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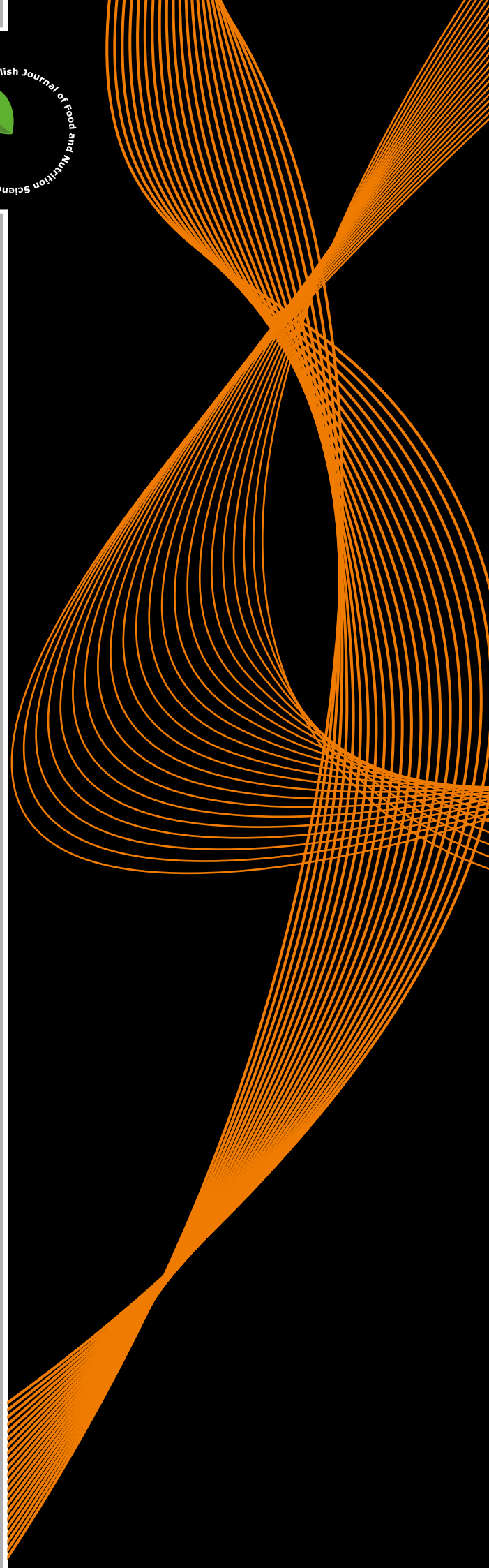

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Effect of Germination Time on the Content of Nutritional and Bioactive Compounds of *Chenopodium quinoa* Wild. Seeds Cultivated in Eastern Morocco

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Germination can be considered an important process for modifying quinoa seeds' nutritional and bioactive compounds. Understanding how germination modifies seed composition is essential to optimize their use in different food preparations and to meet the current trend for a healthy and balanced diet. The present study focuses on the effect of prolonged germination on the composition of quinoa seeds collected from a farm in the eastern Morocco. Seeds were germinated in a growth chamber with a controlled environment for different times (24, 48, 72, 96, and 120 h). At the end of each germination period, the seeds were dried and powdered, and their composition was analyzed. Powder obtained from ungerminated seeds was used as control. The results showed that germination led to a significant enhancement in the content of protein, fiber, total phenolics, and total flavonoids, with the highest increases observed at 96 h by 6.09 g/100 g dry matter (DM), 0.89 g/100 g DM, 50.27 mg/100 g DM, and 73.49 mg/100 g DM, respectively, compared to the control. Tocopherols (α, β, and δ) increased by 1.63, 1.21, and 2.67 µg/g of oil at 24 h, 72 h, and 120 h, respectively, compared to the control. Conversely, carbohydrate, energy, and saponin content decreased significantly relative to the control by 9.43–10.11 g/100 g DM (seeds sprouted for 72–96 h), 20.35 kcal/100 g DM (seeds sprouted for 72 h), and 0.58 g/100 g DM (seeds sprouted for 48 h and 72 h). This suggests that powder from germinated *C. quinoa* seeds subjected to prolonged germination (96 h) could be used as functional ingredients in food formulations, offering high levels of macronutrients, minerals, and bioactive compounds with a reduced saponin content.

Keywords: functional ingredient, germination period, health benefits, nutritional profile, quinoa seeds, tocopherols

INTRODUCTION

Chenopodium quinoa Wild. is an emerging crop worldwide, cultivated for over 7,000 years. It is characterized by its ability to grow in different marginal environments [Choukr-Allah *et al*., 2016], with marked resistance to various abiotic stresses, including salinity, drought, and frost [Jacobsen *et al*., 2003; Nazih *et al*., 2024].

C. quinoa seeds are distinguished by their high nutritional value and bioactive properties [Nowak *et al*., 2016; Vega-Gálvez

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et al., 2010]. They are rich in protein (9.1 to 15.7 g/100 g), lipids (4.0 to 7.6 g/100 g) and crude fiber (7.0 to 14.1 g/100 g), and their average carbohydrate content is 58 g/100 g [Pathan & Siddiqui, 2022]. Quinoa seed proteins contain all the essential amino acids and have a high efficiency ratio. These seeds are also rich in minerals, including potassium, calcium, magnesium, and iron [Pathan & Siddiqui, 2022]; tocopherols (7.29 mg/100 g) [Carciochi *et al*., 2016]; and phenolic compounds (71.7 mg/100 g) with high antioxidant activity [Alvarez-Jubete *et al*., 2010]. Other important phytochemicals of quinoa seeds are saponins with contents between 1.41 and 2.03 g/100 g [Mhada *et al*., 2020]. It should be emphasized, however, that the nutritional and bioactive composition of *C. quinoa* seeds depends on the plant genotype and the environmental conditions in which it grows [Granado-Rodríguez *et al*., 2021a,b]. From a nutritional point of view, regular consumption of quinoa seeds in the diet elicits certain health benefits and can reduce the risk of development of cardiovascular disease, obesity and diabetes [Lan *et al*., 2023]. Quinoa is also a good alternative for patients suffering from celiac disease as it is gluten-free [Alvarez-Jubete *et al*., 2010].

Due to its precious qualities, quinoa, with its multiple uses, certainly has a great future, especially in arid and semi-arid areas severely affected by climate impacts, as is the case in several marginal regions of Morocco. In this context, several studies have shown that germination enhances the nutritional value of quinoa by modifying its composition [Omary *et al*., 2012; Suárez-Estrella *et al*., 2020]. Bhinder *et al*. [2021] showed that germination can modify protein content, starch functionality, and bioactive compound profile, enhance the availability of certain minerals such as copper and zinc, and reduce antinutrient levels, including saponins and phytic acid. Additionally, Ramos-Pacheco *et al*. [2024] observed a notable improvement in proteins, lipids, ash, and fiber and a reduction in carbohydrate contents of quinoa seeds as a result of germination. They also noted increases in phosphorus, iron, manganese, and potassium, as well as in total phenolic and total flavonoid contents and antioxidant capacity. These modifications could be due to the activation of endogenous enzymes, which reduce antinutrients and improve nutrient profile, and antioxidant potential [Darwish *et al*., 2021]. However, most of these studies focused on shorter germination periods (up to 72 h) and did not fully investigate the variations in bioactive compounds, especially tocopherols. Therefore, the current study aims to evaluate the effect of prolonged germination (up to 120 h) on the nutritional and bioactive compound compositions of *C. quinoa* seeds and to determine the optimal germination time that enhances the content of valuable compounds, in order to find alternatives for patients with gluten intolerance or celiac disease and individuals seeking highly nutritious food. In addition, the current study provides new insights into the effect of germination on the contents of macronutrients, minerals, and bioactive compounds of quinoa grown and harvested in the eastern region of Morocco.

MATERIALS AND METHODS

Plant material

To conduct this study, fresh-matured *C. quinoa* seeds of the certified Titicaca cultivar were harvested from a farm in Berkane province in the eastern region of Morocco. Titicaca is a Danish quinoa variety developed by the University of Copenhagen. It is known for its early maturity, tolerance to abiotic stress, and adaptability to marginal soils. Its fruit is an achene containing round seeds about 2 mm in diameter.

Germination of *C. quinoa* seeds

The process of germination of quinoa seeds was carried out according to the methodology of Aguilar *et al*. [2019], with slight modifications. A total of 720 g of *C. quinoa* seeds (120 g *per* germination time, including the control) were soaked in 3.6 L of distilled water (600 mL *per* batch) for 24 h. After soaking, the seeds were placed in plastic boxes (three boxes for each germination time) with filter paper wetted with distilled water to keep moisture conversing. The boxes were then transferred to a growth chamber (Memmert GmbH, Schwabach, Germany), where the temperature, relative humidity, and light/dark cycle were 25°C, 70%, and 16/8 h respectively. The soaked seeds (control, germination time – 0 h) and seeds after 24, 48, 72, 96 and 120 h of germination were dried in a forced-air oven (Pol-Eko Aparatura, Wodzisław Śląski, Poland) at 40°C for 24 h to inhibit the activity of hydrolytic enzymes such as amylases, proteases and phytases without significantly altering heat-sensitive nutrients [Guardianelli *et al*., 2022], then crushed with a mill and sifted with a 500 µm sieve to obtain the powders, which were stored at −20°C until analysis of their nutrient and bioactive compound profiles.

Water activity measurement

Water activity (a_w) of powders from sprouted quinoa seeds was measured using an AW meter (Steroglass, Perugia, Italy). Calibration was performed with pure water of a_w=1. Samples weighing 1 g were used for the a_w reading [Ligarda-Samanez *et al*., 2022].

Dry matter determination

The dry matter content of powdered quinoa seeds after germination and drying was determined using the AOAC International method 934.01 [AOAC, 2005]. The samples (1 g) were heated at 105°C until their mass reached a constant value.

Protein content determination

To determine the total nitrogen content of the sprouted quinoa seeds by the Kjeldahl method [AFNOR, 2002], 1 g of powder was weighed and placed in a mineralization tube containing 2 g of catalyst (K_2SO_4, $CuSO_4 \times 5H_2O$, Se), 10 mL of 30% hydrogen peroxide and 20 mL of 98% sulfuric acid. The heating protocol involved a progressive temperature: 45 min at 190°C, 45 min at 290°C until carbonization, and 3 h at 420°C until a lipid liquid appears. After mineralization, 50 mL of distilled water and 80 mL of 40% sodium hydroxide were added for distillation, followed by collection in 50 mL of 4% boric acid and titration with 0.2 N sulfuric acid. A blank sample was treated in the same way for each series. The total nitrogen content (N, %) was calculated using Equation (1):

$$N\ (\%) = \frac{(V - V_0) \times 14 \times N_A \times 100}{E \times 1{,}000} \quad (1)$$

where: V is volume of H_2SO_4 used for the sample titration in mL, V_0 is volume of H_2SO_4 used for the blank titration in mL, N_A is acid solution normality, and E is sample weight in g.

Protein content was obtained by multiplying N×6.25 [Nascimento *et al*., 2014; Zhou *et al*., 2023] and expressed in g/100 g of dry matter (DM) of sprouted seeds.

Lipid content determination

A Soxhlet extractor (Gerhardt, Konigswinter, Germany) with *n*-hexane as a solvent was used for lipid determination according to the AOAC International method 920.39 [AOAC, 1990]. The procedure consisted of placing 40 g of sprouted quinoa seed powder in a cellulose cartridge. The cartridge was then placed in a ground flask containing 150 mL of *n*-hexane. The flask was fitted with a heating mantle and heated for 6 h. The solvent was then evaporated by distillation using a rotary evaporator (Hahn Vapor, Gimpo, South Korea). Finally, the flask containing the resulting lipids was weighed. The lipid content of sprouted seeds was expressed in g/100 g DM.

Crude fiber determination

The crude fiber was determined according to the AOAC International method 988.15 [AOAC, 1990] using a Fibertech system (VELP Scientifica, Usmate Velate, Italy). The sprouted quinoa seed powder was decarbonated and degreased with 100 mL of 1.25 N sulfuric acid and 100 mL of 1.25 N sodium hydroxide solutions. The residue obtained was then separated by filtration through a glass filter. The crucibles containing the residue were then washed and dried in an oven at 103°C for 12 h to obtain the dry weight (Dw) and then incinerated in a muffle furnace at 550°C for 5 h to obtain the ash weight (Aw). The crude fiber content (CF) was calculated according to the Equation (2) and expressed in g/100 g DM of sprouted seeds:

$$CF = \frac{(T + Dw) - (T + Aw)}{Sw} \times 100 \quad (2)$$

where: T is tare weight and Sw is sample weight.

Ash content determination

The ash content was determined by the AOAC International method 942.05 [AOAC, 2005]. The powders weighing 1 g were incinerated in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 650°C for 8 h. The ashed samples were weighed, and ash content of sprouted seeds was expressed in g/100 g DM.

Carbohydrate content estimation

Carbohydrate content of sprouted quinoa seeds was estimated by difference according to Equation (3) [Abedin *et al*., 2022]:

$$\text{Carbohydrates} = 100 - \text{contents of (moisture + protein + lipid + ash + crude fiber)} \quad (3)$$

The results were expressed in g/100 g DM of sprouted seeds.

Energy value estimation

The energy value of the sprouted quinoa seed powders was calculated using Equation (4) [FAO, 2003]:

$$\text{Energy value} = (\text{carbohydrate content} \times 4) + (\text{protein content} \times 4) + (\text{lipid content} \times 9) \quad (4)$$

Results were expressed in kcal/100 g DM.

Mineral composition analysis

The method described by Granado-Rodríguez *et al*. [2021b], with some modifications, was used to determine the content of K, Mg, Ca, Fe, Zn, Cu, and Mn in sprouted quinoa seeds. Powders weighing 2 g were placed in a muffle furnace at a temperature of 650°C for 4 h. The ash formed was dissolved with 3 mL of concentrated hydrochloric acid (37%) and heated in a boiling water bath until the ash was completely dissolved. The volume was made up of 100 mL with pure water, and the solution was analyzed using an atomic absorption spectrophotometer (PerkinElmer, Waltham, MA, USA). The results were expressed in g/kg DM of sprouted seeds.

Determination of total phenolic content and total flavonoid content

The powders were extracted according to the method proposed by Ollivier *et al*. [2004]. The sample was weighed (500 mg) and added to 3 mL of a mixture of methanol and distilled water (80:20, *v/v*) in Eppendorf tubes. The tubes were vortexed at 1,500 rpm for 15 min and then centrifuged at 1,130×*g* for 15 min. The supernatant was collected into a 10 mL flask, and the extraction was repeated three times. Finally, the samples were spiked with methanol and stored in a freezer at −20°C until analysis.

The total phenolic content was determined with a Folin-Ciocalteu reagent according the method described by Joy Ujiroghene *et al*. [2019], with slight modifications. The extract (2 mL) was mixed with 5 mL of 10% Na_2CO_3, 1 mL of the Folin-Ciocalteu reagent, and 5 mL of distilled water. This mixture was incubated in the dark for 30 min. Absorbance readings were then taken using a spectrophotometer (PG Instruments Ltd, Lutterworth, United Kingdom) at a wavelength of 750 nm. The results were expressed as mg gallic acid equivalent (GAE) *per* 100 g DM of sprouted seeds.

The total flavonoid content was quantified using the aluminum chloride colorimetric method described by Suárez-Estrella *et al.* [2020], with minor modifications. Volume of 1 mL of 2% $AlCl_3$ was added to 1 mL of the extract. After shaking, the mixture was incubated for 10 min. Its absorbance was then

read with a spectrophotometer (PG Instruments Ltd) at a wavelength of 430 nm. The results were expressed as mg quercetin equivalent (QE) *per* 100 g DM of sprouted seeds.

Determination of saponin content

The method used to extract saponins from sprouted quinoa seed powders was the one described by Rafik *et al*. [2021], which involved a Soxhlet extraction with *n*-hexane, repeated three times, to delipidate the powder. Next, 5 g of the delipidated powder was mixed with 50 mL of ethanol and stirred for 30 min. The mixture was then filtered, and the filtrate collected was made up to 50 mL with ethanol. Then, 2 mL of a reagent (mixture of glacial acetic acid and concentrated sulfuric acid (1:1, *v/v*), was added to 250 µL of the extract, and the mixture was vortexed and incubated at 60°C for 30 min in a water bath (Bunzen, Madrid, Spain) [Torrez Irigoyen & Giner, 2018]. After incubation, the mixture was cooled in an ice bath. The absorbance was measured using a spectrophotometer (PG Instruments Ltd) at 527 nm. Saponin (CAS No. 8047-15-2), purchased in Solvachim (Casablanca, Morocco), was used as a reference. The results were expressed as g saponin standard equivalent *per* 100 g DM of sprouted seeds.

Tocopherol analysis

To determine tocopherols, 200 mg of oil, extracted from quinoa powder by solid-liquid extraction (Soxhlet) and previously filtered with a 0.45 µm filter, was dissolved in 1 mL of methanol. The mixture was then vortexed at 1,500 rpm for 2 min. Finally, the sample was injected into a column connected to a high-performance liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany) with fluorescence detector (FLD) for tocopherol analysis. The chromatographic separation was performed on a Poroshell 120 EC-C18 column (4.6×150 mm, 4 µm; Agilent Technologies) in an isocratic elution mode. The mobile phase was a mixture of acetonitrile and methanol (50:50, *v/v*) (both solvents of HPLC grade), with a flow rate of 1 mL/min and an injection volume of 20 µL. Detection was performed at 290 nm for excitation and 330 nm for emission. A calibration curve was plotted using α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol standards. The results were expressed as µg/g of oil.

Statistical analysis

Three replicates were performed for each treatment (germination time) and three samples (one from each replicate) were analyzed by each method. Means and standard deviations were calculated. One-way analysis of variance (ANOVA) was used to determine the effect of germination time (factor) on quinoa composition using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA). The post hoc Tukey's test was used to compare means and determine significant differences between samples. Differences were considered as significant at $p \leq 0.05$. The graph was generated using Excel software for Microsoft 365 MSO, version 2405 (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

Effect of germination time on water activity

Water activity of powders obtained from ungerminated and germinated quinoa seeds varied between 0.22 and 0.27, with insignificant ($p>0.05$) differences between powders from germinated and control seeds as well as between powder from seeds sprouted for different times (**Figure 1**). This slight change in a_w indicated that the powders did not present a risk of proliferation of microorganisms [Pellegrini *et al*., 2018] and can be used as a safe ingredient. Our results were consistent with those reported by Ramos-Pacheco *et al*. [2024], who found values of $a_w<0.3$ in all germinated and ungerminated quinoa seed powders.

Effect of germination time on macronutrient content

Protein content

The protein content of sprouted quinoa seeds varied between 15.73 and 23.64 g/100 g DM (**Table 1**). Compared to the control (17.55 g/100 g DM), a significant ($p \leq 0.05$) decrease was observed in the seeds sprouted for 24 h. However, an important increase

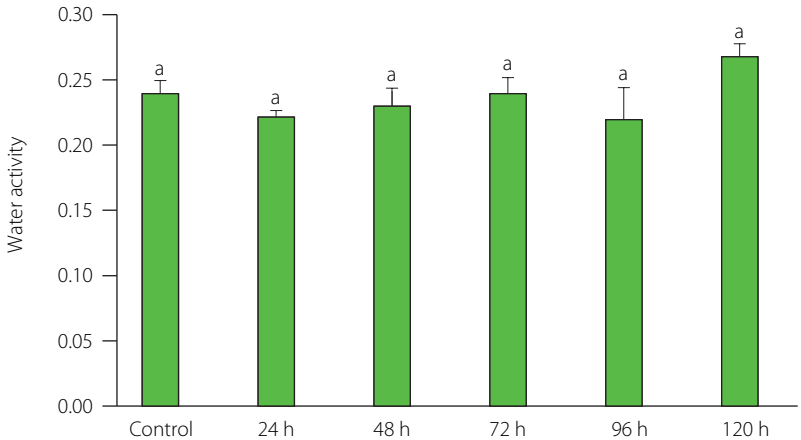

Figure 1. Water activity of powders from quinoa seeds sprouted at different times. Results are shown as means and standard deviation. The same letter above bars indicates no significant differences between means according to Tukey's test ($p>0.05$).

Table 1. Macronutrient content and energy value of quinoa seeds sprouted for different times.

Germination time	Protein (g/100 g DM)	Lipids (g/100 g DM)	Carbohydrates (g/100 g DM)	Crude fiber (g/100 g DM)	Energy value (kcal/100 g DM)
0 h (Control)	17.55±0.07^{c}	3.87±1.09^{ab}	67.23±0.14^{b}	4.49±0.01^{c}	373.0±12.1^{a}
24 h	15.73±0.43^{d}	3.38±0.32^{b}	68.46±0.62^{a}	4.88±0.39^{bc}	366.6±2.3^{ab}
48 h	19.61±0.50^{b}	4.54±0.38^{ab}	62.65±0.83^{c}	4.99±0.50^{ab}	368.1±1.9^{a}
72 h	19.89±0.72^{b}	5.05±0.51^{a}	57.80±0.04^{d}	4.98±0.49^{ab}	352.7±0.3^{b}
96 h	23.64±0.18^{a}	5.02±0.39^{a}	57.12±0.39^{d}	5.38±0.89^{a}	366.1±1.5^{ab}
120 h	20.39±0.33^{b}	3.65±1.21^{ab}	62.74±0.61^{c}	4.98±0.49^{ab}	363.9±13.5^{ab}

Results are shown as mean ± standard deviation (n=3). According to Tukey's test, means with different letters in column differ significantly (p≤0.05). DM, dry matter.

(p≤0.05) was observed in the seeds sprouted for 48–120 h, with the highest protein content determined in the seeds germinated for 96 h (increase of 6.09 g/100 g DM compared to the control). This remarkable augmentation could be explained by the synthesis of new amino acids and the loss of carbohydrates through seed respiration during germination [Bertazzo *et al*., 2011]. In addition, Pilco-Quesada *et al*. [2020] explained the augmentation in protein content during germination by biological synthesis and the mobilization of nutrient reserves. Bewley *et al.* [2013] reported that during the first three days of germination, amino acid content increased, leading to an increase in protein content. In the same context, Jimenez *et al*. [2019] estimated that 2 to 3 days after imbibition, proteolytic enzymes hydrolyzed proteins into peptides and amino acids, increasing the bioavailability of nutrients. Our study results are close to those of Aguilar *et al*. [2019], who observed that *C. quinoa* seeds (Negra Collana variety) displayed a maximum increase of 8% in protein content after 48 h of germination. Similarly, Thakur *et al*. [2021] noted a considerable improvement in protein content from 14.94% (control) to 17.88% after 72 h of germination of quinoa seeds. Pilco-Quesada *et al*. [2020] also observed an enhancement in protein from 9.6 to 26.0% after 72 h of germination. Guardianelli *et al*. [2022] also confirmed a positive correlation between germination time and protein content.

Our findings, in accordance with literature data, suggest that germination is an effective strategy for enhancing the protein content of quinoa, making it a promising approach for improving the nutritional value of quinoa-based food products.

Lipid content

The lipid content of sprouted seeds ranged from 3.38 to 5.05 g/100 g DM, with insignificant (p>0.05) variation observed between germinated and control seeds (**Table 1**). However, the continuous significant (p≤0.05) increase in lipid content was found from 24 to 72 h of processing. This minor uptick in lipid content could be attributed to the abundance of fatty acids released from triglycerides or phospholipids by lipolytic enzymes during germination [Obizoba & Atii, 1991]. On the other hand, during germination, seeds use fatty acids to produce sucrose *via* gluconeogenesis, which is an energy source necessary to complete growth [Lan *et al*., 2023; Nelson *et al*. 2013].

Our study results do not agree with those reported by Obizoba & Atii [1991], who showed an improvement in lipid content in seeds germinated for 96 h, with a value of 5.7% compared to 3.1% for ungerminated sorghum seeds. Darwish *et al*. [2021] also found a 0.8% increase for seeds germinated for 72 h compared to ungerminated quinoa seeds.

Crude fiber content

The crude fiber content of sprouted seeds varied between 4.88 and 5.38 g/100 g DM (Table 1). Compared to the control (4.49 g/100 g DM), a considerable increase (p≤0.05) was observed in the seeds sprouted for 48–120 h. However, it should be noted that the crude fiber content did not differ significantly (p>0.05) between seeds germinated for 48, 72, 96 and 120 h. This significant increase compared to the control could be explained by the abundance of fiber in the early stages of germination [Guardianelli *et al*., 2022]. Pilco-Quesada *et al.* [2020] justified this change in fiber content by the loss of other nutritional components. This augmentation is consistent with the findings of Darwish *et al*. [2021], who highlighted an increase in the crude fiber of 1.77 g/100 g in *C. quinoa* seeds germinated for 72 h compared to the ungerminated seeds. Furthermore, Thakur *et al*. [2021] found that crude fiber content increased significantly with germination time.

Carbohydrate content

The carbohydrate content of sprouted seeds varied between 57.12 and 68.46 g/100 g DM (**Table 1**). Compared to the control (67.23 g/100 g DM), a remarkable increase (p≤0.05) was observed in the seeds sprouted for 24 h. Nevertheless, a noticeably lower (p≤0.05) carbohydrate content was noted in the seeds sprouted for 48 h to 120 h. The maximum reduction compared to the control was 9.43–10.11 g/100 g DM in the seeds germinated for 72–96 h. This substantial decrease could be attributed to the decomposition of complex carbohydrates into simple carbohydrates by enzymes activated during germination [Nelson *et al*., 2013] to provide the energy required for new plants [Ferreira

Table 2. Ash content and mineral composition of quinoa seeds sprouted for different times.

Germination time	Ash (g/100 g DM)	Iron (g/kg DM)	Manganese (g/kg DM)	Calcium (g/kg DM)	Copper (g/kg DM)	Zinc (g/kg DM)	Potassium (g/kg DM)
0 h (Control)	3.87±0.02^{d}	56.8±0.8^{a}	21.24±0.11^{a}	1,244±140^{ab}	2.51±0.13^{a}	23.04±0.93^{a}	2,526±86^{a}
24 h	5.73±0.12^{a}	59.8±15.2^{a}	19.86±2.38^{a}	1,059±196^{abc}	2.72±0.49^{a}	21.74±3.32^{ab}	2,144±6^{b}
48 h	4.02±0.08^{cd}	47.8±5.6^{a}	14.11±0.16^{bc}	762±22^{c}	2.25±0.40^{a}	18.63±0.63^{ab}	1,670±5^{c}
72 h	4.80±0.42^{b}	67.3±1.9^{a}	16.32±0.86^{ab}	1,002±262^{bc}	2.76±0.58^{a}	22.01±0.48^{a}	1,512±167^{c}
96 h	4.32±0.21^{c}	57.6±19.4^{a}	12.47±2.11^{bc}	1,421±136^{a}	2.07±0.80^{a}	16.33±5.12^{b}	438±53^{d}
120 h	3.68±0.07^{d}	56.9±4.2^{a}	9.41±1.74^{c}	944±185^{bc}	2.06±0.11^{a}	18.04±1.41^{ab}	559±123^{d}

Results are shown as mean ± standard deviation (n=3). According to Tukey's test, means with different letters in column differ significantly (p≤0.05). DM, dry matter.

et al., 2009]. Guardianelli *et al*. [2022] explained this reduction by the structure of starch, which facilitates its hydrolysis by endogenous amylolytic enzymes. Likewise, Elkhalifa & Bernhardt [2010] signaled that α-amylase was synthesized at low concentrations during the first hours of germination. As germination progressed, the concentration of α-amylase increased, breaking down starch into glucose.

Our results are in line with those reported by Pilco-Quesada *et al*. [2020], who observed a reduction of 8.1% in carbohydrates in the seeds germinated for 72 h compared with the control. In addition, Ramos-Pacheco *et al*. [2024] observed a non-significant decrease in carbohydrate content in seeds sprouted for 0 to 72 h. Guardianelli *et al*. [2022] reported that the content of starch, the main component of carbohydrates, decreased with germination time (≥24 h), especially in red *C. quinoa*.

To illustrate, the lower carbohydrate content observed in germinated quinoa seeds could be beneficial to health by helping to prevent diseases induced by high carbohydrate consumption, such as diabetes and cardiovascular disease.

Energy value

The energy value varied between 352.7 and 373.0 kcal/100 g DM (**Table 1**). The sprouted seeds tended to have lower energy value than the control, but a significant (p≤0.05) difference was found only between the seeds germinated for 72 h and ungerminated seeds with a decrease of 20.4 kcal/100 g DM. This decrease could be attributed to the low carbohydrate content. Our study results are consistent with those of Thakur *et al*. [2021], who found a 16.4 kcal/100 g reduction in quinoa seeds germinated for 72 h compared to the control.

Effect of germination time on ash content and mineral composition

The ash content fluctuated between 3.68 and 5.73 g/100 g DM with 3.87 g/100 g DM of the control seeds (**Table 2**). However, ash content of the ungerminated seeds did not differ significantly (p>0.05) from that of the seeds sprouted for 48 and 120 h. A noticeably higher (p≤0.05) ash content was determined in the seeds sprouted for 24, 72 and 96 h, with the highest increase compared to the control of 1.86 g/100 g DM noted in the seeds germinated for 24 h. This important enhancement was previously pointed out by Rao & Deosthale [1983] who reported that the high ash content in seeds germinated for 48 to 96 h may result from the treatment method used (reduction of humidity during germination), which concentrates the minerals. In contrast, the decrease in ash content may be due to the soaking and/or the transfer of minerals to the radicles during germination, where they act as co-enzymes in carbohydrate and protein catalysis [Bewley *et al*., 2013]. These radicles are then removed during the drying process.

The mineral composition of germinated quinoa seed varied with germination time. Manganese content varied between 9.41 and 21.24 mg/kg DM. Compared to the control, a marked decline (p≤0.05) was observed in the seeds germinated for 48, 96 and 120 h. The highest potassium content was found in ungerminated seeds (2,526 mg/kg DM) and it decreased significantly (p≤0.05) in the samples for subsequent germination times to a value of 438–559 mg/kg DM determined in the seeds germinated for 96–120 h. Calcium content ranged from 762 to 1,421 mg/kg DM. Relative to the control, a notable reduction of 482 mg/kg DM was observed in the seeds germinated for 48 h (p≤0.05). Regarding zinc, a significant difference (p≤0.05) was only found between the control (23.04 mg/kg DM) and the seeds sprouted for 96 h (16.33 mg/kg DM). No significant differences (p>0.05) were found between the seeds from different germination times with respect to iron and copper contents.

The important decline in the content of certain minerals could be explained by the germination method adopted and the use of distilled water, which allows the minerals to leach out [Bewley *et al*., 2013]. Furthermore, Kajla *et al*. [2017] explained that changes in mineral content during germination are due to the hydrolysis of organic complexes, releasing minerals that act as enzymatic co-factors and support macromolecules catalysis to provide the energy needed for germination. Our study results are close to those of Darwish

Table 3. Total phenolic content, total flavonoid content, and saponin content of quinoa seeds sprouted for different times.

Germination time	Total phenolic content (mg GAE/100 g DM)	Total flavonoid content (mg QE/100 g DM)	Saponin content (g/100 g DM)
0 h (Control)	83.5±4.6^{d}	25.6±0.8^{d}	1.06±0.01^{a}
24 h	92.4±2.6^{cd}	36.6±1.6^{c}	0.99±0.02^{ab}
48 h	97.1±4.1^{c}	36.9±1.5^{c}	0.48±0.04^{b}
72 h	119.6±1.5^{b}	73.5±7.9^{b}	0.48±0.02^{b}
96 h	133.8±7.1^{a}	99.1±1.6^{a}	1.42±0.01^{a}
120 h	94.8±6.7^{cd}	42.3±2.4^{c}	1.22±0.02^{a}

Results are shown as mean ± standard deviation (n=3). According to Tukey's test, means with different letters in column differ significantly (p≤0.05). DM, dry matter; GAE, gallic acid equivalent; QE, quercetin equivalent.

et al. [2021], who observed a 39.43% enhancement in calcium content, in *C. quinoa* seeds. In addition, Bhinder *et al*. [2021] reported a decrease in manganese, zinc, and potassium with increasing germination time in quinoa seeds.

Effect of germination time on bioactive compound content

Total phenolic and total flavonoid contents

The total phenolic content of ungerminated and germinated quinoa seeds fluctuated between 83.5 and 133.8 mg GAE/100 g DM (**Table 3**). The highest value was found in the seeds germinated for 96 h, which was 50.3 mg GAE/100 g DM higher than that determined in the control. A marked augmentation (p≤0.05) was also observed in the seeds germinated for 48 and 72 h relative to the control. Regarding the total flavonoid content, the control seeds had the lowest content (25.6 mg QE/100 g DM). This value gradually increased with the extension of germination time up to 96 h and reached 99.1 mg QE/100 g DM. The total flavonoid content of the seeds germinated for 120 h was higher (p≤0.05) compared to the control, but did not differ significantly (p>0.05) from those of the seeds germinated for 24 and 48 h. The remarkable increases in total phenolic and total flavonoid contents could be attributed to the release of phenolic compounds from the cell walls [Alvarez-Jubete *et al*., 2010]. This increase is one of many metabolic changes that occur during seed germination, mainly due to the increased activity of the hydrolytic action of esterases and glucosidases on non-extractable phenolic compounds [Kim *et al*., 2016]. Kim *et al*. [2016] also noted that soaking seeds in water could activate enzymes, such as phenylalanine ammonia-lyase, which catalyzes the main phenylpropanoid reactions and, therefore, the formation of secondary metabolites.

Our results concur with those found by Thakur *et al*. [2021], who observed a significant enhancement of 34.4% in the total phenolic content in quinoa seeds germinated for 72 h. A similar study by Alvarez-Jubete *et al*. [2010] determined an increase in the total phenolic content by 147.2% in quinoa seeds after 82 h of germination. In turn, Ramos-Pacheco *et al*. [2024] observed that contents of total phenolics and total flavonoids increased with increased germination time, especially in white *C. quinoa*. Therefore, germination can be an efficient means to improve the antioxidant properties of *C. quinoa* seeds.

It is important to note that the initial antioxidant composition of the seeds and their germination response can be influenced by the year of cultivation, the variety, genetic factors, and their interaction [Aguilar *et al*., 2019; Granado-Rodríguez *et al*., 2021a]. In addition, the region where the quinoa is grown and harvested, climatic conditions, the quality of the soil and the water used for irrigation, as well as farming practices determine the content of these compounds in the plant.

Saponin content

The saponin content of the ungerminated seeds was 1.06 g/100 g DM, and that of the germinated seeds varied between 0.48 and 1.42 g/100 g DM (**Table 3**). A significant (p≤0.05) decrease was observed compared to the control in the seeds germinated for 48 and 72 h.

Saponins are mainly found in the pericarp of *C. quinoa* seeds [Suárez-Estrella *et al*., 2020; Yadav *et al*., 2023], which contains about 86% of these secondary metabolites [Ruiz *et al*., 2017]. Considered as anti-nutrients [Granado-Rodríguez, *et al*., 2021b], saponins form insoluble complexes with certain minerals and vitamins, thereby reducing their intestinal absorption [Ruales & Nair, 1993; Zhou *et al*., 2023].

Saponin content in quinoa seeds depends on the variety [Granado-Rodríguez *et al*. 2021a; Mora-Ocación *et al*., 2022] and cultivation conditions such as rainfall, which can reduce saponin content [Lim *et al*., 2020]. The Titicaca, used in our study, is a bitter variety, with a saponin content above the 0.12% threshold set by the Codex Alimentarius [2019] as an acceptable limit to avoid bitterness. Therefore, the seeds of this variety require prior treatment before consumption, such as mechanical processing (polishing or sieving) and/or washing with water [Zhou *et al*., 2023]. However, mechanical treatments can negatively affect the nutritional profile of quinoa [Casalvara *et al*., 2024; Gómez-Caravaca *et al*., 2014], which facilitates treatment by washing or the use of alternative processing, such as germination, to remove saponins [Lan et al., 2024].

Our study results support those of Mhada *et al.* [2020], who found a saponin content of 2.03% in raw quinoa of the Titicaca variety. This level was reduced to 0.07% after polishing and processing into semolina. Furthermore, Nickel *et al*. [2016] reported a saponin content of 3.33% in raw quinoa, which became 2.75% after soaking for 15 min under a stream of running water. Similarly, Chaudhary *et al*. [2024] showed that soaking quinoa seeds in water for 24 and 48 h reduced the saponin content by 0.4% and 0.7%, respectively, compared to 1.9% saponin in raw quinoa. These reductions could be explained by the solubility of saponins in water [Bhinder *et al*., 2021]. Regarding germination, Suárez-Estrella *et al.* [2021] observed a reduction in saponin content from 0.40% to 0.05% in seeds germinated for 72 h. Similarly,

Table 4. Tocopherol content of oil from quinoa seeds sprouted for different times.

Germination time	α-Tocopherol (µg/g oil)	β-Tocopherol (µg/g oil)	γ-Tocopherol (µg/g oil)	δ-Tocopherol (µg/g oil)
0 h (Control)	4.82±0.80^{b}	2.93±0.18^{b}	1.43±0.61^{a}	1.38±0.19^{d}
24 h	6.45±0.68^{a}	2.25±0.15^{cb}	1.16±0.03^{ab}	2.83±0.18^{b}
48 h	3.24±0.04^{d}	1.14±0.18^{d}	0.97±0.02^{ab}	2.52±0.14^{c}
72 h	4.65±0.01^{bc}	4.14±0.01^{a}	0.79±0.01^{b}	1.35±0.01^{d}
96 h	3.25±0.11^{cd}	1.37±0.01^{d}	1.05±0.01^{ab}	2.97±0.04^{b}
120 h	3.54±0.03^{bcd}	1.90±0.01^{cd}	1.20±0.01^{ab}	4.05±0.03^{a}

Results are shown as mean ± standard deviation (n=3). According to Tukey's test, means with different letters in column differ significantly (p≤0.05).

Bhinder *et al*. [2021] reported that germination reduced saponin content in white and black quinoa after 96 h. Moreover, Darwish *et al*. [2021] found a 60% reduction in saponins in quinoa seeds germinated for 72 h.

As a result, it can be concluded that germination, particularly for 48 and 72 h, was an effective technique for partially reducing the saponin content of quinoa seeds, with a reduction of 0.58 g/100 g DM, *i.e.*, a reduction of 54.72%. However, this technique is still not sufficient to completely remove the bitterness. According to Koziol [1991], bitterness is perceived by humans at saponin levels above 0.11%, which necessitates the use of additional techniques to reduce this bitterness, such as prolonged soaking, high-pressure washing, mechanical polishing and boiling of the seeds, to obtain a less bitter powder suitable for various food preparations.

Tocopherol content

The highest α-tocopherol content was determined in oil from the seeds sprouted for 24 h (6.45 µg/g oil) (**Table 4**). It was significantly (p≤0.05) increased by 1.63 µg/g oil compared to the control. Germination beyond 24 h notably (p≤0.05) reduced α-tocopherol content to 3.24–4.65 µg/g oil. In turn, the β-tocopherol content fluctuated between 1.14 and 4.14 µg/g oil, with noticeable differences (p≤0.05) observed in the seeds germinated for 48, 72, 96, and 120 h relative to the control. However, an increase (by 1.21 µg/g oil) was found only in the oil from seeds sprouted for 72 h. Samples analyzed at 48, 96 and 120 h had lower β-tocopherol content than the control. γ-Tocopherol content varied between 0.79 and 1.43 µg/g oil. Oil from the ungerminated seeds had significantly (p≤0.05) higher γ-tocopherol content than the oil from seeds sprouted for 72 h. Differences between the remaining samples were insignificant (p>0.05). Concerning δ-tocopherol, its content ranged from 1.35 to 4.05 µg/g oil. Compared to the control, an important increase was detected in the seeds germinated for 24, 48, 96, and 120 h (p≤0.05). with the highest augmentation of 2.67 µg/g oil found in the seeds germinated for 120 h.

α-Tocopherol, which is the most active form of vitamin E [Žilić *et al*., 2014], was the predominant tocopherol throughout the germination period in our study. This finding is consistent with those reported by Pachari Vera *et al*. [2019], who showed that yellow quinoa varieties had higher α-tocopherol levels compared to other forms. In addition, Tarasevičienė *et al*. [2019] reported an increase in α-tocopherol and a decrease in γ-tocopherol contents during germination in edible seeds such as wheat, radish, sunflower, lentil, and amaranth germinated for 24, 72 and 120 h. In turn, Granda *et al*. [2018] demonstrated that the quinoa varieties Amarilla de Marangani and Titicaca had high levels of α-tocopherol and low levels of γ-tocopherol, while δ-tocopherol content of 3.72 mg/kg and 4.59 mg/kg was found in the quinoa varieties Blanca Dulce and Black Quinoa, respectively. However, these results do not agree with those of Žilić *et al*. [2014], who reported γ-tocopherol as the most abundant tocopherol in quinoa seeds, followed by α-tocopherol, with small amounts of β- and δ-tocopherols, as also reported by Carciochi *et al*. [2016]. These dissimilarities could have been due to varietal differences [Tang *et al*., 2016], seed color [Granda *et al*., 2018], germination and extraction methods, as well as climatic and edaphic growing conditions.

Tocopherols play an important role during germination by scavenging free radicals and preventing lipid peroxidation to protect young seedlings from oxidative stress [Yang *et al*., 2018]. In addition, they have been suggested to elicit many health benefits. In particular, γ-tocopherol inhibits inflammation and the proliferation of prostate and colon cancer [Balakrishnan & Schneider, 2023]. Furthermore, Devaraj *et al*. [2008] highlighted the combined ameliorative effect of α- and γ-tocopherol on oxidative stress in patients with metabolic syndrome. These two tocopherols can readily quench free radicals [Balakrishnan & Schneider, 2023]. Likewise, tocopherols have been linked to the prevention of chronic diseases, disorders, and certain types of cancer [Ryynänen *et al*., 2004]. They are also known to regulate gene expression, signal transduction, and cellular functions [Shahidi & De Camargo, 2016]. In addition, Tarasevičienė *et al*. [2019] reported their role in reducing the production of thromboxane, a cytokine with vasoconstrictive effects, which could affect cardiovascular health. Finally, Rizvi's review [2014] supported and described the various benefits of tocopherols, such

as antioxidant protection, prevention of atherosclerosis, and reduction of prostaglandin levels.

CONCLUSIONS

Germination of quinoa seeds resulted in a remarkable change in the compositions of macronutrients, minerals, and bioactive compounds. Significant improvements in the contents of protein, crude fiber, total phenolics, and total flavonoids were observed in the seeds germinated for 96 h, indicating that prolonged germination was effective. A substantial decrease in carbohydrates was observed from 72–96 h of germination. The seeds germinated for 24 h had high levels of α-tocopherol and ash, while saponin content was reduced after 48 and 72 h of germination. In addition, the energy value of the seeds was reduced in those germinated for 72 h.

In this respect, the powder obtained from germinated quinoa seeds, especially for 96 h, has the potential to be used as a valuable ingredient in various food products *e.g.* in the production of savory snacks, offering new and innovative opportunities. In addition, it will be essential to raise consumer awareness of the benefits of powders obtained from germinated quinoa seeds. However, the development of additional processes to further reduce saponin levels is necessary to ensure their safe use and positive impact on public health.

These results are very promising for selecting quinoa varieties with the best nutritional properties to be grown in the semi-arid climate of eastern Morocco and for new cultivation environments.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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Effects of Carob Flour on Volume, Color, Texture, Sensory Properties, Antioxidant Profile, and Nutritional Quality of Gluten-Free Corn Cake with Buffalo Cream

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The study's objectives were to produce high-quality gluten-free cake, to investigate the effect of carob flour on volume; texture; color; sensory properties; contents of total dietary fiber and α-tocopherol; and composition of macronutrients, minerals, fatty acids, and phenolic compounds of cakes. In this study, corn flour was substituted with carob flour at different levels (10, 20, 30, and 40%, *w/w*). Buffalo cream was used as a fat replacer due to its low lipid content (65 g/100 g). Carob flour caused an increase in cake volume and a decrease in baking loss. The texture properties of gluten-free cakes were improved by carob flour. The gluten-free cakes including carob flour had a lower brightness. The contents of total carbohydrates, total dietary fiber, ash, phosphorous, calcium, potassium, linoleic acid, linolenic acid, α-tocopherol, and phenolic compounds, including caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, *o*-coumaric acid, cinnamic acid, rosmarinic acid, catechin, rutin, resveratrol, quercetin, and naringenin, increased with increasing levels of carob flour in the gluten-free cakes. The highest sensory score (4.3) was given to the gluten-free cake with 20% (*w/w*) carob flour. The best one in terms of the total dietary fiber (5.37 g/100 g), important minerals (phosphorus – 569.8 mg/kg, calcium – 587.0 mg/kg, potassium – 400.6 mg/kg), essential fatty acids (linoleic acid and linolenic acid – 1.08 and 0.36 g/100 g total fatty acids, respectively), and antioxidants was the gluten-free cake with 40% (*w/w*) carob flour. If the protein content and sensory properties were also taken into account, the gluten-free cake including 20% (*w/w*) carob flour could be preferred.

Keywords: bakery product, *Ceratonia siliqua*, fatty acid composition, minerals, phenolics

INTRODUCTION

Corn is an important food source in the world. There has been a growing interest in corn products because they are gluten-free and also dense in antioxidants [Blanch *et al.,* 2023]. Corn is rich in phenolic acids such as gallic acid, ferulic acid, vanillic acid, tannic acid, caffeic acid, *o*-coumaric acid, cinnamic acid, and salicylic acid [Pandey *et al.,* 2013] and also contains flavonoids including anthocyanins in the case of pigmented grain varieties [Magaña-Cerino *et al.*, 2020]. Carob flour, another gluten-free product, has a significant health-promoting value due to its high contents of phenolic compounds and dietary fiber [Ortega *et al.,* 2011]. This insoluble and non-fermentable fiber (cellulose, hemicellulose, and lignin) exerts anti-diabetic and anti-obesity effects [Dahmani *et al*., 2023]. Durazzo *et al.* [2014] reported that carob flour with a high total phenolic content and antioxidant properties offered the potential to be a valuable food ingredient. Carob can also be considered as a natural sweetener [Benković *et al*., 2017].

A growing interest has recently been observed in developing gluten-free bakery products, due to the increasing number of patients with celiac disease and other gluten-related diseases

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in the world [Šmídová & Rysová, 2022; Xu *et al*., 2020]. However, the variety of gluten-free products is limited because of the difficulties in manufacturing products with the desired physicochemical and sensory properties. To improve these properties, new sources of gluten-free flour, such as carob flour, and appropriate technological processes are sought, *e.g.*, Yalcin & Basman [2008] reported that the gelatinization process of corn flour, gum addition (locust bean gum and xanthin gum), and transglutaminase caused an improvement in the quality of corn noodles. Różyło *et al*. [2017] used carob fiber (0–5%) in gluten-free bread made from rice flour, corn flour, and buckwheat flour to increase dietary fiber content because of its generally low content in gluten-free products. They reported that carob fiber increased the volume and springiness while decreasing the hardness of bread. In addition, it affected the color of the bread by decreasing the lightness and yellowness and increasing the redness. Igual *et al.* [2024] investigated the effect of carob flour (5–12.5%) on the quality of gluten-free corn extruded products. They reported that carob flour led to an increase in starch and fiber contents, while reducing hardness. Thus, this product could be a viable source of fiber after extrusion. Preichardt *et al.* [2011] reported that xanthan gum (0.2–0.4%) increased the specific volume and decreased the firmness of cake including rice and maize flour (50/50, *w/w*). In turn, Berk *et al*. [2017] investigated the effects of carob flour (10%, 20%, 30%) and gums (xanthan gum, guar gum) on the quality of the gluten-free cake including rice flour and reported that 30% carob flour and xanthan gum ensured optimum hardness. Ammar *et al.* [2021] optimized a gluten-free sponge cake formulation using combinations of rice flour, maize flour, and whey protein and reported that whey protein caused higher volume, reduced baking loss, and increased hardness. Paesani *et al.* [2021] investigated the effect of stabilized wholegrain maize flour on the quality of gluten-free layer cakes. They determined that the gluten-free cake including extruded whole grain maize flour had a lower specific volume and higher hardness than the gluten-free cake including non-extruded wholegrain maize flour. Previous studies have only investigated a few gluten-free flours including those made of rice, millet, chickpea, buckwheat, and maize starch [Xu *et al*., 2020]. Several gums were also added to the cookies, cakes, and crackers to improve final product quality. Despite the outcomes of these studies, further research is necessary to develop more preferable, and nutritious gluten-free muffins.

In recent years, new gluten-free flours, processing aids, and physical treatments have been used to get closer to gluten-containing products. Flours or starches do not exhibit gluten properties. Adding protein, hydrocolloids, emulsifiers, physical treatments, and enzymatic technology has created a gluten network. Hence, gluten-free products have improved but still differ from wheat-based products in terms of appearance, texture, and sensory properties. Corn flour and carob flour represent good potential in a gluten-free product technology; however, their presence in the market is still limited [Gasparre & Rosell, 2023]. Some studies have been conducted on gluten-free cake production. However, there is no study on the production and quality characteristics of gluten-free cakes including corn and carob flour. Therefore, this research aimed to develop high-volume and score, and good-texture gluten-free cakes with vital minerals, essential fatty acids, and antioxidants at high levels, in order to expand gluten-free food options for celiac patients. Additionally, I used buffalo cream as a fat replacer in the cake to decrease fat content. Buffalo cream is a traditional product of Afyonkarahisar (Turkey). Afyonkarahisar is a city famous for producing poppy products. It is also the place where buffalo breeding is highest. The buffalos here are fed with poppy pulp. Therefore, the flavor of the Afyonkarahisar buffalo cream differs from others and has received a geographical indication.

MATERIALS AND METHODS

Materials

Corn flour (containing 11.4 g of moisture, 4.9 g of protein, 2.5 g of lipids, 79.9 g of total carbohydrates, 3.3 g of total dietary fiber, and 1.3 g of ash *per* 100 g), carob flour (3.8 g of moisture, 4.6 g of protein, 0.6 g of lipids, 89.9 g of total carbohydrates, 39.8 g of total dietary fiber, 2.1 g of ash *per* 100 g), sucrose, baking powder, salt and UHT milk (3.3 g of lipids *per* 100 g) were purchased from a local market in Afyonkarahisar. Buffalo cream (65 g of lipids *per* 100 g) was bought from Afyon Kocatepe University buffalo breeding farm. Egg white powder (8.3 g of moisture, 79.3 g of protein, 1 g of lipids, 5.4 g of total carbohydrates, 6 g of ash *per* 100 g) was supplied from Kor AGRO Organik Gıda Enerji San. Tic. A.Ş. (Izmir, Turkey). The macronutrient composition of the materials was determined using methods described below.

Preparation of cake

The cake was produced according to the method stated by the American Association of Cereal Chemists (AACC) No. 10-91 [AACC, 2000] with some modifications. For this purpose, 100 g of sucrose, 9 g of egg white powder, 112 g of milk, 3 g of salt, 5 g of baking powder, 40 g of buffalo cream, and 100 g of corn flour were used in the control (cake 1). Unlike the control sample, in cake 2, 90 g of corn flour and 10 g of carob flour were used instead of 100 g of corn flour; in cake 3, 80 g of corn flour and 20 g of carob flour were used instead of 100 g of corn flour; in cake 4, 70 g of corn flour and 30 g of carob flour were used instead of 100 g of corn flour; while in cake 5, 60 g of corn flour and 40 g of carob flour was used instead of 100 g of corn flour. Boiled milk, egg white powder, sucrose, and buffalo cream were mixed in a mixer (Arzum, Ningbo, China) at a low speed for 1 min. Afterward, the sides of the container were scraped and mixed at a high speed for 4 min. Then, baking powder, salt, and flour were added to the mixture, which was then mixed at a low speed for 1 min. The sides of the container were scraped and mixed at high speed for 2 min. Finally, the sides of the container were scraped again, and the process was completed by mixing for 1 min at a low speed and for 2 min at a high speed. The resulting dough was weighed in equal amounts (60 g) and divided into molds. It was then baked at 175°C for 35 min in the conventional oven (Seg, Manisa, Turkey). After the baked

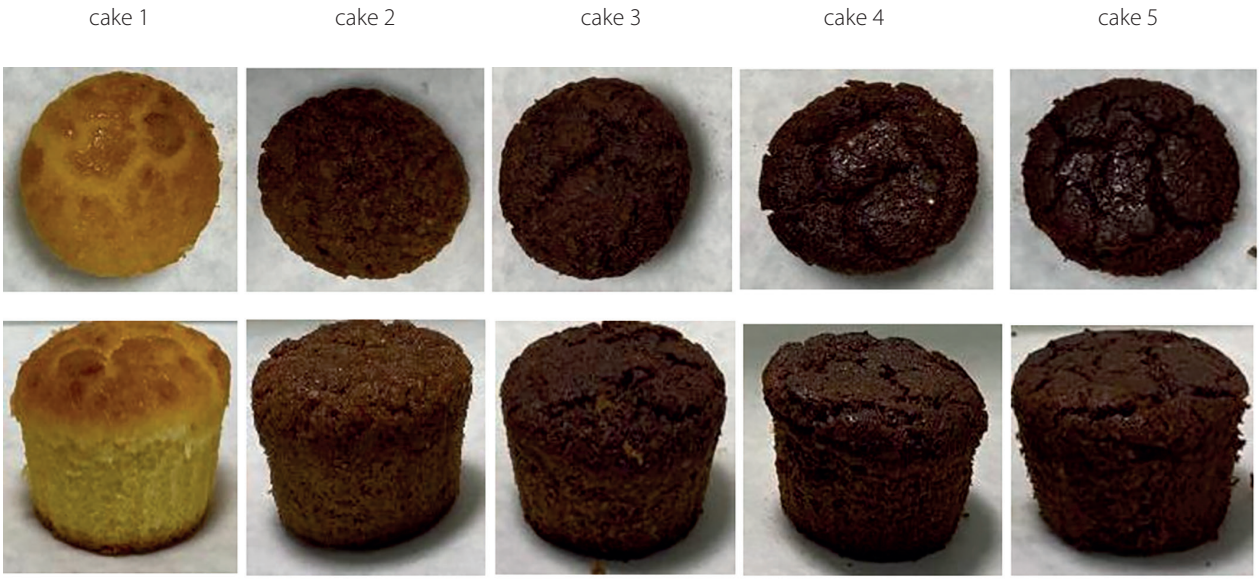

Figure 1. Appearance of corn gluten-free cakes produced without (cake 1) and with carob flour as a corn flour substitute at the weight levels of 10% (cake 2), 20% (cake 3), 30% (cake 4), and 40% (cake 5).

cakes were prepared, they were left in the container to cool for about 30 min. The production was made in duplicate. Six cakes were baked in each batch. The appearance of the gluten-free cakes is shown in **Figure 1**.

Measurement of cake volume

The volume of the cakes was determined according to the rapeseed displacement method. The empty pan was filled with rapeseed. The empty pan volume (V_1) was measured based on the rapeseed volume as determined with a graduated cylinder. The cake was placed in the pan. The rest of the pan's volume (V_2) was filled with rapeseed, and the rapeseed volume was determined with a graduated cylinder. The cake volume was calculated using Equation (1):

$$\text{Cake volume} = V_1 - V_2 \quad (1)$$

Determination of the volume index of cakes

The method of the AACC No. 10-91 [AACC, 2000] was used to determine the volume index of the cakes. The cake was cut vertically through the center. The template was used for measuring the cake heights at three different points (B, C, D) along the cross-sectioned cakes. The volume index was calculated as a sum of B, C, and D, where B was the height of the cake at the points 2.5 cm away from the center towards the left side of the cake, C was the height of the cake at the center point, and D was the height of the cake at the points 2.5 cm away from the center towards the right side of the cake.

Determination of the baking loss of cakes

Percentage baking loss of the cakes during baking was calculated using the weight of the cake batter (W_1) and the weight of the cake after baking (W_2), using Equation (2):

$$\text{Baking loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

Determination of color values of cakes

The color coordinates of the cakes were measured in the CIELab space using a chromameter (Konica Minolta Cr-400/410, Osaka, Japan). The viewing angle of the instrument was 0°, and the light source was a pulsed xenon lamp. The chromameter was calibrated using standard white and black calibration plates. The coordinates L^* describing the brightness, a^* varying from green to red, and b^* varying from blue to yellow were measured. The cake crumb and crust's total color change (ΔE) was determined using Equation (3), where the suffix "0" denotes the control sample (corn cake without carob flour):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (3)$$

Texture analysis of cakes

The texture analysis of the cakes was performed 24 h after baking. The cakes' hardness, springiness, cohesiveness, chewiness, and resilience were determined using a TA.XT plus texture analyzer (Stable Micro Systems, Godalming, England) equipped with a 50 N load cell and a cylindrical probe 36 mm in diameter. Textural parameters were obtained from two-bite force–time curves. Hardness was defined as the peak force of the first compression cycle. Springiness was defined as the height at which the sample sprang back after the first compression. Cohesiveness was defined as the ratio of the work during compression of the second cycle divided by that of the first cycle. Chewiness was defined as the product of hardness, cohesiveness, and springiness. Resilience was defined as the ratio of work returned by the sample as compressive strain is removed to the work required for

compression. Center of cakes was cut into cube shapes having dimensions of 25×25×25 mm and was compressed to 40% strain at a speed of 5 mm/s. The pre-test and post-test speeds were at 1 mm/s and 5 mm/s, respectively.

Measurement of pH of cakes

pH measurement of the cakes was realized according to the method reported by Žilić *et al.* [2016]. Ground cakes (0.4 g) were mixed with 20 mL of deionized water for 3 min. The mixture was kept at an ambient temperature for 60 min to separate solid and liquid phases. The pH of supernatants was measured with a pH meter (Testo 205, Titisee Neustadt, Germany).

Determination of moisture, protein, lipid, total carbohydrate, total dietary fiber, and ash contents of cakes

The moisture, protein, lipid, and ash contents of the cakes were determined according to internationally approved method 44–01.01 [AACC, 2010], Dumas method reported by Shea & Watts [1939], Turkey standard (TS) – ISO 11085 method [TS EN ISO, 2016], and method 08–01.01 [AACC, 2010], respectively. The total carbohydrate content of the cakes was calculated as 100–(g of moisture + g of protein + g of lipids + g of ash *per* 100 g). The total dietary fiber content of the cakes was determined based on the AOAC International 985.29 method [AOAC, 1992] using the total dietary fiber assay kit (Megazyme, Bray, Ireland). The principle of the method was based on the use of α-amylase, protease, and amyloglucosidase under different incubation conditions to remove starch and protein components, followed by gravimetric determination of the total dietary fiber content. Results of analyses were expressed as g *per* 100 g of cakes.

Determination of mineral content of cakes

Ground cakes (1 g) were added to 65% HNO_3 (8 mL) and 30% H_2O_2 (2 mL) and heated to 110°C for 15 min and then left in the microwave oven (Milestone start D, Sorisole, Italy) for 15 min. The cakes' phosphorus, magnesium, calcium, sodium, and potassium contents were determined according to the method reported by Gopalani *et al.* [2007] using an inductively coupled plasma–optical emission spectrometer (ICP-OES) Optima 8000 (Perkin Elmer, Waltham, MA, USA). The phosphorus, magnesium, calcium, sodium, and potassium wavelengths were 214.9, 279.0, 315.8, 589.0, and 766.4 nm, respectively. Mineral contents were expressed as mg *per* kg of cakes.

Analysis of the fatty acid composition of cakes

Lipids from ground cakes were extracted, and their fatty acids were methylated according to the AOAC 996.01 method [AOAC, 1996]. The derivatization solution was the methanolic HCl solution (1.5 M), and the reaction was performed at derivatization 80°C for 2 h. Fatty acid methyl esters were analyzed using a gas chromatography-mass spectrometry (GC-MS) technique by Agilent 7890A GC and Agilent 5975 C XL EI/CI MS (San Diego, CA, USA) equipped with a DB WAX column (50 m × 0.20 mm, 0.20 µm; Agilent). Detector and injector temperature was 240°C. The oven starting temperature was 80°C. After waiting at 60°C for 4 min, it was increased to 175°C with an increase of 13°C *per* min. This temperature was kept for 27 min. Then, 215°C was reached with an increase of 4°C *per* min, and was kept for 5 min. Thereafter, 240°C was reached with an increase of 4°C *per* min and kept for 15 min. Identification of individual compounds was performed using a reference mixture of fatty acid methyl esters (FAME mixture, Supelco, Bellefonte, PA, USA). The content of individual fatty acids was expressed as g *per* 100 g of total fatty acids.

Determination of the α-tocopherol content of cakes

Ground cakes (2 g) were added to 100% *n*-hexane (40 mL), mixed in a vortex for 5 min, sonicated for 120 min, and centrifuged at 8,965×*g* for 5 min. The supernatant was removed and dried in a rotary evaporator at 30°C. The extract was then dissolved in 4 mL of *n*-hexane and used for analysis. α-Tocopherol content was determined by high-performance liquid chromatography (HPLC) analysis. The instrument (Agilent 1260 infinity series) with a diode array detector was connected to a Beckman coulter silica column (250×4.6 mm, 5 µm; Beckman, Brea, CA, USA). The mobile phase consisted of *n*-hexane (997.5 mL) and isopropyl alcohol (2.5 mL) and was used with a flow rate of 1 mL/min. Column temperature was 35°C. The peak was identified by comparing the retention time with the α-tocopherol standard. Quantification of α-tocopherol was performed by using a calibration curve of α-tocopherol standard. α-Tocopherol content was expressed as mg *per* kg of cakes.

Analysis of the phenolic compound profile of cakes

Ground cakes (2 g) were added to 96% ethanol (10 mL), and mixed in a homogenizer for 2 min. The mixture was kept in a water bath at 45°C for 15 h. After that, it was centrifuged at 2,540×*g* for 5 min. The supernatant was removed and dried in a rotary evaporator at 45°C. The extract was then dissolved in 1 mL of methanol and used for analysis. Phenolic compounds of the cakes were analyzed in the gradient system of a mobile phase (solvent A: 0.1% (*v/v*) phosphoric acid and solvent B: 100% acetonitrile, at 0 min volume ratio of 83:17, 7 min – 85:15, 20 min – 80:20, 24 min – 75:25, 28 min – 70:30, 30 min – 60:40, 32 min – 50:50, 36 min – 30:70, 40 min – 83:17) using Agilent 1260 infinity series HPLC equipped with a diode array detector and ACE Generix C18 column (250×4.6 mm, 5 µm; Avantor, Radnor, PA, USA). The flow rate was 0.8 mL/min, and the column temperature was 30°C. The peaks were identified by comparing their retention times with those of phenolic compound standards. Quantitative analysis of phenolic compounds was carried out by using calibration curves of standards. Phenolic compound contents were expressed as mg *per* kg of cakes.

Sensory analysis of cakes

The sensory evaluation of the cakes was performed using a 5-point hedonic scale. To this end, 100 untrained panelists

recruited students of the Afyon Kocatepe University (Afyonkarahisar, Turkey) assessed the cakes for their shape, color, softness, odor, aroma, consistency, and overall acceptability. Based on total scores, consumer acceptance was rated as <2.90 – unacceptable, 3.0–3.50 – acceptable, 3.51–4.50 – good, and 4.51–5.0 – very good [Gambuś *et al.*, 2009].

Statistical analysis

The production of cakes was performed in duplicate. One cake from each batch was analyzed by individual methods. The data was expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was conducted for volume, volume index, baking loss, color coordinates, texture parameters, mineral content, fatty acid composition, contents of α-tocopherol, and phenolic compounds with the Duncan's post hoc test using IBM Statistics SPSS 24 software (IBM Corp. Armonk, NY, USA). The means were considered to be significantly different at $p<0.05$.

RESULTS AND DISCUSSION

Physical properties of cakes

Volume, volume index, and baking loss of corn gluten-free cakes produced with and without carob flour are shown in **Table 1**. Carob flour inclusion into the cake recipe significantly ($p<0.05$) affected these parameters of the analyzed products. Cake volume and volume index increased dramatically with the increasing levels of carob flour used in cake production. Cake including 40% (*w/w*) carob flour (flour-based) had the highest volume and volume index. This finding is consistent with the study by Turabi *et al.* [2008] who reported that locust bean gum (from carob seeds) caused an increase in the specific volume of rice cakes. Statistically similar ($p\geq0.05$) baking loss values were observed for control gluten-free cake, gluten-free cake including 10% (*w/w*) carob flour, and gluten-free cake including 20% carob flour (**Table 1**). The gluten-free cake with 40% (*w/w*) carob flour substitution had the lowest baking loss value.

Color of cakes

The values of the color coordinates of gluten-free cakes produced using corn and carob flours are shown in **Table 1**. Carob flour significantly ($p<0.05$) affected L^*, a^*, b^* crumb and crust color values. L^* and b^* crumb color values decreased, and a^* crumb color values increased, while L^*, a^*, and b^* crust color values decreased with the increasing levels of carob flour used in the gluten-free cakes. The brightness of the control gluten-free cake was significantly ($p<0.05$) higher than that of the gluten-free cakes

Table 1. Volume, volume index, baking loss, texture parameters, and color values of corn gluten-free cakes produced without (control) and with carob flour as a corn flour substitute at the level of 10–40% (*w/w*).

Parameter	Cake 1 (control)	Cake 2 (10% carob flour)	Cake 3 (20% carob flour)	Cake 4 (30% carob flour)	Cake 5 (40% carob flour)
Volume (mL)	71.01±0.85^{e}	78.05±0.63^{d}	85.10±0.41^{c}	92.14±0.59^{b}	99.18±0.96^{a}
Volume index	11.00±0.25^{e}	11.66±0.44^{d}	13.07±0.20^{c}	14.26±0.29^{b}	15.50±0.34^{a}
Baking loss (%)	14.25±0.35^{a}	13.17±0.47^{a}	12.50±0.99^{ab}	10.85±0.92^{bc}	9.65±0.21^{c}
Hardness (N)	18.07±0.54^{a}	15.70±1.06^{b}	13.76±0.08^{c}	11.22±0.06^{d}	8.21±0.56^{e}
Springiness	0.84±0.01^{a}	0.86±0.04^{a}	0.89±0.07^{a}	0.94±0.03^{a}	0.95±0.00^{a}
Cohesiveness	0.65±0.00^{a}	0.66±0.05^{a}	0.67±0.09^{a}	0.72±0.10^{a}	0.75±0.06^{a}
Chewiness	1207±243^{a}	1096±78^{ab}	835±70^{bc}	584±134^{cd}	466±75^{d}
Resilience	0.38±0.02^{c}	0.40±0.01^{bc}	0.42±0.01^{b}	0.47±0.02^{a}	0.49±0.01^{a}
L^* (crumb)	67.83±0.35^{a}	39.45±0.47^{b}	33.33±0.99^{c}	31.10±0.92^{c}	30.29±0.21^{c}
a^* (crumb)	11.79±1.26^{c}	12.89±0.89^{b}	13.97±0.74^{a}	14.08±0.15^{a}	14.51±1.94^{a}
b^* (crumb)	36.45±0.00^{a}	19.59±0.37^{b}	16.92±0.33^{c}	14.93±0.01^{cd}	14.37±0.35^{d}
ΔE (crumb)	–	34.83±0.30^{d}	41.48±2.21^{c}	44.31±1.65^{b}	45.38±3.44^{a}
L^* (crust)	45.78±0.94^{a}	38.87±1.19^{b}	31.68±0.42^{c}	29.95±0.07^{c}	28.14±0.90^{e}
a^* (crust)	18.93±0.14^{a}	16.18±1.12^{b}	14.72±0.86^{c}	13.36±0.06^{cd}	11.63±1.55^{d}
b^* (crust)	31.14±0.19^{a}	18.95±0.70^{b}	11.84±0.80^{c}	10.56±0.03^{c}	7.59±1.10^{d}
ΔE (crust)	–	14.30±1.22^{c}	25.67±2.27^{b}	26.56±0.37^{b}	30.33±1.42^{a}

Data are shown as mean ± standard deviation. Mean values marked with different letters in the row differ significantly ($p<0.05$).

produced with carob flour. The crumb and crust color changes (ΔE) increased with the increasing levels of carob flour in the cake recipe. The darkening, an increase in a^* value, and a decrease in b^* value of the crumb color of the cakes resulting from the use of carob flour were consistent with the literature data. Igual *et al*. [2024] reported such changes in the color of extrudates produced from a blend of corn flour and carob bean flour (5–12.5%). Różyło *et al*. [2017] observed similar trends in the crumb color of breads made from rice, corn, and buckwheat flours with the addition of carob fiber (1–5% of the total flour content). The color of the crumb in the cake, in the present study, was linked to the color of the carob flour, as the temperature inside the cake does not exceed 100°C during baking. In the case of crust, its color could also be influenced by Maillard reaction and caramelization products, which are formed at temperatures above 150°C [Purlis, 2010]. Especially since carob flour is characterized by a high carbohydrate content (above 50% with about 75% of these carbohydrates as sucrose) [El Batal *et al*., 2016].

Texture properties of cakes

The texture parameters of corn gluten-free cakes produced with and without carob flour are shown in **Table 1**. There were significant ($p<0.05$) differences between gluten-free cakes produced with different levels of carob flour in terms of hardness, chewiness, and resilience values. At the same time, the gluten-free cakes did not differ significantly ($p\geq0.05$) in terms of the springiness and cohesiveness values. The hardness values of gluten-free cakes decreased significantly ($p<0.05$) with the increasing levels of carob flour in the recipe. Carob flour caused a decrease in the chewiness values of the gluten-free cakes. The highest chewiness value was obtained in the control gluten-free cake; however, the chewiness of gluten-free cake including 10% (*w/w*) carob flour did not differ significantly ($p\geq0.05$) compared to the control cake. The gluten-free cakes with 30% and 40% (*w/w*) carob flour substitution had significantly higher resilience values compared to other cakes. The lowest resilience value was obtained in the control gluten-free cake and in the cake with 10% (*w/w*) carob flour substitution.

Springiness is related to aerated, fresh, and elastic properties while cohesiveness is negatively associated with crumbliness [Onyango *et al.,* 2010]. The hardness, chewiness, resilience, springiness, and cohesiveness of gluten-free cakes including carob flour were found to be lower than the values reported by Li *et al.* [2020] who found hardness, adhesiveness, resilience, springiness, cohesiveness, and chewiness of gluten-free potato cake to be 1,292.67; −11.40; 35.58; 0.73; 90.07; and 850.03, respectively. The lower the hardness and chewiness are, the softer is the cake [Li *et al.,* 2020]. Gambuś *et al.* [2009] reported that texture parameters like hardness and cohesiveness are essential in gluten-free products. The cited article showed that the control sponge cake had the lowest hardness, while the sponge cake, which included 40 g of potato starch and 60 g of amaranth flour, had the highest cohesiveness. Carrot cake, including 60 g of corn flour and 110 g of linseed meal, had the lowest hardness due to the content of the linseed meal, while carrot cake, including 170 g of corn flour, had the highest cohesiveness. In turn, coconut cake, including 125 g of rice paste and 125 g of linseed meal, had the lower hardness. Román *et al.* [2017] reported that roasting locust bean flour at 100°C for 60, 75, and 90 min increased the activity and consistency of the cake dough since it reduced cake hardness. Some researchers have used the gelling properties of heat-treated and gelatinized starch to improve the texture of gluten-free corn products. Yalcin & Basman [2008] gelatinized corn flour with boiled water to impart the dough a binding feature and reported that the highest gelatinization level (80%) resulted in the best-quality corn noodles. For this reason, in the present study, gelatinization was applied to corn flour with hot milk to improve the gelling properties of the gluten-free corn cake dough. Moreover, carob gum shows its effect in hot water [Mir *et al.*, 2016], while many studies demonstrated that hydrocolloids and gums retain water as well as compete and interact with starches during gelatinization [Mir *et al.*, 2016; Padalino *et al.,* 2013].

pH of cakes

The pH values of gluten-free cakes produced from corn flour and its blends with carob flour are shown in **Table 2**. As the carob flour level increased, the pH values of the gluten-free cakes successively decreased.

Moisture, protein, lipid, total carbohydrate, total dietary fiber, and ash contents of cakes

The moisture, protein, lipid, total carbohydrate, total dietary fiber, and ash contents of the corn gluten-free cakes produced with and without carob flour are shown in **Table 2**. Carob flour caused significant ($p<0.05$) changes in the macronutrient composition of the cakes. Their moisture content significantly ($p<0.05$) increased as the substitution of corn flour with carob flour in the cake recipe increased. A statistically similar protein content was obtained in the control gluten-free cake and the gluten-free cakes with 10%, 20%, and 30% (*w/w*) carob flour substitution. The lipid content of the gluten-free cakes decreased, whereas their contents of total carbohydrates, dietary fiber, and ash increased with the increasing levels of carob flour.

Carob flour substitution reduced the lipid content and increased dietary fiber in the cakes, which is considered healthy. Research has shown that increasing dietary fiber intake can reduce the risk of development of many chronic diseases, such as cardiovascular diseases, diabetes, obesity, colon cancer, and inflammation. These health conditions are major global challenges, making dietary fiber a key focus for disease prevention [Alahmari, 2024]. This increase in dietary fiber content is also valuable for celiac patients, because gluten-free products are usually poor in this dietary constituent [Kupper, 2005].

Compared to the results of this study, higher protein (18.20%), lipid (14.30%), total dietary fiber (9.44%) contents, and a similar total carbohydrate content (56.06%) were observed for gluten-free cake produced using potato flour [Li *et al.,* 2020]. In turn, Gambuś *et al*. [2009] reported that the sponge cake with 50% amaranth flour and 27% corn flour had 35% more protein than

Table 2. pH and contents of moisture, protein, lipids, total carbohydrates, total dietary fiber, ash and minerals of corn gluten-free cakes produced without (control) and with carob flour as a corn flour substitute at the level of 10–40% (*w/w*).

Parameter	Cake 1 (control)	Cake 2 (10% carob flour)	Cake 3 (20% carob flour)	Cake 4 (30% carob flour)	Cake 5 (40% carob flour)
pH	7.56±0.05^{a}	7.04±0.08^{b}	6.93±0.03^{c}	6.84± 0.06^{d}	6.67±0.04^{e}
Moisture (g/100 g)	19.49±0.03^{d}	20.16±0.02^{c}	20.93±0.14^{b}	21.37±0.22^{b}	22.60±0.33^{a}
Protein (g/100 g)	5.17±0.01^{a}	5.11±0.02^{ab}	5.08±0.02^{ab}	5.04±0.01^{ab}	4.97±0.02^{b}
Lipids (g/100 g)	12.98±0.01^{a}	11.05±0.03^{b}	9.82±0.01^{c}	8.53±0.01^{d}	6.57±0.02^{e}
Total carbohydrates (g/100 g)	61.04±0.78^{d}	62.16±0.19^{c}	62.49±0.31^{bc}	63.22±0.06^{ab}	63.88±0.13^{a}
Total dietary fiber (g/100 g)	1.04±0.01^{e}	2.12±0.02^{d}	3.21±0.02^{c}	4.34±0.01^{b}	5.37±0.02^{a}
Ash (g/100 g)	1.32±0.05^{e}	1.52±0.06^{d}	1.68±0.09^{c}	1.84±0.08^{b}	1.98±0.03^{a}
P (mg/kg)	507.1±4.5^{e}	525.4±3.1^{d}	546.4±4.1^{c}	558.5±0.8^{b}	569.8±1.2^{a}
Mg (mg/kg)	137.4±1.9^{a}	107.9±2.4^{b}	104.1±0.7^{b}	89.7±1.9^{c}	83.8±0.9^{d}
Ca (mg/kg)	446.5±3.7^{e}	490.7±6.1^{d}	526.2±6.9^{c}	568.8±0.8^{b}	587.0±4.5^{a}
Na (mg/kg)	1714.3±3.4^{a}	1668.0±24.1^{ab}	1628.4±32.6^{b}	1561.7±1.9^{c}	1477.8±20.8^{d}
K (mg/kg)	156.0±10.0^{e}	255.8±8.1^{d}	311.3±2.5^{c}	345.6±19.0^{b}	400.6±13.5^{a}

Data are shown as mean ± standard deviation. Mean values marked with different letters in the row differ significantly (p<0.05).

the control cake. They also showed that some sponge cakes had the highest total dietary fiber content due to the high content of this fraction of non-starch polysaccharides in amaranth flour and corn flour.

Mineral contents of cakes

The mineral contents of gluten-free cakes produced from corn flour and its blends with carob flour are shown in **Table 2**. Carob flour caused significant (p<0.05) changes in the contents of phosphorous, magnesium, calcium, sodium, and potassium in the gluten-free cakes. The phosphorous, calcium, and potassium contents increased with the increasing levels of carob flour. All these minerals were found in carob flour in significant amounts in previous studies [Dahmani *et al*., 2023; Youssef *et al*., 2013]. The magnesium and sodium contents were significantly (p<0.05) reduced by using carob flour in the cake formula (**Table 2**). A higher content of potassium (630.50 mg/100 g) and lower contents of calcium, sodium, magnesium, and phosphorus (63.00, 107.00, 43.75, and 86.75 mg/100 g, respectively) were determined in potato cake by Li *et al.* [2020].

Fatty acid composition of cakes

The fatty acid composition of the corn gluten-free cakes produced with and without carob flour is shown in **Table 3**. Carob flour significantly affected their fatty acid composition. It caused significant (p<0.05) increases in the contents of butanoic acid, capric acid, 6-heptanoic acid, myristic acid, pentadecanoic acid, undecanoic acid (10-methyl), palmitic acid, heptadecanoic acid, hexadecenoic acid (14-methyl), stearic acid, linoleic acid, and linolenic acid of the gluten-free cakes, while it caused significant (p<0.05) decreases in the contents of caproic acid, caprylic acid, lauric acid, nonanoic acid (9-oxo), palmitoleic acid, 9-12-exadecadienoic acid, and oleic acid. Statistically similar linoleic acid contents were obtained in the gluten-free cakes including 20%, 30%, and 40% (*w/w*) carob flour in the flour blend. In contrast, there was no statistical difference (p≥0.05) between the linolenic acid contents of the gluten-free cakes with 20% and 30% (*w/w*) carob flour in the flour blend. Linolenic acid content was significantly (p<0.05) higher in the gluten-free cake with 40% (*w/w*) carob flour compared to the other gluten-free cakes, which is highly positive due to its nutritional value.

The fatty acid profile of the gluten-free cakes was influenced primarily by the fatty acid profile of the buffalo cream used as a lipid source in the cake recipe, but also by the fatty acid profiles of the corn and carob flours. Felice *et al*. [2021] reported that palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid in buffalo cream accounted for 27.8%, 9.7%, 23.6%, 4.4%, and 0.9% of the total fatty acids, respectively. Similar contents of these fatty acids in buffalo cream (36.0%, 9.85%, 20.3%, 0.9%, and 0.7% of the total fatty acids, respectively) were determined by Ahmad *et al*. [2013]. In turn, Youssef *et al*. [2013] showed that the fatty acid profile of carob flour was characterized by lower contents of palmitic acid and stearic acid (11.01% and 3.08% of the total fatty acids, respectively), and higher contents of oleic acid, linoleic acid, and linolenic acid (40.45%, 23.19%, and 2.47% of the total fatty acids, respectively). In turn, Dunlap *et al*. [1995] analyzed the fatty acid profile of maize flour and reported that

Table 3. Fatty acid composition (g/100 g total fatty acids) of corn gluten-free cakes produced without (control) and with carob flour as a corn flour substitute at the level of 10–40% (*w/w*).

Fatty acid	Cake 1 (control)	Cake 2 (10% carob flour)	Cake 3 (20% carob flour)	Cake 4 (30% carob flour)	Cake 5 (40% carob flour)
Butanoic acid	1.06±0.04^{d}	1.14±0.01^{c}	1.26±0.05^{b}	1.32±0.01^{ab}	1.38±0.10^{a}
Caproic acid	1.02±0.01^{a}	0.99±0.05^{b}	0.97±0.03^{c}	0.96±0.09^{c}	0.94±0.09^{d}
Caprylic acid	1.06±0.04^{a}	0.93±0.01^{b}	0.74±0.02^{c}	0.56±0.05^{d}	0.41±0.05^{e}
Capric acid	1.26±0.05^{d}	1.40±0.09^{c}	1.53±0.04^{b}	1.6±0.01^{b}	1.84±0.03^{a}
6-Heptanoic acid	0.08±0.01^{d}	0.10±0.01^{cd}	0.11±0.01^{c}	0.14±0.01^{b}	0.18±0.02^{a}
Lauric acid	2.18±0.01^{a}	2.16±0.10^{a}	2.12±0.02^{b}	2.09±0.10^{b}	2.04±0.09^{c}
Myristic acid	10.82±0.04^{d}	10.96±0.01^{c}	11.10±0.01^{b}	11.18±0.01^{a}	11.19±1.00^{a}
Pentadecanoic acid	0.52±0.02^{b}	0.56±0.01^{ab}	0.58±0.01^{a}	0.60±0.01^{a}	0.61±0.09^{a}
Nonanoic acid, 9-oxo	0.25±0.01^{a}	0.24±0.01^{ab}	0.23±0.00^{ab}	0.22±0.01^{ab}	0.21±0.04^{b}
Undecanoic acid, 10-methyl	0.86±0.03^{c}	0.90±0.05^{bc}	0.92±0.06^{b}	0.95±0.01^{b}	1.00±0.09^{a}
Palmitic acid	51.02±0.03^{d}	51.16±0.02^{c}	51.41±0.01^{b}	51.56±0.02^{a}	51.64±0.01^{a}
Palmitoleic acid	1.46±0.03^{a}	1.40±0.01^{b}	1.38±0.01^{bc}	1.35±0.01^{cd}	1.32±0.01^{d}
Heptadecanoic acid	0.23±0.01^{d}	0.27±0.01^{c}	0.32±0.01^{b}	0.33±0.03^{b}	0.40±0.05^{a}
Hexadecenoic acid, 14-methyl	0.28±0.02^{d}	0.32±0.05^{cd}	0.35±0.01^{bc}	0.36±0.01^{b}	0.42±0.03^{a}
9,12-Hexadecadienoic acid	0.43±0.03^{a}	0.40±0.01^{b}	0.36±0.01^{c}	0.29±0.01^{d}	0.24±0.01^{e}
Stearic acid	4.50±0.12^{e}	5.34±0.02^{d}	5.56±0.03^{c}	5.77±0.05^{b}	6.14±0.01^{a}
Oleic acid	19.92±0.05^{a}	19.66±0.05^{b}	19.36±0.01^{c}	19.00±0.08^{d}	18.68±0.02^{e}
Linoleic acid	0.98±0.02^{b}	0.99±0.03^{b}	1.03±0.01^{ab}	1.06±0.09^{a}	1.08±0.02^{a}
Linolenic acid	0.14±0.01^{c}	0.18±0.02^{c}	0.26±0.02^{b}	0.29±0.03^{b}	0.36±0.01^{a}

Data are shown as mean ± standard deviation. Mean values marked with different letters in the row differ significantly ($p<0.05$).

oleic acid and linoleic acid accounted for 16.2–43.8% and 39.5–69.5% of the total fatty acids, respectively. Summarizing, the high oleic acid content in the gluten-free cakes could be caused by its contents in buffalo cream, corn flour, and carob flour. Its content decreased in the gluten-free cakes with the increasing levels of carob flour due to the lower oleic acid content in carob flour compared to corn flour. Moreover, the low linoleic acid content in the gluten-free cakes could be attributed to the high baking temperature applied to the batter. According to Hădărugă *et al.* [2006], linoleic acid was degraded at temperatures above 100°C, and aldehydes were formed due to oxidation; in turn the heat treatment at 150°C reduced linoleic acid content to 54.5%.

α-Tocopherol content of cakes

The α-tocopherol content of the corn gluten-free cakes is shown in **Table 4**. Carob flour caused a significant ($p<0.05$) increase in its content in the cakes. The highest α-tocopherol content was determined in the gluten-free cakes including 30% and 40% (*w/w*) carob flour.

α-Tocopherol was determined in both corn flour and carob flour in previous studies [Dunn *et al.*, 2014; Youssef *et al.*, 2013]. Dunn *et al.* [2014] reported its content at 0.13–6.1 mg/kg in corn flour, whereas Youssef *et al.* [2013] demonstrated its content at 5.177 µg/100 g in carob flour. Therefore, a high α-tocopherol content in the gluten-free cakes analyzed in my study could be attributed to its content in corn flour and carob flour. It increased with the increasing levels of carob flour in the cake formula because it was a richer source of this valuable dietary constituent compared to corn flour.

Phenolic compound profile of cakes

The content of phenolic compounds in the corn gluten-free cakes produced without and with carob flour is shown in **Table 4**. Carob flour caused significant decreases in contents of chlorogenic acid, 4-hydroxybenzoic acid, vanillic acid, and naringin, while increases

Table 4. Content of α-tocopherol and phenolic compounds (mg/kg) of corn gluten-free cakes produced without (control) and with carob flour as a corn flour substitute at the level of 10–40% (*w/w*).

Compound	Cake 1 (control)	Cake 2 (10% carob flour)	Cake 3 (20% carob flour)	Cake 4 (30% carob flour)	Cake 5 (40% carob flour)
α-Tocopherol	1.73±0.03^{c}	1.77±0.02^{bc}	1.78±0.03^{bc}	1.82±0.03^{ab}	1.85±0.01^{a}
Chlorogenic acid	0.49±0.03	nd	nd	nd	nd
4-Hydroxybenzoic acid	1.97±0.00^{a}	1.01±0.09^{b}	0.58±0.00^{c}	nd	nd
Vanillic acid	0.83±0.00^{a}	0.66±0.00^{b}	0.64±0.01^{b}	0.22±0.01^{c}	0.12±0.02^{d}
Caffeic acid	0.06±0.01^{d}	0.42±0.01^{c}	0.82±0.15^{b}	0.93±0.31^{b}	1.16±0.35^{a}
p-Coumaric acid	0.35±0.02^{c}	0.36±0.00^{c}	0.38±0.00^{b}	0.39±0.02^{b}	0.44±0.00^{a}
trans-Ferulic acid	0.98±0.36^{c}	1.72±0.02^{d}	3.21±0.12^{c}	4.93±0.19^{b}	6.41±0.01^{a}
o-Coumaric acid	nd	0.36±0.01^{d}	0.46±0.00^{c}	0.50±0.00^{b}	0.60±0.01^{a}
Cinnamic acid	0.07±0.00^{e}	0.42±0.01^{d}	0.72±0.07^{c}	1.46±0.00^{b}	1.84±0.03^{a}
Rosmarinic acid	0.12±0.00^{c}	0.21±0.02^{bc}	0.25±0.03^{c}	0.29±0.01^{b}	0.40±0.03^{a}
Catechin	2.24±0.04^{e}	7.78±0.35^{d}	15.57±1.18^{c}	22.78±0.20^{b}	29.50±0.25^{a}
Rutin	1.06±0.00^{e}	2.04±0.01^{d}	3.04±0.42^{c}	4.18±0.01^{b}	6.76±0.27^{a}
Naringin	1.19±0.01^{a}	nd	nd	nd	nd
Resveratrol	0.08±0.02^{d}	0.20±0.00^{c}	0.24±0.03^{bc}	0.28±0.01^{ab}	0.31±0.00^{a}
Quercetin	0.36±0.01^{d}	0.41±0.01^{d}	0.59±0.07^{c}	0.68±0.07^{b}	0.89±0.02^{a}
Naringenin	0.11±0.00^{d}	0.25±0.00^{c}	0.39±0.02^{b}	0.40±0.02^{b}	0.63±0.03^{a}

Data are shown as mean ± standard deviation. Mean values marked with different letters in the row differ significantly ($p<0.05$). nd, Not detected.

in contents of caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, *o*-coumaric acid, cinnamic acid, rosmarinic acid, catechin, rutin, resveratrol, quercetin, and naringenin in the cakes. The highest caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, *o*-coumaric acid, cinnamic acid, rosmarinic acid, catechin, rutin, quercetin, and naringenin contents were determined in the gluten-free cake including 40% (*w/w*) carob flour in the flour blend. Chlorogenic acid and naringin were detected only in the control gluten-free cake, which additionally had the highest contents of 4-hydroxybenzoic acid and vanillic acid. In turn, the highest resveratrol content was determined in the gluten-free cakes with 30 and 40% (*w/w*) carob flour in the flour blend.

Among the phenolic compounds determined in the gluten-free cakes, chlorogenic acid, caffeic acid, *p*-coumaric acid, and *trans*-ferulic acid were previously identified in corn flour at the contents of 39.52 µg/g, 39.14 µg/g, 35.42 µg/g, and 8.48 µg/g, respectively [Nikolić *et al*., 2019]. In turn, Youssef *et al*. [2013] showed that vanillic acid, ferulic acid, cinnamic acid, and catechin were present in carob flour at the contents of 13.92 ppm, 10.17 ppm, 3.78 ppm, and 27.97 ppm, respectively. High ferulic acid and catechin contents in the analyzed gluten-free cakes could be attributed to a high ferulic acid content in corn flour and to high ferulic acid and catechin contents in carob flour. Their contents in the cakes increased with the increasing levels of carob flour – being their richer source compared to corn flour – in the cake formula.

Sensory properties of cakes

The sensory profile of the gluten-free cakes produced from corn flour and its blend with carob flour is shown in **Figure 2**. The gluten-free cakes with 20% (*w/w*) substitution of corn flour with carob flour had the best shape and color. The softness of the cakes including carob flour was higher than that of the control cake. The best odor and aroma were reported for the gluten-free cake including 20% (*w/w*) carob flour in the flour blend. The consistency of the gluten-free cake including 40% (*w/w*) carob flour was the best among all cakes. In turn, the gluten-free cake including 20% (*w/w*) carob flour received the highest score (4.3) for overall acceptability, however, all gluten-free cakes were of good quality (scores above 3.5). Similar scores were also reported by Gambuś *et al.* [2009] for 3 types of gluten-free sponge cakes (60 g of corn flour and 60 g of potato starch and 60 g of amaranth flour), 2 types of gluten-free carrot cakes (170 g of corn flour or 60 g of corn flour and 110 g of linseed meal) and 2 types of gluten-free coconut cakes (250 g of rice paste or 125 g of rice paste and 125 g of linseed meal).

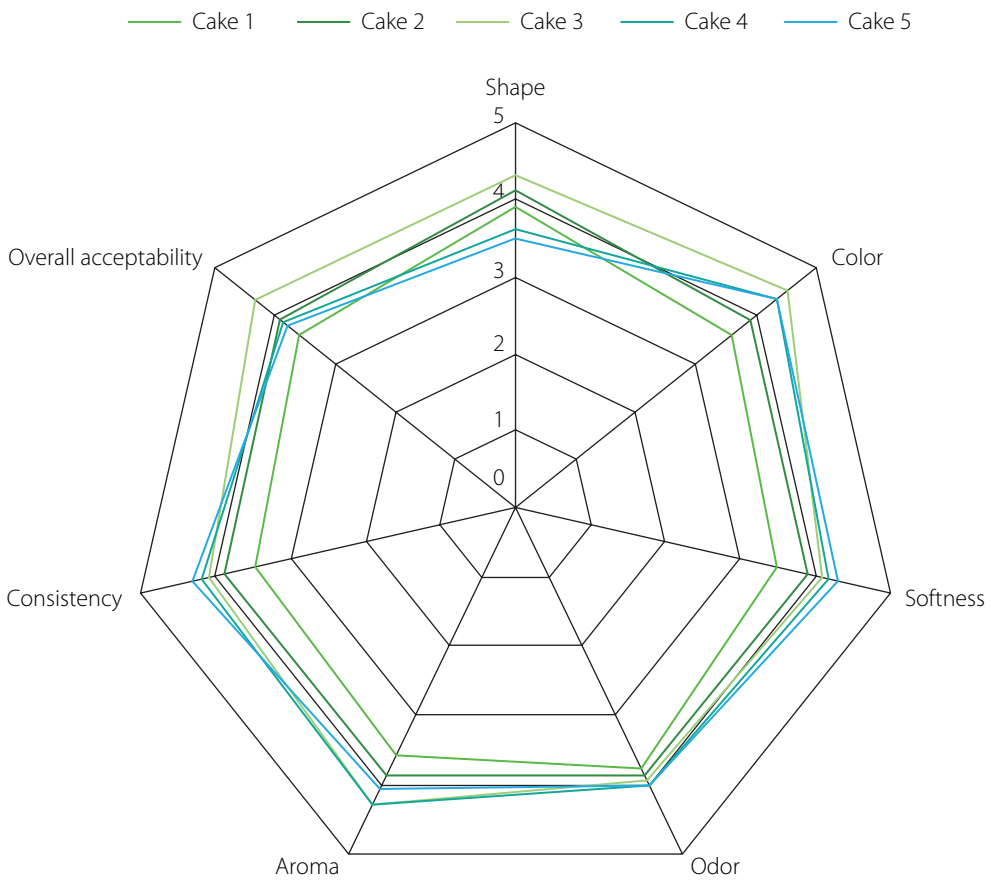

Figure 2. Sensory profile of corn gluten-free cakes produced without (cake 1) and with carob flour as a corn flour substitute at the weight levels of 10% (cake 2), 20% (cake 3), 30% (cake 4), and 40% (cake 5).

My study results suggest that the substitution of corn flour with carob flour in the cake recipe was optimal at 20% (*w/w*) considering the panelists's scores although other properties were superior at higher carob flour substitution, such as volume and contents of total dietary fiber, phosphorus, calcium, potassium, α-tocopherol, caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, *o*-coumaric acid, cinnamic acid, rosmarinic acid, catechin, rutin, resveratrol, quercetin, naringenin, stearic acid, linoleic acid, and linolenic acid.

CONCLUSIONS

Carob flour was generally used in gluten-free cakes made from rice flour. Corn flour and carob flour were used in gluten-free cake production for the first time ever. There are also no studies on the use of buffalo cream in cake products. Carob flour addition to the cake formula caused brown color, increased the volume, nutritional value, and contents of antioxidants as well as improved texture properties of the gluten-free cakes.

This study has shown the feasibility of manufacturing healthy and high-quality gluten-free products by blending corn flour and carob flour in the cake formula. The findings obtained from this study show that the gluten-free cakes produced have a good texture, high volume, sensory properties, and nutritional quality and will be preferred not only by celiac patients but also by healthy persons.

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CONFLCIT OF INTERESTS

I declare that no competing financial interests could influence this work.

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Use of High-Protein Milk Preparations in the Production of Probiotic Fresh Cheeses

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The study on the use of skimmed milk and buttermilk separation products obtained by membrane filtration and the *Lactobacillus acidophilus* LA-5 probiotic culture in the production of fresh cheeses was undertaken. Membrane separation products – micellar casein concentrate (MMC), buttermilk protein concentrate (RMFB), a mixture of micellar casein concentrate and a buttermilk serum protein concentrate (RUFP) – were used in liquid and powder form. Fresh cheeses were produced from fluid protein concentrates or from milk with protein powder addition. A control sample was produced from milk with the addition of skimmed milk powder. Fresh cheeses produced from MCC were characterised by a desirable, high content of protein, calcium, and phosphorus. In turn, magnesium content was highest in fresh cheeses made from RUFP. In all cheeses, *Lb. acidophilus* LA-5 counts exceeded log 6 cfu/g on the last day of storage (day 21), thus satisfying the criteria for probiotic products. Fresh cheese made from MCC was characterised by the greatest difference in colour relative to the control sample. In addition, cheeses produced from MCC fluid or with the addition of MCC powder were characterised by higher firmness (69.58 and 41.67 N, respectively) relative to the cheese produced from RMFB (3.35–3.37 N) or RUFP (5.89–21.96 N). The power law model accurately predicted the rheological properties of the examined cheeses (R^2>0.999). All cheeses displayed pseudoplastic flow behaviour, where the storage modulus (G') was higher than the loss modulus (G") and was not dependent on frequency. The fractal analysis revealed that MCC cheeses had the least irregular microstructure with the lowest values of fractal dimension. The use of high-protein preparations in the production of fresh cheeses generally decreased their sensory acceptability. It can be concluded that probiotic fresh cheeses made from skimmed milk and buttermilk separation products with the addition of the *Lb. acidophilus* LA-5 culture differ in physicochemical properties and sensory attributes.

Keywords: dairy products, milk protein, *Lactobacillus acidophilus*, calcium, phosphorus, colour, rheological characteristics, microstructure, sensory analysis

INTRODUCTION

Due to growing levels of consumer awareness about nutrition and an increased demand for food products with a high nutritional value, foods are enhanced with functional additives that influence their sensory attributes and deliver health benefits. Such additives include probiotic cultures that are used in the production of ripened cheeses [Aljewicz & Cichosz, 2015] and fresh cheeses [Guneser & Aydin, 2022; Kadiya *et al.*, 2014; Soltanzadeh *et al.*, 2019]. Probiotic cultures with confirmed health benefits are applied in cheesemaking to increase the selection of functional

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dairy products. The popularity of high-protein dairy products with an optimal amino acid profile and functional properties is also on the rise [Suthar *et al.*, 2017]. High-protein dairy products are abundant in minerals. Casein is the major protein component in milk. It is a phosphoprotein and contains phosphoric acid esterified with serine and threonine residues that bind bivalent ions, including calcium. Milk contains mostly colloidal calcium; therefore, casein, its α_{s1}, α_{s2}, β, and κ fractions, and colloidal calcium phosphate are the structural components of casein micelles. The colloidal properties of milk are determined by the proportions of casein, whey proteins, and mineral salts, in particular calcium, phosphorus, and citrates [Huppertz, 2013; McMahon & Oommen, 2013].

Milk protein concentrates are used in the production of various dairy foods [Suthar *et al.*, 2017]. Micellar casein concentrate (MCC) is a protein concentrate obtained by microfiltration. Intact casein and whey proteins account for more than 90% and up to 10% of total protein, respectively [Salunke *et al.*, 2021]. A high content of calcium promotes the formation of gel networks in MCC powder, which decreases its solubility. Various physical (*e.g.*, microfluidisation, homogenisation or pressurisation) and chemical (*e.g.*, calcium-binding agents, enzymatic or chemical modifications of protein) techniques are applied to modify the composition of MCC powder and decrease calcium levels to improve its rehydration properties as dispersibility and solubility [Kommineni *et al.*, 2022; McSweeney *et al.*, 2021].

Fresh cheeses constitute a large and highly diverse group of dairy products. During acid coagulation in fresh cheese production, the micronutrients bound to casein micelles, including calcium, are dissolved, released into the soluble phase [Gaucheron, 2005], and partly transferred to whey, thus decreasing the content of minerals, including calcium, in acid curd. Various strategies for enriching dairy products with calcium have been proposed over the years, and milk products appear to be a highly suitable, natural source of calcium in dairy production. Milk proteins and milk powder are abundant in minerals and characterised by a natural taste and aroma. In addition, milk protein concentrates increase the protein content of dairy products, improve the bioavailability of mineral compounds, and enhance the nutritional value of the products [Aljewicz *et al.*, 2018; Kowalska *et al.*, 2012]. Milk proteins are natural ingredients with functional properties, including gelling, emulsifying, and foaming properties, and they can minimise the use of non-protein additives in dairy production [Suthar *et al.*, 2017]. Due to its high nutritional value (high calcium content) and functional properties (thermal stability, gel formation), MCC can be used as an ingredient or an additive in the production of value-added foods [Carter *et al.,* 2021; Kiełczewska *et al.,* 2022]. To modify the composition and properties of fresh cheeses, further research is needed to determine the applicability of protein concentrates and probiotic bacteria in their production. *Lactobacillus acidophilus* LA5 is an example of a probiotic culture that converts lactose to lactic acid by homofermentation and can be applied in the production of acid-coagulated fresh cheeses.

The aim of this study was to determine the importance of the content and composition of milk proteins in the raw material used in the production of fresh cheeses on the counts of probiotic bacteria, content of minerals (calcium, phosphorus, and magnesium), rheological properties, color parameters, and sensory attributes of the product.

MATERIALS AND METHODS

Preparation of dairy raw materials

Raw milk (*ca.* 100 L, acquired from the Research and Education Station in Bałdy, which is a part of the University of Warmia and Mazury in Olsztyn, Poland) was centrifuged at a temperature of 45°C (Spomasz LWG-20 centrifuge, Gniezno, Poland) to obtain skimmed milk. Sweet buttermilk for retentate production was supplied by a dairy plant (*ca.* 100 L, Mlekovita, Poland). Skimmed milk was pasteurised at 72°C for 15 s (Alfa Laval P20-HB plate heat exchanger, Sweden). Skimmed milk and buttermilk were separated by membrane filtration, and the following products were obtained: micellar casein concentrate (MCC), *i.e.* the retentate obtained by microfiltration of skimmed milk which the next was subjected to diafiltration; buttermilk protein concentrate (RMFB), *i.e.* the retentate obtained by microfiltration of buttermilk; and buttermilk serum protein and micellar casein concentrate (RUFP), *i.e.* the retentate obtained by ultrafiltration of a mixture of micellar casein and buttermilk serum proteins (this method has been reported to the Polish Patent Office as: Method of producing a high-protein preparation containing milk serum proteins and buttermilk retentate proteins. P450274 [WIPO ST 10/C PL450274]).

Microfiltration and ultrafiltration were carried out with the use of Membralox ceramic membranes (EP1940GL, AGP1020, 0.1 µ alumina, Pall Corp., East Hills, NY, USA) and Koch membrane systems (3838 HFK-131, Koch Industries, Wichita, KS, USA), respectively, at 50°C, concentration factor (CF) ×3, according to the procedure described by Evans *et al.* [2009]. The concentrates were divided into two portions. One portion was cooled to 4°C, and the other portion was spray dried at an inlet temperature of 185°C (Niro Atomizer, GEA, Søborg, Denmark) according to the procedure described by Dec *et al.* [2023]. The liquid concentrates and powders were used in the production of fresh cheeses.

Production of fresh cheeses

Fresh cheeses were produced from two types of raw materials with the use of: 1) liquid products separated by membrane filtration, and 2) drinking milk with 2% fat content from a dairy plant (Piątnica, Poland) and powdered products separated by membrane filtration (5 g/100 mL). The following raw materials were used: liquid micellar casein concentrate (MCC_F), liquid buttermilk protein concentrate (RMFB_F), a mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate (RUFP_F), milk with the addition of micellar casein powder (M_MCC), milk with the addition of buttermilk protein powder (M_RMFB), and milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder (M_RUFP).

The fresh cheeses produced from the above raw materials are marked with the same symbols. Drinking milk with 2% fat content with the addition of skimmed milk powder (5 g/100 mL) was the control sample.

Milk with the addition of milk powders and liquid products separated by membrane filtration, inoculated with the *Lb. acidophilus* LA-5 probiotic culture (NU-TRISH, Chr. Hansen, Hørsholm, Denmark) at log 5 cfu/mL, were incubated (Memmert ICP500, Schwabach, Germany) in 3 L glass laboratory bottles (Schott, Wolverhampton, UK) at 37°C for 24 h. The obtained curd was separated from whey and left to drain at 4°C for 24 h. Fresh cheeses were packaged and stored at 4°C for 21 days.

Determination of protein and dry matter content

The dry matter content of raw materials and fresh cheeses was evaluated according to AOAC International method no. 990.20 [AOAC, 2007] and the protein content was determined by the AOAC International method no. 991.20 (Kjeldahl method) [AOAC, 2007].

Protein profile analysis by reducing-sodium dodecyl sulphate polyacrylamide gel electrophoresis

The protein profile of the raw materials and fresh cheeses was determined by reducing-sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) [Laemmli, 1970]. Prior to analysis, raw milk samples were defatted by centrifugation at 4,000×*g* for 10 min at 4°C, and the fat layer was carefully removed. Fresh cheese samples were homogenised in ultrapure water at a 1:5 (*w/v*) ratio. The protein fractions were then diluted in Laemmli 2× sample buffer (S3401, Sigma Aldrich, St. Louis, MO, USA) containing 5% β-mercaptoethanol and heated at 95°C for 5 min to denature the proteins. After cooling to room temperature, the samples were centrifuged at 13,000×*g* for 15 min at 21°C to remove insoluble particles. Electrophoresis was conducted on a 4–20% polyacrylamide gel (Mini-PROTEAN® TGX™, Bio-Rad Laboratories Inc., Hercules, CA, USA) in 15-well plates. A Tris-glycine-SDS buffer system (10×, Sigma Aldrich) was used for protein migration. Samples were loaded alongside Precision Plus Protein Dual Colour Standard (Bio-Rad Laboratories Inc.), covering a molecular weight range of 10–250 kDa. The gel was placed in the electrophoresis chamber and initially run at 80 V, with the voltage gradually increased to 120–150 V. Separation was performed using a BIO-RAD Mini-PROTEAN II cell system (Bio-Rad Laboratories Inc). Following electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250, destained, and imaged using the CCD LumiBis imaging system (DNR Bio-Imaging Systems, Modi'in-Maccabim-Re'ut, Israel). Quantification of casein and whey proteins was performed by densitometric analysis using TotalLab Quant 1.0 software (TotalLab, Gosforth, UK). The relative band intensities corresponding to major casein (αs1-casein, αs2-casein, β-casein, κ-casein) and whey protein fractions (β-lactoglobulin, α-lactalbumin) were compared across samples. The results were expressed as the relative percentage of casein and whey proteins in total proteins.

Determination of calcium, magnesium and phosphorus content

The calcium and magnesium content of raw materials and fresh cheeses was determined by flame atomic absorption spectrometry, according to standard method [ISO 8070:2007]. Determinations were performed using the iCE 3000 atomic absorption spectrometer (Thermo-Scientific, Hemel Hempstead, UK) equipped with a deuterium lamp for background correction and cathode lamps for each element. Samples were atomised in an air-acetylene flame at the wavelength of 285.2 and 422.7 nm for Mg and Ca, respectively. The content of phosphorus was determined colourimetrically with ammonium molybdate, sodium sulphate and hydroquinone (Merck, Darmstadt, Germany) according to the method by Pulliainen & Wallin [1994]. Absorbance readings were taken using the Cary 60 UV-VIS spectrophotometer (Agilent, Mississauga, Canada) at a wavelength of 610 nm. Results of analyses were expressed as mg of element *per* 100 g of raw material or fresh cheese. The Ca to P ratio was also calculated. In addition, the coverage of the recommended daily intake (RDI) of Ca, Mg and P by the consumption of 100 g of fresh cheese was estimated based on the dietary guidelines for the Polish population [Wojtasik *et al*., 2020].

pH value determination

The pH value of fresh cheeses was determined directly after production using a Schott Lab 850 pH meter (SI Analytics GmbH, Mainz, Germany).

Microbiological analysis

Bacterial counts in fresh cheeses were determined by streaking. Cheese samples were diluted with a saline solution (1:10; Maximum Recovery Diluent, Merck, Darmstadt, Germany), homogenised (BagMixer 400, Interscience, ST Nom, Saint-Nom-la-Bretèche, France), and plated on De Man-Rogosa-Sharpe (MRS) agar (Merck) for the proliferation, culture, isolation and enumeration of lactic acid bacteria of the genus *Lactobacillus*. Incubation was carried out in Anaerocult C mini bags with a low-oxygen atmosphere (Merck) at 37°C for 48 h. *Lactobacillus* counts in fresh cheeses (expressed as log cfu/g) were determined directly after production and after 7, 14, and 21 days of refrigerated storage.

Colour parameter analysis

The colour parameters of fresh cheeses were measured in the CIELab colour space using the CM-3500d spectrophotometer (Konica Minolta Sensing, Osaka, Japan), which was calibrated using white (CM A120) and black (CM A124) calibration plates. Measurements of L^*, a^* and b^* coordinates were performed with d/8 geometry, 8 mm aperture size, 10° observer angle and D65 illuminant [Kiełczewska *et al.*, 2022]. Coordinate L^* described colour lightness ($L^* = 0$ for black and $L^* = 100$ for white colour). Chromaticity was expressed by coordinates a^* (–a* – greenness and +a* – redness) and b^* (–b^* – blueness and +b^* – yellowness). In addition, the saturation (C) was calculated from Equation (1) [Pathare *et al*., 2013]:

$$C = (a^{*2} + b^{*2})^{0.5} \quad (1)$$

The total difference in colour between the control cheese and other experimental products was calculated with the use of Equation (2):

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5} \quad (2)$$

Rheological properties analysis

The rheological properties of fresh cheeses were determined using an MCR 102 rheometer (Anton Paar, Graz, Austria) equipped with temperature control units, including fluid circulators and a Peltier hood, as described by Aljewicz *et al.* [2021]. A parallel plate measuring system (PP25/S-SN73131) was employed for the tests. Rheological data were recorded using RheoCompass software v. 1.31 (Anton Paar). The linear viscoelastic region (LVER) was determined at 10°C using a strain sweep ranging from 0.001% to 100% strain at a fixed angular frequency of 1 Hz. A logarithmic ramp with six measurement points *per* decade was applied. The sample recovery and temperature stabilization time were set to 60 s. Storage modulus (G') and loss modulus (G") were measured at 10°C using a dynamic frequency sweep within an angular frequency range of 0.1–10 Hz, with a constant strain of 0.1%. A logarithmic ramp with ten measurement points *per* decade was applied. Shear rate, shear stress, and apparent viscosity were calculated using RheoCompass software v. 1.31 (Anton Paar). The flow curve data were fitted to rheological models, including the power law model, described using Equation (3):

$$\tau = \kappa + \gamma^{n} \quad (3)$$

where: τ is the shear stress (Pa), κ is the consistency index (Pa×s^{n}), γ is the shear rate (1/s), n is the flow behaviour index.

G' and G" were utilised by the instrument's software to calculate the complex viscosity (η*). The complex viscosity was computed by means of Equation (4):

$$\eta^* = \frac{\sqrt{G'^2 + G''^2}}{\omega} \quad (4)$$

where: ω represents the angular frequency [Mezger, 2012].

Stress and strain at fracture were determined as the actual stress and strain recorded at the fracture point on the stress-strain curve, corresponding to the point where the cheese cracked [ISO/TS 17996, 2006]. Based on prior studies [ISO/TS 17996, 2006], the strain at fracture was assumed to correspond to 10% of the LVER. Using the determined strain at fracture, the corresponding stress value (Pa) was subsequently read from the stress-strain curve.

Texture analysis

The textural properties of fresh cheeses were determined with the use of the TA.XT.plus texture analyser (Stable Micro Systems, Godalming, UK). Firmness and penetration force were determined in a penetration test with the use of an SMS P/25 aluminium cylindrical probe (25 mm). Penetration depth was 20 mm, probe speed was 1.5 mm/s, and the applied load was 0.049 N. The measurements were conducted at a temperature of 6±1°C [Lis *et al.,* 2021].

Microstructure and fractal analysis

Fresh cheese samples were prepared according to the method described by Smoczyński & Baranowska [2014]. Samples of the tested cheeses were placed in holders and immediately frozen in a microscopic chamber using a Peltier cooler at –18°C. The specimens were then examined under a Quanta 200 scanning microscope (FEI Company, Eindhoven, Netherlands) at 200× magnification. The original microphotographs were analysed with the use of Nis-Elements software (Nikon, Tokyo, Japan). They were first converted into high-contrast images based on the defined contrast parameters (high – 75, low – 74). The perimeter (P) and area (A) of approximately 200 small, medium, and large objects were measured. The analysed objects were self-similar, and their "surface" fractal dimension (D) was calculated from the slope of the log A = f (log P) line [Dziuba *et al.*, 1997], with the use of Equation (5):

$$A \sim P^{2/D} \quad (5)$$

where: A is surface area, P is perimeter, and D is fractal dimension of the contour. Straight lines with the slope of a = 2/D were obtained, which were used to compute the fractal dimensions of the analysed samples [Smoczyński, 2020].

Sensory analysis

The sensory analysis of fresh cheeses was conducted in a sensory laboratory with the use of a standard profiling method [EN ISO 13299:2016-05E] and a five-point descriptive scale. The intensity of 22 sensory attributes (including appearance, aroma, texture, mouthfeel, and taste) was rated, where 1 point denoted the absence of the analysed attribute, and 5 points denoted very high intensity of the analysed attribute. The overall acceptability of fresh cheeses was also evaluated. The sensory panel comprised ten panellists who had been trained to evaluate dairy products and whose sensory sensitivity had been validated according to ISO method [EN ISO 8586:2014–03].

Statistical analysis

The results were verified for normal distribution and homogeneity of variance. Significant differences in the physicochemical properties and sensory attributes of fresh cheeses were determined by one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. In the rheological analysis, the significance of differences between means was estimated by Duncan's test. Data were presented as means ± standard deviation. All results were processed in Statistica 13.5 PL software

(Statsoft 2017, Krakow, Poland) at a significance level of 0.05. The experiment was conducted in duplicate.

RESULTS AND DISCUSSION

Protein and mineral content of raw materials and fresh cheeses

Protein content in membrane separation products of skimmed milk and buttermilk and in milk with their addition was significantly higher ($p \leq 0.05$) compared to the control sample (**Table 1**). Among the liquid fractions used for the production of probiotic cheeses, the highest protein content was found in MCC_F, which accounted for 92% of its dry matter. Protein content in RMFB_F and RUFP_F was lower and accounted for more than half of the dry matter. Milks with the addition of milk protein concentrates in powder were characterised by a significantly lower ($p \leq 0.05$) protein content compared to the analogous fractions of liquid protein concentrates. The use of micellar casein concentrate resulted in a higher protein content compared to the milk with the addition of other high-protein powders. Smaller variations in dry matter content were found between milks with the addition of high-protein powders than in the case of liquid protein fractions. This was reflected in a less varied, although lower, protein content in dry matter, ranging from 45 to 37 g/100 g dry matter depending on the type of high-protein powder added to the milk.

The use of milk protein concentrates, both as a liquid fraction and in the powder form, contributed to the differentiation of the protein content in fresh probiotic cheese in the descending order MCC > RUFP > RMFB (**Table 1**). The highest ($p \leq 0.05$) protein content was achieved in the cheeses produced from MCC_F, which accounted for almost 90% of the dry matter of the product. The use of RUFP also contributed to the increase in the protein content in the cheese, although to a lesser extent compared to the cheese produced from MCC without the addition of other proteins. The protein content in fresh probiotic cheeses produced from M_RMFB did not differ significantly ($p > 0.05$) from the control sample, and in the case of RMFB_F it was lower compared to the control sample. The dry matter content in cheeses produced from MCC_F, M_MCC or M_RUFP was higher compared to the control sample. The dry matter content in the remaining fresh cheese was lower compared to the control sample.

In addition to variations in protein content across the analysed products, distinct protein profiles were observed (**Figure 1**). The electropherograms of the raw material proteins revealed bands corresponding to the major milk proteins, with casein fractions exhibiting molecular weights ranging from 19 to 35 kDa, β-lactoglobulin at ~18.5 kDa, and α-lactalbumin at ~14.5 kDa [Lee & Hong, 2003; Zhang *et al.*, 2022]. Additionally, protein bands around ~80 kDa suggest the presence of milk fat globule membrane (MFGM) proteins, particularly given that these samples were derived from buttermilk subjected to microfiltration. This interpretation aligns with previous research indicating that MFGM proteins, including butyrophilin and PAS6/PAS7, are among the primary proteins in buttermilk and typically appear in this molecular weight range [Miocinovic *et al.*, 2014; Spitsberg, 2005]. These proteins were the most abundant in milk from RMFB_F and in all milk samples with the addition of high-protein powders, including the control sample (**Figure 1A**). In the electropherograms of fresh cheeses, the most distinctive band denoting the presence of a protein with a molecular weight of ≥70 kDa was observed in RMFB_F (**Figure 1B**).

The protein bands in the electropherograms of raw materials and fresh cheeses were subjected to a densitometric analysis, and the results were used to calculate the relative content of casein and whey proteins. The analysis showed that the casein content was the highest in MCC_F (**Figure 1C**). In contrast, the casein content in the other two liquid raw materials,

TABLE 1. Protein and dry matter content of raw materials and fresh cheeses.

Sample	Raw materials		Fresh cheeses	
	Protein (g/100 g)	Dry matter (g/100 g)	Protein (g/100 g)	Dry matter (g/100 g)
C	4.86±0.01^{f}	14.92±0.01^{a}	10.86±0.54^{e}	25.23±0.20^{c}
MCC_F	8.70±0.13^{a}	9.41±0.13^{f}	25.74±0.56^{a}	29.61±0.21^{b}
RMFB_F	7.26±0.01^{b}	13.83±0.01^{d}	9.39±0.13^{f}	16.26±0.07^{f}
RUFP_F	6.34±0.02^{c}	11.73±0.02^{e}	12.42±0.20^{d}	16.92±0.10^{e}
M_MCC	6.45±0.07^{c}	14.42±0.02^{c}	19.33±0.25^{b}	33.25±0.20^{a}
M_RMFB	5.46±0.01^{e}	14.74±0.01^{b}	10.44±0.20^{e}	23.55±0.07^{d}
M_RUFP	5.67±0.07^{d}	14.47±0.02^{c}	16.28±0.25^{c}	29.28±0.02^{b}

The presented values are means with standard deviation. Mean values marked with different letters (a–f) in columns differ significantly at $p \leq 0.05$. Raw materials and fresh cheeses produced from: C, milk with the addition of skimmed milk powder (control sample); MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

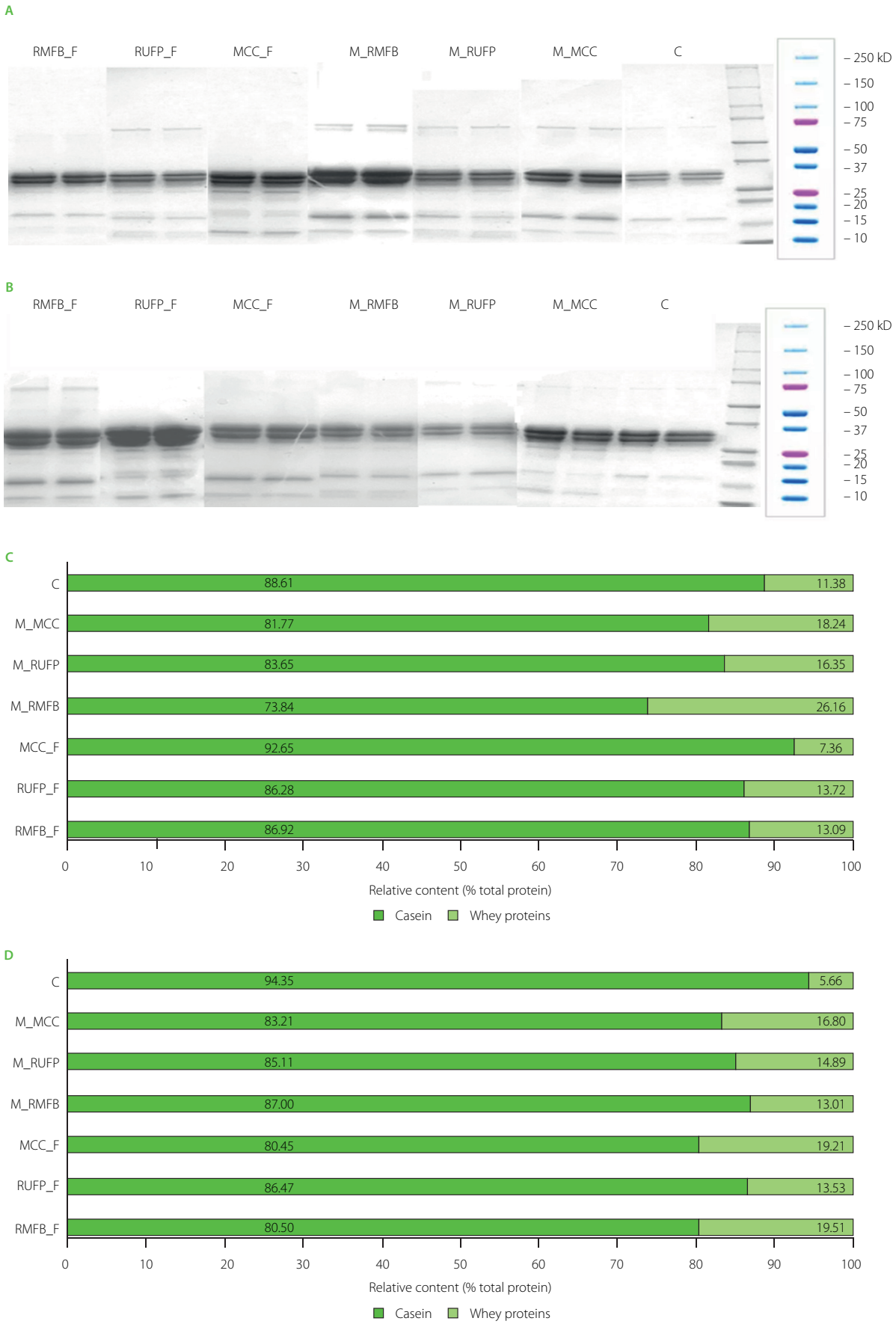

Figure 1. SDS-PAGE electropherograms of raw materials (**A**) and fresh cheeses (**B**) and relative content of casein and whey proteins in total proteins determined based on a densitometric analysis of electrophoretic patterns in raw materials (**C**) and fresh cheeses (**D**) produced from: C, milk with the addition of skimmed milk powder – control sample; MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

obtained through membrane filtration of milk and buttermilk, was lower. In the case of fresh cheeses produced from these raw materials, the casein content was the lowest in MCC_F, as compared to the other cheeses obtained with the use of two liquid membrane materials (**Figure 1D**). These results suggest that processing conditions, including coagulation properties and whey drainage, may have influenced the final protein distribution. The membrane concentration process used in MCC may have altered the protein composition, specifically affecting the efficiency of casein retention in the cheese matrix. This highlights the importance of processing parameters in shaping the protein profile of fresh cheeses.

The tested raw materials differed in the content of calcium, phosphorus, and magnesium (**Table 2**). In the analysed group of liquid fractions, calcium and phosphorus content was highest in MCC_F, where the Ca to P ratio was determined at 1.46. The remaining liquid fractions contained less calcium and more phosphorus than the control sample, and the Ca to P ratio ranged from 1.56 in RMFB_F to 2.23 in RUFP_F. Phosphorus content was significantly ($p \leq 0.05$) lower in the milk with the addition of high-protein powders than in high-protein liquid preparations, which increased the Ca to P ratio. In the milk samples with the addition of high-protein powders, phosphorus content was highest in M_MCC. Calcium and phosphorus levels were higher in raw materials with the addition of casein than in the other high-protein raw materials due to the presence of colloidal calcium phosphate in casein micelles. Magnesium content was lower ($p \leq 0.05$) in the liquid products separated by membrane filtration than in the control sample, but it was higher ($p \leq 0.05$) in the milk with the addition of high-protein powders.

The calcium, phosphorus, and magnesium content of fresh cheeses varied depending on the raw materials applied in the production process (**Table 2**). The phosphorus content in RMFB_F and M_MCC cheeses was significantly ($p \leq 0.05$) higher than in the control cheese. Fresh cheeses produced from the remaining raw materials were characterised by a lower and varied content of calcium and phosphorus in comparison with the control sample. The addition of high-protein powders to milk increased the Ca to P ratio of the resulting cheeses relative to the control sample. Magnesium content was lower ($p \leq 0.05$) in M_RMFB and M_RUFP cheeses than in the control sample. The content of magnesium was significantly ($p \leq 0.05$) higher in the cheeses made from liquid and powdered MCC, as well as in those produced from liquid RMFB and RUFP fractions.

Based on the content of calcium, phosphorus and magnesium in the products, it was calculated how much of them will cover the recommended daily allowance (RDA) of individual groups of consumers [Wojtasik *et al.*, 2020]. In all analysed age groups, the RDA of calcium was best satisfied by a 100 g serving of MCC_F cheese, followed by M_MCC cheese (**Table 3**). In the cheeses produced from milk with the addition of the RUFP powders, the RDA of calcium was similar to that noted in the control sample. The RDA of calcium *per* 100 g of the cheeses produced from liquid buttermilk protein concentrate was similar to that produced from milk with the addition buttermilk protein powder.

TABLE 2. Content of calcium, phosphorus and magnesium, and the calcium to phosphorus ratio in raw materials and fresh cheeses.

Sample	Raw materials				Fresh cheeses			
	Ca (mg/100 g)	P (mg/100 g)	Mg (mg/100 g)	Ca/P	Ca (mg/100 g)	P (mg/100 g)	Mg (mg/100 g)	Ca/P
C	183.69±0.34^{d}	56.43±0.30^{f}	13.16±0.01^{d}	3.26	170.36±0.84^{c}	74.19±0.33^{c}	13.16±0.15^{e}	2.30
MCC_F	299.35±4.82^{a}	205.06±0.72^{a}	8.78±0.17^{g}	1.46	456.75±1.93^{a}	150.05±0.12^{a}	19.64±0.18^{b}	3.04
RMFB_F	161.79±2.36^{f}	103.79±0.35^{b}	10.35±0.17^{e}	1.56	137.67±1.39^{e}	115.04±0.32^{b}	15.59±0.23^{d}	1.20
RUFP_F	169.18±1.07^{e}	75.72±0.17^{d}	8.95±0.05^{f}	2.23	124.32±0.29^{f}	63.60±0.24^{d}	20.47±0.11^{a}	1.95
M_MCC	213.48±0.21^{b}	85.82±0.63^{c}	14.82±0.09^{c}	2.49	195.65±0.68^{b}	61.08±1.06^{e}	15.96±0.32^{c}	3.20
M_RMFB	213.93±0.28^{b}	59.54±0.37^{e}	16.53±0.18^{a}	3.59	136.86±0.74^{e}	41.74±0.52^{f}	11.95±0.12^{g}	3.28
M_RUFP	196.27±0.44^{c}	59.89±0.44^{e}	15.92±0.11^{b}	3.28	165.92±0.49^{d}	31.56±0.74^{g}	12.00±0.12^{f}	5.26

The presented values are means with standard deviation. Mean values marked with different letters (a–g) in columns differ significantly at $p \leq 0.05$. Raw materials and fresh cheeses produced from: C, milk with the addition of skimmed milk powder (control sample); MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

TABLE 3. Daily nutrient intake *per* 100 g of fresh cheese (percentage).

Nutrient	Sex/age (years) group	RDA* (mg/day)	C	MCC_F	RMFB_F	RUFP_F	M_MCC	M_RMFB	M_RUFP
Calcium	Children aged 4–6	1,000	17	46	14	12	20	14	17
	Boys aged 13–15	1,300	13	35	11	10	15	11	13
	Men aged 51–65	1,000	17	46	14	12	20	14	17
	Girls aged 13–15	1,300	13	35	11	10	15	11	13
	Women aged 51–65	1,200	17	46	14	12	20	14	17
Phosphorus	Children aged 4–6	500	15	30	23	13	12	8	6
	Boys aged 13–15	1,250	6	12	9	5	5	3	3
	Men aged 51–65	700	11	21	16	9	9	6	5
	Girls aged 13–15	1,250	6	12	9	5	5	3	3
	Women aged 51–65	700	11	21	16	9	9	6	5
Magnesium	Children aged 4–6	130	10	15	12	16	12	9	9
	Boys aged 13–15	410	3	5	4	5	4	3	3
	Men aged 51–65	420	3	5	4	5	4	3	3
	Girls aged 13–15	360	4	5	4	6	4	3	3
	Women aged 51–65	320	4	6	5	6	5	4	4

Source: own elaboration based on Wojtasik *et al.* [2020]. RDA, recommended dietary allowance based on the dietary guidelines for the Polish population. Fresh cheeses produced from: C, milk with the addition of skimmed milk powder (control sample); MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

The RDA of both cheeses produced on the basis of buttermilk protein concentrate was lower relative to the control sample. In all analysed age groups, the RDA of phosphorus was best satisfied by a 100 g serving of MCC_F cheese, followed by RMFB_F cheese, and the values noted in MCC_F were twice higher than in the control sample. In all age groups, the RDA of phosphorus was lower in 100 g of the remaining cheeses than in the control sample. The analysed products were most effective in satisfying the magnesium RDA of children aged 4-6 years due to lower magnesium requirements in this age group. The type of raw material applied in the production process did not differentiate the target magnesium intake from fresh cheeses in the remaining age groups.

Microbiological quality and pH of fresh cheeses

The pH of fresh cheeses was differentiated by the type of raw material (**Table 4**). This parameter was significantly ($p \leq 0.05$) higher in MCC_F cheese compared to the remaining products for which it ranged from 4.06 to 4.50. The higher pH of MCC_F cheese can probably be attributed to the high buffering capacity of the high-protein matrix. In the production of such fresh cheeses, acid coagulation is accompanied by protein gelation. A decrease in milk pH leads to the solubilisation of calcium [Gaucheron, 2005], therefore the higher pH of fresh cheese is more desirable because it prevents calcium loss during production.

Directly after cheese production and whey separation, *Lb. acidophilus* LA5 counts were higher in the analysed cheeses than in the inoculum. In most products, *Lb. acidophilus* LA5 counts increased on the first day of storage (**Table 4**). In addition, *Lb. acidophilus* LA5 counts continued to increase up to day 7 of storage in the control cheese and in RUFP_F and RMFB_F cheeses. In the remaining cheeses, the abundance of probiotic bacteria decreased on successive days of storage. In all cheeses, *Lb. acidophilus* LA5 counts exceeded log 6 cfu/g on the last day of storage (day 21), thus satisfying the criteria for probiotic products [Szajewska *et.al.*, 2023]. These results indicate that the analysed cheeses contained substrates that are essential for the growth of lactic acid bacteria. *Lactobacillus acidophilus* LA5 cells do not require substrates that are highly abundant in nutrients, and they can utilise milk proteins in fresh cheeses [Bolivar-Jacobo *et.al.*, 2023].

The rate of lactic acid fermentation and lactic acid levels determine the physicochemical (curd formation, syneresis, curd decalcification) and microbiological (autolysis of starter cultures, proliferation of secondary microflora) properties of food products

TABLE 4. pH value and count of *Lactobacillus acidophilus* LA5 in fresh cheeses during storage.

Parameter	Storage time (day)	C	MCC_F	RMFB_F	RUFP_F	M_MCC	M_RMFB	M_RUFP
pH	0	4.06±0.01^{b}	5.46±0.03^{a}	4.25±0.02^{b}	4.19±0.03^{b}	4.50±0.02^{b}	4.10±0.00^{b}	4.56±0.03^{b}
Bacteria count (log cfu/g)	0	7.27±0.20^{ab}	7.26±0.08^{ab}	6.78±0.08^{b}	7.49±0.17^{a}	7.17±0.08^{ab}	7.15±0.12^{ab}	7.25±0.05^{ab}
	1	8.92±0.17^{a}	7.70±0.020^{b}	7.48±0.06^{b}	7.83±0.05^{b}	8.74±0.22^{a}	8.96±0.15^{a}	8.76±0.20^{a}
	7	9.26±0.21^{a}	7.59±0.08^{de}	8.46±0.09^{bc}	8.22±0.04^{c}	7.80±0.10^{cd}	8.77±0.13^{ab}	7.36±0.30^{d}
	14	7.61±0.08^{b}	7.67±0.03^{b}	8.31±0.04^{a}	7.75±0.09^{b}	7.74±0.13^{b}	8.48±0.10^{a}	7.71±0.05^{b}
	21	6.66±0.40^{d}	8.24±0.05^{b}	8.89±0.16^{a}	8.56±0.15^{ab}	6.79±0.31^{d}	7.49±0.12^{c}	7.19±0.30^{cd}

The presented values are means with standard deviation. Mean values marked with different letters (a–d) in rows differ significantly at $p \leq 0.05$. Fresh cheeses produced from: C, milk with the addition of skimmed milk powder (control sample); MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

and, consequently, affect their sensory attributes (colour, texture, taste).

Colour parameters of fresh cheeses

The use of membrane filtration products in the production of fresh cheeses induced a minor, but statistically significant changes in colour. The lightness (L^*) of all cheeses remained within a narrow range of 89.76 to 92.92 (**Table 5**). Fresh cheeses were characterised by a light green ($-3.29 \leq a^* \leq -1.42$) and yellow ($6.89 \leq b^* \leq 12.12$) hue. The differences in the colour parameters of fresh cheeses produced from various raw materials were confirmed by the values of the total colour difference (ΔE) relative to the control sample. The values of ΔE were higher when fresh cheeses were produced with the use of liquid protein fractions than milk with the addition of high-protein powders. The highest values of ΔE were noted in MCC_F cheese (4.04), followed by M_MCC cheese (2.56). The detection of colour difference could be easily possible even by an inexperienced observer if the $\Delta E = 2.0–3.5$ [Dobrzańska & Cais-Sokolińska, 2014]. The application of buttermilk proteins, alone (RUFP_F) or in combination with micellar casein (RMFB_F), decreased the total colour difference in fresh cheeses ($1 < \Delta E < 2$) to a level that was discernible only to highly trained panellists. Despite the low ΔE values of cheeses produced from M_RMFB (0.58) and M_ RUFP (0.93), differences in colour were statistically significant ($p \leq 0.05$) (**Table 5**).

Rheological parameters of fresh cheeses

The pseudoplastic flow behaviour of fresh cheeses was influenced by the type of high-protein preparation used in the production process. The rheological analysis revealed that all products were characterised by pseudoplastic flow behaviour, and that their storage modulus (G') was higher than the loss modulus (G") (**Figure 2A**). In the cheeses made solely from high-protein preparations, G' ranged from 2,147 Pa (RMFB_F) to 13,001 Pa (RUFP_F). The value of G' was significantly higher in the cheeses produced from high-protein preparations with the addition of milk. The increase in G' values was smallest (approx. 50%) in M_RMFB cheese and highest in M_RUFP (312%) and M_MCC (1076%) cheeses.

Cheeses produced from buttermilk protein concentrate were characterised by the lowest value of stress at fracture, which was determined at 622 Pa in RMFB_F and 878 Pa in M_RMFB (**Table 6**). In comparison with the fresh cheeses produced from liquid protein concentrates only, the addition of high-protein powders to milk led to a significant ($p \leq 0.05$) increase in the analysed parameter, by 1,127% in M_MCC and by 830% in M_RUFP. The flow behaviour index of these cheeses was also significantly reduced (**Table 6**). In these products, stress at fracture increased significantly due to the use of milk and the resulting changes in the proportions of different milk proteins. Protein composition is an important factor which affects the spatial structure of the casein gel formed during acidification. Madadlou *et al.* [2006] and Esteves *et al.* [2003] found that the values of stress and strain at fracture were significantly influenced by a product's microstructure and the distribution of casein fibres in casein

TABLE 5. Colour parameters of fresh cheeses.

Fresh cheese	*L**	*a**	*b**	C	Δ*E*
C	91.92±0.08^{b}	−2.20±0.02^{d}	10.77±0.05^{c}	10.99±0.05^{d}	-
MCC_F	91.14±0.08^{d}	−1.42±0.03^{f}	6.89±0.09^{e}	7.03±0.09^{e}	4.04±0.09^{a}
RMFB_F	91.33±0.06^{c}	−3.29±0.02^{a}	10.77±0.03^{c}	11.26±0.04^{c}	1.24±0.04^{d}
RUFP_F	92.92±0.03^{a}	−3.17±0.04^{b}	10.44±0.23^{d}	10.91±0.23^{d}	1.44±0.03^{c}
M_MCC	89.76±0.16^{e}	−2.10±0.09^{e}	12.12±0.08^{a}	12.30±0.07^{a}	2.56±0.14^{b}
M_RMFB	91.83±0.05^{b}	−2.46±0.02^{c}	11.27±0.03^{b}	11.54±0.03^{b}	0.58±0.03^{f}
M_RUFP	91.07±0.04^{d}	−2.11±0.02^{e}	11.14±0.17^{b}	11.34±0.16^{c}	0.93±0.11^{e}

The presented values are means with standard deviation. Mean values marked with different letters (a–f) in columns differ significantly at $p \leq 0.05$. *L**, lightness; *a**, value between green (–) and red (+); b*, value between blue (–) and yellow (+); C, colour intensity; Δ*E*, total colour difference compared to control. Fresh cheeses produced from: C, milk with the addition of skimmed milk powder (control sample); MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

TABLE 6. Textural and rheological parameters of fresh cheeses.

Fresh cheese	Firmness (N)	Penetration force (N×s)	Consistency index (Pa×s^{n})	Flow behaviour index	Stress at fracture (Pa)
C	6.15±0.27^{d}	40.9±2.1^{d}	3,526±150^{e}	0.70±0.03^{c}	2,295±98de
MCC_F	69.58±5.79^{a}	285.6±18.4^{a}	7,450±317^{d}	0.72±0.03^{c}	2,609±111^{d}
RMFB_F	3.35±0.16^{d}	23.1±2.0^{e}	1,850±797^{f}	0.79±0.03^{b}	622±270^{f}
RUFP_F	5.89±0.28^{d}	38.1±0.8^{d}	11,401±485^{c}	0.71±0.03^{c}	4,214±179^{c}
M_MCC	41.67±2.78^{b}	156.2±11.7^{b}	38,842±1,654^{b}	0.82±0.03^{b}	29,412±1,252^{b}
M_RMFB	3.37±0.28^{d}	21.5±1.9^{e}	2,085±89^{f}	0.80±0.03^{b}	878±37^{ef}
M_RUFP	21.96±2.02^{c}	91.6±4.2^{c}	14,233±606^{a}	0.83±0.03^{a}	34,925±1,487^{a}

The presented values are means with standard deviation. Mean values marked with different letters (a–f) in columns differ significantly at $p \leq 0.05$. Fresh cheeses produced from: C, milk with the addition of skimmed milk powder (control sample); MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

gel. The significant increase in the values of stress at fracture was attributed to the formation of larger and less porous casein aggregates and, consequently, a more compact and less elastic casein matrix [Madadlou *et al.*, 2006]. However, a significant increase in the number of bonds between casein molecules not only increases a product's firmness (higher value of G'), but also significantly decreases elasticity and increases susceptibility to damage under exposure to increasing strain [Wium & Qvist, 1998], as demonstrated by the present findings.

The storage moduli (G') of the examined cheeses are compared in **Figure 2B**. In all products, G' values were not highly influenced by frequency, and the curves were characterised by similar shapes and trends. Similar trends were also found for G" values (data not shown). At each tested frequency, the value of G' was higher than the value of G", which implies that all samples had a well-organised, elastic gel structure, where both G' and G" were nearly independent of frequency. These results may point to the high stability of products during storage and packaging [Lucey, 2002].

The results of the rheological analysis were highly accurately predicted (R^2>0.999) by the power law model, which is widely used to describe the rheological properties of foods that are non-Newtonian fluids [Saleh *et al.*, 2020; Zhu *et al.*, 2015]. The flow behaviour index (*n*) of all products was below 1 (0.70–0.83), which indicates that the tested samples had a stable and elastic structure and were moderately susceptible to shear thinning (**Table 6**). The lowest *n* value, with an average of 0.71, was found in samples C, MCC_F and RUFP_F, which could point to the low stability of the protein matrix and susceptibility to internal regrouping that can lead to curd shrinkage and gradual separation of whey during prolonged storage. The consistency index (κ) was significantly influenced by both the type of protein formulation and the form in which it was added. As expected, in general the flow consistency index was

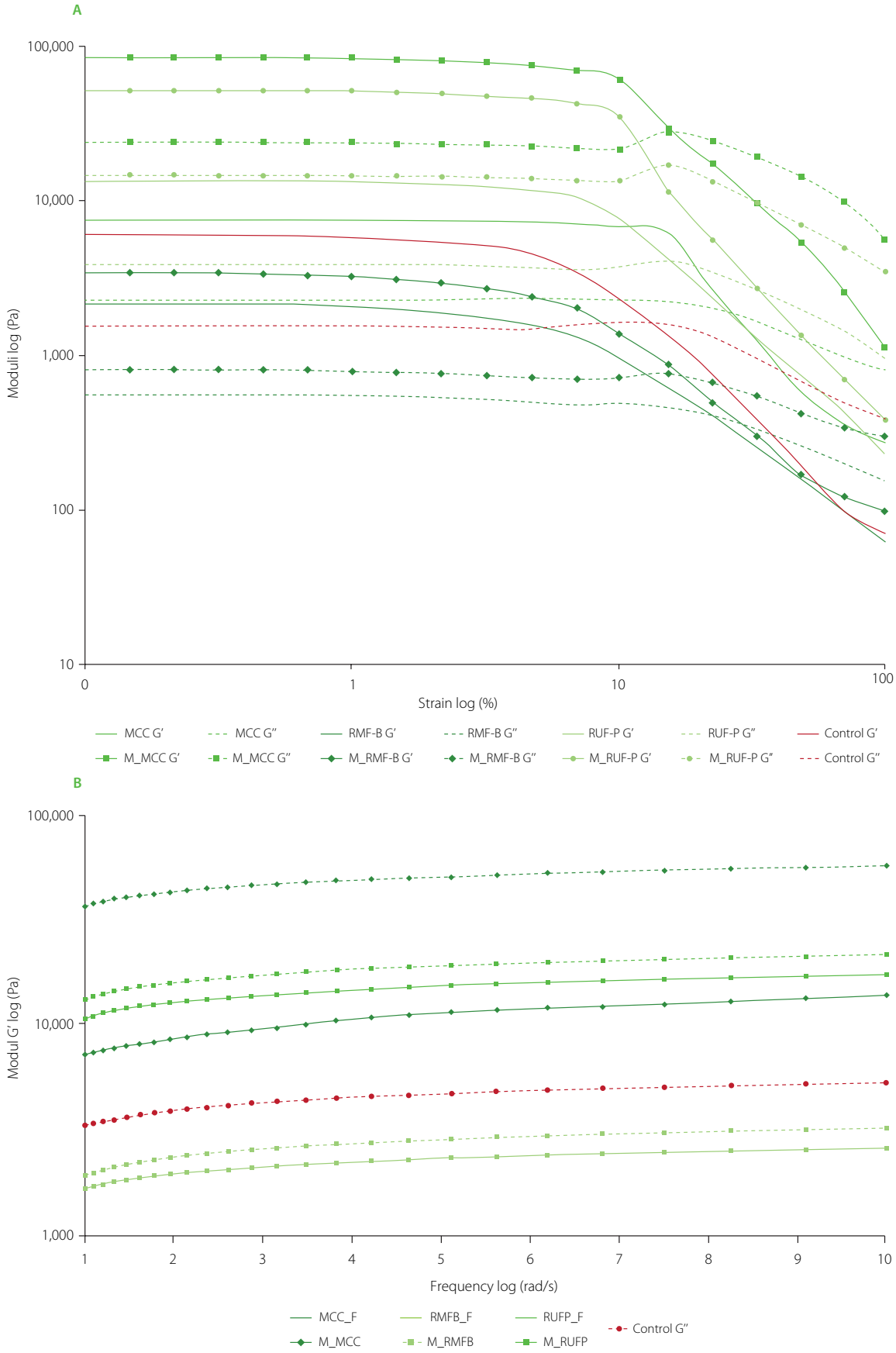

Figure 2. Rheological characteristics of fresh cheeses. (**A**) Strain sweep of modulus in fresh cheeses. The solid line represents the storage modulus component (G′) and the dashed line represents the loss modulus component (G″) of cheeses. (**B**) Frequency sweep test showing variation in G′ of cheeses. Fresh cheeses produced from: C, milk with the addition of skimmed milk powder – control sample; MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

high in the products containing micellar casein concentrate (MCC), as shown in **Table 6**. This can be attributed to the strong water-binding capacity of micellar casein and its ability to form a robust and interconnected protein network, which enhances the viscosity and structural stability of dairy products [Lucey & Singh, 1997]. In contrast, the addition of buttermilk protein concentrate in RMFB products resulted in a significant reduction ($p \leq 0.05$) in κ, with decreases of approximately 75% and 95% for RMFB_F and M_RMFB, respectively. This reduction in κ is likely due to the lower water-binding capacity and weaker network-forming properties of buttermilk proteins compared to micellar casein. Such a reduction may be advantageous for products where a creamier and less viscous texture is desired, improving consumer acceptability, particularly for dessert applications. Interestingly, RUFP products, which included a combination of micellar casein and buttermilk proteins, exhibited a significantly higher κ than RMFB products. This suggests the presence of synergistic interactions between micellar casein and buttermilk proteins, which contribute to the formation of a denser and more cohesive protein network. These interactions may arise from the complementarity of micellar casein's network-forming ability and buttermilk proteins' emulsifying properties, resulting in enhanced rheological performance.

The values of firmness and penetration force were highest in fresh cheeses produced from liquid micellar casein concentrate (MCC_F) or milk with the addition of micellar casein powder (M_MCC) (**Table 6**). Cheeses made from milk micellar casein powder combined with buttermilk serum protein powder (M_RUFP) were characterised by lower firmness (21.96 N) and lower penetration force (91.59 N×s). The values of these texture parameters were lowest in fresh cheeses produced from buttermilk proteins (RMFB_F and M_RMFB). The high values of firmness and penetration force in the fresh cheeses produced from raw materials with a high content of MCC indicate that micellar casein positively affects cheese texture by increasing the strength of the acid curd. The high values of texture parameters in MCC_F cheese confirm the previous hypothesis that gelation and a decrease in pH occur naturally during the production of fresh cheeses.

Microstructure and fractal analysis of fresh cheeses

Fresh cheeses have an irregular and porous surface which is influenced by the raw materials used in the production process. In the image analysis, similarities were observed in the microstructure of cheeses produced from sweet buttermilk proteins (RMFB_F and M_RMFB) or casein concentrate combined with buttermilk serum proteins (RUFP_F and M_RUFP). The micrographs revealed clear differences between MCC_F and M_MCC (**Figure S1** in Supplementary Materials). The fractal dimension was calculated to accurately and objectively describe the microstructure of cheese samples and compare the irregularity and porosity of cheese sample surfaces [Dziuba *et al.*, 1997]. Image analysis is becoming a common methodology in research. Self-similarity, namely the similarity of the observed structure regardless of the scale of magnification (or reduction), may be indicative of its fractal nature. The fractal dimension (D) is calculated to evaluate the degree of structural disorder. The value of D indicates the degree to which space is filled with matter; it describes a product's spatial structure, and enables a comparison of the studied samples [Barrett & Peleg, 1995; Smoczyński & Baranowska, 2014]. In the present study, the fractal dimension of the tested cheeses was calculated based on the results of the image analysis. The values of D were relatively high in the range of 1.40 to 1.65. The coefficient of determination (R^2) ranged from 0.88 to 0.93 (**Table 7**). An exemplary log-log plot for calculating the fractal dimension is presented in **Figure S2** in Supplementary Materials. The fractal dimension was smallest in the cheeses produced from liquid or powdered MCC. Cheese samples M_RMFB and M_RUFP were characterised by similar values of D (1.48–1.50) despite differences in their microstructure. Much greater differences in D values were observed in cheeses made from liquid high-protein fractions, and the analysed parameter was highest in RUFP_F cheese. In addition, the fractal dimension was higher in cheeses produced from liquid high-protein fractions than from milk with the addition of the corresponding high-protein powders. A high value of D is indicative of a rough surface and a complex microstructure. The observed differences in the fractal dimension affected the texture, creaminess, hardness, fluffiness and sensory acceptability of the analysed cheeses.

Sensory analysis of fresh cheeses

Fresh cheeses produced from high-protein milk and buttermilk preparations were evaluated for five groups of attributes: appearance, aroma, texture, mouthfeel, taste, and overall acceptability, in the sensory analysis. The results are presented in **Table 8**. In terms of appearance, all fresh cheeses were characterised by intensively uniform colour, regardless of the applied high-protein preparations ($p > 0.05$). The examined cheeses differed in creamy colour ($p \leq 0.05$), and the cheese made from M_RUFP was characterised by the creamiest colour. The authors' previous study

TABLE 7. Fractal dimensions of the fresh cheeses determined in the image analysis.

Fresh cheese	Coefficient of determination	Fractal dimension
C	0.90	1.49
MCC_F	0.88	1.44
RMFB_F	0.92	1.53
RUFP_F	0.90	1.65
M_MCC	0.93	1.40
M_RMFB	0.92	1.48
M_RUFP	0.93	1.50

Fresh cheeses produced from: C, milk with the addition of skimmed milk powder (control sample); MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

TABLE 8. Mean values of the sensory attributes of fresh cheeses.

Sensory attributes		C	M_MCC	M_RMFB	M_RUFP	MCC_F	RMFB_F	RUFP_F	*p*-Value
Appearance	Creamy colour intensity	2.7^{bc}	3.3^{ab}	1.9^{de}	3.6^{a}	1.3^{e}	3.0^{ab}	2.1^{cd}	0.000
	Colour uniformity	4.6^{a}	4.5^{a}	4.7^{a}	4.5^{a}	4.6^{a}	4.5^{a}	4.6^{a}	>0.05
	Whey leakage	1.0^{c}	1.0^{c}	1.5^{b}	1.0^{c}	1.0^{c}	1.2^{bc}	1.9^{a}	0.000
	Granularity	1.9^{b}	3.6^{a}	1.0^{c}	3.3^{a}	3.5^{a}	2.2^{b}	2.8^{ab}	0.000
Aroma	Overall intensity	2.9^{b}	3.8^{a}	3.3^{ab}	2.7^{bc}	2.3^{c}	3.7^{a}	3.7^{a}	0.000
	Milky	2.3^{ab}	2.4^{ab}	2.5^{ab}	2.3^{ab}	1.8^{b}	2.8^{a}	2.2^{ab}	0.049
	Sour	2.2^{bc}	3.1^{a}	2.9^{ab}	2.5^{abc}	1.8^{c}	2.9^{ab}	2.6^{ab}	0.009
	Atypical	1.3^{b}	1.6^{b}	1.6^{b}	1.5^{b}	1.9^{ab}	1.4^{b}	2.5^{a}	0.046
Texture	Uniformity	3.8^{a}	3.7^{a}	4.1^{a}	3.0^{bc}	2.4^{c}	3.6^{ab}	3.4^{b}	0.019
	Firmness	1.8^{c}	2.8^{b}	1.2^{d}	2.8^{b}	3.5^{a}	1.1^{d}	1.8^{c}	0.000
	Viscosity	4.1^{a}	2.0^{b}	4.4^{a}	2.6^{b}	1.3^{c}	4.1^{a}	3.8^{a}	0.000
	Spreadability	4.8^{a}	1.4^{d}	5.0^{a}	2.2^{c}	1.0^{d}	4.6^{a}	3.9^{b}	0.000
Mouthfeel	Wateriness	2.4^{a}	1.7^{ab}	2.3^{a}	1.9^{ab}	1.4^{b}	2.1^{ab}	1.8^{ab}	0.049
	Mealiness	1.5^{bc}	2.9^{a}	1.1^{c}	2.1^{b}	2.7^{a}	1.7^{b}	2.0^{b}	0.000
	Creaminess	4.0^{a}	1.7^{c}	4.4^{a}	3.0^{b}	1.5^{c}	3.1^{b}	2.2^{c}	0.000
	Adhesiveness	3.7^{a}	2.3^{c}	3.6^{a}	3.3^{a}	1.2^{d}	3.1^{ab}	2.5^{bc}	0.000
Taste	Overall intensity	4.4^{ab}	3.9^{bc}	4.9^{a}	3.7^{c}	1.6^{d}	4.7^{a}	3.9^{bc}	0.000
	Milky	2.3^{a}	1.6^{b}	1.6^{b}	1.8^{ab}	1.2^{b}	2.5^{a}	2.4^{a}	0.003
	Sweet	1.3^{a}	1.1^{a}	1.1^{a}	1.5^{a}	1.3^{a}	1.4^{a}	1.2^{a}	>0.05
	Sour	3.9^{b}	3.8^{b}	4.7^{a}	3.1^{c}	1.7^{d}	4.5^{a}	3.9^{b}	0.000
	Bitter	1.1^{c}	2.0^{ab}	1.5^{bc}	1.8^{ab}	1.2^{c}	2.3^{a}	1.9^{ab}	0.001
	Atypical	1.3^{b}	1.8^{ab}	2.3^{a}	1.9^{ab}	1.6^{ab}	1.8^{ab}	2.1^{a}	0.040
Overall acceptability		3.2^{a}	2.2^{b}	2.0^{b}	3.3^{a}	1.1^{c}	2.6^{b}	2.0^{b}	0.000

Mean values marked with different letters (a–e) in rows differ significantly at $p \leq 0.05$. Fresh cheeses produced from: C, milk with the addition of skimmed milk powder (control sample); MCC_F, liquid micellar casein concentrate; RMFB_F, liquid buttermilk protein concentrate; RUFP_F, mixture of liquid milk micellar casein concentrate and liquid buttermilk serum protein concentrate; M_MCC, milk with the addition of micellar casein powder; M_RMFB, milk with the addition of buttermilk protein powder; M_RUFP, milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder.

investigating the enrichment of non-fermented milk with protein preparations revealed that the addition of serum protein concentrate led to a minor increase in the intensity of the creamy colour [Kiełczewska *et al*., 2022]. Minor whey leakage was observed in the cheeses made from RUFP_F, M_RMFB, and RMFB_F, but not in the remaining products ($p \leq 0.05$). A high degree of granularity was noted in the cheeses made from MCC, regardless of whether it was added in liquid or powdered form during the production process. In turn, granularity was not at all visible in M_RMFB cheese ($p<0.05$).

Fresh cheeses differed in the overall aroma intensity and also in milky, sour, and atypical aromas ($p \leq 0.05$). The highest overall aroma intensity was noted in M_MCC, followed by RMFB_F and RUFP_F. The overall aroma intensity was lowest (but still moderate) in MCC_F cheese. This cheese was also characterised by the least smellable milky and sour aromas. In turn, the sour aroma was most pronounced in M_MCC cheese. These observations indicate that the form of MCC (liquid or powder) used in the production process affected the aroma of the end products. Most cheeses were also characterised by a weak atypical aroma which, according to the panellists, resembled the aroma of boiled milk. The atypical aroma was most distinctive (but moderate) in RUFP_F cheese. A rancid aroma was also detected in this product.

Statistical differences were also observed in texture attributes ($p \leq 0.05$). M_RMFB cheese was characterised by the highest mean scores of uniformity, viscosity, spreadability (with mean scores over 4), and minimal firmness. The distribution of textural attributes was similar in the control cheese and in the cheeses produced from RMFB_F and RUFP_F. However, the cheeses with the addition of both liquid and powdered RMFB were characterised by the lowest firmness. In turn, the cheese with the addition of liquid MCC was characterised by higher firmness and lower viscosity than the remaining products. Moreover, both MCC cheeses were characterised by the lowest spreadability. According to Suthar *et al.* [2017] and Simov *et al.* [2005], micellar casein influences the textural attributes of cheese, in particular firmness. Cheese produced from M_RUFP was rated slightly different in terms of textural attributes. Its uniformity of texture, firmness, viscosity, and spreadability were evaluated as moderate.

Fresh cheeses differed in mouthfeel-associated attributes ($p \leq 0.05$), *i.e.*, wateriness, mealiness, creaminess, and adhesiveness. The control sample and the cheeses produced from M_RMFB and RMFB_F were characterised by moderate wateriness (mean scores between 2.1–2.4), whereas the remaining samples were evaluated as less watery (with mean scores less than 2.0). Cheeses produced from liquid or powdered MCC were characterised by moderate mealiness. Creaminess was most distinctive in the cheese produced from M_RMFB, and least distinctive in the samples produced from liquid or powdered MCC. Adhesiveness was most detectable in the control sample and in M_RMFB cheese, and least detectable in the cheese produced from MCC_F cheese.

Differences were also detected in taste attributes such as overall intensity, milky, sour, bitter, atypical tastes, and overall acceptability ($p \leq 0.05$). Cheeses produced from M_RMFB and RMFB_F were characterised by a very high intensity of the overall taste, with a predominance of a strong sour taste. The overall taste intensity was also very high in the control sample, but a sour taste was moderate. Milky taste was weakly detectable in most cheeses, excluding the control sample, RMFB_F, and RUFP_F samples which had a moderately milky taste. Bitter taste was most intense in RMFB_F sample, and atypical taste was most intense in the cheeses produced from M_RMFB and RUFP_F, where it was described as rancid. Sweet taste intensity was evaluated as low in all analysed products ($p>0.05$). Thus, sweet taste was not enhanced, which is typical of dairy products enriched with whey preparations [Królczyk *et al.,* 2016]. Sample M_RUFP received the highest mean score for overall acceptability (just before the control sample), whereas sample MCC_F received the lowest score due to the highest firmness, lowest spreadability, and lowest overall taste intensity.

CONCLUSIONS

Skimmed milk and buttermilk fractions separated by membrane filtration and the *Lb. acidophilus* LA-5 culture can be used in the production of probiotic fresh cheeses. In the examined cheeses, *Lb. acidophilus* LA-5 counts exceeded log 6 cfu/g on the 21st day of storage. Therefore, the analysed fresh cheeses met the requirements for probiotic products and could be classified as functional foods. In turn, raw materials with a varied content and composition of skimmed milk and buttermilk proteins can be applied to produce fresh cheeses with different physicochemical, rheological, and sensory properties. The examined cheeses differed in pH, which affected their calcium and phosphorus content. Fresh cheeses produced from raw materials with a high content of micellar casein were characterised by the highest pH and the highest retention of calcium and phosphorus. In comparison with the remaining products, these cheeses were also characterised by the highest firmness and the lowest fractal dimension in the microstructure analysis. Regardless of the type of high-protein preparations used in the production process, all cheeses exhibited pseudoplastic flow behaviour characteristic of shear-thinned fluids in the power law model. The rheological analysis revealed that curd quality should not deteriorate during storage and packaging. The flow behaviour index was lowest in the fresh cheese produced from raw material rich in micellar casein, which could suggest that the resulting protein matrix was less stable than in the remaining products.

The sensory analysis revealed significant differences in relation to most of the tested attributes, but the fresh cheese produced from milk with the addition of micellar casein combined with buttermilk serum proteins achieved the highest score for these attributes. The use of only the liquid fraction of micellar casein combined with buttermilk serum proteins resulted in the rejection of the product mainly due to its atypical aroma. The cheeses produced from raw materials rich in milk micellar casein were least spreadable, most granular and mealy. In turn, the products made from buttermilk protein preparations were excessively viscous and sour in taste.

The use of protein concentrates, in particular micellar casein, in the production of fresh cheeses could be an effective strategy for increasing calcium content and improving the Ca to P ratio, which enhances calcium bioavailability. In summary, the present study provides valuable insights for optimising the production of high-protein probiotic foods, enhancing their sensory attributes and health-promoting properties. Special attention should be paid to the changes induced by protein gelation during acid coagulation above the isoelectric point of casein. The resulting knowledge can be used to develop food products that not only meet consumer preferences regarding taste and texture but also deliver health benefits.

SUPPLEMENTARY MATERIALS

The following are available online at **https://journal.pan.olsztyn.pl/Use-of-High-Protein-Milk-Preparations-in-the-Production-of-Probiotic-Fresh-Cheeses,203483,0,2.html**; Figure S1. Micrographs of fresh cheeses produced from: milk with the addition of skimmed milk powder – control sample (A); liquid micellar casein concentrate (B); liquid buttermilk protein concentrate (C); mixture of liquid milk micellar casein and liquid buttermilk serum protein concentrate (D); milk with the addition of micellar casein powder (E); milk with the addition of buttermilk protein powder (F); milk with the addition of a mixture of milk micellar casein and buttermilk serum protein powder (G). Figure S2. Logarithmic relationship between the perimeter and surface area of M_MCC cheese.

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CONFLICT OF INTERESTS

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

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Egg Roll Cookies with Non-Itchy Taro (*Colocasia esculanta* var. Febi521) Flour Prolong Satiation

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Satiety refers to the processes that occur after a meal, which suppress hunger and inhibit further eating, and can be measured by the satiety index. Identifying the satiety index of food could help with the problem of overnutrition. This study aimed to determine the satiety index of non-itchy taro-based egg roll cookies. The non-itchy taro (*Colocasia esculenta* var. Febi521) flour with a high fiber content and its blends with modified cassava flour were used to produce egg roll cookies. Wheat cookies and white bread were reference products. A semi-crossover design was used, with each food provided isocalorically in 1,000 kJ (240 kcal). The satiety score was measured using a visual analogue scale (VAS) along with hunger, desire to eat, and prospective consumption score. Appetite score was also calculated. The total subjects were 11, with an average age of 21.55 years. Satiety scores decreased and appetite scores increased from the 90th min after consumption. The satiety score for non-itchy taro egg roll cookies was significantly higher than for the reference products at the 180th min after consumption, while the appetite score did not show significant differences between the products. The results showed that egg roll cookies from non-itchy taro flour produced a significantly higher satiety index (131.8) than white bread (100.0) and wheat egg roll cookies (103.92). The content of fat, dietary fiber, insoluble fiber, and carbohydrates in a portion of products correlated significantly with the satiety index. These findings suggest that substituting wheat flour with taro flour in egg roll cookies could increase the satiety index.

Keywords: baked product, flour blend, non-itchy taro, satiety index, visual analogue scale

INTRODUCTION

Satiety is the feeling of fullness occurring after a meal which suppresses hunger and prevents further eating, measured by the satiety index [Gerstein *et al.*, 2004]. It is affected by multiple factors, such as consumer beliefs about the food, food sensory characteristics, macronutrients composition, volume, and weight [Yeomans, 2020]. Different macronutrients have different effects on satiety and its intensity. Previous studies showed that the macronutrients that provide the highest and most persistent satiety were protein and fiber, respectively [Ahmed *et al.*, 2014; Akhlaghi, 2024; Drozdowska *et al.*, 2020; Palupi *et al.*, 2024].

The Indonesian dietary pattern is high in carbohydrate but low in protein, fiber, and vitamin. A previous study [Khusun *et al.*, 2023] found that the median intake of fiber among Indonesian adult was 6.2 g/day, not even half of the national dietary recommendation for adult, which was 29.0–37.0 g/day [Ministry of Health of the Republic of Indonesia, 2019]. The macronutrient intake in Indonesia was dominated by carbohydrates, especially from white rice, with its consumption

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reaching 81.1 kg/cap/year in 2022 [Central Statistics Agency of the Republic of Indonesia, 2024]. Beside white rice, the carbohydrate food commodity that was consumed majorly was wheat flour, reaching 24.1 kg/cap/year in 2022 [UNICEF, 2023]. Wheat flour was utilized into many types of food. However, it was very poor in dietary fiber, the content of which was only 0.3 g/100 g [Ministry of Health of the Republic of Indonesia, 2017]. Other than that, wheat was not native to Indonesia and was imported from other countries, including Canada, Australia, and the United States [Anugraheni *et al.*, 2024]. In 2022, Indonesia imported 9.5 million ton to meet the wheat flour demand [UNICEF, 2023]. To overcome the dependence of wheat flour while also overcoming the problem of fiber deficiency, a substitution using local food with a high dietary fiber content can be implemented.

Indonesia has many indigenous foods with a high fiber content that are underutilized. One of them is Bogor taro (*Colocasia esculenta*), which has a fiber content of 0.9 g/100 g [Ministry of Health of the Republic of Indonesia, 2017]. However, Bogor taro production has been decreasing since 2008, from 57.0 thousand ton to 11.2 thousand ton in 2019 [Central Statistics Agency of the Republic of Indonesia, 2020]. Bogor taro has low growing demand due to its high calcium oxalate content. It appears in the form of a needle, which can penetrate skin and cause irritation and itching sensation when eaten [Rashmi *et al.*, 2018]. A new variety of Bogor taro with 90% lower oxalate content has been registered under the name Febi521 (hereafter, non-itchy taro) by the Ministry of Agriculture of The Republic of Indonesia [Nurilmala *et al.*, 2024]. The reduction in oxalate content made the taro not cause itchiness. The water content of this taro variety is 72.4 g/100 g, which was categorized as high, hence reducing the shelf-life. To overcome the short shelf-life, in our previous study, non-itchy taro was processed into flour, and utilized along with modified cassava flour to develop egg roll cookies [Nurilmala *et al.*, 2024]. Cookies are a popular snack among Indonesian, with the consumption of 24.3 kg/cap/year and increasing each year [Central Statistics Agency of the Republic of Indonesia, 2022]. Incorporating non-itchy taro into egg roll cookies could enable reducing wheat flour use, while also utilizing available local food.

Our previous study showed that the egg rolls made from the blends of non-itchy taro flour and modified cassava flour in different proportions had varied macronutrients content and all of them produced a low glycemic index [Nurilmala *et al.*, 2024]. However, the satiety index of non-itchy taro-based egg roll is still unknown. Therefore, the objective of this study was to determine the satiety indices of egg roll cookies from non-itchy taro flour and its blends with modified cassava flour and compare them with wheat egg roll cookies and white bread.

MATERIALS AND METHODS

This research has obtained permission from the Commission on Research Ethics Involving Human Subjects, IPB University Number: 1227/IT3.KEPMSM-IPB/SK/2024.

Subjects recruitment

Fourteen subjects (8 women and 6 men) were recruited using purposive sampling. The subjects would be eligible if they (1) were between 19 to 23 years old, (2) had normal body mass index (BMI) (18.5–22.9 kg/m^2), and (3) had normal fasting blood glucose (FBG) (70–99 mg/dL). The subjects would be excluded if they (1) were allergic to egg and/or taro, (2) had history of diabetes (including parents and grandparents), (3) were on a specific diet, (4) were undergoing a medical treatment, (5) were suffering from chronic diseases or complications, (6) were having indigestion, (7) were pregnant or lactating, (8) were an active smoker, and (9) were not willing to be interviewed and have their nutritional status measured. Informed consent was obtained from each subject before the test. All subjects completed the test; however, 3 subjects were eliminated due to human error in questionnaire filling, making the satiety score abnormally shifting up and down and the satiety index abnormally higher than the rest of the subjects. A sample size of 11 was still considered acceptable for satiety index study [Holt *et al.,* 1995]. While a larger sample size would increase the statistical power and generalizability, the current sample size can still provide valuable insights.

Test foods

Four formulas of egg roll cookies with different flour composition were used in this study. The formulas contained wheat flour (WF), taro flour (TF), and blends of TF with modified cassava flour (MF): F0 (100% WF), F1 (40% TF:60% MF, *w/w*), F2 (60% TF: 40% MF, *w/w*), and F3 (100% TF) [Nurilmala *et al.*, 2024]. WF and MF were commercially bought from a market. The TF was made from Febi521 (non-itchy) taro, which was developed through *in vitro* somaclonal induction, resulting in a new taro variety with a 90% lower oxalate content. The taro was subsequently processed into flour [Nurilmala *et al,* 2024; Nurilmala & Mardiana, 2019]. White bread was used as a standard for satiety index calculation according to Holt *et al.* [1995], and F0 was used to produce standard egg roll cookies. The white bread was made using a recipe from the US Dairy Export Council [US Dairy Council, 2014], which consisted of medium-protein wheat flour 12.7 g/100 g (70.4%), water (10%), granulated sugar (6.3%), yeast (0.8%), and whole milk powder (0.8%). The egg roll cookies were produced from WF, MF, and TF with the respective composition (20.2%), egg (42.1%), margarine (6.7%), butter (13.5%), sugar (16.8%), and powdered milk (0.7%) [Nurilmala *et al.,* 2024]. Each food product was provided isocalorically in 1,000 kJ (240 kcal). According to the energy content, F0 was given 46.7 g, F1 was given 44.7 g, F2 was given 44.0 g, F3 was given 44.6 g, and white bread was given 100.0 g. One cup of water (220 mL) was also provided to aid the ingesting process.

Test procedure

Prior to the first session, subjects' nutritional status was measured, including body weight and body height, followed by BMI calculation. The night before the session, the subjects were asked to fast for 8–10 h and to keep their physical activity as usual. This

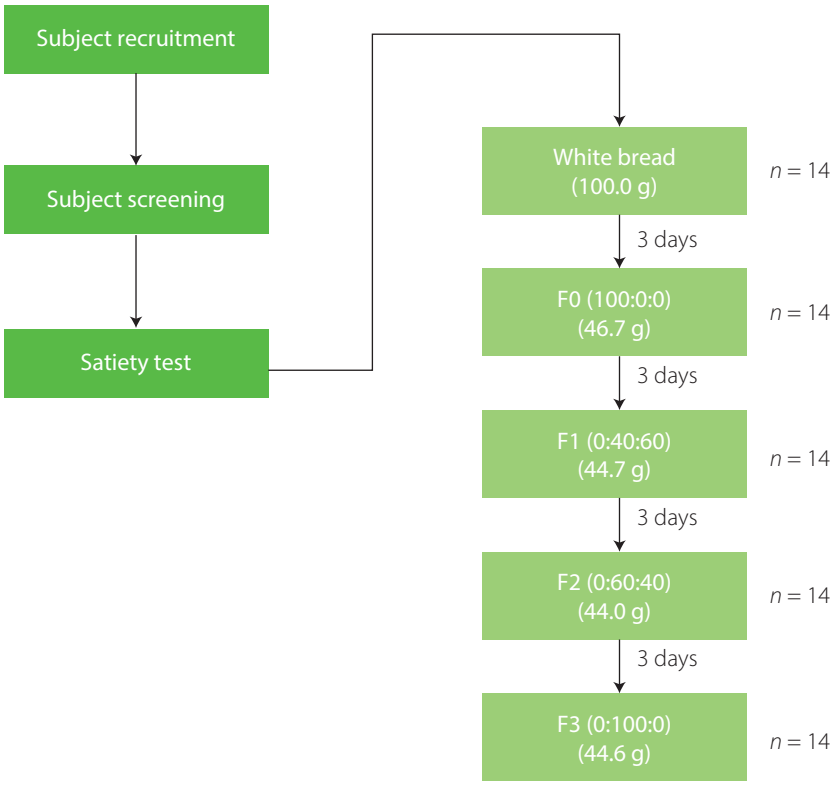

Figure 1. The procedure of subject recruitment and data collection. Egg roll cookies were produced from different flours: Fn (x:y:z), the combination of x% wheat flour, y% non-itchy taro flour, and z% modified cassava flour (*w/w/w*); *n*, number of subjects.

study used a semi-crossover design. On the first session, the subjects were given white bread. They were asked to eat the white bread in 10 min and fill the satiety visual analogue scale (VAS) questionnaire consisting of four indicators: (1) hunger score, (2) satiety score, (3) desire to eat score, and (4) prospective food consumption score. The subjects were told to draw a vertical line between a 100-mm horizontal scale, with the left end indicating least hungry/satiated/desire to eat/prospective food consumed, while the right end indicating the opposites. The satiety VAS questionnaire was filled 7 times with 30 min interval: 0, 30, 60, 90, 120, 150, and 180 min.

After the session, a 3-day washout was conducted to ensure that the physiological condition had returned to normal. The order of the test food products offered were egg roll cookies from F0, F1, F2, and F3. The flow diagram of the test procedure is shown in **Figure 1**.

Data processing and statistical analysis

The results of this study were processed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and analyzed using IBM SPSS version 26.0 for Windows (IBM, Armonk, NY, USA). The homogeneity of subject characteristics was analyzed using the coefficient of variation percentage (%CV). The data of subject characteristics was considered homogenous if the %CV for BMI was equal to or less than 5% [Jamaiyah *et al.*, 2010] and %CV for fasting blood glucose was less than 33% [Mo *et al.*, 2021]. The %CV was calculated using Equation (1) [Ulijaszek & Kerr, 1999]:

$$\%CV = (\text{Standard deviation}/\text{Mean}) \times 100\% \quad (1)$$

The satiety score (SS) was obtained by measuring the distance between the left point and the vertical line drawn by the subject. The result was plotted into a line chart, and the area under the curve (AUC) was calculated to determine the total score. Then, the satiety index was calculated using Equation (2) based on Holt *et al.* [1995]:

$$\text{Satiety index} = \frac{\text{AUC of test food}}{\text{AUC of standard food}} \times 100 \quad (2)$$

For food product with the highest SS at 180 min, the SS was later used together with the regression equation of curve SS *vs.* time for WB to determine the satiety prolonging effect of the tested food product compared to WB as a reference [Palupi *et al.*, 2024]

The scores of the other indicators, including desire to eat score (DES), hunger score (HS) and prospective consumption score (PCS), were obtained using the same way mentioned before. Then, appetite scores were calculated using scores of the four indicators according to Equation (3) [Anderson *et al.*, 2002]:

$$\text{Appetite score} = [\text{DES} + \text{HS} + (100 - \text{SS}) + \text{PCS}]/4 \quad (3)$$

The satiety score and appetite score were analyzed using one-way analysis of variance (ANOVA) and repeated two-way

Table 1. Characteristics of subjects.

Subject code	Sex	Age (years)	BMI (kg/m^2)	FBG (mg/dL)
001	Male	22	22.2	89
002	Female	22	21.2	88
003	Male	22	21.1	96
004	Female	21	21.6	87
005	Female	22	21.6	78
006	Male	23	19.3	99
007	Male	21	22.8	99
008	Female	21	21.8	90
009	Female	22	20.1	82
010	Female	19	22.9	89
011	Male	22	21.0	86
Mean		21.55	21.40	89.36
SD		1.04	1.08	6.58
%CV		4.82	5.00	7.36

BMI, body mass index; FBS, fasting blood glucose; SD, standard deviation; %CV, coefficient of variance percentage.

ANOVA, while the satiety index was analyzed using one-way ANOVA. If there were significant differences, the data were further analyzed using post-hoc Duncan's test. The results were considered significantly different if the *p*-value was lower than the α ($p<0.05$). Correlation between macronutrients and satiety index was analyzed using a Spearman correlation test.

RESULTS AND DISCUSSION

Subjects characteristics

The characteristics of the subjects of this study are presented in **Table 1**. Subjects were 11 adults (6 women and 5 men) with the age ranging from 19 to 23 years (the average age was 21.55 years). The age range was taken from the regulation of the Ministry of Health of the Republic of Indonesia number 28, year 2019 [Ministry of Health of the Republic of Indonesia, 2019]. It was assumed that the subjects in the age range of 19–23 years would have similar nutritional needs. The average BMI of the subjects was 21.40 kg/m^2, while the average FBG of the subjects was 89.36 mg/dL. The inclusion criteria of normal BMI and FBG were determined to prevent bias.

Based on the calculation of %CV, all characteristics were considered homogenous. This meant that the result of this study could represent the satiety index of egg roll cookies.

Nutrient content *per* portion

Holt *et al.* [1995] stated that there was an effect of the type of macronutrients on the level of satiety of the consumer. The egg roll cookies, given to the subjects in our study, varied in the flour composition in the formula and, therefore, also in their nutrient profile. The nutrient contents of egg roll cookies from wheat flour, non-itchy taro flour and blends of non-itchy taro and modified cassava flours *per* 100 g were reported in our previous study [Nurilmala *et al.*, 2024]. **Table 2** shows nutrient contents *per* portion (1,000 kJ or 240 kcal). The substitution of wheat flour with non-itchy taro flour and modified cassava flour increased the contents of fat and dietary fiber, but also decreased the content of protein. The F1, F2 and F3 portions had a lower protein content than F0, but their protein content successively increased as the contribution of non-itchy taro flour in the cookie formula increased. Conversely, both the total carbohydrates and available

Table 2. Nutrient content *per* serving (240 kcal) of white bread (WB) and egg roll cookies from wheat flour (F0), non-itchy taro flour (F3) and blends of non-itchy taro and modified cassava flours (F1 and F2)*.

Nutrient	WB	F0 (100:0:0)	F1 (0:40:60)	F2 (0:60:40)	F3 (0:100:0)
Protein (g)	9.90±0.04^a	4.78±0.02^b	3.34±0.02^d	3.36±0.02^d	3.66±0.04^c
Fat (g)	4.70±0.02^d	12.34±0.21^c	13.61±0.24^b	14.24±0.40^a	14.29±0.26^a
Total carbohydrates (g)	48.38±3.62^a	27.50±0.21^b	26.08±0.26^{bc}	24.52±0.39^{bc}	24.16±0.26^c
Available carbohydrates (g)	39.28±0.32^a	24.81±0.23^b	19.61±0.27^c	18.31±0.38^d	15.26±0.36^e
Dietary fiber (g)	9.10±3.30^a	2.69±0.04^c	6.47±0.11^{ab}	6.21±0.04^b	8.90±0.11^{ab}
Soluble fiber (g)	6.44±2.34^a	0.60±0.02^c	1.76±0.02^b	1.72±0.05^b	1.21±0.02^{bc}
Insoluble fiber (g)	2.66±0.96^{bc}	2.10±0.03^c	4.71±0.10^b	4.49±0.07^b	7.69±0.09^a
Weight *per* 240 kcal (g)	100.00	46.70	44.70	44.00	44.60

*Based on nutrient composition determined in our previous study [Nurilmala *et al.*, 2024]. Fn (x:y:z) – the combination of x% wheat flour, y% non-itchy taro flour, and z% modified cassava flour (*w/w/w*). Numbers followed by different letters in the same row mean that the results are significantly different ($p<0.05$) according to the analysis of variance with the Duncan's post hoc test.

carbohydrates decreased with the increase in taro flour content. These results were due to the nature of wheat flour, with a higher protein and carbohydrate content, but also a lower dietary fiber content compared to modified cassava flour and taro flour [Ministry of Health of the Republic of Indonesia, 2017]. Meanwhile, the portion of white bread made with wheat flour and entirely different recipe had the highest contents of protein, total carbohydrates, available carbohydrates, and dietary fiber.

According to previous studies [Chambers *et al*., 2015; Daly *et al*., 2020; Santo-Hernandez *et al*., 2018], the protein was known to elicit the highest satiety effect, while the effect of carbohydrates and fat on the satiety level was still up to debate [Chambers *et al*., 2015]. Beside protein, fiber was also known to affect satiety [Munekata *et al*., 2021]. Based on the known fact, it was expected that WB would produce the highest satiety index.

Appetite score, satiety score, and satiety index

Appetite score was calculated using four indicators including hunger, satiety, desire to eat, and prospective consumption score. These indicators for white bread and egg roll cookies at different times after consumption are shown in **Tables S1–S3** in Supplementary Materials. The hunger score for non-itchy taro egg roll cookies (F3) was significantly ($p<0.05$) lower than these for WB, wheat flour egg roll cookies (F0), and egg roll cookies with larger contribution of modified cassava flour (F1) at the 180th min after consumption. Sample F3 experienced a significant increase of hunger score the latest, from the 150th min after consumption. There were no significant ($p\geq0.05$) differences in both the desire to eat score and the prospective consumption score across all tested food products at any time point. For both WB and sample F3, desire to eat score increased significantly starting from the 120th min after consumption, while for the rest of the egg roll cookies, it significantly increased from the 90th min after consumption. Meanwhile, no clear pattern was observed regarding the timing of significant prospective consumption score increases.

The appetite score was significantly affected by both the tested food product ($p=0.002$) and the time after consumption ($p=0.000$), but the interaction of food product and time after consumption did not significantly affect its value ($p=1.000$). Consumption of WB and F3 caused a significant appetite score increase at the 90th min, while in the case of the rest of the food products the increase occurred earlier, at the 60th min. Besides that, there was no significant difference in the appetite score detected between all food products each time ($p\geq0.05$) (**Table 3**).

The same effects were found for the satiety score, *e.g.*, the tested food product ($p=0.000$) and the time after consumption ($p=0.000$) significantly affected its value, but the interaction of food product and time after consumption did not ($p=1.000$). All samples experienced a significant decrease of the satiety score at the same time, from the 90th min (**Table 4**). However, it was found that the egg roll cookies from non-itchy taro flour (sample F3) produced a significantly higher satiety score compared to WB and wheat egg roll cookies on the last minutes ($p<0.05$). Then, we compared the ability of WB (as the standard) and egg roll cookies with the highest satiety score at the 180th min after consumption (sample F3) on prolonging satiety. To do that, the satiety score of sample F3 at the 180th min after consumption (41.91) was applied alongside the regression equation established using white bread as a reference (Equation 4), with the axis y as the satiety score and the axis x as the time after consumption:

$$y = -0.361x + 76.332 \quad (4)$$

It was found that WB produced the same satiety score as non-itchy taro egg roll cookies 85 min earlier. As a result, it can be concluded that consuming non-itchy taro egg roll cookies prolonged the feeling of fullness by 85 min compared to WB.

The egg roll cookies, from the lowest to the highest satiety index, were produced from F0 (103.92), F2 (119.44), F1 (121.32), and F3 (131.18). A significant ($p<0.05$) difference was found between the wheat egg roll cookies (F0) and the non-itchy taro egg

Table 3. Appetite score (mm) after the consumption of white bread (WB) and egg roll cookies from wheat flour (F0), non-itchy taro flour (F3) and blends of taro and modified cassava flour (F1 and F2).

Time after consumption (min)	WB	F0 (100:0:0)	F1 (0:40:60)	F2 (0:60:40)	F3 (0:100:0)
0	26 ± 19^{aD}	25 ± 17^{aD}	23 ± 16^{aE}	22 ± 18^{aE}	20 ± 17^{aC}
30	28 ± 20^{aD}	28 ± 15^{aD}	29 ± 15^{aDE}	26 ± 15^{aDE}	22 ± 18^{aC}
60	33 ± 12^{aD}	39 ± 16^{aCD}	34 ± 16^{aCDE}	33 ± 16^{aCDE}	28 ± 21^{aBC}
90	42 ± 16^{aD}	48 ± 20^{aBC}	42 ± 17^{aCD}	42 ± 17^{aBCD}	37 ± 23^{aABC}
120	52 ± 16^{aBC}	56 ± 22^{aB}	48 ± 19^{aBC}	49 ± 20^{aABC}	42 ± 24^{aAB}
150	63 ± 20^{aAB}	64 ± 21^{aAB}	60 ± 17^{aAB}	54 ± 20^{aAB}	48 ± 23^{aAB}
180	73 ± 20^{aA}	74 ± 17^{aA}	71 ± 19^{aA}	62 ± 22^{aA}	56 ± 24^{aA}

Fn (x:y:z) – the combination of x% wheat flour, y% non-itchy taro flour, and z% modified cassava flour (*w/w/w*). Superscripts with lowercase indicate significant differences ($p<0.05$) between columns, superscripts with uppercase indicate significant differences between rows according to one-way analysis of variance (ANOVA) with Duncan's post hoc test. Food product effect: $p=0.000$, time effect: $p=0.000$, formula-by-time effect: $p=1.000$ according two-way ANOVA.

Table 4. Satiety score (mm) after the consumption of white bread (WB) and egg roll cookies from wheat flour (F0), non-itchy taro flour (F3) and blends of non-itchy taro and modified cassava flour (F1 and F2).

Time after consumption (min)	White bread	F0 (100:0:0)	F1 (0:40:60)	F2 (0:60:40)	F3 (0:100:0)
0	75 ± 17^{aA}	73 ± 17^{aA}	78 ± 15^{aA}	77 ± 22^{aA}	80 ± 17^{aA}
30	66 ± 22^{aA}	66 ± 20^{aA}	70 ± 20^{aAB}	72 ± 18^{aAB}	78 ± 16^{aAB}
60	60 ± 19^{aAB}	56 ± 22^{aAB}	70 ± 13^{aAB}	64 ± 19^{aABC}	70 ± 21^{aABC}
90	48 ± 17^{aBC}	45 ± 25^{aBC}	56 ± 19^{aBC}	57 ± 20^{aBCD}	59 ± 25^{aBCD}
120	38 ± 18^{aCD}	40 ± 23^{aBCD}	50 ± 20^{aCD}	49 ± 22^{aCDE}	54 ± 24^{aCD}
150	27 ± 15^{bDE}	34 ± 21^{abCD}	38 ± 18^{abDE}	45 ± 21^{abDE}	49 ± 23^{aD}
180	20 ± 16^{bE}	23 ± 18^{bD}	27 ± 19^{abE}	35 ± 23^{abE}	42 ± 22^{aD}

Fn (x:y:z) – the combination of x% wheat flour, y% non-itchy taro flour, and z% modified cassava flour (*w/w/w*). Superscripts with lowercase indicate significant differences ($p<0.05$) between columns, superscripts with uppercase indicate significant differences between rows according to one-way analysis of variance (ANOVA) with Duncan's post hoc test. Food product effect: $p=0.000$, time effect: $p=0.000$, formula-by-time effect: $p=1.000$ according two-way ANOVA.

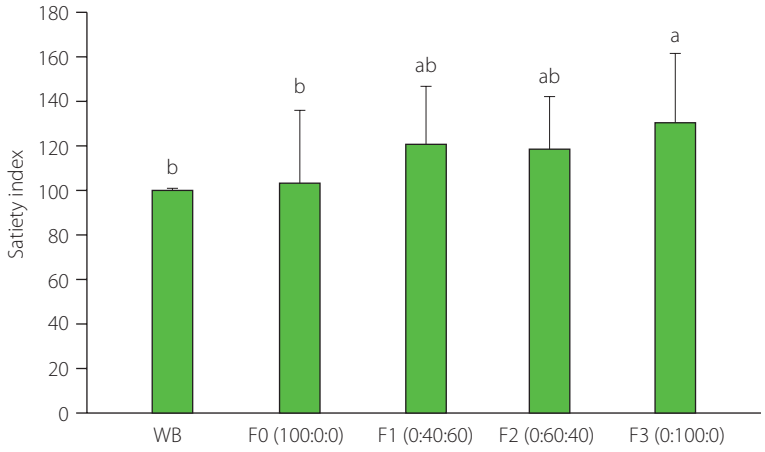

Figure 2. Satiety index of wheat bread (WB) and egg roll cookies from different flours: Fn (x:y:z), the combination of x% wheat flour, y% non-itchy taro flour, and z% modified cassava flour (*w/w/w*). The different lowercase letters above the bar indicating significant differences ($p<0.05$).

roll cookies (F3). Even though numerically each sample produced a higher satiety index than the white bread (100), statistically only the egg roll cookies produced from F3 produced a significantly higher satiety index ($p<0.05$) (**Figure 2**).

As mentioned above, based on the nutrient composition, it was expected that WB would produce the highest satiety index. Instead, the satiety index of WB was the lowest, while the highest satiety index was produced by the non-itchy taro flour egg roll cookies. In contrast with this result, Santaliestra-Pasías *et al.* [2016] and Daliani *et al.* [2019] stated that white bread enrichment with fiber increased fullness and satiety perception, and decreased hunger compared to conventional white bread, indicating that fiber increases satiety. The several factors might explain the differences in our findings and the literature data: the available carbohydrates, types of fiber, types of protein, and types of fat [Chandler-Laney *et al.*, 2019; Granger *et al.*, 2018; Salleh *et al.*, 2019; Szutowska, 2020].

Sensory characteristics and palatability of a product also affect both satiety and appetite [Abeywickrema *et al.*, 2022]. The sensory evaluation of non-itchy taro egg rolls had been conducted previously [Nurilmala *et al.*, 2024]. According to those studies, egg roll cookies from non-itchy taro flour achieved the best total acceptability scores and their characteristics were not significantly different compared to those of wheat egg roll cookies.

Correlation between macronutrient content and satiety index

The results of correlation analysis between macronutrient content *per* portion and satiety index of the egg roll cookies are shown in **Table 5**. There was a significant correlation between fat content and satiety index ($p=0.042$) with a positive correlation coefficient ($r=0.307$) as well as between carbohydrates and satiety index ($p=0.049$) with a negative correlation coefficient ($r=-0.299$). Moreover, the satiety index significantly, positively correlated with the total dietary fiber content ($p=0.024$, $r=0.340$) and with the insoluble fiber content ($p=0.028$, $r=0.332$). Unexpectedly, the correlation of the satiety index with protein

Table 5. Correlation between satiety index and contents of individual macronutrients *per* portion of egg roll cookies.

Macronutrient	r	*p*-Value
Protein	–0.263	0.085
Fat	0.307	0.042*
Carbohydrates	–0.299	0.049*
Total dietary fiber	0.340	0.024*
Soluble fiber	0.200	0.193
Insoluble fiber	0.332	0.028*

*Correlation is significant at $p<0.05$. *r*, Correlation coefficient.

was insignificant ($p=0.085$). These results were not completely in accordance with a previous study by Holt *et al.* [1995] about the satiety index of several types of food, who stated that an increased fat content was correlated with a lower satiety index, while carbohydrate content was not significantly correlated with the satiety index. Meanwhile, Chandler-Laney *et al.* [2014] stated that carbohydrates caused rapid hunger.

Available carbohydrates are related to the glycemic index (GI), where food with a high content of available carbohydrates tends to have a high GI [Afandi *et al.*, 2019]. High GI foods cause a spike in blood glucose, which triggers rapid hunger [Al-Sowayan *et al.,* 2023; Chandler-Laney *et al.*, 2014]. A previously published study from our project demonstrated that each egg roll cookie, regardless of the type of flour used, had low GI [Nurilmala *et al.* 2024]. The egg roll cookies from lowest to highest GI were produced according formulas F3 (39), F0 (47), F1 (49), and F2 (50). This was in contrast with white bread, whose GI was 70 and made it categorized as high GI [Vardhan *et al.,* 2024].

As for fiber, the type of fiber (soluble and insoluble) affects satiety through different mechanisms. Soluble fiber affects satiety through the slowing of the gastric emptying rate and fermentation by gut-microbiota, which triggers the hormone glucose-like--peptide 1 and peptide tyrosine-tyrosine, while insoluble fiber affects satiety through stomach distensing [Granger *et al.*, 2018; Salleh *et al.*, 2019; Szutowska, 2020]. Previous study considered soluble fiber, which was found to preserve satiety better than insoluble fiber [Alhabeeb *et al.*, 2021].

Numerous studies have examined the relationship between satiety and various macronutrients, yielding diverse results [Akhlagi, 2024; Luhovyy *et al.*, 2014; Munekata *et al.*, 2021; Warrilow *et al.*, 2019]. A consensus exists, however, regarding the hierarchy of macronutrients in influencing satiety, with protein being the most satiating, followed by carbohydrates and fats, although their effects remain inconclusive (protein > carbohydrates = fat). Meanwhile, fiber was thought to preserve satiety [Akhlagi, 2024]. However, prior researches proved that fiber affected satiety differently depending on its medium [Munekata *et al.*, 2021]. Research by Luhovyy *et al.* [2014] found that there was no effect of consumption of resistant starch-enriched cookies on subjective appetite. Further investigations into satiety have proposed that, similarly to fiber, the type of fat, depending on its saturation, affects satiety in varying degrees. Polyunsaturated fatty acids are the most satiating, followed by monounsaturated fatty acids, and saturated fatty acids [Monnard & Dulloo, 2021]. In our study, the egg roll cookies produced from F3, with significantly higher contents of insoluble fiber and fat *per* portion than WB produced a higher satiety index (despite their soluble fiber content was significantly lower). Additionally, research by Warrilow *et al.* [2019] suggested that there was no significant effect of the fat-fiber interaction on satiety. These findings, along with the result of this study, suggest that the role of fat in satiety may be more significant than previously recognized, and hence further investigation into the interaction between macronutrients on satiety is needed.

Implications and limitations

Indonesia is a country with diverse foods, although lots of local foods have not been widely utilized. Through this study and a previous one [Nurilmala *et al.*, 2024], we found that the substitution of wheat flour with non-itchy taro flour in egg roll cookies resulted in products with a lower glycemic index and a higher satiety index, and potentially gluten-free. Diverse nutrient contents of the local foods can be used to help overcome nutritional problem. Besides, utilizing local foods is one way to empower local community and contribute to rural development [Stein & Santini, 2022]. Thus, researchers and food manufacturers should feel encouraged to explore other local foods.

This research has several limitations that may impact the generalizability of its findings. Firstly, the sample size was small, reducing the statistical power and potentially limiting the ability to detect significant effects. Additionally, the age range of the subjects was narrow, which means the results may not be applicable to broader, more diverse populations. The method used to measure satiety relied on a subjective questionnaire, which could introduce biases related to self-reporting and individuals' interpretations of their feelings of fullness. Finally, the complexity of satiety was not fully explored in this study. Further research is, therefore, needed to investigate the various factors affecting satiety.

CONCLUSIONS

In conclusion, this study showed that among the egg roll cookies with different flour compositions, those made from non-itchy taro flour (F3) produced a higher satiety index compared to white bread (WB) and wheat-based egg roll cookies (F0) in healthy, normal-weight adults. Furthermore, the F3 egg roll cookies prolonged satiety by 85 min compared to white bread. The portion of F3 egg roll cookies (240 kcal) was high in fat and fiber while being low in available carbohydrates. A positive correlation was observed between the satiety index and fat, total dietary fiber, and insoluble fiber, whereas the carbohydrate content was negatively correlated with satiety. Unexpectedly, the correlation between the satiety index and the protein content was not significant. These findings suggest that substituting wheat

flour with taro flour in egg roll cookies may enhance satiety, highlighting the potential role of fat and fiber in satiety regulation. However, further research is needed to investigate the effect of interaction between macronutrients on satiety.

SUPPLEMENTARY MATERIALS

The following are available online at **https://journal.pan.olsztyn.pl/Egg-Roll-Cookies-with-Non-Itchy-Taro-Colocasia-esculanta-var-Febi521-Flour-Prolong,203705,0,2.html**; Table S1. Hunger score (mm) after consumption of white bread (WB) and egg roll cookies from wheat flour (F0), non-itchy taro flour (F3) and blends of taro and modified cassava flour (F1 and F2). Table S2. Desire to eat score (mm) after consumption of white bread (WB) and egg roll cookies from wheat flour (F0), non-itchy taro flour (F3) and blends of taro and modified cassava flour (F1 and F2). Table S3. Prospective consumption score (mm) after consumption of white bread (WB) and egg roll cookies from wheat flour (F0), non-itchy taro flour (F3) and blends of taro and modified cassava flour (F1 and F2).

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CONFLICT OF INTERESTS

E. Palupi reports a relationship with IPB University that includes: funding grants. F Nurilmala has patent #Ministry of Agriculture of the Republic of Indonesia. Plant Variety Protection Febi521. Center for Plant Variety Protection and Agricultural Licensing Ministry of Agriculture of the Republic of Indonesia. 2023. Licensed to Plant Variety Protection Febi521. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

INFORMED CONSENT

This research has obtained permission from the Commission on Research Ethics Involving Human Subjects, IPB University Number: 1227/IT3.KEPMSM-IPB/SK/2024 (EC).

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Physicochemical Properties, Sensory Profile, and Emotional Perception of Unpolished Organic Rice

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Incorporating unpolished rice into an everyday diet improves overall health and well-being. The objective of this study was to evaluate the physicochemical properties, sensory profiles, and emotional attributes of various unpolished organic rice cultivars and their blends. Three individual samples (brown, red, and black rice) were analyzed for proximate composition, pasting properties, and texture profiles, while sensory evaluation was conducted on cooked rice for both the individual samples and three blend combinations (brown-red, brown-black, and red-black rice). A total of 70 panelists assessed the samples using rate-all-that-apply (RATA) approach, emotional sensory mapping (ESM), and hedonic perception to understand the sensory perception of products and their associations with emotional, need-state, and environmental-state attributes. The rice samples differed in their physicochemical properties, with moisture, ash, lipid, protein, carbohydrate, total energy, and energy from lipids ranging from 10.72–12.56 g/100 g, 2.88–3.57 g/100 g, 5.48–6.69 g/100 g, 9.66–12.06, 67.03–69.73 g/100 g, 365.7–376.3 kcal/100 g, and 49.3–60.2 kcal/100 g, respectively. Although the three unpolished organic rice varieties exhibited similar pasting temperatures and final viscosities, red rice showed the lowest peak viscosity and the highest hardness, which may be attributed to its higher protein content. Moreover, despite notable differences in sensory attributes such as lightness, rice color, and the roughness of appearance and texture, the unpolished organic cooked rice samples did not show significant variations in hedonic liking. A key takeaway from this study is that consumer preference for unpolished rice is not heavily influenced by emotional, need-state, or environmental-state attributes. These findings provide valuable insights for the development of technologies to improve cooked rice, particularly through improving the texture. Furthermore, future studies on the presentation of unpolished rice-based meals could provide insights into developing sensory and emotional profiles that enhance consumer acceptance.

Keywords: black rice, brown rice, emotional sensory mapping, hedonic perception, penalty analysis, rate-all-that-apply approach, red rice

INTRODUCTION

Epidemiological studies have demonstrated that the consumption of whole grain foods and their derivatives can reduce the risk of development of non-degenerative diseases, such as type II diabetes, cardiovascular diseases, cancer, and obesity [Benisi-Kohansal *et al*., 2016; Khan *et al*., 2022; Ye *et al*., 2012]. Unpolished rice is classified as a whole grain because its post-harvest processing involves only the removal of the rice husk, leaving the bran

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layer attached to the endosperm. It is recognized for its health benefits as it contains higher levels of nutrients and bioactive phytochemicals than polished rice [Munarko *et al*., 2020; Ukpong *et al*., 2024; Yan *et al*., 2020]. Meanwhile, the global organic rice market is projected to grow at an annual rate of 8%, driven by consumer preferences for healthier, environmentally friendly, and convenient food choices [Bergman & Pandhi, 2023; Hazra *et al*., 2018]. This trend underscores the strong market potential of unpolished organic rice in the future. There are various types of unpolished rice available, but the most commonly found in the market are brown rice and some pigmented rice varieties, such as red and black rice [Pengkumsri *et al*., 2015]. Brown rice is an important source of phytochemicals including phenolic acids, γ-oryzanol and γ-aminobutyric acid [Munarko *et al*., 2020; Shao *et al*., 2018], while red rice and black rice, in addition, are rich in anthocyanins and proanthocyanins, respectively, which ensures their greater antioxidant capacity [Jantasee *et al*., 2014; Shao *et al*., 2018]. In Indonesia, these rice varieties are commercially available and widely distributed through both physical stores and online platforms. Interestingly, some producers offer blended varieties, such as brown rice mixed with red rice, red rice with black rice, and brown rice with black rice. The physical differences between unpolished rice varieties and their blends can influence sensory attributes of the finished products, potentially affecting consumer acceptance.

Previous studies have involved the sensory profiling of cooked rice, and thus several attributes, including color, glossiness, integrity, stickiness, elasticity, and hardness, as well as various taste, flavor, and aroma attributes, were identified using the quantitative descriptive analysis (QDA) method [Choi & Lee, 2021; Juemanee *et al*., 2018b; Kim & Kim, 2007]. On the other hand, consumer-based sensory science has increasingly driven the development of sensory profiling techniques utilizing direct consumer input. Methods such as check-all-that-apply (CATA), rate-all-that-apply (RATA), polarized sensory positioning (PSP), and projective mapping have been introduced in recent years [Ares & Varela, 2018; Ervina *et al*., 2023; Jariyah *et al*., 2024]. These approaches require no prior training, making them straightforward and adaptable alternatives to traditional sensory characterization methods like QDA [Ares & Varela, 2018; Jariyah *et al*., 2024].

RATA is a rapid sensory profiling method derived from the CATA technique. In this approach, participants identify terms relevant to the sample from a predefined list and subsequently rate the intensity or applicability of each selected term [Ares *et al*., 2014; Jariyah *et al*., 2024]. In the case of cooked rice, sensory profiling using the CATA method has been conducted to investigate the rice profile at different serving temperatures [Pramudya & Seo, 2018]. Interestingly, to date, no research has been conducted on the sensory profiling of unpolished rice products by using RATA method, specifically in organic rice varieties.

Beyond sensory attributes, consumer emotions play a crucial role in product selection. The emotions experienced during interaction with the product can provide valuable insights for the food industry, helping to design attributes that evoke positive or negative feelings [Jariyah *et al*., 2024]. The emotional sensory mapping (ESM) method is used to assess the emotional responses of panelists or consumers and their relationship with a product's sensory attributes [de Melo *et al*., 2021; Jariyah *et al*., 2024; Mora *et al*., 2018; Schouteten *et al*., 2016]. ESM can explore emotions, environmental conditions, and need-states linked to sensory characteristics, all of which are interconnected and perceivable during the consumption of the product.

To date, several researchers have conducted studies evaluating the nutritional value and sensory profile of rice samples from various varieties across the world [Choi & Lee, 2021; Gondal *et al*., 2021; Juemanee *et al*., 2018a; Shobana *et al*., 2011]. However, investigating the physicochemical and sensory attributes of unpolished cooked rice using the RATA method, combined with ESM to capture emotional responses, presents an intriguing research opportunity. Therefore, this study aimed to evaluate the physicochemical properties, sensory profiles and emotional perspectives of unpolished organic rice.

MATERIALS AND METHODS

Materials

This study employed three types of unpolished organic rice, *i.e.,* brown rice var. Berlian, red rice var. A3, and black rice var. Pekat. The rice was purchased from Sirtanio Organics in Banyuwangi, Indonesia. The varieties were selected because they are quite popular in East Java Province, Indonesia, and are available in both offline and online stores. All samples were harvested in 2024.

Determination of the nutritional composition of unpolished rice

The nutritional composition of three samples of each unpolished organic rice variety was determined in duplicate, including moisture, ash, total lipid, protein, total carbohydrate, total energy value and energy value from lipids. Prior to analysis, each rice variety was milled and sieved trough an 80-mesh sieve.

Moisture content was analyzed by the oven method according to the Indonesian National Standard (SNI 01-2891-1992) [BSN, 1992]. Ash content was determined by the dry ashing method, following the procedure described in the SNI 3549:2009 [BSN, 2009]. Total lipid content was analyzed using the Weibull method, with a Soxhlet apparatus [BSN, 1992]. Protein content was measured using the Kjeldahl method, based on the nitrogen content obtained through titration, with a conversion factor of 5.95 [BSN, 1992]. Total carbohydrate content was calculated by difference, subtracting the values of moisture, ash, protein, and total lipid from the total sample weight. The total energy value was calculated as the sum of: (CP × 4) + (TL × 9) + (CHO × 4) [Osborne & Voogt, 1978], while the energy derived from lipids was determined as TL × 9, where CP, TL, and CHO represent g of crude protein, total lipid, and carbohydrate *per* 100 g of rice, respectively.

Determination of pasting properties of unpolished rice flour

Pasting properties of three samples of each type of rice were measured in duplicate by using a Rapid Visco-Analyzer (RVA TecMaster, PerkinElmer Inc., Waltham, MA, USA). Pasting profiles

were determined by evaluating changes in paste viscosity during cooking, cooling, and stirring [Munarko *et al*., 2020]. The main parameters measured included peak viscosity (cP), trough viscosity (cP), breakdown (cP), final viscosity (cP), setback (cP), and pasting temperature (°C). Approximately 3 g of a flour sample and 25 mL of distilled water were placed into the RVA chamber and equilibrated at 50°C for 60 s. Within 4 min, the temperature was increased to 95°C and maintained for 2 min, then cooled down to 50°C within 4 min and held for 2 min. The paddle speed was set at 160 rpm during the measurement [AACC, 1999].

Measurement of texture parameters of cooked rice

The texture profile analysis (TPA) of cooked rice was conducted using a texture analyzer following the procedure of Tao *et al*. [2020] with a modification. A rice sample (50 g) was cooked with 150 mL of distilled water in an automatic rice cooker (Cosmos CRJ-1031 0.3L, Jakarta, Indonesia) for approximately 45–60 min and then left in the warm mode for 10 min. Subsequently, the upper layer of rice was removed, and the remaining rice was gently mixed. Once the sample had cooled to room temperature, three rice grains were placed directly under the probe on the base plate. The TPA was operated using a TX 700 texture analyzer (Lamy Rheology, Champagne au Mont d'Or, France), which was equipped with a 25 mm cylindrical probe. The analysis was conducted using a two-cycle force-*versus*-distance compression program. The TPA settings were as follows: down speed, 0.5 mm/s; force to start, 0.05 N; delay, 5 s; distance, 2 mm; wait position, 5 mm; and up speed, 0.5 mm/s. The parameters of TPA, namely hardness, cohesiveness, springiness, chewiness, and resilience, were calculated using the software provided with the instrument. Twelve texture measurements were tested for each rice sample.

Sensory profile characterization of cooked rice

Cooked rice preparation

Sensory profile and emotional sensory mapping analysis were conducted in six samples, including three samples of individual rice (brown rice: Br, red rice: Re, and black rice: Bl) and three samples of rice blends in a 50:50 (*w/w*) ratio (brown and red rice: Br-Re, brown and black rice: Br-Bl, and red and black rice: Re-Bl). Rice samples (130 g) were cooked using a rice-to-water ratio of 1:3 (*w/v*), following the procedure previously described in the texture measurement. After rice had been cooked, it was then transferred to a container and cooled to room temperature. Once cooled, the samples were prepared for sensory analysis.

Development of sensory attributes

The terms related to sensory, emotional, need-state, and environmental-state profiles were collected from previous research on cooked rice and other products. To identify the attributes for product testing, a total of nine panelists comprising rice researchers and general consumers from the Food Technology Department, Universitas Pembangunan Nasional Veteran Jawa Timur, who were familiar with the product, participated in focus group discussions (FGDs). The FGDs were conducted over three sessions, adopted from Jariyah *et al*. [2024] procedure with modification. In the first and second sessions, panelists were prompted to identify attributes present in the samples open-ended question. The third session focused on determining the sensory, emotional, need-state, and environmental-state attributes based on the responses gathered in the previous sessions.

Based on the findings from literature reviews and discussions of the panelists during the FGDs, 29 sensory attributes were identified, comprising 7 attributes related to appearance, 10 to aroma, 6 to texture, and 6 to taste/flavor. Additionally, 15 emotional attributes, 11 need-state attributes, and 10 environmental-state attributes were established. The complete list of attributes -state in the sensory testing is presented in **Table 1**.

Sample test

A total of 70 panelists were recruited from the Department of Food Technology database to participate in the sensory evaluation. Before testing, all participants provided informed consent and completed a pre-selection questionnaire *via* Google Forms. Eligibility criteria required participants to be at least 18 years old, in good health, and able to consume rice without restrictions.

Initially, panelists were asked to complete a questionnaire containing ideal attributes according to their perceptions. Subsequently, they were provided with samples (approximately 20 g for each sample) labelled with three randomly assigned codes, served sequentially. The panelists tested the samples using RATA and ESM with a list of sensory attributes previously determined in FGDs (**Table 1**). In the RATA test, the panelists indicated whether an attribute was absent by selecting "0" and rated the intensity of the attribute on a 5-point scale (very low to very high) [Ares *et al*., 2014a; Meyners *et al*., 2016]. For the ESM method, the panelists were first instructed to check all terms they found suitable for describing the tested products. Subsequently, they were asked to identify the terms that best represented the emotions, need-states, and environmental-states associated with their ideal product [Jariyah *et al*., 2024]. For hedonic testing, a 7-point scale was used, ranging from "very dislike" to "very like". Hedonic testing was necessary to gain insight into consumer preferences for the product being tested [Ares *et al*., 2014b; Meyners *et al*., 2016]. Mineral water was used to neutralize the mouth prior to testing and at each sample change.

Data analysis

Nutritional, pasting, and texture parameters

The means and standard deviation for results of proximate composition, pasting properties and texture parameters were calculated by SPSS 22.0 statistical software (Statistical Graphics Corp., Princeton, NJ, USA). Significant differences among the samples were evaluated using analysis of variance (ANOVA) with Duncan's multiple range tests ($p<0.05$).

Overall liking and acceptance

Hedonic data was expressed as means and analyzed using XLSTAT software (version 2019, Addinsoft, New York, NY, USA). A non-parametric statistical method, the Kruskal-Wallis test,

Table 1. Sensory, emotional, need-state and environmental-state attributes established by panelists during focus group discussions for unpolished organic cooked rice.

Appearance	Aroma	Texture	Flavor/Taste	Emotional	Need-state	Environmental-state
Lightness (Light)	Vanilla (Van-Ar)	Roughness (Rough-T)	Bitter (Bit-Fl)	Ordinary	Relaxation	Cold
Yellowish white (Ylow-whte)	Floral (Flo-Ar)	Smoothness (Smooth-T)	Sweet (Swt-Fl)	Satisfying	Refreshment	Night
Purplish black (Prpl-blck)	Beany (Bean-Ar)	Stickiness (Stick-T)	Plain (Pln-Fl)	Energetic	Energy	Afternoon
Brownish red (Brwn-red)	Cooked rice (Cook-Ar)	Hardness (Hard-T)	Beany (Bean-Fl)	Healthy	Balance	Evening
Uniformity (Uniform)	Planty (Pln-Ar)	Chewiness (Chew-T)	Nutty (Nut-Fl)	Happy	Experience	Morning
Roughness (Rough)	Grainy (Grain-Ar)	Moisture absorption (Moist-T)	Grainy (Grain-Fl)	Peaceful	Sharing	Quiet
Stickiness (Stick)	Pandan leaf (Pandan-Ar)			Warm	Escape	Crowded
Glossiness (Glos)	Nutty (Nut-Ar)			Comforting	Pleasure	Rainy
	Sweet aromatic (Swt-Ar)			Exciting	Raising the spirit	Summer
	Starchy (Starch-Ar)			Sociable	Health	Windy
				Relaxing	Convenience	
				Confident		
				Flat		
				Plain		
				Discontented		

was employed to determine significant differences between the samples ($p<0.05$). When significant effects at a confidence level of 95% were observed, multiple pairwise comparisons were conducted using Dunn's procedure.

Rate-all-that-apply and emotional sensory mapping

The RATA data was analyzed using a non-parametric Kruskal-Wallis test followed by Dunn's test to determine the differences between the samples with 95% confidence level. Principal component analysis (PCA) was applied to evaluate the distribution of panelists' responses to sensory attributes. PCA transforms the original variables into a new coordinate system through linear combinations, facilitating the interpretation of complex data matrices from sensory evaluations. This analysis was utilized to assess the mean values of appearance, aroma, flavor, and texture attributes [Jariyah *et al*., 2024; Yang & Lee, 2020]. All of the analyses were conducted by XLSTAT software (version 2019, Addinsoft).

ESM data was analyzed using the Cochran's Q test, correspondence analysis (CA), principal coordinate analysis (PCoA), and penalty analysis. The Cochran's Q test was applied to assess significant differences between the samples at a 95% confidence level [Ares & Jaeger, 2015; Jariyah *et al*., 2024]. CA and PCoA were employed to visualize the relationships between attributes and samples, as well as between attributes and the ideal point [Ares & Jaeger, 2015; Jariyah *et al*., 2024]. A chi-square analysis was performed to examine the association between the samples and sensory attributes in CA analysis. Penalty analysis was used to evaluate consumer responses by quantifying the reduction in overall liking (hedonic) associated with deviations from ideal product attributes. This analysis compared consumers' perceptions of the samples with those of the ideal product to assess their impact on liking scores [Ares *et al*., 2014b; Hunaefi *et al*., 2022; Meyners *et al*., 2016].

RESULTS AND DISCUSSION

Nutritional characteristics of unpolished organic rice

Table 2 presents, the proximate composition and energy values of brown, red and black organic rice, including moisture, ash, total lipid, protein, total carbohydrate, total energy value, and energy from lipid.

The moisture content of cereal commodities is a critical determinant of their optimal storage period. Moisture content greater than 12 g/100 g is widely recognized as a primary factor contributing to insect infestation and microbial growth, which compromise the suitability of cereals for long-term storage [Nath *et al*., 2022]. In this study, the moisture content of rice ranged from 10.72 g/100 g in brown rice to 12.56 g/100 g in red rice (Table 2). The higher moisture level in red rice may reduce its suitability for long-term storage. Ash content, indicative of the mineral content in rice, was significantly ($p<0.05$) higher in brown rice (3.57 g/100 g) than in black rice and white rice showing ash contents of 2.88 and 2.94 g/100 g, respectively. The bran layer in unpolished rice is typically rich in minerals, contributing to higher ash content. Moreover, unpolished rice was reported to contain ash levels three to four times greater than polished rice, a characteristic observed in pigmented rice varieties [Reddy *et al*., 2017].

Among the unpolished rice samples, brown rice exhibited significantly ($p<0.05$) higher total lipid content (6.69 g/100 g) than red rice (5.48 g/100 g) (Table 2). Black rice contained 6.02 g lipid/100 g. The total lipid content in the samples in our study was higher compared to that previously reported in Thai organic rice, Southeastern Nigerian rice, and Indian rice varieties [Kraithong *et al*., 2018; Nath *et al*., 2022; Oko & Ugwu, 2011; Reddy *et al*., 2017; Verma & Srivastav, 2017]. In turn, protein content of the samples ranged from 9.66 to 12.06 g/100 g (Table 2), with the highest content determined in red rice, followed by brown rice and black rice. These values are notably higher than those reported for several other varieties, which typically exhibit protein levels below 9 g/100 g [Kraithong *et al*., 2018; Oko & Ugwu, 2011; Reddy *et al*., 2017]. Variations in lipid and protein contents among rice varieties are largely influenced by factors such as plant genotype, agronomic practices, and environmental conditions [Nath *et al*., 2022; Verma & Srivastav, 2017]. Additionally, post-harvest processes like polishing are known to significantly reduce the lipid and protein content of rice grains [Reddy *et al*., 2017].

Table 2. Nutritional composition of unpolished organic rice.

Parameter	Brown rice	Red rice	Black rice
Moisture (g/100 g)	10.72 ± 0.92^{b}	12.56 ± 0.23^{a}	11.66 ± 0.61^{ab}
Ash (g/100 g)	3.57 ± 0.21^{a}	2.88 ± 0.14^{b}	2.94 ± 0.16^{b}
Total lipids (g/100 g)	6.69 ± 0.90^{a}	5.48 ± 0.38^{b}	6.02 ± 0.68^{ab}
Protein (g/100 g)	10.64 ± 0.18^{b}	12.06 ± 0.27^{a}	9.66 ± 0.06^{c}
Total carbohydrates (g/100 g)	68.38 ± 0.45^{ab}	67.03 ± 0.75^{b}	69.73 ± 1.41^{a}
Total energy value (kcal/100 g)	376.3 ± 7.2^{a}	365.7 ± 1.8^{b}	371.7 ± 0.7^{ab}
Energy from lipids (kcal/100 g)	60.2 ± 8.1^{a}	49.3 ± 3.5^{b}	54.1 ± 6.1^{ab}

Data are shown as mean ± standard deviation. Values with different small letters in the same row differ significantly ($p<0.05$).

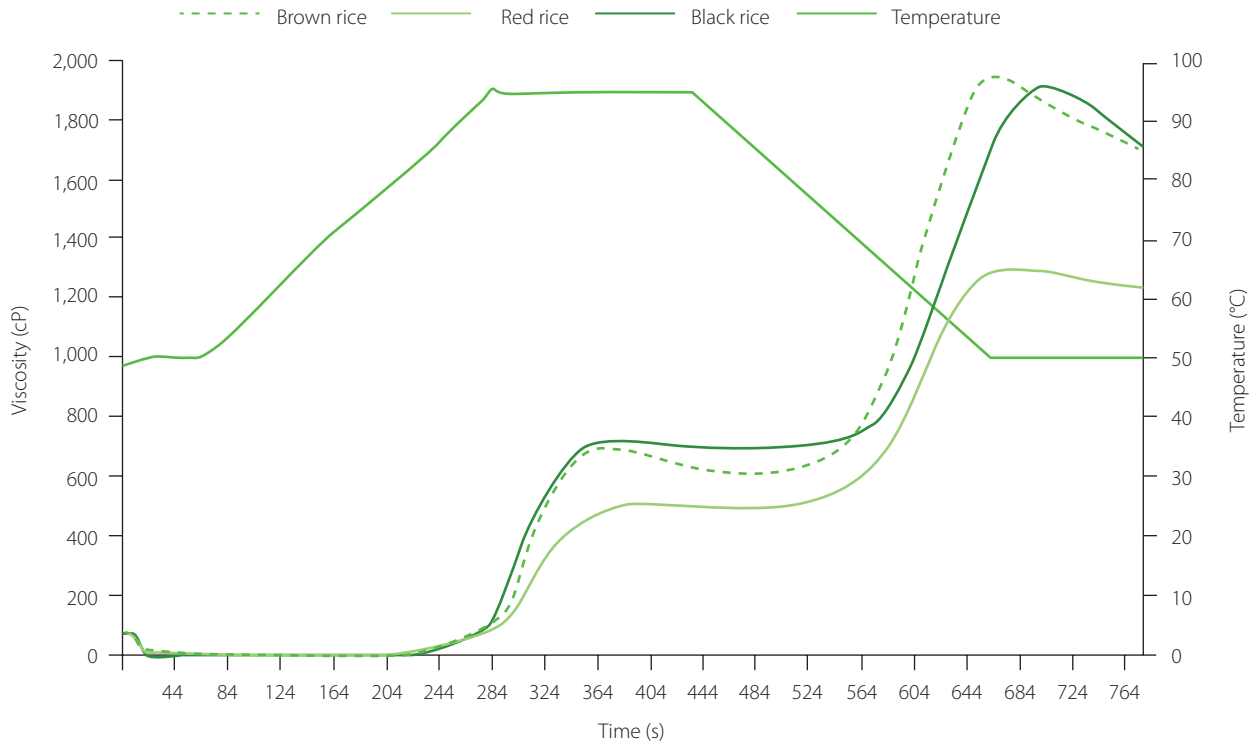

Figure 1. Visco-amylograms of unpolished organic rice.

Rice also serves as the primary carbohydrate source for the global population. The total carbohydrate content in the samples ranged from 67.03 to 69.73 g/100 g, with black rice showing significantly ($p<0.05$) higher total carbohydrate level than red rice (**Table 2**). Total energy values revealed slight but statistically significant differences ($p<0.05$) among the brown and red unpolished organic rice samples. Brown rice demonstrated total energy value of 376.3 kcal/100 g and energy derived from lipids of 60.2 kcal/100 g, and for red rice these values were 365.7 and 49.3 kcal/100 g, respectively. The concept of "food energy" refers to the energy available from food through cellular respiration [Nath *et al*., 2022].

Pasting properties of unpolished rice flour

The pasting profiles of unpolished organic brown rice, red rice, and black rice are presented in **Figure 1**. Pasting temperature demonstrates the temperature at which starch granules undergo gelatinization, a critical process influencing the functional properties of starch-based foods. In this study, the pasting temperatures of the three samples showed no significant differences ($p\geq0.05$), ranging from 94.8°C to 94.9°C. The gelatinized starch paste was swollen until it reached its peak viscosity. Among the samples, organic black rice and brown rice exhibited the peak viscosities of 725 cP and 702 cP, respectively. Red rice showed significantly ($p<0.05$) lower peak viscosity (510 cP). Peak viscosity reflects the ability of starch granules to absorb and bind water [Kraithong *et al*., 2018].

After complete gelatinization, starch granules break down under constant temperature, resulting in a decrease in viscosity known as trough viscosity. Organic red rice demonstrated a significantly lower trough viscosity ($p<0.05$) compared to brown and black rice. The difference between peak viscosity and trough viscosity, referred to as the breakdown value, indicates the stability of the starch paste during cooking [Munarko *et al*., 2020; Thiranusornkij *et al*., 2018]. The breakdown values for organic brown rice, red rice, and black rice were 85 cP, 28 cP, and 38 cP, respectively, with no statistically significant differences ($p\geq0.05$) observed among the samples. Although brown rice and black rice exhibited higher peak viscosity, their trough viscosities were also relatively high, resulting in breakdown values that were not significantly different ($p\geq0.05$) from those of red rice.

During the measurement of the pasting profile, the cooling process following paste heating caused an increase in viscosity, which can be attributed to the reformation of starch bonds. The final viscosity values for all three samples were not significantly different ($p\geq0.05$), ranging from 1,240 to 1,731 cP. Similarly, the setback values, indicative of paste retrogradation, showed no significant differences ($p\geq0.05$), with values ranging from 758 to 1,114 cP. The final viscosity parameter is commonly used as an indicator of starch gel formation during the cooling phase, while the setback value measures the extent of paste retrogradation [Munarko *et al*., 2020]. These parameters provide valuable insights into the functional properties of rice starch, which are critical for understanding its performance in various food applications.

Textural properties of cooked organic rice

The texture and visual appeal of cooked rice are crucial factors in determining its acceptability to consumers. The results

Table 3. Texture parameters of cooked organic rice.

Parameter	Brown rice	Red rice	Black rice
Hardness (N)	19.9±4.2^{b}	25.2±7.6^{a}	9.5±1.8^{c}
Cohesiveness	0.34±0.03^{b}	0.35±0.04^{b}	0.46±0.08^{a}
Springiness	2.1±1.4^{a}	2.2±1.4^{a}	2.5±1.4^{a}
Chewiness	14.0±9.1^{a}	20.0±15.7^{a}	10.4±5.9^{a}
Resilience	0.13±0.01^{b}	0.13±0.02^{b}	0.20±0.04^{a}

Data are shown as mean ± standard deviation. Values with different small letters in the same rows differ significantly (p<0.05).

of texture profile analysis of cooked rice are shown in **Table 3**. Hardness represents the force required to compress rice grains during mastication and is a critical textural property influencing the palatability of cooked rice. Among the samples, red rice exhibited the highest hardness (25.2 N), followed by brown rice (19.9 N) and black rice (9.5 N), with a significant (p<0.05) difference observed between samples. Chewiness measures the energy needed to chew rice grains until they reach a consistency suitable for swallowing. Springiness, on the other hand, refers to how much the rice grains stretch or rebound when in contact with surfaces, such as a plunger or teeth, before being pulled away. For both parameters there was no significant (p≥0.05) difference between brown, red and black rice. Resilience indicates the ability of rice grains to recover their shape after being deformed during biting. Brown rice and red rice displayed similar resilience, with no significant (p≥0.05) difference between them. However, black rice demonstrated a significantly (p<0.05) higher value of this parameter (0.20).

The textural variation may be attributed to differences in chemical composition between rice varieties. Huang *et al*. [2021] compared white and brown rice varieties and showed that cooked grains with a higher protein content had greater hardness. This finding was consistent with our study results. In addition to protein, a higher amylose content contributes to greater hardness and reduced springiness, which directly affect the overall eating experience [Bhat & Riar, 2017; Huang *et al*., 2021]. Previous studies reported that red rice had an amylose content 1.6 times higher than that of black rice [Devi & Badwaik, 2022], with its hardness being three times greater. Additionally, Mogoginta *et al*. [2024] found that black rice cultivated on Java Island, Indonesia, contained lower amylose levels than red rice. Besides protein and amylose, fiber also affects texture. Wholegrain rice contains nearly twice the total dietary fiber (TDF) of white rice, as most fiber is concentrated in the bran [Carcea, 2021]. These variations in fiber content among rice varieties may further influence textural properties.

Hedonic perception of cooked organic rice

The non-parametric Kruskal-Wallis test indicated no significant (p≥0.05) differences in the overall liking between the different samples. The panelists' preferences for unpolished rice varieties ranged from slight dislike to slight like, with mean scores of 4.63 for brown rice, 4.40 for red rice, 4.26 for black rice, 4.68 for the brown–red rice blend, 4.27 for the brown–black rice blend, and 4.47 for the red–black rice blend, as evaluated on a 7-point hedonic scale. Comparable findings were reported by Juemanee *et al*. [2018b], where various Thai unpolished pigmented rice samples achieved an acceptance score of 7 on a 9-point scale. Consumers tend to dislike unpolished rice due to its firm texture and the perception of hay-like and cardboard-like notes, which are regarded as undesirable characteristics [Charoenthaikij *et al*., 2021].

Rate-all-that-apply evaluation

Data of the intensity of sensory attributes of cooked rice are shown in **Table 4**. Based on the evaluation of 29 sensory attributes, nine attributes exhibited significant differences (p<0.05), comprising six appearance attributes, two aroma attributes, and one texture attribute.

Among the appearance attributes, lightness, yellowish white, purplish black, brownish red, uniformity, and roughness showed notable differences across the samples. Brown rice typically exhibited higher lightness, a yellowish white color, and lower roughness. In contrast, red and black rice demonstrated distinctive brownish red and purplish black colors, respectively, with higher roughness and lower lightness. These findings are consistent with previous studies indicating that color parameters of brown rice tend to have higher L^* (lightness) and b^* (yellow-blue) values [Pramai & Jiamyangyuen, 2016]. Color of red rice showed the highest a^* (red-green) values, while black rice had the lowest L^* values [Pramai & Jiamyangyuen, 2016]. The differences in appearance among rice samples are influenced by the accumulation of phenolic compounds in the bran layer [Kaur *et al*., 2018; Shao *et al*., 2018]. Moreover, the abundant anthocyanin content in red and black rice strongly correlates with their distinct coloration [Shao *et al*., 2018]. Meanwhile, the appearance characteristics of cooked rice blends consisting of two rice types reflected traits similar to the individual rice types, although with lower uniformity than the single-cooked rice samples.

Table 4. Intensity of sensory attributes of organic cooked rice.

Attributes		Brown rice	Red rice	Black rice	Brown-red rice	Brown-black rice	Red-black rice	*p*-Value
Appearance	Lightness	4.26^{a}	2.36^{b}	0.40^{d}	2.83^{b}	1.41^{c}	0.89^{cd}	<0.0001
	Yellowish white	4.24^{a}	1.21^{c}	0.04^{e}	2.49^{b}	0.69^{cd}	0.36^{de}	<0.0001
	Purplish black	0.00^{d}	1.01^{c}	4.66^{a}	0.57^{cd}	3.57^{b}	4.03^{ab}	<0.0001
	Brownish red	0.03^{c}	3.21^{a}	2.37^{ab}	2.06^{b}	2.36^{b}	2.49^{ab}	<0.0001
	Uniformity	3.39^{a}	2.41^{bc}	3.20^{ab}	2.10^{c}	1.77^{c}	1.80^{c}	<0.0001
	Roughness	1.87^{cd}	2.43^{abc}	2.57^{ab}	1.77^{d}	1.96^{bcd}	2.59^{a}	<0.0001
	Stickiness	2.76	2.53	2.96	2.43	2.83	2.91	0.079
	Glossiness	1.31	1.50	1.27	1.39	1.20	1.19	0.862
Aroma	Vanilla	0.90	1.16	0.89	1.41	1.06	1.26	0.388
	Floral	0.39^{b}	0.60^{ab}	0.73^{ab}	0.51^{b}	0.63^{ab}	1.26^{a}	0.002
	Beany	1.14	1.20	1.51	1.04	1.27	1.41	0.534
	Cooked rice	3.80^{a}	3.19^{ab}	2.90^{b}	3.39^{ab}	3.13^{ab}	2.76^{b}	0.003
	Planty	1.04	1.36	1.67	1.26	1.34	1.29	0.243
	Grainy	2.69	2.46	2.74	2.74	2.80	2.99	0.441
	Pandan leaf	1.23	1.10	1.10	1.36	1.57	1.53	0.335
	Nutty	1.86	2.06	1.87	2.27	2.33	2.33	0.222
	Sweet aromatic	1.81	1.93	1.87	1.93	1.91	1.89	0.996
	Starchy	3.31	3.17	2.90	3.26	2.91	3.16	0.501
Texture	Roughness	1.86^{c}	2.56^{ab}	2.53^{abc}	1.94^{bc}	2.13^{bc}	2.80^{a}	<0.0001
	Smoothness	2.03^{a}	1.61^{a}	2.01^{a}	2.06^{a}	2.16^{a}	1.57^{a}	0.046
	Stickiness	2.47	2.39	2.51	2.49	2.21	2.40	0.839
	Hardness	2.01	2.21	1.94	1.74	1.73	2.33	0.123
	Chewiness	2.10	2.11	1.83	2.40	2.07	2.04	0.260
	Moisture absorption	2.60	2.46	2.47	2.64	2.29	2.33	0.448
Taste/Flavor	Bitter	0.17	0.31	0.69	0.19	0.33	0.41	0.058
	Sweet	1.71	1.84	1.59	2.29	1.94	1.83	0.678
	Plain	3.43	2.90	2.97	2.93	2.93	2.87	0.245
	Beany	0.96	0.96	1.27	0.99	1.06	1.10	0.767
	Nutty	1.53	2.00	1.87	2.04	1.99	2.01	0.395
	Grainy	2.29	2.50	2.20	2.57	2.59	2.61	0.462

Values with different small letters in the same row differ significantly ($p<0.05$).

The intensity of aroma attributes of floral and cooked rice and texture attribute of roughness exhibited significant ($p<0.05$) differences between the rice samples. Brown rice showed significantly stronger cooked aroma than black rice and red-black rice blends. Floral aroma intensity was identified as the significantly more pronounced in the red-black rice blend than

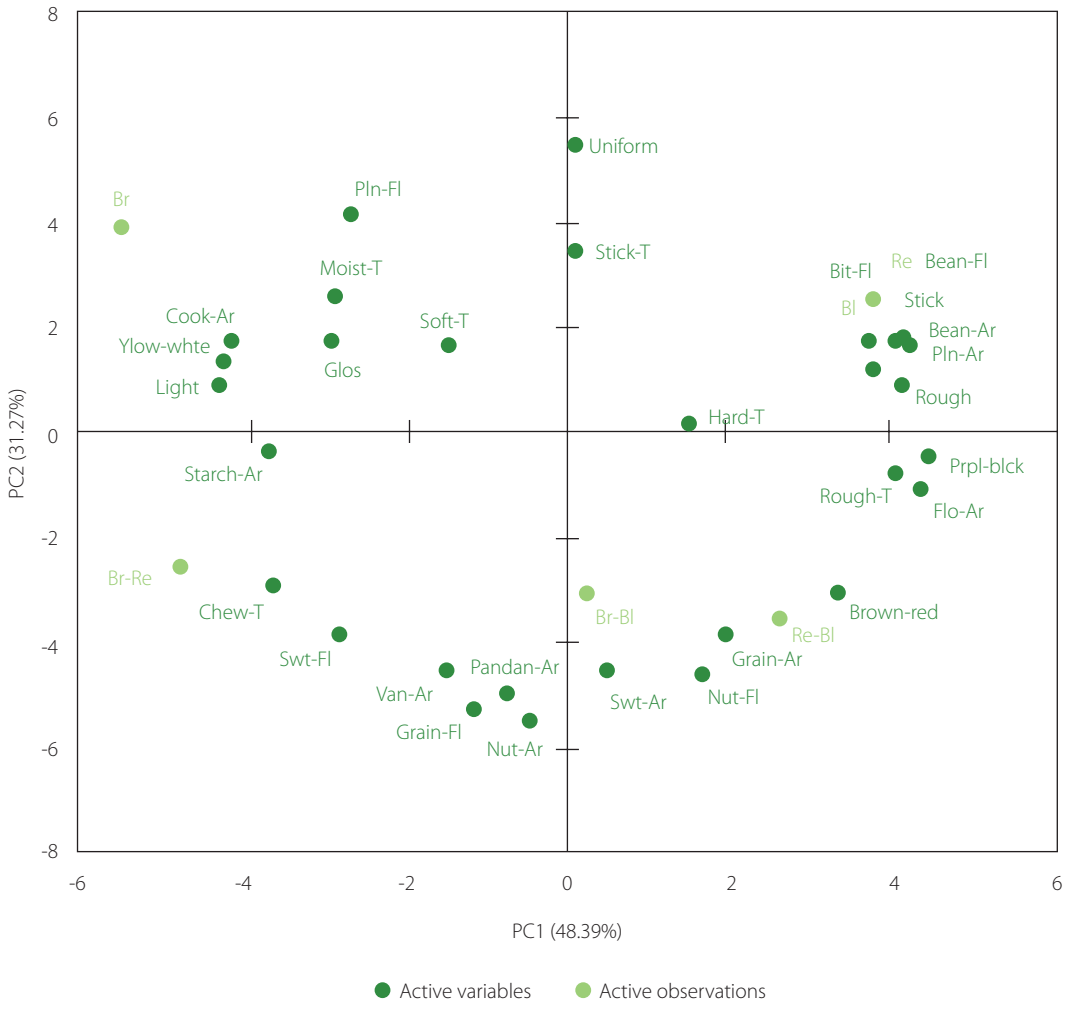

Figure 2. Principal component analysis (PCA) biplot showing the association between cooked rice samples and their sensory attributes. Br, brown rice; Re, red rice; Bl, black rice; Br-Re, brown and red rice blend; Br-Bl, brown and black rice blend; Re-Bl, red and black rice blend. Attribute abbreviations correspond to the full names shown in Table 1.

the brown rice and brown-red rice blend. For the rice texture attributes, red rice, black rice, and their blends demonstrated higher roughness levels. The red-black rice blend exhibited the highest roughness, perhaps due to the panelists' perception of its non-uniform appearance.

In the rate-all-that-apply method, PCA was used to see correlations between samples and their attributes. As shown in **Figure 2**, the cooked rice samples were grouped into clusters based on identical sensory attributes. Brown rice tended to exhibit attributes such as lightness, yellowish-white color, glossiness, cooked aroma, moisture absorption, smooth texture, and typically a plain flavor with floral notes. Meanwhile, cooked red rice and black rice showed the same sensory profile in roughness appearance and stickiness appearance, beany aroma and flavor, and had a planty aroma. Otherwise, the cooked rice from the blends indicated a different sensory profile than that in the single cooked rice. A blend of brown and red rice showed more chewy, sweet in flavor and typically starchy aroma, while the brown and black rice, and red and black rice blend had typical attributes especially in the grainy aroma and nutty flavor.

The sensory profile of cooked rice can be influenced by several factors, such as rice type and variety, physicochemical properties, and post-harvest processing technologies. Juemanee *et al*. [2018b] reported sensory differences between 12 samples of Thai unpolished pigmented rice (black rice, red rice, and glutinous red rice). In their study, consumers generally preferred unpolished rice types with lower hardness, texture and lower glossiness, plumpiness, and bursting appearance. Gondal *et al*. [2021] also emphasized that texture is the most critical sensory attribute in determining the sensory acceptance of cooked rice. Differences in the color of pigmented rice play a less significant role in consumer acceptance, although darker colors are often associated with health-promoting effects [Juemanee *et al*., 2018b]. These findings align with the results of the present study. Although the six rice samples exhibited distinct sensory profiles, particularly in terms of appearance, no significant differences were observed in their hedonic acceptance. This is perhaps due to all six samples having similar hardness intensity as perceived by the panelists (**Table 4**). Therefore, improving the texture quality of unpolished

Figure 3. Frequency (%) of panelists' evaluations for emotion (**A**), need-state (**B**), and environmental-state (**C**) attributes across organic cooked rice samples. For attributes labeled with (*), significant differences between rice samples were determined at $p<0.05$.

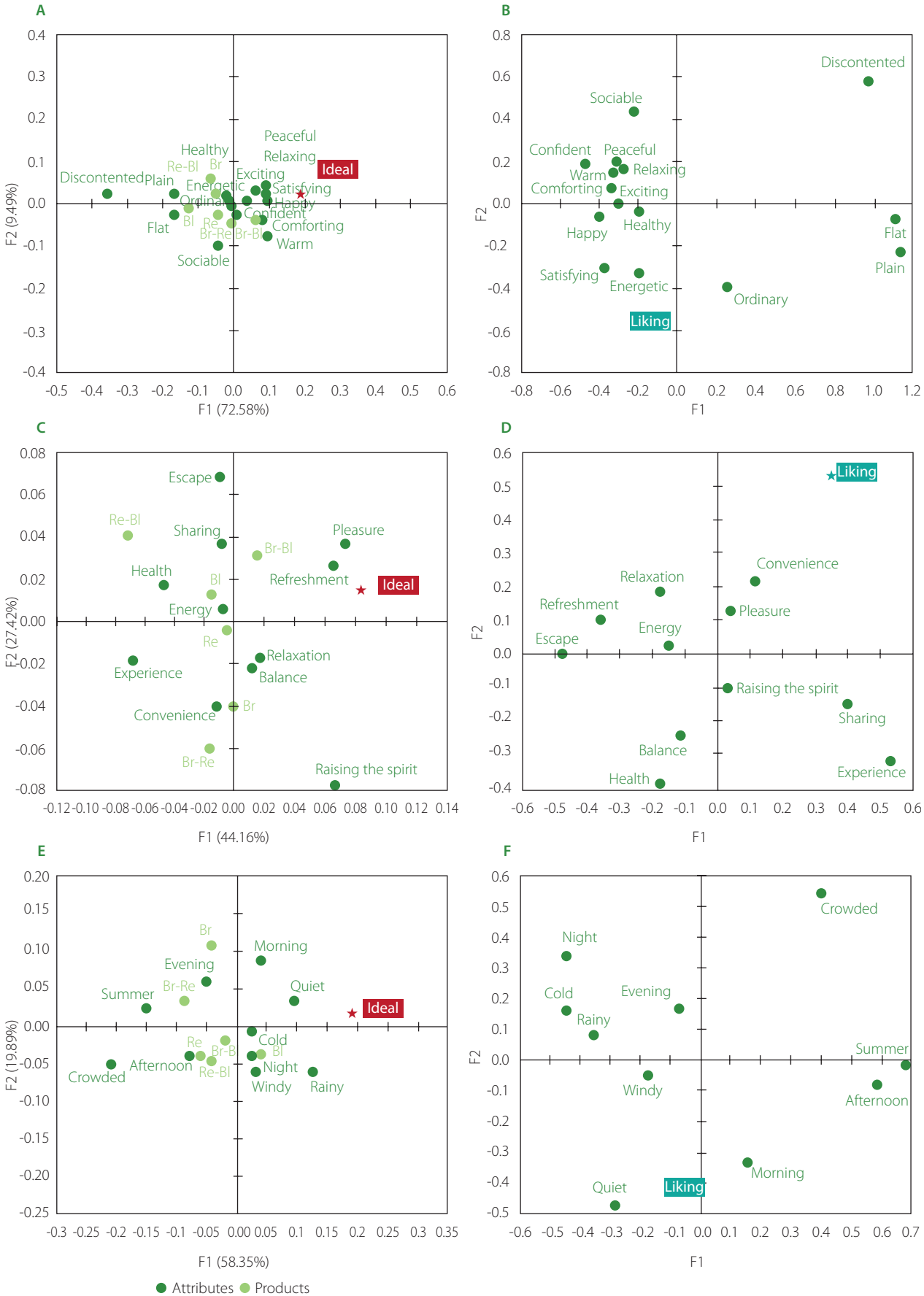

Figure 4. Plots of correspondence analysis (CA) and principal coordinate analysis (PCoA) of cooked rice. (**A**) CA of emotion attributes, (**B**) PCoA of emotion attributes, (**C**) CA of need-state attributes, (**D**) PCoA of need-state attributes, (**E**) CA of environmental-state attributes, (**F**) PCoA of environmental-state attributes. Br, brown rice; Re, red rice; Bl, black rice; Br-Re, brown and red rice blend; Br-Bl, brown and black rice blend; Re-Bl, red and black rice blend.

rice is crucial for enhancing its overall consumer acceptance in the future.

Emotional attribute profiles of unpolished organic cooked rice

The results of the emotional sensory mapping analysis for six unpolished organic cooked rice samples included 15 emotional attributes, 11 need-state attributes, and 10 environmental-state attributes (**Figure 3**). Overall, both individual cooked rice samples and their blends exhibited similar patterns. The panelists predominantly selected emotional attributes such as: ordinary, satisfying, energetic, healthy, happy, peaceful, and plain; need-state attributes including relaxation, energy, balance, sharing, and health; and environmental-state attributes such as evening, morning, and quiet, each chosen by more than 50% of the panelists. The testing using Cochran's Q test showed that the samples differed significantly ($p<0.05$) in two emotional attributes (comforting and plain) and three environmental-state attributes (afternoon, evening, and morning). However, no significant differences ($p\geq0.05$) were observed in the need-state profiles across the samples.

Plots of correspondence analysis (CA) and principal coordinate analysis (PCoA), which were performed to explore the correlation between products, measured attributes, liking points, and the ideal product are shown in **Figure 4**. The results of the chi-square significance test between the products and attributes indicated that the profiles of emotion, need-state, and environmental-state showed no significant associations ($p\geq0.05$). This suggested the absence of strong correlations between the tested products and the evaluated attributes. From the CA and PCoA graphs, it was evident that none of the products closely aligned with the ideal product or the desired liking based on the tested emotional, need-state, and environmental-state profiles.

According to the CA analysis for emotional profiles (**Figure 4A**), an ideal product was characterized by attributes such as happy, satisfying, and relaxing However, none of the cooked rice samples exhibited an emotional profile similar to the ideal product. All products were positioned relatively close to one another, although in different quadrants. The brown-black rice blend was associated with emotional attributes such as exciting, comforting, confident and warm. Cooked rice samples of red, black, and brown-red blend were linked to flat and sociable emotions, whereas black and red-black cooked rice were associated with energetic, healthy and ordinary emotions.

The CA analysis for need-state profiles also showed no strong associations between the ideal profile and the tested products (**Figure 4C**). Consumers tended to prefer an ideal product that provided pleasure and refreshment over one associated with health benefits. While the six products exhibited diverse need-state attributes, none was positioned close to the ideal product. A similar trend was observed in the environmental-state profiles, where no ideal environmental profile was identified by panelists (**Figure 4E**). All samples were distant from the ideal product and clustered into three distinct groups.

The first two dimensions of PCoA were sufficient to interpret the relationships among attributes. The PCoA results showed no association between liking and the attributes in the emotion, need-state, and environmental-state profiles (**Figure 4**). This interpretation was supported by the lack of proximity between liking and the attributes within each profile.

Analyzing the emotional, need-state, and environmental-state profiles of food products provides valuable insights. Emotion is a critical factor influencing consumers' purchasing decisions [de Melo *et al*., 2021; Hunaefi *et al*., 2023]. Need-states represent psychological needs and self-perceptions, forming the basis for marketing strategies that align products with consumers' needs [Clark *et al*., 2021; Hunaefi *et al*., 2023]. Additionally, environmental factors serve as external influencers that shape the context of consumer behavior, eliciting specific experiential feelings [Jariyah *et al*., 2024].

This study on organic cooked rice products presents intriguing findings, indicating that the ideal product and liking levels for cooked unpolished rice were not strongly dependent on emotional profiles. Nevertheless, the analysis provided valuable insights into the types of emotional, need-state, and environmental-state attributes that influence product liking, which can be further explored through penalty analysis.

Penalty analysis identifies sensory attributes that may decrease or increase consumer preference. The classification results of penalty analysis are divided into five groups: must-have, nice-to-have, does not influence, does not harm, and must-not-have. The must-have and nice-to-have attributes based on the penalty analysis are shown in **Figure 5**. It is evident that the attributes represented by P(No)/(Yes), a condition in which the attributes are absent in the product but desired in the rice sample, are located in the upper-right quadrant, indicating that these must-have attributes should be present in the product but are currently missing. Penalty analysis identified "must-have" attributes that enhance liking, including emotional attributes such as satisfying, happy, peaceful, comforting, exciting and relaxing; need-state attributes such as relaxation, refreshment, energy, balance, sharing, pleasure, and raising the spirit; and the morning environmental-state attribute.

The findings of this study provide valuable insights for the development of unpolished rice products, particularly in enhancing consumer acceptance and improving some sensory attributes. Technological approaches such as germination, pre-gelatinization, and partial milling could be considered to refine the texture and taste of unpolished rice. Additionally, blending unpolished rice with polished rice at specific ratios may serve as an alternative strategy to enhance sensory properties while retaining its functional benefits. Furthermore, consuming unpolished cooked rice in combination with various condiments and side dishes may positively influence both sensory acceptance and emotional perception.

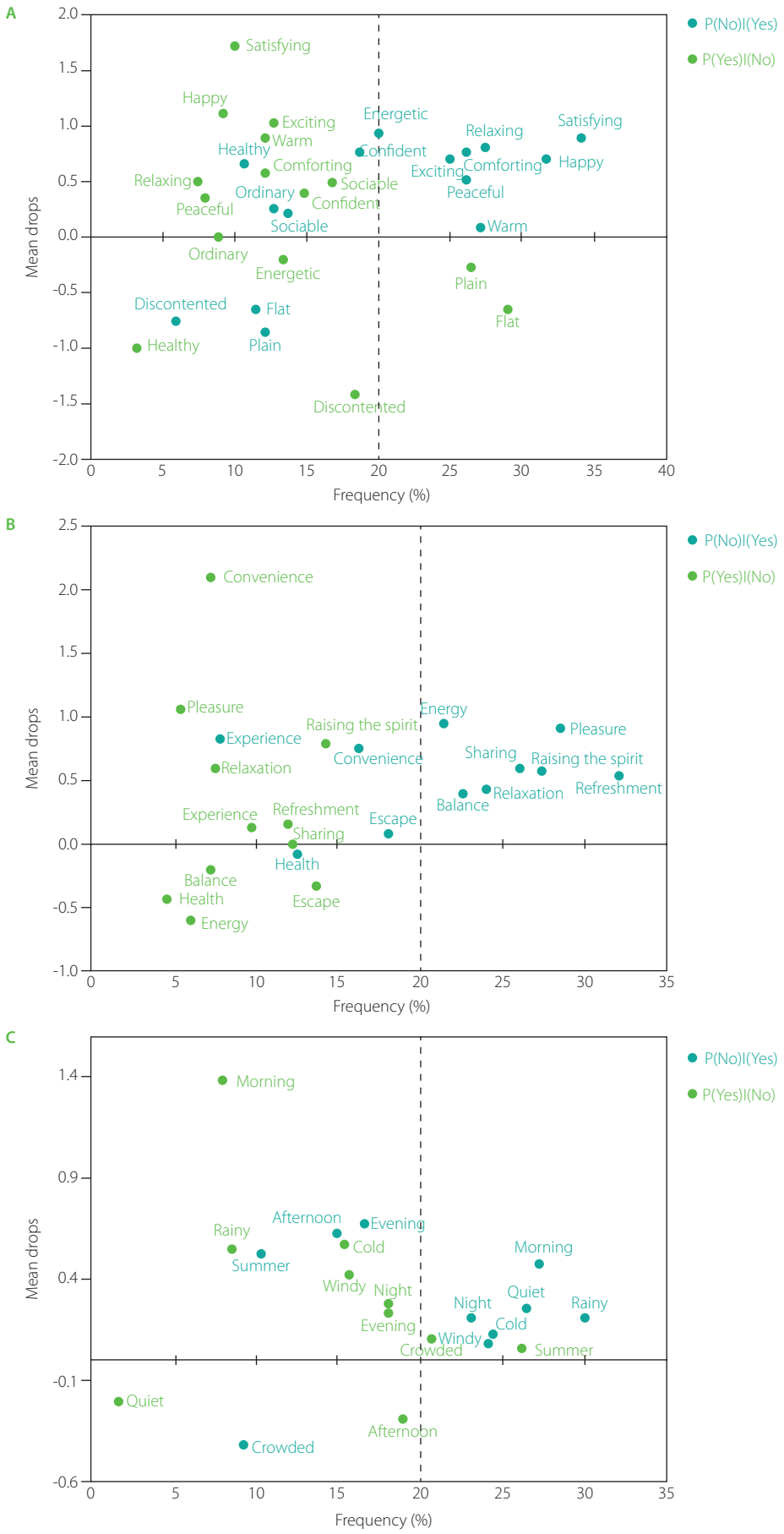

Figure 5. Mean drop in (**A**) emotion attributes, (**B**) need-state attributes, (**C**) environmental-state attributes as a function of the frequency of panelists' evaluations that checked an attribute differently than for the ideal products. P(No)/(Yes) refers to a condition in which a sensory attribute is absent in the tested product but is desired in the ideal product; P(Yes)/(No) represents a condition in which a sensory attribute is present in the tested product but absent in the ideal product.

CONCLUSIONS

The variations in types of unpolished organic rice were associated with differences in proximate characteristics, pasting properties, and texture profiles. Hedonic perceptions of consuming unpolished organic cooked rice, whether as individual types (brown, red, or black rice) or as blends (brown-red, brown-black, and red-black), did not show significant differences. However, sensory profiling revealed some variations in attributes such as color, uniformity, roughness, floral and cooked aroma, and rough texture. Additionally, the findings highlighted that consumer preference for unpolished organic cooked rice was not strongly influenced by its emotional profile. These findings provide a valuable foundation for the future development of unpolished rice products. Optimizing unpolished rice processing technologies is essential to enhance sensory attributes, improve consumer acceptability, and influence both sensory perception and emotional response to unpolished rice.

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CONFLICT OF INTERESTS

The authors state no conflict of interests.

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Enhancing Beef Meat Emulsion: The Role of Banana Peel Albedo Powder

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This study aimed to evaluate the effects of banana peel albedo powder (BPAP) on the physicochemical and microstructural properties of beef meat emulsions. The emulsions were formulated with BPAP added at levels of 0% (control), 0.5%, 1.0%, and 1.5% of the weight of the raw beef used. Due to the formulation process, the final BPAP contents in the emulsions were 0%, 0.013%, 0.025%, and 0.038% (*w/v*), respectively. BPAP, 100 g, was found to contain 25.19 g insoluble and 6.20 g soluble dietary fiber. Among the minerals analyzed, calcium and zinc showed the highest content in BPAP. Incorporation of BPAP at a level of 1.5% of raw beef (*w/w*) to the emulsion resulted in the higher water holding capacity and the lowest cooking loss compared to control. Furthermore, the addition of BPAP up to a 1.0% of raw beef (*w/w*) level enhanced the emulsion capacity, emulsion stability, and apparent viscosity of the samples. Confocal laser scanning microscopy micrographs revealed that the emulsions containing BPAP at 0.5% and 1% of raw beef (*w/w*) exhibited a more stable and homogeneous microstructure. These findings suggest that BPAP, particularly at 1.0% of raw beef (*w/w*) level (0.025% in emulsion, *w/v*), can be effectively utilized as a natural functional ingredient to improve the quality of meat emulsions in food formulations.

Keywords: beef meat, dietary fiber, emulsion, food by-product, functional meat

INTRODUCTION

Emulsion-type meat products come into existence through the emulsification of muscle proteins, lipids, water, and assorted non-meat additives. The emulsification mechanism is explained by two hypotheses. The initial theory, termed oil-in-water, involves the formation of a myofibrillar protein film around fat particles to prevent the accumulation of fat globules. The second theory, known as the physical entrapment theory, explains that fat globules are embedded in a protein network and that the stabilization of fat particles occurs through the myofibrillar protein gel network [Gordon *et al*., 1992]. To prepare meat emulsion, myofibrillar proteins, which can be soluble in water with salt and act as emulsifiers, are first extracted from lean meat through the chopping process, then animal fat is also finely chopped and added to the meat batter. The chopping process is used to produce a meat emulsion from protein extracted from lean meat, fat and water [Gordon *et al*., 1992; Zhao *et al*., 2019]. The fat particles stabilized in this matrix affect the sensory properties, technological characteristics (water-holding capacity, cooking yields) and textural attributes of the meat [Kumar *et al*., 2016; Zhao *et al*., 2019]. However, concerns about the adverse health effects of excessive fat consumption have prompted extensive research around the world into the production of low-fat and healthier meat products [Chappalwar *et al*., 2020; Choi *et al*., 2014; Kumar *et al*., 2016].

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One of the ways to produce meat products that are both healthy and functional is to use different plant materials rich in dietary fiber as a functional ingredient [Das *et al*., 2020]. Consuming sufficient dietary fiber has been reported to reduce the risk of developing some chronic diseases, such as colon cancer, obesity and cardiovascular disease, by lowering cholesterol and triglyceride levels [WHO/FAO, 2003]. Various dietary fibers, with different non-starch polysaccharide compositions (pectins, hemicelluloses, lignins, *etc*.), show not only positive effects on health, but also have different technological characteristics, including gelling properties, that can improve the emulsification, and can influence the sensory rheological, and the desirable textural properties of processed meat products [Das *et al*., 2020; Unal *et al*., 2022].

The banana is widely recognized as one of the most commonly consumed tropical fruits, known for its high carbohydrate, dietary fiber, mineral, and vitamin content. It is also rich in several compounds with antioxidative activity, such as carotenoids, phenolics, ascorbic acid, and tocopherols [Singh *et al*., 2016]. The banana peel, a major by-product of the food industry, accounts for approximately 30% of the fruit's total weight [Schieber *et al.,* 2001]. Many studies have highlighted the potential of banana peels, which are a burden on the environment, to be used in the food industry, especially in the production of bakery products and pasta [Gomes *et al*., 2022; Puraikalan, 2018; Salama *et al*., 2019], although recently also a meat product (chicken sausage) with the addition of banana peel powder has been formulated [Zaini *et al*., 2020]. They are of interest due to a high dietary fiber content (40–50 g/100 g dry matter, dm), mainly the insoluble fraction, which accounts for 75% of the total dietary fiber [Emaga *et al*., 2007]. Another component of banana peels, important in terms of their use as a food ingredient, are phenolic compounds, including dominant proanthocyanidins with a high degree of polymerization, procyanidin dimers, flavan-3-ols and flavonol glycosides, which are responsible for their higher antioxidative potential compared to banana pulp [Rebello *et al*., 2014; Someya *et al*., 2002]. Banana peels are also a source of other bioactive compounds like carotenoids and biogenic amines [Pereira & Maraschin, 2015]. Additionally, they contain essential minerals like potassium, protein (8–11 g/100 g dm), polyunsaturated fatty acids, and essential amino acids [Emaga *et al*., 2007]. Despite attempts to use this cheap and easily accessible material in food production, there is a lack of research on the use of banana peel albedo as a functional ingredient in emulsion-type meat products. Therefore, this study aimed to explore the effects of banana peel albedo powder (BPAP) on the technological and microstructural properties of beef meat emulsions.

MATERIALS AND METHODS

Banana peel albedo powder preparation

Banana fruits (approximately 5 kg) were collected from the Mediterranean Region of Türkiye (Antalya). The banana albedo used in this study was obtained by carefully removing the white inner layer (albedo) from the banana peel surrounding the fruit. The albedo was then air-dried at room temperature (at approximately 25°C) for 48 h. After drying, the albedo was pulverized using a grinder and sifted through a 60-mesh screen to obtain a fine powder. The resulting banana peel albedo powder (BPAP) was stored in airtight packaging at 15±2°C in cold storage until further analysis.

Preparation of beef meat emulsions

Fresh raw beef, with the moisture content of 72.01±0.25 g/100 g, protein content of 19.21±0.08 g/100 g, fat content of 8.29±0.47 g/100 g, and pH of 5.89±0.01, was purchased from a butcher in Konya, Türkiye. To prepare the beef meat emulsions, the methodology detailed in our previous study [Unal *et al*., 2022] was used, with slight modifications such as mixing time and oil temperature. To this end, 25 g of raw ground beef without BPAP (0.0%, control) or with BPAP at different levels (0.5%, 1.0%, and 1.5% of the weight of raw ground beef, *w/w*) were mixed with 100 mL of an NaCl (2.5%) + K_2HPO_4 (0.5%) solution and blended for 120 s in a Waring Commercial 8011 blender (Stamford, CT, USA), resulting in four separate slurries. In the next step, the slurries (12.5 g) were blended with 37.5 mL of an NaCl (2.5%) + K_2HPO_4 (0.5%) solution and 50 mL of corn oil (9°C). An oil was added at a stable rate using a system described by Ockerman [1985] with a cold water-jacketed burette (4°C). The dispersion was carried out at 13,000 rpm for 20 s until a uniform mixture was obtained, ensuring consistent texture. The BPAP contents in the final emulsions were 0.0%, 0.013%, 0.025%, and 0.038% (*w/v*), respectively.

The experiment was conducted with three replications, *e.g.*, emulsions for each formulation were prepared separately in triplicate, and all analyses were conducted on the three independent emulsions for each formulation. The prepared emulsions were stored under controlled conditions until further analysis.

Determination of dietary fiber content and mineral profile of banana peel albedo powder

The dietary fiber content (soluble and insoluble) of BPAP was determined according to the AACC International method [AACC, 1999]. The soluble and insoluble dietary fiber contents were expressed in g/100 g BPAP.

For the determination of mineral content, 1 g of dry BPAP was subjected to wet ashing using sulfuric acid and hydrogen peroxide, as described by Babiker *et al.* [2021]. The mineral content in the resulting ash was analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Varian-Vista ICP-AES instrument (Varian, Inc., Mulgrave, Victoria Australia) was used, which operated under the following conditions: radio frequency plasma power was maintained at 0.7–1.5 kW (1.2–1.3 kW for axial); plasma Ar flow rate was 10.5–15 L/min (radial) and 15 L/min (axial); auxiliary Ar flow rate was kept at 1.5 L/min; the viewing height was 5–12 mm; the copy and reading time was set at 1–5 s (max. 60 s), and the copy time was 3 s (max. 100 s) [Babiker *et al.,* 2021]. Quantification of minerals was carried out using data on similar minerals obtained by analysis of certified reference samples from the National Institute of Standards and Technology

(NIST; Gaithersburg, MD, USA) [Skujins, 1998]. The mineral contents were expressed as mg *per* 100 g of BPAP.

Determination of total phenolic content, total flavonoid content and antioxidative activity of banana peel albedo powder

The procedure suggested by Cetin-Babaoglu *et al*. [2024] was modified to acquire the extract from the BPAP to determine the total phenolic content (TPC), total flavonoid content (TFC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, and ferric reducing antioxidant power (FRAP). Five grams of ground BPAP were suspended in 100 mL of 70% methanol (*v/v*) for this purpose, and incubated for 1 h at 25°C in a shaking water bath (250 rpm). Following incubation, the supernatant was separated using centrifugation at 13,400×*g* for 10 min.

To determine the TPC, 0.1 mL of the extract, 0.4 mL of distilled water, and 2.5 mL of a 10% Folin-Ciocalteu's reagent (*v/v*) were placed in a tube and kept in the dark for 2 min [Škerget *et al*., 2005]. The sample tube was then filled with 2 mL of a 7.5% Na_2CO_3 (*w/v*) solution and incubated at 50°C for 5 min. The absorbance of the reaction mixture was measured at 760 nm using a UV–Vis spectrophotometer (UV-160 A, Shimadzu, Tokyo, Japan). The TPC of the BPAP was ascertained using a gallic acid standard curve, and results were expressed as mg gallic acid equivalent (GAE)/g.

To determine the TFC, 150 µL of 5% $NaNO_3$ (*w/v*), 2 mL of distilled water, and 0.5 mL of the extract were put in a tube and left in the dark for 6 min [Eyiz *et al*., 2020]. The sample tube was then filled with 200 µL of distilled water, 2 mL of 1 M NaOH, and 150 µL of a 10% $AlCl_3$ (*w/v*) solution, and it was left to stand for 15 min. The absorbance of the mixture was measured at 510 nm using a spectrophotometer (UV-160 A, Shimadzu). The TFC of the BPAP was calculated using a catechin standard curve, and results were expressed as mg catechin equivalent (CE)/g.

The DPPH radical scavenging activity of the BPAP was determined using the method described by Brand-Williams *et al*. [1995]. Briefly, the BPAP extract (50 µL) was added to 2,950 µL of a 100 µM methanolic DPPH radical solution, and the reaction mixture was incubated in the dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer (UV-160 A, Shimadzu). The DPPH radical scavenging activity of BPAP was expressed as Trolox equivalents (µmol Trolox/g), calculated using a calibration curve prepared with standard Trolox solutions.

Then, 10 mL of a 2,4,6-tri(2-pyridyl)-*s*-triazine solution (10 mM in a 40 mM hydrochloric acid solution), 100 mL of sodium acetate buffer (300 mM, pH 3.6), and 10 mL of a ferric chloride hexahydrate solution (20 mM) were combined to achieve the FRAP reagent [Benzie & Strain, 1996]. After that, 225 µL of distilled water, 2.25 mL of the FRAP reagent, and 75 µL of the extract were vortexed and allowed to stand at room temperature for 30 min. The samples' absorbance was measured at 593 nm. Iron(II) sulfate heptahydrate was used to plot the standard curve, and the findings were reported as mg Fe^{2+}/kg BPAP.

pH measurement of beef meat emulsions

The pH of the beef meat emulsions was measured following the procedure outlined by Zorba *et al*. [1993]. A pH meter (WTW series pH 720, Weilheim, Germany) was used to assess the pH values. To this end, 10 g of each emulsion sample was homogenized with 100 mL of distilled water, and the pH was directly measured after the calibration of the instrument with standard buffer solutions at pH 4.0 and 7.0. The readings were taken immediately after calibration to ensure accuracy.

Measurements of color coordinates of beef meat emulsions

The color coordinates of the raw and cooked (cooking conditions as for determining cooking loss) beef meat emulsions were measured in the CIELab space using a Minolta chromameter CR-400 (Konica Minolta, Osaka, Japan), in accordance with the method described by Hunt *et al*. [1991]. The instrument had a measurement area diameter of 8 mm and a range of values from 0 to 100 for L^* (lightness), ±60 for a^* (red/green), and ±60 for b^* (yellow/blue). The measurements were taken under D65 illuminant with a 2° observer angle, ensuring high accuracy for surface color analysis. Each sample was measured five times to ensure consistency, and the average values were recorded. The results reflect the surface color characteristics of the emulsions, providing comprehensive data on their visual appearance.

Determination of beef meat emulsion cooking loss and water holding capacity

The cooking loss (CL) of the beef meat emulsions was determined following the method described by Mejia *et al.* [2018]. For each formulation, exactly 30 g of the emulsion were portioned into 50 mL polypropylene tubes. The samples were then subjected to thermal treatment in a water bath (Nüve, Ankara, Türkiye), beginning at 50°C and gradually increasing the water temperature until the internal temperature of each sample reached 72°C, which was monitored using a thermometer probe. Following heating, the tubes were cooled in ice water for 5 min, inverted, and stored at 4°C for 14 h to collect the released exudate. The difference in weight before and after cooking was used to determine CL, expressed as a percentage based on the initial sample mass.

The water holding capacity (WHC) of the beef meat emulsions was evaluated using a modified version of the method by Hughes *et al.* [1997]. Ten grams of uncooked emulsion batter were placed into glass jars and heated in a 90°C water bath for 10 min. After cooling to room temperature, the samples were wrapped in cotton cheesecloth and centrifuged at 7,245×*g* for 15 min. WHC was determined by measuring the amount of water retained after heating and centrifugation, relative to the total moisture content of the sample. Results were expressed as a percentage of retained water.

Analysis of emulsifying properties of beef meat emulsions

The emulsifying properties, including emulsifying capacity (EC) and emulsion stability (ES), were determined according to

the method of Ockerman [1985]. The endpoint for emulsification was identified using the procedure described by Webb *et al*. [1970]. To measure EC, the corn oil was added to the beef meat emulsion from a burette, and the electrical conductivity was simultaneously monitored by an ohmmeter (YX-360TR N Multitester Fuse and Diode Protection, Sunwa, Tokyo, Japan) with an electric potential (mV) recorder (Labsco Laboratory Supply, Louisville, KY, USA). At the emulsification endpoint, the conductivity suddenly decreased, a sudden reduction in resistance was observed in the ohmmeter reading. The oil addition was stopped, and the total volume of emulsified oil (added to beef meat emulsion) was recorded from the burette. EC was calculated as mL of oil retained *per* g of protein. The protein content of the beef meat was determined by the Kjeldahl method [AACC, 1999].

To determine ES, an emulsion with corn oil was formed as described above. However, the emulsification was stopped when 110 mL of oil was used. Then, 20 g of the emulsion were weighed into test tubes and heated in a water bath (Nüve) at 80°C until the core temperature reached 72°C. The tubes were centrifuged and drained into a volumetric cylinder for 10 h to collect the unbound oil and water. ES was calculated according to Equation (1):

$$ES\ (\%) = 100 - (SO + SW) \quad (1)$$

where: SO is the amount of separated oil released by the emulsion and SW is the amount of separated water released by the emulsion [Ockerman, 1985].

Determination of apparent viscosity, flow behavior index and consistency index of beef meat emulsions

Apparent viscosity (AV) of the newly formed beef meat emulsions (approximately 25 g), kept at a constant temperature of 20°C using a thermostatically controlled water bath, was measured at four different rotational speeds (10, 20, 50, and 100 rpm) using a rotational viscometer (Brookfield, DV2TRVTJ0, Middleborough, MA, USA). Then, the flow curves, *e.g.*, apparent viscosity *vs.* shear rate, were plotted. Using linear regression analysis, the data were fitted to the power law model presented in Equation (2) to determine the flow behavior index (n), consistency index (k), and determination coefficients (r^2) [Barnes *et al*., 1989]:

$$\eta_a = k \times \dot{\gamma}^{(n-1)} \quad (2)$$

where: η_a is the apparent viscosity (mPa×s), k is the consistency index (Pa×s^n), $\dot{\gamma}$ is the shear rate (1/s), and n (dimensionless) is the flow behavior index. For $n<1$, the fluid is pseudoplastic (shear-thinning); for $n>1$, the fluid is dilatant (shear-thickening).

Texture profile analysis of beef meat emulsions

Texture profile analysis (TPA) of the beef meat emulsions was performed using a Stable Micro Systems Texture Analyser (model: TA.XT Plus, Godalming, UK). For each formulation, exactly 30 g of the emulsion were portioned into 50 mL Falcon tubes, which were then cooked in a water bath (Nüve) at 80°C until the core temperature reached 72°C. This process took approximately 20 min. After cooling to room temperature, the samples were removed from the tubes and cut into cylindrical shapes with a diameter equal to the tube's internal diameter (28 mm) and a height of 15 mm for TPA measurements. Seven representative samples were randomly selected from each analyzed emulsion to ensure adequate representation of the overall texture profile. A cylindrical plate with a diameter of 36 mm and a 50 kg load cell were used. Each sample was compressed twice, with a 0.1 s delay between the descents, at a distance of 5 mm. The following test parameters were applied: pre-test speed of 1 mm/s, test speed of 5 mm/s, post-test speed of 5 mm/s, and 50% compression. TPA was conducted at room temperature (21°C). The following textural parameters were determined: hardness (N), adhesiveness (N×s), springiness (mm), cohesiveness, and gumminess (N). Hardness was determined by the maximum force required to compress the sample, cohesiveness was calculated as the ratio of the positive force area during the second compression to that during the first compression, and springiness was determined as the distance the sample recovered after the first compression. Adhesiveness was calculated as the negative force area during the first compression, while gumminess was defined as the product of hardness and cohesiveness [Herrero *et al*., 2007].

Microstructural analysis of beef meat emulsions

Microstructural analysis, including the acquisition of confocal laser scanning microscope (CLSM) images, was conducted using a Nikon A1R1 instrument (Nikon, Tokyo, Japan), equipped with a helium/neon laser at the fluorescence excitation of 520–530 nm in accordance with the method specified by Zhu *et al*. [2018]. The resolution of the images was 1,024×1,024 pixels, and 10 images were captured for each sample.

Statistical analysis

The differences were considered significant at $p<0.05$. Results related to the BPAP itself, such as dietary fiber content, mineral profile, total phenolic content, total flavonoid content, and antioxidative activity, were expressed as mean ± standard deviation (SD). In contrast, results for the emulsions (including both BPAP-added and control samples) were presented as mean ± standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among the beef meat emulsions with different contents of BPAP. The statistical analysis was performed using Minitab software, version 16.0 (Minitab, LLC, State College, PA, USA). Post-hoc comparisons were made using Tukey's honestly significant difference (HSD) test. The assumption of normality and homogeneity of variances was checked prior to conducting ANOVA.

RESULTS AND DISCUSSION

Dietary fiber and mineral contents of banana peel albedo powder

The dietary fiber content as insoluble and soluble fractions of banana peel albedo powder is shown in **Table 1**. The content of insoluble dietary fiber of BPAP was found as 25.19 g/100 g.

Table 1. Mineral composition, dietary fiber content, total phenolic content (TPC), total flavonoid content (TFC) content, and antioxidative activity of banana peel albedo powder (BPAP).

Parameter	Content/activity
Ca (mg/100 g)	3.85±0.11
Fe (mg/100 g)	0.27±0.01
K (mg/100 g)	90.83±2.05
Mg (mg/100 g)	2.85±0.04
Na (mg/100 g)	1.11±0.04
P (mg/100 g)	9.47±0.13
Zn (mg/100 g)	0.10±0.01
Insoluble dietary fiber (g/100 g)	25.19±1.23
Soluble dietary fiber (g/100 g)	6.20±0.01
DPPH radical scavenging activity (µmol Trolox/g)	97.90±10.29
FRAP (µmol Fe^{+2}/g)	45.40±0.01
TPC (mg GAE/g)	9.16±8.43
TFC (mg CE/g)	2.67±0.74

Results are shown as mean ± standard deviation. DPPH radical, 1,1-diphenyl-2--picrylhydrazyl radical; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; CE, catechin equivalent.

Moreover, its soluble dietary fiber was determined as 6.20 g/100 g. Emaga *et al*. [2007] analyzed the dietary fiber content in banana and plantain peels from different varieties and fruits of different ripeness and reported higher soluble and insoluble dietary fiber content in dessert banana peels (36.3–42.9% and 6.8–13.7%, respectively) compared to the content of these fractions in powdered banana peel albedo found in our study. In another study, the soluble and insoluble dietary fiber content of banana peels was found as 9.60 and 32.00%, respectively [Salama *et al*., 2019]. In turn, Zaini *et al*. [2020] prepared banana peel powder with total dietary fiber content of 44.03%.

The mineral content of banana peel albedo powder is presented in **Table 2**. Contents of individual minerals were found as: 3.85 mg/100 g for calcium, 0.27 mg/100 g for iron, 90.83 mg/100 g for potassium, 2.85 mg/100 g for magnesium, 1.11 mg/100 g for sodium, 9.47 mg/100 g for phosphorus, and 0.1 mg/100 g for zinc. Romelle *et al*. [2016] found the Ca, Zn, Fe, and Mn contents of banana peel as 19.86, 1.72, 15.15, and 9.05 mg/100 g dry peel, respectively. It is thought that various factors like soil condition, state of fruit ripeness, plant variety, irrigation regime, soil type, and which part of the fruit is used can cause differences in the mineral contents of a given type fruit sourced from different regions [Bartual *et al.,* 2022; Emaga *et al*., 2007]. However, it was concluded that banana peel albedo is a source of dietary fiber and some minerals, so when used in product formulations, it will increase the dietary fiber and mineral content of the final product.

Table 2. Cooking loss and water holding capacity of beef meat emulsions with banana peel albedo powder (BPAP) added at different levels.

BPAP level (% raw beef, *w/w*)*	Cooking loss (%)	Water holding capacity (%)
0.0 (Control)	10.47±2.61^{a}	15.71±0.41^{b}
0.5	6.10±0.14^{ab}	17.96±0.77^{ab}
1.0	5.24±0.18^{ab}	18.31±0.56^{ab}
1.5	4.76±0.93^{b}	18.95±1.34^{a}

*The final BPAP content in the emulsions was 0.013%, 0.025%, and 0.038% (*w/v*) for BPAP added at 0.5%, 1.0%, and 1.5% of raw beef (*w/w*), respectively. Results are shown as mean ± standard error. Values with different letters (a–b) within the column differ significantly ($p<0.05$).

Total phenolic content, total flavonoid content and antioxidative activity of banana peel albedo powder

The TPC and TFC of BPAP were found as 9.16 mg GAE/g and 2.67 mg CE/g, respectively (**Tab**le 1). The DPPH radical scavenging activity of BPAP was determined to be 97.90 µmol Trolox/g, and the FRAP was 45.40 µmol Fe^{+2}/g.

BPAP was a rich source of phenolic compounds compared to by-products from other tropical fruits, *e.g*., Can-Cauich *et al*. [2017] found a TPC of 210 mg GAE/100 g in mamey sapote peel, Selani *et al*. [2016] reported TPC in guava and passion fruit by-products to be 254.7 and 175.6 mg GAE/100 g, respectively, and the TPC determined by Lopez- Martinez *et al*. [2023] for guava, mamey sapote, and passion fruit peels ranged from 185.2 to 365.7 mg GAE/100 g. Regarding banana fruit, Someya *et al*. [2002] showed that banana peel contained four times more total phenolics than pulp.

In turn, Rebello *et al*. [2014] reported that TPC of banana peel was 29.2 mg GAE/g. According to Salama *et al*. [2019], the banana peel had TPC of 71.685 mg GAE/g. These values were higher than the TPC shown in **Table 1**; however, the whole banana peel was analyzed in the cited studies. To the best of our knowledge, the TPC of only the albedo part of the peel has not been determined so far. In addition to TPC, also the TFC found in our study for the BPAP was lower than that reported for banana peel expressed as rutin equivalents (3.354 mg RE/g) [Salama *et al*., 2019].

Cooking loss and water holding capacity of beef meat emulsions

The results shown in **Table 2** indicate that the incorporation of BPAP into the beef meat emulsion affected its CL and WHC. The WHC tended to increase, while the CL tended to decrease with an increasing level of BPAP in the beef meat emulsion; however, significant differences ($p<0.05$) were found only between the emulsion with the highest level of BPAP (1.5% of raw beef meat, *w/w*, corresponding to 0.038% of emulsion, *w/v*) and the control. These changes could be due to the present of dietary fiber in banana peel. Overall, these findings were in agreement with our previous research, wherein the use of pumpkin

Table 3. pH and color parameters of raw beef meat emulsions with banana peel albedo powder (BPAP) added at different levels.

BPAP level (% raw beef, *w/w*)*	pH	*L**	*a**	*b**
0.0 (Control)	7.25±0.01^{a}	85.57±0.03^{a}	−1.00±0.04^{b}	10.97±0.04^{a}
0.5	7.26±0.01^{a}	85.27±0.05^{a}	−0.41±0.02^{a}	11.07±0.12^{a}
1.0	7.26±0.01^{a}	85.37±0.20^{a}	−0.62±0.16^{ab}	11.11±0.03^{a}
1.5	7.26±0.01^{a}	85.18±0.29^{a}	−0.68±0.15^{ab}	11.02±0.01^{a}

*The final BPAP content in the emulsions was 0.013%, 0.025%, and 0.038% (*w/v*) for BPAP added at 0.5%, 1.0%, and 1.5% of raw beef (*w/w*), respectively. Results are shown as mean ± standard error. Values with different letters (a–b) within the column differ significantly ($p<0.05$). *L**, lightness (0 – black, 100 – white); *a**, redness-greenness (+*a** – red, –*a** – green); *b**, yellowness-blueness (+*b** – yellow, –*b** – blue).

powder decreased the cooking loss of beef by 17.38%–21.99% [Unal *et al*., 2022].

pH value of raw beef meat emulsions

The pH values of raw emulsions were 7.25–7.26 (**Table 3**). BPAP use in emulsion formula had no significant ($p\geq0.05$) effect on pH. There are conflicting reports regarding the pH values of meat products and the addition of different sources of fibers. Fernández-Ginés *et al*. [2003] reported that the pH values of bologna sausages did not change significantly as a result of the addition of citrus fiber. In contrast, Chappalwar *et al*. [2020] reported that the incorporation of banana peel flour in chicken patties caused a decrease in pH. Sarıçoban *et al*. [2008] also found a gradual decrease in pH of model meat emulsions with the increase in the level of lemon albedo added to them.

Color parameters of raw and cooked beef meat emulsions

Color parameters of raw and cooked emulsions are shown in **Table 3** and **4**, respectively. The incorporation of BPAP did not affect the lightness and yellowness of raw emulsions ($p\geq0.05$). The *L** values were in the range of 85.18–85.57 and were higher than those measured for the cooked beef emulsions (71.46–76.13). The cooked emulsion with BPAP added at a level of 1.5% of raw beef meat (*w/w*) was characterized by significantly ($p<0.05$) lower lightness compared to the control. A similar effect of reducing the lightness of corn pasta and extrudate after replacing 5% of flour with banana peel powder was observed by Puraikalan [2018].

Regarding the *a** value, the addition of BPAP caused significant ($p<0.05$) changes only in raw emulsions, for which the *a** values ranged from −1.00 to −0.41 (**Table 3**). The BPAP addition at the level of 0.5% of raw beef meat (*w/w*) resulted in a higher ($p<0.05$) *a** value in raw sample compared to the control emulsion, whereas no significant ($p\geq0.05$) differences in *a** values were observed among the emulsions with BPAP content. Additionally, there was no significant ($p\geq0.05$) effect of BPAP incorporation on *b** values of both raw and cooked emulsions.

Huang & Bohrer [2020] reported that the addition of commercial banana flour at 1%, 2%, and 4% levels increased *a** values and decreased *b** values in beef emulsions after cooking. Conversely, Kumar *et al.* [2013] observed that replacing 3% and 4% of lean meat with green banana flour in cooked chicken nuggets did not affect *a** values, while a 5% substitution significantly reduced them. These findings indicate that banana flours may enhance the red color of cooked and cured meat products, although the effects appear to vary depending on the flour origin.

Table 4. Color parameters of cooked beef meat emulsions with banana peel albedo powder (BPAP) added at different levels.

BPAP level (% raw beef, *w/w*)*	*L**	*a**	*b**
0.0 (Control)	76.13±1.15^{a}	−2.44±0.15^{a}	15.47±0.71^{a}
0.5	76.08±0.47^{a}	−2.28±0.02^{a}	14.56±0.22^{a}
1.0	74.38±0.30^{a}	−2.28±0.03^{a}	14.58±0.53^{a}
1.5	71.46±0.11^{b}	−2.22±0.05^{a}	15.41±0.48^{a}

*The final BPAP content in the emulsions was 0.013%, 0.025%, and 0.038% (*w/v*) for BPAP added at 0.5%, 1.0%, and 1.5% of raw beef (*w/w*), respectively. Results are shown as mean ± standard error. Values with different letters (a–b) within the column differ significantly ($p<0.05$). *L**, lightness (0 – black, 100 – white); *a**, redness-greenness (+*a** – red, –*a** – green); *b**, yellowness-blueness (+*b** – yellow, –*b** – blue).

Beef meat emulsion capacity and stability

The ES and EC of beef meat emulsions with different contents of BPAP are shown in **Figure 1**. The control sample had the lowest EC and ES. Adding BPAP to the emulsions at the levels up to 1.0% of raw beef meat, *w/w* (up to 0.025% of emulsion, *w/v*), gradually increased their EC and ES. A further increase of the additive content caused a decrease of both parameters. Similar results were obtained by dos Santos Alves *et al*. [2016], who investigated the effect of pork skin and green banana flour blends as fat substitutes in bologna-type sausages. They reported greater emulsion stability when up to 60% fat substitute was included to the product. In contrast to our results, Chappalwar *et al*. [2020] found that banana peel powder did not affect the ES of chicken patties.

Apparent viscosity of cooked beef meat emulsions

The flow curves of cooked beef meat emulsions are shown in **Figure 2**. In addition, the parameters of power law model, including flow behavior index and consistency index, determined for all emulsions are presented in **Table 5**. The consistency

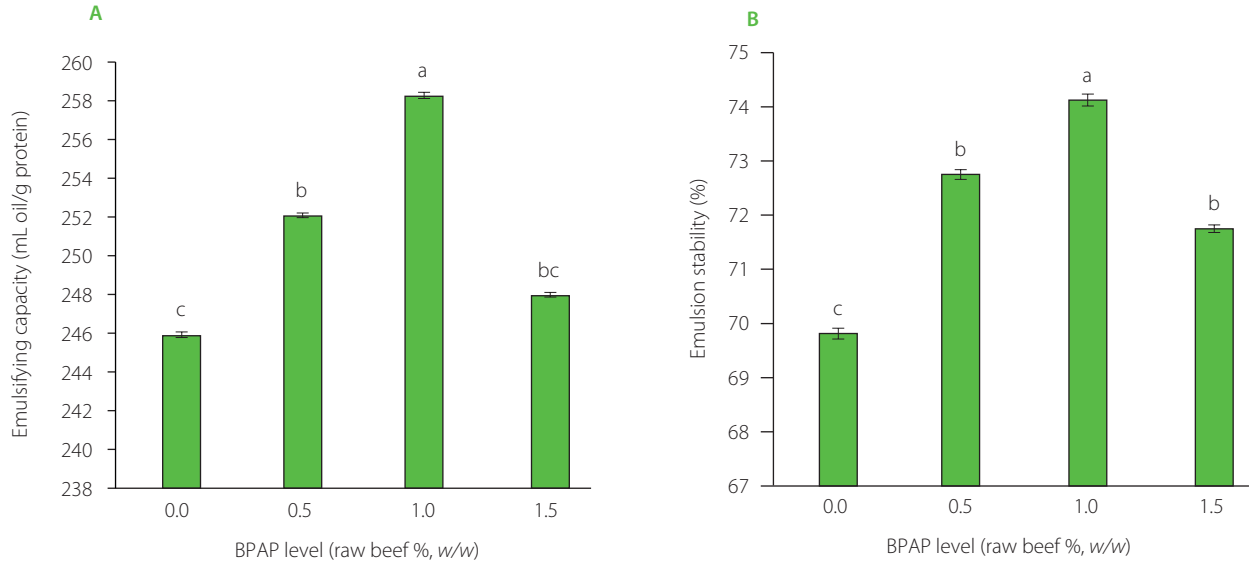

Figure 1. Emulsifying capacity (EC) (**A**) and emulsion stability (ES) (**B**) of beef meat emulsions with banana peel albedo powder (BPAP) added at 0.0%, 0.5%, 1.0%, and 1.5% based on the weight of the raw beef used. The actual contents of BPAP in the emulsions were 0.0%, 0.025%, 0.038%, and 0.075% (*w/v*), respectively.

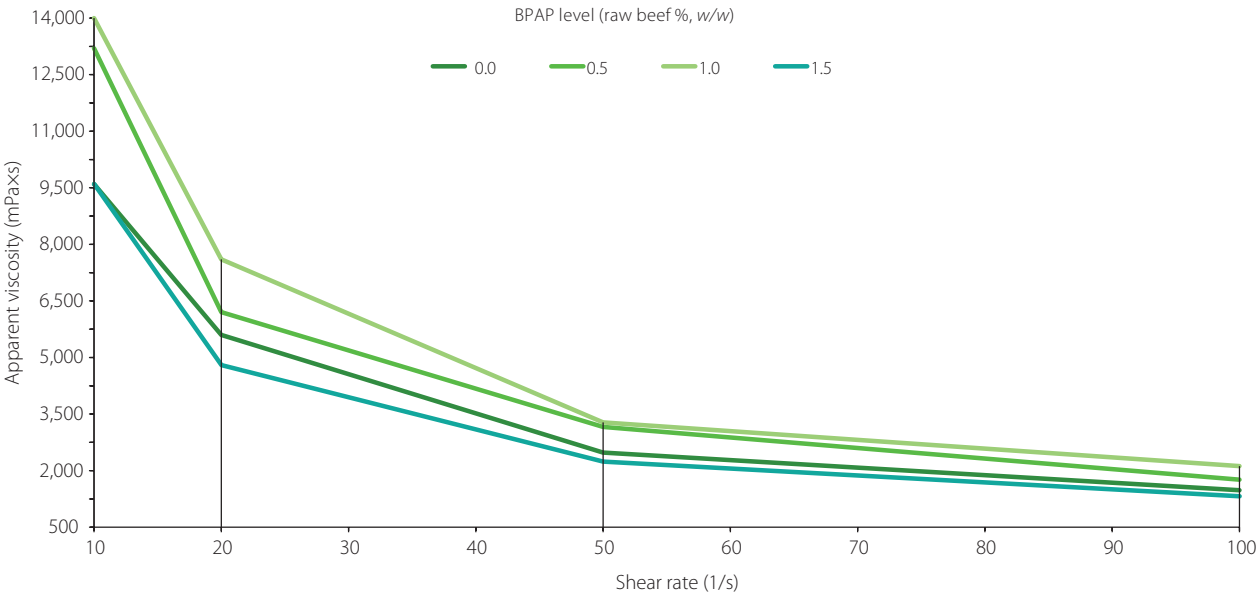

Figure 2. Apparent viscosity of beef meat emulsions with banana peel albedo powder (BPAP) added at 0.0%, 0.5%, 1.0%, and 1.5% based on the weight of the raw beef used. The actual contents of BPAP in the emulsions were 0.0%, 0.025%, 0.038%, and 0.075% (*w/v*), respectively.

index was in the range of 64,206 and 92,667 mPa×s^n. The emulsion with BPAP added at the level of 1.0% of raw beef meat, *w/w* (0.038% of the emulsion, *w/v*) had the highest consistency index of 92,667 mPa×s^n, while the lowest flow behavior index of 0.137. Therefore, the flow behavior index showed completely reverse trend with consistency index. Similar findings were reported by Sarıçoban *et al.* [2010], who investigated rheological properties of beef emulsions with the addition of sunflower head pith.

Incorporation of BPAP at different levels affected the AV of the beef meat emulsions (**Figure 2**). While the addition of BPAP at the levels of 0.5% and 1.0% of raw beef meat (*w/w*), which corresponded to 0.013% and 0.025% of the emulsion (*w/v*), respectively, increased AV compared to the control (0.0% BPAP), the addition at the level of 1.5% of raw beef, *w/w* (0.038% of the emulsion, *w/v*) decreased AV. The AV of all emulsions diminished with shear rate.

Choi *et al.* [2009] investigated the quality characteristics of low-fat meat emulsions by replacing pork fat with various vegetable oils (olive, corn, soybean, canola, and grape seed) and rice bran fiber. They stated that AV values of all groups decreased as the rotation period increased. Therefore, the highest viscosity values were found in the meat samples including rice bran fiber.

In addition, Choi *et al.* [2014] found that the inclusion of makgeolli lees fiber and varying fat contents had an impact on the frankfurter meat batters' perceived viscosity. All raw batters were reported to be thixotropic, with apparent viscosity decreasing with rotation time. It was also determined that higher

Table 5. Consistency index (*k*) and flow behavior index (*n*) of beef meat emulsions with banana peel albedo powder (BPAP) added at different levels.

BPAP level (% raw beef, *w/w*)*	*n*	*k* (mPa×s^n)	r^2
0.0 (Control)	0.177±0.00^a	64,206±979^c	0.999
0.5	0.144±0.02^b	88,823±4,233^b	0.990
1.0	0.137±0.01^c	92,667±1,578^a	0.998
1.5	0.142±0.01^b	66,068±537^c	0.996

*The final BPAP content in the emulsions was 0.013%, 0.025%, and 0.038% (*w/v*) for BPAP added at 0.5%, 1.0%, and 1.5% of raw beef (*w/w*), respectively. Results are shown as mean ± standard deviations. Values with different letters (a–c) within the column differ significantly ($p<0.05$). r^2, determination coefficient.

levels of fat and macgeolli fiber in the samples caused more pronounced changes in the apparent viscosity of the batters.

Apparent viscosity is an important parameter affecting the quality characteristics of meat, and emulsions with high apparent viscosity are preferred, since high apparent viscosity emulsions are not easily broken [Choi *et al.*, 2009, 2014].

Texture properties of cooked beef meat emulsions

The texture parameters of cooked beef meat emulsions with different contents of BPAP are shown in **Table 6**. The hardness, adhesiveness, springiness, cohesiveness, and gumminess were in the ranges of 3.65–4.00 N, (−1.80)–(−1.38) N×s, 0.89–0.94, 0.72–0.77, and 2.63–3.04 N, respectively. When increasing the addition level of BPAP from 0.0% to 1.5% of raw beef meat, *w/w* (which corresponds to 0.0–0.038% in the emulsions, *w/v*), the hardness of the beef emulsions decreased, while there was a fluctuation in the springiness values. On the other hand, adhesiveness, cohesiveness and gumminess of the emulsions were not significantly ($p\geq0.05$) affected by the addition of BPAP.

The decrease in hardness observed with increasing levels of BPAP in this study is consistent with the findings of Bastos *et al.* [2014], who reported that the addition of green banana flour to meat burgers resulted in lower hardness compared to the control samples, likely due to the water-binding capacity of the fiber. Similarly, García *et al.* [2006] found that the incorporation of inulin in a jelly form into sausages led to a reduction in hardness and an improvement in elasticity. However, in contrast to our results, several other studies have reported an increase in hardness following the addition of various dietary fibers. Choi *et al*. [2007] found that the incorporation of wheat fiber into meat products significantly increased hardness. Additionally, Zaini *et al.* [2020] showed that banana peel powder increased hardness and chewiness in chicken sausages depending on the inclusion level. These conflicting findings may be explained by differences in the type and structure of the fiber used, its concentration, degree of solubility, and processing method (*e.g.*, fresh or dried). Furthermore, the interaction of dietary fiber with the protein matrix and the fat content of the meat product can also influence textural outcomes. As highlighted by Yadav *et al.* [2018], such factors are critical in shaping the textural properties of meat products, as they can alter key texture parameters like hardness, springiness, and chewiness depending on the fiber's characteristics and the processing conditions.

Microstructure of beef meat emulsions

In our research, the effects of BPAP on the microstructural properties of meat emulsions were observed using a CLSM. The CLSM is a suitable method to scan the distribution of oil particles and the interactions between oil and protein. The CLSM micrographs showed that the particle sizes of the emulsions containing BPAP at levels of 0.5% and 1% of raw beef (*w/w*), corresponding to 0.013% and 0.025% in the emulsions (*w/v*), were smaller than of the emulsions without banana peel powder and had a more stable, homogeneous appearance (**Figure 3**). In addition, the oil particles of the emulsion containing 0.025% BPAP were more equally and better dispersed, and there were fewer voids in the emulsion system. Zhao *et al*. [2019] reported that in the CLSM micrographs, the meat emulsions without regenerated cellulose fiber had considerably larger particles, while the emulsions with higher fiber ratios had smaller particle sizes. In agreement with our results, the emulsion with 0.8% regenerated cellulose fiber had the smallest particle size, and its oil particles were more homogeneously dispersed. Qi *et al*. [2021] suggested that the citrus fiber was mainly imbibed as well-dispersed particles in the oil-water interface of the emulsion particles, thus stabilizing the emulsion.

Câmara *et al*. [2020] reported that the addition of chia mucilage to emulsified meat products caused changes in their structure assessed by CLSM. In the case of 2% chia mucilage powder and 2% chia mucilage gel addition treatments, while a definite grade of cohesion was observed in the protein structures, the fat particles, which had a well-described view, were imaged as looser and less connected in the protein matrix, particularly in the samples added with 2% chia mucilage powder. The authors suggested that this result, seen in the images of the samples with 2% chia mucilage powder added, could be straightly associated with the lower emulsion stability. In the current study, the stability of the beef meat emulsions with BPAP at 0%, 0.5% and 1.5% of raw beef (*w/w*) was found to be lower than that of the samples with BPAP at 1% of raw beef (*w/w*). In addition, the CLSM micrographs of these samples with BPAP at 0%, 0.5% and 1.5% of raw beef (*w/w*) showed they were less bound, looser, and inhomogeneous. Câmara *et al*. [2020] reported that when the emulsified meat samples contained the double amount of chia mucilage, the protein structures in the CLSM micrographs were more porous, discontinuous, and non-uniform.

Zeeb *et al*. [2018] found that the use of pectin substantially modified the microstructure of raw fermented and emulsion-type sausages. Other authors also observed that dietary fibers influenced the microstructure of meat [Han *et al*., 2018]. Tong *et al*. [2018], using CLSM micrographs, found that konjac glucomannan formed homogeneous gels with myofibrillar protein and showed good conformity with it. The conclusion was that a matrix-like structure was formed.

Table 6. Textural properties of beef meat emulsions with banana peel albedo powder (BPAP) added at different levels.

BPAP level (% raw beef, *w/w*)*	Hardness (N)	Adhesiveness (N×s)	Springiness (mm)	Cohesiveness	Gumminess (N)
0.0 (control)	4.00±0.07^{a}	−1.62±0.71^{a}	0.93±0.01^{ab}	0.76±0.04^{a}	3.04±0.20^{a}
0.5	3.82±0.09^{ab}	−1.80±0.04^{a}	0.89±0.02^{b}	0.74±0.02^{a}	2.83±0.03^{a}
1.0	3.89±0.01^{ab}	−1.38±0.36^{a}	0.94±0.01^{a}	0.77±0.01^{a}	2.99±0.02^{a}
1.5	3.65±0.06^{b}	−1.51±0.14^{a}	0.89±0.01^{b}	0.72±0.02^{a}	2.63±0.08^{a}

*The final BPAP content in the emulsions was 0.013%, 0.025%, and 0.038% (*w/v*) for BPAP added at 0.5%, 1.0%, and 1.5% of raw beef (*w/w*), respectively. Results are shown as mean ± standard error. Values with different letters (a–b) within the column differ significantly ($p<0.05$).

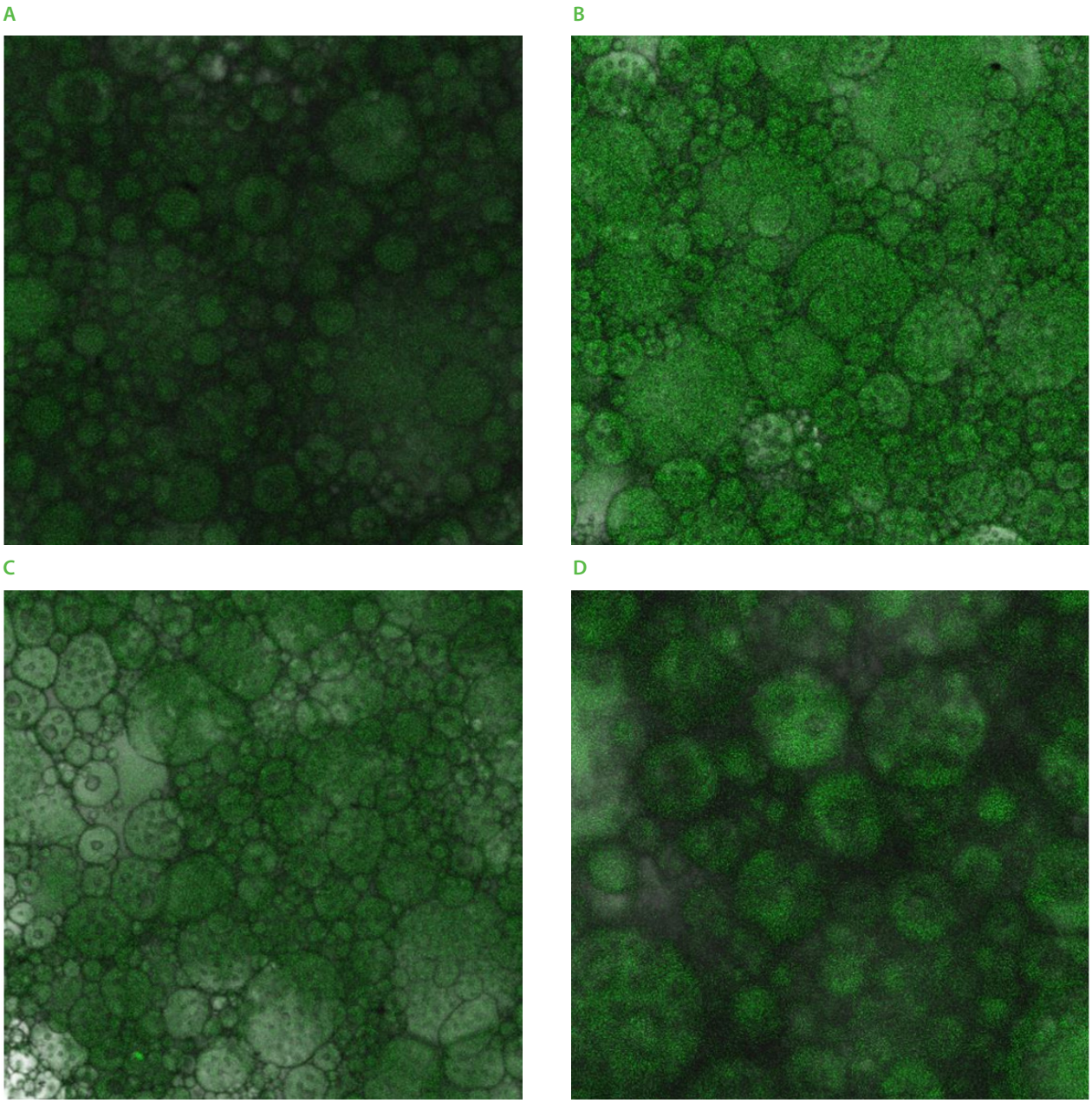

Figure 3. Confocal laser scanning microscope micrographs of beef meat emulsions with banana peel albedo powder (BPAP) added at 0.0% (**A**), 0.5% (**B**), 1.0% (**C**), and 1.5% (**D**) based on the weight of the raw beef used. The actual contents of BPAP in the emulsions were 0.0%, 0.025%, 0.038%, and 0.075% (*w/v*), respectively.

CONCLUSIONS

The present study results indicate that the physicochemical, emulsification, and microstructural characteristics of meat emulsions could be improved by using of BPAP. The addition of BPAP up to 1.5% of raw beef (*w/w*) level, corresponding to content of 0.038% (*w/v*) in the emulsion, increased the WHC and decreased the CL, hardness, and chewiness, which could be due to the presence of dietary fiber in the banana peel. However, the incorporation of BPAP up to 1.0% of raw beef (*w/w*) increased the emulsion capacity, emulsion stability values, and the AV of the samples. Regarding the CLSM micrographs, BPAP up to 1% of raw beef (*w/w*) (0.025% in the emulsion, *w/v*) led to more stable and homogenous meat emulsions. Consequently, it is recommended to use BPAP as a cheap source of dietary fiber in meat emulsions at a level of 1% of raw beef (*w/w*) (0.025% in the emulsion, *w/v*) to improve technological, textural, and microstructural properties. Further studies should be undertaken to clarify the effect of banana peel albedo powder on the microbiological stability and bioactive potential of meat products.

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CONFLICT OF INTERESTS

The authors affirm that they do not have any known competing financial interests or personal relationships that could be perceived as influencing the work reported in this paper.

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Evaluation of the Physicochemical, Antioxidant and Sensory Properties of Wheat Bread with Partial Substitution of Wheat Flour with *Cordyceps sinensis* Powder

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Wheat bread is one of the most popular and widely consumed cereal products. The aim of this study was to determine the impact of replacing wheat flour with *Cordyceps sinensis* (CS) powder at levels of 3, 6, 9, and 12% (*w/w*) on dough rheological properties, as well as bread baking parameters, physical characteristics, nutritional value, and sensory attributes. The study also assessed the effect of CS powder on the total phenolic content, the total flavonoid content, and the antioxidant capacity of the bread. The results demonstrated that already 3% (*w/w*) substitution of wheat flour with CS powder affected a significantly higher content of the total dietary fiber (9.49 g/100 g dry matter (d.m.)) and protein (13.82 g/100 g d.m.) in the bread compared to the control sample (without CS powder). Increasing substitution levels resulted in an increase in the total phenolic content from 1.09 to 5.16 mg GAE/g d.m. and consequently increased the antioxidant capacity of the bread. The CS powder influenced the rheological properties of the dough, notably increasing the flour water absorption and the dough development time. However, it also led to a deterioration in dough consistency by reducing dough stability and the farinograph quality number, while increasing the degree of softening. The texture profile analysis showed no significant changes in hardness or elasticity after 48 h of storage, indicating good textural stability of the enriched breads. Compared to control, the enriched bread exhibited a more intense colour and similar sensory overall acceptability. The obtained results indicate the considerable potential of CS as a functional additive that enhances the bioactive compound content and the nutritional value of bread.

Keywords: bakery products, dietary fiber, functional foods, medicinal mushrooms, minerals, polyphenols fiber

INTRODUCTION

In light of contemporary health challenges, such as the increasing prevalence of lifestyle diseases, functional foods are gaining growing importance as tools for disease prevention and the overall improvement of public health. There is a growing consumer demand for innovative food products that combine high nutritional quality, sensory appeal, and health-promoting properties [Topolska *et al*., 2021]. Wheat bread, being a staple food in many countries worldwide, represents a promising matrix for the enrichment with bioactive compounds. The source of these bioactive compounds may be, among others, mushrooms [Łysakowska *et al*., 2023].

Cordyceps sinensis (Berk) Sacc. (CS) (synonym: *Ophiocordyceps sinensis* (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora) is a parasitic mushroom, also known as "winter worm, summer grass", naturally occurring in the high-altitude regions of Tibet and the Himalayas [Sharma *et al*., 2024]. It has been valued for centuries in traditional Asian medicine for its broad spectrum of health-promoting properties. This mushroom is a source of bioactive compounds such as nucleosides

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(mainly cordycepin and adenosine), polysaccharides (including β-glucans), sterols (including ergosterol), and flavonoids, which exhibit multi-faceted biological activities [Liu *et al*., 2015; Sharma *et al*., 2024]. Notably, cordycepin, a nucleoside structurally similar to adenosine, demonstrates anti-inflammatory, immunomodulatory, anticancer, and neuroprotective properties [Chen *et al*., 2013; Sharma *et al*., 2024]. By modulating key signalling mechanisms, such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and the mitogen-activated protein kinase (MAPK) pathway, cordycepin reduces neuroinflammatory processes, which are a crucial factor in the pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [Chen *et al*., 2013]. Moreover, in animal studies, polysaccharides derived from *C. sinensis* have been shown to regulate intestinal immunity and modulate gut microbiota composition in mice with cyclophosphamide-induced intestinal injury, which may indirectly contribute to the maintenance of gut-brain axis balance [Ying *et al*., 2020]. In addition, the polysaccharides present in CS may have beneficial effects on cognitive functions and nervous system health [Zhang *et al*., 2024]. CS is also characterised by a high content of nutritional compounds; *per* 100 g, it contains 21.9–30.4 g of protein, 24.2–24.9 g of carbohydrates, and 8.62–9.09 g of fat [Hsu *et al*., 2002]. The relatively high protein content, balanced amino acid profile, presence of unsaturated fatty acids, as well as B-group vitamins (B_1, B_2, and B_{12}), vitamin E, and essential minerals further enhance the functional properties of *C. sinensis* [Sharma *et al*., 2024]. Due to its low energy value (50–70 kcal/100 g of fresh matter) and a high fiber content, CS may support weight management and metabolic regulation [Jędrejko *et al*., 2024; Liu *et al*., 2015].

Previous studies have indicated that the enrichment of bread with medicinal mushrooms, such as *Inonotus obliquus* (chaga), *Lentinula edodes* (shiitake), *Hericium erinaceus* (lion's mane), and *Ganoderma lucidum* (reishi), leads to significant improvements in its nutritional and health-promoting properties [Kobus *et al*., 2024; Lu *et al*., 2021; Łysakowska *et al*., 2024, 2025]. For instance, wheat starch supplemented with β-glucan derived from *L. edodes* has been identified as a product with a reduced glycaemic index (GI), making it a suitable component of functional bread and pasta for individuals with carbohydrate metabolism disorders [Zhuang *et al*., 2017]. Furthermore, the complete genome analysis of *L. edodes* enabled identifying genes involved in aroma formation and fruiting body autolysis. These genetic features highlight the potential of *L. edodes* as a valuable additive for enhancing the sensory qualities of bakery products [Salwan *et al*., 2021]. Additionally, *H. erinaceus*, which is rich in phenolic compounds and was incorporated into bread by replacing up to 12% of the wheat flour (*w/w*), significantly increased its antioxidant potential [Łysakowska *et al*., 2025]. Our previous study further indicated that replacing 3% of wheat flour with *G. lucidum* powder (*w/w*) resulted in a significantly higher content of dietary fiber and minerals, particularly calcium and copper, in the bread [Łysakowska *et al*., 2024]. However, the incorporation of mushrooms into bread may be associated with a deterioration in its baking quality and sensory characteristics. Various biologically active compounds (such as chitin and β-glucans) of mushrooms may hinder gluten network formation in the dough, impede the fermentation process, and negatively affect the expansion of dough pieces, crumb structure, and crust colour [Zhao *et al*., 2022]. For these reasons, determining the optimal level of supplementation of bread with mushroom, ensuring that, under appropriate technological conditions, obtaining a product with a high nutritional value and health-promoting potential, is required. To date, no studies have evaluated the impact of CS supplementation on wheat dough rheology, bread quality parameters, and its nutritional value. Therefore, the aim of this study was to comprehensively evaluate the effect of replacing wheat flour with CS powder at various levels (3–12%, *w/w*) on wheat dough rheology, bread baking performance, physical properties, and sensory characteristics, as well as on the chemical composition (including macronutrients, dietary fiber and minerals), total phenolic and flavonoid content, and antioxidant capacity. The study also aimed to determine the optimal substitution level that ensures both technological feasibility and enhancement of the nutritional and health-promoting potential of the bread.

MATERIALS AND METHODS

Materials

Wheat flour type 750, used for bread production, was supplied by Polskie Młyny (Warsaw, Poland). Its gluten index was 99.0±0.3, a falling number was 304±6 s, and an average particle size was 120 µm. Powdered *C. sinensis* fruiting bodies (CS), used as wheat flour substitute in bread, were sourced from a certified supplier (NatVita, Mirków, Poland). The CS powder had a particle size of 200–300 µm and underwent microbiological control and heavy metal content analysis prior to use. Both raw materials were stored in airtight, dark containers at temperatures below 25°C and a relative humidity (RH) of 60–65%, in accordance with the guidelines from previous studies [Łysakowska *et al*., 2024].

Determination of farinographic characteristics of dough

The farinographic characteristics were determined using a Farinograph-E (model 8110142, Brabender, Duisburg, Germany), following the Cereals & Grains Association, AACC method 54-21 [AACC, 2010]. The study involved the analysis of dough prepared from wheat flour (control, CON) and from wheat flour substituted with CS powder at levels of 3, 6, 9, and 12% (*w/w*) (BC3, BC6, BC9, BC12, respectively). The following parameters were assessed: water absorption (WA), defined as the sample's ability to absorb water; dough development time (DDT), representing the time required to achieve optimal consistency; dough stability (ST), indicating how long the dough maintains its consistency; degree of softening (DS), referring to the loss of consistency during mixing; and farinograph quality number (FQN), which serves as an indicator of the dough's technological quality. Each measurement was repeated three times.

Bread production

Five bread variants were produced including the control bread (CON from wheat flour, and four types of bread from wheat flour with 3, 6, 9, and 12% (*w/w*) substitution with CS powder (BC3, BC6, BC9, BC12, respectively). The recipe for each variant included 600 g of flour or a mixture of flour and powder, 9 g of salt (1.5%), 18 g of fresh yeast (3%), and the amount of water determined farinographically according to AACC method 54-21 as water absorption (WA) [AACC, 2010].

The process started with mixing the ingredients in a BEAR Varimixer Teddy 5 L mixer (Varimixer A/S, Copenhagen, Denmark). Mixing was carried out at low speed for 3 min, followed by higher speed until the gluten structure was fully developed. After mixing, the dough was transferred to a proofing chamber (Tefi Klima pro 100, Debag, Bautzen, Germany) and fermented for 90 min at 30°C and an RH of 85±2%. During fermentation, after 60 min, a degassing process was performed to improve dough structure. After fermentation, the dough was divided into portions weighing 290±5 g, which were then manually shaped and placed into baking pans measuring 18×7.5×7 cm. The pans with the dough were left for 30 min in the proofing chamber at 30°C and an RH of 85±2% to ensure optimal volume development. Baking was carried out in a Helios pro 100 oven (Debag) at 230°C for 30 min. Two batches of bread were baked, with each batch consisting of three loaves of each bread variant. After baking, the bread was cooled for 1 h at room temperature, then packed in polyethylene bags and stored at 20°C with an RH of 50% before further analysis.

Chemical composition analysis of raw materials and bread

The chemical composition of the wheat flour, CS powder and bread was analysed, starting with the determination of moisture content using the AACC method 44-15A by drying the samples (3 g) in a laboratory oven (model KBC-100W, WAMED, Warsaw, Poland) at 103±1°C until a constant weight was achieved [AACC, 2010]. The results were expressed as g *per* 100 g of sample. Next, the ash content was determined using the combustion method (AACC method 08-01) in a muffle furnace [AACC, 2010]. Samples weighing 3 g were placed in porcelain crucibles and ashed at 590°C for 7 h. The protein content was determined using the Kjeldahl method according to the AOAC International standard (method 979.09) [AOAC, 2016] with the Kjeltec™ 8400 apparatus (Foss Analytical AB, Höganäs, Sweden). The nitrogen present in the samples was converted to protein using a conversion factor of *N*×5.7. The fat content was measured after acid hydrolysis using the Soxtec™ 8000 extraction apparatus (Foss Analytical AB) and *n*-hexane as the solvent (AOAC method 2003.05) [AOAC, 2016]. The dietary fiber content was analysed using enzymatic methods, following AOAC International guidelines (method 991.43) [AOAC, 2016]. The process involved sequential enzymatic hydrolysis of a 1 g sample using α-amylase, protease, and amyloglucosidase provided by Megazyme Ltd. (Bray, Ireland). The total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) contents were determined. The contents of nutrients were expressed in g *per* 100 g dry matter (d.m.) of wheat flour, CS powder or bread. All analyses were carried out in triplicate. The content of digestible carbohydrates was calculated as the difference between 100 and the sum of the protein, fat, dietary fiber, and ash content in 100 g d.m. The energy value of the raw materials and bread was determined in kcal *per* 100 g of product, using the Atwater factors: protein and carbohydrates were assigned a value of 4 kcal/g, fat 9 kcal/g, and dietary fiber 2 kcal/g.

Evaluation of bread baking quality parameters

The bread baking quality was analysed 5 h after cooling. Dough yield was calculated as a percentage by dividing the total weight of the prepared dough by the weight of the flour used. The bread yield was expressed as the percentage ratio of the mass of the baked bread to the mass of flour used for its preparation. Baking loss was determined based on the difference in mass of dough before baking (W_2) and the mass of the finished bread (W_1). The results were calculated as the percentage mass loss relative to the dough mass according to Equation (1):

$$\text{Baking loss (\%)} = \frac{W_2 - W_1}{W_2} \times 100 \quad (1)$$

The bread volume was assessed by the mustard seed displacement method, AACC method 10-05.01, which allowed for determination of the crumb aeration degree [AACC, 2010]. The specific volume (mL/100 g) was calculated as the ratio of the bread volume to its mass.

To assess the crumb porosity, the microscopic images were taken using a digital microscope VHX-7000N (Keyence, Osaka, Japan) at magnifications ranging from ×20 to ×100. Bread slices approximately 10 mm thick were cut from the central part of the loaf and left to stabilise at room temperature prior to imaging. For each sample, images were acquired from randomly selected areas measuring 3×3 cm in the crumb cross-section under consistent lighting conditions. The analysis included the evaluation of pore size, shape, and distribution, with particular attention to pore connectivity and the presence of irregular voids. Uniformity of pore distribution was also assessed as an indicator of crumb consistency and aeration.

Evaluation of bread colour parameters

The analysis of bread crumb colour was performed using a CR-5 chroma meter (Konica Minolta, Sakai, Osaka, Japan) in the CIELab colour space under D65 light and at a 10° observation angle. The values of lightness (L^*), representing the perception of reflected light, the red-green component (a^*), and the yellow-blue component (b^*) were recorded. Measurements were performed ten times for each sample at different locations within the crumb to ensure the reliability of the results and minimise the impact of material heterogeneity.

Based on the obtained L^*, a^* and b^* data, secondary colour parameters were calculated. The colour intensity (chroma, C) was

expressed as the vector modulus in the a^*, b^* space and was calculated according to Equation (2):

$$C = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

The hue angle (h°), defining the tonal character of the colour, was calculated using the arctangent function of the ratio of the b^* and a^* components. Saturation (S), which defines the vividness and purity of the colour relative to its lightness, was calculated as the ratio of C to L^*. The colour difference (ΔE) between the bread with CS powder (coordinates) and the control bread (coordinates) was calculated according to Equation (3):

$$\Delta E = \sqrt{(L^*_c - L^*_t)^2 + (a^*_c - a^*_t)^2 + (b^*_c - b^*_t)^2} \quad (3)$$

The browning index (BI) was calculated using Equation (4):

$$BI = \frac{100\,(x - 0.31)}{0.17} \quad (4)$$

where x was obtained according to Equation (5):

$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 0.012b^*)} \quad (5)$$

Texture profile analysis of bread

The texture profile analysis (TPA) of the bread was conducted 24 and 48 h after baking. Samples in the form of rectangular prisms with dimensions of 30×30×20 mm were subjected to a double compression test using a Zwick/Roell Z0.5 machine (BT1-FR0.5TN. D14, Ulm, Germany), equipped with a flat cylindrical plunger with a diameter of 50 mm. Compression was performed to 50% of the initial height of the sample at a constant head speed of 1 mm/s. The parameters assessed during the analysis included hardness (expressed in N), elasticity, cohesiveness, and chewiness (expressed in N).

Determination of the element contents of raw materials and bread

The contents of magnesium (Mg), calcium (Ca), potassium (K), iron (Fe), copper (Cu), and zinc (Zn) in wheat flour, CS powder and bread were determined by flame atomic absorption spectrometry (FAAS). The analysis was performed using a Varian SpectrAA 280FS spectrometer (Varian, Mulgrave, VIC, Australia) after microwave-assisted mineralisation of the samples. The digestion was carried out in a CEM Mars Xpress microwave system (CEM Corporation, Matthews, NC, USA) using 4 mL of nitric acid for each 0.5 g of the sample. The mineralised solutions were diluted to 50 mL with deionised water. The FAAS determinations were conducted with an air-acetylene flame and element-specific conditions (wavelength, slit width and lamp current) optimised for each element. Calibration curves were prepared using standard solutions. Selenium (Se), lead (Pb), and cadmium (Cd) were determined using inductively coupled plasma mass spectrometry (ICP-MS) (ICP, Palo Alto, CA, USA). The samples digested as described above were analysed using Varian MS-820 mass spectrometer operated with the following settings: plasma gas flow – 16 L/min, nebuliser gas flow – 0.98 L/min, RF power – 1.38 kW, and sampling depth – 6.5 mm. No collision/reaction cell was used. Selected isotopes were monitored for identification and quantification (78Se, 114Cd, 206Pb, 207Pb, 208Pb). Quantification was performed using external calibration with certified standard solutions. The results for all analysed elements were expressed in mg/kg on a dry matter basis. Quality control included the analysis of blanks, duplicates, and certified reference material (NIST-1577c Bovine Liver, National Institute of Standards and Technology, Gaithersburg, MD, USA) to ensure accuracy and precision.

Extraction and determination of total phenolic content, total flavonoid content and antioxidant capacity of raw materials and bread

To determine the total phenolic content (TPC), the total flavonoid content (TFC) and the antioxidant capacity, the wheat flour, CS powder and bread were extracted using 70% (*v/v*) ethanol as the solvent. Ten-gram samples were mixed with 90 mL of the solvent and incubated in a water bath at 40°C for 10 h, according to the methodology of Kozłowska *et al*. [2015]. After extraction, the samples were filtered through filter paper, and the obtained extracts were used for further determinations.

The TPC was determined using the method of Singleton & Rossi [1965] with a Folin-Ciocâlteu reagent. Briefly, 0.1 mL of the extract was mixed with 0.1 mL of the Folin-Ciocâlteu reagent and 1 mL of a 20% (*w/w*) sodium carbonate solution. After 30-min incubation, absorbance was measured at a wavelength of 700 nm using a Thermo Spectronic Helios Epsilon spectrophotometer (Thermo Electron, Waltham, MA, USA). The results were expressed as mg gallic acid equivalents (GAE) *per* g of product d.m.

The TFC was determined using the aluminium chloride method according to Quettier-Deleu *et al*. [2000]. Two mL of the extract were mixed with 0.4 mL of a 5% (*w/w*) aluminium chloride solution in test tubes. The mixtures were incubated for 30 min at room temperature in the dark. Absorbance was measured at a wavelength of 405 nm using a Thermo Spectronic Helios Epsilon spectrophotometer (Thermo Electron). The results were expressed as mg quercetin equivalents (QE) *per* g of product d.m.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using the method of Brand-Williams *et al*. [1995]. An extract (0.5 mL) was mixed with 2 mL of an ethanolic DPPH radical solution (0.025 g DPPH radical in 100 mL ethanol). The absorbance of the reaction mixture was measured at 515 nm using a Thermo Spectronic Helios Epsilon spectrophotometer (Thermo Electron). Trolox (6-hydroxy-2,5,7,8--tetramethylchroman-2-carboxylic acid) was used as a standard, and the results were expressed in mg Trolox equivalents (TE) *per* g of product d.m.

To determine 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity, the ABTS radical cation solution was prepared by mixing 0.1920 g of ABTS with 0.0343 g of potassium persulfate, followed by incubation for 16 h in the dark [Re *et al*., 1999]. The solution was then diluted with methanol to an absorbance of 0.75 at a wavelength of 734 nm. Next, 100 µL of the extract were added to 1.8 mL of the ABTS radical cation solution, mixed, and incubated for 3 min. Absorbance was measured at a wavelength of 734 nm Results were calculated based on a calibration curve plotted for Trolox and expressed as mg TE *per* g of product d.m.

All analyses were performed in triplicate for each sample.

Sensory analysis

The sensory analysis of bread was conducted by a nine-member panel of experts selected based on their experience in food product evaluation, regular consumption of bakery products, and absence of gluten allergies. The panellists were trained in accordance with a standard method [ISO 8586:2012] and familiarised with the principles of sensory evaluation using a five-point scale. Assessments were carried out under controlled laboratory conditions, ensuring standardised lighting, temperature, and humidity [ISO 8589:2007]. The study received approval from the Bioethics Committee of the University of Life Sciences in Lublin, Faculty of Food Sciences and Biotechnology (Resolution No. UKE/09/2023).

The evaluation included the following sensory attributes: external appearance, aroma, crumb colour, crumb elasticity, taste, and overall rating. The appearance was assessed based on shape, degree of crust browning, and surface uniformity. Aroma was analysed upon unpacking the samples, considering intensity and specific olfactory notes associated with the presence of the CS powder. Elasticity was evaluated by applying pressure to the crumb and observing its ability to return to its original shape, while porosity was determined based on pore size and distribution. Taste was assessed for intensity, balance, and the presence of characteristic notes, whereas overall acceptability incorporated all analysed attributes. Bread was sliced into 1 cm-thick pieces, coded, and presented to panellists in a random order to eliminate systematic errors. Panellists were allowed to cleanse their taste receptors with water and coffee between evaluations. Results were recorded on evaluation sheets using a five-point scale, where 5 indicated the highest quality and 1 the lowest. Each assessed attribute was assigned corresponding reference points, defining specific criteria for each value on the scale. The inclusion of these reference points standardised assessments, enhancing their objectivity and reproducibility.

Statistical analysis

The obtained data were analysed using one-way analysis of variance (ANOVA) with Tukey's test to assess the significance of differences in physical parameters, chemical composition, sensory attributes, content of elements and phenolics, and antioxidant capacity of bread produced from wheat flour with different levels of CS powder substitution. The differences were considered significant at $p \leq 0.05$. The ANOVA of data for wheat flour and CS powder (chemical composition, content of elements and phenolics, and antioxidant capacity) was conducted in a separate set.

Results for each parameter were presented as means from repetitions with standard deviations. The data were analysed using STATISTICA 13 software (StatSoft, Kraków, Poland).

RESULTS AND DISCUSSION

Farinographic properties of the dough

The partial substitution of wheat flour with CS powder significantly affected the rheological properties of dough (**Table 1**). The observed extension of dough development time compared to the control dough (by approximately 111-121%) indicated a disruption in the kinetics of gluten network formation, which can be attributed to the specific physicochemical properties of the additives [Liu *et al*., 2020]. Similar results were observed for the dough enriched with other powdered mushrooms including *G. lucidum*, *Agaricus bisporus*, and *Pleurotus ostreatus* (oyster mushroom) [Łysakowska *et al*., 2024; Majeed *et al*., 2017; Zhang *et al*., 2019]. The authors attributed the observed changes in dough development time to the interactions of mushroom-derived polysaccharides and proteins with wheat gluten.

Table 1. Farinographic characteristics of doughs from wheat flour (control, CON) and from wheat flour substituted with *Cordyceps sinensis* powder at levels of 3, 6, 9, and 12%, *w/w*, (BC3, BC6, BC9, BC12, respectively).

Dough	DDT (min)	WA (%)	ST (min)	DS (FU)	FQN
CON	2.18 ± 0.12^{c}	57.9 ± 0.3^{b}	9.0 ± 0.2^{a}	33 ± 2^{d}	94 ± 5^{a}
BC3	4.62 ± 0.05^{b}	58.0 ± 0.4^{b}	7.0 ± 0.3^{b}	42 ± 1^{c}	81 ± 3^{b}
BC6	5.17 ± 0.05^{a}	58.3 ± 0.2^{b}	6.7 ± 0.2^{bc}	46 ± 2^{c}	78 ± 2^{bc}
BC9	5.17 ± 0.05^{a}	58.9 ± 0.3^{ab}	6.1 ± 0.2^{c}	55 ± 2^{b}	73 ± 3^{c}
BC12	4.83 ± 0.05^{ab}	59.6 ± 0.1^{a}	6.2 ± 0.1^{c}	60 ± 1^{a}	73 ± 2^{c}

Results are shown as mean ± standard deviation (n=3). Different letters within a column indicate significant differences according to Tukey's test ($p \leq 0.05$). DDT, dough development time; WA, water absorption; ST, dough stability; DS, degree of softening; FQN, farinograph quality number.

As shown in **Table 2**, CS powder was significantly richer in protein and dietary fiber compared to wheat flour. The substitution of wheat flour with such a high-fiber and high-protein ingredient led to a significant ($p \leq 0.05$) increase in the water absorption (WA) of the flour (**Table 1**), which could, in turn, result in higher dough and bread yield (**Table 3**). The increase in WA was in line with findings from a previous study by Majeed *et al*. [2017] who prepared wheat dough with oyster mushroom powder.

An important farinographic parameter defining the quality of flour is dough stability (ST), which measures the dough's resistance to prolonged mixing. The addition of CS powder to wheat flour reduced values of this parameter, with the most significant changes observed in the samples with substitution levels of 9 and 12% (*w/w*) (**Table 1**). A similar trend was observed by Majeed *et al*. [2017] when adding oyster mushroom powder to wheat flour, as well as by other authors who used various high-fiber additives such as aronia berry powder [Cacak-Pietrzak *et al*., 2023] and roasted barley coffee [Cacak-Pietrzak *et al*., 2024]. These changes in dough stability are most likely due to the disruption of the gluten matrix continuity and are positively correlated with the amount of mushroom or other high-fiber additives. When using such additives in wheat dough, it is important to strictly control the mixing time to ensure that, on the one hand, the gluten network develops sufficiently strong and, on the other, it does not suffer mechanical damage.

The substitution of wheat flour with CS powder negatively affected dough softening. The degree of dough softness increased by 91 and 150% for the 3 and 12% (*w/w*) mushroom powder-supplemented samples, respectively (**Table 1**). This suggests a weaker protein matrix in the dough prepared with mushroom powder and a greater susceptibility to degradation during mixing. Majeed *et al*. [2017] also observed this phenomenon and noted the greatest degree of softening in the dough with a 15% oyster mushroom powder addition, while the least softening occurred in the dough made with 100% wheat flour. Similarly, when adding raw materials rich in phenolic compounds (green tea extract) or plant proteins (hemp seed cake), which negatively affect the structure of the gluten network, dough stability was reduced, leading to poorer bread quality [Capcanari *et al*., 2023; Qin *et al*., 2022].

The use of CS powder in wheat dough preparation also led to a decrease in the farinograph quality number (FQN). Replacing 9–12% of wheat flour with the mushroom powder resulted in a decrease in the FQN from 94 to 73.

Chemical composition of raw materials and bread

The production of bread from wheat flour with CS powder substitution ranging from 3 to 12% (*w/w*) resulted in significant changes in its chemical composition, proportional to the enrichment level (**Table 2**). Due to the high dietary fiber content of the CS powder, enrichment of bread with this powder resulted in a significant ($p \leq 0.05$) increase in the total dietary fiber (TDF) content by up to 131% in the BC12 sample. This change was mainly attributed to the increase in the content of insoluble dietary fiber (IDF). In the BC12 sample, the IDF content was more than 2.7 times

Table 2. Chemical composition of raw materials including wheat flour and *Cordyceps sinensis* (CS) powder, and bread from wheat flour (control, CON) and from wheat flour substituted with CS powder at levels of 3, 6, 9, and 12%, *w/w*, (BC3, BC6, BC9, BC12, respectively).

Product	Moisture (g/100 g)	Ash (g/100 g d.m.)	Protein (g/100 g d.m.)	Fat (g/100 g d.m.)	TDF (g/100 g d.m.)	IDF (g/100 g d.m.)	SDF (g/100 g d.m.)	Digestible carbohydrates (g/100 g d.m.)	Energy value (kcal/100 g)
Wheat flour	9.40 ± 0.10^{A}	0.69 ± 0.01^{B}	8.79 ± 0.04^{B}	0.45 ± 0.03^{B}	5.30 ± 0.13^{B}	2.40 ± 0.10^{B}	2.90 ± 0.20^{B}	84.77 ± 0.03^{A}	352.33 ± 0.11^{A}
CS powder	3.35 ± 0.09^{B}	5.69 ± 0.60^{A}	33.73 ± 1.40^{A}	5.64 ± 0.11^{A}	48.13 ± 0.80^{A}	44.83 ± 0.83^{A}	5.30 ± 0.08^{A}	3.46 ± 0.05^{B}	199.56 ± 0.40^{B}
CON	41.46 ± 0.71^{d}	2.20 ± 0.01^{c}	10.83 ± 0.04^{d}	1.23 ± 0.03^{d}	7.21 ± 0.19^{e}	4.26 ± 0.17^{e}	2.95 ± 0.02^{d}	78.53 ± 0.42^{a}	224.16 ± 0.17^{a}
BC3	43.34 ± 0.23^{c}	2.50 ± 0.05^{b}	13.82 ± 0.10^{c}	1.42 ± 0.08^{c}	9.49 ± 0.53^{d}	5.97 ± 0.31^{d}	3.52 ± 0.12^{c}	72.77 ± 0.54^{b}	214.24 ± 0.24^{b}
BC6	43.82 ± 0.30^{bc}	2.56 ± 0.02^{b}	14.89 ± 0.06^{bc}	1.67 ± 0.11^{b}	11.42 ± 0.28^{c}	7.12 ± 0.53^{c}	4.30 ± 0.25^{b}	69.46 ± 0.62^{c}	210.83 ± 0.22^{c}
BC9	44.50 ± 0.20^{b}	2.69 ± 0.08^{ab}	15.54 ± 0.07^{ab}	1.75 ± 0.14^{ab}	13.93 ± 0.12^{b}	9.15 ± 0.82^{b}	4.78 ± 0.12^{b}	66.09 ± 0.22^{d}	205.42 ± 0.42^{d}
BC12	45.70 ± 0.15^{a}	2.78 ± 0.03^{a}	16.41 ± 0.15^{a}	1.81 ± 0.09^{a}	16.71 ± 0.19^{a}	11.54 ± 0.94^{a}	5.17 ± 0.24^{a}	62.29 ± 0.32^{e}	197.93 ± 0.19^{e}

Data are shown as mean ± standard deviation (n=3). Different lowercase letters (a–e) and uppercase letters (A–B) within a column indicate significant differences according to Tukey's test ($p \leq 0.05$). TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; d.m., dry matter.

Table 3. Parameters of baking quality of bread from wheat flour (control, CON) and from wheat flour substituted with *Cordyceps sinensis* powder at levels of 3, 6, 9, and 12%, *w/w*, (BC3, BC6, BC9, BC12, respectively).

Bread	Dough yield (%)	Bread yield (%)	Baking loss (%)	Bread volume (mL/100 g)
CON	162.17±0.51^{c}	140.78±1.31^{e}	13.19±0.99^{a}	350.8±2.7^{a}
BC3	162.67±0.23^{bc}	147.09±0.20^{d}	9.57±0.71^{b}	329.0±2.6^{b}
BC6	162.83±0.34^{bc}	149.68±0.90^{c}	8.64±0.91^{c}	316.5±3.4^{bc}
BC9	163.50±0.32^{b}	151.26±0.40^{b}	8.05±0.31^{c}	307.6±4.5^{c}
BC12	165.00±0.21^{a}	151.65±0.72^{a}	8.29±0.46^{c}	306.2±2.3^{c}

Results are shown as mean ± standard deviation (n=3). Different letters within a column indicate significant differences according to Tukey's test (p≤0.05).

higher than in the control bread. Much smaller, but statistically significant (p≤0.05) changes were observed in the soluble dietary fiber (SDF) content. It should be emphasised that different medicinal mushroom powders are excellent sources of dietary fiber, especially insoluble fiber, and their addition to bread consistently leads to an increase in fiber content of the final product [Łysakowska *et al*., 2023]. A similar trend was observed when analysing the protein content changes (**Table 2**). The lowest 3% (*w/w*) substitution of wheat flour with the mushroom powder resulted in a bread protein content increase of about 28%, while the 12% (*w/w*) substitution led to a 51% increase compared to the control sample. CS is distinguished among other medicinal mushrooms due to its particularly high protein content, which in our study was 33.73 g/100 g d.m. For comparison, the protein content of *H. erinaceus* was reported to be 21.16% d.m. and *G. lucidum* – 15.49% d.m. [Łysakowska *et al*., 2024, 2025]. The dominant amino acids in CS proteins are glutamic acid, aspartic acid, lysine, arginine, and histidine [Hsu *et al*., 2002]. Therefore, the addition of this mushroom can effectively balance the lysine deficiencies in bread, which are typical for cereal-based products. Although not directly demonstrated for CS, phenolic compounds such as chlorogenic acid, caffeic acid, and other hydroxycinnamic derivatives – known to occur in this mushroom [Babotă *et al*., 2022] – may enhance the bioavailability of basic amino acids, including lysine, by inhibiting Maillard reactions in which these amino acids are consumed as substrates. This mechanism has been observed in polyphenol-rich plant extracts such as those from *Coffea arabica*, which reduce protein glycation by trapping reactive carbonyl species and protecting amino groups [Rebollo-Hernanz *et al*., 2019. This is particularly important for individuals on plant-based diets, who often face deficiencies in essential amino acids [Mariotti & Gardner, 2019]. Up until now, protein from legume seeds has been most commonly added to improve the nutritional value and amino acid profile of bread [Bosmali *et al*., 2025; Han *et al*., 2024]. However, it is worth noting that there is currently a lack of studies on the digestibility of CS protein, and researching its bioavailability could provide valuable insights into the full nutritional value of this mushroom.

The introduction of CS powder into the recipe also led to an increase in the fat and ash contents of the bread (**Table 2**). The changes in fat content were slight, but statistically significant (p≤0.05), with the fat content of the bread produced from wheat flour with the highest (12%, *w/w*) substitution with mushroom powder not exceeding 2 g/100 g d.m. These increases were due to the higher fat and ash contents naturally present in the CS powder compared to wheat flour.

The increase in fiber, protein, and fat contents was correlated with a decrease in the amount of digestible carbohydrates. Compared to the control bread, their levels decreased in the range of 7.34–20.69%, proportionally to the increasing level of mushroom powder replacement (**Table 2**). The reduction in carbohydrates, which are the primary source of energy in wheat bread, suggests a potential reduction in its glycaemic load. The decrease in carbohydrate content, along with the higher moisture content of the enriched bread, also resulted in a reduction in its energy value. Compared to the control sample, the energy value of the bread with the mushroom powder decreased in the range of 4.42–11.71%, proportionally to the enrichment level (3–12% of what flour, *w/w*). The reduction in the energy value of bread is particularly significant in the context of the growing demand for products with lower energy values, especially in diets aimed at controlling or reducing body weight [Karunarathna *et al*., 2024].

Bread baking quality

The substitution of wheat flour with CS powder led to a significant (p≤0.05) increase in bread yield ranging from 4.5 to 7.7%, and the highest bread yield was determined for the BC12 sample (151.65%) (**Table 2**). This increase can be attributed to the higher water absorption observed in the dough enriched with the mushroom powder. It is important to highlight that the CS powder was rich in dietary fiber and protein, both of which have a high water absorption capacity and likely contribute to the improved water retention in the bread [Łysakowska *et al*., 2023]. The water absorption of flour plays an important role in the economic aspect of bread production, as it leads to a higher bread yield, which reduces raw material costs and improves production efficiency [Różyło *et al*., 2015]. Our results are in line with findings from studies that used powders from other mushroom species, such as *Flammulina velutipes* and *A. bisporus*, and showed enhanced water absorption in the dough with these powders, leading to an increase in bread yield [Nie *et al*., 2019; Zhang *et al*., 2019].

The baking loss, reflecting the mass loss of bread due to evaporation during baking, was lower by 27–39% in the case of the bread enriched with the mushroom powder compared to the control, for which it was 13.19% (**Table 3**). However, the baking loss of the bread produced from flour containing the mushroom powder at levels of 6, 9 and 12% (*w/w*) did not differ significantly (p>0.05). The reduced baking losses observed in the study may result from increased moisture retention in the bread, which is consistent with the results of Ammar *et al.* [2016] and Bojňanská *et al.* [2024], who showed that additives such as *Boerhavia elegana*

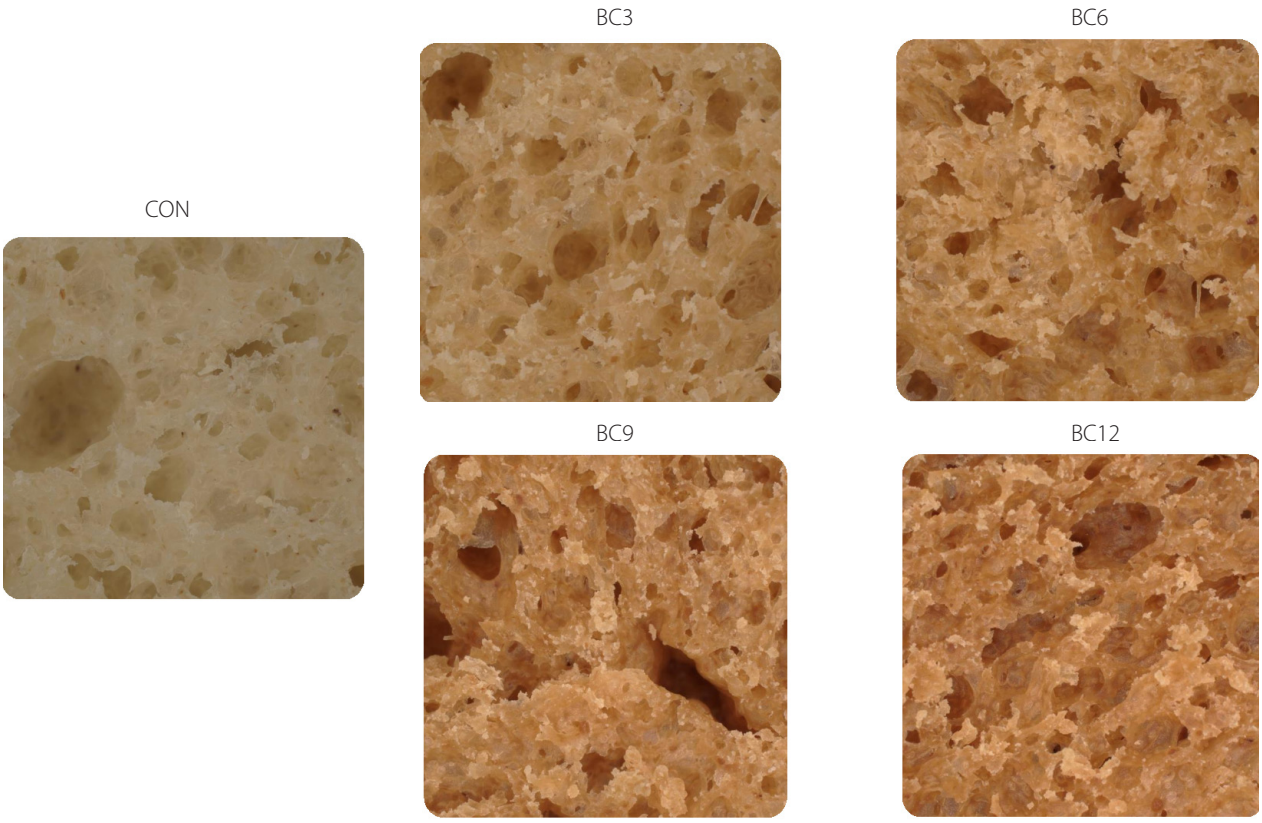

Figure 1. Microscopic images showing porosity of fresh crumb of bread from wheat flour (control, CON) and from wheat flour substituted with *Cordyceps sinensis* powder at levels of 3, 6, 9, and 12%, *w/w*, (BC3, BC6, BC9, BC12, respectively). Magnification ×2,000.

Choisy seed flour, rich in fiber, and turmeric extracts, rich in phenolic compounds can significantly impact dough's ability to retain water. Similarly, in the case of CS powder, dietary fiber, protein, and bioactive compounds such as phenolics may determine the internal structure of the gluten network. These compounds can interact with proteins and starch, increasing the ability of the bread to bind and retain water. Higher water retention positively affects bread crumb texture, reduces staling, and extends freshness [Dong & Karboune, 2021].

The addition of CS powder negatively affected the bread volume (**Table 3**). The highest bread volume (350.8 mL/100 g) was determined for the bread prepared with 100% wheat flour. Partial substitution of wheat flour with the mushroom powder led to a decrease in bread volume by 6.2–12.7%, with the most significant changes observed in the BC12 sample. The reduction in bread volume can be explained by the weakening of the gluten network, caused by the introduction of components that do not contain gluten proteins but are rich in ingredients such as fiber and phenolic compounds, which, as studies have shown, may negatively affect the gluten structure [Welc-Stanowska *et.al*., 2023]. Phenolic compounds in plant extracts can interact with gluten proteins, disrupting their intermolecular and intramolecular interactions, which may lead to the weakening of the gluten network in the dough. According to the research by Hanuka-Katz *et al*. [2022], these interactions may involve non-covalent bonds, such as hydrogen bonds and hydrophobic interactions, which influence dough properties. Furthermore, non-gluten proteins present in the CS powder may compete with gliadins and glutenins, hindering the formation of a strong gluten network and reducing the ability to trap gases during dough fermentation and bread baking. The microscopic images of crumbs showed that enriching bread with the CS powder reduced its porosity (**Figure 1**). Samples with a higher substitution level of the mushroom powder (BC9 and BC12) had visibly lower porosity than those with lower levels of wheat flour substitution (BC3 and BC6). At the same time, the pores in the BC9 and BC12 samples were more heterogeneous, thick-walled, and relatively densely packed. The crumb of bread with lower CS powder additions (BC3 and BC6) exhibited a more regular pore structure, with a large number of larger, thin-walled pores, indicating slightly better textural properties of these products.

Bread colour

The substitution of wheat flour with the mushroom powder led to a significant ($p \leq 0.05$) reduction in lightness (L^*) and an intensification of red and yellow tones (higher a^* and b^* values) in the bread crumb (**Table 4**). This effect was further confirmed by the increase in the browning index (BI), which indicated a progressive darkening of the crumb with a higher mushroom powder content. A significant ($p \leq 0.05$) increase in chroma (C) and a decrease in hue angle (h°) were observed in the samples BC3 to BC12, indicating a progressive intensification and shift of crumb colour towards warmer, red-yellow tones. Additionally, saturation (S) increased with the addition of the CS powder, with significant differences ($p \leq 0.05$) noted between the control sample and BC6, BC9, and BC12 samples, and the highest S

Table 4. Colour parameters of bread from wheat flour (control, CON) and from wheat flour substituted with *Cordyceps sinensis* powder at levels of 3, 6, 9, and 12%, w/w, (BC3, BC6, BC9, BC12, respectively).

Bread	*L**	*a**	*b**	BI	Δ*E*	C	h°	S
CON	61.49±3.71^{a}	0.51±2.30^{e}	12.27±3.74^{e}	0.68±0.09^{e}	–	12.28±0.21^{e}	87.62±2.40^{a}	0.20±0.02^{c}
BC3	61.21±4.34^{b}	2.81±1.55^{d}	22.40±1.26^{d}	3.42±0.82^{d}	10.39±1.20^{d}	22.58±0.36^{d}	82.85±3.04^{b}	0.37±0.04^{bc}
BC6	53.65±2.00^{c}	6.88±0.58^{c}	24.20±0.24^{c}	9.20±1.23^{c}	15.63±0.80^{c}	25.16±0.13^{c}	74.13±1.82^{c}	0.47±0.03^{ab}
BC9	47.93±2.98^{d}	9.25±0.51^{b}	25.73±0.74^{b}	13.64±1.13^{b}	21.01±1.50^{b}	27.34±0.27^{b}	70.23±0.93^{d}	0.57±0.04^{ab}
BC12	43.81±3.36^{e}	10.18±0.80^{a}	27.71±2.31^{a}	16.31±1.23^{a}	25.39±1.80^{a}	29.52±0.43^{a}	69.83±1.23^{e}	0.67±0.06^{a}

Results are shown as mean ± standard deviation (n=10). Different letters within a column indicate significant differences according to Tukey's test (p≤0.05). *L**, lightness; *a**, red-green spectrum; *b**, blue-yellow spectrum; Δ*E**, colour difference compared to the control; C, chroma; h°, hue angle; S, saturation; BI, browning index.

Figure 2. Appearance of bread from wheat flour (control, CON) and from wheat flour substituted with *Cordyceps sinensis* powder at levels of 3, 6, 9, and 12%, *w/w*, (BC3, BC6, BC9, BC12, respectively).

value recorded for BC12, confirming the enhancement of colour intensity in the enriched breads, as also visually presented in **Figure 2**. These results are consistent with findings from other studies on the impact of mushroom additions on bread colour, where similar trends were observed; the addition of mushrooms such as *I. obliquus*, *A. bisporus*, and *G. lucidum* to bread formulations resulted in darker product colours, with noticeable changes in the intensity of red and yellow hues [Kobus *et al*., 2024; Łysakowska *et al*., 2024; Zhang *et al*., 2019].

The changes in the colour of the crumb in the wheat bread enriched with CS (**Table 4**) can be attributed to the presence of natural high-molecular weight pigments, such as melanins [Karmańska & Karwowski, 2022] and phenolic compounds [Babotă *et al*., 2022]. The phenolic compounds present in the CS powder may inhibit the Maillard reaction and reduce the formation of melanoidins by binding to amines, thereby decreasing their availability for reactions with reducing sugars [Rebollo-Hernanz *et al*., 2019]. Additionally, phenolic compounds can inhibit oxidoreductases, such as polyphenol oxidase (PPO) and peroxidase (POD), thereby limiting enzymatic browning of the crumb [Karmańska & Karwowski, 2022; Sui *et al*., 2023; Zhang *et al*., 2019]. However, in our study, these possible reactions of phenolic compounds did not significantly affect the course of colour changes, which was primarily determined by the high content of pigments (melanins) in the CS powder.

Texture profile of bread

The texture parameters of wheat bread enriched with the CS powder did not show statistically significant differences (p>0.05) after 24 and 48 h of storage (**Table 5**). The hardness of the crumb, measured 24 h after baking, increased with the growing addition of mushroom powder and was higher compared to the control bread by 4.7–46.9%. This trend may result from the decreased porosity of the bread, which is linked to the deterioration of the gluten network structure and its reduced elasticity [Jødal & Larsen, 2021]. The weakening of the gluten network could be directly due to the lower content of gluten proteins, while the increasing proportion of non-gluten proteins, dietary fiber, and polyphenols may contribute to the disruption and weakening of the gluten network, as shown in previous studies [Franceschinis *et al*., 2024; Marinopoulou *et al*., 2024; Zhang *et al*., 2019]. No significant (p>0.05) changes in bread hardness were observed after 48 h of storage as a function of increasing replacement of wheat flour with the CS powder (**Table 5**). An earlier study on the influence of bioactive ingredients, such as inulin and fiber from *Helianthus tuberosus* L., showed that their presence increased the hardness

Table 5. Parameters of texture profile of bread from wheat flour (control, CON) and from wheat flour substituted with *Cordyceps sinensis* powder at levels of 3, 6, 9, and 12%, *w/w*, (BC3, BC6, BC9, BC12, respectively), determined 24 and 48 h after baking.

Bread	Hardness (N)		Elasticity		Chewiness (N)		Cohesiveness	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
CON	17.01±1.50^{bA}	19.03±0.67^{aA}	6.33±1.50^{aA}	6.19±0.67^{aA}	6.58±0.45^{dA}	6.43±1.31^{bA}	0.86±0.05^{aA}	0.72±0.07^{aA}
BC3	17.81±0.17^{bA}	17.52±0.92^{aA}	6.27±0.17^{aA}	6.31±0.14^{aA}	8.69±0.24^{cdA}	8.06±0.48^{abA}	0.72±0.06^{aA}	0.69±0.04^{aA}
BC6	19.47±1.25^{bA}	18.92±1.92^{aA}	6.30±0.10^{aA}	6.32±0.08^{aA}	11.36±0.56^{bcA}	9.29±1.55^{abA}	0.68±0.02^{aA}	0.68±0.01^{aA}
BC9	22.42±0.71^{abA}	21.76±1.53^{aA}	5.48±0.52^{aA}	6.34±0.18^{aA}	12.85±0.80^{abA}	11.57±1.41^{abA}	0.64±0.02^{aA}	0.64±0.02^{aA}
BC12	24.98±0.54^{aA}	24.03±2.78^{aA}	5.47±0.12^{aA}	6.24±0.10^{aA}	14.58±0.20^{aA}	14.44±0.55^{aA}	0.63±0.04^{aA}	0.57±0.02^{aA}

Data are shown as mean ± standard deviation (n=7). Different lowercase letters (a–d) within a column indicate significant differences according to Tukey's test (p≤0.05). No significant (p>0.05) differences were found between determinations performed 24 and 48 h after baking (separately for each parameter and product).

of the crumb through modifications of the water-starch-protein interactions [Franceschinis *et al*., 2024]. Similar effects were observed with the introduction of hemp proteins, which induced a denser crumb structure of the bread, while simultaneously reducing its elasticity [Marinopoulou *et al*., 2024]. The changes in crumb elasticity and cohesiveness observed after 24 and 48 h of storage, resulting from the use of the CS powder in bread production, were not statistically significant (p>0.05) (**Table 5**). Compared to the control bread, chewiness, measured 24 h after storage, was significantly (p≤0.05) higher for the bread produced from flour containing 6% (*w/w*) CS powder and more, while after 48 h, statistically significant differences in this parameter were observed only at the 12% (*w/w*) fortification level. The increase in chewiness in the bread enriched with the CS powder correlated with a previous report on the impact of high-fiber additives, such as carob extract, on the textural properties of bread [Zahorec *et al*., 2024].

Element content of raw materials and bread

The introduction of the CS powder into bread significantly enriched its mineral profile (**Table 6**). Compared to wheat flour, the mushroom powder contained approximately 10 times more magnesium, 9 times more calcium, and 7.5 times more potassium. It also exhibited significantly higher levels of trace elements such as iron, copper, zinc, and selenium, while showing only trace amounts of heavy metals (lead and cadmium), which ensures its health safety. Compared to the control sample, the bread produced with 12% (*w/w*) wheat flour substitution with the mushroom powder contained over three times more magnesium, which constitutes 14.7% of the recommended daily allowance (RDA) for this element, as established by the Institute of Medicine of the National Academies, USA [Institute of Medicine, 2006]. Similarly, the calcium content increased 2.8 times, meaning that the consumption of BC12 bread covers 5.5% of the RDA for this mineral. The potassium content increased 1.5 times, reaching 15% of the RDA. A significant increase (p≤0.05) was also observed in the trace elements. Even at the lowest 3% (*w/w*) substitution level of wheat flour with the CS powder, the bread became a good source of iron, zinc, and selenium (covering >15% of the RDA for these elements). Fortification at levels of 6–12% (*w/w*) resulted in bread with a high copper content (>30% RDA). At the same time, the content of lead and cadmium, remained at safe levels. The Pb content in the bread produced from flour containing 12% (*w/w*) of the CS powder was 0.0104 mg/100 g, which corresponded to only 2.43% of the acceptable daily intake (ADI) established by the joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) expert committee [FAO/WHO, 2001]. The Cd content of this bread type was minimal (0.0032 mg/100 g), accounting for 5.33% of the ADI. This indicates that the use of CS in bread production does not increase the risk associated with the presence of these metals and ensures the full health safety of the product. The fortification of bread with elements such as iron and zinc, whose bioavailability in wheat-based products is limited due to the presence of phytates, becomes especially significant in the context of preventing deficiencies of these elements in groups at risk of their shortages [Gupta *et al*., 2024].

Total phenolic content, total flavonoid content and antioxidant capacity of raw materials and bread

The incorporation of the CS powder into bread significantly enhanced the TPC, TFC and antioxidant capacity in DPPH and ABTS assays compared to the control bread (**Table 7**). Flavonoids were undetectable in both wheat flour and the control bread but TFC exhibited an increase in the bread produced from flour with increasing levels of the mushroom powder. For instance, the sample with the highest mushroom powder substitution (BC12) demonstrated an over 25-fold increase in TFC compared to the samples with lower supplementation level (*e.g.*, BC3). TPC also increased markedly in the enriched breads. BC12 showed an 11.2-fold increase in TPC relative to the control and a 39.7-fold increase compared to wheat flour. Statistical analysis confirmed significant (p≤0.05) differences between samples. Antioxidant capacity, assessed *via* the DPPH

Table 6. Content of selected elements (mg/100 g d.m.) in raw materials including wheat flour and *Cordyceps sinensis* (CS) powder, and bread from wheat flour (control, CON) and from wheat flour substituted with CS powder at levels of 3, 6, 9, and 12%, *w/w*, (BC3, BC6, BC9, BC12, respectively).

Product	Macroelements			Microelements				Heavy metals	
	Mg	Ca	K	Fe	Cu	Zn	Se	Pb	Cd
Wheat flour	22.3±0.5^{B}	22.8±0.5^{B}	171±3^{B}	2.50±0.05^{B}	0.16±0.00^{B}	0.70±0.01^{B}	0.01±0.01^{B}	ND	ND
CS powder	227.0±4.5^{A}	198.0±4.0^{A}	1,270±25^{A}	22.30±0.45^{A}	0.94±0.02^{A}	4.27±0.09^{A}	0.03±0.03^{A}	0.02±0.00	0.00±0.00
CON	13.5±0.2^{d}	11.6±0.4^{d}	121±1^{e}	1.36±0.05^{d}	0.17±0.01^{d}	0.54±0.20^{d}	0.01±0.01^{b}	ND	ND
BC3	47.5±0.4^{c}	35.4±0.9^{c}	222±2^{d}	2.32±0.08^{c}	0.24±0.08^{c}	1.80±0.09^{bc}	0.03±0.00^{a}	0.0094±0.0002^{a}	0.0032±0.0003^{a}
BC6	52.7±0.3^{b}	37.7±0.7^{bc}	242±2^{c}	2.54±0.21^{bc}	0.32±0.11^{b}	1.84±0.05^{bc}	0.03±0.00^{a}	0.0096±0.0001^{a}	0.0032±0.0002^{a}
BC9	53.2±0.5^{b}	39.5±1.2^{b}	263±2^{b}	2.70±0.18^{b}	0.33±0.06^{ab}	1.89±0.10^{b}	0.03±0.01^{a}	0.0096±0.0002^{a}	0.0032±0.0003^{a}
BC12	55.1±0.4^{a}	43.8±0.9^{a}	304±2^{a}	3.06±0.27^{a}	0.33±0.08^{ab}	2.18±0.12^{a}	0.03±0.00^{a}	0.0104±0.0011^{a}	0.0032±0.0030^{a}
RDA (mg/day)	375	800	2,000	14	1	10	0.055	–	–
ADI (mg/day)	–	–	–	–	–	–	–	0.428	0.06

Results are shown as mean ± standard deviation (n=3). Different lowercase letters (a–e) and uppercase letters (A–B) within a column indicate differences according to Tukey's test ($p \leq 0.05$). d.m., Dry matter; ND, not detected; RDA, recommended dietary allowance [Institute of Medicine, 2006]; ADI, acceptable daily intake [FAO/WHO, 2001].

Table 7. Total phenolic content, total flavonoid content and antioxidant capacity of raw materials including wheat flour and *Cordyceps sinensis* (CS) powder, and bread from wheat flour (control, CON) and from wheat flour substituted with CS powder at levels of 3, 6, 9, and 12%, *w/w*, (BC3, BC6, BC9, BC12, respectively).

Product	Total flavonoid content (mg QE/g d.m.)	Total phenolic content (mg GAE/g d.m.)	Antioxidant capacity (mg TE/g d.m.)	
			DPPH assay	ABTS assay
Wheat flour	ND	0.13±0.02^{B}	1.03±0.00^{B}	1.42±0.03^{B}
CS powder	0.73±0.05	13.06±0.30^{A}	3.24±0.01^{A}	5.49±0.13^{A}
CON	ND	0.46±0.04^{e}	1.42±0.02^{d}	1.48±0.14^{d}
BC3	0.08±0.01^{c}	1.09±0.20^{d}	1.73±0.01^{c}	1.83±0.08^{c}
BC6	0.10±0.01^{c}	2.26±0.10^{c}	1.89±0.01^{b}	2.36±0.13^{b}
BC9	0.18±0.02^{b}	4.08±0.30^{b}	1.94±0.03^{b}	2.85±0.18^{a}
BC12	0.26±0.01^{a}	5.16±0.40^{a}	2.16±0.02^{a}	3.05±0.12^{a}

Results are shown as mean ± standard deviation (n=3). Different lowercase letters (a–e) and uppercase letters (A–B) within a column indicate significant differences according to Tukey's test (p≤0.05). QE, quercetin equivalent; GAE, gallic acid equivalent; TE, Trolox equivalent; DPPH assay, assay with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals; ABTS assay, assay with 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cations; d.m., dry matter; ND, not detected.

assay, improved proportionally with the increase in the amount of mushroom used in the production of the breads. BC12 exhibited a 52% higher DPPH radical scavenging activity than the control and a 110% increase over wheat flour. A comparable pattern was observed in the ABTS assay, where antioxidant capacity in BC12 more than doubled compared to the control and increased nearly fourfold relative to wheat flour. Although the antioxidant capacity of the enriched breads remained lower than that of pure CS powder (*e.g.*, approximately 33% lower for BC12), the results highlight the viability of enriching bread with mushroom, even at low levels of flour replacement, in enhancing its functional properties. These trends align with studies on bread enriched with other medicinal mushrooms (*e.g.*, chaga and shiitake) or plant-derived antioxidants (*e.g.*, green tea) where phenolic enrichment correlated with improved free radical-scavenging capacity [Chen *et al.*, 2022; Kobus *et al.*, 2024; Lu *et al.*, 2021]. Notably, the magnitude of improvement observed here exceeds reports for *Terfezia boudieri* (desert truffle), where a 5% supplementation increased TPC fivefold [Najjaa *et al.*, 2021], underscoring CS as a potent functional ingredient for bakery applications.

Sensory scores for bread

The substitution of wheat flour with the CS powder in amounts ranging from 3–12% (*w/w*) did not significantly impair the sensory quality of the bread (**Table 8**). Although generally, an increase in the level of the mushroom powder was associated with a slight deterioration in external appearance, aroma, taste, and crumb elasticity of the bread, leading to a decrease in the overall rating, the differences in the evaluation of individual sensory attributes were statistically insignificant (p>0.05). Moreover, according to expert evaluations, the use of the mushroom powder in bread recipe did not significantly (p>0.05) change the crumb colour. However, even a wheat flour substitution with CS powder at a level of 12% (*w/w*) resulted in bread that was still fully acceptable from a sensory perspective. The characteristic mild taste of CS contributed to its high acceptability. Comparing these results with studies on bread enriched with *G. lucidum* powder, where doses above 6% led to a drastic decline in sensory scores due to a bitter taste [Łysakowska *et al.*, 2024], CS showed a significantly higher potential for bread substitution and, therefore, can be used in relatively high doses. Similarly, 5–10% flour replacement with *P. ostreatus* powder ensured high acceptability of the bread, but its higher contents negatively impacted the texture [Wahab *et al.*, 2022]. Furthermore, studies on bread enriched with *H. erinaceus* powder suggested that the best-quality bread could be obtained using a moderate substitution level (6–9%) [Łysakowska *et al.*, 2025].

CONCLUSIONS

The research has shown that *C. sinensis* exhibits high potential as a functional ingredient in wheat bread. The substitution of 3–12% (*w/w*) of wheat flour with the mushroom powder modified the nutritional value of the bread by increasing its dietary fiber, protein, and selected mineral contents, while also lowering the levels of digestible carbohydrates. Incorporation the CS powder into bread recipe increased the content

Table 8. Sensory evaluation of bread from wheat flour (control, CON) and from wheat flour substituted with *Cordyceps sinensis* (CS) powder at levels of 3, 6, 9, and 12%, w/w, (BC3, BC6, BC9, BC12, respectively).

Bread	External appearance	Aroma	Crumb colour	Crumb elasticity	Taste	Overall rating
CON	4.71±0.73^{a}	4.71±0.70^{a}	4.29±0.70^{a}	4.29±0.88^{a}	4.57±0.49^{a}	4.51±0.26^{a}
BC3	4.33±0.45^{a}	4.29±0.88^{a}	4.29±0.73^{a}	4.57±0.35^{a}	4.43±0.57^{a}	4.38±0.24^{a}
BC6	4.39±0.49^{a}	4.71±0.76^{a}	4.57±0.49^{a}	4.57±0.35^{a}	4.43±0.45^{a}	4.53±0.24^{a}
BC9	4.57±0.35^{a}	4.43±0.45^{a}	4.57±0.45^{a}	4.86±0.88^{a}	4.57±0.35^{a}	4.60±0.24^{a}
BC12	4.36±0.45^{a}	4.00±0.76^{a}	4.71±0.45^{a}	4.14±0.42^{a}	4.43±0.49^{a}	4.33±0.31^{a}

Results are shown as mean ± standard deviation (n=9). Different letters within a column indicate significant differences according to Tukey's test (p≤0.05).

of phenolic compounds of the product, significantly enhancing its antioxidant capacity. The texture parameters of the bread generally did not change significantly with increasing levels of the mushroom powder in the recipe, with only a notable increase in hardness occurring at the level of 12% (*w/w*) wheat flour substitution, while a 6–12% (*w/w*) substitution improved its chewiness. Sensory analysis confirmed the high acceptability of the enriched bread, with even the highest 12% (*w/w*) wheat flour substitution with the mushroom powder resulting in bread with good sensory quality, as indicated by high sensory scores. The use of the CS powder in bread fortification highlights its great potential in the production of foods with improved health-promoting properties that can help prevent lifestyle-related diseases. The research findings encourage further application of this ingredient in various bakery products and other categories of functional foods.

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CONFLICT OF INTERESTS

The authors confirm that they have no competing interests related to the research described in this manuscript.

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Changes in the Quality of Dried Vacuum-Infused Strawberries Enriched with Encapsulated Pomegranate Peel Extract During Storage

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The low stability of phenolic compounds restricts their use in the food industry. Encapsulation can help protect these compounds during food processing. In this study, pomegranate peel extract (PPE) was encapsulated and then used to produce dried vacuum-infused strawberries. The quality changes of strawberry products enriched with encapsulated pomegranate peel extract (EPPE) were investigated during 90 days of storage at 25°C and compared to those infused with non-encapsulated PPE. During storage, the dried strawberries enriched with EPPE exhibited higher contents of total phenolics and individual phenolics, such as ellagic acid and punicalagin, than the dried strawberries with PPE. The total phenolic content of the strawberry product with EPPE was preserved at 9.40% better after 90 days of storage compared to the product enriched with PPE. Additionally, after storage, the strawberry product with EPPE showed significantly lower microbial counts (total aerobic mesophilic bacteria, yeast, and mold) and reduced browning index compared to the product with PPE. Moisture content changes during storage were controlled to the greater extent in the product with EPPE, contributing to its structural integrity. These results indicate that encapsulation, combined with vacuum impregnation, can effectively enhance the stability, microbial safety, and shelf-life of dried infused strawberries.

Keywords: encapsulation, pomegranate by-product, storage vacuum impregnation, strawberry snack

ABBREVIATIONS

AJC, apple juice concentrate; BI, browning index; EPPE, encapsulated pomegranate peel extract; GAE, gallic acid equivalent; GMO, glycerol monooleate; IQF, individually quick frozen; PPE, pomegranate peel extract; PPs, pomegranate peels; SFO, sunflower oil; TPC, total phenolic content; TSS, total soluble solids; VI, vacuum impregnation.

INTRODUCTION

Researchers have focused on using pomegranate peels (PPs) to extract valuable phenolic compounds and produce natural antioxidant additives for use in the food industry [Kaderides *et al*., 2021]. *In vitro* and *in vivo* studies have shown that these compounds elicit health-promoting effects due to their anti-inflammatory, antimutagenic, anticarcinogenic, and antihypertensive properties [Kandylis & Kokkinomagoulos, 2020].

As a popular processing method that enhances the stability and bioavailability of bioactive compounds, encapsulation was also used to improve PPE, offering improved health benefits and making it a viable functional food ingredient [Hady *et al*., 2022; Marcillo-Parra *et al*., 2021; Rashid *et al*., 2022]. PPE has been encapsulated by different methods, such as spray drying,

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electro-blow spinning, ionic gelation and various emulsion systems [Sanhueza *et al*., 2022; Wilkanowicz & Saud, 2023].

Strawberries are soft fruits with a high water content, susceptible to microorganisms and prone to spoilage. Therefore, various processes involving the use of nanoparticles are applied to extend their shelf-life. Li *et al*. [2023] examined the impact of solid lipid nanoparticles encapsulating cinnamaldehyde (SLN-CA) compared to non-encapsulated cinnamaldehyde on the freshness of strawberries stored for a week. The application of SLN-CA significantly decreased decay and softness, reduced the loss of organic acids, and enhanced the sensory qualities of the strawberries. In another study conducted by Yin *et al*. [2024], a nanoemulsion containing eugenol and citral was developed, exhibiting favorable physicochemical properties and antimicrobial efficacy for strawberry preservation. The findings indicated that the bilayer emulsion demonstrated significant antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger*, and was proved effective in preserving the quality of strawberries during storage. Moreover, Zhu *et al*. [2025] produced cinnamon essential oil nanoemulsions (CEO NEs) and investigated their effect on the shelf-life of strawberries during storage at room temperature. The results indicated that the shelf-life of strawberries can be prolonged for up to 7 days at room temperature, with the group treated with CEO NEs exhibiting a reduced rate of weight loss and mold growth compared to the control groups. Notably, strawberries in the ultrapure water group deteriorated more quickly and became contaminated with mold after just 3 days.

In our previous study, the nanoemulsions with PPE characterized by a high phenolic content and strong antioxidant properties were prepared and their stability during storage was investigated [Ertek *et al*., 2023]. After obtaining the encapsulated pomegranate peel extract (EPPE), the process conditions to produce phenolic-infused strawberries were optimized. As a result, we maintained constant process conditions at 480 bar and 20°C for 20 min. This study continues our previous research and looks at how the quality of strawberry product obtained by vacuum impregnation with EPPE and drying changes over storage time. To the best of our knowledge, no studies have evaluated the quality characteristics of strawberry products containing an encapsulated phenolic-rich extract during storage. Therefore, the aim of this study was to determine the physicochemical parameters, phenolic contents, and microbiological stability of dried vacuum-infused strawberries enriched with EPPE during 90 days of storage and to compare their properties with those enriched with non-encapsulated PPE.

MATERIALS AND METHODS

Materials

Individually quick frozen (IQF) strawberry (var. *Camarosa*) and apple juice concentrate (AJC) (70°Brix) were supplied from Işık Organic Food Company (Kemalpaşa, İzmir, Turkey). AJC was used as the osmotic impregnation solution. PP powder was used to obtain the phenolic extracts. For this purpose, PPs were purchased from a local market (Bornova, İzmir, Turkey). The raw materials were stored at −18°C until processing. Tween 80 (T80, Merck, Rahway, NJ, USA), an encapsulating emulsifier, was purchased from Sigma-Aldrich (Saint Louis, MO, USA).

Preparation of the pomegranate peel extract

First, the PPs were washed and drained. The samples were then dried in an air-circulation oven at 50°C until the moisture content was below 10 g/100 g. The dried peels were ground using a grinder (SCM 2934, Sinbo, İstanbul, Turkey), soaked, and mixed in water at room temperature for 24 h. The solid to solvent ratio was adjusted to 3:100 g/mL. The mixture was then centrifuged using a Universal 320 R centrifuge (Hettich, Kirchlengern, Germany) at 4°C and 12,000×*g* for 15 min to separate the supernatant. Then the liquid extract was freeze-dried (Christ Alpha 1-2LD, lyophilization system, Osterode am Harz, Germany) at −40°C and 0.002 mbar. Finally, the pomegranate peel extract (PPE) was milled and stored at 4°C until encapsulation. The extraction yield was 94.5% as was determined in our previous study [Ertek *et al*., 2023].

Encapsulation of pomegranate peel extract

PPE was encapsulated with T80 and glyceryl monooleate (GMO). Our previous work optimized the encapsulation process [Ertek *et al*., 2023], and emulsions containing PPE were prepared according to the formulation: 1% (*w/w*) PPE, 1% (*w/w*) T80, 1% (*w/w*) GMO, 3% (*w/w*) sunflower oil (SFO), and 94% distilled water. Initially, the organic phase consisting of PPE and SFO and the aqueous phase containing emulsifiers and distilled water were prepared using a magnetic stirrer. Then, both phases were combined and mixed with a homogenizer (Ultra Turrax T25, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 24,000 rpm for 5 min [Donsi *et al*., 2012]. Then, a high-pressure homogenizer (ML-100L microfluidizer, Microfluidics, Westwood, MA, USA) was used to obtain a nanoemulsion from the coarse emulsion (150 MPa, 5 cycles).

Vacuum impregnation of strawberries

The vacuum impregnation (VI) process was conducted using a system available at the Ege University, Department of Food Engineering, where both the vacuum level and temperature can be controlled, and the samples are agitated. The parameters that varied during the vacuum impregnation process included the vacuum pressure (100 to 480 mbar), the duration of the vacuum (10 to 30 min), and the processing temperature (between 20 and 40°C). The process was performed by keeping the optimum processing conditions constant, as determined in a previous study [Ertek *et al*., 2023]. The mass ratio of the strawberries (after being thawed at 4°C for 18 h) to the impregnation solution (AJC) was maintained at 1:1 (*w/w*). PPE solution (1%, *w/w*) or PPE nanoemulsion was added to the AJC at a ratio of 1% (*v/v*). A vacuum pressure of 480 bar was applied to the system for 10 min at 20°C. The mixtures were then kept at atmospheric pressure for 1 h. After impregnation, both groups of vacuum-infused strawberries (with PPE and with EPPE) were drained and dried in a tray dryer (50°C, Weintek, İstanbul, Turkey) until the water activity of the strawberry products was 0.60.

Storage of dried vacuum-infused strawberries

After production of the dried vacuum-infused strawberries with PPE and with EPPE, they were stored as 100 g *per* serving in 175 g polypropylene bags at 25°C (53% relative humidity) for 90 days. Just before storage (day 0) and on days 15, 30, 60 and 90 of storage, samples were taken from both groups, and quality analyses were performed.

Quality analyses of dried vacuum-infused strawberries

Total soluble solid content, moisture content, and water activity

The total soluble solid (TSS) content of the dried vacuum-infused strawberries was measured using a digital refractometer (HI96801, Hanna, Cluj-Napoca, Romania) after the strawberry products were homogenized with water at 1:5 (*w/w*) ratio. Water activity was measured using a digital water activity meter (Testo 645, Lenzkirch, Germany) [Taze & Ünlütürk, 2018]. To determine moisture content (g/100 g), the weighed samples were dried in a vacuum oven at 65±2°C, and drying was completed when the samples achieved a constant weight [AOAC, 2000].

pH and titratable acidity

The pH and titratable acidity were determined using a pH meter (Orion 2 Star, Thermo Fisher Scientific, Waltham, MA, USA) according to the method of AOAC International [AOAC, 2000]. The strawberry products were soaked in water at a 1:10 g/mL ratio and homogenized for 15 s using a homogenizer (Ultra Turrax T25, IKA-Werke GmbH & Co. KG, Staufen, Germany). Titratable acidity was measured using 0.1 M NaOH until the pH of the sample reached 8.1. The titratable acidity was expressed as g citric acid equivalents/100 g of strawberry product.

Firmness

The firmness of the vacuum-infused strawberry products was analyzed using a texture analysis device (TA XT Plus Texture Analyzer, Stable Micro Systems, Godalming, Surrey, UK), as outlined by Tylewicz *et al.* [2019]. The system was equipped with a 5 kg load cell and an 8 mm diameter stainless-steel probe. The test involved penetrating the samples to 90% of their thickness. Analysis was conducted with 8 replicates, and the results were reported as the maximum force (N) values.

Total phenolic content

In the extraction, 10 g of strawberry products were mixed with 10 mL of aqueous methanol (80%, *v/v*) and homogenized (8,000 rpm for 2 min) (Ultra Turrax T25, IKA). Then, the mixture was centrifuged at 4°C for 12,000×*g* for 15 min (Universal 320 R centrifuge, Hettich). The supernatants were stored at 4°C until analyzed.

The total phenolic content (TPC) was determined using the Folin-Ciocalteu'a reagent according to the procedure described by De Ancos *et al.* [2017]. The supernatant (200 µL) was mixed with 1 mL of the Folin-Ciocalteu'a reagent (0.2 N) in a tube and shaken for 5 min. Then, 800 µL of 3.5% (*w/v*) sodium carbonate were added, and the mixture was kept for 1 h at 25°C. After reaction, 300 µL was taken and transferred to a 96-well plate. The absorbance was measured using a microplate reader (Multiskan Go UV/VIS, Thermo Scientific, Waltham, MA, USA) at 750 nm. TPC was expressed as mg gallic acid equivalents (GAE)/100 g dry matter (dm) of strawberry product.

Punicalagin and ellagic acid content

Punicalagin and ellagic acid standards were purchased from Sigma-Aldrich (Sant Louis, MO, USA). The aqueous methanolic extract obtained for the TPC analysis was also used for punicalagin and ellagic acid determination. The high-performance liquid chromatography (HPLC) system (Agilent 1200, Santa Clara, CA, USA) was equipped with a Zorbax C18 (250×4.5 mm, 5 µm, Agilent) column and a diode array detector (DAD) to quantify the phenolics. Analysis was performed using water/acetic acid (98:2, *v/v*) (A) and methanol (B) as the mobile phase at the 1 mL/min flow rate [Çam & Hışıl, 2010]. The gradient was as follows: 5% B for 5 min, 5–70% B for 25 min, and 70–5% B for 10 min. The column temperature was maintained at 35°C. Punicalagin was detected at 280 nm, whereas ellagic acid was detected at 378 nm. Standard curves were prepared at 0–100 mg/L for punicalagin and 0–200 mg/L for ellagic acid. The results of punicalagin and ellagic acid content were expressed as mg/kg dm of strawberry product.

Browning index

The browning index (BI) of vacuum-infused strawberry products was analyzed according to the method previously used by Coskun *et al.* [2013]. For the extraction, a 2.5 g sample was combined with 25 mL of a 2% (*v/v*) acetic acid solution (Sigma Aldrich) and homogenized using an IKA T25 Ultra Turrax at 25,000 rpm for 2 min. The mixture was then centrifuged at 4,032×*g* for 10 min using a Hettich Universal 320 R centrifuge. The supernatant was collected, and the extraction process was repeated under the same conditions. Then, 2.5 mL of the combined supernatants were taken, and an equal volume of ethanol (Merck) was added. The resultant mixture was centrifuged again. Absorbance measurements against a solution of ethanol and 2% acetic acid (1:1, *v/v*) were recorded at 420 nm using a spectrophotometer (Genesys 10S Vis, Thermo Fisher Scientific). The results were expressed as A_{420}/g of strawberry product.

Microbiological analysis

Total aerobic mesophilic bacteria and total yeast and mold enumeration in dried vacuum-infused strawberries with EPPE and with PPE was performed using the AOAC International methods 990.12 and 997.02 [AOAC, 2000]. Total aerobic mesophilic bacteria were determined using plate count agar (PCA, Merck) with the pour-plate method. The plates were incubated for mesophiles at 37°C for 24–48 h. The enumeration of total yeast and molds was carried out in potato dextrose agar (acidified to pH 3.5 with 100 g/L tartaric acid) (PDA, Merck) after incubation at 25°C for 3–5 days. The results were expressed as log CFU *per* 100 g of strawberry product.

Statistical analysis

All treatments were performed in triplicate, and results were expressed as the mean and standard deviation. Statistical analysis was conducted using SPSS 18 software (SPSS Inc., Chicago, IL, USA). The analysis of variance (ANOVA) with Duncan's post hoc test was performed to evaluate the effects of encapsulation and storage time. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Total soluble solid content, moisture content, water activity, pH, and titratable acidity of dried vacuum-infused strawberries during storage

The total soluble solid (TSS) and moisture content, water activity, pH, and titratable acidity values of dried vacuum-infused strawberries with PPE and EPPE are shown in **Table 1**.

The TSS is an important parameter that reflects the quality of fruits during storage, as it is closely related to sugar content. An increase in TSS is contributes to the ripening process by enhancing the sweetness and overall flavor profile of the fruit. The more significant the decrease in TSS, the faster the deterioration of fruit quality tends to occur. While this relationship is well established in fresh fruits, where TSS reduction often reflects increased respiration and loss of sweetness, similar trends may also be observed in dried fruits during storage, potentially indicating degradation of sugars and overall product quality under certain conditions [Ghinea *et al*., 2022; Zhang *et al*., 2019]. In our study, there were no significant ($p\geq0.05$) differences in TSS between dried vacuum-infused strawberries at the beginning of storage (day 0) and on day 15 of storage in both groups (**Table 1**). However, a significant ($p<0.05$) increase was observed from day 15 to the end of storage. Strawberries of the EPPE group had significantly ($p<0.05$) higher TSS content then strawberries of the PPE group throughout the storage, which could be attributed to variations in the physical characteristics of the strawberry tissue, such as cell structure, wall integrity, and porosity. Castelló *et al*. [2006] observed differences in TSS contents in distinct tissue regions (especially pores) of the strawberry, indicating the formation of concentration gradients, which may be associated with structural and physiological changes during impregnation.

Strawberries have a short shelf-life because of their delicate tissue and high moisture content. Drying reduces the content of free water, making them microbiologically safer. Water activity of dried vacuum-infused strawberries ranged from 0.58–0.61 (**Table 1**). These values were below the critical a_w required for the growth of microorganisms (>0.65) [Gautam *et al*., 2024]. Similarly to our study, Corrêa *et al*. [2010] observed a notable decrease in the water activity of guava slices subjected to osmotic dehydration, influenced by the application of vacuum pulses and solution concentration. Previous studies have also shown that vacuum impregnation followed by drying can significantly reduce water activity. For instance, Nawirska-Olszańska *et al*. [2020] reported much lower a_w values (0.144–0.207) in impregnated chokeberry fruits, which were notably lower than the a_w range observed in our study (0.58–0.61), though both remain below the microbial growth threshold.

The moisture content of dried strawberries containing EPPE changed from 11.5 to 13.4 g/100 g during storage (**Table 1**). It significantly ($p<0.05$) increased during the 60 days of storage, but no significant ($p\geq0.05$) difference was observed in the moisture

Table 1. The total soluble solid content, moisture content, water activity, pH, and titratable acidity of dried vacuum-infused strawberries with pomegranate peel extract (PPE) and encapsulated pomegranate peel extract (EPPE) during storage.

Parameter	Group	Storage time (day)				
		0	15	30	60	90
Total soluble solid content (°Brix)	Strawberry product with EPPE	58.7 ± 0.16^{Ad}	59.3 ± 0.09^{Ad}	65.5 ± 0.19^{Ac}	72.2 ± 0.50^{Ab}	73.3 ± 0.52^{Aa}
	Strawberry product with PPE	55.2 ± 0.22^{Bd}	55.0 ± 0.05^{Bd}	60.2 ± 0.12^{Bc}	70.0 ± 0.52^{Bb}	71.9 ± 0.52^{Ba}
Moisture content (g/100 g)	Strawberry product with EPPE	11.5 ± 0.02^{Bd}	11.8 ± 0.01^{Bc}	12.5 ± 0.03^{Ab}	13.3 ± 0.14^{Aa}	13.4 ± 0.10^{Ba}
	Strawberry product with PPE	12.5 ± 0.02^{Ac}	12.1 ± 0.06^{Ad}	11.8 ± 0.02^{Be}	13.6 ± 0.08^{Ab}	14.0 ± 0.12^{Aa}
Water activity	Strawberry product with EPPE	0.60 ± 0.005^{Aa}	0.60 ± 0.005^{Aa}	0.58 ± 0.005^{Ac}	0.59 ± 0.005^{Ab}	0.59 ± 0.005^{Ab}
	Strawberry product with PPE	0.60 ± 0.005^{Ab}	0.60 ± 0.005^{Ab}	0.60 ± 0.005^{Ab}	0.60 ± 0.005^{Ab}	0.61 ± 0.009^{Aa}
pH	Strawberry product with EPPE	4.2 ± 0.01^{Aa}	3.8 ± 0.02^{Ab}	3.9 ± 0.01^{Ab}	3.8 ± 0.01^{Ab}	3.9 ± 0.02^{Ab}
	Strawberry product with PPE	4.0 ± 0.01^{Aa}	3.9 ± 0.03^{Aa}	4.0 ± 0.01^{Aa}	3.9 ± 0.01^{Aa}	4.3 ± 0.01^{Bb}
Titratable acidity (g CA/100 g)	Strawberry product with EPPE	8.3 ± 0.46^{Ab}	9.3 ± 0.35^{Aa}	9.7 ± 0.69^{Aa}	9.9 ± 0.23^{Aa}	9.8 ± 0.10^{Aa}
	Strawberry product with PPE	8.7 ± 0.40^{Ab}	9.6 ± 0.45^{Aa}	9.3 ± 0.08^{Aa}	9.1 ± 0.12^{Bb}	9.1 ± 0.07^{Bb}

Results are shown as mean ± standard deviation ($n=3$). Different uppercase letters (A and B) in the same column, separately for each parameter, indicate significant differences between groups ($p<0.05$). Different lowercase letters (a–e) in the same line for each group indicate significant differences between storage times ($p<0.05$). CA, citric acid equivalent.

content during the last month of storage. At the end of storage, the moisture content of strawberries of the EPPE group (13.4 g/100 g) was lower than that of the PPE group (14.0 g/100 g). This difference suggests that the use of EPPE may have contributed to a more stable moisture environment, potentially limiting excessive moisture uptake. In other words, encapsulation may result in a more effective barrier to moisture, thereby preventing excessive hydration of a dry product during storage.

EPPE and PPE-treated strawberries exhibited significant changes ($p<0.05$) in pH relative to their initial levels at the end of storage (**Table 1**). In strawberries of the EPPE group, the pH was highest on the first day, decreased on the 15th day and remained stable until the end of storage. This trend was similar for the strawberry product in the PPE group, although pH decreases continued until 60 days of storage. The pH of strawberries with PPE was significantly ($p<0.05$) higher than that of the product with EPPE after 90 days of storage. The difference in pH between the EPPE and PPE groups can be attributed to the encapsulation process. In the EPPE group, encapsulation may have slowed the release of active compounds, leading to an initial pH stability followed by a gradual decrease. In contrast, the PPE group showed a continuous pH decrease until the 60th day, likely due to the faster release of extract compounds. This suggests that encapsulation helped stabilize the pH during storage. Encapsulation has been shown to enhance the stability of bioactive compounds, including maintaining pH levels during storage, like in a study by Cruz-Molina *et al.* [2021], which demonstrated that maltodextrin encapsulation improved the thermal and pH stability of green tea extract catechins, preserving their chemical structure and antioxidant capacity under varying pH conditions over time.

Similar to the pH values, changes also occurred in the TA of the dried vacuum-infused strawberries during storage (**Table 1**). Moreover, the strawberries with EPPE had significantly ($p<0.05$) higher TA than the strawberries with PPE after 90 days of storage. This may be related to the effect of encapsulation, which has been reported to help maintain TA stability during storage in various food products [Yin *et al*., 2024; Zeng *et al*., 2024]. For instance, in a study on pineapple ready-to-serve beverages enriched with folic acid, the encapsulated form led to a more stable TA over a two-month period compared to the free form, suggesting that encapsulation can help protect organic acids or slow down their degradation during postharvest fruit storage [Pamunuwa *et al.,* 2021]. The TA values obtained in our study (8.3–9.9 g CA/100 g) were high compared to literature data, *e.g.*, the TA of chitosan-coated strawberries containing propolis extract were examined, and the acidity value of the control and treated fruits was determined to be 1.65–1.94 g CA/100 g at the beginning of storage [Akkuzu *et al*., 2024]. The variability in the results is considered due to the variety of the strawberries used in the study.

Firmness of dried vacuum-infused strawberries during storage

Texture is one of the key attributes that shapes the sensory properties of a product and influences consumer perception. The texture quality of food can be assessed by instrumental analysis, the measurements of which often correlate with sensory evaluation [Ribes & Talens, 2023]. Among texture parameters, firmness was determined in our study. Overall, an increase in the firmness of dried vacuum-infused strawberries was observed with increasing storage time in both groups (**Table 2**). There was a significant ($p<0.05$) difference in firmness between the strawberries with EPPE and with PPE on all storage days except for the 15th and 90thday. The absence of a significant difference on the 15th day may be due to a temporary structural equilibrium between strawberries of the EPPE and PPE groups, possibly resulting from similar rates of moisture redistribution or cell wall softening occurring at that specific point in storage. Additionally, reduced enzymatic activity during storage may have slowed down cell wall degradation, helping enhance firmness.

Biegańska-Marecik & Czapski [2007] found that the firmness of vacuum-impregnated apple slices decreased during storage. However, similarly to our study, no significant differences were observed between the treatment groups at certain time points. Similarly, Maslov Bandić *et al*. [2025] did not observe any significant differences in the firmness of untreated strawberries, chitosan-coated strawberries and strawberries coated with chitosan with added apple pomace extract during 9 days of storage.

Total phenolic, punicalagin and ellagic acid contents of dried vacuum-infused strawberries during storage

The changes in the TPC, ellagic acid and punicalagin contents of dried vacuum-infused strawberries during storage are shown in **Table 3**. The results indicate a significant ($p<0.05$) decrease in TPC during the storage. At the end of storage, the TPC was

Table 2. The firmness (N) of dried vacuum-infused strawberries with pomegranate peel extract (PPE) and encapsulated pomegranate peel extract (EPPE) during storage.

Group	Storage time (day)				
	0	15	30	60	90
Strawberry product with EPPE	3.6±0.30^{Bd}	5.0±0.10^{Ac}	4.8±0.30^{Bc}	5.7±0.11^{Bb}	6.4±0.29^{Aa}
Strawberry product with PPE	4.6±0.19^{Ae}	5.1±0.46^{Ad}	5.6±0.20^{Ac}	6.2±0.30^{Ab}	6.6±0.10^{Aa}

Results are shown as mean ± standard deviation (n=3). Different uppercase letters (A and B) in the same column indicate significant differences between groups ($p<0.05$). Different lowercase letters (a–e) in the same line for each group indicate significant differences between storage times ($p<0.05$).

Table 3. The content of total phenolics, punicalagin and ellagic acid of dried vacuum-infused strawberries with pomegranate peel extract (PPE) and encapsulated pomegranate peel extract (EPPE) during storage.

Parameter	Group	Storage time (day)				
		0	15	30	60	90
Total phenolic content (mg GAE/100 g dm)	Strawberry product with EPPE	409 ± 12^{Aa}	325 ± 61^{Ac}	380 ± 4.5^{Ab}	255 ± 14^{Ad}	231 ± 6.6^{Ae}
	Strawberry product with PPE	313 ± 15^{Ba}	250 ± 13^{Bb}	218 ± 15^{Bc}	115 ± 13^{Be}	147 ± 6.6^{Bd}
Punicalagin content (mg/kg dm)	Strawberry product with EPPE	$13{,}367\pm85^{Aa}$	$11{,}333\pm1247^{Ab}$	$9{,}479\pm157^{Ac}$	$8{,}138\pm120^{Ad}$	$6{,}248\pm37^{Ae}$
	Strawberry product with PPE	$9{,}450\pm83^{Ba}$	$8{,}403\pm51^{Bb}$	$7{,}274\pm49^{Bc}$	$7{,}209\pm54^{Bc}$	$5{,}128\pm85^{Bd}$
Ellagic acid content (mg/kg dm)	Strawberry product with EPPE	186 ± 0.8^{Aa}	155 ± 3.9^{Ab}	146 ± 0.3^{Ac}	131 ± 1.3^{Ad}	126 ± 0.5^{Ae}
	Strawberry product with PPE	141 ± 5.0^{Ba}	117 ± 1.7^{Bb}	112 ± 1.5^{Bc}	95 ± 0.2^{Bc}	83 ± 2.8^{Bd}

Results are shown as mean ± standard deviation (n=3). Different uppercase letters (A and B) in the same column, separately for each parameter, indicate significant differences between groups (p<0.05). Different lowercase letters (a–e) in the same line for each group indicate significant differences between storage times (p<0.05). GAE, gallic acid equivalent, dm, dry matter.

230.8 mg GAE/100 g dm for strawberries of the EPPE group and 147.2 mg GAE/100 g dm for strawberries of the PPE group. This could result from phenolic compound degradation due to oxidation, enzymatic activity, or interactions with other food components during storage. Hassanein *et al*. [2024] studied the possibility of shelf-life extension of strawberries using encapsulated pomegranate seed oil, and found that the TPC of the strawberries containing nanoemulsion was higher at the end of storage compared with the samples treated with coarse emulsion (non-encapsulated oil). In turn, Amiri *et al*. [2022] demonstrated that the incorporation of catechin nanoemulsions encapsulated within *Aloe vera* gel effectively preserved the TPC of strawberries during storage by limiting the degradation of phenolic compounds. Pomegranate seed oil-enriched carboxymethyl cellulose coatings effectively preserved the phenolic content of strawberries during storage, enabling to maintain up to 70% of total phenolics, which highlights their potential to reduce oxidative degradation and support antioxidative stability [Melikoğlu *et al*., 2022].

At the end of storage, the strawberries of the EPPE group had a higher TPC than the strawberries of the PPE group (**Table 3**), which clearly demonstrates the positive effect of encapsulation. Kaderides *et al*. [2020] reported that encapsulation significantly affected phenolic retention; the encapsulated pomegranate peel extract in orange juice industry by-products exhibited significantly higher antioxidative activity, TPC, and punicalagin content than the crude PPE. Similarly, Xu *et al*. [2019] reported that the phenolic compounds of the crude mulberry extract degraded more rapidly than the encapsulated mulberry extract and that approximately 80% of the phenolic compounds were preserved in mulberry microcapsules after storage at room temperature for 20 days. Furthermore, the encapsulation was found to improve the stability of phenolic compounds of grape pomace after storage at 60°C for 45 days [Tsali & Goula, 2018].

The phenolic profile of dried vacuum-infused strawberries was related not only to the phenolic profile of strawberries, but also to the phenolic profile of the pomegranate peel extract. The major phenolic compounds in PPs are punicalagin and ellagic acid, which exhibit strong antioxidative and antibacterial activities [Çam & Hışıl, 2010; Firuzi *et al*., 2019; Qu *et al*., 2012a; Soleimanzadeh *et al*., 2024]. Notably, punicalagin isomers constitute most tannins in PPs [Çam & Hışıl, 2010]. However, the content of these compounds in extracts varies depending on factors such as cultivation conditions and plant variety [Faria & Silva, 2024], and also extraction method used [Al-Zoreky, 2009; Zaki *et al*., 2015]. In our study, initially, the punicalagin content in strawberries of the EPPE group was significantly (p<0.05) higher than that in strawberries of the PPE group, which contained approximately 70% of punicalagin determined in product with the EPPE (**Table 3**). As the storage period continued, the amount of punicalagin decreased in both groups, with a significant difference observed compared to the initial values (p<0.05). Although encapsulation can offer some protection to phenolic compounds like punicalagin, preventing their degradation entirely during storage remains challenging. The reduction in punicalagin content observed in both groups during storage may be attributed to several factors, including the ongoing oxidation and hydrolysis reactions, which can still occur despite encapsulation. Furthermore, environmental factors such as temperature, light, and oxygen exposure during storage may contribute to the gradual loss of phenolic compounds [Akkuzu *et al*., 2024; Qu *et al*., 2012b; Xu *et al*., 2019]. While encapsulation helps slow down these processes, it does not completely prevent the degradation of bioactive compounds over time. Another potential reason for the decrease in punicalagin content during storage could be the presence of punicalagin α and β anomers, which may undergo different degradation rates over time, as suggested by Rakshit & Srivastav [2022]. In the cited study, punicalagin, as a compound of the pomegranate peel hydrolysable tannin fraction, dissociated up to 58.48 and 88.34% during storage at neutral and alkaline pH, respectively [Rakshit & Srivastav, 2022]. Qu *et al*. [2012b] found that the punicalagin

Table 4. The browning index (A_{420}/g) of dried vacuum-infused strawberries with pomegranate peel extract (PPE) and encapsulated pomegranate peel extract (EPPE) during storage.

Group	Storage time (day)				
	0	15	30	60	90
Strawberry product with EPPE	4.8 ± 0.25^{Aa}	4.0 ± 0.67^{Ab}	4.2 ± 0.45^{Bb}	4.2 ± 0.13^{Bb}	4.3 ± 0.06^{Bb}
Strawberry product with PPE	4.3 ± 0.13^{Bd}	4.1 ± 0.15^{Ac}	4.5 ± 0.21^{Ab}	4.5 ± 0.04^{Ab}	4.8 ± 0.02^{Aa}

Results are shown as mean ± standard deviation (n=3). Different uppercase letters (A and B) in the same column indicate significant differences between groups (p<0.05). Different lowercase letters (a–e) in the same line for each group indicate significant differences between storage times (p<0.05).

Table 5. The total aerobic mesophilic bacteria and total yeast and mold counts (log CFU/g) of dried vacuum-infused strawberries with pomegranate peel extract (PPE) and encapsulated pomegranate peel extract (EPPE) during storage.

Parameter	Group	Storage time (day)				
		0	15	30	60	90
Total aerobic mesophilic bacteria	Strawberry product with EPPE	1.72 ± 0.10^{Ad}	1.82 ± 0.06^{Bc}	1.91 ± 0.03^{Bb}	1.98 ± 0.01^{Bab}	2.01 ± 0.01^{Ba}
	Strawberry product with PPE	1.85 ± 0.05^{Ac}	1.99 ± 0.04^{Aab}	1.99 ± 0.03^{Ab}	2.03 ± 0.02^{Aa}	2.06 ± 0.04^{Aa}
Total yeasts and molds	Strawberry product with EPPE	1.73 ± 0.03^{Ac}	1.80 ± 0.03^{Ac}	1.98 ± 0.03^{Ab}	2.04 ± 0.03^{Bb}	2.14 ± 0.04^{Ba}
	Strawberry product with PPE	1.76 ± 0.03^{Ae}	1.83 ± 0.03^{Ad}	2.00 ± 0.03^{Ac}	2.13 ± 0.02^{Ab}	2.23 ± 0.02^{Aa}

Results are shown as mean ± standard deviation (n=3). Different uppercase letters (A and B) in the same column, separately for each parameter, indicate significant differences between groups (p<0.05). Different lowercase letters (a–e) in the same line for each group indicate significant differences between storage times (p<0.05).

content in a PP extract significantly decreased even after 1 day of storage at 4°C, independent of pH.

The decrease in ellagic acid content in the vacuum-infused strawberries followed a similar trend to that of punicalagin (**Table 3**). After 90 days, the ellagic acid content was determined at 125.9 mg/kg in the dried strawberries containing EPPE, whereas it was 83.4 mg/kg in the strawberries of the PPE group.

Browning index of dried vacuum-infused strawberries during storage

The changes in color of fruits can be evaluated using the browning index based on the loss of red pigment and an increased content of yellow pigment [Wigati *et al*., 2024; Zeng *et al*., 2024]. The BI of dried vacuum-infused strawberries ranged from 4.0 to 4.8 A_{420}/g (**Table 4**). The browning index of the strawberries containing EPPE was significantly (p<0.05) lower than that of the strawberries of the PPE groups after 30–60 days of storage. These findings indicate that encapsulation of PPE improved the protection of strawberries against environmental factors, particularly oxygen, thereby inhibiting their browning [Wigati *et al*., 2024]. Despite its high content of antioxidants, PPs is sensitive to oxidation at high pH, and its browning rate has been correlated with its antioxidant content [Silveira *et al*., 2023].

A similar browning phenomenon was observed during the storage of grape pomace extract [Tsali & Goula, 2018]. This effect was attributed to the loss of anthocyanin-derived red pigments. In contrast to our results, for strawberries coated with starch and calcium propionate and stored in cold conditions (4°C and 85% RH), the highest BI was observed in the control fruits (uncoated) after 16 days of storage [Wigati *et al*., 2024]. The BI values of uncoated and coated strawberries in the cited study ranged from 0.525 to 1.524 and were lower than those of vacuum-infused strawberries in our study, probably due to the drying process we used after the VI.

Counts of total aerobic bacteria and total yeast and mold in dried vacuum-infused strawberries during storage

Due to short shelf-life of strawberries, which is caused by high water activity and the associated vulnerability to microbial and enzymatic degradation, strawberries are frequently subjected to various processing techniques such as drying, impregnation, and coating [Kowalska *et al*., 2018; Maslov Bandić *et al*., 2025; Yin *et al*., 2024]. In our study, the total aerobic mesophilic bacteria and mold-yeast counts of dried vacuum-infused strawberries were 2.06 and 2.23 log CFU/g after 90 days of storage, respectively (**Table 5**). These counts of microorganisms were low, due to the low moisture content and water activity of products. Although the effect of PPE encapsulation was not observed on the first day of storage of dried vacuum-infused strawberries, the antimicrobial effect of EPPE against aerobic bacteria became significantly (p<0.05) better than that of EPP after the 15th day. The trend of mold and yeast count in strawberries during storage was similar to that of total aerobic bacteria count.

The antimicrobial effect was likely due to the presence of punicalagin in PPE. Phenolic compounds possess various

antifungal and antimicrobial properties [Kaderides *et al*., 2020]. The antimicrobial effect of phenolic compounds is attributed to their hydroxyl groups, which lower the pH of the bacterial cell membrane surface and disrupt bacterial metabolism, leading to bacterial death [Pisoschi *et al*., 2018].

In a review encompassing studies on the use of PPE as an edible film, it was noted that films containing PPE exhibited excellent antioxidative and antimicrobial properties [Soleimanzadeh *et al*., 2024]. In turn, Yin *et al*. [2024] demonstrated that strawberry samples coated with a bilayer emulsion containing eugenol/citral exhibited strong antimicrobial activity against *E. coli*, *S. aureus*, and *A. niger*.

CONCLUSIONS

In conclusion, this study effectively highlights the impact of PPE encapsulation on the preservation and quality of dried vacuum-infused strawberries over a 90-day storage period. The findings demonstrate that while total soluble solids remained stable between days 0 and 15, a significant increase was observed thereafter, indicating the beneficial effects of strawberry processing on maintaining sugar content during storage. The relatively low water activity and moisture content changes reinforce the efficacy of the drying process, while also supporting the viability of the product over time.

Notable changes in pH and titratable acidity provide additional insight into the varying retention of active compounds in strawberries of the EPPE and PPE groups. The encapsulation promoted a slow release of these components, maintaining a stable pH in strawberries of the EPPE group while resulting in a more significant decrease in the PPE group. Moreover, the findings indicate a continued decrease in the total phenolic content during storage; however, strawberries in the EPPE group retained significantly higher levels of phenolics by the end of the study, which highlights the importance of encapsulation in enhancing antioxidant properties. After 90 days, strawberries in the EPPE group showed lower browning indices, indicative of better protection against oxidative damage when compared to the PPE group. This attribute may be particularly advantageous for maintaining product appeal and quality in storage conditions. Furthermore, the microbial analysis confirmed the safety of the dried strawberries, with low total aerobic mesophilic bacteria, and mold and yeast counts detected during the storage.

Overall, this study presents clear evidence that the use of encapsulated PPE plays a crucial role in enhancing the quality and shelf-life of vacuum-infused strawberries, suggesting significant potential for commercial applications in fruit preservation and value-added processing. Future studies may explore different encapsulation techniques and their effects on other fruit varieties, thus expanding the knowledge in this essential area of food science.

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CONFLICT OF INTERESTS

The authors confirm that they have no competing interests related to the research described in this manuscript.

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