Çukurova Tarım Gıda Bil. Der. Çukurova J. Agric. Food Sci.
39(2) : 296-306
doi: 10.36846/CJAFS.2024.151

Research Article

The Utilization of Chasteberry (*Vitex agnus-castus* L.) Seed Powder as a Natural Antioxidant in the Production of Beef Meatball

Sevgi ARSLAN[1], Cem Okan ÖZER[1*]

ABSTRACT
This study examined the impact of incorporating varying concentrations (2.5%, 5%, 7.5%, and 10%) of chasteberry (*Vitex agnus-castus* L.) seed powder on the quality, shelf life, and sensory attributes of beef meatballs. The findings revealed a notable enhancement in antioxidant capacity and phenolic content with the incorporation of chasteberry. Lipid oxidation was markedly reduced, particularly in the 10% chasteberry group. However, the addition of chasteberry powder significantly influenced sensory acceptability, with higher concentrations resulting in a decline in taste and odor scores. The inclusion of chasteberry at 2.5% appears to provide an optimal equilibrium between antioxidant stability and sensory properties.
Keywords: chasteberry, meatball, antioxidant, phenolic content

Sığır Köfte Üretiminde Doğal Bir Antioksidan Olarak Hayıt (*Vitex agnus-castus* L.) Tohumu Tozunun Kullanımı

ÖZ
Bu çalışmada, farklı konsantrasyonlarda (%2,5, %5, %7,5 ve %10) hayıt (*Vitex agnus-castus* L.) tohumu tozu kullanımının sığır köftelerinin kalitesi, raf ömrü ve duyusal nitelikleri üzerindeki etkisi incelenmiştir. Bulgular, hayıt tohumunun eklenmesinin antioksidan kapasite ve fenolik içerikte dikkate değer bir artış olduğunu ortaya koymuştur. Özellikle %10 hayıt tohumu içeren grupta lipid oksidasyonu belirgin şekilde azalmıştır. Ancak, yüksek konsantrasyonlarda hayıt tohumu tozunun kullanımı duyusal kabul edilebilirliği önemli ölçüde etkilemiş, tat ve koku puanlarında düşüşe neden olmuştur. Hayıt tohumunun %2,5 oranında eklenmesinin oksidasyon stabilitesi ve duyusal özellikler arasında optimum bir denge sağladığı belirlenmiştir.
Anahtar Kelimeler: hayıt, köfte, antioksidan, fenolik içerik

ORCID ID (Yazar sırasına göre)
0000-0002-0092-9974, 0000-0002-2030-1412

Yayın Kuruluna Geliş Tarihi: 17.09.2024
Kabul Tarihi: 16.10.2024
[1] Nevşehir Hacı Bektaş Veli Üniversitesi, Mühendislik ve Mimarlık Fakültesi, Gıda Mühendisliği Bölümü
*E-posta: cemokanozer@nevsehir.edu.tr

The Utilization of Chasteberry (*Vitex agnus-castus* L.) Seed Powder as a Natural Antioxidant in the Production of Beef Meatball

Introduction

In recent decades, consumers have become increasingly interested in consuming natural antioxidants due to the growing awareness of the benefits of a healthy lifestyle. Therefore, the incorporation of natural antioxidants in foods has become more in demand (Mendonça et al., 2022). Antioxidants play a crucial function in human health by counteracting oxidative stress caused by free radicals produced during metabolic processes and by external factors, which can cause cellular aging and oxidative DNA damage, leading to various health problems such as cardiovascular disease, cancer and Alzheimer's disease (Boccellino, 2023; Burle et al., 2023). Plant-based antioxidants are in high demand and preferred by consumers because they are easy to use and improve the taste, flavor, and shelf life of food (Lorenzo et al. 2018; Mahassen and Hamdy 2023).

In the current scenario, where there is a growing demand for healthy and safe food, natural antioxidant sources are used to improve meat products quality, functioning as a significant alternative. This approach is in alignment with the increasing demand for natural and sustainable food practices that appeal to the discerning preferences of today's health-conscious consumers (Kumar et al., 2015; Bellucci et al., 2022; Olivas-Méndez et al., 2022; Aguiar Campolina et al., 2023). It is common practice to use the antioxidant capacity of aromatic plants, particularly spices and herbs, to improve the sensory properties of meat products, particularly in terms of flavor and aroma. Studies have shown that the ability to extend product shelf life and preserve quality parameters by using a variety of herbs and spices, including rosemary, thyme, cumin, turmeric, ginger and garlic, or essential oils, plant extracts, or powders obtained from these foods, in meat products (Atia et al., 2022; Olivas-Méndez et al., 2022; Ji et al., 2023; Šojić et al., 2023). Chasteberry seed is one of the natural antioxidant sources that has a high potential for use in the formulation of meat products.

Chasteberry or chaste tree (*Vitex agnus castus* L.) is a shrub widely grown in Europe, Central Asia, and the United States (Niroumand et al., 2018). Chasteberry are enclosed in purple-blackberry-shaped fruits and are used in many regions instead of black pepper because their grain structure is similar to that of black pepper, and they even have a similar taste and aroma (Niroumand et al., 2018). The seeds contain high levels of flavonoids and flavonoid glycosides which have antioxidant effects (Sezik et al., 2013; Zahid et al., 2016). Additionally, chasteberry seeds exhibit antimicrobial, anti-inflammatory, and antitumor properties (Gül 2007; Sezik et al., 2013; Souto et al. 2020). Therefore, it has already been used in many societies as part of traditional medicine, cosmetics and skincare products for nearly 2500 years (Dülger et al., 2002; Eryigit et al., 2015; Zahid et al., 2016; Niroumand et al. 2018).

The bioactive effects of chasteberry seeds, especially their antioxidant effects, indicate that they have significant potential in using various food formulations. Consequently, chasteberry seeds are used in foods produced for medical, health and dietary purposes (Fukahori et al., 2014). Nevertheless, the utilization of chasteberry seeds in the formulation of meat products is limited. However, it is predicted that chaste seeds, which have a taste and aroma similar to black pepper, a widely used and preferred spice in meat products, can be used in meat products without causing any negative effects on the sensory properties. In comparison to some natural antioxidant source components whose utilization is restricted due to their adverse impact on sensory properties, chasteberry seeds have a considerable advantage in this regard. Consequently, the aim of the present study was to determine the effect of the addition of chasteberry seed powder to a beef meatball formulation on product quality parameters.

Materials and Methods

Materials

The post-mortem beef meat (*M. Longissimus dorsi)* that had completed a 24-hour resting period, animal fat, and spices used in the production of meatballs were sourced from a local supplier (İtimat Et Galerisi, Nevşehir, Türkiye). The sun-dried chasteberry (*Vitex agnus castus* L.) seeds were obtained as vacuum packed (Attar Dünyası, Manisa, Turkey). The seeds were ground into a fine flour (Yücebaş Machinery, Izmir, Turkey) and the powder that passed through a 100-mesh sieve was used in meatball production. All ingredients were supplied separately for each of the two replicates.

Beef meatball production

The manufacturing of meatball was performed as previously stated by Özer and Secen (2018) with the following ingredients; beef meat (70%), beef fat (15%), breadcrumbs (7%), onion (4%), salt (2%), garlic (1%) and red pepper (1%). Chasteberry seed powder was not used in the formulation of the control group samples. In the other treatment groups, chasteberry seed powder was added in place of a portion of the beef meat, in the proportions of 2.5, 5, 7.5 and 10%, respectively. All the ingredients were mixed and kneaded to prepare the meatball mix. Pieces of the mixture weighing 50 g each were taken to obtain meatballs with a thickness of 1 cm and a diameter of 90 mm. The meatballs were cooked on a hot plate for 6 min, with both sides cooked evenly and the center point temperature reaching 70±2 °C. After cooking, the meatballs were cooled to 20±2 °C and then vacuum-packed. They were then shelf-stored at +4°C for 15 days.

Total phenolic compound analysis

The total phenolic compound in the chasteberry seed powder and meatballs was analyzed using the Folin-Ciocalteu method (Slinkard and Singleton, 1977). A 4.3 mg sample of the meatball was stirred with 10 ml methanol for 2 hours in the dark. Then, 300 µl of this mixture was pipetted into to a test tube and mixed with 3.16 ml distilled water, 200 µl Folin-Ciocalteu reagent and 1 ml methanol. The mixture was allowed to stand at 20±2 °C for 8 min. Next, 600 µl sodium carbonate solution (10%) was added and then vortexed, and kept at 40 °C for 30 min. A blank was prepared using the same volume of methanol and following the same procedure as for the sample. The absorbance of the prepared solution at a wavelength of 765 nm was measured. The total amount of phenolic compounds was expressed as mg of gallic acid equivalent per gram of sample (mg GAE/g sample).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity analysis

The DPPH radical scavenging activity for chasteberry seed powder and meatball samples was determined by minor changes in the procedure description by Brand-Williams et al. (1995). A DPPH solution with 0.98±0.02 absorbance at 517 nm wavelength was prepared for analysis. 100 µl sample extract (1 mg/ml) and 3 ml of the DPPH solution were thoroughly mixed. The antioxidant activity (%) was determined by measuring the absorbance at a wavelength of 517 nm after 30 min.

Beta-carotene bleaching activity analysis

Beta-carotene bleaching analysis was performed on chasteberry seed powder and meatballs by the method outlined by Matthäus (2002) with some modifications. In total, 4 ml β-carotene solution, 400 µl Tween 40 and 40 µl linoleic acid were mixed and then evaporated to separate the chloroform. Then, 100 ml of distilled water was added to prepare the test solution. Sample extracts were prepared at specified concentrations and 3 ml of test solution was mixed with each extract. The first absorbance was determined at 470 nm, then the samples were kept at 50°C for 100 min and the absorbance was determined again. The same procedure was applied to the control group prepared without using β-carotene, and the antioxidant activity was calculated.

TBARS analysis

The formation of thiobarbituric acid reactive substances (TBARS) was measured to assess the stability of lipid oxidation (Kilic and Richards 2003). Briefly, a 1 g sample homogenized with 6 mL of an extraction solution containing trichloroacetic acid, EDTA and propyl gallate. Then, mixture was filtered through Whatman No:1 filtrate paper. Thereafter, 1 mL filtrate and 1 mL thiobarbituric acid solution (TBA) were mixed and heated at 100°C for 40 min. The mixture was centrifuged (2000xg for 5 min) after cooling. Absorbance was detected at 532 nm wavelength. TBARS levels were calculated as µmol MDA/kg sample.

Physico-chemical composition and cooking properties

The protein, moisture, ash and fat composition of chasteberry seed powder and meatballs were determined by the methods of AOAC (2005). The total dietary fiber content of chasteberry seed powder was determined (de Almeida Costa et al., 2006). In addition, the overall amount of carbohydrates in the samples was determined through the difference in the total proximate compositions. A digital pH meter was used to analyze the pH of the meatball samples.

The surface color values (L^*, a^* and b^*) of the samples were determined by a Hunter Lab colorimeter (CR-400, Konica Minolta, Japan) in the specular component excluded (SCE) mode under a D65 light source, with a circle of 8 mm aperture diameter and an angle of observation of 10°. The color differences (ΔE^*) in meatballs were calculated using color values. The weight, diameter and thickness of the meatball samples were determined before and after cooking. The cooking yield, reduction in thickness and diameter and total shrinkage values were determined (Murphy et al. 1975; El-Magoli et al., 1996).

Texture Profile Analysis

Texture Analyzer (TA.XT2 Plus, Stable Micro Systems, UK) with circular probe (Ø10 cm) and a 50 kg load cell was used for texture profile analysis (TPA) of the samples. During the analysis, a compression of 0.5 mm (50%) was applied to the samples at 0.5 mm/s speed. The probe speed was 5 mm/s before and after the test. Texture profile analysis results were used to determine the hardness, chewiness, cohesiveness, springiness, and resilience values of the samples.

Sensory Analysis

Sensory analyses of cooked meatball samples were conducted by a panel of 13 individuals consisting of 5 male and 8 female students and academic staff from the Food Engineering Department. Panelists were trained prior to the panel on the analysis method, sensory characteristics of meatballs and other parameters to be considered during sensory analysis. The samples were prepared by heating them on an electric heating surface for 45 seconds and presented to each panelist for sensory analysis. Salt-free bread and water were served to panelists to clean the mouth and palate between samples. The panelists evaluated the meatballs using a 9-point hedonic scale for color, ease of fracture, juiciness, greasiness, taste, odor, and overall acceptability.

Statistical analysis

The study was repeated twice and each measurement in all analyses was conducted at least in duplicate. The statistical analysis was carried out with the use of a statistical program (SPSS 22.0.0, SPSS Inc., USA). A fully randomized design with five treated (control and four experimental groups) and two replicates was used. Treatment groups and storage time as fixed effects and replicates as random effects were treated, and all data were analyzed using the general linear model (GLM). One-way analysis of variance (ANOVA) and Duncan post-hoc test were performed on the chemical composition, physico-chemical, sensory and textural data of the meatballs. Differences between means were considered significant if $P<0.05$. Results are presented as mean values and standard errors of means (SEM).

Results and Discussion

It was determined that the chasteberry seed powder used in meatball production contained 9.2% moisture, 13.5% protein, 7.3% fat, 10.3% ash, and 38.7% dietary fiber. In addition, the DPPH radical scavenging activity and β-carotene bleaching value for chasteberry seed powder were determined to be 74.8% and 61.8%, respectively. The total phenolic content of chasteberry seed powder was determined to be 83.6 mg GAE/g. The chemical composition and bioactive properties of chasteberry are subject to variation depending on parameters such as variety and growing environment (Dülger et al., 2002; Maltaş et al., 2010; Souto et al., 2020).

The proportion of chasteberry seed powder used in the meatball formulation exhibited a significant ($P<0.05$) effect on the phenolic content and antioxidant capacity of the meatballs, increasing both parameters. (Table 1). The phenolic content for the control samples, which was thought to be mainly derived from the spices used, was determined to be 0.42 mg GAE/g sample.

Table 1. The total phenolic content and antioxidant properties of meatballs

Groups	DPPH (%)	β-carotene bleaching (%)	Total phenolic content (mg GAE/g)
Control	44.05^{e}	31.45^{e}	0.42^{e}
2.5%	55.10^{d}	38.35^{d}	0.73^{d}
5%	64.71^{c}	42.92^{c}	1.35^{c}
7.5%	70.75^{b}	48.86^{b}	1.91^{b}
10%	82.06^{a}	54.04^{a}	2.31^{a}
SEM	2.99	1.82	0.16

a-e (↓) different letters in the same column indicate statistical differences.
SEM: standard error of the means

It is known that spices such as pepper, onion, and garlic contain different levels of phenolics such as gallic, coumaric, cinnamic, ferulic, caffeic and vanillic acid (Zheng and Wang, 2001). Conversely, the total phenolic content for the meatballs increased approximately 5.5 times when 10% chasteberry seed powder was used in the meatball formulation (P<0.05). As anticipated, the antioxidant capacity of the meatballs was also found to be significantly enhanced when chasteberry seed powder was incorporated (P<0.05). This increase in phenolic content also resulted in a significant enhancement in antioxidant capacity, as shown by the DPPH and β-carotene bleaching assays (Table 1). The DPPH and β-carotene bleaching activities of the meatballs increased by approximately 1.9 and 1.7 times, respectively, when chasteberry seed powder was added to the meatball formulation. It is demonstrated that the flavonoid and tannin content, along with iridoids and diterpenoids, which provide potent antioxidant properties to chasteberry, can effectively inhibit lipid oxidation in meat products (Sağlam et al., 2007; Latoui et al., 2012). The study results showed that meatballs prepared with chasteberry seed powder, which is rich in phenolic, significantly increased the content of phenolic in meatball samples. The findings indicate that chasteberry seed can be used as an alternative natural antioxidant in the meat products formulation.

The enhanced antioxidant capacity and total phenolic content of the samples resulting from the incorporation of chasteberry seed powder notably influenced the oxidation level of lipids throughout the storage period (Table 2). The TBARS level for the control samples was considerably higher than meatballs produced with chasteberry seed during the storage period (P<0.05). Due to the increase of chasteberry powder in the meatball formulation, lipid oxidation in meatball samples was limited (P<0.05).

Table 2. The changes in TBARS values for meatballs during storage

Groups	Storage (days)				SEM
	1	5	10	15	
Control	4.82^{aD}	5.29^{aC}	6.09^{aB}	6.25^{aA}	0.22
2.5%	3.40^{bD}	3.90^{bC}	4.16^{bB}	4.23^{bA}	0.13
5%	3.13^{cD}	3.50^{cC}	4.03^{cB}	4.31^{bA}	0.18
7.5%	2.82^{dD}	3.18^{dC}	3.48^{dB}	3.54^{cA}	0.11
10%	2.59^{eC}	2.65^{eC}	2.80^{eB}	2.93^{dA}	0.05
SEM	0.18	0.20	0.25	0.37	

a-e (↓) different letters in the same column indicate statistical differences.
A-D (→) different letters in the same column indicate statistical differences.
SEM: standard error of the means

The impact was most significant in the 10% chasteberry group, where TBARS values were approximately 50% lower than those observed in the control group after 15 days of storage (P<0.05). The relationship between the increase in phenolic content and the decrease in lipid oxidation is significant. This reduction can be attributed to the high phenolic content of the chasteberry, which includes flavonoids and iridoids that have been evidenced to scavenge free radicals and inhibit lipid peroxidation. They also have an inhibitory effect on lipid peroxidation by disrupting the polymer chain reactions that occur during lipid peroxidation (Okamura et al., 1993; Van Acker et al., 1996; Masuoka et al., 2012). In the present study, the increased phenolic content due to adding chasteberry powder resulted in a notable reduction in TBARS values, indicating better oxidation stability during storage. It is also reported that phenolic acids, anthocyanins, flavonals, and flavonoids inhibit the formation of hydroxyl radicals formed from hydrogen peroxide (Gök, 2006). This finding is consistent with the results of previous studies that have investigated the use of other natural antioxidants, such as flaxseed and peach peel powder, pomegranate, citrus and mango peel extract, and olive leaf extract, in meatballs (Turp, 2016; Zhang et al., 2016; Turgut et al., 2017; Nishad et al., 2018; Mokhtar and Eldeep, 2020; Rubel et al., 2020). These studies have demonstrated a correlation between the high phenolic content of these antioxidants and a reduction in lipid oxidation

The utilization of chasteberry seed powder in the production of meatballs resulted in considerable alterations to the cooking properties of the meatballs, except for cooking yield (Table 3). While the addition of chasteberry seed powder in the formulation of meatballs increased the reduction in diameter and total shrinkage rate of the meatballs (P<0.05), the thickness reduction was reduced (P<0.05).

Table 3. The cooking characteristics of meatballs

Groups	CY (%)	RD (%)	RT (%)	S (%)
Control	94.39^{a}	14.45^{c}	26.50^{a}	-26.77^{a}
2.5%	94.95^{a}	16.11^{c}	21.75^{b}	-29.61^{b}
5%	91.45^{a}	14.17^{c}	15.00^{c}	-26.31^{b}
7.5%	94.39^{a}	20.55^{b}	10.75^{c}	-36.81^{c}
10%	93.86^{a}	24.17^{a}	6.25^{d}	-42.48^{d}
SEM	1.59	4.35	7.83	7.06

CY: cooking yield; RD: Reduction in diameter; RT: Reduction in thickness; S: Shrinkage
a-e (↓) different letters in the same column indicate statistical differences.
SEM: standard error of the means

It has been reported in the literature that the use of components containing dietary fiber is used in meat products, water and fat retention properties and textural parameters are improved and cooking losses are reduced (Erkan, 2010; Foster et al., 2010).

The proximate composition of chasteberry seed powder significantly affected the composition of the meatballs as well as their cooking properties (Table 4). While the fat and ash contents of the meatballs containing more than 5% chasteberry seed powder increased significantly and the moisture and protein content decreased (P<0.05). The change in the proximate composition of meatballs is associated with chemical composition and amount of the ingredient added to the formulation. Studies indicated that the addition of 3% *Mentha pulegium* to the meatball formulation had no significant effect, however, 37.5% soybean pulp addition changed the proximate composition of meatballs (Gün, 2014; Guliyeva, 2020).

Table 4. The proximate composition of meatballs

Groups	pH	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Control	5.88^{ab}	62.38^{a}	18.66^{a}	16.15^{c}	1.98^{c}	0.83^{e}
2.5%	5.87^{b}	62.07^{a}	17.61^{b}	15.75^{c}	2.22^{c}	2.35^{d}
5%	5.90^{a}	60.02^{ab}	16.37^{c}	17.97^{b}	3.02^{b}	3.12^{c}
7.5%	5.90^{a}	59.01^{bc}	15.90^{cd}	18.39^{ab}	3.12^{ab}	3.68^{b}
10%	5.82^{c}	58.11^{c}	15.37^{d}	19.21^{a}	3.25^{a}	4.06^{a}
SEM	0.02	0.32	0.42	0.12	0.17	0.11

a-e (↓) different letters in the same column indicate statistical differences.
SEM: standard error of the means

The texture profile analysis indicated that there were notable alterations in the textural properties of the meatballs with the incremental incorporation of chasteberry powder (Table 5). While the use of 2.5% chasteberry powder in the formulation of meatballs did not cause any textural change except hardness. However, springiness and cohesiveness values were most affected by the highest chasteberry concentration (10%).

Table 5. Texture profile analysis results of meatballs

Groups	Hardness (N)	Springiness	Cohesiveness	Chewiness	Resilience
Control	293.07^{b}	0.65^{b}	0.63^{b}	121.17^{b}	0.28^{b}
2.5%	365.68^{a}	0.70^{b}	0.65^{b}	165.20^{ab}	0.29^{b}
5%	337.51^{ab}	0.82^{a}	0.73^{a}	201.36^{a}	0.37^{a}
7.5%	390.53^{a}	0.77^{a}	0.71^{a}	210.38^{a}	0.34^{a}
10%	315.08^{ab}	0.78^{a}	0.71^{a}	172.82^{ab}	0.35^{a}
SEM	16.44	0.06	0.01	39.15	0.04

a-e (↓) different letters in the same column indicate statistical differences.
SEM: standard error of the means

It has been indicated that the use of different extracts, powders and fibers of plants in meatball formulations causes an increase in parameters such as hardness, springiness, cohesiveness and resilience (Çarşı, 2019; Özabravci, 2019; Özdemir, 2019; Guliyeva 2020). The changes can be attributed to the high fiber content of chasteberry seed powder, which is likely to have increased water retention, thus altering the textural properties. It is established that dietary fiber improves water and fat retention in meat products, resulting in a firmer texture (Özer and Secen 2018). However, it was stated by the panelists that the effect of chasteberry powder on textural parameters determined by analytical was insignificant sensory (Figure 1).

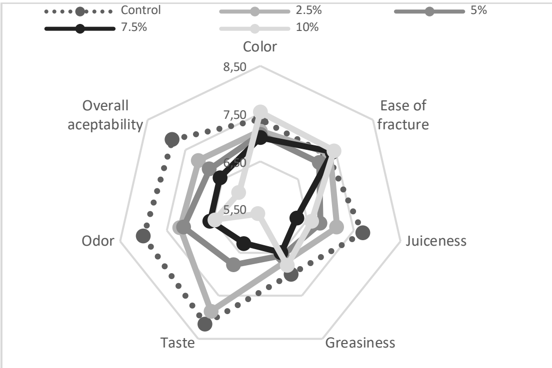

Figure 1. Sensory evaluation of cooked meatballs

The panelists indicated that incorporating chasteberry powder into the meatball formulation insignificant minor alters in the color, bite character and ease of fracture parameters of the products. However, significant differences were determined in other sensorial parameters, especially in taste and odor ($P<0.05$). The panelists determined that the control group meatballs were more highly appreciated in terms of taste and odor. Furthermore, the taste and odor of the meatballs decreased as the usage rate of chasteberry powder in the meatball formulation increased ($P<0.05$). The strong and unique aroma and taste of chaste seed, which can be described as bitter, sour and spicy, affected the sensory properties of the meatball samples, although it was used at low usage rates in formulation. Panelists stated that chasteberry powder gave the meatball samples the taste and odor characteristics that they described as acrid, astringent, bitter, sour and tart, and that these characteristics could be felt very easily, especially at high doses. This also affected the overall acceptability of the meatballs. The control group samples exhibited the highest mean overall acceptability score, while the meatballs containing 7.5% and 10% chasteberry powder showed the lowest overall acceptability scores ($P<0.05$). In many studies, it has been reported that when components with high content of essential oil and phenolic compounds, such as chasteberry seeds, are used in the formulations of meat products, their overall acceptability decrease (Nassu et al., 2003; Gök, 2006; İlhan, 2010; Guliyeva, 2020). Furthermore, the dose-dependent effect of chasteberry seed powder on lipid oxidation indicates that higher concentrations could yield more pronounced antioxidant benefits. However, as observed in the present study, sensory acceptability decreased as the concentration increased, indicating the desirability of achieving a balance between antioxidant capacity and consumer preferences. Similar findings have been reported for rosemary and garlic, where high concentrations also resulted in sensory rejection despite the antioxidant benefits they offer (Olivas-Méndez et al., 2022). Therefore, this balance is of particular importance in food formulations, especially when developing products with extended shelf life (Bellucci et al., 2022). To minimize these effects, further investigation could be conducted into the potential application of encapsulating sources with dominant aromatic sensory properties, with the aim of masking their strong aroma while preserving their antioxidant properties.

The color differences of the products was observed throughout their storage (Figure 2). While the color differences in the control group samples increased during storage compared to the production day, the samples containing chasteberry seed powder initially demonstrated a decrease and then an increase. It was found that the samples containing chasteberry seed powder that were stored for 10 days exhibited color characteristics that were similar to those observed on the day of production. However, after the storage period, it was determined that there were notable differences in the color values of all samples when compared to the samples produced on the initial day.

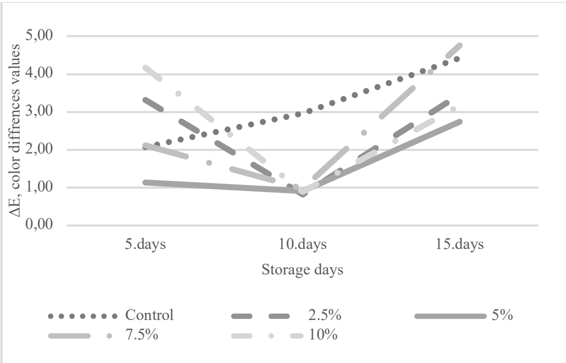

Figure 2. Color differences (ΔE^*) of meatballs during storage

The differences in product color properties (ΔE^*) may be attributed to the phenolic compounds, flavonoid content, and antioxidant capacity of chasteberry seed powder, which stabilizes color by decelerating oxidative processes (Souto et al., 2020). The initial decrease in color difference may be attributed to interactions between these components and lipid oxidation. However, as storage continues, reducing antioxidant capacity may result in color changes. The differences determined after the storage period indicate that the stabilization of color properties in chasteberry seed powder may be relatively limited over extended periods.

Conclusion

The incorporation of chasteberry powder into the formulation of meatballs has resulted in considerable increases in total phenolic content and antioxidant capacity of the product, thereby contributing to the development of enhanced oxidation stability. However, higher concentrations of chasteberry were found to have an adverse effect on the sensory properties of the product, indicating that 2.5% represents the optimal level for achieving a balance between antioxidant capacity and consumer acceptability. It would be beneficial for future studies to investigate the potential of encapsulation or other techniques to enhance the applicability of chasteberry seed powder without compromising the sensory appeal of the final product.

References

Aguiar Campolina, G., M. das Graças Cardoso, A. Rodrigues-Silva-Caetano, D. Lee Nelson and E. Mendes Ramos (2023). Essential oil and plant extracts as preservatives and natural antioxidants applied to meat and meat products: a review. *Food Tech Biotech & Biotech Equip* 61(2): 212-225.

AOAC (2005). *Official methods of analysis of AOAC International*, AOAC Int.

Atia, T. S., E. A. Moustafa and S. I. Ibrahim (2022). Improving Bacterial, Oxidative, and Sensory Quality of Meat Steaks Using Cumin, Garlic, and Thyme Essential Oils. *World's Vet J* 12(3): 274-283.

Bellucci, E. R. B., C. V. Bis-Souza, R. Domínguez, R. Bermúdez and A. C. D. Barretto (2022). Addition of Natural Extracts with Antioxidant Function to Preserve the Quality of Meat Products. *Biomol* 12(10).

Boccellino, M. (2023). Health Effects of Natural Antioxidants. *Int J Mol Sci* 24(13): 10792.

Brand-Williams, W., M.-E. Cuvelier and C. Berset (1995). Use of a free radical method to evaluate antioxidant activity. *LWT* 28(1): 25-30.

Burle, S. S., K. R. Gupta, S. N. Lade, S. W. Rangari and M. J. Umekar (2023). *Antioxidants Obtained from the Natural Sources: Importance in Human Health*. Biochemistry, IntechOpen.

Çarşı, Ş. C. (2019). Çördük otu tozu ilavesinin sığır eti köftelerinin bazı kalite özellikleri üzerine etkilerinin belirlenmesi, Selçuk Üniversitesi

de Almeida Costa, G. E., K. da Silva Queiroz-Monici, S. M. P. M. Reis and A. C. de Oliveira (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chem* 94(3): 327-330.

Dülger, B., E. Uğurlu and F. Gücin (2002). Vitex agnus-castus L (Hayıt)'un Antimikrobiyal Aktivitesi. *Çev-Kor* 11(45): 1-5.

El-Magoli, S. B., S. Laroia and P. J. M. S. Hansen (1996). Flavor and texture characteristics of low fat ground beef patties formulated with whey protein concentrate. 42(2): 179-193.

Erkan, T. (2010). Gıdalardaki katkı maddeleri. *Türk Ped Arş* 45(4): 315-318.

Eryigit, T., A. Çig, N. Okut, B. Yildirim and K. Ekici (2015). Evaluation of chemical composition and antimicrobial activity of Vitex agnus castus L. fruits' essential oils from West Anatolia, Turkey. *J Ess Oil Bearing Plants* 18(1): 208-214.

Foster, E., J. C. Mathers and A. J. Adamson (2010). Packaged food intake by British children aged 0 to 6 years. *Food Add Cont* 27(3): 380-388.

Fukahori, M., S. Kobayashi, Y. Naraki, T. Sasaki, H. Oka, M. Seki, S. Masada-Atsumi, T. Hakamatsuka, Y. J. C. Goda and P. Bulletin (2014). Quality evaluation of medicinal products and health foods containing chaste berry (Vitex agnus-castus) in Japanese, European and American markets. 62(4): 379-385.

Gök, V. (2006). Antioksidan kullanımının fermente sucukların bazı kalite özellikleri üzerine etkileri. Doktora Tezi, Ankara Üniversitesi.

Guliyeva, F. (2020). Yarpuz (Mentha pulegium L.) Kullanılarak Üretilen Köftelerin Soğukta Muhafaza Süresince Lipit Oksidasyonu ve Renk Kararlılığının Değerlendirilmesi. Yüksek Lisans, Ondokuz Mayıs Üniversitesi.

Gül, A. (2007). Kadın Hastalıklarında Kullanılan Droglar, Vitex agnus-castus. Yüksek Lisans Tezi, Gazi Üniversitesi.

Gün, M. (2014). Sığır eti köftelerinin bazı fiziksel kimyasal tekstürel ve duyusal özellikleri üzerine çeşitli sütçülük yan ürünlerinin etkisi. Yüksek Lisans, Selçuk Üniversitesi

İlhan, E. Y. (2010). Farklı oranlarda dana kırıntı eti ile formüle edilen hamburger köftelerinde biberiye ekstraktı ilavesinin raf ömrüne etkisi. Yüksek Lisans, Ankara Üniversitesi

Ji, J., S. Shankar, F. Royon, S. Salmieri and M. Lacroix (2023). Essential oils as natural antimicrobials applied in meat and meat products-A review. *Crit Rev Food Sci Nutr* 63(8): 993-1009.

Kilic, B. and M. Richards (2003). Lipid oxidation in poultry döner kebab: Pro-oxidative and anti-oxidative factors. *J Food Sci* 68(2): 686-689.

Kumar, Y., D. N. Yadav, T. Ahmad and K. Narsaiah (2015). Recent trends in the use of natural antioxidants for meat and meat products. *Compr Rev Food Sci Food Saf* 14(6): 796-812.

Latoui, M., B. Aliakbarian, A. A. Casazza, M. Seffen, A. Converti and P. Perego (2012). Extraction of phenolic compounds from Vitex agnus-castus L. *Food Bioprod Process* 90(4): 748-754.

Lorenzo, J. M., M. Pateiro, R. Domínguez, F. J. Barba, P. Putnik, D. B. Kovačević, A. Shpigelman, D. Granato and D. Franco (2018). Berries extracts as natural antioxidants in meat products: A review. *Food Res Int* 106: 1095-1104.

Mahassen, M. S. and A. S. Hamdy (2023). Natural Antıoxıdants From Herbs And Spıces And Theır Effects For Improvıng The Functıonalıty And Shelf Lıfe Of Food And Daıry Products: A Review. *J Multidiscip Res* 3(1).

Maltaş, E., A. Uysal, S. Yildiz and Y. Durak (2010). Evaluation of antioxidant and antimicrobial activity of Vitex agnus-castus L. *Fresen Environ Bull* 19: 3094-3099.

Masuoka, N., M. Matsuda and I. Kubo (2012). Characterisation of the antioxidant activity of flavonoids. *Food Chem* 131(2): 541-545.

Matthäus, B. (2002). Antioxidant activity of extracts obtained from residues of different oilseeds. *J Agri Food Chem* 50(12): 3444-3452.

Mendonça, J. d. S., R. d. C. A. Guimarães, V. A. Zorgetto-Pinheiro, C. D. P. Fernandes, G. Marcelino, D. Bogo, K. d. C. Freitas, P. A. Hiane, E. S. de Pádua Melo and M. L. B. Vilela (2022). Natural antioxidant evaluation: A review of detection methods. *Mol* 27(11): 3563.

Mokhtar, S. M. and G. S. S. Eldeep (2020). Impact of Mango Peel Extract on the Physicochemical Properties, Microbiological Stability and Sensory Characteristics of Beef Burgers During Cold Storage. *Egyp J Food Sci* 48(2 (In Progress)): 245-258.

Murphy, E. W., P. E. Criner and B. Gray (1975). Comparisons of methods for calculating retentions of nutrients in cooked foods. *J Agri Food Chem Adv* 23(6): 1153-1157.

Nassu, R. T., L. A. G. Gonçalves, M. A. A. P. da Silva and F. J. Beserra (2003). Oxidative stability of fermented goat meat sausage with different levels of natural antioxidant. *Meat Sci* 63(1): 43-49.

Niroumand, M. C., F. Heydarpour and M. H. Farzaei (2018). Pharmacological and therapeutic effects of Vitex agnus-castus L.: A review. *Pharmacognosy Rev* 12(23).

Nishad, J., T. K. Koley, E. Varghese and C. Kaur (2018). Synergistic effects of nutmeg and citrus peel extracts in imparting oxidative stability in meat balls. *Food Res Int* 106: 1026-1036.

Okamura, H., A. Mimura, Y. Yakou, M. Niwano and Y. Takahara (1993). Antioxidant activity of tannins and flavonoids in Eucalyptus rostrata. *Phytochem* 33(3): 557-561.

Olivas-Méndez, P., A. Chávez-Martínez, E. Santellano-Estrada, L. Guerrero Asorey, R. Sánchez-Vega, A. L. Rentería-Monterrubio, D. Chávez-Flores, J. M. Tirado-Gallegos and G. Méndez-Zamora (2022). Antioxidant and antimicrobial activity of rosemary (Rosmarinus officinalis) and garlic (Allium sativum) essential oils and chipotle pepper oleoresin (Capsicum annum) on beef hamburgers. *Foods* 11(14): 2018.

Özabravci, A. (2019). Tekirdağ köftesi üretiminde bezelye proteini ve lifinin kullanım olanaklarının araştırılması. Yüksek Lisans Tezi, Namık Kemal Üniversitesi.

Özdemir, S. (2019). Köfte üretiminde kurutulmuş koruk (vitis vinifera l.) posası kullanımının hca oluşumu ve ürün raf ömrü üzerine etkilerinin incelenmesi. Yüksek Lisans Tezi, Ege Üniversitesi.

Özer, C. O. and S. M. Secen (2018). Effects of quinoa flour on lipid and protein oxidation in raw and cooked beef burger during long term frozen storage. *Food Sci Tech* 38: 221-227.

Rubel, S. A., Z. Yu, H. Murshed, S. A. Islam, D. Sultana, S. Rahman and J. Wang (2020). Addition of olive (olea europaea) leaf extract as a source of natural antioxidant in mutton meatball stored at refrigeration temperature. *J Food Sci Tech*: 1-9.

Sağlam, H., A. Pabuçcuoğlu and B. Kıvçak (2007). Antioxidant activity of Vitex agnus-castus L. extracts. *Phytotherapy Research: International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 21(11): 1059-1060.

Sezik, E., G. Özkök and M. Sezik (2013). Vitex agnus castus L. preparatı ile retrospektif bir çalışma. *Med J Suleyman Demirel Uni* 20(2).

Slinkard, K. and V. L. Singleton (1977). Total phenol analysis: automation and comparison with manual methods. *American J Enol Viti* 28(1): 49-55.

Šojić, B., S. Milošević, D. Savanović, Z. Zeković, V. Tomović and B. Pavlić (2023). Isolation, bioactive potential, and application of essential oils and terpenoid-rich extracts as effective antioxidant and antimicrobial agents in meat and meat products. *Mol* 28(5): 2293.

Souto, E. B., A. Durazzo, A. Nazhand, M. Lucarini, M. Zaccardelli, S. B. Souto, A. M. Silva, P. Severino, E. Novellino and A. Santini (2020). Vitex agnus-castus L.: main features and nutraceutical perspectives. *Forests* 11(7): 761.

Turgut, S. S., F. Işıkçı and A. Soyer (2017). Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. *Meat Sci* 129: 111-119.

Turp, G. Y. (2016). Effects of four different cooking methods on some quality characteristics of low fat Inegol meatball enriched with flaxseed flour. *Meat Sci* 121: 40-46.

Van Acker, S. A., M. N. Tromp, D. H. Griffioen, W. P. Van Bennekom, W. J. Van Der Vijgh and A. Bast (1996). Structural aspects of antioxidant activity of flavonoids. *Free Radical Bio Med* 20(3): 331-342.

Zahid, H., G. H. Rizwani and S. Ishaqe (2016). Phytopharmacological review on Vitex agnus-castus: a potential medicinal plant. *Chinese Herb Med* 8(1): 24-29.

Zhang, Y., I. Han, W. Bridges and P. Dawson (2016). Peach skin powder inhibits oxidation in cooked turkey meat. *Poultry Sci* 95(10): 2435-2440.

Zheng, W. and S. Y. Wang (2001). Antioxidant activity and phenolic compounds in selected herbs. *J Agri Food Chem* 49(11): 5165-5170.