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DEVELOPMENT OF GLUTEN-FREE MEATBALLS USING OLEASTER AND COCONUT FLOURS AS BREADCRUMB REPLACERS

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ABSTRACT
This study explored the development of gluten-free meatballs by substituting traditional breadcrumbs (BC) with oleaster flour (OF) and coconut flour (CF). Five meatball formulations were prepared: 8% BC (control, M1), 8% OF (M2), 8% CF (M3), 6% CF + 2% OF (M4), 4% CF + 4% OF (M5). The flour and meatball samples were evaluated for some of their physical, chemical, physicochemical and sensorial properties. OF addition notably enhanced the antioxidant capacity of the meatballs (52.93 mg Trolox equivalent/100g dry weight). OF and CF utilization increased cooking loss and altered textural properties ($P<0.05$). Sensory analysis revealed that M2 showed comparable scores to the control, while CF-containing samples received lower scores. Overall, OF and CF show potential as gluten-free ingredients in meatball formulations, with OF (M2) emerging as the most promising substitute for BC in terms of both nutritional quality and sensory acceptance.
Keywords: Gluten-related disorders, gluten-free diet, meatball, oleaster flour, coconut flour

GALETA UNU YERİNE İĞDE VE HİNDİSTAN CEVİZİ UNU KULLANILARAK GLUTENSİZ KÖFTE GELİŞTİRİLMESİ

ÖZ
Bu çalışmada, geleneksel galeta unu (GU) yerine iğde unu (İU) ve hindistan cevizi unu (HCU) kullanılarak glutensiz köfte geliştirilmesi araştırılmıştır. Beş köfte formülasyonu hazırlanmıştır: %8 GU (kontrol, M1), %8 İU (M2), %8 HCU (M3), %6 HCU + %2 İU (M4), %4 HCU + %4 İU (M5). Un ve köfte örnekleri bazı fiziksel, kimyasal, fizikokimyasal ve duyusal özellikleri açısından değerlendirilmiştir. İU ilavesi, köftelerin antioksidan kapasitesini belirgin şekilde artırmıştır (52.93 mg Troloks eşdeğeri/100 g kuru ağırlık). İU ve HCU kullanımı pişirme kaybını artırmış ve tekstürel özellikleri değiştirmiştir ($P<0.05$). Duyusal analiz sonuçlarına göre, M2 grubu kontrol grubuyla benzer puanlar alırken, HCU içeren formülasyonlar daha düşük puanlar almıştır. Genel olarak, İU ve HCU,

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glutensiz köfte formülasyonlarında kullanılabilecek potansiyele sahip bileşenlerdir ve özellikle İU (M2), hem besinsel kalite hem de duyusal kabul açısından GU yerine en umut verici alternatif olarak öne çıkmaktadır.
Anahtar kelimeler: Glutenle ilişkili rahatsızlıklar, glutensiz beslenme, köfte, iğde unu, hindistan cevizi unu

INTRODUCTION

Meat and meat products play a vital role in human nutrition due to their high content of protein, vitamins, and essential fatty acids (Domínguez et al., 2019). Among these, meatballs are widely enjoyed globally for their appealing taste and ease of preparation and cooking. Traditionally, meatballs are made using minced meat (from beef, chicken, ham, fish, or a combination), breadcrumbs (BC), chopped onion, eggs, butter, and spices. Breadcrumbs are a key ingredient in meatball recipes, helping to bind the mixture and prevent it from breaking apart during cooking. However, the gluten content in BC renders meatballs unsuitable for those with gluten sensitivity (Babaoğlu, 2022).

Gluten is a protein naturally found in certain cereals, including wheat, barley, rye, and oats that contributes to the structural and sensory characteristics of products made from these grains. However, gluten-related disorders, including celiac disease (CD), wheat allergy, and non-celiac gluten sensitivity, have become increasingly prevalent (Canabillas, 2020; Šmídová and Rysová, 2020; Sebença et al., 2021). CD is one of the most common and serious gluten-related conditions, presenting as an autoimmune enteropathy triggered by gluten in genetically susceptible people (Niewinski, 2008). The global prevalence of CD is estimated to exceed 1% and is on the rise, with variations in prevalence ranging from 0% to 3.1% depending on the country (Bradauskiene et al., 2023; Jansson-Knodell et al., 2023). Based on five different diagnostic methods, wheat allergy prevalence is predicted to be less than 1% for each method (Liu et al., 2023). A range between 0.5-15% is estimated for the prevalence of non-celiac gluten sensitivity. For individuals with gluten-related disorders, lifelong gluten elimination from the diet is the primary step for treating the disease and alleviating its symptoms (Miranda et al., 2014; Kaur, 2023). This necessitates the removal of almost all bakery goods (bread, pasta, cookies, cakes, etc.), including those sold in the market and made at home, from the diet. Furthermore, other products containing gluten and their derivatives should be avoided. Therefore, it is necessary to replace the ingredients in these products with their gluten-free counterparts. Gluten-free alternatives for these ingredients include gluten-free cereals, pseudocereals, legumes, tubers, nuts, oilseeds, fruits, vegetables, and their flours (El Khoury et al., 2018; Šmídová and Rysová, 2020). Additionally, commercially available gluten-free flour mixes are commonly used in gluten-free formulations (Stoin, 2016).

While conceptually straightforward, removing gluten from the diet can be challenging due to various economic and logistical factors, such as individuals' financial means and the limited availability of gluten-free products. These specialty products are often costly and may not be available in all markets. Additionally, the absence of gluten complicates the task of achieving desired textural, sensory, and nutritional qualities, often requiring the addition of other functional ingredients like hydrocolloids, emulsifiers, fibers, and enzymes (El Khoury et al., 2018). To expand the variety and accessibility of gluten-free products, a growing number of studies are being conducted by the food industry and academic researchers (Oyeyinka et al., 2018; Babaoğlu, 2022; Oyeyinka et al., 2022).

With the increasing demand for gluten-free meat products, the use of alternative flours (such as quinoa, buckwheat, chickpea, corn, millet, maize, rice, soy, linseed, amaranth, and hemp) in meatball production has garnered scientific interest (Mastanjević et al., 2014; Prabhu et al., 2016; Bağdatlı, 2018; Augustyńska-Prejsnar et al., 2022; Babaoğlu, 2022). For instance, Yazdanpanah et al. (2022) developed gluten-free sausages using chickpea flour, corn flour, and hydroxypropyl methylcellulose. Other studies, like those by de Carvalho et al. (2018), optimized gluten-free nuggets with ingredients like oregano, basil, egg, soy oil, and amaranth flakes, while Devatkal et al. (2011) explored the quality of gluten-free chicken nuggets formulated with sorghum flour. Similarly, Romero et al. (2018), developed gluten-free fish patties using rice, corn, amaranth, or quinoa flours. Despite the growing literature, no study to date has explored the use of coconut flour (CF) and oleaster flour (OF) as binding ingredients in meatball formulations to replace BC.

Coconut is a tropical fruit known for its rich dietary fiber, vitamins, and minerals, and is widely used in various industries, including culinary, cosmetics, and pharmaceutical (Agyemang-Yeboah, 2011; Shankar et al., 2014). Coconut flour (CF), a by-product of coconut oil or coconut milk production, is derived from coconut residue and holds potential as a functional ingredient in foods. Studies suggest that adding CF to the diet may help reduce the risk of chronic diseases and regulate blood cholesterol and sugar levels (Jiamjariyatam et al., 2022). Oleaster is a nutrient-dense fruit with a floury texture, rich in essential fatty acids, vitamins, minerals, carotenoids, flavonoids, and other bioactive components (Sahan et al., 2015; Karkar and Şahin, 2022). It has been valued for its medicinal properties such as antipyretic, antidiarrheal, diuretic, and tonic effects; and is traditionally used for various treatments in Turkish, Jordanian, and Iranian folk medicine (Sahan et al., 2015). Oleaster flour (OF) is gluten-free and serves as a functional ingredient in diverse food applications, including bakery goods, dairy products, beverages, and confectionery, due to its dietary fiber, mineral content, phenolic compounds, and antioxidant activity (Sahan et al., 2013; 2015).

One of the primary challenges in producing gluten-free products is replicating the quality characteristics of gluten-containing counterparts. For this reason, the ingredients must perform similar functions to gluten. Dietary fiber, for instance, plays a crucial role in gluten-free formulations by binding water, increasing viscosity, and promoting gel formation (El Khoury et al., 2018). Given the high fiber content of OF (Sahan et al., 2015) and CF (Trinidad et al., 2006), these flours may be promising substitutes for gluten-containing flours in meatball production, potentially fulfilling the same role as BC. This study aimed to develop gluten-free meatballs by replacing BC with gluten-free OF and CF and to evaluate their physical, chemical, and sensory properties. Furthermore, this research assessed the potential of OF and CF as innovative, functional ingredients in meatball formulations.

MATERIALS AND METHODS

Materials

Minced beef and spices (salt, cumin, red pepper, and black pepper) were sourced from the local market (File Markets) in Sakarya, Türkiye. The OF (flour of *Elaeagnus angustifolia* L.) obtained from the whole oleaster fruit including peel and seed also (Aktarmarka, İzmir, Türkiye) and the CF (flour of *Cocos nucifera*) (TheLifeCo, İstanbul, Türkiye) were procured from local suppliers. BC was obtained from local producer Bağdat Baharat (Bağdat Marketing Trade. Co. Ltd., Ankara, Türkiye) for control samples. OF, CF, and BC were purchased in powder form and used without pretreatment in the meatball formulations. Methanol was procured from Sigma Aldrich (St. Louis, MO, USA). Trolox (Acros, Thermo Fisher Scientific, MA USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (TCI, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), gallic acid, trichloroacetic acid (Isolab Laborgeräte GmbH, Eschau, Germany), acetone (VWR, Avantor, Radnor, US), sodium carbonate (AFG Scientific, Northbrook, USA), petroleum ether (Fluka, Honeywell Research Chemicals, North Carolina, US), nitric acid (Carlo Erba Reagents GmbH, Emmendingen, Germany), diethyl ether (Macron Fine Chemicals, Avantor, Radnor, US), and 1,1,3,3-Tetraethoxypropane (Cas no: 122-31-6, Sigma Aldrich, Germany) were used in the study. All other chemicals (such as Folin Ciocalteu's phenol reagent, acetic acid, etc.) used in the study were purchased from Merck (Merck KGaA, Darmstadt, Germany) unless otherwise specified.

Methods

Flour analyses

Proximate composition. The moisture (934.01), ash (923.03), protein (960.52), crude fat (920.39) content of flour samples were determined according to Association of Official Analytical Collaboration (AOAC) International method (AOAC, 2000). The method specified by the International Association for Cereal Science and Technology (ICC) was used for crude fiber analysis. Additionally, coconut flour was analyzed for crude fiber content following a defatting process (ICC, 1972).

Water and oil absorption. Water (WAC) and oil absorption (OAC) capacities of the flour were determined according to the method of Marchetti et al. (2018). One gram of flour samples was mixed with 10 ml of water and sunflower oil for WAC and OAC, respectively. The mixtures were held at room temperature for 30 min after vortexing for 2 min. After centrifuging at 3000 × g for 20 min, supernatants were discarded, and the tubes were weighed. The experiments were conducted with four replicates, and the results were expressed as "g water/g flour" for WAC or "g oil/g flour" for OAC on a dry weight (dw) basis.

Total phenolic content and radical scavenging activity. Total phenolic content analysis of the flour samples was conducted following the method outlined by Ramírez-Maganda et al. (2015), with modifications to the extraction procedure as demonstrated by Handa et al. (2016). For extraction, each flour sample (0.6 g) was combined with methanol (50:50, methanol:water, w/w) until the mixture reached a total weight of 6 g. The mixture was shaken by vortexing which was repeated at 15 minutes intervals during incubation at 25 °C for one hour. After incubation, the mixtures were placed in an ultrasonic bath (CLS ULT.4010, Türkiye) at 25 °C for 15 minutes and then centrifuged (3000 rpm, 10 minutes). The supernatant was filtered through a filter paper (4-7 μm pore size) and a 0.45 μm cellulose-acetate-based syringe filter (Isolab Laborgeräte GmbH, Germany), respectively. Total phenolic content results were expressed as mg Gallic acid equivalent/100 g dry sample (mg GAE/100 g dw) using a calibration curve (y=11.55x+0.0113, R^2=0.9998) of Gallic acid (5-80 mg/L).

The extracts obtained for the total phenolic content analysis were also used to determine the 2,2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging activity (Akdeniz et al., 2018). The radical scavenging effect was determined with Equation (1). The results were expressed as both inhibition percentage and mg Trolox equivalent per 100 g dry sample (mg TE/100 g dw) using the calibration curve (y=0.0778x-2.6201, R^2=0.9992) of Trolox (40-1200 μM).

$$\text{Radical scavenging effect (\%)} = \frac{(ABS_{Blank} - ABS_{Sample\ or\ Trolox})}{ABS_{Blank}} * 100 \quad (1)$$

Meatball analyses

Preparation of meatballs

The minced beef meat was divided into five portions. As shown in Table 1, five different meatball formulations were prepared as follows: M1 (control group - 8% BC), M2 (8% OF), M3 (8% CF), M4 (6% CF and 2% OF) and M5 (4% CF and 4% OF). These groups were formulated based on preliminary laboratory studies to determine the optimal formulation. To prepare the meatball dough, all ingredients were weighed separately, mixed, and kneaded until homogeneous. A portion of the meatball dough weighing 25±0.5 g was taken and shaped into round meatballs using a meatball press, resulting in meatballs with a diameter of 6 cm. The shaped meatballs were placed in commercial polypropylene food containers, wrapped with stretch film, and then stored in a refrigerator (+4 ºC) until analysis. Meatballs were produced with three independent repetitions, and samples from these three replicates were analyzed in duplicate.

Proximate composition. The moisture (950.46), ash (920.153), crude protein (955.04), and crude fat (991.36) content of the meatballs were determined according to the AOAC International method (AOAC, 2000). For pH analysis, the meatballs were diluted 10 times with distilled water, and the resulting mixture was measured at room temperature (24±2ºC) in a calibrated digital pH-meter (WTW, pH315i, Germany) (Haskaraca et al., 2017). The salt content of the meatballs was determined using the Mohr precipitation titration method as described by Lee et al. (2022).

Table 1. Formulations used in meatball production

Ingredients	Formulations				
	M1	M2	M3	M4	M5
Minced beef meat	89%	89%	89%	89%	89%
BC	8%	-	-	-	-
OF	-	8%	-	2%	4%
CF	-	-	8%	6%	4%
Seasoning mix (1.25% salt, 1% cumin, 0.5% red pepper, 0.25% black pepper)	3%	3%	3%	3%	3%

OF: oleaster flour, CF: coconut flour, BC: breadcrumbs, M1: control group meatball including 8% breadcrumbs, M2: meatball including 8% oleaster flour, M3: meatball including 8% coconut flour, M4: meatball including 6% coconut flour and 2% oleaster flour, M5: meatball including 4% coconut flour and 4% oleaster flour

Cooking loss and diameter reduction

In order to determine cooking loss, 25 grams of meatballs were placed in a polyethylene bag and kept in a water bath at 80 °C until the internal temperature reached 72 °C. Then, the cooked meatballs were weighed after cooling, and cooking losses were calculated with Equation (2) (Murphy et al., 1975). The diameter reduction of the meatballs was calculated with Equation (3), which involved measuring the diameter of both raw and cooked meatballs. Photos of the cooked and raw meatball samples are provided in Figure 1.

$$\text{Cooking Loss (\%)} = \frac{(\text{Raw meatball weight} - \text{Cooked meatball weight})}{\text{Raw meatball weight}} * 100 \quad (2)$$

$$\text{Diameter Reduction (\%)} = \frac{(\text{Raw meatball diameter} - \text{Cooked meatball diameter})}{\text{Raw meatball diameter}} * 100 \quad (3)$$

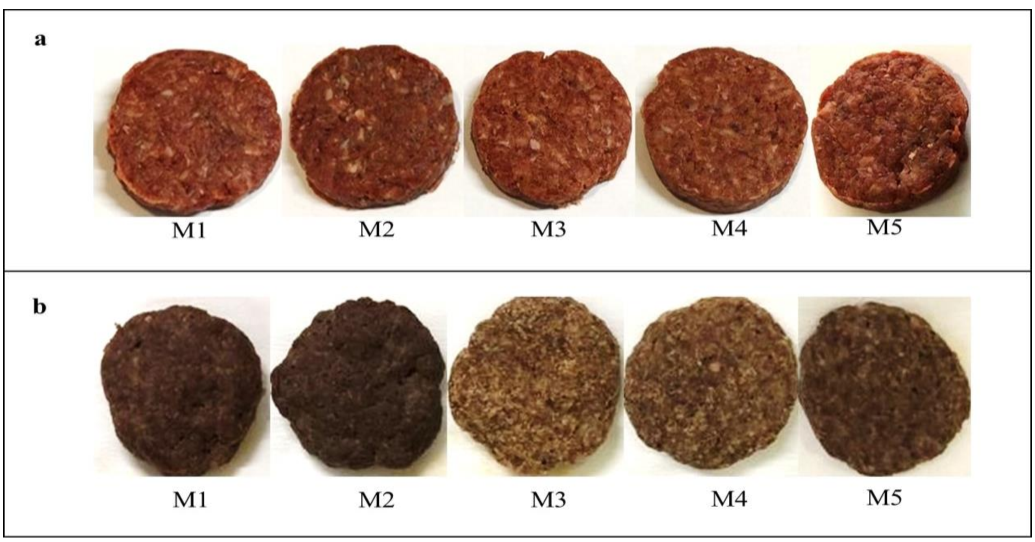

Figure 1. Appearance of raw (1a) and cooked (1b) meatballs prepared with different flour formulations.

Color measurement

Color values of the raw and cooked (cooked according to the procedure outlined in the cooking loss analysis) meatball samples were analyzed using CIE L* (lightness), a* (redness), and b* (yellowness) color scale with a colorimeter (PCE-CSM 7, PCE Instruments, Meschede, Germany) (Turgut et al., 2017). For each group, measurements were taken from six different points on the surfaces of three randomly selected meatballs. The results are presented as the mean L*, a*, and b* values.

DPPH radical scavenging activity

Radical scavenging activity analysis of the meatballs was conducted using the DPPH method (Akdeniz et al., 2018). The meatballs were extracted following the procedure outlined by Ergezer and Serdaroğlu (2018). For this purpose, two grams of meatball were vortexed with 10 ml of methanol and kept overnight for extraction at refrigeration temperature. After the extraction period, the mixture was centrifuged at 10000 rpm for 15 minutes (Nüve NF 1200R, Ankara, Türkiye), and the supernatants were stored at 4°C until analysis.

The DPPH radical scavenging activity, expressed as inhibition percentage (%), was determined for the meatball samples using Equation (1). Additionally, the DPPH radical scavenging activity was calculated as mg Trolox equivalent per 100 g of dry sample (mg TE/100 g dw) using the calibration curve of Trolox ($y = 0.2998x+0.5004$, $R^2=0.9982$) for concentrations ranging from 5 to 125 ppm.

Thiobarbituric acid reactive substances (TBARs)

The TBARs values were determined according to the method applied by Mielnik et al. (2006) with a minor modification (the only modification was in the centrifugation step; samples treated with 7.5% trichloroacetic acid were centrifuged at 10000 rpm for 5 minutes). The absorbance of the samples was measured spectrophotometrically (Shimadzu UV-mini 1240, Kyoto, Japan) at 532 nm against a blank sample, and the results were calculated as "mg malonaldehyde/kg meatball (mg MA/kg)" using a calibration graph obtained with 1,1,3,3-Tetraethoxypropane as the standard material.

Texture profile analysis (TPA)

TPA was performed using the TA-TX Plus texture analyzer (Stable Micro System, Surrey, UK) equipped with a 50 mm diameter cylindrical probe. The parameters included a test speed of 5 mm/s and a 30 kg load cell, operating under double compression mode at 50% compression. For this purpose, three cooked meatballs used in the cooking loss analysis were used for the TPA after cooling to room temperature. Two independent measurements were taken for each meatball, and the results were calculated as the mean of the measurements. The results were expressed in terms of hardness (N), adhesiveness (N.s), springiness, cohesiveness, gumminess (N), chewiness (N), and resilience (Crehan et al., 2000; Erdem et al., 2020).

Sensory evaluation

The sensory evaluation of the meatballs prepared with different gluten-free flours was conducted with the participation of 8 experienced panelists, consisting of academic staff and students (aged 20–40) from the Department of Food Engineering at Sakarya University. The sensory analyses of the meatballs took place after grilling both surfaces of the meatballs in a pan for 5 minutes. Panelists were asked to evaluate the meatballs in terms of their appearance/color, odor, flavor, texture, juiciness, and overall acceptance using a 9-point hedonic scale, ranging from 1 (extremely bad) to 9 (excellent) for each attribute (with 5 representing neither bad nor good) (Altuğ Onoğur and Elmacı, 2005).

Statistical analysis

Statistical differences between the flour types and the effect of different flour types on the physical, chemical, and sensory characteristics of the meatballs were determined by performing a one-way ANOVA using SAS System Software (SAS On Demand for Academics). Duncan's multiple range test was applied when the difference between samples was statistically significant ($P<0.05$). All results are expressed as mean ± standard deviation in all tables. Meatballs were produced with three independent repetitions and all analyses were performed in duplicate.

RESULTS AND DISCUSSION

Characterization of oleaster flour, coconut flour and breadcrumb

The characteristics of oleaster flour (OF), coconut flour (CF), and the control flour (BC), are presented in Table 2. Significant differences were observed across the flour samples for all properties (*P*<0.05). OF exhibited the highest total phenolic content and radical scavenging activity, while CF had the highest values for crude fiber, ash, fat, WAC, and OAC. The protein content was the highest in BC (12.81%), followed by CF (11.50%) and OF (7.69%).

Table 2. Characterization of oleaster flour, coconut flour and breadcrumb used in meatball production

	OF	CF	BC
Moisture (%)	5.72±0.11^{a}	3.76±0.03^{b}	5.60±0.08^{a}
Ash (%)	1.83±0.07^{b}	2.46±0.06^{a}	1.70±0.05^{c}
Lipid (%)	3.26±0.05^{b}	17.58±0.27^{a}	0.62±0.03^{c}
Protein (%)	7.69±0.00^{c}	11.50±0.00^{b}	12.81±0.00^{a}
Crude fiber (%)	15.12±0.27^{b}	22.25±0.54^{a}	0.74±0.07^{c}
WAC (g/g dw)	1.68±0.04^{c}	6.89±0.09^{a}	2.22±0.02^{b}
OAC (g/g dw)	1.17±0.01^{b}	1.23±0.01^{a}	1.04±0.02^{c}
TPC (mg GAE/100 g dw)	341.81±6.92^{a}	33.42±1.70^{c}	55.12±1.12^{b}
DPPH radical scavenging activity (as inhibition %)	70.07±2.81^{a}	9.58±0.34^{b}	6.13±0.59^{c}
DPPH radical scavenging activity (mg TE/100 g dw)	270.90±10.19^{a}	45.23±1.29^{b}	32.07±2.40^{c}

(a-c): Means with different letters in the same row indicate significant differences among the flour samples (*P*<0.05)
OF: oleaster flour, CF: coconut flour, BC: breadcrumbs, WAC: water absorption capacity, OAC: oil absorption capacity, TPC: total phenolic content, GAE: Gallic acid equivalent, TE: Trolox equivalent, dw: dry weight

Sahan et al. (2013) studied OF derived from two different oleaster genotypes, prepared by milling the dried fruit flesh with and without the peel after removing the seed. They reported the ash and protein contents for OF (with peel) from different genotypes ranged between 1.87 - 2.57 g/100 g and 4.49 - 4.65 g/100 g on a dry basis, respectively. Kouhanestani et al. (2019) produced OF from unpeeled fruit flesh and reported the ash, fat, protein, and crude fiber contents as 2.44, 4.38, 6.30, and 4.47 g/100 g dw, respectively. Differences observed between these studies and the data shown in Table 2 likely stem from variations in oleaster genotype, growing conditions, and processing methods. The OF used in this study was a commercial product containing peel, flesh, and seed. Kouhanestani et al. (2019) and Yavuz et al. (2021) reported the total phenolic contents for OF as 436.60 mg GAE/100 g and 3957.06 mg GAE/g, respectively, with the higher phenolic content in the study of Yavuz et al. (2021) likely due to the absence of a drying process. They reported antiradical activity at 6.48% using the DPPH method, whereas the activity observed in this study was approximately 70% (Table 2). Karkar and Şahin (2022) also reported higher total phenolic content for oleaster flour dried at room temperature compared to OF used in this study. Although the drying temperature for the OF sample is unknown, the lower moisture levels in Table 2 might suggest higher drying temperatures or longer drying times.

Different production methods for CF, including those with or without the defatting process, have been reported in the literature (Bawalan, 2000; Hopkin et al., 2022). Gunathilake et al. (2009) and Mihiranie et al. (2017) reported the protein, fiber, fat, and ash content of CF between 21.65% and 22.10%, 10.45% and 17.69%, 8.42% and 8.04%, and 5.96% and 6.17%, respectively. In comparison, CF's fat content in this study was nearly double, while the protein content was approximately half of their findings, which may be attributed to differences in CF production methods. The antioxidant activity (%) of defatted CF ranges between 20-65%, depending on extraction temperature (Du et al., 2019), whereas the radical scavenging activity in the current study for CF was 9.6%.

The moisture, protein, fat, ash, total phenolic content, and radical scavenging activity of BC were determined as 9.59 g/100 g, 10.30 g/100 g, 0.46 g/100 g, 0.53 g/100 g, 0.99 mg GAE/g, and 21.64%, respectively by Bahmanyar et al. (2021). Given the influence of growth conditions and wheat varieties on chemical composition (Kaur et al., 2021), some variations in results are expected. Additionally, the extraction method also affects the total phenolic content and radical scavenging activity. In the present study, the radical scavenging activity of BC was determined as 6.12% (Table 2).

WAC and OAC values are critical parameters in flour applications for achieving high-quality food products. Flour with excessively high or low WAC and OAC values can negatively impact food quality (Awuchi et al., 2019). Optimal OAC values enhance flavor retention and improve mouthfeel (Chandra and Samsher, 2013). As shown in Table 2, CF exhibited a significantly higher WAC than the other flours, likely due to its high fiber content, as reported by other researchers (Trinidad et al., 2006; Dat and Phuong, 2017; Du et al., 2021). Significant differences in OAC values were observed among the flour samples ($P<0.05$). While Dat and Phuong (2017) determined the WAC and OAC values of different particle-sized CF samples to range between 7.91-11.88 g/g and 3.28-3.93 g/g, respectively, Mihiranie et al. (2017) found these values for defatted CF to be approximately 3g/g and 1 g/g, respectively. In contrast to the WAC values of OF shown in Table 2, Sahan et al. (2015) reported the WAC values of unpeeled oleaster flour to range between 4.1 and 4.3 g/g, obtained from two different oleaster fruit genotypes. However, they obtained the flour after removing the seed, which might have a decreasing effect on WAC in our study.

Physicochemical properties of meatballs

Proximate composition

The proximate composition and pH values of raw meatballs are presented in Table 3. Different flours used in meatball production did not lead to statistically significant changes in moisture, fat, ash, protein, salt contents, or pH of the meatballs ($P>0.05$). Similarly, previous studies have reported that the addition of flours such as buckwheat, chickpea, corn, and millet had no effect on moisture, fat, ash content, or pH, though differences in protein content were noted due to the inherent protein levels of each flour type (Babaoğlu, 2022). Another study reported quinoa flour led to statistically significant differences in the proximate composition of meatballs, although pH remained unaffected (Bağdatlı, 2018). The salt content of meatballs in this study was comparable to that of meatballs made with toasted BC, at 1.53% (Yılmaz and Dağlıoğlu, 2003).

Color

The color parameters L*, a*, and b* for raw and cooked meatballs are illustrated in Figure 2. Flour type significantly influenced the color values L*, a*, and b* ($P<0.05$). The meatball sample containing 100% CF (M3) had the highest L*, a*, and b* values in both raw and cooked states. Generally, the inclusion of CF increased the color values, which can be attributed to its naturally whiter and brighter appearance, while increasing the OF content led to decreased values, likely due to the darker pigmentation and higher fiber content of oleaster flour, which may reduce the lightness and brightness of the meatball surface. Bağdatlı (2018) reported that quinoa flour increased the L* value of the meatballs, and Babaoğlu (2022) found that meatballs with buckwheat flour had the highest a* values for both raw and cooked states. Similarly, buckwheat and chickpea flour increased the L* values in raw and cooked meatballs, respectively. These findings align with current results, indicating that variations in meatball ingredients can influence color characteristics (El Khoury et al., 2018; Kaur, 2023).

Table 3. Physicochemical properties of meatballs

	Formulations				
	M1	M2	M3	M4	M5
Moisture (%)	61.11±0.71	60.69±0.36	60.48±0.09	61.15±0.55	61.80±1.88
Ash (%)	2.26±0.06	2.27±0.07	2.41±0.17	2.40±0.15	2.30±0.07
Lipid (%)	11.18±0.71	11.52±0.75	13.00±0.08	11.90±0.42	12.18±1.00
Protein (%)	14.89±0.33	14.12±0.51	14.52±0.25	14.30±0.16	14.73±0.30
pH	5.95±0.04	5.90±0.02	5.92±0.00	5.92±0.04	5.88±0.01
Salt (%)	1.70±0.08	1.73±0.18	1.69±0.08	1.71±0.11	1.59±0.13
Cooking loss (%)	12.82±0.24^{b}	16.24±0.51^{a}	16.28±1.13^{a}	16.34±0.37^{a}	15.70±0.33^{a}
Diameter reduction (%)	11.97±4.22	13.38±2.64	10.00±1.53	12.38±1.47	12.93±1.28
DPPH radical scavenging activity (mg TE/100 g dw)	46.68±1.08^{bc}	52.93±1.78^{a}	38.40±1.43^{d}	43.89±2.44^{c}	50.55±3.55^{ab}
TBARs (mg MA/kg meatball)	1.46±0.13^{c}	2.44±0.18^{a}	1.58±0.11^{c}	2.01±0.16^{b}	2.12±0.24^{b}

(a-c): Means with different letters in the same row indicate significant differences among the meatball groups (*P*<0.05)

M1: control group meatball including 8% breadcrumbs, M2: meatball including 8% oleaster flour, M3: meatball including 8% coconut flour, M4: meatball including 6% coconut flour and 2% oleaster flour, M5: meatball including 4% coconut flour and 4% oleaster flour

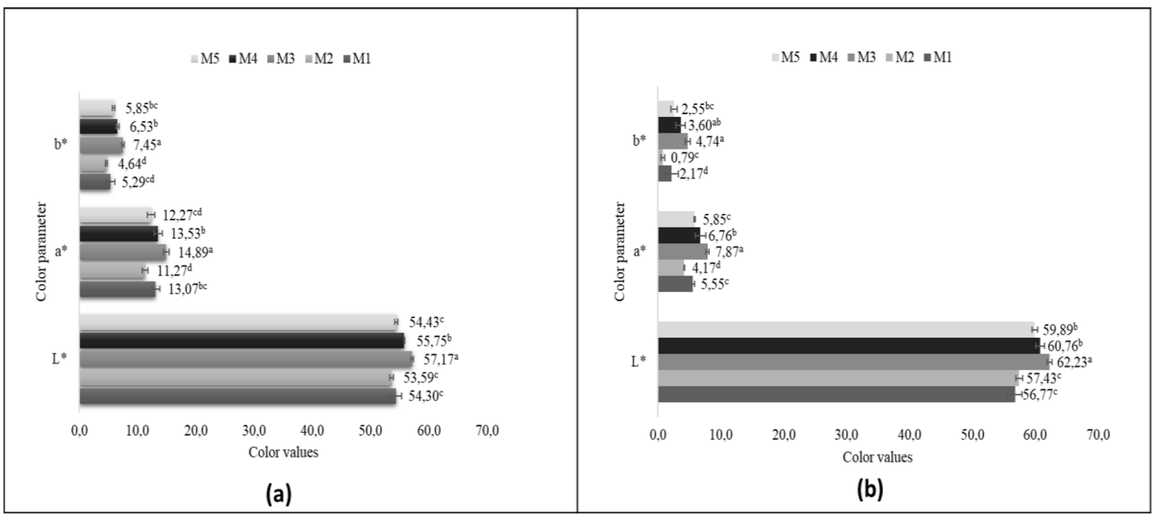

Figure 2. L*, a*, and b* color values of raw (2a) and cooked (2b) meatballs formulated with various gluten-free flours.

(a-d): Means with different letters indicate significant differences among different meatball groups for given color parameter (*P*<0.05).

Cooking loss and diameter reduction

The cooking loss and diameter reduction of the meatball samples are presented in Table 3. Given the high crude fiber, WAC, and OAC of OF and CF, it was initially expected that these meatballs would demonstrate lower cooking loss. However, the inclusion of OF and CF actually resulted in increased cooking loss compared to the control ($P<0.05$), possibly due to molecular interactions during cooking that altered water absorption behavior. But in the meantime, no significant differences were observed in the cooking loss of meatballs made with gluten-free flours. Similarly, Bağdatlı (2018) indicated that meatballs made with quinoa flour had lower cooking yields than those made with BC. Ergezer et al. (2014) found that meatballs prepared with potato puree exhibited higher cooking loss than those prepared with BC, while another study reported decreased cooking loss with gluten-free acorn flour (Akcan et al., 2024). In contrast, gluten-free meatballs made with buckwheat, chickpea, corn, and millet flours generally had lower cooking losses than BC-containing meatballs (Babaoğlu, 2022). In the literature, it has been observed that the cooking loss of meat products changes depending on the type of flour added. This variation is thought to be due to the chemical composition of the added flour, the contributions of other components in the formulation, or the applied cooking method.

Cooking loss is generally attributed to the water and fat loss occurring during meatball processing (Wang et al., 2016). Gluten, which typically forms a cohesive network, can reduce the mass transfer of proteins in flour and prevent excessive water loss (Kilincceker, 2013). The higher cooking loss values observed in meatballs containing OF and CF are likely due to the lack of gluten in these flours (Table 2). Moreover, protein molecules, especially those with polar amino acids, have a high affinity for water; approximately 31% of total water absorption in flour is attributed to proteins (Awuchi et al., 2019). As shown in Table 2, the protein content of OF and CF is lower than that of BC, which may contribute to the increased cooking loss in meatballs with OF and CF. Literature suggests that gluten-free meatballs made with flours containing protein levels similar to or higher than BC generally exhibit lower cooking losses.

As expected, the diameter of all meatballs decreased with the cooking due to moisture and fat loss (Salarkarimi et al., 2019). However, no statistically significant differences in diameter reduction were observed among the groups in this study ($P>0.05$). Prior studies indicate that gluten-free flours tend to reduce the diameter reduction in cooked meatballs compared to gluten-containing controls (Salarkarimi et al., 2019; Babaoğlu, 2022). In meatballs made with various legume flours, diameter reduction ranged from 6.9% to 10.6% (Serdaroğlu et al., 2005). In comparison, meatballs made with buckwheat, chickpea, corn, and millet flours had diameter reductions below 16.73%, which is the value observed for BC-containing meatballs (Babaoğlu, 2022). The maximum and minimum diameter reductions in this study were obtained for the M2 group (13.38%) and the M3 group (10.00%), respectively.

DPPH radical scavenging activity

Table 3 shows that the highest radical scavenging activity was found in M2 (11.07% inhibition; 52.93 mg TE/100 g dw), likely due to the high DPPH radical scavenging activity of OF compared to CF and BC. This was followed by M5 (12.28% inhibition; 50.55 mg TE/100 g dw), M1 (11.36% inhibition; 46.68 mg TE/100 g dw), M4 (10.81% inhibition; 43.89 mg TE/100 g dw), and M3 (9.70% inhibition; 38.40 mg TE/100 g dw), respectively ($P<0.05$). Although CF has higher radical scavenging activity than BC, M3 did not exhibit higher antioxidant activity than M1 ($P<0.05$). differences in the stability or action duration of antioxidant compounds within different matrices, suggesting that CF's antioxidant compounds may exert a delayed effect within the meat matrix. The antioxidant activities of OF and CF are primarily attributed to their phenolic compounds (Adeloye et al., 2020; Karkar and Şahin, 2022).

Babaoğlu (2022) reported that cooked control meatballs exhibited lower radical scavenging activity (around 10%) than those made with gluten-free flours (approximately 11-18%), Likewise, other researchers found that the addition of gluten-free tapioca and sorghum flours increased the antioxidant activity in meatballs (Ristanti et al., 2023). In a different study, chicken meatballs formulated with corn starch, quinoa starch, and quinoa seeds showed radical scavenging activities ranging from 20.28% to 34.84% (Park et al., 2021), values higher than those observed in our study. The presence of ingredients such as onion, garlic, and ginger in their formulation could explain the enhanced antioxidant activity in those meatballs.

TBARs

The TBARs values, an indicator of lipid oxidation, are presented in Table 3. Among the samples, M1 exhibited the lowest TBARS value (1.46 mg MA/kg meatball), followed by M3 (1.58 mg MA/kg meatball), M4 (2.01 mg MA/kg meatball), M5 (2.12 mg MA/kg meatball), and M2 (2.44 mg MA/kg meatball). Similar findings have been reported, showing that TBARS values in raw meatballs increase with the inclusion of gluten-free acorn flour (Akcan et al., 2024). Although OF demonstrated higher DPPH radical scavenging activity, meatballs containing OF had higher TBARS values compared to other samples. This could be attributed to the intrinsic characteristics, matrix interactions, and the effect of additives on antioxidant activity duration (Brewer, 2011).

In the present study, obtaining radical scavenging activity equivalent to or higher than the control group was a great achievement for developing gluten-free meatballs. However, further research is needed to examine the effects of OF and CF on antioxidant activity and TBARS values throughout storage.

Faki et al. (2022) reported that oleaster endocarp tissues contain approximately 90% unsaturated fatty acids, predominantly of the polyunsaturated type. Lipid oxidation in meat involves polyunsaturated fatty acids reacting with reactive oxygen species, suggesting that the high polyunsaturated fatty acid content in OF may contribute to the elevated TBARS levels observed in M2 (Amaral et al., 2018). Despite the higher crude fat content of CF compared to BC, TBARS values in the M1 and M3 groups were not significantly different (P>0.05). Previous studies have found that meatballs with BC showed higher TBARS values than those containing oat flour, but lower values than those with pepper seed flour (Kılınççeker, 2015). The TBARs values of meatballs with breadcrumbs were higher than those of the meatballs with hemp cake (Kotecka-Majchrzak et al., 2021). For high-quality meat products, TBARS values are recommended to be below 3 mg MA/kg (Cadun et al., 2008). All meatball samples in this study are within this quality threshold, indicating acceptable lipid oxidation levels. Despite the differences in polyunsaturated fatty acid and crude fat content between OF and CF, TBARS values did not exceed the critical limit for quality.

Texture profile analysis (TPA)

TPA results of cooked meatball samples are shown in Table 4. The ranges of hardness (N), adhesiveness (N.s), springiness, resilience, gumminess (N), chewiness (N), and cohesiveness of meatballs are 208.03 - 342.87 N, (-5.54) - (-1.23) N.s, 0.27 - 0.30, 0.21 - 0.34, 118.11 - 263.71 N, 35.44 - 74.10 N, and 0.57 - 0.77, respectively. No statistical difference was observed in the adhesiveness and springiness values of the meatballs made from different flours (P>0.05). However, there was a statistically significant difference in the hardness, resilience, gumminess, chewiness, and cohesiveness values of the meatballs (P<0.05). The M3, M4, and M5 groups had higher hardness, resilience, gumminess, chewiness, and cohesiveness values than the M1 and M2 groups. However, there is no significant difference among the M3, M4, and M5 groups (P>0.05). The addition of OF or CF increased the hardness of meatballs compared to those with BC. This increment could be due to the higher cooking loss in the OF and CF-added groups. The change in textural properties is mainly related to the water binding and cooking loss properties of meatballs (Babaoğlu, 2022). Among all formulations, M1, which had the lowest cooking loss (Table 3), was the best regarding textural properties.

Table 4. Texture profile analysis of meatballs

	Formulations				
	M1	M2	M3	M4	M5
Hardness (N)	208.03±11.42^{c}	311.78±9.74^{b}	330.10±14.24^{ab}	342.87±10.32^{a}	329.58±15.52^{ab}
Adhesiveness (N.sec)	-3.86±3.19	-3.24±1.59	-5.54±5.01	-2.82±1.18	-1.23±1.47
Springiness	0.30±0.01	0.29±0.02	0.27±0.01	0.28±0.02	0.28±0.00
Resilience	0.21±0.01^{c}	0.29±0.01^{b}	0.34±0.00^{a}	0.34±0.00^{a}	0.33±0.00^{a}
Gumminess (N)	118.11±6.43^{c}	213.61±8.38^{b}	252.02±13.55^{a}	263.71±6.23^{a}	248.39±11.49^{a}
Chewiness (N)	35.44±3.40^{b}	64.82±67.39^{a}	67.39±4.16^{a}	74.10±7.53^{a}	70.25±2.91^{a}
Cohesiveness	0.57±0.02^{c}	0.70±0.02^{b}	0.76±0.01^{a}	0.77±0.00^{a}	0.75±0.00^{a}

(a-c): Means with different letters in the same row indicate significant differences among the meatball groups (P<0.05)

M1: control group meatball including 8% breadcrumbs, M2: meatball including 8% oleaster flour, M3: meatball including 8% coconut flour, M4: meatball including 6% coconut flour and 2% oleaster flour, M5: meatball including 4% coconut flour and 4% oleaster flour

Ikhlas et al. (2011) used cassava, corn, wheat, sago, and potato flour in meatball production. They determined that meatballs produced with cassava flour had the best textural properties, while those with potato flour had the worst. Babaoğlu (2022) found that meatballs made with chickpea flour had better textural properties and were not different from the control meatballs. They also stated that the meatballs produced with corn flour showed the poorest textural properties. In the study of Bağdatlı (2018), hardness and cohesiveness values were higher in meatballs containing quinoa flour, while springiness values were higher in the control group. Mastanjević et al. (2014) concluded that hardness values were higher in gluten-free meatballs compared to the control samples. Salarkarimi et al. (2019) stated that hardness, springiness, cohesiveness, chewiness, gumminess, and resilience values were higher in meatballs made with toasted flour compared to those formulated with chestnut flour. They also suggested that these texture parameters decreased as the amount of chestnut flour substituted for toasted flour increased.

Sensory evaluation

The sensory attributes of the cooked meatball are shown in Figure 3. Flour types did not significantly affect the appearance/color of the meatballs (P>0.05). A statistically significant difference was observed only between the M1 and M3 groups in terms of odor, flavor, and texture parameters (P<0.05). The juiciness scores of the meatballs decreased with the addition of gluten-free flours but this reduction was only found significant in groups M3, M4, and M5, compared to the control group (P<0.05). However, overall acceptance scores were significantly influenced by the flour types and concentrations used (P<0.05). The M1 group received the highest scores for flavor, texture, juiciness, and overall acceptance, while the CF-containing meatballs had the lowest ratings. Notably, the sensory scores of the M2 group were close to those of the control group (M1) (P>0.05). Although the inclusion of CF led to lower sensory scores, the meatballs were still deemed acceptable by the sensory panel, with overall acceptance scores as follows: M3-5.33, M4-6.29, and M5-6.38. Despite the lower sensory acceptability of CF, it still holds potential to be used in combination with other gluten-free flours in meatball formulations as an alternative to traditional breadcrumbs, due to its comparable protein content and antioxidant capacity to BC. To observe the single effects of the flours, the meatball formulations were held constant in this study, limiting improvements in texture and sensory attributes. Nevertheless, it was observed that with optimized formulations (adjusting fat, water, and spice levels), these flours could be well-suited for commercial gluten-free meatball production.

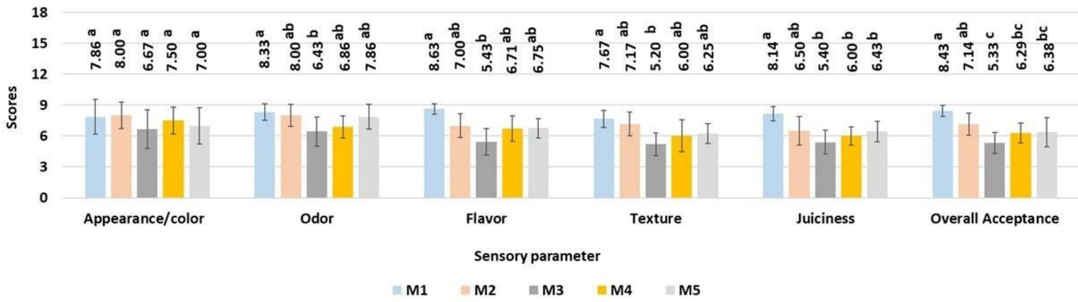

Figure 3. Sensory evaluation scores of meatball samples prepared with different flour formulations. (a-c): Means with different letters indicate significant differences among different meatball groups for same sensory parameter (P<0.05).

Salarkarimi et al. (2019) found that increasing chestnut flour content in meatballs significantly improved texture, flavor, juiciness, and overall acceptance scores, although color scores declined. Mastanjević et al. (2014) reported no discernible differences in sensory properties among meatballs made with wheat, maize, and rice, while those with soy had the lowest scores for odor, consistency, and taste. It was reported that meatballs with potato puree attained higher flavor scores than those made with BC, albeit with lower texture scores (Ergezer et al., 2014). Bağdatlı (2018) observed no significant differences in odor, texture, or overall acceptance between meatballs containing equal portions of BC and quinoa flour, though taste scores improved with increasing quinoa flour content. Babaoğlu (2022) concluded that gluten-free flours did not significantly impact the appearance, odor, or texture of the meatballs.

While no study related to the addition of coconut flour and/or oleaster flour in meatballs could be found, cassava-coconut composite flour was evaluated in chicken sausages by Ayandipe et al. (2022), and 4.8% coconut flour and 15.2% cassava flour were determined as the optimum blend ratio to replace wheat flour. They observed no significant differences in the sensory acceptance of the optimal sausage over the wheat flour-containing sausage. A recent study introduced coconut flour to fish sausages (James and Krishnamoorthy, 2025) and found it useful for improving the nutritional, textural, and sensory properties compared to the control, corn flour. On the other hand, Khalid et al. (2021) used young and mature coconut flesh as fat replacers in meatballs, and reported that using young coconuts in meatballs resulted in better cooking yield, texture, and sensory properties, and they recommended young coconut flesh as a potential fat replacer.

CONCLUSIONS

This study evaluated the use of OF and CF as gluten-free alternatives in meatball formulations, focusing on their effects on the physicochemical, antioxidant, and sensory properties of the final product. While the OF prominently led to higher antioxidant activity in the meatballs, CF exhibited much better results in terms of lipid oxidation similar to the control sample. The gluten-free nature of these flours, combined with lower protein content, led to increased cooking loss in OF- and CF-based meatballs when compared to traditional breadcrumb (BC) formulations.

The texture profile analysis revealed that gluten-free formulations with OF and CF increased hardness, gumminess, and chewiness, likely due to the elevated cooking losses. Sensory evaluations further indicated that BC-based meatballs received the highest scores for flavor, texture, juiciness, and overall acceptance, while OF received the same scores and CF-containing meatballs had comparatively lower overall acceptance scores. Among the gluten-free formulations, the meatballs containing only oleaster flour (M2) demonstrated the most favorable sensory attributes, making it the most suitable alternative to traditional breadcrumbs.

In conclusion, both oleaster and coconut flours hold potential as functional, gluten-free ingredients in meatball production, particularly given their beneficial antioxidant properties. Future studies focusing on optimization and refining production methods could facilitate the development of gluten-free meat products with enhanced sensory appeal and improved functional attributes, contributing to healthier alternatives in gluten-free diets.

CONFLICT OF INTEREST

The authors report that there are no competing interests to declare.

ETHICAL STATEMENTS

Ethical approval for the involvement of human subjects in this study was granted by Sakarya University Research Ethics Committee, with reference number E-61923333-050.99-232826, 03/22/2023.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Esra Bostanci Selbeş: Formal analysis, data curation, methodology, conceptualization, writing - original draft, visualization, Guliz Haskaraca: Formal analysis, data curation, methodology, conceptualization, writing - review & editing, visualization, Fundagül Erem: Formal analysis, data curation, methodology, conceptualization, statistics, writing - review & editing, Zehra Ayhan: technical support, writing - review & editing.

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