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Development of Gluten-Free Biscuits with Alternative Sweeteners Enriched with Medicinal Herbs: Mint, Rose, Thyme, and Oregano

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This study aimed to develop gluten-free biscuits enriched with 10% of mint, rose, thyme, or oregano and sweetened with alternative sweeteners. Oat flour was combined with each herb, and the biscuits were manufactured under lab conditions. Proximate composition, mineral enrichment, and antioxidant activity were analyzed. The nutritional benefits and functional value that herbal addition brought to the biscuits were also studied to determine its actual impact on human health. The results showed that by adding herbs, the nutritional quality of the biscuits was raised obviously. Mint and oregano-incorporated biscuits were found to have markedly increased protein content, while thyme-infused ones were particularly high in iron, as shown by their mineral content. Hence, the incorporation of herbs brought clear benefits to the mineral composition of an herb at each level. Overall, the addition of herbs also improved biscuits' total phenolic and flavonoid content, with rose-injected biscuits showing the highest levels of antioxidant action. However, traces of heavy metal species were detected in thyme-incorporated biscuits, indicating the necessity for purity and careful procurement of medicinal herbs. This study showed that adding medicinal herbs into gluten-free biscuit formulations was an effective way of meeting consumer demands for healthier and more functional snacks. The nutrient density, antioxidant power, and sensory attractiveness of the enhanced biscuits were all enhanced. By combining traditional ingredients with herbs rich in phenolic compounds and essential mineral compounds, biscuit makers can create an array of gluten-free products catering to health-conscious consumers looking for healthy and antioxidant-rich snacks.

Keywords: herbs, bakery products, antioxidant activity, phenolic compounds, nutritional enhancement

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Introduction

Gluten plays a crucial role in wheat-based products by establishing a strong protein network that imparts the desired viscoelasticity to dough, enhancing gas retention, and contributing to the structure of baked goods (Irigoytia et al., 2023; Preda et al., 2024). Gluten intolerance involves a range of conditions initiated by the consumption of gluten, a protein found in grains like wheat, barley, rye, etc. Celiac disease, non-celiac gluten sensitivity, and wheat allergies (Balakireva and Zamyatnin, 2016; Asri et al., 2021). Creating products without gluten for individuals affected by gluten-related illnesses is crucial to meet the nutritional necessities (Jossen & Lebwohl, 2023; Wall and Semrad, 2024). The rising prevalence of gluten-related disorders has increased the demand for gluten-free (GF) products. Additionally, growing consumer interest in healthier eating habits and sustainable food systems has propelled the GF market forward. Providing safe dietary options for those with gluten intolerance and reducing the risk of associated health issues such as nutrient malabsorption, obesity, cardiovascular disease, and insulin resistance (Taraghikhah et al., 2020; Bianchi et al., 2024). Plus, the importance of nutritional enhancement by addressing the nutritional deficiencies is often found in GF products, which can be higher in fat, sugar, and sodium and lower in protein and essential mineral compounds. And improving the taste, texture, and nutritional quality of GF products to match their gluten-containing counterparts, thereby increasing acceptance among consumers (Dale et al., 2018). The growth of the market is also not less important. The global gluten-free products market continues to grow, fueled by rising consumer interest not just from those with gluten-related issues, but also from health-conscious individuals (Grand View Research, 2020; Khairuddin and Lasekan, 2021; Palomares-Navarro et al., 2023). Differences in calorie content between GF and gluten-containing foods are not significant in most cases. However, GF flours, breads, and pastry products may contain more calories due to added fats (Mármol-Soler et al., 2022). GF products may contain more fat, especially saturated fat, due to the addition of oils and emulsifiers to improve taste and texture (Miranda et al., 2014). The absence of gluten proteins can result in reduced overall protein content (Segura and Rosell, 2011). Some GF foods differ in carbohydrate content, with pasta groups showing lower sugar levels, indicating starch as the main carbohydrate component. GF diets often result in a lower intake of non-starchy carbohydrates (Maghaydah et al., 2024). GF foods are often low in fiber (Myhrstad et al., 2021) due to the absence of whole grains in production. Sodium Content GF products may add more salt to make up for the missing protein bundle and taste enhancement (Vergeer et al., 2021). Recent research has revealed that many gluten-free foods are deficient in important nutrients such as fiber, iron, and B vitamins, necessitating fortification strategies (Vici et al., 2016; Bianchi et al., 2024). GF products can exhibit variations in volume, crumb grain, color, and texture compared to gluten-containing products. Different formulations affect these properties and, consequently, sensory acceptability (Matos and Rosell, 2012; Sciarini et al., 2012; Ferreira et al., 2015). Consumer preferences can be influenced by the appearance and taste of GF products. Bread with heterogeneous, coarser crumb grains and darker colors are generally preferred (Matos and Rosell, 2012; Tóth et al., 2022). The availability of starch to hydrolytic enzymes can be affected by the specific volume and physical structure of GF bread. Few studies have analyzed the glycemic index of GF products, with conflicting results. Some GF products may cause blood sugar spikes due to higher starch content (Culetu et al., 2021; Romão et al., 2021). Strategies to lower the glycemic index include increasing fiber content or using sourdough fermentation (Wolter et al., 2014). Removing gluten can decrease taste and acceptance due to its role in dough elasticity and viscosity. Replicating these properties requires innovative approaches (Cappelli et al., 2020). Combining traditional flours and starches with alternative flours like amaranth, teff, or brown rice can increase the nutrient density of GF foods. These formulations not only improve nutritional value but also maintain consumer acceptance (Culetu et al., 2021). However, avoiding unhealthy strategies such as replacing carbohydrates with lipids or proteins should be avoided, as GF products often serve as the main carbohydrate sources in the diet of celiac patients (Šmídová and Rysová, 2022). According to a previous study, high-pressure processing (HPP) has been explored as a method to improve the texture and shelf-life of products (Culetu et al., 2021).

Oats (*Avena sativa* L.) are unique among cereals due to their high nutritional value. However, their inclusion in GF diets is controversial. Some studies indicate that avenins, the prolamin fraction in oats, can trigger immune reactions in celiac patients, and the immunogenicity may vary depending on the oat cultivar (Real et al., 2012). Oats are often grown, stored, or processed alongside gluten-containing grains, leading to contamination. Ensuring uncontaminated, pure oats is essential for safety. Previous research has confirmed that consumption of pure, uncontaminated oats is safe for most individuals with celiac disease (Gilissen et al., 2016). Among the most significant types relied upon globally for gluten-free foods are oats and oat flour. Oats offer significant nutritional advantages with a high nutrient content. Rich in carbohydrates (53–66%), protein (9–20%), dietary fiber (3–11%), vitamins, and mineral compounds like calcium, phosphorus, potassium, and zinc. And it is high in unsaturated fatty acids, thiamine, folate, and notably beta-glucans, which have been linked to cholesterol reduction and improved heart health (Leszczyńska et al., 2023). Sugars, primarily found in fruits and vegetables, balance the flavor of food and provide the energy required for survival (Grembecka, 2015). However, excessive sugar intake has been linked to various health issues, including obesity, type 2 diabetes, and cardiovascular diseases (Malik et al., 2010). To address these concerns, alternative sweeteners have been developed to replicate the sweetness of sugar without the associated negative health effects.

Mint is widely appreciated for its refreshing flavor and aroma. It contains significant amounts of phenolic compounds, particularly rosmarinic acid, which contribute to its strong antioxidant activity (Dorman et al., 2003). Studies have reported that the antioxidative properties of mint can improve the shelf life and nutritional value of biscuits by inhibiting the oxidative degradation of fats and oils (Bajaj et al., 2016). Research indicates rose petals are rich in flavonoids and anthocyanins, compounds known for their antioxidant and anti-inflammatory activities (Akram et al., 2019). Providing health benefits such as reducing oxidative stress and potentially improving mood and anxiety levels (Nazıroğlu et al., 2012). The importance of rose extracts has antioxidative properties (Chroho et al., 2022) that may enhance the stability of bakery products by delaying rancidity. Thyme is a culinary herb known for its distinctive flavor and potent antimicrobial properties due to its high content of thymol and carvacrol (Marchese et al., 2016). Adding thyme to biscuits can enhance their sensory qualities and extend shelf life by inhibiting microbial growth (Chouliara et al., 2007). Moreover, thyme exhibits strong antioxidant activity, which can protect against oxidative damage and contribute to overall health (Tohidi et al., 2017). Oregano is rich in phenolic compounds and is recognized for its robust antioxidant and antimicrobial activities (Kosakowska et al., 2020). The primary active constituents, carvacrol, and thymol, have been shown to inhibit the growth of bacteria, yeasts, and molds, thereby enhancing food safety and preservation (Nostro et al., 2007). Mixing oregano into biscuits not only adds a savory flavor but may also confer health benefits such as anti-inflammatory effects and support for cardiovascular health (Sharifi-Rad et al., 2020).

This study aimed to develop gluten-free biscuits that are medicinally enriched with 10% of mint (*Mentha piperita* L.), rose (*Rosa damascena* L.), thyme (*Thymus vulgaris* L.), or oregano (*Origanum vulgare* L.) and sweetened with alternative sweeteners.

Material and Methodology

Materials

All ingredients were sourced from local markets in Nitra, Slovakia. The following is a list of ingredients used for each type of biscuit:

Control sample:

- 300 g oat flour, 8 ml liquid sweetener, 80 g butter, sodium carbonate, salt
- Sample enriched with 10% of dried herbs (*Mentha piperita*, *Rosa damascena*, *Thymus vulgaris*, *Origanum vulgare*):
- 270 g oat flour, 30 g herbs, 8 ml liquid sweetener, 80 g butter, sodium carbonate, salt.

Preparation of Biscuits

The samples were prepared under laboratory conditions, each variant (control, sample with mint, sample with rose (Figure 1, 2 and 3), sample with thyme and sample with oregano) was prepared separatory:

1. Grinding the herbs: Rose petals, mint, thyme, and oregano were ground using a Tefal electric grinder (GT110838, Germany) to obtain a fine powder.
2. Mixing ingredients: The oat flour, ground herb (specific to each sample), butter, sodium carbonate, salt, and liquid sweetener were combined and thoroughly mixed to form a dough.
3. Forming the dough: The dough was divided into equal portions and shaped into biscuits.
4. Baking: The biscuits were baked in a convection oven (MIWE Condo, Germany) at 180 °C for 15 minutes, with the heat source from the bottom only.
5. Cooling: After baking, the biscuits were allowed to cool to room temperature before further analysis.

Figure 1 Sample of rose-enriched biscuit dough
Photo: Jawa Hassan

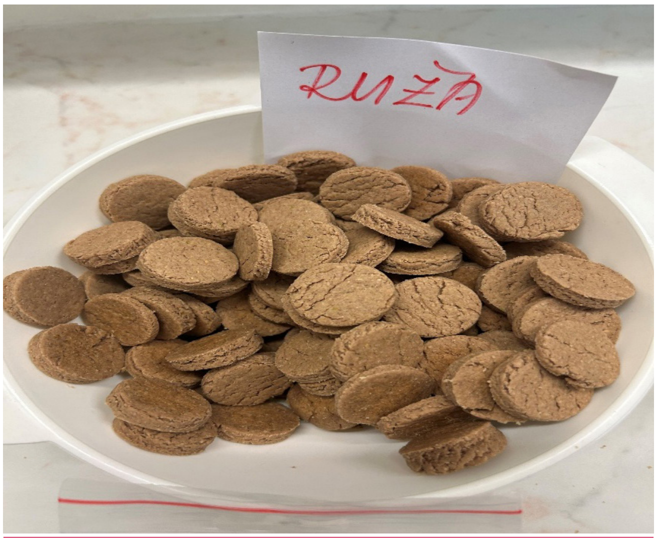

Figure 3 Rose-enriched biscuits after baking
Photo: Jawa Hassan

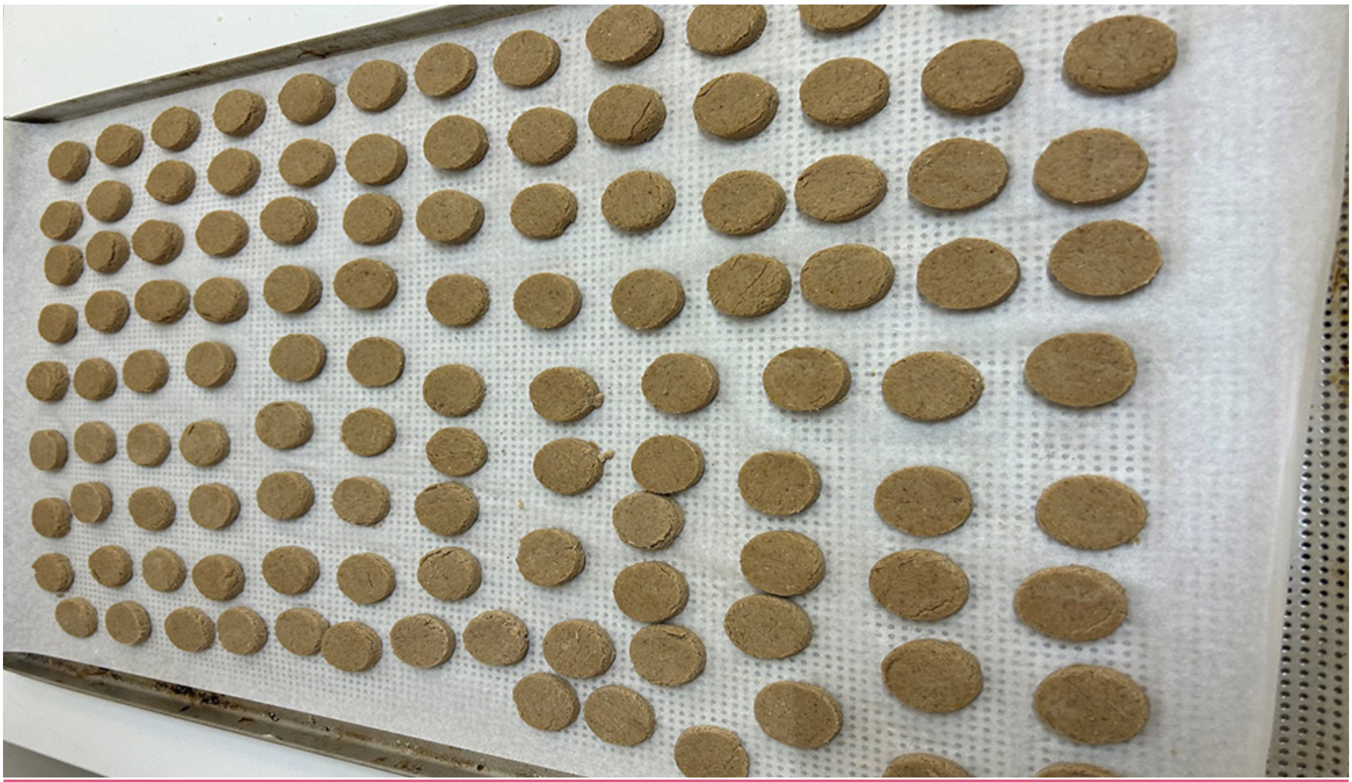

Figure 2 Rose-enriched biscuits before baking
Photo: Jawa Hassan

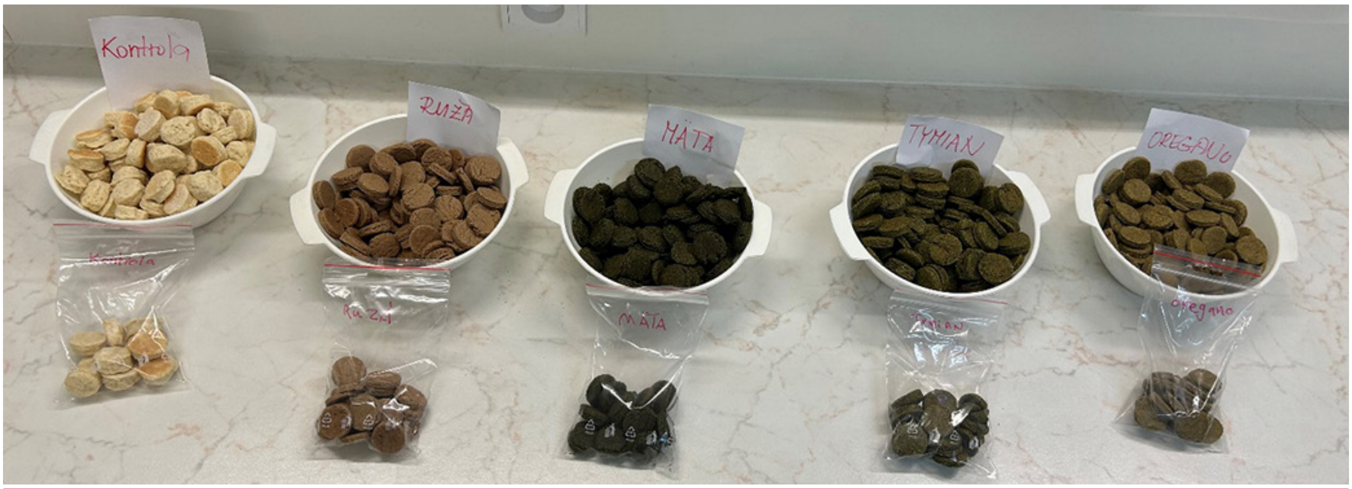

Figure 4 Samples of the biscuits after baking
kontrola – control; ruža – rose; mäta – mint; tymian – thyme; oregano – oregano
Photo: Jawa Hassan

The samples were prepared under laboratory conditions (Figure 4). All herbs-enriched biscuit samples were compared to the control sample to determine the effect of each herb on the proximate composition and other physicochemical properties.

Methods

Proximate Composition

Dry Matter, Ash, and Protein Content

AACC method 08-01 will be used to determine the dry matter, ash, and crude protein content (AACC, 1996). The semi-micro-Kjeldahl method was used to calculate the nitrogen content. The usual factor of 5.7 was used to convert nitrogen to protein. According to manufacturer guidelines, the Ancom XT15 Fat Extractor (USA) will be used to measure the amount of fat in the sample.

Energy Value

The corresponding energy was calculated according to the Atwater system:

$$\text{energy (kcal.100 g}^{-1}) = 4 \times (\text{g proteins} + \text{g carbohydrates}) + 9 \times (\text{g fat})$$

(Arraibi et al., 2021).

Reducing Sugars Content

The reducing sugars content was determined spectrophotometrically (Jenway 6320D, UV VIS, UK) by dinitrosalicylic colorimetric method according to the procedures described by Wang (2005). Glucose was used as a standard.

Mineral Compounds Analysis

With a D2 lamp background correction system and an air-acetylene flame (air 13.5 L.min^{-1}, acetylene 2.0 L.min^{-1}; Varian, Ltd., Mulgrave, Australia), the Varian model AA 240 FS will be used to analyze mineral compounds. The outcomes were compared to the multielementary GF AAS standard, CertiPUR® (Merck, Germany).

Antioxidant Characteristics

DPPH – Free Radical Scavenging Activity

According to the steps outlined by Sanchéz-Moreno et al. (1998), the 2,2-difenyl-1-picrylhydrazyl (DPPH) assay will be used to measure the samples‘ capacity to scavenge free radicals using spectrophotometer (Jenway 6320D, UV VIS, UK). Trolox was used as a standard.

Total Polyphenol Content

The total polyphenol content of samples will be determined spectrophotometrically (Jenway 6320D, UV VIS, UK) using the Singleton et al. (1965) modified Folin-Ciocalteu method. Gallic acid was used as a standard.

Total Flavonoid Content

Total flavonoids will be determined spectrophotometrically (Jenway 6320D, UV VIS, UK) using a modified method of Willett (2002). Quercetin was used as a standard.

Total Phenolic Acid Content

Total phenolic acids content will be determined (Jenway 6320D, UV VIS, UK) using the method of Jain et al. (2017). Caffeic acid was used as a standard.

Statistical Analysis

The results are expressed as mean values of 5 replications ±standard deviation (SD). Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

Results and Discussion

Proximate Composition of Biscuits

The analysis of the proximate composition of biscuits (Table 1) shows that different samples vary greatly in their proportions of moisture, protein, ash, fat, and carbohydrate. Alphabetical superscripts show statistically significant differences ($p < 0.05$) between samples within a row. They indicate which herbs affect each measured parameter. A one-way ANOVA test was used for statistical analysis. These results reflect the nutritional influences that each herb addition has, as supported by previous research findings.

Moisture Content

The biscuits enriched with rose petals show the highest moisture content (9.55 ±0.01%), with a significant difference from other formulations. This higher moisture content aligns with findings from studies on herbal formulations that suggest fiber-rich herbs, such as rose, can absorb and retain more water in food matrices, thereby increasing overall moisture content (Arun et al., 2015; Suriya et al., 2017; Gül and Tekeli, 2019). Enhanced moisture may contribute to better texture and mouthfeel, which can improve consumer acceptance of herbal biscuits. Previous studies have shown that when rose powder is added at a rate of 7.5% (Królak et al., 2017; Gül and Tekeli, 2019).

Protein Content

Biscuits enriched with mint and oregano (Table 1) have significantly higher protein content (9.12 ±0.02 and 9.05±0.02%, respectively) than the control biscuit sample. In a study conducted in 2024 on dried mint leaves, it was noted that the percentage of crude protein was 3.8 grams.100 $grams^{-1}$ (Afshiya and Anil, 2024), which means that our biscuits enriched with mint contribute more to the protein content. A previous study conducted to analyze dried oregano reported that the protein content was 6.06% (John et al., 2024). Although mint and oregano are not considered a primary source, which improve the nutritional quality of biscuits and their amino acid profile.

Ash Content

Biscuits enriched with thyme have the highest ash content (2.44 ±0.05%), compared to other samples (Table 1). This research suggests higher mineral content contributed by thyme. This is what previous research has confirmed thyme's substantial mineral presence, especially iron and calcium (Hammoudi Halat et al., 2021). The use of thyme is particularly beneficial for increasing the content of essential minerals such as potassium and magnesium, this enhances the nutritional profile of the biscuits (Dauqan and Abdullah, 2017). With a high concentration of ash in samples, it could be brought in a form that is more beneficial to human nutrition and fills the specific needs of micronutrients.

Fat Content

The fat content is significantly higher in the mint and thyme samples compared to the control (Table 1). This

Table 1 Proximate composition of prepared biscuits

Parameter	Biscuits				
	control	10% rose	10% oregano	10% mint	10% thyme
Moisture (%)	$6.16±0.01^{e}$	9.55±0.01	$8.41±0.03^{b}$	$6.92±0.05^{d}$	$7.65±0.03^{c}$
Crude Protein (%)	$6.62±0.01^{b}$	$8.16±0.04^{a}$	$9.05±0.02^{a}$	$9.12±0.02^{a}$	$8.26±0.03^{a}$
Ash (%)	$0.75±0.03^{e}$	$1.88±0.07^{d}$	$2.01±0.04^{c}$	$2.12±0.07^{b}$	$2.44±0.05^{a}$
Fat (%)	$11.33±0.05^{c}$	$15.916±0.05^{b}$	$17.715±0.05^{ab}$	$18.27±0.05^{a}$	$17.18±0.05^{ab}$
Reducing sugars (mg GE.g^{-1})	$75.12±0.09^{a}$	$64.489±0.05^{b}$	$62.810±0.06^{b}$	63.562±0.08	$64.45±0.06^{a}$
Total energy (kcal.100 g^{-1})	$428.51±0.05^{b}$	$433.84±0.07^{b}$	$446.90±0.11^{a}$	$455.18±0.11^{a}$	$445.55±0.15^{a}$

Notes: Results expressed as mean ±SD on a dry basis (n = 5). Different superscript letters within identical rows significantly differ ($p < 0.05$); GE – glucose equivalent presence of essential oils and healthy fats in these herbs, A previous study indicates the oil composition of mint and thyme (Vlaicu et al., 2021). The content of fat in mint could enhance the flavor profile and palatability of the biscuits, creating a balanced energy source (Saqib et al., 2021). Additionally, it was noted in a previous study that thyme provided the highest number of antioxidants in fats (Vlaicu et al., 2021) improve the oxidative stability of baked products, thus extending shelf life.

Reducing Sugars and Energy Content

Comparing the reducing sugar content in the control sample, we noticed that herb-enriched samples have lower content (TabLe 1). The total energy values remain elevated due to higher protein and fat levels in these formulations. This was confirmed by previous studies, where carbohydrates were replaced with protein and fats (Olagunju et al., 2022; Olagunju et al., 2024). Formulations containing herbal ingredients are suitable for consumers seeking balanced, low-glycemic index snacks. The energy profile of these biscuits is sustained, making them suitable for consumers seeking lower carbohydrate intake without sacrificing calorie content.

Mineral Compound Composition

The analysis of mineral content (Table 2) reveals that the addition of certain herbs can significantly enrich biscuits with essential minerals such as copper, zinc, and iron. While, minor levels of heavy metals such as lead and cadmium are also noted, which require further attention. The addition of herbs like mint and thyme effectively contributes to essential trace elements, providing nutritional benefits (Nazıroğlu et al., 2012; Saqib et al., 2021).

Mint-enriched biscuits demonstrated the highest copper content at 2.97 ±0.01 mg.kg^{-1}, while thyme-enriched biscuits exhibited the highest zinc concentration, at 13.19 ±0.01 mg.kg^{-1}. Copper and zinc are essential micronutrients critical for several physiological functions. Previous studies show that copper is an essential component of many enzymes, therefore playing a significant role in a wide range of physiological processes, including iron utilization, free radical elimination, bone, and connective tissue development, and melanin production (Dghaim et al., 2015). Nevertheless, excessive intake of copper can cause dermatitis, irritation of the upper respiratory tract, abdominal pain, nausea, diarrhoea, vomiting, and liver damage (Martin and Griswold, 2009; Ullah, 2012). Also, zinc is an essential trace element for proper growth, blood clotting, thyroid function, and protein and DNA synthesis (Dghaim et al., 2015). The FAO/WHO permissible limit 50 mg.kg^{-1} (PL) set for zinc in herbal medicines while the regulatory limits of the WHO/FAO have not been established yet for copper in herbal medicines (Ullah, 2012). There is not enough information available on Zn toxicity; however, high zinc intake beyond permissible limits produces toxic effects on the immune system, blood lipoprotein levels, and copper levels (Fosmire, 1990). The results of a previous study showed Zn, Cu, and Fe content varies depending on the aromatic herb (García-Galdeano et al., 2020). This research shows the highest iron concentration (83.94 ±0.01 mg.kg^{-1}), in thyme-enriched biscuits. This is what recent research has confirmed regarding the percentage of iron found in thyme iron (Fe) was found in the highest concentrations. The highest iron content was determined in *Thymus serpyllum* L. (Ivanišová et al., 2023). Iron (Fe) is involved as

Table 2 Micronutrients including risk elements

Parameter (mg.kg^{-1})	Biscuits				
	control	10% rose	10% oregano	10% mint	10% thyme
Copper (Cu)	0.72 ±0.03^{e}	2.16 ±0.01^{d}	2.36 ±0.01^{c}	2.97 ±0.01^{a}	2.45 ±0.01^{b}
Zinc (Zn)	3.09 ±0.02^{e}	12.52 ±0.01^{d}	12.82 ±0.02^{c}	12.85 ±0.01^{b}	13.19 ±0.01^{a}
Manganese (Mn)	16.64 ±0.01^{e}	16.64 ±0.02^{d}	18.12 ±0.01^{c}	19.02 ±0.03^{b}	24.02 ±0.02^{a}
Iron (Fe)	6.76 ±0.03^{e}	33.80 ±0.01^{d}	73.10 ±0.01^{b}	54.17 ±0.02^{c}	83.94 ±0.01^{a}
Nickel (Ni)	nd	0.68 ±0.01^{b}	1.46 ±0.01^{a}	0.49 ±0.01^{c}	0.37 ±0.01d
Cobalt (Co)	0.19 ±0.01^{c}	0.33 ±0.01^{b}	0.34 ±0.01^{b}	0.41 ±0.03^{a}	0.34 ±0.02^{b}
Chromium (Cr)	nd	nd	nd	nd	0.11 ±0.01
Lead (Pb)	nd	nd	nd	nd	0.10 ±0.03
Cadmium (Cd)	nd	nd	nd	nd	0.03 ±0.01

Notes: Results expressed as mean ±SD on a dry basis (*n* = 5). Different superscript letters within identical rows significantly differ (p <0.05); n.d. – not detected a significant component in the formation of red blood cells and plays an important role in the treatment of iron deficiency anaemia (IDA) (Dghaim et al., 2015). Iron is a crucial element in all living organisms. It is involved in a wide range of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. Studies prove iron has several key functions in the human body, including oxygen supply, energy production, and immunity. systems (Martin and Griswold, 2009; Dghaim et al., 2015). Iron deficiency is a very common problem in humans, usually caused by insufficient intake of this element with food or excessive menstrual bleeding. Anaemia is a common disease (Lokhande et al., 2010). Iron overdose is associated with symptoms of dizziness, nausea and vomiting, diarrhoea, joint pain, shock, and liver damage. Iron toxicity has an adverse effect on various metabolic functions and cardiovascular (Martin and Griswold, 2009; Dghaim et al., 2015). The concentration in body tissues must be regulated because iron can generate free radicals and its high concentration can lead to tissue damage (Abbaspour et al., 2014). The WHO limit for iron in medicinal herbs has not been established yet. The results of the current study show a wide variation of iron in different herb samples. These results are comparable to values of iron found in Egyptian spices and medicinal plants that ranged between 26.96 and 1,046.25 $mg.kg^{-1}$ (Abou-Arab and Donia, 2000). Low levels found of lead and cadmium, in the thyme biscuits. No percentage was recorded in the remaining samples. Previous studies have shown that the content of lead (Pb) in the analyzed. The maximum concentrations of lead in mint, oregano, and thyme were found to be 9.24, 18.06, and 23.52 $mg.kg^{-1}$, respectively (Dghaim et al., 2015). And another research shows the highest mean concentration of lead in individual herbs was detected in thyme 19.3 $mg.kg^{-1}$ (Ababneh, 2017). The percentage of lead in this study in thyme was 0.10 ±0.03 $mg.kg^{-1}$. While FAO/WHO maximum permissible limit of lead in consumed medicinal herbs is 10 $mg.kg^{-1}$ (WHO, 2005; WHO, 2006). High concentration of lead above permissible limits in medicinal plants and herbs has been reported in other Middle Eastern countries (Dghaim et al., 2015). Lead is known to be one of the highly toxic environmental pollutants. It can be complex with various biomolecules and adversely affect their functions. Lead exposure may have an adverse effect on the blood, nervous, immune, renal, skeletal, muscular, reproductive, and cardiovascular systems (Dghaim et al., 2015). Exposures to lead at early childhood and prenatally are associated with slowed cognitive development, learning deficits, and many other effects (Dghaim et al., 2015). High levels of cadmium exceeding 0.3 $mg.kg^{-1}$, the permissible limit (PL) set by FAO/WHO for medicinal herbs and plants in different countries (WHO, 2005; WHO, 2006). Similar results of high levels of cadmium in Egyptian and Iranian medicinal herbs and plants have been reported in earlier studies (Abou-Arab and Donia, 2000; Ziarati, 2012). Cadmium (Cd) is released into the environment through natural activities (e.g., volcanic eruptions), weather conditions, and some human activities (Dghaim et al., 2015). Cadmium is a highly toxic element for the kidneys, a Inhaling excessive amounts of this element can lead to serious lung damage. Oral intake of higher amounts of this element leads to stomach irritation, vomiting, and diarrhoea. Excretion of cadmium is very slow, and it accumulates in human kidney for a relatively long time, resulting in an irreversible impairment of the renal tract (Li et al., 2012; Maobe et al., 2012). At high concentrations, cadmium produces serious effects on the liver and vascular and immune system (Maobe et al., 2012). Prolonged exposure to lower concentrations can lead to cadmium deposition in the kidneys, a condition that can lead to kidney diseases, bone thinning, and lung damage (Ivanišová et al., 2023). Regarding herb purity and sourcing, environmental contamination can introduce heavy metals into plant-based products (Ivanišová et al., 2023). Lead and cadmium are toxic elements for human; they perform no beneficial biological roles and can be very dangerous even at low concentrations(Ababneh, 2017).

Antioxidant Characteristics

As indicated in (Table 3), adding medicinal herbs to the biscuit formulations significantly enhances phenolic and flavonoid content, along with antioxidant activity. These characteristics are essential for antioxidant defence and contribute to the health-promoting effects of the final product.

The biscuits with oregano and mint exhibit the highest levels of total phenolics (1,370.2±0.13 mg $GAE.100\ g^{-1}$) and flavonoids (252.4 ±0.04 mg $QE.100\ g^{-1}$), respectively (Table 3). According to previous study. The presence of these compounds is well-documented in mint and oregano, which are known to contain flavonoid and phenolic acids compounds with effective antioxidant activities. These antioxidants are not only providing health benefits but also enhance the sensory attributes of baked goods. The Augmentation of phenolic compounds is profitable as it contributes to the biscuit's shelf life and nutritional quality

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Table 3 Phytochemicals and antioxidant activity

Parameter	Biscuits				
	control	10% rose	10% oregano	10% mint	10% thyme
Polyphenol (mg GAE.100 g^{-1})	185.80 ±0.11d	772.40 ±0.09b	1,370.20 ±0.13a	701.30 ±0.88b	519.10 ±0.07c
Flavonoids (mg GE.100 g^{-1})	147.01 ±0.03b	156.89 ±0.02b	220.97 ±0.07a	252.40 ±0.04	213.21 ±0.00a
Phenolic acid (mg CAE.100 g^{-1})	426.35 ±0.00d	451.06 ±0.00c	469.88 ±0.00b	565.20 ±0.00a	482.80 ±0.00b
DPPH (mg TEAC.100 g^{-1})	14.56 ±0.00c	294.59 ±0.00a	269.72 ±0.00b	258.91 ±0.00b	254.23 ±0.00b

Notes: Results expressed as mean ±SD on a dry basis (*n* = 5). Different superscript letters within identical rows significantly differ ($p < 0.05$); GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent; TEAC – trolox equivalent antioxidant capacity

(Brown et al., 2019). The highest phenolic acid levels (565.2 ±0.00 mg.100 g^{-1}) in mint-enriched samples have been marked by comparison to the all tested samples (Table 3). Phenolic acids are known to exhibit powerful antioxidant properties, which can contribute to reducing oxidative stress when consumed and This agrees with these results high phenolic acid content in herbal-enriched foods is compatible with reduced markers of oxidative stress, which has a potential role in functional food applications. Thus, the high content of phenolic acid in mint biscuits suggests that they may offer considerable health benefits while at the same time inhibiting oxidative degradation (Kiokias et al., 2020).

Rose-enriched biscuits exhibit the strongest radical scavenging activity (294.59 ±0.00%), supporting rose's known antioxidant properties. The antioxidant properties of rose can significantly improve the oxidative stability of baked products, by enhancing both shelf life and nutritional value. High DPPH inhibition values suggest a potent capacity to combat free radicals, essential for reducing oxidative stress and prolonging the product's shelf life. Plus, the high DPPH values in roses make them suitable for functional foods that are aimed at enhancing antioxidant intake (Foss et al., 2022).

Conclusion

Changing over to a flour-free biscuit by adding 10% of medicinal herbs like mint, rose, thyme and oregano has greatly improved nutrition and corporate performance. The enrichment of herbs obviously led to protein levels in general, essential mineral content (particularly iron), and the antioxidant activity of phenolic or flavonoid compounds of rose. However, the presence of trace heavy metals in some herb-enriched samples underscores the importance of using pure, high-quality herbs to ensure product safety and compliance with health standards. In the future, it is necessary to focus on adjusting the levels of herb enrichment and developing more herb varieties to improve the health benefits while always keeping in mind safety and quality considerations. Thus, the results of the study demonstrate that eating biscuits enriched with medicinal herbs with a gluten-free base is not only a health improvement strategy but also provides functional snacks. This is what consumers want: delicious, nutritious, and full of antioxidants.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethical Statement

This article doesn't contain any studies that require an ethical statement.

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