stickiness values of the spaghetti samples supplemented with 20% RS4 and bran was not significant. This is probably due to the more pronounced deteriorative effects of bran as compared to RS4. Bran and RS4 supplementation caused increases in the stickiness on the cooked spaghetti surface as they weaken protein matrix during the cooking process. Liu and Shepherd (1996) stated that high quality cooked pasta had good textural properties, high surface disintegration resistance, low TOM value and did not show increased surface stickiness. In the present study, the stickiness values of the samples were highly correlated with the TOM values ($p < 0.001$, $r = 0.953$). Supplementation of RS4 and bran caused significant decreases in the firmness values of the cooked spaghetti samples. The lowest firmness value was observed for the spaghetti sample supplemented with bran. The textural parameters including firmness and stickiness of cooked pasta are very important in terms of consumer acceptability. Good quality pasta should have high firmness and elasticity values (Dhiraj & Prabhasankar, 2013). In other words, good quality cooked pasta should be firm to the bite (*al dente*) and not sticky (Carini, Curti, Minucciani, Antoniazzi, & Vittadini, 2014). In the present study, RS4 supplementation resulted in higher firmness value as compared to bran supplementation indicating better quality spaghetti. However, they all had lower firmness values as compared to that of the control spaghetti.

The color parameters of the spaghetti samples supplemented with different levels of RS4 and wheat bran are presented in Table 4. L*, a*

Table 3
Textural properties of spaghetti samples supplemented with different levels of RS4 and wheat bran.

Sample	Flexure		Stickiness (g)	Firmness (g·cm)
	Breaking force (g)	Distance (mm)		
CS	44.80 ± 3.17[a]	37.4 ± 2.28[a]	67.07 ± 4.26[d]	10.59 ± 1.03[a]
RS15	36.44 ± 4.70[b]	28.35 ± 4.94[b]	91.58 ± 4.34[c]	9.09 ± 1.30[b]
RS20	33.93 ± 3.67[c]	26.29 ± 2.73[c]	103.22 ± 6.13[b]	7.50 ± 1.80[c]
RS25	31.43 ± 3.24[d]	20.44 ± 1.42[d]	116.02 ± 6.47[a]	6.74 ± 1.37[d]
WB15	34.04 ± 4.85[c]	25.14 ± 2.15[c]	102.60 ± 5.72[b]	5.77 ± 0.81[e]

CS; Control spaghetti, RS15; spaghetti supplemented with RS4 at 15% level, RS20; spaghetti supplemented with RS4 at 20% level, RS25; spaghetti supplemented with RS4 at 25% level, WB15; spaghetti supplemented with wheat bran at 15% level, S; Semolina.
[a,e]Means with different small letters within each column are significantly different ($p < 0.05$).

and b* values of the control spaghetti sample was 54.04, 2.12 and 16.12, respectively. RS4 supplementation did not caused a significant change in the L* values of the spaghetti samples. Significant decreases were observed in the a* values of the spaghetti samples with RS4 addition ($p < 0.05$). On the contrary, wheat bran supplementation caused a significant decrease in L* value and a significant increase in the a* value of the control spaghetti sample. Similar to L* values, RS4 supplementation did not cause a significant decrease in the b* value of the spaghetti sample. However significant ($p < 0.05$) decrease in the b* value of the spaghetti sample was observed with bran supplementation. Besides the cooking quality and textural properties, color properties are also important parameters for the quality of pasta and pasta with a bright yellow color is usually more desirable in terms of consumer acceptability (Biernacka et al., 2018; Debbouz, Pitz, Moore, & D'appolonia, 1995). Among the spaghetti samples, the darkest color, in other words the lowest L* and the highest a* value was observed in the spaghetti supplemented with 15% wheat bran. It can be concluded that RS4 supplementation resulted in a spaghetti with much better color properties as compared to bran supplemented one.

SEM images of surface of the control spaghetti samples and the spaghetti samples supplemented with 15% bran, 15% RS4 and 25% RS4 are shown in Fig. 1. Distinct starch granules entrapped in a matrix could be identified in control sample (Fig. 1a) and RS4 supplemented samples (Fig. 1c and d). However, in the bran supplemented spaghetti (Fig. 1b), starch granules are less distinct, possibly disrupting the continuity of the protein starch matrix. Furthermore, as can be seen from Fig. 1b, bran supplemented spaghetti had a more irregular surface with a greater number of cracks and holes compared to control and RS4 supplemented spaghetti samples. Similar observations were also reported in the literature. Ramy, Salama, and Shouk (2002) and Aravind et al. (2012) observed similar cracks and holes in the pasta samples supplemented with dietary fiber. As stated before, bran addition appears to alter protein matrix continuity (Aravind et al., 2012), while resistant starch was easily incorporated into pasta structure probably due to its lower particle size while maintaining the integrity of protein matrix (Bustos et al., 2013; Manthey & Schorno, 2002).

Table 4
Color parameters of spaghetti samples supplemented with different levels of RS4 and wheat bran.

Sample	Color paramaters		
	L*	a*	b*
CS	54.04 ± 2.28[a]	2.12 ± 0.17[a]	16.12 ± 0.54[a]
RS15	56.14 ± 2.46[a]	1.37 ± 0.36[b]	15.15 ± 0.46[a]
RS20	57.00 ± 2.14[a]	1.27 ± 0.29[b]	14.92 ± 0.31[a]
RS25	58.59 ± 1.98[a]	1.20 ± 0.21[b]	14.66 ± 0.29[a]
WB15	48.32 ± 1.25[b]	4.74 ± 0.23[c]	7.60 ± 0.32[b]

CS; Control spaghetti, RS15; spaghetti supplemented with RS4 at 15% level, RS20; spaghetti supplemented with RS4 at 20% level, RS25; spaghetti supplemented with RS4 at 25% level, WB15; spaghetti supplemented with wheat bran at 15% level, S; Semolina.
[a,c]Means with different small letters within each column are significantly different ($p < 0.05$).

TDF contents and *in vitro* GI values of the spaghetti samples are presented in Table 5. TDF content of the cooked control spaghetti sample was 7.6%. Sobota and Zarzycki (2013) reported that the TDF contents of the spaghetti samples cooked for 16 min was 7% which was similar to the value obtained for the control spaghetti sample in the present study. TDF contents of RS4 and bran were 85.4% and 60.0%, respectively. The supplementation of RS4 and wheat bran significantly ($p < 0.05$) increased the TDF content of the spaghetti samples. The highest TDF content was observed for the spaghetti sample supplemented with 25% RS4. TDF content of the bran supplemented sample was significantly lower than the sample supplemented with RS4 at the same level. Food and Drug Administration suggest that dietary fiber intake should be 25–30 g per day (FDA, 2016). Our study indicated that one portion of cooked control pasta (around 150 g) can provide around 35–45% of the suggested dietary fiber intake, while one portion of cooked pasta supplemented with 25% RS4 can easily provide more dietary fiber intake than the suggested amount.

The *in vitro* GI value of the control spaghetti sample was 82.75. Bran and RS4 supplementation caused significant ($p < 0.05$) decreases in the *in vitro* GI values of the spaghetti samples. While bran supplementation caused a decrease in *in vitro* GI value, GI value of the bran supplemented spaghetti sample was not significantly different from the 15% and 20% RS4 supplemented samples. As a result of protein matrix disruption with bran/RS supplementation, more gelatinized starch leach out from starch during the pasta cooking. Besides this, concentration of digestible starch in the spaghetti samples decrease with the supplementation of spaghetti with bran/RS4. The resistance of bran/RS4 to digestion enzymes and the decrease in the starch concentration cause decreases in the *in vitro* GI values of spaghetti samples. Foods are classified as low (GI ≤ 55), medium (GI 56–69), and high (GI ≥ 70) glycemic index foods (Kumar, Sahoo, Baisakha, Augustine Okpani, Ngangkham, Basak, & Sharma, 2018). The results of the present study indicated that the spaghetti samples supplemented with 15 and 20% RS4 as well as 15% bran can be categorized as medium GI food. The spaghetti sample supplemented with 25% RS4 had a GI value of 56.50 which is very close to the GI value of low GI (≤55) foods. Gelencsér et al. (2008) reported that although, the durum samples supplemented with 10% and 20% RS4 had significantly lower total area under curve [AUC (mg glucose/g sample) × min] values compared to the control sample, there was no significant difference between the samples supplemented with 10% and 20% RS4.

Bile acids have an important role in lipid metabolism because of facilitating fat absorption. They are synthesized in the liver and then secreted into bile. After secretion, they are stored in the gall bladder. Then they are secreted into the duodenum for assisting numerous physiologically functions such as digestion of lipids (Dziedzic et al., 2016). *In vitro* bile acid binding values of the spaghetti samples are shown in Table 6. The results are stated both as the amount of BA

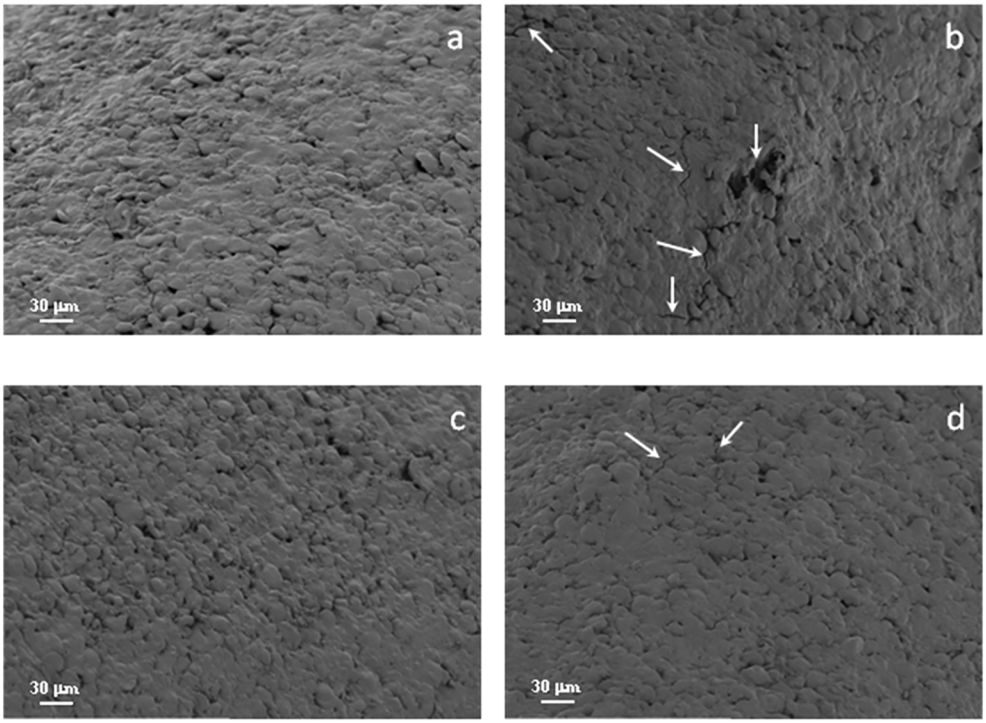

Fig. 1. SEM of surface of spaghetti samples. (a) control; (b) 15% bran supplemented; (c) 15% RS4 supplemented; (d) 25% RS4 supplemented. Magnification 250×. Arrows show the cracks.

Table 5
TDF contents and *in vitro* GI values of spaghetti samples.

Sample	Total Dietary Fiber (%)	*In vitro* GI
CB	7.6 ± 0.24^{e}	82.75 ± 0.32^{a}
RS15	21.3 ± 0.30^{c}	69.65 ± 0.34^{b}
RS20	26.8 ± 0.21^{b}	68.40 ± 0.43^{c}
RS25	31.3 ± 0.29^{a}	56.50 ± 0.63^{d}
WB15	17.3 ± 0.50^{d}	69.05 ± 0.02^{bc}

CS; Control spaghetti, RS15; spaghetti supplemented with RS4 at 15% level, RS20; spaghetti supplemented with RS4 at 20% level, RS25; spaghetti supplemented with RS4 at 25% level, WB15; spaghetti supplemented with wheat bran at 15% level, S; Semolina.
^{a,e}Means with different small letters within each column are significantly different ($p < 0.05$).

Table 6
In vitro bile acid binding capacity of spaghetti samples.

Sample	Bile Acid (BA) Binding Capacity	
	Bound BA (µmol/100 g)	Bound BA Relative to Cholestyramine (%)
CB	0.55 ± 0.01^{a}	5.5 ± 0.01^{a}
RS15	0.84 ± 0.02^{b}	8.4 ± 0.02^{b}
RS20	0.86 ± 0.01^{c}	8.6 ± 0.01^{c}
RS25	0.89 ± 0.03^{d}	8.9 ± 0.03^{d}
WB15	0.83 ± 0.01^{b}	8.4 ± 0.01^{b}

CS; Control spaghetti, RS15; spaghetti supplemented with RS4 at 15% level, RS20; spaghetti supplemented with RS4 at 20% level, RS25; spaghetti supplemented with RS4 at 25% level, WB15; spaghetti supplemented with wheat bran at 15% level, S; Semolina.
^{a,d}Means with different small letters within each column are significantly different ($p < 0.05$).

bound (µmol/100 g spaghetti) and as percent BA bound relative to Cholestyramine (%). BA binding capacity of spaghetti samples increased significantly with increasing level of RS4 supplementation ($p < 0.05$). The sample supplemented with 25% RS4 had the highest BA binding capacity. Both the bran supplementation and 15% RS4 supplementation caused the same BA binding capacity values. Bile acids are the end products of cholesterol catabolism and are synthesized from cholesterol in the liver. After their function they are reabsorbed and returned to the liver (Chiang, 2013; Dawson, Lan, & Rao, 2009). Dietary fibers have been reported to bind bile salts in the duodenum and this results the synthesis of additional bile salts from cholesterol which lowers blood cholesterol. Similar to other types of dietary fibers, RS binds to bile acids; prevents their reabsorption and removes them from the body (Grubben et al., 2001; Hinkle, 2013; Sharma, Yadav, & Ritika, 2008; Simsek & El, 2012; Trautwein, Kunath-Rau, & Erbersdobler, 1999). However, further research is needed to explain bile acid binding mechanism of resistant starch.

In vitro mineral bioavailability of the spaghetti samples supplemented with 15% RS4 and wheat bran are presented in Table 7. 800 mg Ca/100 g, 11.7 mg Fe/100 g and 10 mg Zn/100 g were added to the spaghetti formulation according to Turkish Food Codex Legislation (Anonymous, 2017). The Ca and Zn bioavailability values of the spaghetti sample supplemented with 15% of RS4 were significantly higher than those of the control sample enriched with minerals (CSM). The difference between the Fe bioavailability of RS4 supplemented spaghetti and CSM was not significant. Fe bioavailability of the sample supplemented with 15% of RS4 was slightly higher than that of CSM. However, the difference was not significant. Ca, Fe and Zn bioavailability of the spaghetti sample supplemented with bran was significantly lower than the sample supplemented with 15% RS4. Control spaghetti sample prepared from semolina with an ash content of 0.93% indicating that there was bran contamination and some phytic acid in the control spaghetti sample. On the other hand, RS4 sample used in the present study does not have any phytic acid to bind metallic cations. Hence, RS4 supplementation is expected to cause a dilution effect on phytic acid content of spaghetti while bran supplementation expected to increase phytic acid. Phytate is a salt form of phytic acid and located in the cereal bran. Phytate has six negatively charged phosphate groups which strongly bind metallic cations such as Ca, Fe, Zn, rendering them insoluble. They become unavailable for nutritional absorption because

Table 7
Bioavailability of calcium, iron and zinc of the spaghetti samples supplemented with 15% RS4 or wheat bran.

Sample	Minerals		
	Ca (%)	Fe (%)	Zn (%)
CSM	38.3 ± 0.14[b]	9.50 ± 0.51[a]	21.8 ± 0.72[b]
RSM15	43.5 ± 1.27[a]	10.0 ± 0.14[a]	29.5 ± 0.29[a]
WBM15	27.2 ± 2.16[c]	6.0 ± 0.12[b]	10.3 ± 0.31[c]

CSM; Spaghetti samples enriched with $CaCO_3$ and Fe-Zn mixture, RSM15; Spaghetti samples enriched with RS4 at 15% level and $CaCO_3$ and Fe-Zn mixture, WBM15; Spaghetti samples enriched with wheat bran at 15% level and $CaCO_3$ and Fe-Zn mixture.
[a,c]Means with different small letters within each column are significantly different ($p < 0.05$).

they are in insoluble form (Emanuelli, Milbradt, da Kolinski, Callegaro, & Augusti, 2014). Hence, bran supplementation caused lower mineral bioavailability values in spaghetti.

4. Conclusion

The results of the present study proved that RS4 had some advantages in terms of quality and nutritional properties in spaghetti. RS4 has a bland taste/flavor and did not have deteriorative effects on taste and flavor. RS4 supplementation resulted in better appearance and texture and sensory properties than wheat bran supplementation in spaghetti. Color of spaghetti is a quite important quality parameter in terms of consumer acceptability and bright yellow color is desirable in spaghetti. RS4 supplementation caused an increase in L* value of spaghetti. However, it caused decreases in a* and b* values. While bran supplementation caused an increase in a* value, it caused a decrease in L* and b* values of spaghetti. Resistant starch type 4 caused much better color values than the wheat bran, and the wheat bran had substantial deteriorative effect on the color of spaghetti. Bran and RS4 supplementation caused increase in cooking loss values of spaghetti. The highest cooking loss value was observed in bran supplemented spaghetti. Cooking loss was affected less from RS4 supplementation as compared to bran supplementation. RS4 supplementation resulted in significantly lower TOM and stickiness values and higher firmness value than the bran supplementation at the same level ($p < 0.05$). It also caused significantly better sensory properties than the wheat bran ($p < 0.05$). In addition to its lower detrimental effects on quality parameters, significant increases in total dietary fiber and mineral bioavailability values were observed in spaghetti samples supplemented with RS4 compared to the spaghetti sample supplemented with wheat bran. The decrease in *in vitro* glycemic index value and increase in bile acid binding capacity of the sample supplemented with wheat bran were also comparable with RS supplementation at 15% level. Besides these effects, increase in the RS4 supplementation level caused an increase in nutritional properties of spaghetti samples. The spaghetti sample supplemented with 25% RS4 has better nutritional properties such as *in vitro* GI, BA binding capacity and TDF content compared to the samples supplemented with RS4 at the other levels. Overall results indicated that RS4 can be an alternative ingredient in terms of lower *in vitro* GI and higher TDF content, *in vitro* BA binding capacity and mineral bioavailability values compared to wheat bran. Hence, RS supplementation might be an advantageous fiber source for high fiber spaghetti production.

Author contributions

H. Koksel, M. Aribas designed the study.

M. Aribas - Chemical and physicochemical analysis, spaghetti processing and drying, texture analysis of uncooked spaghetti, spaghetti quality analysis, *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 - *in vitro* glycemic index value, total dietary fiber content analysis, SEM analysis, statistical analysis, and manuscript writing.

Ethical statement

- This article does not contain any studies involving animals performed by any of the authors.
- This article does not contain any studies involving human participants performed by any of the authors.

CRediT authorship contribution statement

Merve Aribas: Conceptualization, Software, Validation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Kevser Kahraman:** Formal analysis, Software, Validation, Writing - original draft, Writing - review & editing, Visualization. **Hamit Koksel:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision, Project administration, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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