Yuzuncu Yıl University
Journal of the Institute of Natural & Applied Sciences

https://dergipark.org.tr/en/pub/yyufbed

Research Article

Effect of Mulberry Fortification on Functional, Physical and Sensory Properties of Gluten-free Muffin

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Article Info

Received: 21.03.2022
Accepted: 15.07.2022
Online December 2022
DOI: 10.53433/yyufbed.1090898

Keywords
Chestnut flour,
Gluten-free muffin,
Mulberry

Abstract: The present study investigated the effects of mulberry on some quality parameters of gluten-free muffins. The muffins were produced containing mulberry 0 (MB0), 5 (MB5), 10, (MB10), and 15% (MB15), respectively. The average specific volume of the MB0 muffin was 2.22 mL g^{-1}, but the muffin volumes decreased to 2.18, 2.06, and 1.99 mL g^{-1} for other samples. The firmness increased with increased mulberry levels. While the firmness of MB0 was 0.61 kg, it increased to 0.64, 0.65, and 0.71 kg in the MB5, M10, and MB15. The addition of mulberry increased the total phenolic content (TPC) from 8.10 to 31.95 mg GAE g^{-1}. ABTS and DPPH values for MB0 were 32.26 µmol TEAC g^{-1} and 138.8 µmol TEAC g^{-1}, respectively. They increased to 80.79 and 225.61 µmol TEAC g^{-1} at MB15 samples. The rutin content of the muffin prepared with 15% mulberry (5.62 mg 100 g^{1}) had five times higher than those of the MB0 muffin (0.94 mg 100 g^{1}). The catechin and quercetin content of gluten-free muffins was very high compared to MB0 samples. Sensory analysis results revealed that mulberry can be successfully incorporated into gluten-free muffin formulations up to 15% ratio without any negative effects on all tested sensory properties.

Dut Takviyesinin Glutensiz Muffinin Fonksiyonel, Fiziksel ve Duyusal Özelliklerine Etkisi

Makale Bilgileri

Geliş: 21.03.2022
Kabul: 15.07.2022
Online Aralık 2022
DOI: 10.53433/yyufbed.1090898

Anahtar Kelimeler
Dut,
Glutensiz muffin,
Kestane unu

Öz: Bu çalışma, dutun glutensiz muffinlerin bazı kalite parametreleri üzerindeki etkilerini araştırmayı amaçlamıştır. Dutu 0 (MB0), 5 (MB5), 10, (MB10) ve %15 (MB15) içeren muffinler üretilmiştir. Kontrol örneğinin ortalama özgül hacminin 2.22 mL g^{-1} olduğu, glutensiz örnekler için hacmin 2.18, 2.06, 1.99 mL g^{-}1'e düştüğü belirlenmiştir. Artan dut seviyeleri ile sertlik artmıştır. Dut içermeyen örneğin (MB0) sertliği 0.61 kg iken, bu değer MB5, M10 ve MB15'te 0.64, 0.65 ve 0.71 kg'a yükselmiştir. Dut ilavesi toplam fenolik madde konsantrasyonunu (TFM) 8.10'dan 31. mg GAE g^{-1}' e yükseltmiştir. MB0 örneği için ABTS ve DPPH değerleri sırasıyla 32.26 µmol TEAC g^{-1} ve 138.8 µmol TEAC g^{-1} olarak belirlenmiştir. Bu değerler, MB15 örneklerinde 80.79 ve 225.61 µmol TEAC g^{-1}' e yükselmiştir. %15 dut ile hazırlanan muffinin rutin içeriği (5.62 mg 100 g^{1}) MB0 örneğine göre (0.94 mg 100 g^{1}) beş kat daha yüksek bulunmuştur. Glutensiz örneklerin kateşin ve kuersetin içeriği, kontrol örneklerine kıyasla yüksek çıkmıştır. Duyusal analiz sonuçları dutun, duyusal özellikler üzerinde herhangi bir olumsuz etki yapmadan %15 oranına kadar glutensiz muffin formülasyonuna başarıyla dahil edilebileceğini ortaya koymuştur.

1. Introduction

Celiac disease (CD) is an autoimmune disorder that occurs in the upper small intestine after the consumption of gluten. The symptoms of CD include anemia, malnutrition, diarrhea, growth retardation, and fatigue (Makovicky et al., 2020). The U.S. Food and Drug Administration (FDA) classifies gluten-free food as follows: 1) The gluten-free foods do not contain gluten-including cereals (wheat, rye) and gluten-containing flour (wheat flour); 2) It does not contain an ingredient derived from gluten-containing cereals (eg, wheat starch). if there is lower than 20 ppm of gluten in the food in which any of these ingredients are used, the food can be defined as gluten-free food. In summary; gluten-free foods should not contain gluten or the amount of gluten should not exceed 20 ppm (Xu et al., 2020).

A systematic review and meta-analysis were conducted by Singh et al. (2018) to determine the prevalence of CD. According to blood and biopsy results, the incidence of CD is 1.4%, and it is 0.7%, respectively. CD prevalence values for South America, Africa, North America, Asia, Europe, and Oceania were determined as 0.4%, 0.5%, and 0.6%, respectively. Because there is no found pharmaceutical treatment for CD, the gluten-free diet is currently the only available treatment for CD or non-celiac gluten sensitivity. . For this reason, gluten protein should completely be removed from the diet. All these conditions are factors making it difficult for celiac patients to adapt to a gluten-free diet. Hence, the development of a gluten-free product range that patients can consume is of great importance. Therefore, the number of GF products will increase worldwide. Globally, the GF product market size reached 5.6 billion USD in 2020. The expected market size will reach USD 8.3 billion in 2025, with an annual growth rate of 8.1% (Köten, 2021; Marketsandmarkets, 2022).

The nutritional value, flavor, and mouthfeel of gluten-free (GF) bakery products are often poor also their price are high compared to traditional products. Therefore, the enrichment of GF products has been investigated. In recent years, there is a trend toward enriched bakery products. The enrichment of bakery products with natural antioxidants has become a topic of research for food technologists (Dogan et al., 2016; Doğan & Meral, 2019; Kutlu & Meral, 2022). Although there are many studies related to the production of gluten-free bakery products, the enrichment of gluten-free products with natural antioxidants is limited. Enriched gluten-free products have been suggested to improve the quality of coeliac patients' diets. The addition of mulberry is one of the many ways that could be used to increase the antioxidant properties of gluten-free cakes.

The mulberry has high bioactive ingredients because of the high abundance of different types of ingredients namely, anthocyanins, polysaccharides, phenols, alkaloids, and flavonoids. The mulberry contains anthocyanins, phenolic acids, fibers, and some phytochemicals having a role in reducing the risks of diseases, such as inflammation and certain cancers (Wen et al., 2019). In this sense, mulberry has been used for the fortification of bakery products (Meral & Dogan, 2012). Gluten-free products generally are produced by using starch or refined flour and they are not fortified. Therefore, they may not contain the same levels of nutrients compared to their gluten-containing counterparts. Also, although there are many gluten-free products on the market for individuals with celiac and gluten sensitivity. However, it is a problem that these products are not suitable for the palate and their nutritional and functional properties are low. This study was planned to increase the functional properties of muffins by adding mulberry to gluten-free muffins.

2. Material and Methods

2.1. Materials

Chestnut flour with 5.4% protein and 2.4% (Kafkas Sekerleme San. ve Tic. A.S., Bursa) and potato starch (Soyyigit Gıda San. ve Tic. A.S., Istanbul) were used as starch sources. A commercial mixture (Puratos, Istanbul) containing mono-diglyceride (E 471), propylene glycol alginate (E 405), polyglycerol esters of fatty acids (E 475), sorbitol (E 420), and anti-foaming fatty acids (E 570), was used as an emulsifier. In the study, xanthan (1500-1600 cps), guar gum (3000-3500 cps) (Dairy Gold Co., Ireland), emulsified shortening (Puratos, Istanbul), dried egg white powder (A.B Gıda San. ve Tic. A.S., Balıkesir), skimmed milk powder (Göktürk Gıda San. Dis Tic. Ltd. Sti., Istanbul), baking powder (Ülker, Istanbul), drinking water (Palandöken Desni, Erzurum), refined table salt (Billur Tuz, Istanbul) and commercially available crystal sugar were also used in the formula. Gallic acid, rutin, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS), Trolox, methanol were obtained from Sigma-Aldrich Company (St. Lois, MO, USA).

Black mulberry (*Morus nigra*) fruits were collected from the İskele region of Van, Turkey. The fruits were manually separated from stalks, cleaned, crushed, and fresh fruits were used in the muffin formulation.

2.2. Muffin preparation

The preparation of muffins was based on AACC Method 10-90 (2000). The formulation of gluten-free muffin enriched with four different mulberry levels was shown in Table 1. Gluten-free muffins enriched with freshly mulberry by 0, 5, 10, and 15% levels were designated as MB0, MB5, MB10, and MB15, respectively. The water content of experimental formulas was adjusted by calculating the amount of water in mulberry. The high paper moulds that have 80 mm diameter×50 mm were filled with 60 g batter. The batters were baked for 17 min at 175°C.

Table 1. Ingredients used in the formulation

Ingredients (g)	MB0	MB5	MB10	MB15
Chestnut flour	70	70	70	70
Potato starch	30	30	30	30
Mulberry	-	5	10	15
Water	103	98.8	93	88
Emulsifier	6	6	6	6
Xanthan gum	0.225	0.225	0.225	0.225
Guar gum	0.075	0.075	0.075	0.075
Sugar	80	80	80	80
Shortening	50	50	50	50
Milk powder	12	12	12	12
Egg white powder	8	8	8	8
Baking powder	3	3	3	3
Sodium chloride	1.5	1.5	1.5	1.5
Vanillin	1	1	1	1

MB0: samples no-added mulberry: MB5: samples contain 5% mulberry, MB10: samples contain 10% mulberry, MB15: samples contain 15% mulberry

Batter density was the ratio of the weight of the batter to the weight of distilled water present in the cup having equal volume. A penetrometer was used to determine the consistency of the batters. A digital penetrometer (Model K95590; Koehler Instrument Company, Inc., Bohemia, NY, USA) equipped with a 35-g aluminum cone (K20090) was used for measurement. After cooling for 2 h, muffin volumes (mL) were measured by the displacement method (AACC Method 10-05) using a volume meter (Şimşek Laborteknik, Ankara, Turkey) (AACC, 2000). The specific volume of muffins was determined as used muffin volume to weight (mL g^{-1}). Three replicates were carried out.

The texture profile analysis (TPA) was done for each muffin with a texture analyzer (TA.XT2. Stable Micro Systems Ltd. Godalming Surrey, UK). The TPA was performed using a 25 mm diameter cylinder probe (P/25). With pre-test, test, and post-test speeds of 10 mm/s, 2.0 mm/s, and 2.0 mm/s, respectively, a compression mode texture profile analysis was performed. The samples were compressed until they reached a target strain of 40% (Gómez et al., 2007).

External (crust) and internal (crumb) attributes of layer cakes were graded according to modified AACC Method 10-90 (AACC, 2000). Crumb cells (10 points), texture (10 points), color (10 points), and flavor (15 points) were given a total of 100 points for internal cake (crumb) attributes, and crust color (10 points), symmetry (10 points), and crust texture (10 points) were given a total of 30 points for external (crust) attributes (AACC, 2000).

2.3. The extraction of antioxidant compounds

200 mL of hexane was added to 20 g of the sample and left for 24 hours. At the end of the period, the hexane was filtered and the sample was dried at 40°C. The cakes were ground into a fine powder using a laboratory mill (Perten LM 120, Sweden). An approximate 10 g defatted sample from each type of cake was mixed with 30 mL methanol and stirred for 22 h in the dark and at 35° C by a shaking incubator (Heidolph Unimax 1010, Schwabach, Germany). After this, the homogenates in the tube were centrifuged at 12000 *g* for 15 min at 20° C and the supernatant was transferred into an amber volumetric flask. The precipitate was extracted again with the same solvent and at the same conditions and made up to a final volume of 100 mL. Extracts were stored at -18° C for 3 weeks (Bakkalbasi et al., 2015; Meral & Erim Köse, 2019).

2.4. Determination of total phenolic content (TPC)

The method used by Meral (2016) was used to determine the TPC concentration. Briefly, 150 µL sample extract and 3.0 mL of 2% sodium carbonate (w/v in water) were added to a test tube. After about 2 min 150 µL Folin-Ciocalteu's reagents (1:1, v/v in water) were added and mixed thoroughly. The mixture was left to stand for 45 min at room temperature in the dark. The absorbance was measured at 765 nm using a spectrophotometer (PG Instrument T80 UV/VIS Spectrophotometer, Wibtoft, England). All spectrometric measurements were carried out in triplicate. The concentration of TPC was determined using the Folin-Ciocalteau method. The results were expressed as mg of gallic acid equivalents (mg GAE g^{-1}).

2.5.Antioxidant activity

ABTS and DPPH tests were used to determine the antioxidant activities of muffin samples. The free radical scavenging activity of the extract was measured using DPPH free radical scavenging method (Brand-Williams et al., 1995) with some modifications. DPPH (4 mg L-1) was dissolved in pure methanol. The radical stock solution was prepared daily as fresh. The DPPH solution (3 mL) was added to an aliquot of sample extract (1 mL). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 517 nm. Methanol was used instead of the sample for control measurement. Scavenge of DPPH was determined according to the following equation. All determinations were performed in triplicate.

$$\text{\% Inhibition} = \left[\frac{(A\ control - A\ sample)}{A\ control}\right] * 100$$

A control: Absorbance of control at 517 nm
A sample: Absorbance of the sample at 517 nm

The ABTS value was determined using a method described by Meral & Doğan, (2013a). Briefly, ABTS•+ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate via incubation at room temperature in the dark for 12-16 h. The ABTS•+ solution was diluted with phosphate-buffered saline (PBS, pH 7.4) to an absorbance of 0.700±0.05 at 734 nm. To 3 mL of diluted ABTS•+, 50 µL of each sample extract solution was added and mixed. The reactive mixture was allowed to stand at room temperature for 6 min and the absorbance was recorded immediately at 734 nm. Trolox standard solutions in methanol were prepared and assayed using the same conditions. The percent of inhibition was calculated and plotted as a function of the concentration of Trolox for the standard reference data. Triplicate analyses were performed for DPPH and ABTS tests and results were given as µmol Trolox equivalent antioxidant capacity (µmol TEAC g^{-1} sample)

2.6. Analysis of the polyphenol composition by using HPLC

The phenolic composition of the samples was determined by HPLC described by Meral & Dogan (2013a). The samples were filtered through a 0.45 μm membrane Millipore chromatographic filter before injection. The HPLC analysis was performed on an HP 1100 Agilent Chromatographic system (Agilent Technologies, Palo Alto, CA, USA) equipped with a pump, degasser, and diode array detector (DAD) (Agilent Technologies, Palo Alto, CA, USA). Separations were conducted on an ODS Hypersil column (HiChrom, Theale, PA, USA) with 250x4.6 mm, and 4 μm particle size. The chromatographic separation was carried out using methanol-acetic acid-water (10:2:88, v/v) as solvent A mobile phase and methanol-acetic acid-water (90:2:8, v/v) as solvent B. The solvent gradient program was set as follows: initial conditions 100% A, 0% B; 0-15 min, 85% A, 15% B; 15-25 min, 50% A, 50% B; 25-35 min, 15% A, 85% B; 35-45 min, 0% A, 100% B; 45-50 min. The wavelength was set at 280 and 320 nm, the flow rate was1 mL/min, and the injection volume was 20 μL.

2.7. Sensory evaluation

The thirteen panelists (7 male and 6 female) were trained before sensory evaluation until they were familiar with the evaluated attributes. The randomly numbered 25 g muffin samples were served to the panelists. Each sample was evaluated for sensory attributes using a 10 cm-long divided scale (1 extremely disliked, 10= extremely liked) in the sensory evaluation form (Bakkalbasi et al., 2015).

2.8. Statistical methods

The Stat Graphics Centrum 15.1 for one-way ANOVA and Duncan's Multiple Comparison test procedures were used to determine significant differences (P< 0.05).

3. Results and Discussion

3.1. Physical characteristics of batter and muffin

Table 2 demonstrates the changes in batter properties enriched with mulberry. The batter density ranged from 0.87 (MB5) to 0.91 (MB15) g $mL^{-1.}$ The density of the batters did not change at the level of 5, 10%, and 15% compared to the MB0 sample. Dough density refers to the amount of air added to the dough during mechanical mixing and therefore affects the volume, crispness, and texture of cakes. Therefore, it must be continuously controlled in cake production. If the batter density is too high (that means lower air cell volume incorporated into the batter and low batter viscosity), the cake will have a lower volume and dense grain. On the contrary, if it’s too low (that means many air cells are incorporated into the batter and it has high batter viscosity), the cake will be fragile and have a crumbly crust (Gómez et al., 2007). Thus, it could be said that mulberry increased the air incorporation during mixing, with no significant differences between batters at the level of 0%, 5%, 10%, and 15% of mulberry substitution. According to Dhen et al. (2016), the muffin batter density had changed between 1.00 and 1.12, and they stated that soy flour incorporation resulted in a decrease in batter density. Similar findings were also found by other researchers. Román et al. (2015) found that the density of cake batters was 0.89-1.11 g mL^{-1}. According to Hedayati & Mazaheri Tehrani (2018), batter density changed between 1.02-1.10 g mL^{-1}.

The batter consistency is an important physical property determining the final muffin volume. Because the batter consistency gives information about the retained air in the batter. Low cake volumes are attributable to low consistency values (Herranz et al., 2016). On the other hand, the excessive consistency can also decrease muffin quality, since it will limit the expansion of batter in the oven (Marchetti et al., 2018). Too low consistency causes the collapse because air bubbles cannot be retained in the dough. A highly viscous batter can keep air bubbles inside, but the expansion of the batter is restricted because of its higher viscosity. In the present study, the consistency values ranged between 239-247 (mm/10), and significant differences in the consistency of the muffin batters were noted (Table 2). All batter formations containing mulberry had lower consistency values than the MB0 batter. However, batter containing 15% mulberry showed similar characteristics to the MB0 batter.

Specific volume is an important quality property for cake and muffins. The cakes having greater volume as more desirable. Obtaining cake with a high specific volume is difficult due to the absence of gluten.

Table 2. The physical and textural properties of gluten-free muffin batter and muffin

	MB0	MB5	MB10	MB15
Density (g mL^{-1})	0.89±0.01^{ab}	0.87±0.00^{b}	0.89±0.00^{ab}	0.91±0.00^{a}
Consistency (mm/10)	247±0.70 ^{a}	243±1.41^{b}	239±1.41^{c}	245±0.70^{ab}
Specific volume (mL g^{-1})	2.22±0.01 ^{a}	2.18±0.00^{b}	2.06±0.00^{c}	1.99±0.00^{d}
External attributes	25.00±0.00^{a}	25.00±0.00^{a}	25.00±0.00^{a}	24.00±0.00^{a}
Internal attributes	94.00±0.70^{a}	94.00±0.70^{a}	93.00±0.00^{a}	91.00±0.00^{a}
Firmness (kg)	0.61±0.08^{c}	0.64±0.03^{b}	0.65±0.07^{b}	0.71±0.04^{a}
Cohesiveness	0.37±0.00^{b}	0.39±0.00^{ab}	0.40±0.01^{a}	0.41±0.00^{a}
Chewiness	0.24±0.02^{d}	0.27±0.00^{c}	0.28±0.02^{b}	0.30±0.01^{a}
Resilience	0.10±0.00^{b}	0.12±0.00^{b}	0.14±0.00^{a}	0.13±0.00^{a}

a-d: Means with different letters within a row are significantly different from each other ($P<0.05$). MB0: samples no-added mulberry: MB5: samples contain 5% mulberry, MB10: samples contain 10% mulberry, MB15: samples contain 15% mulberry

The specific volume of gluten-free muffins obtained from the present study is shown in Table 2. The specific volume was reduced due to the increasing levels of mulberry in the formula. The MB0 muffin had an average specific volume of 2.22 mL g^{-1}, but the muffin volumes decreased to 2.18, 2.06, and 1.99 mL g^{-1} for MB5, MB10, and MB15 formulas, respectively. The addition of mulberry decreased muffin-specific volume by about 1.8-10.3% compared to the MB0. This decrease in the muffin volume may be caused by an increase in fiber content due to increasing levels of mulberry. Aydogdu et al. (2018) reported that the specific volume of cakes prepared with oat, pea, apple, and lemon fibers varied between 1.32 and 1.91 mL g^{-1}. Sahagún et al. (2018) demonstrated that the specific volume of gluten-free cakes changed between 1.99 and 3.45 mL g^{-1}. Kırbaş et al. (2019) determined increasing levels of fiber decreased the specific volume of gluten-free cakes. Türker et al. (2016) also reported a decrease in the gluten-free cake volume with increasing green banana peel flour levels. They stated that while the specific volume of the control cake was 2.35 mL g^{-1}, it was reduced to 1.91 mL g^{-1} in the cake including 25% green banana peel flour. Consequently, it was expected that mulberry incorporation would decrease the specific volume, but the decrease was very low in this study. It means that gluten-free muffins can be made with little modification in desired qualities such as volume.

One of the most important factors affecting consumer acceptance is the external attributes including crust color and homogeneity, crust thickness, and cake symmetry. The scores of external attributes varied between 24/30 and 25/30 (Table 2). Significant differences were not determined in the external scores of muffins. Other important criteria affecting consumer acceptance and can give information about the stage of process and ingredients used in the formula is the internal attributes such as the size and uniformity of pore, texture, internal color, flavor, and aroma. The scores of internal attributes varied between 91/100 and 94/100. A significant decrease in internal attributes was not observed between the MB0 and gluten-free muffins.

As a result, it was demonstrated that the mulberry incorporation would change batter density, consistency, and muffin volume due to its chemical composition such as fiber. As known, mulberry fruits contain dietary fiber (Zhang et al., 2018; Kadam et al., 2019) It has been reported that freeze-dried powder of mulberry fruits contains 243.0 mg/g of dietary fiber (Yang et al., 2010). When the comparison was done with the MB0 sample, it was revealed that the change was very low, and acceptable muffins could be produced with the addition of mulberry.

The textural properties of gluten-free muffins are demonstrated in Table 2. The firmness increased with increased mulberry levels. While the firmness of MB0 was 0.61 kg, it increased to 0.64, 0.65, and 0.71 kg in the MB5, M10, and MB15. The increase was associated with increased fiber content due to an increased mulberry incorporation level in the muffin formula. The firmness is a textural parameter that measures the peak force during the first compression cycle. The firmness of muffins can be related to the density of the muffin batters (Lu et al., 2010). The increase in firmness might also be due to the lower density of batter containing mulberry. Majzoobi et al. (2016) revealed that the firmness value increased with increased carrot pomace levels in gluten-free cakes. Levent et al. (2021) determined an increase in the firmness value of gluten-free cakes with the addition of grape seed, pomegranate seed, flaxseed, poppy seed, and turmeric. Sung et al. (2020) used chia seed flour to prepare a gluten-free rice layer cake. They found that firmness rose from 8.02 N to 16.56 N.

The addition of mulberry at different levels increased the cohesiveness, chewiness, and resilience of muffins. The chewiness is a parameter dependent on the firmness value. The chewiness is a metric that is influenced by the hardness level. As a result, it was expected that the chewiness would rise as the firmness rose.

Gularte et al. (2012) reported an increase in cake firmness and cohesiveness and a decrease in resilience when fibers from different sources were incorporated into the formula. Park et al. (2021) determined that the hardness value of rice cakes increased with the increase of mulberry (*Morus alba* L.) leaf powder supplementation. According to Sung et al. (2020), with the addition of chia seed flour to rice flour, springiness did not change, while resilience and cohesiveness decreased gradually in cake samples. The TPA analysis is one of the most important analyses that give important information about the textural properties of foods. Consequently, although TPA results obtained from this study demonstrated that the textural properties of muffins could change significantly with the addition of mulberry to the formula, these changes can be acceptable levels. According to sensory analysis results, huge differences in firmness values were not observed among muffin samples (Figure 2).

3.2. The functional properties

The TPC and antioxidant activities of muffins are given in Table 3 and Figure1. The TPC value of muffins varied from 8.10 to 31.95 mg GAE kg^{-1}. As expected, there was a significant increase in the TPC values of muffins with increasing mulberry levels. The MB0 muffin had the lowest TPC. The TPC increased when the mulberry level increased. The highest TPC was determined in MB15 The phenolic compounds have antioxidant activity, meaning that there are positive correlations between TPC and antioxidant action (Meral & Dogan, 2013; Bakkalbasi et al., 2015). In the literature, different results have been given regarding the TPC in cakes. For example; while it was 51.8 mg GAE 100 g^{1} in the cakes prepared with wheat flour, it was 185.6 mg GAE 100 g^{-1} in the samples prepared with defatted Baru flour (Pineli et al., 2015). According to Alifakı et al. (2019) guar and xanthan gum-added batters with 40 % buckwheat flour had total phenolic content of 1.046 and 1.053 mg GAE g^{-1} DW batter, respectively. Bakkalbasi et al. (2015) determined 299-2228 mg GAE kg^{-1} TPC in cakes containing walnut press cake. These differences are due to sampling differences, flour difference, and extraction method (Meral, 2016).

The antioxidant activities of muffins were determined by using ABTS and DPPH radical scavenging activity assays. Table 3 and Figure 1. contain the antioxidant properties. The antioxidant activities of muffins increased due to increasing levels of mulberry. The ABTS values of muffins enriched with mulberry ranged between 32.36-80.79 µmol Trolox g^{-1}. The ABTS values of muffins were in the descending order MB15>MB10>MB5>MB0. The ABTS value of the muffin enriched with 15% mulberry is approximately three times higher than that of the MB0 muffin. The TPC and ABTS values of muffins increased with increasing levels of mulberry. In a study conducted by Drabińska et al. (2018) examining the functional properties of cakes containing broccoli by-products, ABTS values were found to be 1.15-4.19 µmol Trolox g^{-1}.

The muffins enriched with mulberry (0, 5, 10, and 15%) showed 138.82-226.61 µmol TEAC g^{-1} DPPH radical scavenging (Table 3 and Figure 1). DPPH radical scavenging activity increased with the addition of mulberry. The best antioxidant activity was observed in the sample including 15% mulberry. DPPH scavenging effect increased with the increase of mulberry addition. DPPH activity, which was 138.82 µmol TEAC g^{-1} in the MB0 sample, increased to 225.61 µmol TEAC g^{-1} in the MB15. The mulberry exhibited strong concentration-dependent DPPH radical scavenging activity giving higher antioxidant activity. Similar findings were obtained from other researchers who investigated the effects of natural antioxidants on the quality of bakery products. Meral & Erim Köse, (2019) found that grape and pomegranate seeds improved the antioxidant properties of bread. It was determined that *Morus nigra* (Meral & Doğan, 2012) and flaxseed (Meral & Dogan, 2013b) enhanced the antioxidant activity of bread. Pineli et al. (2015) showed that Baru (Brazilian Almond) flour increased the total phenolic content and total flavonoids of gluten-free cakes. Walnut press cake could be successfully incorporated into the cake formula by Bakkalbasi et al. (2015). According to Wang et al. (2012) adding 50 % green banana flour to snacks boosted the nutritional content, including fiber, minerals, polyphenols, and antioxidant capacity. The present results reflected that adding mulberry greatly enhanced the antioxidant properties of gluten-free muffins. The improved antioxidant properties of muffins enriched with mulberry are due to the addition of phenolic components having higher antioxidant activity.

The phenolic composition of different samples is also illustrated in Table 3. Higher phenolic content was found in the MB15 prepared with 15% mulberry. In this study, to reveal the phenolic profile of gluten-free muffin samples, different twelve phenolic compounds were used as standard compounds in HPLC analysis. But three of them (rutin, catechin, and quercetin) were determined in the muffin samples. The phenolic content of muffin samples significantly increased with increasing levels of mulberry. The available data showed that the rutin content in the muffin enriched with 15% mulberry (5.62 mg 100 g^{1}) was six times higher than the MB0 muffin (0.94 mg 100 g^{-1}) indicating that the mulberry was rich in phenolics.

The muffin prepared from chestnut flour only without mulberry had 0 mg 100 g^{1} of catechin, 0.07 mg 100 g^{-1} of quercetin, and 0.94 mg 100 g^{1} of rutin, whereas the muffin prepared with 15% mulberry had 50.98 mg 100 g^{1} of catechin, 5.62 mg 100 g^{1} of rutin and 5.30 mg 100 g^{1} of quercetin. With the addition of mulberry, the amount of rutin, quercetin, and catechin in the muffins increased significantly. In the enriched muffins, the amount of rutin increased 4 to 6 times, and the amount of quercetin increased 63 to 75 times compared to the MB0 samples. Catechin, which was not present in the MB0 sample, was also detected in mulberry-containing muffins. Catechin amounts were 0, 39.30, 45.26, and 50.98 mg 100 g^{1} for C, MB5, MB10, and MB15, respectively. The mulberry added to the muffin increased the phenolic components of the muffin. Meral & Doğan (2012), in their study of adding mulberry to bread, it was determined that the amount of gallic acid and catechin increased in bread with the addition of mulberry and that rutin and quercetin, which were not found in the control bread, were found in mulberry-added bread. Phenolic compounds are secondary metabolites commonly found in plants. The efficacy of these ingredients has been confirmed in recent studies in combating cardiovascular diseases, cancer, several metabolic syndrome, respiratory tract disorders, toxicities, microbial diseases, and oxidative damage. In this sense, gluten-free muffins enriched with mulberry will be a healthy food alternative for people suffering from celiac disease and gluten sensitivity.

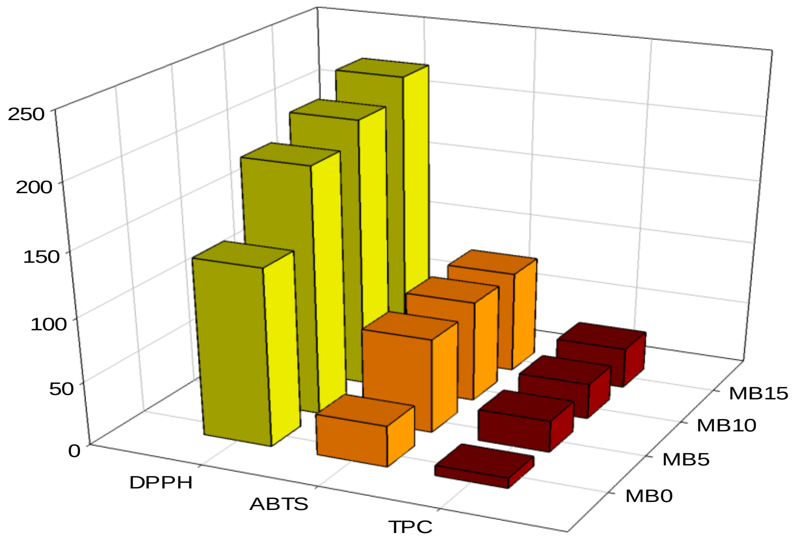

Figure 1. Functional properties of muffin (DPPH and ABTS values were given as µmol TEAC g^{-1}, TPC was given as mg GAE kg^{-1}).

Table 3. Effects of mulberry on the TPC, antioxidant activity, and phenolic composition of gluten-free muffins

	MB0	MB5	MB10	MB15
TPC (mg GAE kg^{-1})	8.10±0.14^{c}	24.86±1.04^{b}	28.06±0.33^{b}	31.95±2.04^{a}
ABTS (μmol TEAC g^{-1})	32.36±1.19^{c}	74.37±0.77^{b}	79.95±0.36^{b}	80.79±0.00^{a}
DPPH (μmol TEAC g^{-1})	138.82±5.09^{d}	193.73±7.51^{c}	209.68±5.00^{b}	225.61±2.50^{a}
Rutin (mg 100 g^{-1})	0.94±0.14^{d}	3.84±0.05^{c}	4.94±0.02^{b}	5.62±0.07^{a}
Catechin (mg 100 g^{-1})	0.00±0.00^{d}	39.30±1.16^{c}	45.26±1.90^{b}	50.98±0.43^{a}
Quercetin (mg 100 g^{-1})	0.07±0.02^{c}	4.44±0.50^{b}	5.06±0.05^{ab}	5.30±0.09^{a}

a-d. This means with different letters within a row are significantly different from each other ($P<0.05$).

3.3. Sensory properties

Sensory evaluation scores of gluten-free muffins with chestnut flour enriched with mulberry at the levels of 0, 5, 10, and 15% are shown in Figure 2. Increasing the level of mulberry in the formula caused very significant differences in the sensorial properties of gluten-free muffins. Muffins were evaluated in terms of appearance, pore structure, moistness, flavor, aftertaste, and general acceptability. In terms of all properties evaluated, sensory properties increased with increasing mulberry levels by up to 15%. As can be seen from the figure, samples containing up to 15% mulberry got higher scores than the MB0 sample. According to Singh et al. (2016), there was no significant difference between gluten-free muffins containing 0, 3, 6, and 9% black carrot pomace fiber. Sudha et al. (2007), on the other hand, discovered that increasing the amount of apple pomace in the cake reduced the dessert's sensory qualities. The inclusion of up to 30% carrot pomace powder (CPP) and 20% CPP+HC (a mixture of hydrocolloids including pectin and xanthan (1.5%) each) improved the quality and sensory qualities of gluten-free cakes according to the findings obtained by (Majzoobi et al., 2017). In the study conducted by Levent et al. (2021), it was found that in gluten-free cake samples, flaxseed and poppy seeds offered the same or higher sensory scores than the control sample. In comparison to the control samples, turmeric-enriched samples got the lowest sensory scores, except for appearance, whereas flaxseed and poppy seeds received the greatest value. In the present study, muffin samples containing 15% MB gained lower scores than the MB0 and other muffins. The most desired muffins were the samples containing 5% mulberry. As a result, the addition of a 5 to 10% proportion of mulberry was found much more suitable in terms of sensorial characteristics.

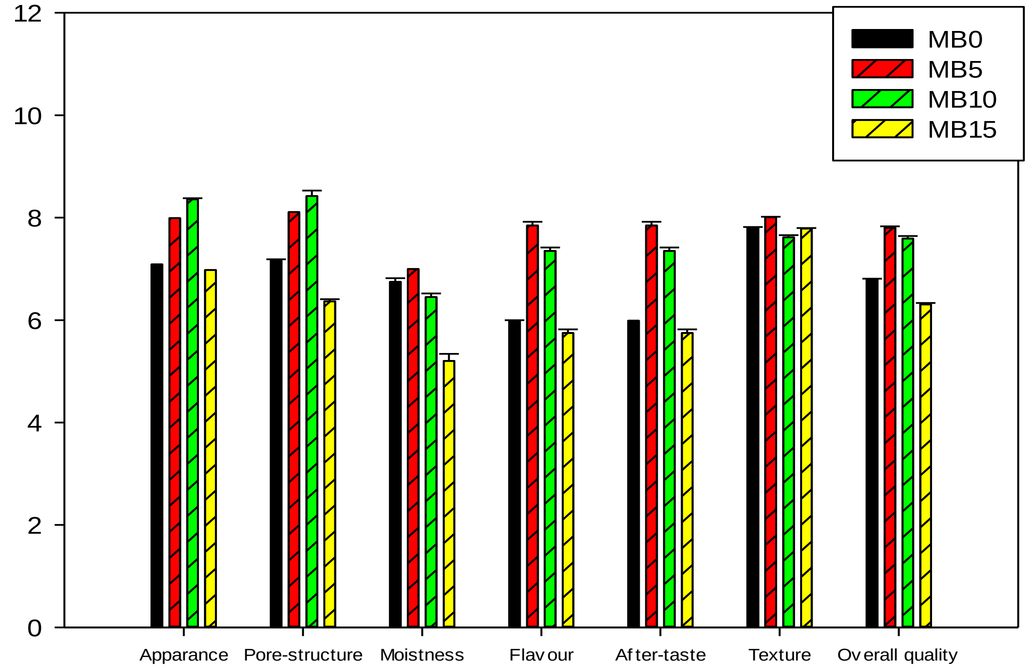

Figure 2. Sensory properties of muffin.

4. Conclusion

In this study, the effect of the mulberry on the physical, functional, and sensory properties of the gluten-free muffin was investigated. In general, the results indicated that it could be possible to obtain gluten-free muffins with very similar physical characteristics to the MB0 sample. Moreover, a significant increase in TPC, TEAC values, DPPH scavenging activity, and phenolic composition of the gluten-free muffins was observed in the study. Sensory analysis results revealed that mulberry can be successfully incorporated into gluten-free muffin formulations up to 15% ratio without any negative effects on all tested sensory properties.

The phenolic composition of the gluten-free muffins significantly increased with increasing mulberry levels by enriching the muffins with some of the bioactive compounds such as phenolics. These gluten-free muffins with mulberry can be suggested to health-conscious individuals.

Acknowledgment

The author thanks the Scientific Research Project Chairmanship of YYU (FYL-2019-8767) for financial support.

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