



Research Article

Bioaccessibility of Phenolic and Antioxidant Substances in Gluten-Free Cookies Prepared with Sorghum Flour, Rice Bran, and Quinoa

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Abstract: The main objective of this study was to determine the bioaccessibility of bioactive components in gluten-free cookies fortified with rice bran (RB) and quinoa (Q). The data set includes measurements of total phenolic content (TPC), antioxidant activity (DPPH and ABTS radical scavenging), and sensory evaluation scores. Cookies containing 15% RB exhibited the highest total phenolic content (0.634 mg GAE g⁻¹) and antioxidant activity (88.13% DPPH inhibition) before digestion. After simulated gastrointestinal digestion, these cookies showed a remarkable increase in antioxidant activity, with a 4.3-fold improvement in DPPH radical scavenging and a 2.53-fold increase in ABTS radical inhibition. Additionally, the addition of 15% quinoa resulted in a 1.58-fold increase in total phenolic content bioaccessibility. In this study, cookie hardness ranged from 35.89 to 62.92 N and increased significantly with the incorporation of RB and Q.

A progressive rise in TBA values was noted with increasing levels of RB and Q substitution. Notably, RB15 showed a significantly higher TBA value compared to RB5 and RB10, with a similar pattern also observed in the quinoa group. Sensory evaluation highlighted that cookies with 5% quinoa were the most preferred, receiving the highest scores in overall acceptability. While higher enrichment levels (15% RB or Q) led to superior antioxidant potential and bioaccessibility, these formulations were less favored in sensory evaluation, suggesting a trade-off between nutritional enhancement and consumer acceptance. This study demonstrates that incorporating RB and Q into gluten-free cookies not only enhances their nutritional value and antioxidant properties but also improves their sensory appeal.

Keywords: Bioaccessibility, Cookie, Gluten-free, Rice bran

Sorgum Unu, Pirinç Kepeği ve Kinoa ile Hazırlanan Glutensiz Bisküvilerde Fenolik ve Antioksidan Bileşiklerin Biyoerişilebilirliği

Öz: Bu çalışmanın temel amacı, pirinç kepeği (RB) ve kinoa (Q) ile zenginleştirilmiş aromasız bisküvilerdeki biyolojik olarak aktif bileşenlerin biyoerişilebilirliğini belirlemektir. Veri seti; toplam fenolik madde içeriği (TPC), antioksidan aktivite (DPPH ve ABTS radikal süpürme kapasitesi) ve duyuşal değerlendirme skorlarını içermektedir. %15 RB içeren bisküviler, sindirim öncesinde en yüksek toplam fenolik madde içeriği (0.634 mg GAE g⁻¹) ve antioksidan aktiviteye (%88.13 DPPH inhibisyonu) sahip olmuştur. Simüle edilmiş gastrointestinal sindirim sonrası, bu bisküviler antioksidan aktivitede dikkat çekici bir artış göstermiştir; DPPH radikal süpürme kapasitesinde 4.3 kat, ABTS radikal inhibisyonunda ise 2.53 kat artış kaydedilmiştir. Ayrıca, %15 kinoa ilavesi toplam fenolik madde biyoerişilebilirliğinde 1.58 kat artış sağlamıştır. Bisküvi sertliği 35.89-62.92 N arasında değişmiştir. RB ve Q ilavesi arttıkça TBA değerlerinde kademeli bir artış gözlemlenmiş, özellikle, RB15 örneğinin, RB5 ve RB10'a kıyasla daha yüksek bir TBA değerine sahip olduğu belirlenmiştir. Duyusal değerlendirme sonuçlarına göre, %5 kinoa içeren bisküviler genel beğeni açısından en yüksek puanı alarak en çok tercih edilen ürün olmuştur. Yüksek düzeyde zenginleştirilen örnekler (15% RB veya Q), daha yüksek antioksidan kapasite ve biyoerişilebilirlik göstermesine rağmen, duyuşal değerlendirmede daha düşük puan almıştır. Bu durum, besinsel iyileştirme ile tüketici kabulü arasında bir denge gerekliliğini ortaya koymaktadır. Bu çalışma, RB ve Q'nun glutensiz bisküvilere eklenmesinin yalnızca besin değerini ve antioksidan özellikleri artırmakla kalmayıp aynı zamanda duyuşal açıdan da kabul edilebilirliğini geliştirdiğini ortaya koymuştur.

Anahtar Kelimeler: Bisküvi, Biyoerişilebilirlik, Glutensiz, Pirinç kepeği

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1. Introduction

Celiac disease is a chronic autoimmune disorder triggered by the ingestion of gluten in genetically predisposed individuals, affecting approximately 1–2% of the global population (Lebwohl et al., 2018). Celiac disease (CD), whose prevalence has increased rapidly around the world in recent years, has unquestionable dimensions. Public awareness related to the disease, which has an impact on health and living standards, is growing daily (Green & Cellier, 2007). As the most prevalent autoimmune and chronic illness in the world, CD is viewed as a problem for global health. Today, the only treatment for the disease is a gluten-free diet, which the person must adhere to for the rest of their life. The biggest challenge for celiac patients is their inability to adapt to a gluten-free diet, which is why it is so important to create a wide variety of gluten-free products that patients can consume. Gluten-free products are often criticized for having poor texture, low protein content, and reduced sensory quality, which can limit dietary adherence and consumer satisfaction (Gobbetti et al., 2018). Finding high-quality gluten-free food is one of the most important challenges celiac patients face in following a gluten-free diet (Theethira & Dennis, 2015). Developing technology offers solutions in many areas (Kına & Biçek, 2023; 2024). In this context, solutions have been developed for celiac patients. Everyone needs to consume gluten-free products, including those with gluten sensitivity, celiac disease, and those who want to adopt a gluten-free diet as part of their lifestyle. Numerous studies have shown that alternative flours from cereals and legumes can be used to create delicious gluten-free products while also improving nutritional value, antioxidant activity, and glycaemic index (Di Cairano et al., 2018). In particular, quinoa (*Chenopodium quinoa* Willd.) (Q) is rich in essential amino acids, fiber, and phenolic compounds, making it a valuable functional ingredient in gluten-free formulations (Vega-Gálvez et al., 2010). Similarly, rice bran (RB) contains dietary fiber, γ -oryzanol, vitamins, and antioxidants, which contribute to its nutritional and health-promoting properties (Gul et al., 2015; Moongngarm & Saetung, 2010).

Gluten-free diets have gained significant popularity in recent years due to the growing awareness of gluten-related sensitivities and celiac disease. This dietary trend has prompted the development of a wide range of gluten-free alternatives for various food products, including biscuits. One such alternative is the incorporation of quinoa and rice bran into gluten-free cookie recipes, offering not only a tasty and satisfying treat but also a nutritious option for individuals seeking gluten-free alternatives.

Bioaccessibility refers to the proportion of a substance released from a food, a material, or a product that can potentially be absorbed by the body and become available for biological processes. Following consumption of food, gastrointestinal digestion and colonic bacterial metabolism are likely to significantly affect the bioaccessibility and bioavailability of cereal polyphenols. Therefore, understanding the interaction between phenolic components and the gastrointestinal digestive environment will help elucidate the positive health outcomes observed with the consumption of phenolics-rich cereal-based products.

Although past studies have elucidated the amount of phenolics derived from cereal sources and the effect of processing on these components, there are limited studies on how phenolics of cereal-based products are affected during digestion. In this context, the objectives of this study are to enrich gluten-free cookies with rice bran and quinoa, which are known to be rich in antioxidants. Another aim of the study is to determine the bioaccessibility of the antioxidant substances in these cookies and to reveal how much of the functional properties of these cookies are accessible after ingestion and to create consumer awareness.

2. Material and Methods

2.1. Material

The cookie formula is presented in Table 1. Sorghum flour was substituted with rice bran (RB) and quinoa (Q) at the level of 5, 10, and 15% level. Seven cookies were made in all, including the control cookies and cookies with 5, 10, and 15% RB (RB5, RB10, and RB15) and 5, 10, and 15% Q, respectively (Q5, Q10, and Q15).

Table 1. Cookie formulation

Ingredients	(g)
Sorghum flour	100
Sugar	41.94
Shortening	39.95
Water	21.84
Sodium bicarbonate	1.02
Amonium bicarbonate	0.49
Salt	1.24
Vanillin	0.3

2.2. Cookie production

The AACC (10-53.1) (AACC, 2000) method was modified to make cookie dough using a KitchenAid mixer (Model 5KSM45). Shortening, sugar, salt, vanilla, and sodium bicarbonate were added to the mixture, which was then mixed at 60 rpm for three minutes. Then, ammonium bicarbonate, and water were added, and the mixture was mixed at 90 rpm for one minute. The flour was then added to the recipe and mixed for a further 2 minutes at 60 rpm. Doughs shaped on double-layered insulated cookie trays greased with standard-compliant frying oil were baked in an electric oven (Öztiryakiler, Istanbul) at 185 °C for 13 minutes under a non-fan setting and 85% relative humidity.

2.3. Physical properties of cookies

Using measured wooden equipment, the width (W) and thickness (H) values of the cookie samples were calculated. Four measurements were taken with the cookie in various positions and locations to obtain an average value. The cookie spread ratio was then estimated using the (W/H) formula.

2.4. Texture

The texture of the cookie samples was determined using the AACC (74-09.01) (AACC, 2000) method, and a three-point bend rig was utilized using the TA.XT plus Texture Analyzer (TA.TX2. Stable Micro Systems Ltd. Godalming Surrey, England). According to the fracture test procedure (load cell: 3 kg, pre-test speed: 1.0 mm/s, test speed: 5.0 mm/s, final -test speed: 10.0 mm/s, distance: 10 mm, trigger force: 50 g, apparatus width: 40 mm), the force (hardness, N) was determined.

2.5. Phenolic extraction

To remove the fat from the cookie samples, 100 mL of hexane was added to 10 g of each sample, and the mixture was shaken and left in a shaking water bath at 25 °C for 24 hours. Afterward, the hexane was filtered, and the remaining sample was dried in an oven at 40 °C to ensure complete removal of hexane. Phenolic extraction was then performed on the defatted samples. According to a method described by Zhang et al. (2010), phenolic compounds were extracted. 10 mL of methanol and 0.1 N HCl (85:15) were added to 5 g of the cookie samples that had been weighed into tubes. The tubes were then combined for 20 minutes in a water bath at 25° C and 250 rpm (Julabo SW23 shaking water bath; Julabo GmbH, Seelbach, Germany). The samples were mixed, then centrifuged at 3000 g for 15 minutes, collecting the supernatant into 50 mL amber-colored flasks. Four times this technique was carried out. Until analysis, samples were stored at -18 °C.

2.6. Total phenolic content (TPC)

In test tubes, 300 µL of the sample and 3 mL of Na₂CO₃ (2%) were added. Following the addition of 150 µL of Folin-Ciocalteu reagent diluted 1:1 with distilled water to the tubes, the mixture

was stirred with the aid of a vortex and left to stand at room temperature for 45 minutes in a dark area. After this time, a reading at 765 nm was taken using the spectrophotometer (UV-1900PC, Aoelab, CN). The gallic acid calibration curve was used to compute the total phenolic material content, and the results were represented as mg gallic acid equivalent (mg GAE g⁻¹).

2.7. Antioxidant activity (AA)

The antioxidant activity was assessed using DPPH and ABTS assays. For the DPPH method, 500 µl of each extract was mixed with 3 ml of 0.004% DPPH solution. After incubation in the dark (30 min), absorbance was measured at 517 nm, and % inhibition was calculated based on the difference between control (a sample without antioxidant compounds) and sample absorbance.

A 7 mM ABTS⁺ solution was prepared using 2.45 mM potassium persulfate and diluted with ethanol to an absorbance of 0.70±0.02 at 734 nm. Then, 20 µl of the sample was added to 1,980 µl of the ABTS⁺ solution, vortexed, and absorbance was measured at 734 nm after 6 minutes. The percentage of inhibition was calculated by measuring the difference in absorbance between the sample and a control sample that did not contain any antioxidant compounds.

2.8. Bioaccessibility of phenolic and antioxidant substances (BE-TFM and BE-DPPH, BE-ABTS)

The in-vitro gastrointestinal digestion method was used to extract the samples to assess the TPC and AA bioaccessibility of cookies. The procedure described by [Chen et al. \(2019\)](#) was applied. A defeated cookie sample (1.5 g) was combined with 1.5 mL of pepsin solution in 30 mL of distilled water (20 g of pepsin dissolved in 0.1 mol of HCl). The pH was brought down to 2 using 6 mol/L HCl, and it was then left in a water bath that was shaking at 37 °C for an hour. 1 mol/L of NaHCO₃ was added to this mixture to change the pH to 7.2. The mixture was then incubated for a further 2.5 hours with 7.5 mL of bile/pancreatin solution (2 g/L pancreatin and 12 g/L bile salt with 0.1 mol/L NaHCO₃) and 7.5 mL of NaCl/KCl (120 mmol/L NaCl and 5 mmol L⁻¹ KCl). The mixture was centrifuged at 5000 ×g for 10 min at 20 °C using a Hettich Rotina 380R centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). After the addition of 5 mL methanol to 1 mL of the obtained supernatant, the mixture was centrifuged again at 5000 g for 10 minutes. The resulting supernatant was subsequently used to determine total phenolic content (TPC) and antioxidant activity (AA) using the Folin–Ciocalteu colorimetric method, as well as the DPPH and ABTS assays. The percentages of BE-TFM and BE-AA were calculated using the calculations below (Equation 1, 2, and 3).

$$BE - TPC (\%) = \frac{(TPC - after\ digestion)}{(TPC - before\ digestion\ (chemical\ extracts))} \times 100 \quad (1)$$

$$BE - AA(\%) = \frac{(DPPH - after\ digestion)}{(DPPH - before\ digestion\ (chemical\ extracts))} \times 100 \quad (2)$$

$$BE - AA(\%) = \frac{(ABTS - after\ digestion)}{(ABTS - before\ digestion\ (chemical\ extracts))} \times 100 \quad (3)$$

2.9. Thiobarbituric acide (TBA)

For TBA analysis, the method reported by [Tarladgis et al. \(1960\)](#) was used and the results were calculated as malondialdehyde/kg (mg MDA kg⁻¹).

2.10. Sensory

Thirty panelists, ranging in age from 23 to 60 (17 women and 13 men), participated in the study. Sensory characteristics were rated using a five-point hedonic scale (1 = "dislike very much," 5 = "like very much"). The study was reviewed and approved by Van Yüzüncü Yıl University, and informed consent was obtained from each participant prior to their involvement.

2.11. Statistical analyses

An ANOVA test was applied to examine the differences between the groups. This analysis was performed using Python-based tools according to the method proposed by [Alav et al. \(2024\)](#); [Meral et al. \(2024\)](#). Integrating Python tools into statistical analysis can facilitate the processing of complex data patterns and increase the reliability of results. This is because AI-based methods can contribute to the analysis process by providing significant support in data preprocessing, automation of modeling tasks and improvement of data visualization, especially when working with large and complex datasets ([Kina, 2025](#)). Subsequent comparisons were made using the Tukey HSD test to determine differences between samples

3. Results and Discussion

3.1. Physical properties of cookies

Figure 1 displays the photos of the cookies. Cookie top grain, or the cracking that appears on the cookie's top surface, is another indicator of cookie quality. Due to the recrystallization of sucrose at the cookie's surface during baking, the top grain of the cookie (with many surface cracks) is considered good ([Barak et al., 2014](#); [Meral et al., 2025](#)). In this regard, all cookie samples had regular surface cracks, indicating good quality.



Figure 1. Appearance of cookies.

The diameter, thickness, and spread ratio are crucial quality characteristics of the cookies. The findings are shown in Table 2. It has been found that the diameter varies between 43.06 and 46.06 mm and the thickness varies between 8.90 and 9.40 mm. The addition of quinoa and rice bran had a substantial impact on the diameter and thickness ($P < 0.05$). It was found that the samples containing 5% quinoa had the highest diameter and the lowest thickness. Better quality flours are thought to yield cookies with a wider diameter and a shorter thickness ([Barak et al., 2014](#)). The sample prepared with 15% quinoa had the smallest diameter. The RB-10 sample containing 10% rice bran showed the maximum thickness. It was discovered that when quinoa was introduced at a level of 5%, the diameter first increased and subsequently decreased. The diameter was reduced when rice bran was added. Similarly, several studies have reported that the type of flour and the addition of various ingredients significantly influence the diameter and thickness of gluten-free biscuits. For instance, [Sarabhai et al. \(2017\)](#) measured the diameter by aligning the biscuits edge-to-edge and the thickness by stacking. The average diameter and thickness values for the control cookies were reported to be between 5.5–5.6 cm and 0.50–0.53 cm, respectively.

Teshome et al. (2017) reported that the diameter of Teff-based gluten-free biscuits ranged from 52.7 to 54.0 mm. Similarly, Man et al. (2014) observed a decrease in diameter from 1.95 cm to 1.85 cm and a corresponding increase in thickness. These findings collectively highlight the influence of flour quality and formulation strategies on the dimensional characteristics of gluten-free cookies.

The spread ratio is a quantitative characteristic that is used to assess the quality of the biscuits. The quality and acceptability of the biscuits increase with the increase in spread ratio (Barak et al., 2014). Table 2 provides the impacts of RB and Q addition on the spread ratio. These components significantly influenced how the biscuits spread during baking. With the addition of quinoa at a 15% addition level and rice bran at two addition levels (10% and 15%), the spread ratio values were dramatically lowered ($P < 0.05$). But compared to the control, the addition of 10% quinoa and 5% rice bran had no appreciable impact on the spread ratio ($P > 0.05$). The spread ratio was greatly increased by adding 5% quinoa. As indicated in Table 2, the results of this investigation revealed that cookies made from the formula that included 5% quinoa had the highest spread ratio, while cookies made from the formula that contained 15% quinoa and 15% rice bran had the lowest spread ratio.

Table 2. The physical properties and TBA values of cookies

Sample	Diameter (mm)	Thickness (mm)	Spread ratio	Hardness (N)	TBA (mg MDA kg ⁻¹)
Control	45.36±0.15 ^b	8.90±0.10 ^c	5.09±0.07 ^b	35.89±7.76 ^d	2.08±0.06 ^f
RB-5	45.06±0.60 ^b	8.80±0.10 ^c	5.12±0.08 ^b	51.23±1.70 ^{bc}	2.32±0.03 ^e
RB-10	43.60±0.17 ^{cd}	9.40±0.10 ^a	4.63±0.05 ^d	57.99±3.67 ^{ab}	2.84±0.07 ^d
RB-15	43.83±0.28 ^c	8.93±0.32 ^{bc}	4.90±0.08 ^c	62.92±2.80 ^a	3.99±0.01 ^a
Q-5	46.06±0.60 ^a	8.43±0.32 ^d	5.46±0.13 ^a	44.89±1.48 ^c	3.35±0.08 ^e
Q-10	44.90±0.30 ^b	8.76±0.05 ^c	5.12±0.04 ^b	44.28±1.92 ^{cd}	3.46±0.12 ^e
Q-15	43.06±0.11 ^d	8.93±0.11 ^{ab}	4.66±0.12 ^d	56.25±0.80 ^{ab}	3.72±0.09 ^b

Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$) according to one-way ANOVA followed by Tukey's post hoc test. RB5: Cookies containing 5% rice bran, RB10: Cookies containing 10% rice bran, RB15: Cookies containing 15% rice bran, Q5: Cookies containing 5% quinoa, Q10: Cookies containing 10% quinoa, Q15: Cookies containing 15% quinoa

Similar results were found in earlier research. The spread ratio ranged from 7.07 to 8.26 when Hamdani et al. (2021) examined the effects of various gums on biscuit quality. According to Simons and Hall (2018), the spread ratio in gluten-free biscuits ranged from 6.1 to 6.6. In another study, Jan et al. (2018a) found that the spread ratio of biscuits made with quinoa flour ranged from 5.89 to 7.26. According to Paesani et al. (2020), the spread ratio of gluten-free biscuits ranges from 6.43 to 12.20. The present study revealed that rice bran and quinoa affect the spread ratio; also, high levels of rice bran and quinoa may not be preferred to achieve a high spread ratio.

Although the addition of quinoa had a more modest impact on the hardness than did the addition of rice bran, these cookies nevertheless showed a statistically significant rise. It has been acknowledged that hardness is a crucial aspect of cookie quality since it influences consumer acceptance and repeat sales (Jan et al., 2018b). Our results are consistent with the results previously reported. For example, the biscuit's hardness ranged from 34.69 to 48.14 N (Hamdani et al., 2021). According to Jan et al. (2018a) the hardness of gluten-free biscuits ranges from 34.05 N to 58.09 N. Increasing levels of rice bran (RB) and quinoa (Q) significantly influenced the hardness of the cookie samples ($p < 0.05$). The higher hardness values observed in RB-10, RB-15, and Q-15 compared to lower substitution levels (RB-5, Q-5, and Q-10) are likely due to the increased fiber and protein content, which enhance matrix compactness by limiting moisture retention and increasing structural rigidity. This increase in hardness can be attributed to the high fiber and protein content of rice bran and quinoa, which enhance the structural integrity of the cookie matrix by reducing its porosity and increasing density (Dhingra & Jood, 2002; Sudha et al., 2007). Moreover, the water-binding capacity of dietary fibers may limit water availability for starch gelatinization, leading to firmer textures. The reduction of free water in the dough caused by the binding of the water of the proteins in the quinoa and rice bran proteins was linked to the increase

in hardness when compared to the control cookie. The amount of fiber in the finished product also influences hardness. The hardness of food products increases as the fiber content increases.

In conclusion, adding 5% rice bran and 5% and 10% quinoa did not result in a decrease in the spread ratio of the cookies. It was determined, these additional levels shouldn't be exceeded to get a spread ratio that is comparable to the control cookie. Their hardness tended to increase with the addition of quinoa and rice bran, but this increase was more constrained in cookies prepared with rice bran, and the hardness of cookies prepared with 5% and 10% quinoa was comparable to the control group. It has been demonstrated that quinoa performs better than rice bran in terms of producing softer cookies.

3.2. TPC and antioxidant activity

TPC and antioxidant activity results of cookie samples are shown in Figure 2. TPC levels before simulated gastrointestinal digestion (chemical or methanolic extracts) ranged from 0.366 to 0.634 mg GAE g⁻¹. According to [Hamdani et al. \(2021\)](#), gluten-free biscuits have a total phenolic content that ranges from 0.37 to 0.48 mg GAE g⁻¹. The cookie sample manufactured from rice flour has a TPC value of 0.9 mg GAE g⁻¹, according to [Molinari et al. \(2018\)](#). TPC of the millet flour-based combinations ranged from 18.34 to 52.01 mg GAE 100 g⁻¹ ([Sharma et al., 2016](#)). When we compare the results of our study to those of earlier studies, we find that our findings are consistent with those documented in the literature.

In the present study, the addition of quinoa and rice bran to the cookies significantly increased the TPC. The TPC decreased with increasing levels of quinoa addition, while the amount of TPC increased with the addition of rice bran. TPC levels in these cookies, however, were higher than in the control. The decrease in the amount of TPC with increasing quinoa addition was attributed to the difficulty of phenolic extraction as a result of interactions between proteins and phenolics.

The DPPH radical scavenging effect ranged from 60.45% to 88.13%, with the control group having the lowest radical scavenging effect. All chemical extracts of all cookies displayed very high radical inhibition. Sorghum flour has more antioxidant components than wheat flour. Also, quinoa and rice bran have rich content of phenolic and antioxidant compounds ([Antognoni et al., 2021](#)).

The cookie prepared with 15% rice bran contained the highest value with 88.13%. Although quinoa had a lower influence on DPPH radical scavenging than rice bran, it nevertheless had a higher radical scavenging capability than the control group.

Using the ABTS method, the scavenge capacity of ABTS radicals was evaluated to determine their antioxidant activity. It was found that quinoa and rice bran enhanced the ABTS scavenge capacity of cookies and that cookies with 15% rice bran had the highest degree of ABTS inhibition (38.01%). According to [Jan et al. \(2018b\)](#), adding more quinoa flour to combinations of wheat and quinoa flour raised their DPPH radical scavenging activity from 12.46% to 16.59%. As epidemiological studies have shown that regular consumption of antioxidants is linked to a lower risk of cancer and cardiovascular disease, antioxidants have drawn more consumer interest. In this regard, quinoa and rice bran can serve as excellent sources of antioxidants in gluten-free foods.

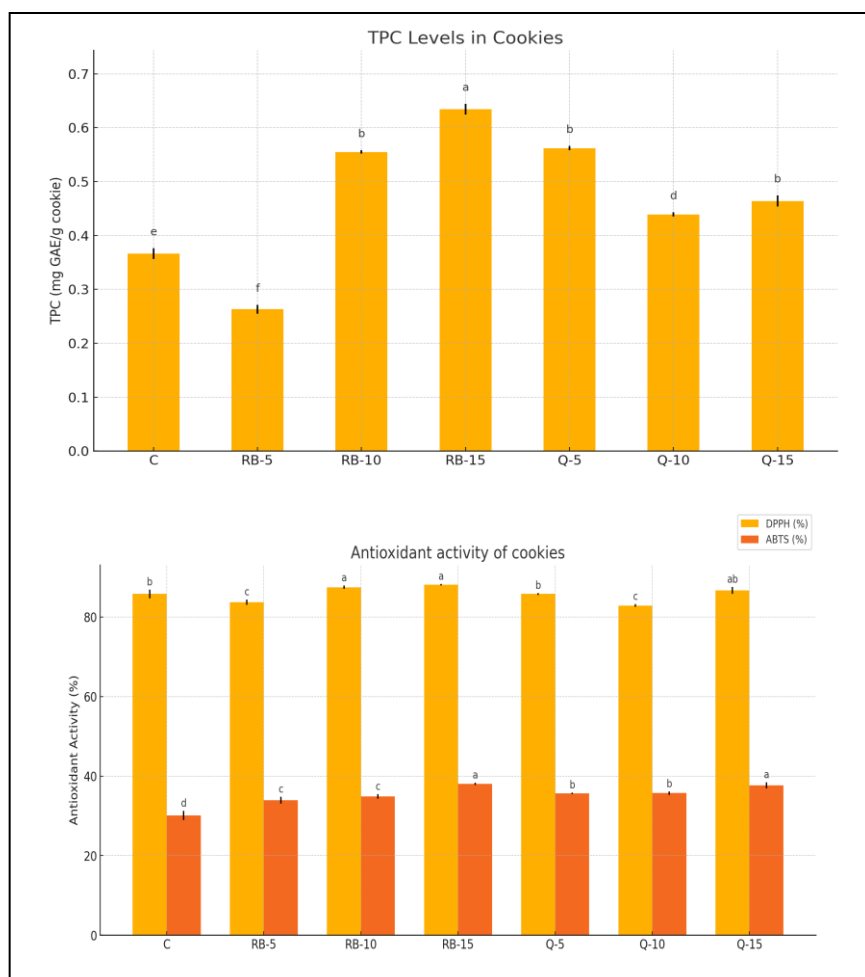


Figure 2. TPC and antioxidant activity of chemical extracts of the cookie (before digestion) (Different superscript letters indicate statistically significant differences ($p < 0.05$)).

3.3. Changes in TPC and antioxidant properties after the simulated digestion and bioavailability of TPC and antioxidant compounds

The potential bioavailability of phenolics present in samples, as well as the changes in their antioxidant properties were examined by using the simulated gastrointestinal system. Results are given in Figure 3.

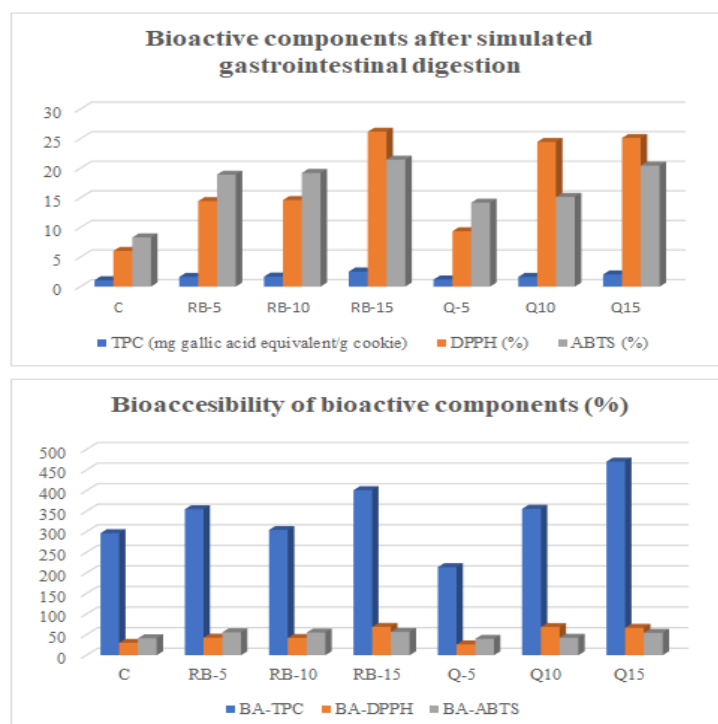


Figure 3. TPC, antioxidant activity, and bioaccessibility of the bioaccessible fraction of cookie (after digestion).

Compared to the initial methanolic extract, the amount of total phenolics significantly increased after the simulated digestion. Probably, certain bound phenolic acids were transformed into soluble forms after the digestion of the cookie sample. TPC increased by 2.14 to 4.7 times compared to the methanolic extract (before digestion). This increase is evidence that the extraction of phenolics is better with the addition of enzymes. According to numerous studies, phenolics are boosted by heat, acid, and enzymes. The addition of RB and Q resulted in significant increases in the TPC values of the bioaccessible fractions. Cookies enriched with %15 RB contained on average a 2.34-fold higher content of phenolic compounds than control cookies. The total content of polyphenols in RB15 reached 2.544 mg GAE g⁻¹. Already at 5% RB, the mean content of phenolics was approximately 1.51-fold higher compared with the control cookie, while the 10% addition of rice bran caused a 1.55-fold increase. Similarly, in the cookies enriched with quinoa, the content of phenolics was 5, 10%, and 15% higher than in the control cookie. The increases were 1.10, 1.52, and 1.90-fold for Q5, Q10, and Q15, respectively. All fold change and percentage differences mentioned in the text were calculated based on the control sample values and are summarized in Table 3.

Table 3. Fold change and percentage increase in total phenolic content (TPC) after gastrointestinal digestion of enriched cookies compared to control

Sample	TPC (mg GAE/g)	Fold Change vs Control	% Increase vs Control
Control	1.09	1.00×	0.00%
RB5	1.65	1.51×	+51.38%
RB10	1.69	1.55×	+55.05%
RB15	2.54	2.34×	+133.03%
Q5	1.20	1.10×	+10.09%
Q10	1.66	1.52×	+52.29%
Q15	2.07	1.90×	+89.91%

RB5: Cookies containing 5% rice bran, RB10: Cookies containing 10% rice bran, RB15: Cookies containing 15% rice bran, Q5: Cookies containing 5% quinoa, Q10: Cookies containing 10% quinoa, Q15: Cookies containing 15% quinoa

The amount of food components that are liberated from the solid matrix during digestion and transit through the gut is referred to as bioaccessibility. The BE-TPC in the control cookie was found to be 296%. RB and Q addition experienced a considerable increase in BE-TPC. The greatest BE-TPC was measured as 470% in samples (Q15) containing 15% quinoa. In the present study, phenolics were highly bioaccessible, as indicated by the improved cookies' estimated relative bioaccessibility index. The cookies prepared with 15% Q and 15% RB had the highest bioaccessibility index, which may mean that these additives helped to increase the release of the phenolic compounds examined during in vitro digestion. In this regard, a 1.58-fold increase in TPC bioaccessibility was detected when 15% quinoa was added to the cookie formulation. The findings may also point to an increase in phenolic content caused by the addition of the Q and RB as functional additives, which led to a higher relative bioaccessibility of the sorghum-based cookies under investigation and is a sign of the additive's efficiency. When green coffee extracts (Świeca et al., 2017), and free and microencapsulated powders from *Amelanchier alnifolia* Nutt (Lachowicz et al., 2020) were added to bread, similar results were seen. The relative bioaccessibility index value calculated for antioxidant activity showed that these breads had a high in vitro relative bioaccessibility. The results as mentioned above could be attributed more easily extraction of phenolics via the enzymatic digestion pathway. As was previously observed for wheat bread enriched with green coffee beans or microencapsulated powders from *Amelanchier alnifolia* Nutt, the digestion process itself can also facilitate the release of phenolic compounds, and the low relative bioaccessibility of the compounds may indicate interactions with the components of the dough matrix (Świeca et al., 2017; Lachowicz et al., 2020).

The results of antioxidant activity after simulated gastrointestinal digestion are shown in Figure 3. According to the findings, after simulated digestion, DPPH radical scavenging ability was 6.04% in the control samples, whereas it was 26.17 in RB15. Similarly, the inhibition of the ABTS radical varied between 8.31 and 21.45%. In both radical scavenging tests, while the highest antioxidant activity after simulated gastrointestinal digestion was found in the sample containing 15% rice bran, the control cookie had the lowest radical inhibition rate.

However, the antioxidant potential of bioaccessible parts of cookies was significantly lower than that of the chemical extracts when measured with the DPPH assay, that is, by 84.75%, 87.5% and 88.13% in the RB5, RB10, and RB15 in the case of chemical extraction; 14.45%, 14.64%, and 26.17% in the case of gastrointestinal digestion; and 85.78%, 85.89%, and 86.71% in the Q5, Q10, and Q15 in the case of chemical extraction. The effects of the bioaccessible fractions on DPPH radical scavenging were 14.19 %, 15.1%, and 20.45% for Q5, Q10, and Q15, respectively. A similar trend was observed when compared with the ABTS radical scavenging effects of cookies. The radical scavenging effect of bioaccessible fractions was lower than that of chemical extracts. Earlier research demonstrated that interactions with the food product matrix might reduce the antioxidant activity of bioactive substances. Lachowicz et al. (2021) obtained similar results in the control group bread. These researchers revealed that the FRAP of the chemical extract was higher than the FRAP of the bioaccessible part obtained after simulated in-vitro digestion. Also, it is known that phenolics and antioxidant compounds can interact with protein groups. A more nuanced understanding of the observed decrease in antioxidant activity in bioaccessible fractions, compared to chemical extracts, can be gained by considering recent literature. Protein–polyphenol interactions are known to affect the antioxidant potential of food matrices. For example, Elias et al. (2008) reported that proteins and peptides exhibit inherent antioxidant activity, but these effects may be altered or diminished through interactions with polyphenols, depending on their structural properties and surrounding matrix conditions (Elias et al., 2008). Furthermore, Lam Hon Wah et al. (2024) demonstrated that certain peptides, such as pea pentapeptides, can antagonize the antioxidant potential of polyphenols like quercetin and rutin.

Additionally, the structure and composition of the food matrix play a critical role in regulating antioxidant bioaccessibility. Neji et al. (2023) observed that incorporating legume proteins into cereal-based matrices reduced the antioxidant activity of bioaccessible fractions despite high phenolic content (Neji et al., 2023). Therefore, besides protein-antioxidant compound interactions, the physicochemical properties of the matrix and additive composition significantly influence antioxidant activity and bioaccessibility in enriched bakery products.

The antiradical compounds were highly bioaccessible based on the values of the bioaccessibility index determined for all of the investigated cookies. Świeca et al. (2017) made the opposite observation for wheat bread enriched with green coffee, finding that while the bioaccessibility was lower, the

antioxidant activity was higher after in vitro digestion than it was for raw products. In the present study, it was observed that cookies enriched with higher levels of rice bran and quinoa exhibited greater antioxidant bioaccessibility after in vitro digestion compared to those with lower levels of enrichment.

RB and quinoa had a positive impact on cookie quality. Due to their widespread consumption, cookies are a fantastic example of a food product that can be used as a perfect carrier for functional substances that may be incorporated at any point in the technological process. The in vitro bioaccessibility of compounds present in functional additives—and their impact on the nutritional value of the final product—are often overlooked when evaluating the true benefit of fortified foods. However, these are critical considerations that must be taken into account during the formulation of such products (Lachowicz et al., 2020).

3.4. TBA

TBA results are given in Table 2. In comparison to the control, TBA values increased when rice bran and quinoa were added. Due to the lipolytic enzymes in rice bran and the unsaturated fatty acids in quinoa, oxidation will accelerate when enhanced foods containing these ingredients are designed.

In both rice bran (RB) and quinoa (Q) supplemented groups, a gradual increase in TBA values was observed with higher substitution levels. Specifically, the TBA value of RB15 (3.99 mg MDA/kg) was markedly higher than those of RB5 and RB10, while a similar trend was evident in the quinoa group, where Q15 (3.72 mg MDA kg⁻¹) exceeded both Q5 and Q10. This increase can be attributed to the elevated levels of unsaturated lipids and bioactive compounds present in rice bran and quinoa, which are more prone to oxidative degradation during baking and storage. At higher concentrations (15%), the increased surface exposure and lipid content may have accelerated lipid oxidation, resulting in significantly elevated TBA values. These findings suggest that although rice bran and quinoa contribute to the nutritional enhancement of cookies, their optimal inclusion level should be carefully determined to avoid promoting oxidative instability. A number of studies have explored thiobarbituric acid (TBA) analysis in cookies and similar baked goods to assess lipid oxidation, which is a key factor in determining shelf life and product stability. One such study investigated the incorporation of microencapsulated fish oil into cookies and evaluated lipid oxidation through thiobarbituric acid (TBA) analysis. The results showed that encapsulation significantly reduced lipid oxidation levels in cookies ($P < 0.05$), suggesting enhanced oxidative stability during storage (Jeyakumari et al., 2016). In the study conducted by Meral et al. (2025), thiobarbituric acid (TBA) values were used to evaluate lipid oxidation in cookies formulated with maltodextrin-based nanoemulsions as fat replacers. The results indicated a gradual increase in TBA values from 1.47–2.51 mg MDA/kg on day 0 to a maximum of 4.13 mg MDA/kg by day 90. However, the increase was significantly lower in cookies containing nanoemulsions, especially those with sesame and coconut oil. The sesame oil group exhibited the smallest increase (29.4%), suggesting that nanoemulsions enriched with natural antioxidants can effectively delay lipid oxidation and may serve as clean-label alternatives to synthetic preservatives.

In a study employing potato peel extract and BHA in biscuits had TBA values of 0.382–0.625, 0.378–0.597, and it varied between 0.376–0.578 and 0.376–0.550 mg MDA kg⁻¹, respectively, over the 6-month storage period. The TBA value was reported to range between 0.383 and 0.669 in biscuits with BHA and 0.385 to 0.805 MDA kg⁻¹ in biscuits from the control group. The samples used in our analysis produced TBA findings that were higher than those noted in the literature. The qualities of the flour utilized in the research and/or the procedures used in the extraction of cookies oils are assumed to be the reason why the TBA value is higher than the literature data.

3.5. Sensory

Figure 4 shows the findings of the sensory analysis. A one-way ANOVA followed by Tukey's post hoc test ($P < 0.05$) was applied to evaluate significant differences among the sensory scores. As a result, all cookies received high scores for all rating attributes. Control cookies, however, were the most widely desired cookies. Among fortified cookies, cookies made with a combination of 95% sorghum and 5% quinoa flour received the highest overall approval ratings, followed by cookies made with 5% rice bran and 95% sorghum ($P < 0.05$). In the present study, the cookies containing quinoa were more preferred than those containing rice bran, and the overall acceptability scores decreased when the level

of both rice bran and quinoa increased. The lowest scores were given to cookies made with 15% rice bran substitution (RB15), and panelists noted some bitterness in these cookies. This bitterness may be linked to the higher concentration of fiber and phenolic compounds in rice bran, which are known to impart astringent or bitter flavors in baked goods (Ertürk and Meral, 2019). Texture and mouthfeel also influenced sensory preferences. Cookies enriched with higher levels of rice bran or quinoa exhibited a firmer and denser structure, which may have negatively affected their acceptability. In contrast, the 5% quinoa formulation maintained a crisp yet tender texture, which was positively received by the panelists. Probably due to the high protein, fiber, and ash content of quinoa, cookies with a slightly darker appearance and color than the control were produced, resulting in a decrease in taste, odor, and overall acceptability scores. Color differences were also noted to play a role in perception, as darker cookies were sometimes perceived as overbaked or less appealing, especially in higher substitution levels.

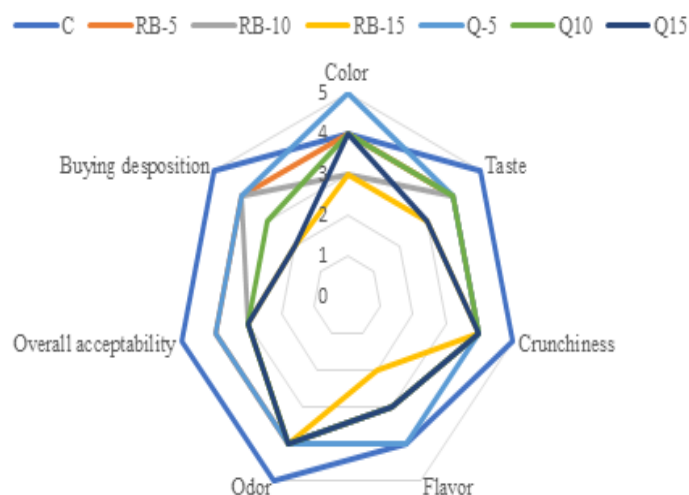


Figure 4. Sensory scores of cookies.

The cookies with the greatest sensory ratings were those produced with 100% sorghum flour (control cookies) and cookies prepared with a combination of 5% quinoa flour and 95% sorghum flour. In this study, sorghum flour was used as a starch source, as it has been used in many food processes

Ciacci et al. (2007) also confirmed the favorable flavor profile of sorghum-based products in gluten-free diets. Similarly, Rai et al. (2014) found that cookies made with sorghum flour demonstrated superior sensory properties. These findings are consistent with the present study and support the suitability of sorghum as a base flour in gluten-free cookie formulations. Therefore, cookies enriched with sorghum and small amounts of quinoa may provide an acceptable gluten-free alternative for individuals with gluten intolerance. In addition, as these formulations rely on locally sourced and relatively low-cost ingredients, they may offer a more accessible option for broader populations.

4. Conclusion

The fortification of gluten-free cookies was carried out using rice bran and quinoa, two functional ingredients known for their richness in fiber, polyphenols, and other bioactive compounds. The enriched cookies exhibited acceptable aesthetic and sensory properties, with especially favorable responses observed for formulations containing lower substitution levels. This suggests that rice bran and quinoa can be successfully incorporated into gluten-free baked products without compromising consumer acceptability.

In addition to their sensory appeal, the inclusion of these ingredients significantly enhanced the nutritional profile of the cookies by increasing total phenolic content and antioxidant capacity. However, antioxidant activity measured after in vitro digestion was lower than that of chemical extracts, indicating that bioaccessibility of antioxidant compounds remains limited despite high total content. These findings are of particular relevance to individuals with gluten intolerance or celiac disease, who often face challenges in accessing nutritionally balanced gluten-free products.

From a practical standpoint, this study offers valuable insights for the food industry, especially in the development of functional bakery products that align with current trends in health-conscious and allergen-free eating. The use of sorghum flour, combined with rice bran and quinoa, not only supports the formulation of gluten-free alternatives but also allows for the utilization of underused or by-product materials, contributing to sustainable food processing. Further studies are recommended to investigate encapsulation techniques or alternative formulation strategies aimed at improving the release and stability of antioxidants during digestion.

Furthermore, the affordability and nutritional enhancement potential of these ingredients make them promising candidates for addressing dietary needs in lower-income populations. Therefore, the findings of this research have broader implications for public health nutrition and can inform future innovations in gluten-free product development aimed at improving both health outcomes and food accessibility.

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