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The Study and comparison of the effect of replacing semolina with black and red rice flours on qualitative properties and antioxidant activity of pasta

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ABSTRACT

Black and red rice are rich in bioactive compounds, especially polyphenols, which exhibit strong antioxidant activity and may reduce the risk of chronic diseases. Pasta, as a widely consumed food, can be fortified with these rice flours to enhance its nutritional and functional properties. In this study, semolina was partially replaced with black and red rice flour (5, 10, and 15%), while 100% semolina pasta served as the control. The replacement levels were selected based on a preliminary trial, which indicated that levels up to 15% maintained acceptable dough handling properties and pasta structure. Chemical composition, rheological behavior, texture, cooking quality, color indices (a, b, L*), total phenolic content (TPC), and antioxidant activity (DPPH radical scavenging) were evaluated before and after cooking. Results showed that increasing rice flour levels significantly enhanced crude fiber, TPC, and antioxidant activity ($p \leq 0.05$). Rheological tests indicated higher water absorption, gluten softening, and starch gelatinization, while amylase activity and dough stability decreased. Chemical analysis revealed increased fat, protein, and ash contents. Cooking loss and weight gain rose with higher rice flour levels, whereas maximum cutting force decreased. Color indices shifted, with L* and b* decreasing in black rice samples, and a* increasing in red rice samples. Sensory evaluation indicated that pasta fortified with 15% black rice flour was most preferred for texture and taste.

1- INTRODUCTION

Pasta, a staple food with global consumption, is traditionally manufactured from durum wheat semolina, which imparts the desired amber color, firm texture, and cooking quality [1]. However, from a nutritional standpoint, conventional semolina-based pasta is often considered a source of carbohydrates with relatively low levels of dietary fiber, proteins, and bioactive compounds [2]. In recent years, growing consumer awareness of the link between diet and health has driven significant research interest in developing functional pasta by incorporating alternative flour sources rich in phytonutrients. Among these, pigmented rice varieties, such as black and red rice, have emerged as promising ingredients due to their exceptional nutritional profiles. These grains are abundant in phenolic compounds, flavonoids (notably anthocyanins like cyanidin-3-glucoside), and proanthocyanidins, which confer potent antioxidant activities linked to potential health benefits, including anti-inflammatory and anti-carcinogenic effects [3]. The selection of black and red rice flours was specifically justified by their distinct anthocyanin profiles (black rice) and proanthocyanidin content (red rice), which provide a comparative basis for studying their unique bioactive contributions to fortified pasta. Furthermore, these pigmented varieties are underutilized in pasta applications despite their high antioxidant potential compared to conventional cereal flours.

The partial or complete substitution of semolina with these novel flours presents a viable strategy for enhancing the nutritional value of pasta [4]. Nevertheless, this incorporation fundamentally alters the protein matrix and gluten network,

inevitably affecting critical technological and sensory attributes such as cooking loss, texture, firmness, and color stability [5]. The replacement levels (5%, 10%, and 15%) were chosen based on preliminary trials and literature reports indicating that higher levels (>20%) often lead to significant technological defects, such as increased cooking loss and loss of firmness, without a proportional gain in antioxidant activity.

Therefore, a systematic investigation is required to balance the nutritional enhancement with the preservation of acceptable quality parameters [6]. While several studies have explored the use of individual non-conventional flours in pasta, a direct comparative analysis of black and red rice flours, focusing on their distinct phytochemical contributions and their consequent impact on the multifaceted quality of the final product, remains less explored [7]. This study aims to comprehensively evaluate and compare the effects of replacing semolina with black rice flour and red rice flour on the qualitative properties (cooking characteristics, textural attributes, and color) and the *in vitro* antioxidant activity (measured by DPPH, ABTS, and FRAP assays) of fortified pasta. The findings will provide crucial insights for the food industry in formulating nutritionally superior pasta with optimized technological performance.

2- Materials and Methods

For the preparation of experimental treatments, varying proportions of black rice flour (BRF) and red rice flour (RRF) were separately used to replace semolina at three levels (5%, 10%, and 15%). To this end, whole black rice and red rice grains were milled using a hammer mill and subsequently passed through a 250-mesh sieve to obtain fine flour. For each

formulation, the specified proportion of BRF or RRF was thoroughly blended with semolina. To maintain a constant gluten content across all treatments, vital wheat gluten was added as a supplement (Table 1). The dry solid mixture was initially blended in a mixer for 3 minutes. Following this, water was gradually added, and mixing continued for a further 10 minutes in a pilot-scale mixer to achieve a homogenous, non-sticky, and properly hydrated dough with optimal consistency. The amount of water added was adjusted for each formulation to reach a constant dough consistency of approximately 450 Brabender Units (BU), as determined by a farinograph. The final dough was then extruded through a bronze die (penne rigate shape) at a temperature of 45°C and under a pressure of 120 mmHg. The extrusion screw speed was maintained at 30 rpm throughout the process to ensure consistent shearing and product uniformity. During extrusion, the temperature of the extrudate exiting the die was controlled by circulating water at 20°C around the die head. This step was crucial to prevent the freshly extruded pasta strands from sticking together and losing their shape. The extruded penne pasta was first

deposited onto plastic trays. A fan positioned beneath the trays operated continuously to facilitate initial surface drying and prevent cohesion of the pasta pieces. The pasta was then transferred to wooden trays with wire mesh for drying in an automatic dryer. The drying process was conducted in two distinct stages. The primary drying stage was carried out at a low temperature (approximately 50°C) and high relative humidity (55%) for 2 hours. This gentle initial phase aimed to prevent rapid surface drying and the consequent risk of checking (crack formation). The secondary drying stage employed a higher temperature (75°C) and lower relative humidity (20--30%). During the drying process, 2--3 trays containing pasta scraps were placed inside the dryer to generate sufficient humidity within the chamber. Upon completion of drying, the finished pasta was packaged and stored for subsequent analysis. For cooking, 20 g of pasta was boiled in 300 ml of distilled water for 8 minutes (the optimal cooking time determined for the control sample), then drained and rinsed with cold water for 10 seconds to stop the cooking process.

Table 1 Formulation of treatments.

Test treatments	Formulation
Control sample (C)	Semolina: 100%
First treatment (R5)	Semolina: 95% Red rice flour: 5%
Second treatment (R10)	Semolina: 90% Red rice flour: 10%
Third treatment (R15)	Semolina: 85% Red rice flour: 15%
Fourth treatment (B5)	Semolina: 95% Black rice flour: 5%
Fifth treatment (B10)	Semolina: 90% Black rice flour: 10%

Sixth treatment (B15)

Semolina: 85% Black rice flour: 15%

2-1 The investigated parameters

2-2-1 Assessment of Dough Rheological Properties Using a Mixolab

To characterize the rheological properties of the samples, a Mixolab device was employed. This test was conducted in accordance with the standard ICC method No. 173 [8]. All measurements were performed in triplicate, and the device was calibrated before each run.

2-2-2 Color measurement test

The color of the samples was measured by a Hunter lab device, which was measured by reflection on a Hunter lab colorimeter with parameters a^* , b^* and L^* , and the color parameter was determined [9]. Measurements were taken on both raw and cooked pasta samples, and each reading was performed in triplicate at different points on the sample surface.

2-2-3 Product weight after cooking

This test was performed by Iranian Standard Method 213 [10]. The weight of the cooked pasta was recorded after draining for 2 minutes.

2-2-4 Test for percentage of solids in cooking water

This test was performed by Iranian standard method 213 [10]. Cooking water was collected, dried in an oven at 105°C, and the residue was weighed to determine the percentage of solids lost.

2-2-5 Fiber Measurement

This test was performed according to the Iranian National Standard Test Method No. 3105 [11].

2-2-6 Texture Assessment

This procedure is derived from AACC Standard No. 50.01 66 based on the method of Oh et al. in 1983 [12]. A texture analyzer was used to measure the maximum cutting force (firmness) of cooked pasta strands.

2-2-7 Total phenolic content determination test

In calculating the total phenolic content, 1 ml of the extracted extract of each sample was mixed with 0.5 ml of Folin color reagent 0.5 normal, then the reaction mixture was mixed with 2 ml of 20% sodium carbonate and the absorbance of each was read at 760 nm using a spectrophotometer. Finally, the polyphenol content was reported based on milligrams of gallic acid in 100 g of sample [13]. All extractions were performed in triplicate, and results were expressed as mean $\pm$ standard deviation.

2-2-8 DPPH free radical scavenging test

For this purpose, a reaction mixture containing 1.5 ml of prepared DPPH solution (containing 4.73 mg of DPPH in 100 ml of HPLC grade ethanol) and 300 μ l of rice extract was prepared. The resulting mixture was shaken and incubated in a dark room at room temperature for 40 minutes. And the absorbance at 515 nm was read in comparison with the control (as a 100% sample) using a spectrophotometer [13]. The DPPH assay is a widely used method for evaluating the free radical scavenging activity of antioxidants, though it should be noted that it measures only the activity of lipophilic antioxidants and may not fully represent the in vivo antioxidant potential. The percentage of radical scavenging ability was calculated using the following formula:

$100 \times \frac{\text{Absorption at 515 nm sample} - \text{Absorption at 515 nm control}}{\text{Absorption at 515 nm control}} = (\%)$
scavenging ability

2-2-9 Sensory test

For the sensory evaluation of pasta samples, the cooking test method presented by (Larmond & Voisey, 1973) was used [14]. A panel of 10 trained semi-trained panelists evaluated the samples for appearance, color, taste, and overall acceptability using a 5-point hedonic scale (1=dislike extremely, 5=like extremely). The samples were presented in a randomized order in a sensory evaluation room under white light.

2-3 Statistical analysis

In this study, black and red rice flour were used as variables in this model with percentages of 5, 10, 15 percent instead of semolina, and we had a total of 6 combined treatments and one control treatment. The experiments of this study were conducted in a completely randomized design with three replications. Data analysis was performed using SAS version 9.2 software and comparison of data means was performed by Duncan's test using MSTAT-C software. Statistical significance was defined as $p < 0.05$. Graphs were also drawn using Excel software.

3- Discussion and Results

3-1 Results of the effect of different treatments on rheological properties

3-1-1 Comparing average dough consistency

The results from the comparison of dough consistency among the various treatments indicated significant differences ($p < 0.05$) (Figure 1). The dough consistency values for the samples ranged from 1.11 to 1.13. The highest value was observed for treatment R15, with a mean of 1.13, while the lowest values were recorded for treatments R5, B5, and the control sample. No significant difference was found among treatments R5, B5, and the control.

3-1-2 Gluten Softening Degree

The results comparing the mean gluten softening degree among the different treatments revealed significant variations ($p < 0.05$) (Figure 2). The gluten softening degree of the samples ranged from 0.55 to 0.58. The highest values were observed for treatments R15 and R10, both with a mean of 0.58, whereas the lowest value was recorded for the control treatment. Compared to the control, treatments R15, R10, R5, B15, B10, and B5 exhibited increases of 5%, 5%, 2%, 3%, 1%, and 1%, respectively.

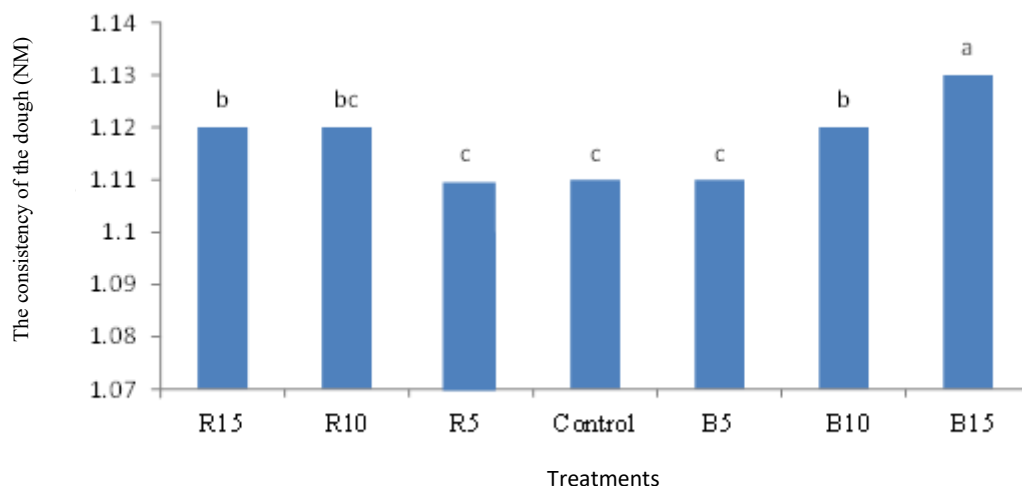


Figure 1: Comparison of average dough consistency between different treatments

The comparison of dough consistency among the different treatments showed significant variation ($p < 0.05$). According to Figure 1, the dough consistency of samples increased in treatments containing black and red rice flours, with the highest value observed for treatment R15, averaging 1.13. Dough consistency reflects the water absorption capacity of the flour during mixing. Throughout the mixing process, water is absorbed by flour particles, and a three-dimensional gluten network is formed. Given that the Mixolab mixer operates under torque, the force required for mixing indicates the dough's consistency. The results of this study show that increasing the proportion of black and red rice flours led to higher dough consistency. Several factors influence flour water absorption, including protein and starch characteristics, flour particle size and distribution, and bran content. Generally, as protein content increases, so does water absorption. Therefore, considering the protein content of the treatments, the increase in dough consistency is expected. On the other hand, for producing high-quality pasta, it is essential that semolina particles have a uniform size distribution. During the mixing of semolina with water, finer particles tend to absorb water more

readily, requiring greater mixing force. However, excessive water absorption by these fine particles can leave insufficient water for coarser particles, resulting in a non-uniform dough. Such dough often requires prolonged mixing to achieve homogeneity, which can adversely affect the final product quality. Thus, uniformity in particle size is more critical than particle size itself for semolina. Water absorption capacity depends on flour particle size; finer particles generally absorb more water, and vice versa. However, with relatively uniform particle size, higher protein content leads to greater water absorption [15-18].

According to studies by Hedayati et al. (2011), whole grain flour exhibits higher water absorption due to the presence of bran particles, which contain hydrophilic compounds and have a strong affinity for water [19]. Sosulski and Wu (1988) reported that mixing wheat flour with fiber increases its water absorption capacity proportionally with the fiber content [20]. Therefore, since black and red rice flours are derived from whole grains, they contain bran particles, resulting in distinct water absorption properties. Overall, differences between black/red rice flours and semolina—such as starch granule types, varying milling conditions, and the

presence of bran particles—lead to non-uniform particle distribution and varying water absorption capacities among particles

in the treatments, which could explain the differences observed in dough consistency.

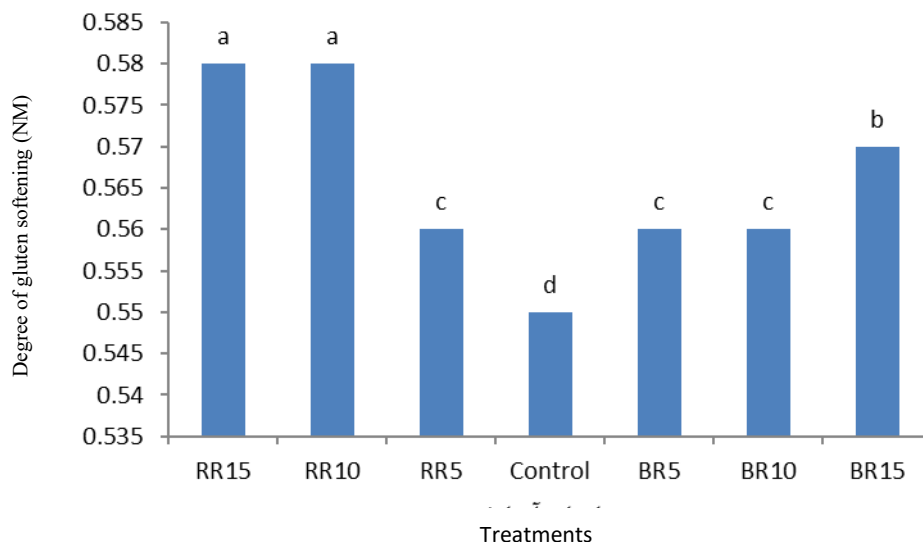


Figure 2: Comparison of the average degree of gluten softening between different treatments

The comparison of gluten softening degree among the different treatments showed significant variation ($p < 0.05$). According to Figure 2, the gluten softening degree of samples increased in treatments containing black and red rice flours, with the highest values observed for treatments R15 and R10, averaging 0.58.

Protein denaturation occurs under heat. In the Mixolab, the dough softening degree indicates the torque required to break down the protein network due to mechanical and thermal stress; this parameter is primarily influenced by the quantity and quality of gluten. A stronger gluten protein network is more resistant to breakdown, resulting in a lower softening degree. In the treatments of this study, despite equal gluten content, an increasing trend in softening degree was observed with higher proportions of black and red rice flours. The presence of bran particles in flour impedes the formation of a strong gluten network [19]. Consequently, the resulting dough exhibits less resistance to mechanical and thermal stress, leading to an increased gluten softening degree.

Therefore, given the presence of bran particles in black and red rice flours, the increased softening in these treatments can be attributed to the role of these particles in weakening the gluten structure.

3-1-3 Starch Gelatinization

The comparison of mean starch gelatinization levels among the treatments indicated significant differences ($p < 0.05$) (Figure 3). Starch gelatinization values for the samples ranged from 1.97 to 2.07. The maximum value was recorded for treatment R5, with a mean of 2.07, while the minimum value corresponded to the control treatment. Relative to the control, treatments R15, R10, R5, B15, B10, and B5 resulted in increases of 1%, 4%, 5%, 1%, 2%, and 3%, respectively.

3-1-4 Gel Consistency

The comparison of mean gel consistency among the treatments revealed significant differences ($p < 0.05$) (Figure 4). The formed gel consistency values for the samples ranged from 1.57 to 2.02. The highest value was observed for the control treatment, with

a mean of 2.02, while the lowest value was recorded for treatment B15. Compared to the control, treatments R15, R10, B15, B10, and B5 resulted in decreases

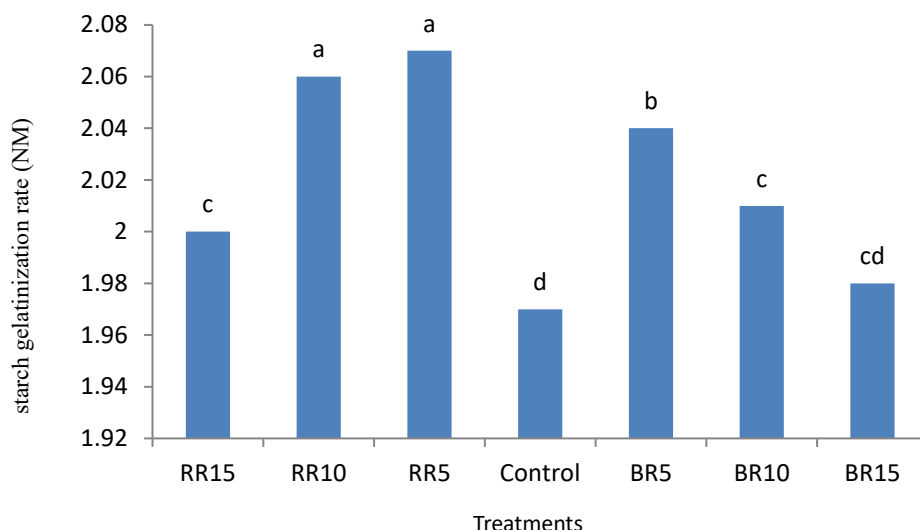


Figure 3: Comparison of the average starch gelatinization rate between different treatments

The comparison of starch gelatinization among the different treatments showed significant variation ($p < 0.05$). According to Figure 3, the starch gelatinization of samples decreased with increasing proportions of black and red rice flours, with the highest value observed for treatment R5, averaging 2.07. However, overall, all treatments exhibited higher starch gelatinization compared to the control. Starch granules are insoluble in cold water. During the mixing of starch and water under heat, water penetrates the starch granules, typically causing them to swell at temperatures above approximately 50°C. As swelling progresses, the protein membrane surrounding the granules ruptures, and the starch granules disintegrate. Part of the expanded starch molecules leach out from the granules, merge with each other, and form a homogeneous, viscous mass, resulting in gelatinized starch [20]. The C3 index in the Mixolab is a measure of the viscosity of the gel formed after the starch gelatinization

stage. Starch gelatinization depends on starch characteristics, aging, granule dimensions, milling process, flour extraction rate, and other factors [20]. The role of protein in this stage is less significant, with starch granules playing a key role. According to a study by Hedayati et al. (2018), comparing rice flour and wheat flour, rice flour has a higher gelatinization rate due to its higher starch content [21]. Therefore, the higher starch gelatinization in treatments containing black and red rice flours compared to the control can be attributed to their higher starch content and differences in milling processes. Conversely, the same study noted that, compared to whole wheat flour, refined wheat flour exhibits higher gelatinization. This is due to the presence of bran particles in whole wheat flour and their high-water absorption capacity, which competes with starch for water. Additionally, whole wheat flour has a higher lipid content, and the formation of amylose-lipid complexes can inhibit

amylose leaching, thereby reducing viscosity compared to refined wheat flour [22]. Hence, the decrease in gelatinization observed in samples containing black and red rice flours with increasing flour proportion may result from the increased

bran particles and lipid content in these treatments.

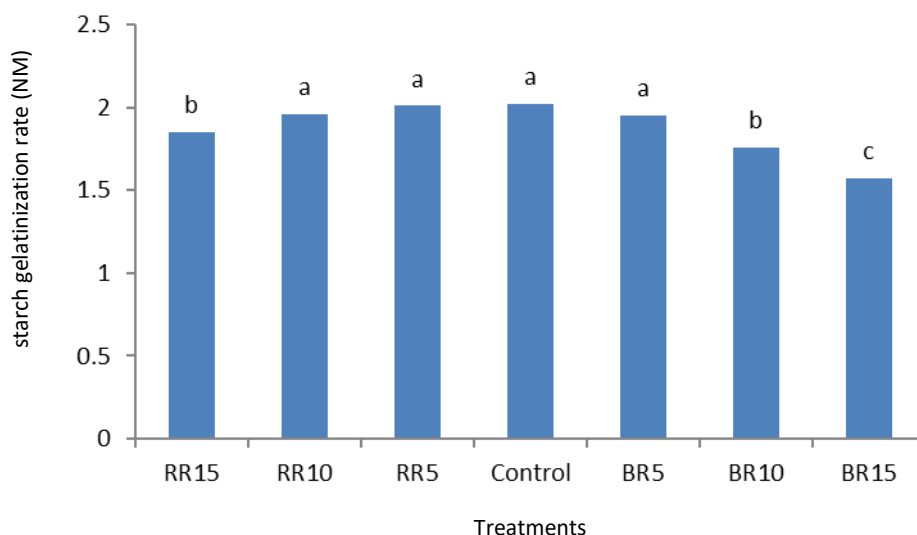


Figure 4: Comparison of the average gel consistency formed between different treatments

The comparison of gel consistency among the different treatments showed significant variation ($p < 0.05$). According to Figure 4, the former gel consistency of the samples decreased with increasing proportions of black and red rice flours, with the highest value observed for the control, averaging 2.02. Flour contains various enzymes, including alpha-amylase. This enzyme hydrolyzes starch to form dextrins, which contribute to dough stickiness and water release. Additionally, amylase cleaves the internal branches of starch, markedly reducing the viscosity and consistency of the dough [23]. Natural starch is slowly degraded by amylase; however, gelatinized starch is readily attacked by amylase, leading to starch breakdown (Rajabzadeh, 2010). Therefore, the torque measured at this stage is directly related to the viscosity and amylolytic activity of the dough. Enzyme activity is not uniform throughout the grain; after milling, enzymes are unevenly distributed among different parts.

In general, the endosperm has the lowest amylase activity, while increased extraction rate and the incorporation of the aleurone layer, germ, and bran into the flour enhance its enzymatic activity [20]. Given the presence of bran particles in black and red rice flours, higher alpha-amylase activity compared to the control would be expected. However, a decreasing trend was observed in the treatments with increasing proportions of rice flour. According to studies by Tadera et al. (2006) and Padilla-Camberos et al. (2014), polyphenols possess inhibitory effects on carbohydrate-digesting enzymes, including alpha-amylase [24,25]. Thus, the results of this test can be attributed to the inhibitory effect of polyphenols on alpha-amylase activity. However, given the lack of research on this effect in cereal products, further investigation in this area is needed.

3-3 Colorimetry:

The comparison of the mean colorimetric b^* (yellowness-blueness) index among the different treatments showed significant variations ($p < 0.05$) (Figure 5). The b^* index values for the samples ranged from

0.095 to 27.0. The highest value was recorded for the control treatment, with a mean of 27.0, while the lowest value corresponded to treatment B15. Compared to the control, treatments R15, R10, R5, B15, and B10 exhibited reductions of 25%, 14%, 14%, 99%, and 97%, respectively.

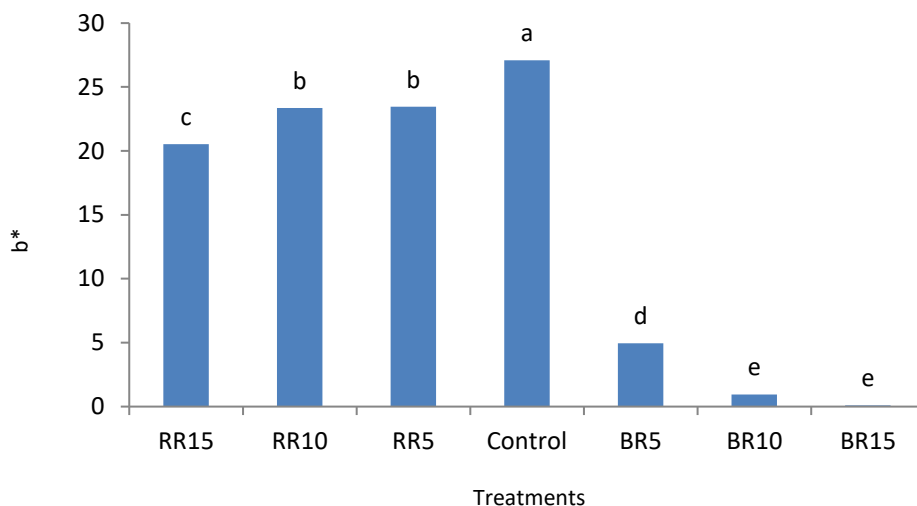


Figure 5 Comparison of the average colorimetric value b^* (yellow and blue) between different treatments

The comparison of the mean colorimetric a^* (redness-greenness) index among the different treatments revealed significant variations ($p < 0.05$) (Figure 6). The a^* index values for the samples ranged from 1.57 to 8.75. The highest values were

observed for treatment R15, with a mean of 8.65, and treatment B15. Compared to the control, treatments R15, R10, and R5 exhibited increases of 2.62, 2.35, and 1.81 times, respectively.

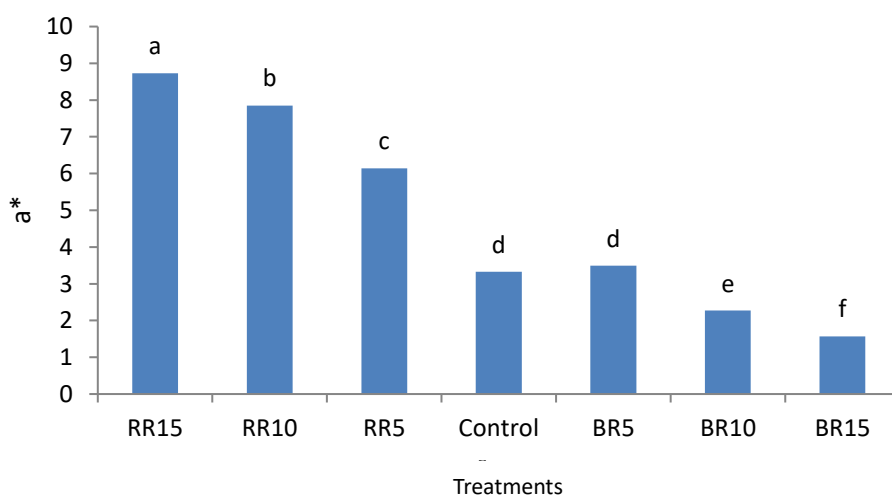


Figure 6: Comparison of the average colorimetric value of the a^* index (redness and greenness) between different treatments

The comparison of the mean colorimetric L^* (lightness) index among the different treatments revealed significant variations ($p < 0.05$) (Figure 7). The L^* index values for the samples ranged from 1.57 to 8.75. The highest values were observed for treatment

R15, with a mean of 8.65, and treatment B15. Compared to the control, treatments R15, R10, and R5 exhibited increases of 2.62, 2.35, and 1.84 times, respectively.

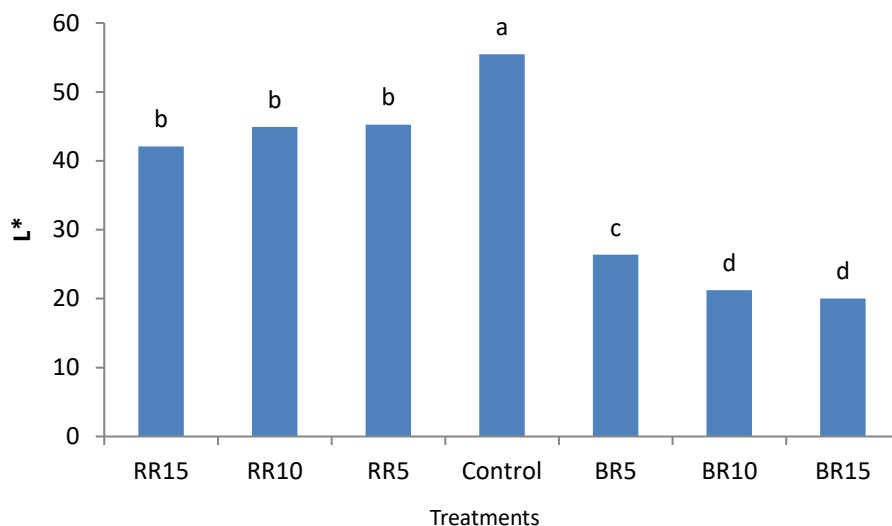


Figure 7: Comparison of the average colorimetric value L^* (brightness) between different treatments

The comparison of colorimetric parameters a^* , b^* , and L^* among the different treatments revealed significant variations ($p < 0.05$). With an increase in the proportion of black and red rice flours, the b^* (yellowness-blueness) index decreased, particularly in treatments containing black rice flour, with the lowest value observed for treatment B15. The a^* (redness-greenness) index showed an increasing trend compared to the control in treatments containing red rice flour, while a decreasing trend was observed in those with black rice flour. The highest a^* value was recorded for treatment R15. The L^* (lightness) index also exhibited a decreasing trend relative to the control, with a more pronounced reduction in treatments containing black rice flour. The presence of anthocyanin and anthocyanidin pigments in the various bran layers of pigmented rice varieties is responsible for their distinct black and red

colors. Consequently, it is expected that treatments containing colored rice flours would display different color profiles compared to the control.

3-4 Cooking Loss

The comparison of maximum total solids in cooking water among the different treatments showed significant variation ($p < 0.05$) (Figure 8). The maximum percentage of total solids in the cooking water ranged from 7.58% to 10.24%. The highest value for this index was observed for treatment R15, with a mean of 10.24%, while the lowest value corresponded to the control treatment. Compared to the control, treatments R15, R10, R5, B15, B10, and B5 resulted in increases of 35%, 30%, 21%, 11%, 5%, and 4%, respectively.

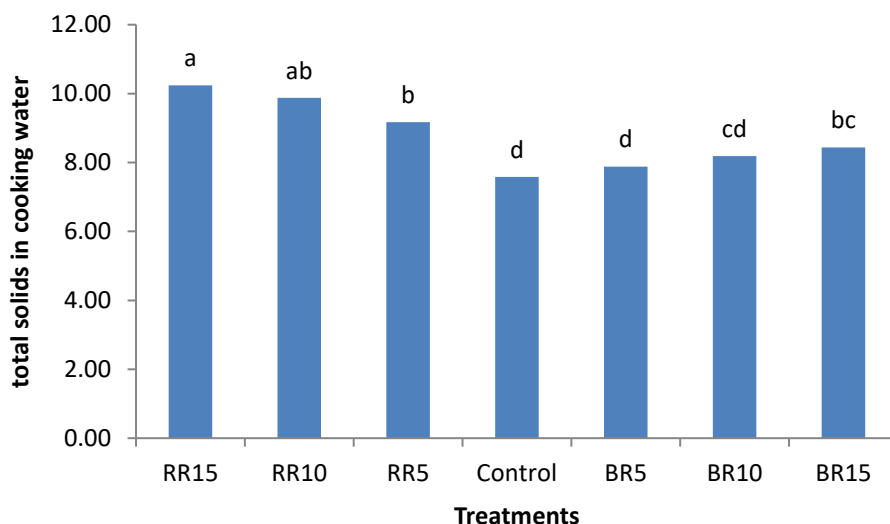


Figure 8. Comparison of the mean percentage of total solids in cooking water among different treatments.

The comparison of the maximum percentage of total solids in the cooking water among the different treatments showed significant variation ($p < 0.05$). According to Figure 8, the total solids in the cooking water increased with higher proportions of black and red rice flours, with the highest value observed for treatment R15. In general, the solid matter released into the cooking water is referred to as cooking loss. The strength of the starch granules influences this loss; a stronger gluten network and greater starch granule integrity result in less solid matter leaching and, consequently, lower cooking loss. The protein network entraps gelatinized starch granules, preventing surface rupture of the pasta and the leaching of starch granules and protein into the cooking water [26].

The presence of bran particles in flour increases the leaching of compounds into the cooking water due to two main factors:

(1) the disruption of the gluten network by bran particles, and (2) the presence of water-soluble compounds in the aleurone layer and bran. The disruption of the gluten network, combined with gaps formed between bran particles and the surrounding matrix in the dried pasta, can increase water absorption. This greater absorption exposes starch granules more readily to swelling and disintegration [27,28].

3-5 Cooked Weight

The comparison of the minimum cooked weight of 20 g of pasta among the different treatments showed significant variation ($p < 0.05$) (Figure 9). The minimum cooked weight of 20 g of pasta ranged from 48.8 g to 52.38 g. The highest value for this index was observed for treatment B15, with a mean of 52.38 g, while the lowest value corresponded to the control treatment. Compared to the control, treatments R15, R10, R5, B15, and B10 resulted in increases of 6%, 4%, 3%, 7%, and 3%, respectively.

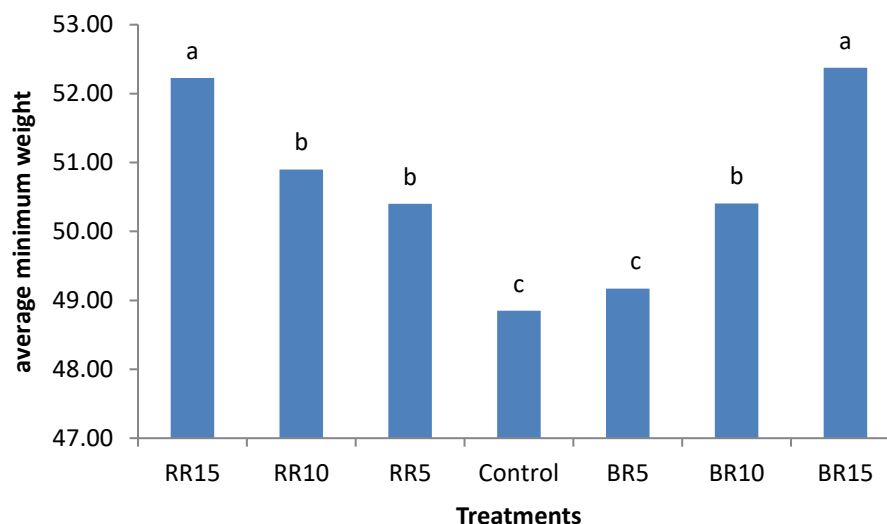


Figure 9: Comparison of the average minimum weight after cooking of 20 grams of pasta between different treatments

The comparison of the minimum cooked weight of 20 g of pasta indicated that the cooked weight increased with higher proportions of black and red rice flours. The highest value for this index was observed for treatment B15. The characteristics of starch granules and the particle size of flour significantly influence water absorption capacity [18]. The differences between black/red rice flours and semolina, along with their distinct milling processes, appear to contribute to the increased cooked weight of the pasta. Furthermore, enhanced water absorption may be attributed to the increased fiber content in the treatments. According to studies by Chen et al. (1998), the strong water-binding capacity of fibers can increase water absorption [29]. Additionally, the disruption of the gluten network by bran particles increases the swelling capacity of starch granules and promotes water absorption [16]. Therefore,

the increase in cooked weight observed in the treatments of this study may result from the presence of bran and aleurone layer particles in black and red rice flours, as well as the potential existence of damaged starch in these flours.

3-6 Total Phenolic Content (Raw Pasta)

The comparison of mean total phenolic content (TPC) in raw pasta among the different treatments revealed significant variations ($p < 0.05$) (Figure 10). The TPC values of the samples ranged from 97.92 to 178.17 mg GAE/100 g (or equivalent unit, depending on the standard used in the study). The highest TPC was observed for treatment B15, with a mean of 178.17, while the lowest value corresponded to the control treatment. Compared to the control, treatments R15, R10, R5, B15, B10, and B5 resulted in increases of 19%, 32%, 62%, 81%, 65%, and 26%, respectively.

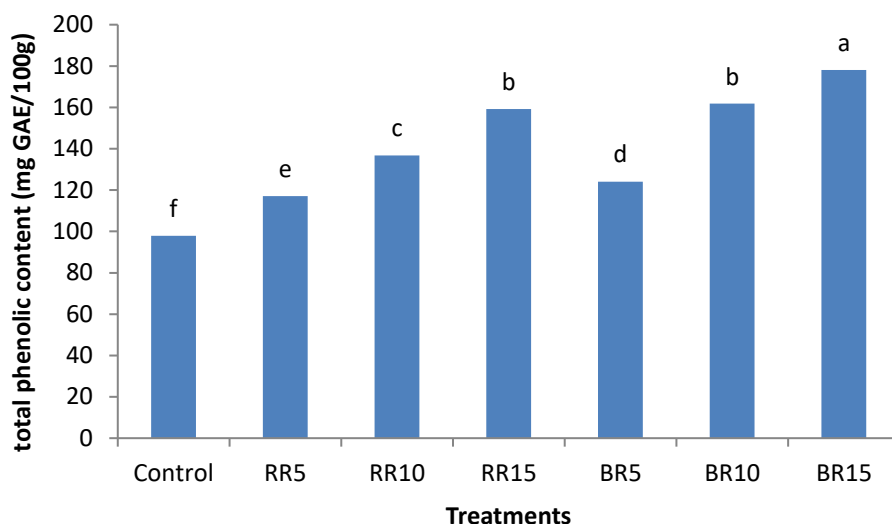


Figure 10: Comparison of average total phenolic content between different treatments.

The comparison of total phenolic content (TPC) among the different treatments revealed significant variation ($p < 0.05$). According to Figure 10, the TPC of the samples increased with higher proportions of black and red rice flour, with the highest value observed for treatment B15 (mean 178.17) and the lowest for the control treatment. Whole black and red rice grains contain anthocyanin and anthocyanidin pigments in their various bran layers. These pigments belong to the phenolic and flavonoid compound classes [30,31]. Consequently, flours derived from these rice varieties are rich in phenolic compounds. The TPC of black rice flour (699.46 ± 5.8 mg GAE/100g) and red rice flour (540.61 ± 11.17 mg GAE/100g) is several times higher than that of semolina (96.64 ± 3.64 mg GAE/100g). Therefore, the increase in TPC in the pasta samples

with increasing levels of black and red rice flour was expected and aligns with the results obtained in this study.

3-7 Total Phenolic Content (Cooked Pasta)

The comparison of mean total phenolic content (TPC) in cooked pasta among the different treatments revealed significant variations ($p < 0.05$) (Figure 11). The TPC values of the cooked samples ranged from 80.23 to 131.38 mg GAE/100 g (or equivalent unit). The highest TPC was observed for treatment B15, with a mean of 131.38, while the lowest value corresponded to the control treatment. Compared to the control, treatments R15, R10, R5, B15, B10, and B5 resulted in increases of 16%, 28%, 51%, 63%, 49%, and 19%, respectively.

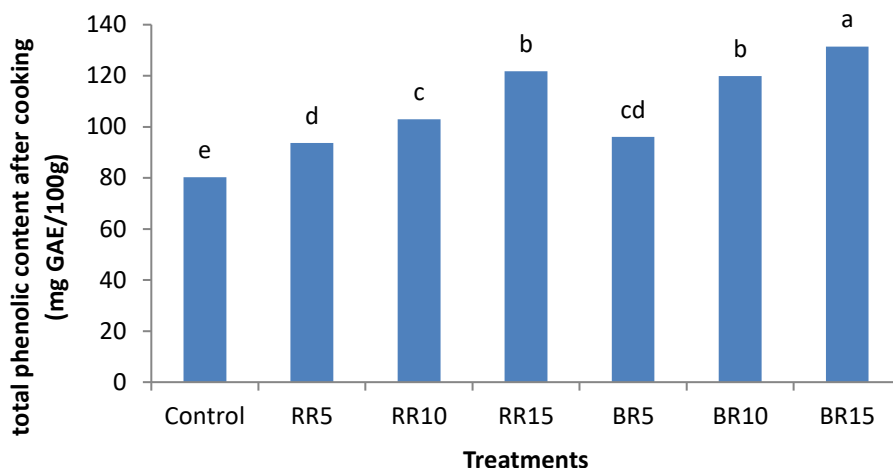


Figure 11: Comparison of the average total phenolic content after cooking between different treatments

The comparison of TPC after cooking among the different treatments also showed significant variation ($p < 0.05$). According to Figure 11, TPC increased with higher proportions of black and red rice flour, although it decreased overall compared to the raw pasta. Anthocyanins are water-soluble pigments [32]. Therefore, it is reasonable to assume that a portion of these compounds leached into the cooking water during pasta preparation, leading to a reduction in TPC after cooking.

3-8 Antioxidant Activity: DPPH Radical Scavenging Assay

3-8-1 Raw Pasta

The comparison of mean antioxidant activity (DPPH radical scavenging) in raw pasta among the different treatments revealed significant variations ($p < 0.05$) (Figure 12). The antioxidant activity values of the raw samples ranged from 12.7% to 24.8%. The highest activity was observed

for treatment B15, with a mean of 24.8%, while the lowest value corresponded to the control treatment. Compared to the control, treatments R15, R10, R5, B15, B10, and B5 exhibited increases of 38%, 43%, 60%, 89%, 51%, and 39%, respectively.

3-8-2 Cooked Pasta

The comparison of mean antioxidant activity (DPPH radical scavenging) in cooked pasta among the different treatments also revealed significant variations ($p < 0.05$) (Figure 13). The antioxidant activity values of the cooked samples ranged from 10.7% to 18.5%. The highest activity was recorded for treatment B15, with a mean of 18.5%, while the lowest value corresponded to the control treatment. Compared to the control, treatments R15, R10, R5, B15, B10, and B5 resulted in increases of 36%, 37%, 50%, 72%, 43%, and 33%, respectively.

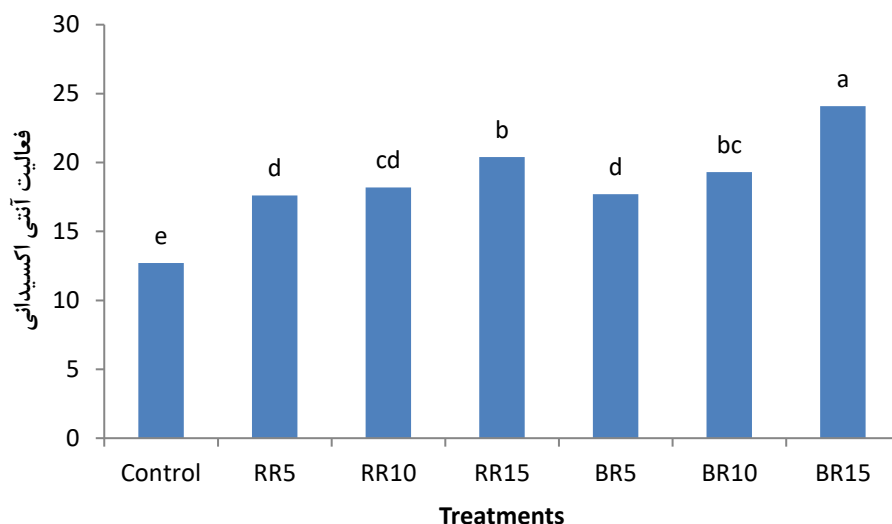


Figure 12: Comparison of average antioxidant activity levels between different treatments

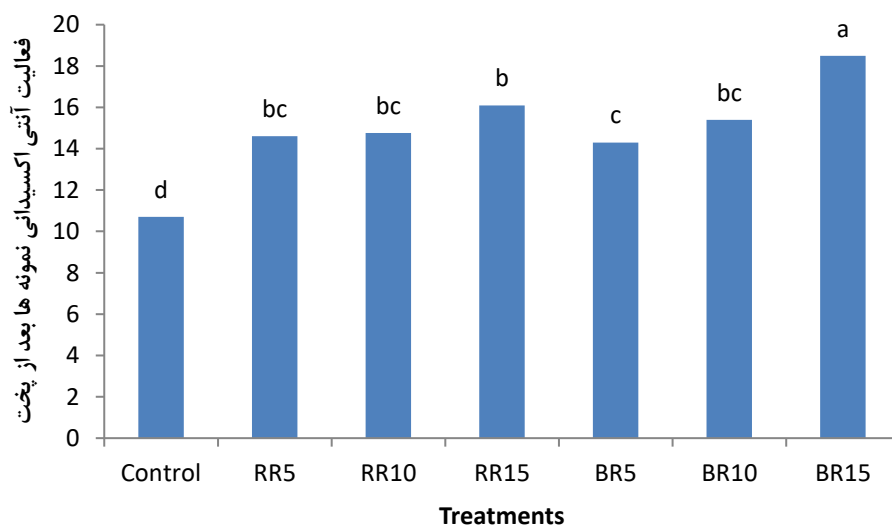


Figure 13: Comparison of the average antioxidant activity after cooking between different treatments

The comparison of antioxidant activity among the different treatments revealed significant variation ($p < 0.05$). According to Figure 12, antioxidant activity before cooking increased with higher proportions of black and red rice flour, with the highest value observed for treatment B15 and the lowest for the control.

Whole black and red rice grains contain anthocyanin and anthocyanidin pigments in

their bran layers (pericarp, aleurone, and tegmen). These pigments are phenolic and flavonoid compounds known for their potent antioxidant effects [33]. To measure the antioxidant activity of various compounds, different methods are employed; one effective method is the DPPH radical scavenging assay. Based on this method, the antioxidant activity of black rice flour ($75.5 \pm 0.88\%$) and red rice

flour ($62.1 \pm 1.35\%$) is several times higher than that of semolina ($14.1 \pm 0.62\%$). Hence, the increase in antioxidant activity in the pasta samples with increasing levels of black and red rice flour was anticipated.

The comparison of antioxidant activity after cooking among the different treatments also showed significant variation ($p < 0.05$). According to Figure 13, antioxidant activity increased with higher proportions of black and red rice flour, with the highest value observed for treatment B15 (mean 18.5%) and the lowest for the control. However, overall, the activity decreased compared to the raw pasta. As anthocyanins are water-soluble pigments, it is logical that a portion of these compounds leached into the cooking water, resulting in reduced antioxidant activity after cooking.

3-9 Sensory Evaluation

3-9-1 Appearance

The comparison of appearance scores among the different treatments revealed significant variation ($p < 0.05$) (Figure 14). The appearance scores ranged from 4.12 to 5.0 (on a 5-point scale). The highest score was observed for the control treatment (mean 5.0), while the lowest score was recorded for treatment B5. Treatments R5 and R10 were statistically grouped with the control. Therefore, based on appearance, treatments R5, R10, and the control were considered the best.

3-9-2 Color

The comparison of color scores among the different treatments showed significant variation ($p < 0.05$) (Figure 15). The color scores ranged from 2.87 to 5.0. The highest score was obtained by the control (mean 5.0), while the lowest score was for treatment B5. Treatment B15 was statistically grouped with the control. Thus, regarding color, treatments B15 and the control were considered the best.

3-9-3 Taste and Flavor

The comparison of taste and flavor scores among the different treatments indicated significant variation ($p < 0.05$) (Figure 16). The taste scores ranged from 3.62 to 4.5. The highest score was observed for treatment B15 (mean 4.5), while the lowest scores were recorded for treatments R10 and B10. Consequently, based on taste and flavor, treatment B15 was rated as the best.

3-9-4 Overall Acceptability

The comparison of overall acceptability scores among the different treatments revealed significant variation ($p < 0.05$) (Figure 17). The overall acceptability scores ranged from 3.87 to 4.5. The highest scores were observed for treatments R15 and B15 (both with a mean of 4.5), while the lowest score was for the control treatment. Therefore, regarding overall acceptability, treatments R15 and B15 were considered the best.

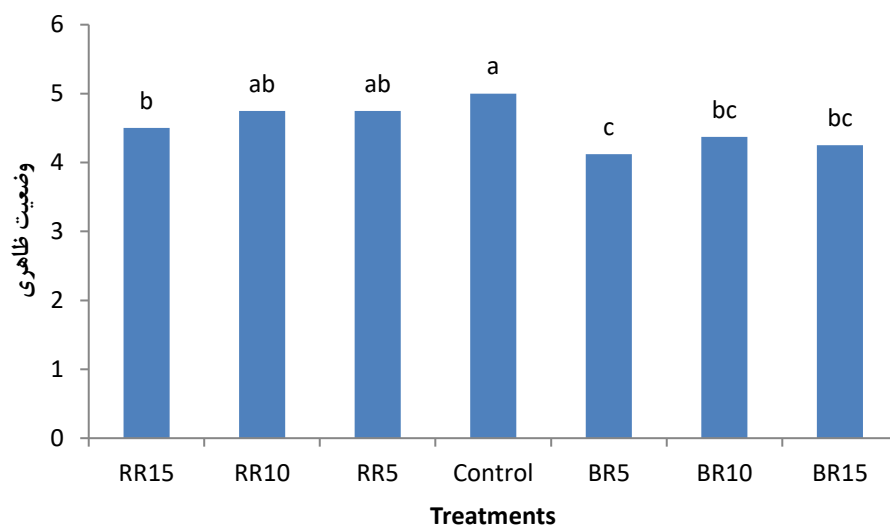


Figure 14: Comparison of average visual condition scores between different treatments

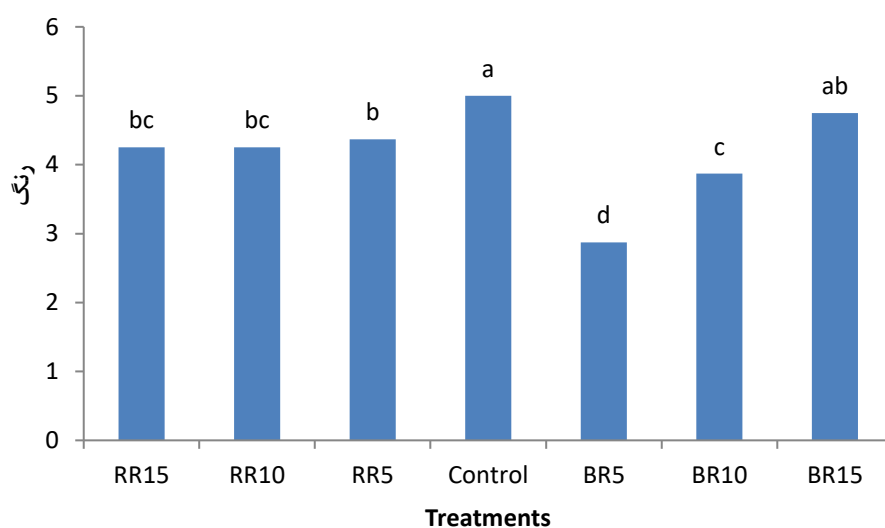


Figure 15: Comparison of average color scores between different treatments

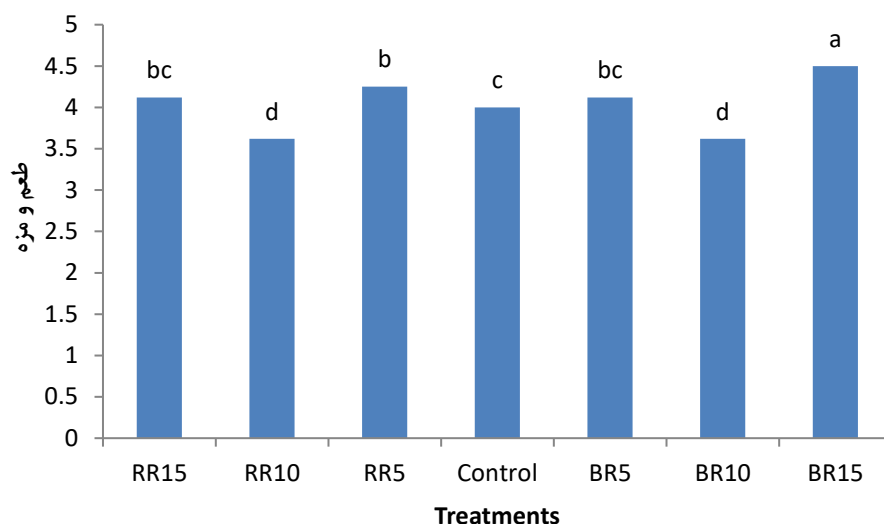


Figure 16: Comparison of average taste satisfaction scores between different treatments

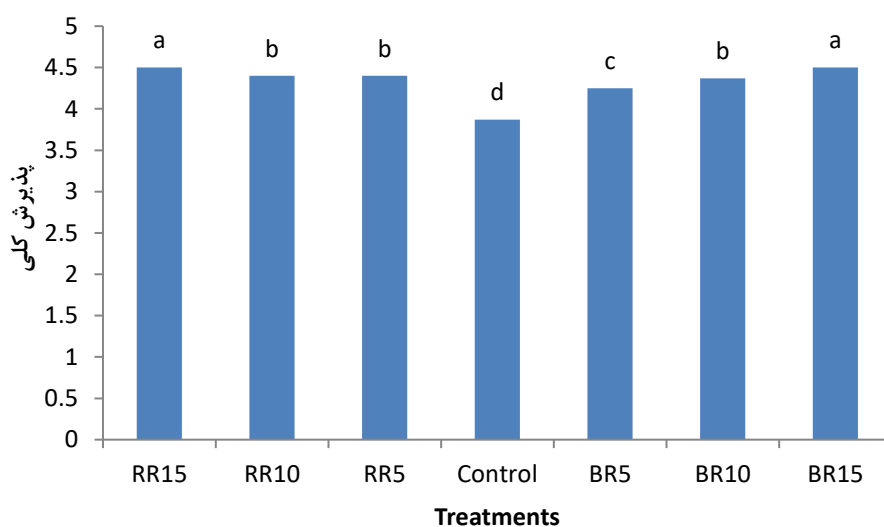


Figure 17: Comparison of average overall acceptance scores between different treatments

The sensory evaluation results for the various treatments, including appearance, color, taste and flavor, and texture, showed significant differences among the treatments ($p < 0.05$). The results of the sensory test in different treatments including appearance, color, taste and texture showed significant changes among different treatments ($p < 0.05$). In terms of appearance, treatments R5 and R10 and the control were considered the best treatments. In terms of color, treatments B15 and the

control, and in terms of taste, treatment B15 had the highest score. It is noteworthy that despite the significant color changes, panelists found the unique pigmentation of the B15 treatment acceptable, which is a promising result for marketability.

4-Conclusion

While the primary objective of producing pasta enriched with black and red rice flours was to enhance nutritional properties, and the secondary objective was to produce a

product with acceptable quality, this study aimed to optimize the formulation and process to achieve both nutritional (higher fiber and antioxidant properties) and quality aspects of the final product. The results demonstrated that the total phenolic content, antioxidant activity, and dietary fiber percentage of the different treatments showed significant differences ($p < 0.05$), increasing with higher proportions of black and red rice flour. Colorimetric results also revealed significant differences in product color. Specifically, with increasing levels of black and red rice flour, the b^* (yellowness) and L^* (lightness) indices decreased, particularly in treatments containing black rice flour. The a^* (redness) index increased significantly in treatments containing red rice flour compared to the control, while it decreased significantly in those containing black rice flour. The different treatments exhibited significant ($p < 0.05$) and distinct behaviors in various rheological parameters measured using the Mixolab. With increasing proportions of black and red rice flour, parameters such as dough consistency, gluten softening degree, and starch gelatinization increased, while parameters like gel consistency, stability time, and starch retrogradation decreased. Regarding cooking quality, both the cooked weight and the percentage of total solids in the cooking water showed significant differences ($p < 0.05$), increasing with higher levels of black and red rice flour. Furthermore, sensory evaluation results indicated that pasta prepared with 15% black rice flour (B15) received the highest scores for most sensory attributes, including odor, taste, color, texture, and overall acceptability. In most cases, these differences were significant compared to other treatments. It is important to note that research conducted in line with the present study has been very limited, and in some cases, suitable reports are lacking.

Therefore, further research is warranted to provide more definitive interpretations of the findings extracted from this study. In summary, the study successfully demonstrated that partial replacement of semolina with up to 15% pigmented rice flour, particularly black rice flour, can significantly enhance the nutritional and antioxidant properties of pasta while maintaining acceptable sensory qualities. The B15 treatment emerged as the optimal formulation. One of the main limitations of this study is the loss of phenolic compounds and antioxidant activity during the cooking process. Future research should therefore focus on strategies to mitigate this loss, such as the use of encapsulation techniques or the application of surface coatings to protect bioactive compounds during hydrothermal treatment. Furthermore, while this study focused on technological and sensory attributes, *in vivo* studies are necessary to validate the health benefits of the enriched pasta. Additionally, the study of the effect of different extrusion conditions and drying temperatures on the retention of bioactive compounds could be a valuable future research direction. It is also important to note that research conducted in line with the present study has been very limited, and in some cases, suitable reports are lacking. Therefore, further research is warranted to provide more definitive interpretations of the findings extracted from this study.

Author Contributions

All activities were performed by the authors.

Data availability

All data supporting the findings of this study are available within the article.

Conflict of Interest

The author confirms that there are no financial conflicts of interest or competing interests in this study.

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5-Reference

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