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SCIENTIFIC PAPER

UDC

# GLUTEN AND GLUTEN-FREE BISCUITS WITH FUNCTIONAL COMPONENTS: PHYSICOCHEMICAL, NUTRITIONAL AND ANTIOXIDANT PROPERTIES

## Highlights

- Functional additives for gluten and gluten-free biscuits with enhanced nutritive status
- Utilization of byproducts for the preparation of fine bakery products and better sustainability and circular economy concepts.
- Gluten-free biscuits rich in fiber

## Abstract

*This study aims to determine the effect of different compounds on the nutritional, antioxidant, microstructural, and color characteristics of biscuits classified as gluten and gluten-free. Namely, biscuits are enriched with dietary fibers, acacia fibers, spent coffee grounds, and anthocyanins. The addition of these functional components to biscuit matrix affected the physical properties of the biscuits; namely, the spread factor value of all biscuits ranged from 2.98 to 7.88, the content of total polyphenols increased, the highest polyphenol content was obtained in the gluten-free biscuits with added coffee grounds (77.98 mg), while in the biscuits with wheat flour has in the range of 44.62–128.63 mg. All gluten-free biscuits can be labeled as products with "rich in fiber" (6.32-7.68 g/100 g) and with a higher antioxidant content compared to biscuits without added ingredients. The total number of microorganisms in the tested cookies is below acceptable limits. The findings of this study show that the inclusion of raw nutritional components in the recipe of traditional gluten and gluten-free biscuits leads to an improvement in the nutritional value and other quality characteristics of the fortified food products.*

*Keywords: Antioxidant activity, polyphenol content, biscuits, gluten-free biscuits.*

## INTRODUCTION

Consumer demand for health-enhanced products is constantly increasing due to socio-economic trends and environmental concerns, such as longer life expectancy, rising health care costs, pursuit of a better quality of life, as well as better planetary health [1-4]. To develop value-added and functional food products, the addition of nutritive components could be a potential solution. The benefits are even greater when the added functional compounds are originally coming from biowaste or byproducts, e.g., from the food sector with functional properties. Most of them are already reported in the literature and are a valuable source of dietary fibers, vitamins, minerals, etc. [5-9].

The bakery products are an important part of the human diet. At a global level, bakery products constitute an essential part of human nutrition. The development of innovative products using value-added ingredients has become an important trend in the bakery sector to meet the demand of a new generation of consumers seeking healthier lifestyles [9]. Thanks to their ready-to-eat, long shelf life, and affordable price, biscuits are one of the most popular bakery products consumed worldwide [10-13]. In

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most cases, biscuits are high in sugar and fat but low in vitamin content, minerals, fiber, and phenolic compounds [14]. Based on the literature, there are a lot of studies on the enrichment of biscuits, e.g., with a collagen peptide [10], olive stone powder [15], and green tea [16], that are mainly used as sources of dietary fiber, proteins, and phenolic compounds to fortify the biscuits.

On the other hand, gluten-free biscuits tend to be low in protein and high in starch compared to traditional gluten biscuits, according to many scientific findings, for example, by Cervini *et al.* [17]. Other authors have also reported that gluten-free biscuits have a different protein and starch content compared to traditional biscuits, etc. [18–20] On the other hand, Hopkin *et al.* [21] suggests that alternative flours, such as coconut and almond, that can improve the functionality of gluten free products; namely, they can be used in gluten-free biscuit recipes to achieve a similar texture and taste to traditional biscuits. Moreover, the use of plantain and amaranth flours could serve as a rich source of polyphenols and dietary fibers in gluten-free bread formulation [22].

The author's position is that incorporating functional additives such as acacia fiber, citrus fiber, spent coffee grounds, and anthocyanins into traditional and gluten-free biscuits can improve their nutritional value and sensory properties. Namely, *Robinia pseudoacacia* L. (acacia) belongs to the *Fabaceae* family; it is a honey tree. Acacia flowers are white and form inflorescences that are 10–15 cm long; it is edible and nutritious, rich in proteins and trace nutrients, flavonoids, robinin, polysaccharides, zinc, magnesium, iron, calcium, β-carene, linalool, anthranylated aldehyde, and (Z)-β-farnesene provide a pleasant aroma. Acacia flower contains bioactive components – phenols and ascorbic acid. Therefore, it is used in the food industry for the preparation of a variety of sweets as well as in functional products as a source of antioxidants [23]. In addition, acacia flower flour [24] is rich in dietary fiber, and it has a beneficial effect on blood sugar and cholesterol levels.

On the other hand, the use of biowaste and byproducts from the food industry as ingredients in the development of new products represents an important approach for a better economic and environmental qualification of these materials [25–27]. Therefore use of extracted nutritive components from them and their utilization in bakery products becomes very attractive nowadays. There are a lot of studies on this topic. For example, dietary fibers from different fruit waste, like citrus ones, anthocyanins from certain fruit peels, or spent coffee grounds, are only a few examples that are considered in this study. Namely, citrus fibers have a low glycemic index and can reduce the glycemic content of foods. Citrus fibers possess high water and fat-binding capacities due to their physicochemical properties, which are similar to those of the original citrus fruits [28]. Furthermore, coffee is a rich source of antioxidants and has been shown to have anti-inflammatory effects [29]. Coffee by-products have significant potential to be used as additives in food products. Coffee by-products have relatively high antioxidant properties, and they are rich in fiber and nutrients, making them potential functional components in many products [30]. Despite their high antioxidant activity, fiber source, and polyphenol content, the use of coffee by-products is relatively scarce [31,32].

In addition, Acacia fiber in the form of flour is often used as a thickener or emulsifier in food processing. It is also a good source of fiber and has been shown to have prebiotic properties that help maintain gut health [33]. On the other hand, anthocyanins are water-soluble pigments found in various fruits and vegetables, such as berries, red cabbage, and black rice, responsible for their red, purple, and blue colors. These compounds have been shown to have antioxidant and anti-inflammatory effects [34].

The color of the biscuits can be an indicator of their nutritional value, taste, as well as customer acceptance, like the orange color is often seen in food with high vitamin C and carotenoid content, while green foods are often rich in chlorophyll and antioxidants [34].

Coffee has a brown color due to changes during heat treatment, while anthocyanins are responsible for the red, blue, or purple color, depending on their origin, fruit/vegetable types. These pigments are powerful antioxidants that have been linked to numerous health benefits, including reduced inflammation and improved heart health [31,32]; the addition of acacia to the biscuits will provide a high fiber content, which has the function of improving the health of the intestines while the coffee grounds addition will give a rich flavor and aroma (and possibly some caffeine) to the biscuits. In other words, the newly developed biscuits will not only be tasty but also will have high nutritional potential for the consumer; they could be classified as functional products. In this context, the addition of functional components can be associated with distinctive biscuit colors that may serve as visual indicators of their health benefits. For example, biscuits with anthocyanins may appear violet/purple and could appeal to children while indicating antioxidant content. In this context, gluten and gluten-free biscuits could be color-coded based on the addition of functional components, making them easily recognizable-especially for children. This color coding could also serve as an indicator of health benefits; for instance, one color could signify improved brain function, while another could indicate benefits for eye health, helping consumers easily identify the specific advantages of each product. In other words, the newly developed biscuits will not only be tasty but also will have high nutritional potential for the consumer; they could be classified as functional products.

The aim of this study is to compare traditional gluten-containing and gluten-free biscuits enriched with citrus fibers, acacia fiber, spent coffee grounds, and anthocyanins in terms of their physicochemical, nutritional, and antioxidant properties.

## MATERIALS AND METHODS

### Materials

The compounds used in this study are: flour type 400 (Zito Luks, North Macedonia), rice flour-gluten free (Vitalia, North Macedonia). Functional additives to the traditional recipes of gluten and gluten-free biscuits are:

Citri-Fi®100FG (Fiberstar Inc., River Falls, Wisconsin, United States), is finely ground citrus fiber, with more than 95% (±4%) of the particles passing through a 100-mesh sieve, and spent coffee grounds (KaffeBueno, Denmark) were used in the formulation; acacia powder (Nexira, France); anthocyanins (Currantcraft® 11%, Iprona AG S.p.A., Italy); and stevia, a commercially available blend purchased from the company Vitalia (Skopje, North Macedonia), was also included due to its role as a natural, low-calorie sweetener contributing to the functional profile of the biscuits.

### Biscuit preparation method

Two types of biscuits have been prepared: gluten and gluten-free. The process involves multiple stages, each specifying time duration, temperature, and designation. The designation refers to specific labels assigned to each processing stage in order to clearly identify and differentiate steps such as mixing, baking, and cooling. This system allows for precise control, consistent replication, and easy tracking of the production process.

The initial steps (Stages 1 and 2) focus on the preliminary preparation and dosage of raw materials at a temperature of 20-22 °C. Subsequently, Stage 3 includes the mixing of ingredients for 5 to 10 minutes at 18-20 °C to prepare a crumbly butter pastry base, which is then used to form the shaped pastry biscuit dough. Stage 4 includes the resting and cooling of the prepared dough for 30-40 minutes at 3-4 °C to stabilize the structure and improve the handling properties before further shaping, and Stages 5 to 7 include cooling, rolling out, and formation, all at 18-20 °C. The biscuits are then arranged in baking trays in Stage 8, with a baking time of 10-12 minutes at 200 °C (Stage 9). Afterward, the biscuits cool again (Stage 10) before proceeding to the final stage of packaging at 20-22 °C (Stage 11). Different amounts of functional components are added to gluten and gluten-free biscuits: citrus fibers, acacia powder, coffee grounds, and anthocyanins; compositions are presented in Table 1.

The developed formulations are based on the partial replacement of wheat flour with various functional additives, while the amounts of the remaining raw materials are constant. The amount of wheat flour for gluten-free biscuits is replaced with rice flour, while the amounts of the functional components are preserved. The biscuits were evaluated for their antioxidant activity, physicochemical characteristics, and sensory qualities.

### Methods

#### *Physical characteristics of biscuits.*

The thickness of the biscuits was determined using an electronic digital calliper, and the diameter was measured by the method of Chopra *et al.* [35]. Three biscuits are placed next to each other, and their diameter were measured. Then, the biscuits were rotated 90°, and their diameter (cm) was measured again. An average value was taken from the measurements. The ratio between the diameter and thickness of the biscuits (biscuit expansion factor) was also determined [36].

#### *Color characteristics of biscuits*

An instrumental measurement of the color of the crust biscuits was made, with a digital colorimeter for quality control, model PCE-CSM 5 (PCE Deutschland GmbH, Meschede, Germany). The indicators were reported according to the CIE L*a*b* system. The colorimeter was calibrated with a black-and-white color standard [37]. The color parameters $L^*$, $a^*$, and $b^*$ from Lab, as well as $C^*$ and h* from the LCh color models, were determined.

The color difference $\Delta E$ was determined by Eq. (1):

$$\Delta E = \sqrt{(L_c - L_a)^2 + (a_c - a_a)^2 + (b_c - b_a)^2} \quad (1)$$

where $L_c$, $a_c$, and $b_c$ are color components of the control sample, and $L_c$, $a_a$, and $b_a$ are color components of the enriched samples. $\Delta E$ varies in the range of 0-100, with the closer to 0, the closer the colors of the enriched biscuits were to those of the control sample, and the closer it was to 100, the more strongly they differed.

#### *Water activity of biscuits*

The water activity ($a_w$) of the biscuits was determined with a water activity meter with a sensor device, model HP23-AW-A (ROTRONIC AG, Bassersdorf, Switzerland).

#### *The moisture content of biscuits*

Moisture content of the biscuits was determined by the methods of AOAC (2000) [35]. Moisture was analyzed using a moisture analyzer based on the principle of infrared dry heating (via a halogen lamp).

#### *Microbiological analysis of biscuits*

The total number of microorganisms and the number of molds and yeasts in the biscuits were determined according to the AACC method [38]. Microbiological assay results were expressed as log(CFU/g).

#### *Antioxidant activity (DPPH) of biscuits*

The determination of the antioxidant activity of the biscuits using the DPPH free radical was done according to the methodology described earlier [39]. Biologically active substances were extracted with methanol solution (methanol:water 80:20 v/v). 1 g of sample extract was mixed with 10 mL of methanol solution. The mixture was placed on an electromagnetic stirrer at room temperature for 2 h, then centrifuged at 4300 rpm for 10 min. The antioxidant activity of the extracts was expressed as milligrams of Trolox equivalents (TE) per 100 grams of dry weight (mg TE/g dw).

#### *Total polyphenols of biscuits*

The content of total polyphenols was determined by the method with a modification [40]. In a test tube, 0.1 mL of sample extract was successively mixed with 0.5 mL of Folin reagent (diluted 1:4 with distilled water) and 1.5 mL of aqueous sodium carbonate solution (7.5% w/v), with the volume made up to 10 mL with distilled water.

The reaction mixture was allowed to stand for 2 h in the dark at room temperature before the absorbance at 750 nm was measured. The results obtained are presented as gallic acid equivalents (GAE) in mg per 100 g of sample.

### *Determination of the nutritional value of biscuits*

Crude protein content was determined by the micro-Kjeldahl method by taking 1.0 g of the sample as described in the AOAC (2000) method 920.87 [35]. The crude fat content was determined by taking 1.5 g of the sample by the Soxhlet extraction method using petroleum ether as solvent (the AOAC (2000) method 920.39) [35]. Crude fiber content was determined by the AOAC (2000) method 962.09 [35] after mixing with 1.25% $H_2SO_4$ and 28% KOH, sieving through 75 μm, drying, and firing in a muffle furnace to remove the ash from the raw fibers. The difference determined the total carbohydrate content [41]. The amount of carbohydrate fractions was calculated by subtracting the sum of the amounts of fat, protein, and ash from 100%. Carbohydrate content was expressed as % dry matter.

### *Statistical analysis*

Principal component analysis (PCA) [42] is a statistical technique that is used to find patterns in data. PCA is more commonly used to reduce the dimensionality of data, which can make it easier to analyze and interpret. In this study, PCA was used to investigate the relationships between the contents of acacia, citrus fibers, coffee grounds, and anthocyanins in biscuits and their main characteristics. Before the implementation of PCA, the data were normalized in the interval [0,1]. One-way ANOVA was used to evaluate differences among group means for the relevant variables. Where ANOVA indicated statistically significant differences ($p < 0.05$), a post hoc LSD (Least Significant Difference) was performed.

Additionally, for datasets that did not meet the assumption of normality, the Kruskal-Wallis test, a non-parametric alternative to ANOVA, was applied to ensure the validity of the statistical inferences.

The MATLAB software system (The Mathworks Inc., Natick, MA, USA) was used in the processing of the obtained data. All data were processed at an accepted level of statistical significance, $\alpha < 0.05$.

## RESULTS AND DISCUSSION

### Physical and color characteristics

Two types of biscuits, gluten and gluten-free, with four functional components, were observed. In Figure 1, the gluten and gluten-free biscuits are presented in a general, stylized form. Usually, the gluten biscuits had a brownish color, which was consistent with their production.

However, there were some notable variations between samples, to which finely ground citrus fibers, spent coffee grounds, anthocyanins, and acacia were added. They appeared noticeably darker in color than the control sample. This observation suggested that these additives might have affected the baking process in a way that resulted in a more intense coloration of the biscuits. In contrast, the acacia biscuits appeared closer in color to the control sample, suggesting that this additive had less effect on the baking process, fewer pigments, and a color close to that of wheat flour.

Also, the gluten-free biscuits were presented in a general, stylized form. Noticeable color variation was observed between the different samples, which included a control sample as well as those supplemented with citrus fibers, acacia, coffee grounds, and anthocyanins. The control sample and those with citrus fibers and acacia were fairly similar in color, suggesting that these additives had relatively little effect on the overall appearance of the biscuits. In contrast, samples with coffee grounds and anthocyanins were significantly darker in color, indicating that these additives had a more pronounced effect on the biscuit's pigmentation. The visual presentation of color differences between samples of gluten-free biscuits might reflect differences in their composition, processing methods, or interactions between these factors. These results may be of interest to food producers seeking to optimize the visual appeal of their gluten-free products.

The change in color characteristics of the gluten-free biscuits corresponds to their visible presentation above. The addition of acacia did not result in a color change compared to the control, while samples with citrus fibers, coffee grounds, and anthocyanins appeared darker.

Table 2 shows data on gluten and gluten-free biscuits with various additives. The main effect of the addition of citrus fibers, acacia, coffee, and anthocyanins in gluten biscuits appeared to be related to their effect on the coloring of the final product. Specifically, the samples with citrus fibers, coffee grounds, and anthocyanins appeared darker in color than the control biscuits, while the acacia sample was similar in color to the control. However, it was important to note that these natural additives might have other effects on the physicochemical characteristics of biscuits. For example, Citri-Fi®100FG is a natural citrus fiber that has been shown to improve moisture retention and texture in biscuits. Acacia, also known as gum arabic, is a common food additive that improves the viscosity and stability of biscuits. Coffee and anthocyanins are sources of antioxidants that have potential health benefits.

From Table 2, it is evident that the mean values for the thickness of gluten biscuits decrease in the presence of the additive. A significant difference in thickness was observed for the sample with citrus fiber, while greater thickness was reported for the gluten-free samples, except for the coffee biscuits. All functional cookies were increased in diameter compared to the control. A significant difference in spread ratio was observed for all biscuits. The increase in the spread ratio in the gluten-free cookies with coffee addition might be due to a significant decrease in cookie thickness.

The value of the spread factor of all cookies ranged from 2.98 to 7.88. Clear differences in the spread factor were observed across all biscuit samples. In terms of physico-chemical characteristics, the moisture content of gluten-free biscuits varied depending on the type of additive used. Acacia biscuits had similar levels of moisture content to the control, while those with citrus fibers, coffee grounds, and anthocyanins showed increased moisture content. In addition, adding these additives to gluten-free biscuits increased their antioxidant activity, which might provide potential health benefits.

*Polyphenol content*

The content of total polyphenols for the gluten-free samples was in the range of 29.74–37.95 mg GAE/100 g. The content of total polyphenols was highest in gluten-free biscuits with coffee (77.98 mg), while in biscuits with wheat flour and supplement, it was in the range of 44.62-128.63 mg. The lowest content of these compounds was found in the control biscuits. It is worth noting that all the various types of polyphenols, the most abundant in phenolic acids, flavonoids, and anthocyanins, are acacia biscuits and coffee biscuits.

Table 1. Raw materials used for gluten and gluten-free biscuits.

| Gluten biscuits | | | | | |
|---|---|---|---|---|---|
| Functional Additive | Control | Citrus fibers, 2% | Acacia, 3% | Coffee grounds, 4% | Anthocyanin, 1% |
| Flour type 400, g | 100 | 98 | 97 | 96 | 99 |
| Sugar, g | 20 | 20 | 20 | 20 | 20 |
| Additive, g | - | 2 | 3 | 4 | 1 |
| Water, ml | 40 | 40 | 40 | 40 | 40 |
| Sunflower oil, ml | 30 | 30 | 30 | 30 | 30 |
| Vanilla, g | 2 | 2 | 2 | - | 2 |
| Gluten-free biscuits | | | | | |
| Functional Additive | Control | Citrus fibers, 2% | Acacia, 3% | Coffee grounds, 4% | Anthocyanin, 1% |
| Rice flour, g | 100 | 98 | 97 | 96 | 99 |
| Stevia, g | 10 | 10 | 10 | 10 | 10 |
| Additive, g | - | 2 | 3 | 4 | 1 |
| Water, ml | 50 | 50 | 50 | 50 | 50 |
| Sunflower oil, ml | 30 | 30 | 30 | 30 | 30 |
| Vanilla, g | 2 | 2 | 2 | - | 2 |

Table 2. Characteristics of gluten and gluten-free flour biscuits with various additives.

| Parametar | Control | Acacia | Citrus fibers | Coffee grounds | Anthocyanins |
|---|---|---|---|---|---|
| Color characteristics | | | | | |
| *L* | 56.70±0.40$^{*}$ | 43.00±4.16$^{**}$ | 25.68±3.13$^{*}$ | 23.55±0.61$^{*}$ | 25.02±3.97$^{*}$ |
| *a* | 3.41±0.28$^{**}$ | 2.09±0.95$^{*}$ | 0.99±0.45$^{**}$ | 5.55±0.61$^{*}$ | 4.74±0.93$^{*}$ |
| *b* | 16.33±0.98$^{*}$ | 11.78±2.25$^{*}$ | 7.64±2.51$^{**}$ | 9.88±1.14$^{*}$ | 4.79±0.65$^{*}$ |
| *c* | 16.42±0.58$^{*}$ | 11.97±2.38$^{*}$ | 7.70±2.54$^{*}$ | 11.34±1.28$^{*}$ | 6.30±1.68$^{**}$ |
| *h* | 78.11±0.54$^{**}$ | 80.26±2.44$^{**}$ | 82.80±1.03$^{*}$ | 60.66±1.09$^{*}$ | 40.09±5.40$^{*}$ |
| Physico-chemical characteristics | | | | | |
| WA | 0.82±0.00$^{***}$ | 0.83±0.01$^{**}$ | 0.84±0.00$^{***}$ | 0.86±0.06$^{*}$ | 0.85±0.00$^{***}$ |
| MC, % | 14.51±0.08$^{**}$ | 15.25±0.07$^{**}$ | 12.89±0.04$^{*}$ | 12.08±0.04$^{*}$ | 15.36±0.12$^{*}$ |
| *D*, cm | 4.67±0.15$^{**}$ | 5.03±0.15$^{**}$ | 4.93±0.12$^{*}$ | 4.77±0.25$^{**}$ | 5.07±0.06$^{*}$ |
| *T*, cm | 1.57±0.06$^{*}$ | 1.53±0.06$^{*}$ | 1.27±0.06$^{*}$ | 1.40±0.10$^{*}$ | 1.48±0.03$^{**}$ |
| SR | 2.98±0.04$^{*}$ | 3.28±0.04$^{*}$ | 3.90±0.24$^{**}$ | 3.41±0.07$^{*}$ | 3.42±0.03$^{**}$ |
| Antioxidant activity | | | | | |
| TP, GAE/100g | 44.62±0.02$^{*}$ | 66.29±0.30$^{*}$ | 50.93±0.23$^{*}$ | 128.63±0.11$^{*}$ | 58.46±0.57$^{*}$ |
| DPPH, TE/100g | 250.85±0.54$^{**}$ | 174.90±0.43$^{*}$ | 265.98±0.07$^{*}$ | 887.11±0.82$^{**}$ | 490.25±0.06$^{**}$ |
| Gluten-free biscuits | | | | | |
| Parametar | Control | Acacia | Citrus fibers | Coffee grounds | Anthocyanins |
| Color characteristics | | | | | |
| *L* | 31.82±2.65$^{*}$ | 29.20±1.22$^{*}$ | 38.08±1.22$^{*}$ | 18.21±0.84$^{**}$ | 16.45±4.55$^{*}$ |
| *a* | 1.13±0.06$^{*}$ | 0.95±0.12$^{*}$ | 0.59±0.10$^{*}$ | -0.21±0.18$^{*}$ | -0.06±1.67$^{**}$ |
| *b* | 6.77±0.33$^{**}$ | 6.22±0.69$^{*}$ | 7.44±1.10$^{*}$ | 1.36±0.26$^{**}$ | -1.96±0.27$^{*}$ |
| *c* | 6.86±0.33$^{*}$ | 6.28±0.73$^{*}$ | 7.49±1.16$^{**}$ | 1.39±0.26$^{**}$ | 2.38±0.38$^{*}$ |
| *h* | 80.54±0.45$^{*}$ | 83.02±2.38$^{*}$ | 83.11±2.87$^{*}$ | 90.41±14.56$^{*}$ | 238.36±8.88$^{*}$ |
| Physico-chemical characteristics | | | | | |
| WA | 0.84±0.01$^{**}$ | 0.86±0.06$^{**}$ | 0.85±0.00$^{***}$ | 0.89±0.00$^{***}$ | 0.90±0.00$^{***}$ |
| MC, % | 8.99±0.05$^{*}$ | 8.94±0.11$^{*}$ | 9.71±0.09$^{*}$ | 9.15±0.03$^{*}$ | 9.50±0.06$^{*}$ |
| *D*, cm | 4.97±0.06$^{*}$ | 5.07±0.12$^{*}$ | 5.00±0.00$^{***}$ | 4.97±0.06$^{*}$ | 5.10±0.10$^{*}$ |
| *T*, cm | 0.77±0.06$^{*}$ | 0.81±0.01$^{*}$ | 0.81±0.01$^{*}$ | 0.63±0.06$^{*}$ | 0.90±0.10$^{**}$ |
| SR | 6.51±0.56$^{*}$ | 6.28±0.20$^{**}$ | 6.17±0.08$^{**}$ | 7.88±0.64$^{**}$ | 5.71±0.53$^{*}$ |
| Antioxidant activity | | | | | |
| TP, mg GAE/100 g | 29.74±0.08$^{*}$ | 46.91±0.02$^{**}$ | 31.96±0.19$^{*}$ | 77.98±0.61$^{**}$ | 37.95±0.57$^{*}$ |
| DPPH, mg TE/100 g | 104.86±0.5$^{*}$ | 174.80±0.2$^{*}$ | 170.84±0.53$^{**}$ | 348.25±0.08$^{*}$ | 422.09±1.25$^{*}$ |

*WA-Water activity; MC-Moisture content; D-Diameter; T-Thickness; SR-Spread ratio; TP-Total polyphenols; DPPH - DPPH assay. Values are represented as mean ± standard deviation of triplicate samples. Mean values bearing different columns with superscript in the same column differ significantly (p<0,05). All data have statistically significant differences at p<0,05*

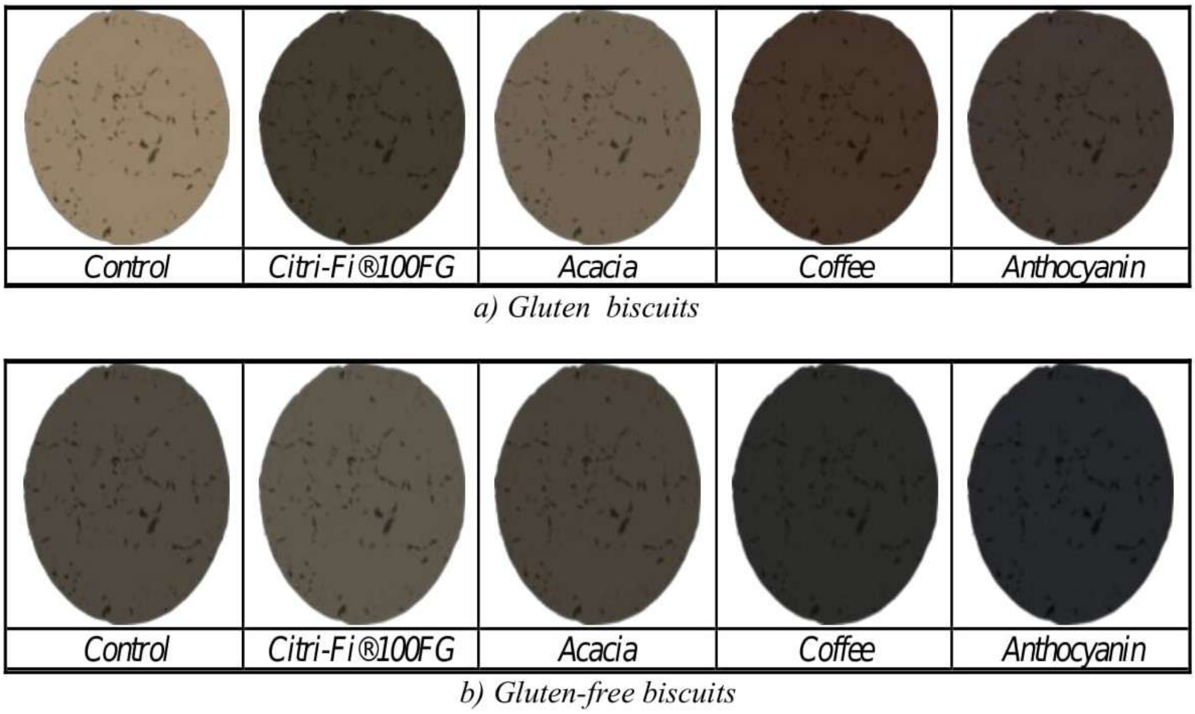


*Figure 1. General view of biscuits with additives: a) gluten biscuits and b) gluten-free biscuits.*

The baking process at high temperatures (>180-200 °C) can have a significant impact on polyphenolic molecules, leading to some changes in their content and structure [43]. For example, as a result of heat treatment, anthocyanins can be converted to chalcone glycosides and then hydrolyzed to chalcones, which are sequentially cleaved to form various end products such as aldehydes and acids [44]. In addition, metal ions present in food (iron, copper, and manganese) can be involved in the conversion of anthocyanins into quinones, which can react with proteins and other polyphenols to form condensation products that contribute to the browning of food [45]. In wheat grain, mostly insoluble fractions (77%) are found, followed by bound soluble acids (22%), including free and soluble fractions (0.5-1%) [46]. Baking increases the level of free phenolic acids in bread, cakes, and buns, but decreases the amount of bound phenolic acids [47], suggesting that baking may promote the release of phenolic acids from bound to free.

### *The antioxidant activity of biscuits*

The antioxidant activity of wheat flour biscuits was between 250 and 490 mg TE/100 g, while gluten-free samples ranged from 105 to 422 mg TE/100 g (DPPH method). Nevertheless, using the measurement method, the antioxidant activity of all cookies enriched with healthy additives was greater than that of the control biscuits, except for the wheat flour and acacia samples. The observed differences in the antioxidant activity of the studied biscuits were the result not only of the differences between the plant sources of the additives but also of the antioxidant activity of the individual phenols. The structure of polyphenolic compounds also plays a significant role; the greater the number of hydroxyl groups, the better the antioxidant properties [48].

### *Color characteristics of gluten biscuits*

Color parameters are important indicators of quality and influence consumers' willingness to buy a food product. As a result of the instrumental color analysis, it was found that the control was the lightest, with a high value for color brightness, $L^*$=56.27 (Table 3); higher $L^*$ values do not necessarily mean a deterioration in product quality, rather it may be the dilution effect of the wall materials refers to the presence of light-colored components (such as dietary fibers or polysaccharides) derived from encapsulating agents, which reduce the visual intensity of natural pigments in the dough, resulting in higher $L^*$ values (i.e., a lighter appearance).

With the darkest coloring were the samples with coffee. From Table 2 and normalized values in Table 3, it can be seen that the biscuits containing coffee have the highest values for parameter $a^*$ (5.55). The values for the parameter $a^*$ of the other types of biscuits are in the range from 0.99 to 4.74. This shows that all types of biscuits are in the brown-red range. From the research, we found that the lowest values for parameter $b^*$ have the biscuits containing anthocyanins (4.79), which proves the blue-purple color characteristic of anthocyanins. The highest values for $b^*$ were recorded for the control biscuits (16.33), which characterized them with a yellow color.

The hue angle ($H^*$) reflects the characteristic color of the samples. Hue angles of 0 °, 90 °, 180 °, and 270 ° represent red, yellow, green, and blue colors, respectively [50]. All samples showed values from the yellow coordinate (40.90-82.80).

Color saturation ($C^*$) of biscuits is a measure of color purity. The most saturated and uniform color was the control because the additives were of different particle sizes and had different color saturation in the cookies.

Figure 2 shows the color difference between a control sample and those with additives. In gluten flour biscuits, the addition of citrus fiber resulted in smaller color differences with the control sample. The samples with added coffee spent grounds and anthocyanins showed the biggest differences.

In the case of gluten-free biscuits, due to the specificity of the flour used, the control sample had a darker color. For this reason, samples with coffee spent grounds and anthocyanins had a small color difference from it. The biggest difference was seen in the acacia sample, as it was lighter in color.

### *Color characteristics of gluten-free biscuits*

The biscuits containing citrus fibers have the highest value for the $L^*$ parameter (38.08). The biscuits produced with the addition of anthocyanins (16.45) had the lowest values for this parameter due to the dark color of the addition. The values for the parameter $a^*$ in biscuits made with coffee and anthocyanins were the lowest, which characterized their dark brown and blue color, as well as the absence of gluten. The highest values for the same parameter were reported for the control biscuits.

The parameter $b^*$ indicates whether a studied object has a yellow or blue color. The lowest values for this parameter have been obtained for the biscuits produced with anthocyanins, while the highest values for the $b^*$ parameter were reported for the biscuits with citrus fibers.

The most saturated color ($C^*$) is the citrus sample. The hue angle ($H^*$) in the gluten-free samples was quite variegated; the control and the acacia citrus biscuits were in yellow shades, the coffee sample was in brown shades, and the anthocyanin biscuits were in purple-blue shades.

Measured data for biscuits made from gluten and gluten-free flour with different additives was processed with the PCA method. Table 3 shows the quality characteristic values of gluten and gluten-free biscuits with different additives. Values were normalized in the interval [0,1]. These values were most affected by the various additives in the biscuits. The number of components required was determined under the condition that the sum of the principal components should describe more than 95% of the variance in the data. Up to 11 principal components could be calculated in rows, and up to 4 in columns.

Figure 3 shows principal component analysis (PCA) results for biscuits with gluten and gluten-free and various additives. Collectively, the three principal components describe over 95% of the variance in the experimental data. The control sample differed from the other samples with additives in the content of polyphenols and the $L^*$ (CIE Lab) color component. Regardless of the additive, they affect the color component. A significant difference in the $h$ (CIE Lch) color component appeared with anthocyanin and coffee supplements. Additions of citrus fibers and acacia had a significant impact on the main characteristics of the biscuits.

The specific characteristics of the composition of citrus fibers, coffee grounds, anthocyanins, and acacia, which lead to changes in the colors, physicochemical characteristics, and antioxidant activity of the biscuits, may vary for each additive. Citri-Fi®100FG is a natural citrus fiber that can function as a water-binding agent, increasing the moisture content and viscosity of biscuits. This can also potentially affect the color and texture of the biscuits. Citrus

Table 3. Normalized mean values of quality characteristics of gluten and gluten-free biscuits with various additives.

| Gluten biscuits | | | | | |
|---|---|---|---|---|---|
| Parametar | Control | Acacia | Citrus fibers | Coffee grounds | Anthocyanins |
| *L* | 0.22 | 0.24 | 0.09 | 0.03 | 0.05 |
| *a* | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 |
| *b* | 0.06 | 0.06 | 0.03 | 0.01 | 0.01 |
| *c* | 0.06 | 0.06 | 0.03 | 0.01 | 0.01 |
| *h* | 0.31 | 0.45 | 0.31 | 0.07 | 0.08 |
| WA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MC | 0.05 | 0.08 | 0.05 | 0.01 | 0.03 |
| *D* | 0.02 | 0.02 | 0.02 | 0.00 | 0.01 |
| *T* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SR | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 |
| TP | 0.17 | 0.37 | 0.19 | 0.14 | 0.12 |
| DPPH | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Gluten-free biscuits | | | | | |
| Parametar | Control | Acacia | Citrus fibers | Coffee grounds | Anthocyanins |
| *L* | 0.30 | 0.16 | 0.22 | 0.05 | 0.04 |
| *a* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *b* | 0.06 | 0.03 | 0.04 | 0.00 | 0.00 |
| *c* | 0.06 | 0.03 | 0.04 | 0.00 | 0.01 |
| *h* | 0.76 | 0.47 | 0.48 | 0.26 | 0.57 |
| WA | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 |
| MC | 0.08 | 0.05 | 0.05 | 0.03 | 0.03 |
| *D* | 0.04 | 0.02 | 0.03 | 0.01 | 0.02 |
| *T* | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 |
| SR | 0.05 | 0.03 | 0.03 | 0.02 | 0.02 |
| TP | 0.28 | 0.26 | 0.18 | 0.22 | 0.09 |
| DPPH | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 |

*WA - Water activity; MC - Moisture content; D - Diameter; T - Thickness; SR - Spread ratio; TP - Total polyphenols; and DPPH - DPPH assay.*

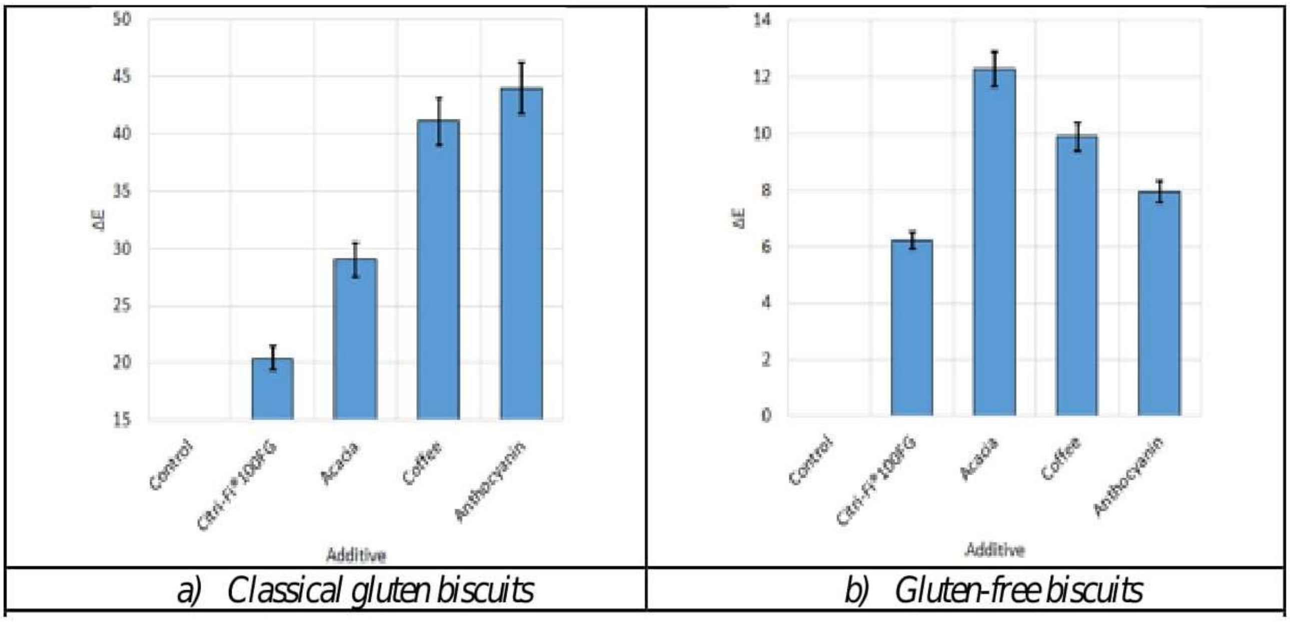


*Figure 2. Color difference ΔE: a) gluten biscuits and b) gluten-free biscuits.*

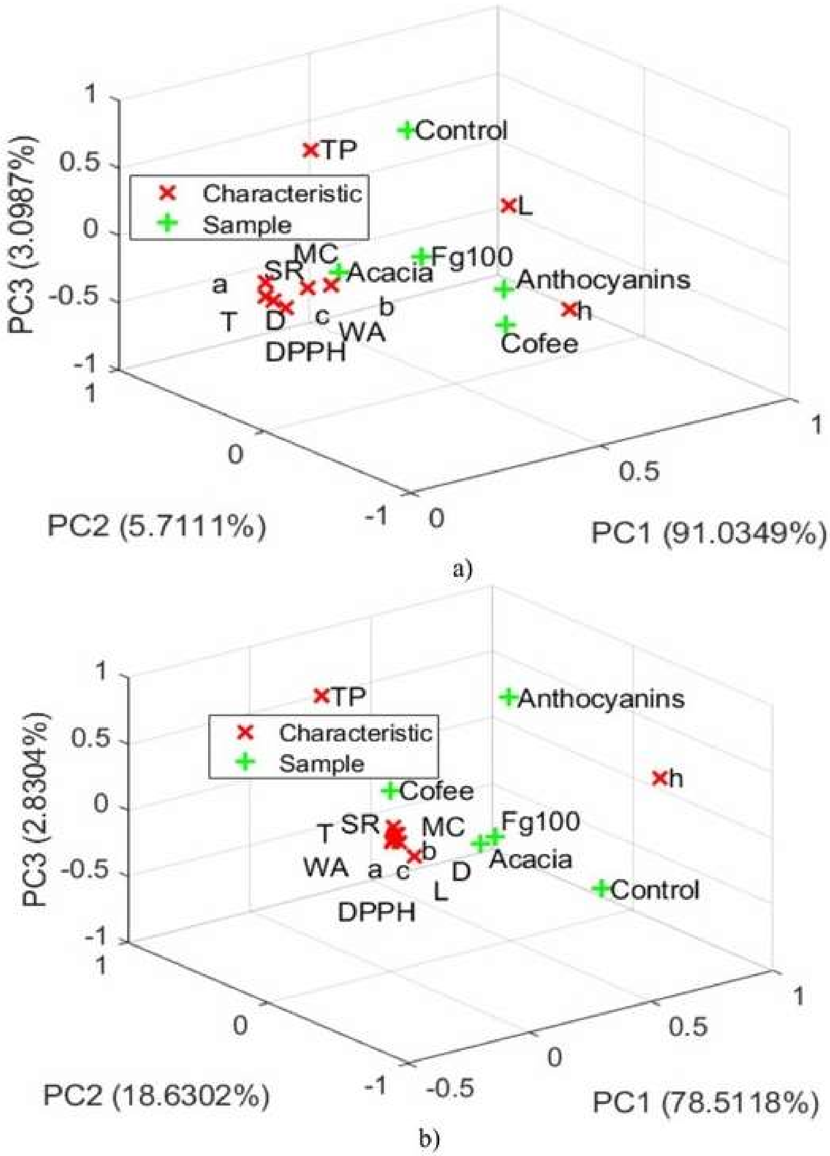


*Figure 3. PCA of: a) gluten biscuits and various additives and b) gluten-free biscuits and various additives*

fibers also have antioxidant properties that may contribute to the increased antioxidant activity of the biscuits.

The results of the analysis (PCA) for gluten-free biscuits also showed a significant difference in the *h* (Lch) color component for all additives because the control sample differed from the supplemented ones precisely on this component. The sample with anthocyanins significantly changed the polyphenol content of the biscuits. The citrus fibers, coffee grounds, and acacia samples had an equally significant effect on the other biscuit characteristics.

The findings of this study complement the work of Cervini *et al.* [17], who investigated how specific formulations influence the composition and characteristics of gluten-free biscuits compared to their gluten-containing counterparts. They identified significant differences in moisture content and color attributes between the two types, highlighting the impact of gluten on sensory properties and overall product quality. These insights provide valuable guidance for biscuit manufacturers, enhancing the understanding of how formulation choices affect key product characteristics. Furthermore, this study reinforces and expands the existing body of research on gluten-free biscuit development.

Specifically, studies by Kohli *et al.* [18] and Sharoba *et al.* [19] were included in the present analysis, which found moisture content to be a crucial characteristic in the formulation of gluten-free biscuit recipes. They suggested using alternative flours rich in bioactive compounds to improve the characteristics of gluten-free biscuits.

To improve the characteristics of gluten-free biscuits, it is suggested to use alternative flours, such as coconut and almond, as recommended by Hopkin *et al.* [21]. By incorporating alternative flours, biscuit manufacturers can potentially improve the sensory characteristics and overall quality of gluten-free products.

The presence of citrus fibers and acacia indicates that the biscuits are high in fiber and may be beneficial in promoting gut health. Similarly, coffee grounds impart a rich flavor to biscuits and, together with anthocyanins, improve the antioxidant activity of the final product. In other words, the resulting biscuits are not only tasty but also useful for the consumer, thus complementing the research of Elhassaneen *et al.* [24] and Azuan *et al.* [29].

The present study provides valuable information for the characterization of gluten and gluten-free biscuits with different functional additives. Its results can serve as preliminary baseline data to be used for future evaluations and studies related to the express automated analysis of biscuits. Based on them, researchers and the food sector in general can have a better understanding of the properties of these types of biscuits and develop more efficient and effective methods for their production. Also, the results can significantly contribute to the development of high-quality biscuits with added value - improved nutritional value and sensory characteristics.

The results from the nutritional profile and energy value of biscuits with gluten and gluten-free biscuits are presented in Table 4. It can be seen that the control samples have the highest amount of carbohydrates (49.20 g/100 g), and the biscuits containing acacia have the least amount (47.98 g/100 g). There is a tendency that with the addition of a functional component in the recipe composition of biscuits, the amount of carbohydrates decreases. The protein and fat content show no statistically significant differences among the biscuit samples containing wheat flour. In addition, adding fiber-rich ingredients increases the content of dietary fiber in biscuits. Products with the additive have a reduced energy value compared to the control sample.

The nutritional composition of gluten-free biscuits is also presented in Table 4, including the content of fat, protein, dietary fiber, and carbohydrates, as well as the energy value. Biscuits mainly contain carbohydrates (45.60-47.06 g/100 g), followed by fat (~18 g/100 g). Dietary fiber contains a variety of carbohydrates that are not hydrolyzed or absorbed by the human small intestine and whose daily intake is associated with health benefits, particularly for gastrointestinal function [51]. According to European regulations [52], food can use the nutrition claim 'source of fibre' if it contains at least 3 g/100 g, and can claim rich in fiber' if it contains 6 g/100 g or more. In this sense, all samples of gluten-free biscuits could be labeled as rich in fiber" (6.32-7.68 g/100 g). The protein content was found to range from 3.39 to 3.46 g /100 g. The energy value of the gluten-free and supplemented biscuits was lower compared to the control sample. Microbiological analysis of biscuits with and without gluten

Table 5 shows the microbiological determination of biscuits with and without gluten, in which the total number of microorganisms and the presence of molds and yeasts were analyzed. Regarding the group of filamentous molds and yeasts, no presence was detected; therefore, good manufacturing practices were used during the production process of the biscuits.

The percentage of additives did not significantly affect the total number of yeasts, molds, and microorganisms. The established values of aw correspond to the obtained results of the microbiological analysis. The microbial load of the biscuit samples was compared to microbiological standards for fortified mixed products, and the total number of microorganisms was found to be less than $1 \cdot 10^5$ CFU/g. This value falls within acceptable limits.

## CONCLUSION

In this study, a comparative analysis was conducted between traditional gluten and gluten-free biscuits enriched with citrus fibers and functional additives including citrus fibers, acacia, coffee grounds, and anthocyanins. The results revealed notable differences in the composition and properties of gluten and gluten-free biscuits:

**Citrus fibers:** Most effective in increasing dietary fiber content. At a 5% addition level, gluten-free biscuits meet the criteria for the nutritional claim 'high in fiber,' making this the simplest and fastest option for implementation in the biscuit industry.

Table 4. Nutritional profile and energy value of biscuits with gluten and gluten-free (per 100 g of product).

| Gluten biscuits | | | | | |
|---|---|---|---|---|---|
| Indicators | Control | Acacia | Citrus fibers | Coffee grounds | Anthocyanins |
| Carbohydrates, g/100 g | 47.81 | 45.24 | 46.16 | 46.48 | 47.40 |
| Dietary fiber, g/100 g | 1.39 | 2.74 | 2.22 | 2.05 | 1.38 |
| Proteins, g/100 g | 6.44 | 6.28 | 6.37 | 6.42 | 6.37 |
| Fats,g/100 g | 18.26 | 18.21 | 18.23 | 18.20 | 18.24 |
| Energy value, kcal/100 g | 378.56 | 364.49 | 369.75 | 371.30 | 376.48 |
| Gluten-free biscuits | | | | | |
| Indicators | Control | Acacia | Citrus fibers | Coffee grounds | Anthocyanins |
| Carbohydrates, g/100 g | 46.24 | 43.85 | 44.49 | 44.48 | 45.75 |
| Dietary fiber, g/100 g | 0.82 | 1.75 | 1.60 | 1.47 | 0.81 |
| Proteins, g/100 g | 3.46 | 3.39 | 3.45 | 3.44 | 3.42 |
| Fats, g/100 g | 16.86 | 16.82 | 16.83 | 16.85 | 16.84 |
| Energy value, kcal/100 g | 352.18 | 343.84 | 346.43 | 346.27 | 349.86 |

Table 5. Microbiological indicators of biscuits.

| Microorganisms | Control | Acacia | Citrus fibers | Coffee grounds | Anthocyanins |
|---|---|---|---|---|---|
| Total number of microorganisms Total Plate Count CFU/g | < 10 | < 10 | < 10 | < 10 | < 10 |
| Molds and yeasts CFU/g | Absence | Absence | Absence | Absence | Absence |

**Acacia**: Contributes to increased antioxidant activity and fiber content. A 3% addition of gluten-free biscuits yielded good results without negatively affecting texture or processing.

**Coffee grounds**: Enhances both antioxidant activity and dietary fiber content. A 2% addition was found to be effective without complicating the production process.

**Anthocyanins**: Improve the visual color characteristics of the biscuits, especially in gluten-free formulations, with limited effect on antioxidant activity.

Based on the balance between nutritional benefit and ease of application, citrus fibers at 5% in gluten-free biscuits are recommended as the most effective option for industrial use. Further research is needed to investigate the effect of these additives on product shelf life.

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NAUČNI RAD

# BISKVITI SA GLUTENOM I BEZ GLUTENA SA FUNKCIONALNIM KOMPONENTAMA: FIZIČKOHEMIJSKA, NUTRITIVNA I ANTIOKSIDATIVNA SVOJSTVA

*Cilj ovog rada je bio da se utvrdi uticaj različitih jedinjenja na nutritivne, antioksidativne, mikrostrukturne i karakteristike boje biskvita klasifikovanih kao biskviti sa glutenom i bez glutena. Naime, biskviti su obogaćeni dijetetskim vlaknima, vlaknima akacije, talogom kafe i antocijaninima. Dodavanje ovih funkcionalnih komponenti u matricu biskvita uticalo je na fizička svojstva biskvita; naime, vrednost faktora širenja svih biskvita kretala se od 2,98 do 7,88, sadržaj ukupnih polifenola se povećao, najveći sadržaj polifenola dobijen je kod biskvita bez glutena sa dodatkom taloga kafe (77,98 mg), dok je kod keksa sa pšeničnim brašnom bio u rasponu od 44,62 do 128,63 mg. Svi bezglutenski biskviti mogu se označiti kao proizvodi „bogati vlaknima" (6,32-7,68 g/100 g) i sa većim sadržajem antioksidanasa u poređenju sa biskvitima bez dodatih sastojaka. Ukupan broj mikroorganizama u testiranim biskvitima je ispod prihvatljivih granica. Rezultati ove studije pokazuju da uključivanje sirovih nutritivnih komponenti u recepturu tradicionalnih glutenskih i bezglutenskih biskvita dovodi do poboljšanja nutritivne vrednosti i drugih karakteristika kvaliteta obogaćenih prehrambenih proizvoda.*

*Ključne reči: Antioksidativna aktivnost, sadržaj polifenola, biskviti, biskviti bez glutena*.