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Original article

The Effect of Purple Carrot Powder on the Microbiological and Physicochemical Properties of Vacuum-Packed Meatballs During Storage (4°C)

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Abstract

The aim of this study is to determine the microbiological and physicochemical properties of vacuum-packed meatball samples prepared with aqueous solutions of purple carrot powder (MHT) at two concentrations: 5 g (mh5) and 10 g (mh10) in 50 ml, stored at refrigerator temperature (4°C) for 7 days. The total phenolic content of MHT, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, and disk diffusion analyses against various pathogens were performed. The total aerobic mesophilic bacteria (TAMB), yeast-mold, and *Staphylococcus aureus* counts, internal and external color (L*, a*, b*), pH, thiobarbituric acid reactive substances (TBARS), and water holding capacity (WHC) of meatballs with and without PCP were analyzed during storage. No significant effect of PCP on TAMB, yeast-mold, and *S. aureus* was observed during storage. The total phenolic content of MHT was found to be 602.2±2.33 mg/100ml gallic acid equivalent (GAE/100ml), and the DPPH activity was 151.43±3.06 mg/100ml. On storage days 0, 2, and 7, no significant differences were observed in TBARS values among the groups, whereas on day 4, the TBARS values of the mh5 and mh10 groups were found to be lower compared to the control group ($P < 0.05$). The L*, a*, and b* values (internal and external), WHC, and pH values (except for day 4 in the mh5 group) showed no significant changes during storage.

Keywords: Meatballs, *Staphylococcus Aureus*, Functional Food, Purple Carrot Powder, Cross Contamination.

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INTRODUCTION

Purple carrot (*Daucus carota L. ssp. sativus var. atrorubens Alef.*) is a root vegetable native to Turkey, the Middle East, and the Far East, rich in phenolic compounds (Kammerer et al., 2004; Mizgier et al., 2016; Ekici et al., 2015). Its bluish-purple color is derived from its high anthocyanin content, which is used as a natural food coloring due to its high stability under heat, light, and pH conditions (Ekici et al., 2015).

The anthocyanins in purple carrot are a subgroup of polyphenols found in various plant species' organs and tissues. Known for their free radical scavenging activities, these compounds provide protection against pathogens, UV radiation, pollination, and biotic and abiotic stresses during plant growth (Pereira-Caro et al., 2021). In addition, they exhibit strong antioxidant and anti-inflammatory activity, reduce the risk of cardiovascular diseases and cancer, regulate blood glucose levels, and help prevent neurological disorders (Perez et al., 2022).

Compared to other carrot varieties, purple carrot has higher total sugar, organic acid, polyphenolic content, total dry matter, and antioxidant activity, demonstrating health-promoting effects such as antioxidant, antiproliferative, and anti-inflammatory properties due to its bioactive compounds (Ekici et al., 2015; Pereira-Caro et al., 2021; Yusuf et al., 2021). Due to its dry matter content, purple carrot has a higher water-holding capacity than other carrot varieties. Certain chemicals extracted from its root are known to exhibit antibacterial activity against *Staphylococcus aureus, Streptomyces scabies, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa,* and *Escherichia coli*, Staphylococcal food poisoning in particular is the most common food poisoning worldwide, with symptoms appearing 0.5-8 hours (average 3 hours) after ingestion of contaminated food, with children and adolescents experiencing symptoms earlier than adults. Symptoms include nausea and severe vomiting, often accompanied by watery diarrhoea, abdominal pain, fever and chills. The illness usually resolves spontaneously within 24 hours. However, in rare cases, fatal dehydration and electrolyte imbalances can occur; mortality rates range from 0.03% in the general population to 4.4% in children and the elderly. (Sallam et al., 2021). Purple carrot, which demonstrates antimicrobial properties against numerous food pathogens, improves food quality by reducing microbial spoilage in foods (Nath et al., 2022; Yusuf et al., 2021).

Interest in using natural antioxidants and antimicrobial substances instead of synthetic food additives to extend the shelf life of meat and meat products and reduce microbial load is increasing. Additionally, studies focusing on the antioxidant effects and color properties of various plants such as rosemary, pomegranate, orange, and green tea have found that these products exhibit higher antioxidant activity, lower TBARS values, and more acceptable texture, color, aroma, and taste characteristics compared to samples prepared without additives (Pereira-Caro et al., 2021).

Accordingly, this study aims to investigate the microbiological and physicochemical stability of beef patties enriched with purple carrot powder, prepared without additives, and stored at refrigerator temperature (+4°C). Furthermore, the study examines the effect of purple carrot powder, added as an aqueous solution to patties, on *Staphylococcus aureus,* one of the most common causes of foodborne infections and intoxications associated with gastrointestinal symptoms.

MATERIALS and METHODS

Materials

The ground beef used for meatball preparation was purchased from a local butcher in Antalya, Muratpaşa, and purple carrot powder was procured from Fx Food (Istanbul, Turkey).

Methods

Total Phenolic Content, DPPH (2,2-Diphenyl-1-picrylhydrazyl) Antioxidant Activity, and GC-MS Chemical Composition Analysis of Purple Carrot Powder

The total phenolic content of purple carrot powder was determined using the Folin-Ciocalteu method (Özdemir et al., 2014), and DPPH analysis was conducted according to Furkan Erdoğan (2022). GC-MS (Gas Chromatography-Mass Spectrometry) analysis was performed as a service by Akdeniz University Food Safety and Agricultural Research Center.

Meatball Production

The meatball formulation consisted of 1 kg medium-fat ground beef, 0.8% black pepper, and 1.5% salt. The first 1 kg batch served as the control group (without purple carrot powder). For the other two groups, aqueous solutions were prepared by dissolving 5 g and 10 g of purple carrot powder in 50 ml of sterile distilled water using a magnetic stirrer for 6 hours. The emulsion was prepared in a bowl chopper (bowl mixer, Arzum, Turkey by adding minced meat and the other ingredients of the formulation in sequential order at a specified time interval. During chopping, the temperature of the emulsion was maintained at 10-12° C by the addition of broken ice. The control group was produced as described above without the addition of purple carrot powder. The remaining two 1 kg batches of meatballs were mixed with these solutions to form the mh5 group (5 g purple carrot powder solution) and mh10 group (10 g purple carrot powder solution). All meatball samples were portioned into 25 g patties, shaped into 1 cm thickness, and approximately 40 patties were obtained for each group. Meatball samples for physicochemical analyses were vacuum-packaged and stored at 4°C. Samples designated for microbiological analysis were inoculated with *Staphylococcus aureus* under a biosafety cabinet.

Staphylococcus aureus Inoculation

Staphylococcus aureus ATCC 25923 strains were cultured on Müller Hilton Agar (MHA) at 37°C for 24 hours. The pathogens were prepared at a turbidity of 0.5 McFarland ($0.5x10^8$ CFU) and inoculated

onto the surface of the meatballs using a pipette (100 µL) and spread with a spatula. After incubation for 20–30 minutes under a safety cabinet to allow the pathogen to adhere to the meatballs, the samples were vacuum-packed and stored at 4°C for subsequent analyses.

Antimicrobial Activity

Disk Diffusion Test

The cultured pathogen (*Staphylococcus aureus* ATCC 25923) was prepared at a turbidity of 0.5 McFarland ($0.5x10^8$ CFU) and inoculated onto Müller Hilton Agar (MHA) plates using a swab. Sterile blank disks were impregnated with 16 µL of purple carrot powder dissolved in alcohol (1/4) and placed on the MHA agar. The plates were incubated at 37°C for 24 hours, and inhibition zone diameters (mm) were measured (Akarca et al., 2020).

Microbiological Analyses

For the microbiological analyses, 25 g of inoculated meatballs from the control, mh5, and mh10 groups were homogenized in 225 ml of 0.1% peptone water using a stomacher for 2 minutes. Serial dilutions (-1 to -6) were prepared, and 100 µL of each dilution was plated using the spread plate method. The following media were used for enumeration: Plate Count Agar (PCA) for total aerobic mesophilic bacteria (TAMB), Baird Parker Agar for *Staphylococcus aureus,* and Dichloran Rose Bengal Chloramphenicol Agar for yeast and mold counts. Results were expressed as $\log_{10}$ CFU/g. Analyses were performed on days 0, 2, 4, and 7 in duplicate and with two replicates.

Physicochemical Analyses

Physicochemical analyses, including TBARS, color, water-holding capacity (WHC), and pH, were performed on meatball samples stored at 4°C on days 0, 2, 4, and 7.

Lipid Oxidation (TBARS)

Thiobarbituric acid reactive substances (TBARS) were measured following modifications of the method described by Kılıç and Richards (2003). Samples (3 g) were homogenized with 18 ml TCA, filtered through Whatman No. 1 paper, then 0.22 µm membrane filters. Filtrates were mixed 1:1 with TBA and incubated at 100°C for 40 minutes. Absorbance was measured at 532 nm using a spectrophotometer (ONDA V-10 Plus VIS, China).

pH

pH measurements were performed using a probe-specific pH meter (Hanna HI 9024, Romania) suitable for meat products (Kaynakcı, 2012).

Water-Holding Capacity (WHC)

Five grams of meatball samples were placed in falcon tubes with cotton to absorb water. The tubes were centrifuged at 9000 rpm for 12 minutes. The WHC was calculated using the following formula (Köker et al., 2024).

WHC=(w2-w1)/w1×100

where **w1** is the initial sample weight, and **w2** is the weight after centrifugation.

Color Measurement

Color parameters (CIE L*, a*, b*) were measured using a colorimeter (LS172, China) calibrated with a white plate. L* indicates lightness, a* indicates redness or greenness, and b* indicates yellowness or blueness (Kaynakcı, 2012). Measurements were taken in quadruplicate for each sample.

Statistical Analysis

Results were analyzed using SPSS 23.0 statistical software. Means were compared using Tukey's multiple comparison test ($\alpha = 0.05$). Data were presented as mean ± standard deviation for all variables. Each analysis was conducted in duplicates with two replicates.

RESULTS and DISCUSSION

In recent years, the consumer trend towards naturally processed and naturally additive foods has led to research into the use of natural preservatives in processed foods. Accordingly, many studies have been carried out on the use of natural preservatives instead of artificial preservatives to extend the shelf life of meat products. Looking at the literature in recent years, fresh spices and extracts (paprika powder and extracts, clove powder and extracts, Piper cubeba powder and extracts (Zaher et al, 2024), chitosan from shrimp scin (Gita et al, 2024), bay leaf (Syzygium polyanthum (Wight) Walp.) (Satar and Hidayati, 2024), various natural extracts (onion peel, potato peel, marjoram, fennel, cinnamon, black seed and olive leaf) (Elhassaneen et al, 2023), sesame oil and sesamol (Sallam et al, 2021), mai-mai mangrove leaf flour (Novitasari et al, 2024), durian rind smoke powder (Faisal, 2023) are used in studies to extend the shelf life of meatballs.

In this study, the effects of purple carrot powder, which is widely grown in Turkey and is known for its high content of phenolic compounds, on microbial growth, lipid oxidation and colour properties of meatballs were evaluated.

Disc Diffusion Results of Purple Carrot Powder

The average results obtained by determining the effects of ethanol extracts of purple carrot powder on some food pathogenic bacteria according to the disc diffusion method are given in Table 1.

Table 1. Disc diffusion results of purple carrot powder against some pathogens

Pathogenic bacterial strain	**Diameter length (mm)**
Listeria monocytogenes ATCC 19118	13mm±0.05
Staphylococcus aureus ATCC 25923	13.5mm±0.34
Escherichia coli ATCC 25922	10mm±0.20

Table 1. shows that ethanol solution of purple carrot powder was highly effective against *Staphylococcus aureus* (Gr +) and *Listeria monocytogenes* (Gr +), followed by *Escherichia coli* (Gr -). The zone diameters of *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* were 13 mm ± 0.05, 14 mm ± 0.34 and 10.00 ± 0.20 mm, respectively. The cell walls of Gram-negative bacteria are more resistant to antimicrobial agents because they contain lipoproteins and lipopolysaccharides. Therefore, it is an expected result that purple carrot powder is less effective against *E. coli*, a gram-negative pathogenic bacteria (Abbasi et al., 2023). As the disc diffusion results indicated that the purple carrot solution was more effective against S. aeurus, the study was continued with this bacterium and artificial contamination of meatballs was performed.

Total Phenolic Matter, DPPH Antioxidant, GC-MS Chemical Content Analysis Results of Purple Carrot Powder

The content analysis of purple carrot powder used in the study is given in Table 2.

Table 2. MHT Chemical Content Analysis

	Compounds (%)	**Purple Carrot Powder**
1	α-thujene	TE
2	α-pinene	0.73
3	Camphene	0.45
4	Trans-2- heptenal	4.21
5	1,1-diethoxyisopentane	2.06
6	2- α-pinene	0.16
7	Benzaldehyde	TE
8	2,2,4,6- pentamethylheptane	0.21
9	2,4-heptadienal	0.48
10	2,6-dimethylnoname	0.14
11	4,6-dimethylundecane	0.28
12	1,8-cineole (eucalyptol)	2.52
13	n-dodecane	1.35
14	4,7-dimethyllundecane	0.39
15	1,1- diethoxyhexane	1.07
16	2,7,10-trimethyldodecane	0.40
17	n-tetradecane	7.67
18	4-methyltetradecane	1.30
19	n-hexadecane	5.87
20	n-heptadecane	8.75
21	Phytane	5.87
22	n-eicosane	TE
23	n-heneicosane	9.04
24	1-monopalmitin(Dihydroxypropyl hexadecanoate)	13.62
25	Camphor	1.57
26	Ethyl palmitate	20.62
27	Other compounds	6.84
	Total	100

TE: not detected

Analysis of the chemical content of purple carrot powder (Table 2.) shows that the major fatty esters are 20.62% ethyl palmitate, 13.62% monopalmitin and 8.75% n-heptadecane. Various fatty acids and esters are known to be important for vitamin D synthesis (Kamel et al, 2023). Although the purple carrot powder used in the study was stirred in distilled water in a magnetic stirrer for a long time, the dissolution process could not be completed. As can be seen from the chemical content, purple carrot powder is rich in fatty esters and complete dissolution can only be achieved in alcohol derivatives. As the test groups were foodstuffs, water was preferred to alcohol as a solvent in the study.

Determination of the total phenolic content of foods is important to give an idea of the hydroxyl groups that provide antioxidant activity. In general, there is a very good linear correlation between total phenolic content and antioxidant activity (Seyhan, 2019). The fact that black carrots have strong antioxidant activity is thought to be due to its reducing and antiperoxidant capacity with the effect of hydroxyl groups attached to phenolic structures and their degree of glycosylation. In addition, it has been reported that black carrot powder is a complex matrix, which should be considered to show synergistic or antagonistic effects with many other components (Smeriglio et al., 2018). In our study, the total phenolic content of purple carrot powder was determined to be 602.2±2.33 mg GAE/100 ml as gallic acid equivalents and the antioxidant activity (DPPH) was determined to be 151.43±3.06 mg/100 ml. Aksu and Turan (2022) determined the total phenolic content of lyophilised black carrot powder to be 2788.89 mg GAE/100 g. The total phenolic content of black carrot powder used in the study to make bread into a functional product was determined to be 2337.10 ± 18.11 mg GAE/100 g and the DPPH (%) antioxidant activity to be 84.30 ± 1.31 (Pandey et al., 2024). In the light of the studies, it is seen that the total phenolic content and antioxidant activity of purple carrot are high due to the bioactive compounds it contains, although the type, drying methods and analytical methods are different (Aslan et al., 2023, Uyan et al., 2004). It is thought that the differences in the values obtained in the studies (total phenolic content, DPPH analysis) may be due to factors such as the extraction method used, solvent, difference in harvest time.

Physicochemical Properties

Colour is an important component of food as it is one of the first properties perceived by the senses and is used by consumers for rapid identification and final acceptance of food (Giusti, & Wrolstad, 2003). The colour values (especially the a* value) of fresh meat and meat products are related to myoglobin, oxymyoglobin and metmyoglobin. Due to increased oxidation of myoglobin during storage in vacuum and/or modified atmosphere packaged products, the typical colour of fresh meat is lost and the meat loses its attractive colour (Aksu and Turan, 2022). The colour of purple carrot solution is known to be affected by pH. Perez et al (2022) observed that the colour of the solution changed from red (pH 2.5) to purple (pH 4.5) and blue (pH 7.0) with increasing pH. These colour changes were found to be due to the different molecular structures of anthocyanins. In our study, the colour values (L, a, b)

were measured separately as inner and outer surface and their changes were monitored during storage (Table 3).

Table 3. Effect of Purple Carrot Powder On L*, a* and b* Internal and External Values of Meatball Experiments

		d0	d2	d4	d7
a*in	Control	7.00±1.45aA	7.16±2.69aB	7.03±2.22aB	7.27±2.26aB
	mh5	7.18±3.32aA	10.69±4.53aA	11.32±3.39aA	9.75±1.3aA
	mh10	7.75±1.01aA	8.42±2.86aAB	8.92±3.53aA	8.79±1.19A
a* out	Control	8.56±1.76aB	8.47±1.26aA	3.54±3.07aB	2.43±5.75aB
	mh5	11.22±1.09aA	12.13±2.42aA	11.43±5.72aAB	10.58±3.22aA
	mh10	11.42±2.37aA	12.24±2.53aA	13.28±3.59aA	6.66±5.43aAB
b* in	Control	8.73±1.65aA	8.35±1.92aA	5.96±1.74aA	7.33±3.38aAB
	mh5	4.24±2.8aAB	7.47±5.18aA	9.44±4.51aA	7.45±2.28aA
	mh10	6.6±2.2aB	5.56±2.34aA	6.42±3.58aA	4.37±1.17aB
b* out	Control	9.9±2.41aA	10.48±1.99aA	7.14±1.84aA	6.41±4.7aA
	mh5	10.08±1.20aA	11±3.27aA	11.95±8.67aA	10.75±4.09aA
	mh10	6.52±2.77aA	10.69±2.88aA	15.74±4.28aA	9.39±5.34aA
L* in	Control	12.11±0.86aA	11.73±3.9aA	10.36±0.46aA	13±3.46aA
	mh5	14.76±5.55aA	12.67±1.63aA	11.08±3.04aA	11.55±3.09aA
	mh10	12.38±7.16aA	10.6±2.04aA	13.44±0.94aA	9.44±4.51aA
L* out	Control	34.7±3.94aA	33.88±4.19aA	35.69±2.84aA	30.84±11.35aA
	mh5	31.51±11.91aA	33.41±2.14aA	31.26±2.4aAB	35.43±4.22aA
	mh10	35.43±4.72aA	35.45±0.85aA	30.83±1.90aB	31.81±5.28aA

*Statistical differences in the same row are indicated by lower case letters (a, b, c); statistical differences in the same column are indicated by upper case letters (A, B, C). mh: Purple carrot powder

No difference was found between the groups for L* internal, b* external values of meatballs fortified with purple carrot (P >0.05). However, there was a difference between the groups for other colour values (p<0.05). As can be seen from Table 3, purple carrot powder increased the A* outer value on the first day of storage and on the fourth day, the a* outer value of the MH10 group was higher than that of the control group (P<0.05). There was no difference in the L* outer values of the groups on the days of storage analysis (except day 4). Looking at the inner b* values of the experiments, purple carrot powder decreased the b* value on the first day of storage (p<0.05). As a result, when the significance between the external colour values of the groups was examined, it was found that the a* value increased while the b* and L* values did not change (except for day 4).

Fernandez-Lopez et al (2005) reported that the a* value is expected to decrease due to oxidation in meat and meat products, but the colour interpretation due to oxidation is not appropriate because the citrus extract used in their study has red pigment. In our study, a* was expected to decrease despite the increase in TBARS during storage, but a* increased due to the intense red pigment of the purple carrot extract used. Therefore, TBARS values and the a* colour were not interpreted comparatively. Ekici et

al. (2015) reported that the addition of black carrot concentrates (BBC) to sausage, a traditional meat product, decreased the external a* values of the samples, contrary to our study, and the lowest a* value ($p < 0.05$) was determined in the sausage sample containing 2 g/100 g BCC and no nitrite. Contrary to the values in our study, yoghurt with 0.25% fibre addition was reported to have the highest L* and b* values in probiotic yoghurt made with frozen black carrot fibre (Say et al., 2022). This is thought to be due to the conversion of anthocyanins to pink as yoghurt is a more acidic food than meat.

Table 4. Effect of purple carrot powder on physicochemical properties of meatball experiments during storage

		d0	d2	d4	d7
pH	Control	5.66±0.02aA	5.60±0.25aA	5.69±0.13aA	6.77±0.19aA
	mh5	5.65±0.08aA	5.52±0.08 aA	5.29±0.14bB	6.66±0.20aA
	mh10	5.61±0.03aA	5.27±0.13aB	5.60±0.13aA	6.66±0.20aA
Water holding capacity	Control	94.91±3.04aA	96.08±1.10 aA	96.74±2.24 aA	95.95±1.16 aA
	mh5	93.71±0.80 aA	92.98±1.64 aA	94.15±3.27 aA	95.65±1.92 aA
	mh10	92.48±5.43 aA	91.42±2.47 aA	93.99±2.78 aA	96.11±1.30 aA
TBARS (µmol MDA/kg)	Control	1.61±0.17bA	3.22±1.03bA	7.11±1.07aA	8.77±0.89aB
	mh5	1.39±0.18bA	3.07±0.59abA	4.27±0.58bB	8.54±1.93aAB
	mh10	1.77±0.26dA	3.90±0.48cA	4.85±0.34bB	8.05±0.54aB

*Statistical differences in the same row are indicated by lower case letters (a, b, c); statistical differences in the same column are indicated by upper case letters (A, B, C). mh: Purple carrot powder

There was no difference ($p > 0.05$) between the pH values (except for the 2nd day of the mh10 group and the 4th day of the mh5 group) on the storage days of the meatball experiments (Table 4). The mh10 group had the lowest pH value (5.27) on day 2 of storage and the mh5 group had the lowest pH value (5.29) on day 4 ($p < 0.05$). In the storage course of the groups, except for the mh5 group ($p<0.05$), the course of the other groups did not show any significance ($p>0.05$). Carrot pulp was also reported to have no effect on the pH of meatballs (Richards et al., 2024). At the end of storage, pH was higher in all groups than on the first day. One study reported that the treatment of black carrot extract with meat decreased pH values during storage (except on day 7) compared to the control, in agreement with our study, and this decrease was due to the acidity of the extract ($p < 0.05$) (Aksu & Turan, 2022). In addition, it has been noted that an increase in pH can occur because of microbial origin and proteolysis during storage (Hernandez-Hernandez et al., 2009). As the pH approaches the isoelectric point of proteins (5.4), the electrical charge decreases, and moisture loss increases due to protein denaturation (Richards et al., 2024). Table 4 shows that as the pH moved away from 5.4, the water holding capacity of the trials increased. However, there was no statistically significant effect of purple carrot powder on the water holding capacity of meatballs during storage and between groups ($p>0.05$) (Table 4). Although the control group (94.91%) had a higher water holding capacity than the mh5 (93.71%) and mh10 (92.48%) groups on day 0 of storage, there was no statistical difference. The day 0 water retention of all groups increased towards the last day of storage ($P > 0.05$). However, in a study in which rice flour was

replaced with black carrot flour at ratios of 0, 0.5, 1, 2, 3 and 4% in the production of sulgidduck (rice cake), it was reported that black carrot powder increased water holding capacity, with the group containing 4% black carrot powder having the highest water holding capacity (237.11%) (Song et al., 2017). The fact that the black/purple carrot powder used in the study was not found to be effective on water holding capacity may be due to the limited dissolution of purple carrot powder in water.

Lipid oxidation leads to the formation of oxidative products as a result of degradation by environmental pro-oxidants during processing and storage of fats in meat. As a result of oxidation, quality deterioration is observed, resulting in the formation of a highly bitter (rancid) taste (Karabudak, 2002). There is an increasing number of studies in the literature on the prevention of lipid oxidation in meat and meat products with fruit and vegetable extracts (grape seed, pomegranate peel extract, rosemary) containing high levels of bioactive compounds (Devatkal et al., 2010, Carpenter et al., 2007, Hernández-Hernández et al., 2009). Purple carrot powder, which has a high phenolic content, was found to be effective on TBARS levels of meatballs ($P < 0.05$) (Table 4.). While there was no difference in TBARS levels between the groups on day 0 and day 2 of storage, TBARS levels of the mh5 and mh10 groups were lower than the control on day 4 and day 7 ($P < 0.05$) (Table 4.). In a study investigating the effect of rosehip powder on the TBARS values of meatballs produced by enrichment with rosehip powder, the TBARS values of the meatball group to which 2% was added were found to be lower than the control group in both cooked and uncooked products ($P < 0.05$) (İlyasoğlu, 2014). In another study on beef steak, it was reported that all samples treated with lyophilised black carrot powder (100, 200, 300 ppm) aqueous solution were exposed to less oxidation (TBARS) during storage than the control group ($P < 0.05$) (Aksu & Turan, 2022). In our study, the high content of phenolic compounds in purple carrot powder may have caused lipid oxidation to proceed slowly, especially up to day 4.

Microbiological Analysis

An important factor in the safety and shelf life of meat products is their microbiological quality. Various technological processes are used in the production of ready-to-eat meat products. Despite the use of heat treatment, the microbial count in the final product can be relatively high. The microbiological quality of a meat product is mainly influenced by the condition of the raw materials (Cegiełka et al., 2022).

The addition of 10% (mh5) and 20% (mh10) 50 ml aqueous solution of purple carrot powder to the meatballs did not cause any difference ($P > 0.05$) in the mean numbers of TAMB, yeasts and S.aureus between the groups during storage (7 days at 4°C). The microbial loads of meatballs prepared with different proportions of purple carrot solutions during storage are shown in Figure 1a, Figure 1b. and Figure 1c.

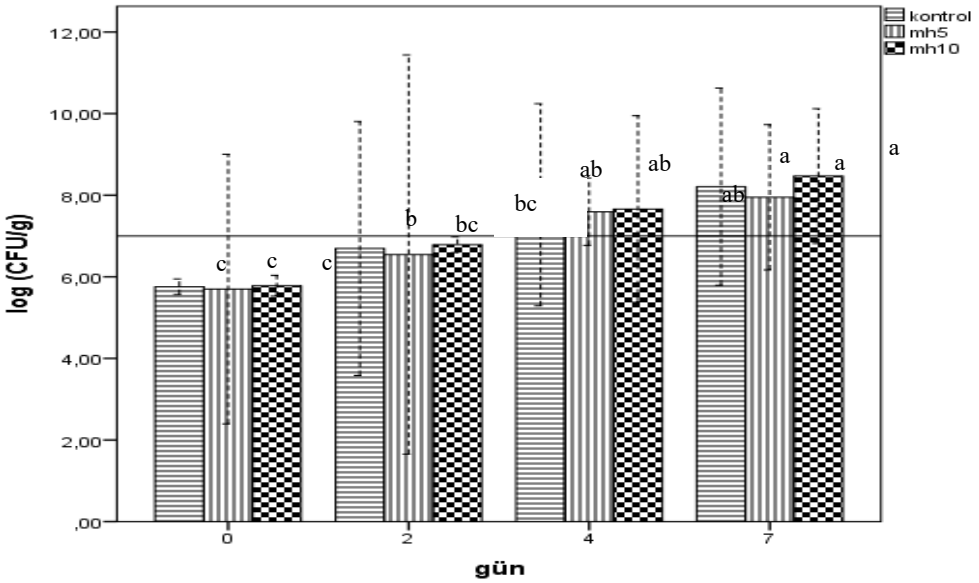

Figure 1a. Effect Of Purple Carrot Powder on Storage TAMB Count (log CFU/g)

Values are expressed as mean standard deviation. Different lowercase letters in the same column indicate a significant difference between the days by Tukey's test (P<0.05). Different capital letters on the same line indicate a significant difference between treatments by Tukey's test (P<0.05).

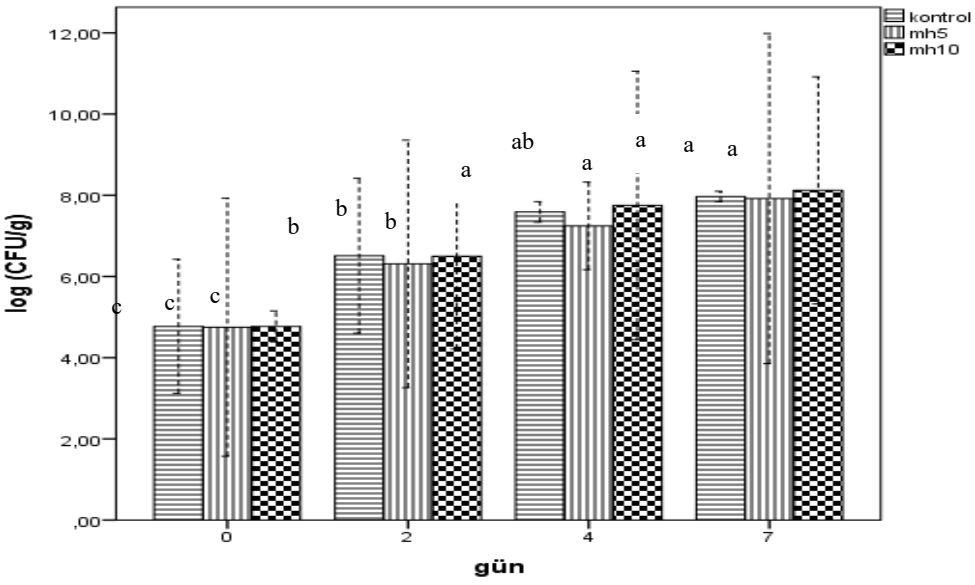

Figure 1b. Effect of Purple Carrot Powder on Storage Total Yeast and Mold Count (log CFU/g)

Values are expressed as mean standard deviation. Different lowercase letters in the same column indicate a significant difference between the days by Tukey's test (p<0.05). Different capital letters on the same line indicate a significant difference between treatments by Tukey's test (p<0.05).

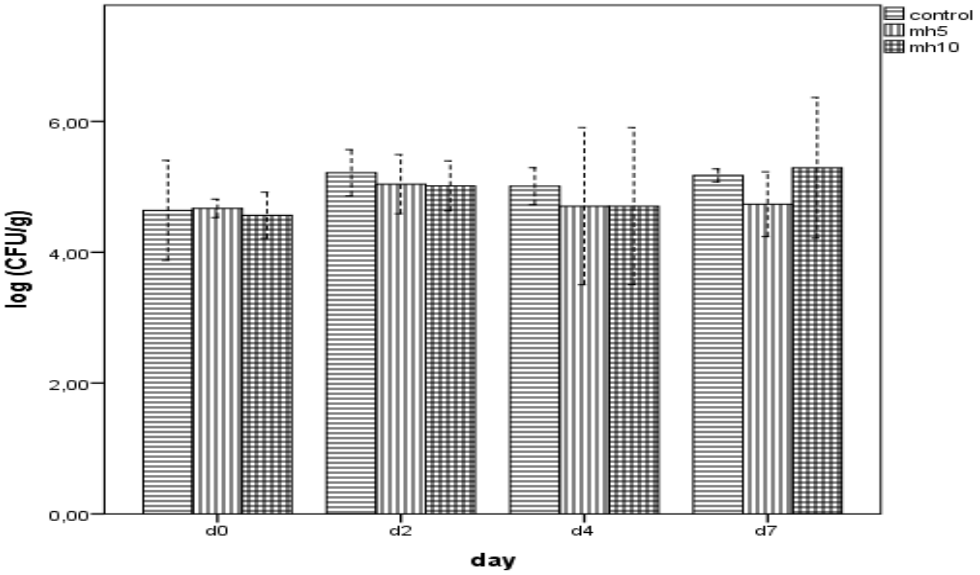

Figure 1c. Effect of Purple Carrot Powder on S.Aureus Count (Log CFU/G) During Storage

Values are expressed as mean standard deviation. Different lowercase letters in the same column indicate a significant difference between the days by Tukey's test (P<0.05). Different capital letters on the same line indicate a significant difference between treatments by Tukey's test (p<0.05).

During storage, there was a significant increase in TAMB (Figure 1a.), yeast moulds (Figure 1b.) (P < 0.05) and S.aureus (Figure 1c.) (p > 0.05). All experimental groups exceeded the 7.00 log Kob/g value set as the limit for TAMB in the Turkish Food Codex Communiqué on Microbiological Criteria on day 4, which is shown as the limit line in Figure 2 (Anonymous, 2009). At the end of the 7th day, the control, mh5 and mh10 groups showed 2.82, 2.49 and 2.98 log increases respectively. Although not statistically significant, the increase in TAMB, Staphylococcus aureus, yeast and mould counts of the mh5 group at the end of storage was less than that of the other groups. In contrast to our study, Kamel et al. (2023) reported that the total mesophilic aerobic count of the groups that added 0.2, 0.4 and 0.6% carrot powder to soft cheese was lower than the control (p< 0.05). Fernandez et al (2005) reported that such conditions may reduce the efficacy of antimicrobial agents due to physical interactions with the food matrix. Rabel et al. (2021) reported that TAMB counts in meatballs produced with olive leaves (0.1 and 0.3%) increased during storage in agreement with our study. Low yeast and mould counts are expected to prevent foodborne illness. The initial yeast counts of the control, mh5 and mh10 meatballs were 4.77±0.18, 4.75±0.36 and 4.77±0.04, respectively, and increased by 3.20, 3.17 and 3.35 log at the end of storage (day 7) (p<0.05) (Figure 1b.).

In our study, approximately 7.7 log S. aureus were infected on the outside of the meatballs and stored for some time to allow the bacteria to adhere. On the first day of storage, the counts of the control,

mh5 and mh10 groups were 4.64±0.38, 4.67±0.22 and 4.57±0.31, respectively, and the increases at the end of storage were 0.60, 0.43 and 0.90 log, respectively. Although not statistically significant, the lowest increase was observed in the mh5 group and the highest in the mh10 group ($p>0.05$). As can be seen in Figure 1c, especially on day 4, the number of S.aureus in the mh5 and mh10 groups was lower than in the control group. The values on day 4 for the control, MH5 and MH10 groups were 5.24 ± 0.08, 4.70 ± 0.59 and 5.02 ± 0.14, respectively. Akhtar et al (2015) stated that pomegranate peel extract added to meatballs as a natural preservative had a clear effect on the bacteriological quality of meatballs by reducing the number of aerobic plate counts, S. aureus, total coliforms and psychrotrophic bacteria, and this effect was related to the presence of polyphenolic chemicals (flavonoids, tannins) with antimicrobial activity.

As a result, purple carrot powder did not have a negative effect on microbial stability. However, it is thought that the fact that microbial stability was not at the desired level in our study may be due to the loss of phenolic compounds because purple carrot concentrates, which is an oily component, could not be completely dissolved in aqueous solution.

Conclusion

Natural plant extracts have been widely used in the food industry in recent years as natural colourings, antioxidants and antimicrobials due to consumer health concerns. Purple carrot powder, which is known to have health benefits due to its phenolic compounds, was not found to have a negative effect on the microbial and physicochemical properties of the test meatballs.

Studies on the use of purple carrot powder, which has high phenolic and antioxidant capacity and is widely grown in our country, to make meat and meat products healthier and have a long shelf life are important. Looking at the literature, there is a limited number of studies on the treatment of meat and meat products with purple carrot powder and it is believed that this study makes important contributions to the literature. The limitation of the study is that purple carrot powder cannot be completely dissolved in water. Studies with different concentrations and solubility enhancing treatments are needed to better understand the effects of purple carrot powder. The effect of purple carrot powder on phenolic compounds, TBARS (lipid oxidation), colour values (L*, a*, b*) after cooking and the treatment of meat and meat products are among the topics to be investigated in the future.

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