Vol. 22, No. 2(2023) Alim2311
Revista Mexicana de Ingeniería Química

Exploring the impact of acorn extract on the quality and taste of beef meat burgers

Explorando el impacto del extracto de bellota en la calidad y sabor de las hamburguesas de carne de res

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Received: January 25, 2023; Accepted: May 5, 2023

Abstract
This study aimed to investigate the effects of extracts obtained from valonia (*Quercus ithaburensis*), sessile (*Quercus petraae*) and holy oak (*Quercus ilex*) acorns on the total phenolic content and antioxidant activity (DPPH radical scavenging activity), physicochemical (color, pH, lipid and protein oxidation) and sensory properties of raw refrigerated beef meat burgers stored at 2ºC for 14 days. Incorporation of acorn extracts significantly increased pH values of the meat burgers on every analysis day (except day 7) ($p<0.05$). Color values of the burgers did not change with the addition of extracts. The phenolic contents and antioxidant activities of the samples containing acorn extracts were significantly higher than that of the control samples throughout the storage ($p<0.05$). Acorn extracts were found to be effective in preventing lipid and protein oxidation. It was concluded that the extracts of acorn fruits have the potential to be used as natural antioxidants to minimize oxidation problems in the meat burgers.
Keywords: Acorn extract, Natural antioxidant, Oxidation, Meat burger.

Resumen
El objetivo de este estudio fue investigar los efectos de los extractos obtenidos de bellotas de valonia (*Quercus ithaburensis*), sésil (*Quercus petraae*) y roble santo (*Quercus ilex*) sobre el contenido fenólico total y la actividad antioxidante (actividad de barrido de radicales DPPH), las propiedades fisicoquímicas (color, pH, oxidación de lípidos y proteínas) y sensoriales de hamburguesas de carne de vacuno cruda refrigerada almacenada a 2ºC durante 14 días. La incorporación de extractos de bellota aumentó significativamente los valores de pH de las hamburguesas de carne en todos los días de análisis (excepto el día 7) ($p<0.05$). Los valores de color de las hamburguesas no cambiaron con la adición de extractos. Los contenidos fenólicos y las actividades antioxidantes de las muestras que contenían extractos de bellota fueron significativamente superiores a los de las muestras de control durante todo el almacenamiento ($p<0.05$). Los extractos de bellota resultaron eficaces para prevenir la oxidación de lípidos y proteínas. Se concluyó que los extractos de frutos de bellota tienen potencial para ser utilizados como antioxidantes naturales para minimizar los problemas de oxidación en las hamburguesas de carne.
Palabras clave: extracto de bellota, antioxidante natural, oxidación, hamburguesas de carne.

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https://doi.org/10.24275/rmiq/Alim2311
ISSN:1665-2738, *issn-e*: 2395-8472

1 Introduction

Meat and meat products are concentrated nutrient sources including proteins, lipids, vitamins (A, B, etc.), minerals (iron, zinc, etc.) and some other bioactive compounds. Although meat and meat products are indispensable elements of a well-balanced nutrition, they might cause some types of cancer and cardiovascular diseases, owing to certain components (saturated fatty acid, cholesterol and salt) in their composition (Toldrá and Reig, 2011). However, certain oxidation reactions can occur depending on the presence of heme pigments, unsaturated fatty acids, metal catalysts and some reactive oxygen species in the muscle tissue (Hadidi *et al*., 2022; Choe *et al*., 2017). Lipid and protein oxidation are the main chemical changes which are responsible for losses in palatability, quality characteristics and nutritional value of products (Mariutti and Bragagnolo, 2017). Recently, artificial antioxidants such as butylated hydroxytoluene (BHT), propyl gallate (PG), butylated hydroxy anisole (BHA) and tertiary butylhydroquinone (TBHQ) have been used to prevent oxidation reactions. Due to their potential toxic effects on human health, consumers have preferred plant-based natural antioxidant sources such as fruits, vegetables, spices, grains etc. to limit oxidation. These plant-based additives are also known with their health benefits due to their bioactive components like phenolic and antioxidant compounds (chlorogenic acids, coumarin, sinapic acids, syringic acids, caffeic acids, etc.) (Mezni *et al*., 2022; Selani *et al*., 2011). Acorn is an oak fruit traditionally used as a food component (Oracz *et al*., 2023). It contains substantially carbohydrates, lipids, amino acids, fibers, vitamins and minerals (Šálková *et al*., 2011). Studies show that the acorns have strong antioxidant effects since they have phenolics and tocopherols (mainly α- and γ- tocopherol). The main phenolic compounds are gallic acid and its derivatives (Akcan *et al*., 2017). Therefore, researchers have considered the beneficial effects of acorn on human health. In Turkey, there are 18 native species of acorns. Some species are used for animal feeding owing to their high energy content (Özcan, 2006), while some others are used in leather industry as they are rich in tannins (Özcan, 2007). So far, very few studies have been conducted on the use of the acorns in food systems, particularly meat and meat products.

Briefly, the aim of this study was to evaluate the effects of the extracts obtained from holy (*Quercus ilex*), valonia (*Quercus ithaburensis*) and sessile oak (*Quercus petraae*) acorns on some physicochemical (pH, color, phenolic content and antioxidant activity, lipid and protein oxidation) and sensory properties of meat burgers throughout refrigerated conditions at 2ºC for 14 days.

2 Materials and methods

2.1 Materials

In this study, fresh boneless beef meat was supplied from a local meat packing company in Denizli, in Türkiye. The acorns (valonia oak, sessile oak and holy oak) were collected manually from the oak trees in Denizli during October in 2022 and transported to the laboratory for obtain extracts.

2.2 Preparation of acorn extracts

The acorns fruits were washed with tap water, dried with paper towel, dehulled with a nutcracker and kernels (cotyledons) were ground with an electric grinder (Arnica Prokit 444, plus, Turkey) into meal. Then, for each species of the acorns, 400 ml 80% ethanol was added onto 200 g powdered cotyledons and the mixtures were stand for extraction processes (72 h, room temperature), stirred regularly. Solutions were filtered through a filter paper (Whatman No. 40). Then, the filtrates were dried in a vacuum rotary evaporator (Buchi Rotavapor R-144, Switzerland) at 45ºC until ethanol was totally evaporated and an aqueous extract was obtained. The aqueous extract was stored at -40°C in amber bottles until usage. Then, 500 mg/L acorn extracts (gallic acid equivalent) were prepared from each acorn type with distilled water according to their total phenolic contents (TPC). TPC's of concentrated acorn extracts were previously shown as gallic acid equivalent for valonia (2.85 g GAE/100 g), sessile (6.03 g GAE/100 g) and holy oaks (6.32 g GAE/100 g) by Özünlü *et al*. (2018).

2.3 Preparation and storage of meat burger

Approximately, 3 kg fresh minced meat was used for burger preparation. The beef rib meat was minced (3 mm plate) with a meat mincer (Arnica Promeat W-1800, Istanbul, Turkey). The minced meat samples were treated with three different acorn extracts treatments respectively A1 (500 mg GAE equivalent valonia acorn phenolics per 1000 g meat); A2 (500 mg GAE equivalent sessile acorn phenolics per 1000 g meat) and A3 (500 mg GAE equivalent holy acorn phenolics per 1000 g meat). Also, a control group (meat without any extract) was used in all assays. In addition, 1.5% NaCl was added to each treatment group. To meat burger preparation, ground meat was manually blended with salt and the extracts for 10 min. The burgers (40±2 g) were molded with the help of a small (50 mm diameter) glass Petri dish. Then, they were placed into polystyrene boxes and were stored for 14 days at 2ºC under atmospheric conditions.

2.4 pH Value and instrumental color measurement

Briefly, ten g meat burgers were combined with 90 ml of distilled water. Then, the solution was blended in a homogenizer (HG-15A WiseTis, Korea) for the determination of pH. The measurements were recorded using a digital pH meter, which is attached to a calibrated electrode (Crison Basic 20, Spain). Color analyses were performed instrumentally using a Hunterlab Miniscan XE Plus colorimeter. CIE L*, a*, and b* (lightness, redness and yellowness, respectively) values were recorded randomly on the surface of the meat burger samples.

2.5 Total phenolic content

The each of the acorns extracts and also meat burgers containing three different acorn extracts (10 ml of pure

methanol was mixed with 1 g of meat burgers and the mixture was homogenized to extract polyphenols) were analyzed for total phenolics (TP) content using the Folin-Ciocalteus (F-C) according to Li *et al*. (2006) and Piñeros-Guerrero *et al*. (2020). Briefly, 0.5 ml extract of the each of the acorn extracts were added to 2.5 ml F-C reagent (0.20 N) and 2 ml Na2CO3 (7.5%, W/V). After being kept in total darkness for 30 min (until occur color reaction), the absorbance was recorded at 760 nm spectrophotometrically (EMC 11 UV, Germany). The amounts of TP were calculated as mg gallic acid equivalents (GAE) /100 g of meat.

2.6 DPPH radical scavenging activity

The free radical scavenging activity of the meat burgers was estimated according to Ergezer and Serdaroğlu (2018) by DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Analysis was performed by adding 0.1 ml meat burger extracts to 4.9 ml DPPH methanol solution. Then the absorbance was measured using a spectrophotometer (EMC-11-UV, EMC Lab, Germany) at 517 nm after 20 min. The scavenging activity was calculated as follows.

$$\text{Scavenging activity } \% = \frac{\text{Absorbance of blank-Absorbance of sample}}{\text{Absorbance of blank}} \times 100 \quad (1)$$

The absorbance of the control (containing all reagents without the test compound) called as “absorbance of blank" and “absorbance of sample" is the absorbance of the test compound recorded at 517 nm.

2.7 Determination of lipid and protein oxidation

Secondary products of the lipid oxidation were monitored by Thiobarbituric acid reactive substances (TBARS) analysis according to Ergezer and Serdaroğlu (2018) with slight modifications during storage. For the extraction of TBARS, 5 g of the burger samples were homogenized in 50 mL TCA (20%, w/v) by using an Ultra Turrax (HG-15A, WiseTis, Korea) for 75 s at 12175 g. Then, they were filtered through a Whatman No. 1 filter paper. 5 mL of 20 mM TBA (thiobarbituric acid) was added on 5 mL of filtrate in a screw capped test tube. These tubes were hold in a boiling water bath (Nüve, BS402, Ankara, Türkiye) for 35 min, then were cooled to ambient temperature to yield a pink color. The absorbances of the mixtures were recorded in the tubes at 532 nm in the spectrophotometer. Pink color development of mixtures was compared with a calibration curve prepared with known concentration of an external standard as 1,1,3,3-tetraethoxypropane. The TBARS values were given as mg of malonaldehyde/kg meat sample.

Protein carbonyls were measured according to the method described by Ferreira *et al*., (2017), for protein oxidation. Meat burgers (1g) were minced and then homogenized 1:10 (w/v) in 20mmol/L sodium phosphate buffer containing 6mol/L NaCl (pH 6.5) using an ultraturrax homogenizer (HG-15A, WiseTis, Korea) for 30s. Two equal aliquots of 0.2mL were taken from the homogenates and dispensed in 2mL eppendorf tubes. Proteins were precipitated by cold 10% TCA (1mL) and subsequent centrifugation (Eppendorf MiniSpin, Hamburg, Germany) for 5min at 4200×g. One pellet was treated with 1mL 2mol/L HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH in 2mol/L HCl (carbonyl concentration measurement). Both samples were incubated for 1h at room temperature. Afterwards, samples were precipitated by 10% TCA (1mL) and washed three times with 1mL ethanol:ethyl acetate (1:1, v/v) to remove excess DNPH. The pellets were then dissolved in 1.5mL of 20mmol/L sodium phosphate buffer containing 6mol/L guanidine HCl (pH 6.5), stirred and centrifuged for 2min at 4200×g to remove insoluble fragments. Protein concentration was calculated from the absorption at 280nm using BSA as standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of $21.0 \text{nM}^{-1}\text{cm}^{-1}$ at 370nm for protein hydrazones.

2.8 Sensory analysis

All the samples were cooked in a household oven (Termikel 13007, Türkiye) at 135ºC for 25 min until the internal temperature reached 80ºC. Then, the samples were warmed and served to the panelists randomly coded with three-digit numbers. Unsalted biscuits and water were given to panelists for rinsing the mouth between samples. The panelists evaluated each sample for on five-point hedonic scales, ranging from dislike extremely (score: 1) to like extremely (score: 5) for color and overall acceptability, extremely dry (score: 1) to extremely juicy (score: 5) for juiciness and not all astringent (score: 1) to very astringency (score: 5). A semi trained panel of 20-30 people (the panelists were trained with using a description of attributes and terminology about chicken meat for three sessions before they started on real samples on the days of analysis) was used to evaluate the meat burger at 1, 4, 7 and 14 days. The panelists scored samples for color, juiciness, astringency, and overall acceptability characteristics.

2.9 Statistical analysis

The effects of different the acorn extracts in the meat burgers were analyzed via a one-way ANOVA in terms of color and pH value, TBARS value, carbonyl content, total phenolic content, DDPH radical scavenging activity and sensory analysis, where the measured variables were set as dependent variables and replicated as a random effect. Experimental data were presented as the means and standard deviation (± SD) of two experiments conducted at different times. All analyses were performed in triplicates in each experiment. Experimental data were analyzed statistically by using SPSS package program (SPSS version 15,0 for Windows). One-way ANOVA procedure was carried out to analyze the effect of the treatments and storage periods. Also, Duncan’s Multiple Range Tests were used to determine the statistical difference at probability of $p<0.05$ between the means.

3 Results and discussion

3.1 The pH and instrumental color values of meat burgers

The pH and instrumental color values of the meat burgers during storage at 2ºC are shown in Table 1. The pH value helps us to understand microbial and chemical changes occurred during food deterioration. The highest and the lowest pH values were determined for the A2 and control samples, respectively, at the beginning and at the end of the storage ($p<0.05$). During the first 7 days of storage, some fluctuations in the pH values of the samples were generally observed and this situation may be attributed to microbiological and chemical changes. However, from day 7 to 14, the pH values of the samples, with no exceptions, were increased possibly, due to a microbial deterioration caused by proteolytic microorganisms. Contrary to our study, it is observed that the gradual increment in the pH values of air packaged beef meat treated with rosemary extract (*Rosmarinus officinalis*) with concentrations of 800 and 1600 ppm happened during storage (4C, 28 days) periods (Abandansarie *et al*., 2019). Virgili *et al*. (2007) stated that the pH value might be affected by low-molecular weight compounds formed from endogenous and exogenous activities in the product. Moreover, Choe *et al*. (2017) reported that an increment in the pH values of pork patties treated with persimmon peel extract occurred during the 12 days of storage.

Color plays an important role in both quality and consumers' acceptance of the meat and meat products. The CIE L* values (lightness) of the meat burgers varied from 39.85 to 45.57. Addition of acorn extracts did not cause any significant differences in the CIE L* values of the burger samples ($p>0.05$). Although some slight differences were observed in the lightness values of the samples among the sampling days, there were no significant differences before and after storage period. Similar to the CIE L* values, redness (a*) values of the burger samples were not significantly affected by the incorporation of the acorn extracts ($p>0.05$). The only exception to this trend, the CIE a* values of the control samples were statistically different from the other samples on the last day. For all samples, the redness values were significantly higher at the beginning ($p<0.05$) and significantly lower at the end of storage period ($p<0.05$). The data obtained by different authors, incorporation of natural antioxidants to meat samples did not change the CIE L* values in several studies (Aksu *et al*., 2022; Babaoğlu *et al*., 2022). It indicates that a discoloration took place during storage, and this can be attributed to the oxidation of myoglobin to metmyoglobin similar to our results (Faustman *et al*., 2010). On the last sampling day, the redness values were significantly higher in the extract-treated samples compared to the control. It shows that the phenolics of acorns can limit the oxidation processes in the burgers due to their high antioxidant properties. This effect was observed for all extract-treated samples regardless of the acorn type. Previously, various plants extract used in the meat and its products had been determined fairly effective preservation on the redness of products (Gahruie *et al*., 2017; Jia *et al*., 2012).

The CIE b* values of (yellowness) the meat burgers varied from 8.14 to 12.41. From the results presented on Table 1, it was observed that the yellowness values of the samples on day 0, 4 and 7 are very much similar to each other ($p>0.05$) and increases within the range of 11.71 to 12.41 at the end of the storage ($p<0.05$). Addition of acorn extracts did not cause any significant differences in the CIE b* values of the burger samples ($p>0.05$) on all sampling days. On the contrary, Gahruie *et al*. (2017) reported that addition of different spice extracts (cinnamon, rosemary and thyme) significantly increased the yellowness of beef patties. It might be attributed to the presence of pigments in spice extracts.

Table 1. pH and color parameters (L*, a* and b*) of meat burgers during storage at 2ºC

pH				
Storage Time (day)				
Samples	1st day	4th day	7th day	14th day
Control	5.70±0.01cBC	5.75±0.01bB	5.65±0.03bC	7.22±0.09cA
A1	5.72±0.01bBC	5.76±0.01abB	5.64±0.05bC	7.40±0.10bA
A2	5.75±0.02aB	5.77±0.01aB	5.53±0.02cC	7.52±0.02aA
A3	5.72±0.01bB	5.76±0.01abB	5.78±0.01aB	7.39±0.05bA
L* (lightness)				
Control	39.85 ± 1.75aB	44.06 ± 1.41aA	43.44 ± 2.12aAB	42.58 ± 3.61aAB
A1	41.47 ± 2.47aB	43.02 ± 1.02aAB	45.14 ± 0.5aA	41.44 ± 1.93aB
A2	41.02 ± 2.37aA	43.49 ± 1.75aA	43.41 ± 3.41aA	42.63 ± 3.52aA
A3	41.22 ± 1.62aA	42.98 ± 4.13aA	45.57 ± 2.57aA	41.71 ± 3.32aA
a* (redness)				
Control	11.34 ± 1.61aA	6.40 ± 0.63aB	6.02 ± 0.42aB	4.56 ± 1.46bC
A1	10.09 ± 0.69aA	6.69 ± 0.41aB	6.08 ± 0.11aB	5.66 ± 1.15aC
A2	10.92 ± 1.12aA	5.56 ± 0.80aB	5.40 ± 0.64aB	5.16 ± 1.46aC
A3	10.91 ± 0.83aA	6.27 ± 1.13aB	5.93 ± 0.67aB	5.20 ± 0.99aC
b* (yellowness)				
Control	8.71 ± 1.31aB	8.35 ± 0.73aB	8.94 ± 0.70aB	12.41 ± 0.91aA
A1	9.34 ± 0.76aB	8.86 ± 1.47aB	8.80 ± 0.35aB	11.48 ± 1.37aA
A2	9.02 ± 1.10aB	8.41 ± 1.55aB	8.14 ± 0.60aB	12.41 ± 0.91aA
A3	9.05 ± 0.44aB	8.30 ± 0.54aB	9.23 ± 1.03aB	11.71 ± 0.56aA

a-c Means within a column with different letters are significantly different ($p<0.05$).
A-C Means within a row with different letters are significantly different ($p<0.05$).
± Standard deviations
A1: Valonia oak acorn extract A2: Sessile oak acorn extract A3: Holy oak acorn extract

3.2 Total phenolic content (TPC) and DPPH radical scavenging activity of meat burgers

The TPC and DPPH radical scavenging activities of the meat burgers during storage at 2ºC are shown in Table 2. TPCs of all extract-treated samples were higher than the control samples on all sampling days ($p<0.05$). Among the extract-treated samples, the A1 and A3 had significantly higher TPCs than that of the A2. It can be seen from the Table 2. that TPCs of the samples gradually decreased with the increasing storage periods ($p<0.05$). Contrary to our results, Devatkal *et al*. (2011) reported that the phenolic contents increased in chicken patties treated with kinnow and pomegranate rind extracts during the last period of chilled storage. According to the authors, this was a result of phenylpropanoid pathway involving phenylalanine ammonia-lyase enzyme, the activity of which increases during chilled storage. In a similar study, the phenolic content of beef patties declined along with the addition of artichoke extracts during storage (Ergezer and Serdaroğlu, 2018).

Similar to TPCs, the DPPH values of the samples also decreased throughout the storage period. On all sampling days, the extract-treated samples had higher DPPH values compared to the control samples. The differences among extract-treated samples were not significant on the first sampling day; however, the A2 and A3 samples had higher DPPH values than the A1 samples for the rest of storage. The DPPH values of these samples were approximately 2-fold higher than that of the control samples at the end of storage. According to our results, the A3 samples had the highest TPC and DPPH value among all the samples tested. This situation may be attributed to chlorogenic acids, coumarin, sinapic acids, syringic acids, caffeic acids, etc. which is found in holy acorn extracts. Therefore, this extract could be recommended as a potential antioxidant additive for the meat burgers. The sequences of TPCs and DPPH values were determined as A3≥A1>A2> control and A3≥A2>A1>control, respectively. According to this finding, the A2 samples had statistically the same radical scavenging activity with A3, on the other hand, had lower TPC compared to the A1 samples.

Table 2. Total phenolic content (TPC), DPPH, TBARS value and carbonyl content of meat burgers during storage at 2ºC.

TPC (Total phenolic content) (mg gallic acid/100 g meat)				
	Storage Time (day)			
Samples	1st day	4th day	7th day	14th day
Control	45.34 ± 1.03cA	38.89 ± 0.89cB	33.55 ± 0.49cC	24.37 ± 0.93cD
A1	77.41 ± 2.45aA	62.46 ± 1.31aB	54.48 ± 0.52aC	49.47 ± 1.05aD
A2	67.68 ± 1.84bA	55.32 ± 0.94bB	47.71 ± 1.20bC	42.69 ± 0.78bD
A3	75.03 ± 1.24aA	62.13 ± 1.18aB	55.88 ± 1.89aC	49.22 ± 0.61aD
DPPH (1,1-diphenyl-2-picrylhydrazyl) (%ARA)				
Control	27.11 ± 0.45bA	24.27 ± 0.61cB	23.35 ± 0.55cB	14.18 ± 1.03cC
A1	47.58 ± 0.71aA	33.08 ± 0.58bB	27.95 ± 0.31bC	23.41 ± 1.66bD
A2	46.17 ± 1.4aA	41.44 ± 0.39aB	31.62 ± 0.45aC	25.09 ± 0.99aD
A3	46.51 ± 0.63aA	40.05 ± 0.91aB	30.56 ± 0.87aC	27.13 ± 0.76aD
TBARS (Thiobarbituric acid reactive substances) values (mg malonaldehyde/kg meat)				
Control	0.07 ± 0.01aD	0.15 ± 0.01aC	0.21 ± 0.01aB	0.26 ± 0.01aA
A1	0.06 ± 0.01bD	0.07 ± 0.01cC	0.08 ± 0.01cB	0.11 ± 0.01cA
A2	0.07 ± 0.01aC	0.09 ± 0.01bB	0.10 ± 0.01bB	0.13 ± 0.01bA
A3	0.07 ± 0.01aC	0.08 ± 0.01bBC	0.09 ± 0.01bB	0.12 ± 0.01bcA
Protein Carbonyl Content (nmolcarbonyl/mg protein)				
Control	1.94 ± 0.01aD	2.36 ± 0.23aC	2.71 ± 0.14aB	3.02 ± 0.18aA
A1	1.71 ± 0.10bB	1.86 ± 0.12bcB	2.13 ± 0.13bcA	2.20 ± 0.15cA
A2	1.53 ± 0.13cC	1.71 ± 0.09cC	1.99 ± 0.10cB	2.29 ± 0.23cA
A3	1.76 ± 0.05bC	1.95 ± 0.12bC	2.24 ± 0.12bB	2.47 ± 0.18bA

a-c Means within a column with different letters are significantly different ($p<0.05$).
A-C Means within a row with different letters are significantly different ($p<0.05$).
± Standard deviations
A1: Valonia oak acorn extract A2: Sessile oak acorn extract A3: Holy oak acorn extract

Then, the high radical scavenging activity of the A2 could be explained not only by phenolics but also by tocopherol content of the extract. Hawashin *et al*. (2016) reported that the addition of different concentrations of destoned olive cake powder into beef patties were found effective against free radical formation. In another study, *Hypericum perforatum* extract showed similar effects on pork meat against radical formation (Sánchez-Muniz *et al*., 2012).

3.3 TBARS value and determination of total carbonyl

The TBARS values and carbonyl content of the meat burgers during storage at 2ºC have been presented in Table 2. The TBARS value is the most prominent parameter which can determine the secondary products of lipid oxidation in meat products. The TBARS values of the samples increased during storage, with the highest rate observed for the control samples. Except for the first day, the TBARS values of the extract treated samples were lower than that of the control on all sampling days ($p<0.05$). Among all samples, the A1 has the lowest TBARS values, which is attributed to high antioxidant activity of the extract. The TBARS values of all extract-treated samples were at least 50% less than the control samples at the end of storage. These results are consistent with those present Muíño *et al*. (2017) who reported that addition of different concentration of olive oil waste extracts successfully prevent the lipid oxidation in beef patties. Also, Azman *et al*. (2015) determined that addition of different concentrations of Gentiana lutea root have a positive effect against the lipid oxidation in beef patties.

Protein carbonyls are one of the well-known indicators of protein oxidation in the muscle tissues (Flores-Silva *et al*., 2022). The total carbonyl content increased significantly in the samples throughout the storage ($p<0.05$). The extract-treated samples had lower carbonyl content than the control samples on all sampling days. At the end of storage period, the carbonyl content of A3 was significantly lower than the control ($p<0.05$), but higher than the A1 and A2 samples ($p< 0.05$). Morcuende *et al*. (2020) reported that the addition of different fruit extracts (*Arbutus unedo* L., *Crataegus monogyna* L., *Rosacanina* L., and *Quercus ilex* subsp. *ballota*) significantly reduced the protein oxidation in lamb cutlets. In that study, acorn extract had shown the most efficient in protecting lamb cutlets against protein carbonylation Rodríguez-Carpena *et al*. (2011) determined that the addition of different avocado by-products (seed and peel) extracts (Hass and Fuerte) in raw porcine patties inhibited to some extent the formation of the protein carbonyls. In contrast; Jia *et al*. (2012) stated that the addition of different concentration black currant extracts (*Ribes nigrum* L.) in pork patties have no significant effect on the protein oxidation. This result is explained by the different chemical structure of phenolic compounds Rodríguez-Carpena *et al*. (2011) reported that the lipid oxidation products (aldehydes, ketones etc.) accelerate the protein oxidation. It is consistent with our findings, which display simultaneous increments in both the lipid and protein oxidation. The acorn extracts used in the present study were

found to be effective in limiting both oxidation reactions.

Phenolic compounds fulfill their ability to act as free radical scavengers and DPPH analysis is routinely used for assessment of free radical scavenging potential of phenolics and considered as an easy colorimetric method for the evaluation of antioxidant properties of phenolic compounds. So, with the use of different acorn extracts had high levels of phenolics as well as high antioxidant activity (DPPH analysis), it is observed that retarding of lipid and protein oxidation, without any damage to the sensory or nutritional properties, resulting in maintaining quality and shelf-life of beef burgers (Oswell *et al*., 2018).

3.4 Sensory analysis

Sensory analysis (color, juiciness, astringency and overall acceptability) scores of the meat burgers are given in Table 3.

Table 3. Sensory analysis values of meat burgers during storage at 2ºC

Storage Time (day)				
Color				
Samples	1st day	4th day	7th day	14th day
Control	5.00± 0.00aA	4.00± 0.82aAB	4.00± 0.82aAB	3.25 ± 0.50aB
A1	4.50 ± 0.58abA	4.25 ± 0.50aA	4.00± 0.82aA	3.75 ± 0.50aA
A2	4.00± 0.82bA	4.00± 0.82aA	3.50 ± 0.58aA	3.00± 0.82aA
A3	4.50 ± 0.58abA	4.00± 0.82aA	3.75 ± 0.50aA	3.50 ± 0.82aA
Juiciness				
Control	3.00± 0.82aA	3.00± 0.82aA	2.75 ± 0.50bA	2.50 ± 0.58aA
A1	3.00± 0.82aA	3.25 ± 0.50aA	3.50 ± 0.58abA	3.00± 0.82aA
A2	3.00± 0.82aA	3.50 ± 0.58aA	3.25 ± 0.50abA	3.50 ± 0.58aA
A3	4.00± 0.82aA	3.75 ± 0.50aA	3.75 ± 0.50aA	3.50 ± 0.58aA
Astringency				
Control	1.00± 0.00bC	1.25 ± 0.50aC	1.00 ± 0.58bB	1.00 ± 0.50aA
A1	1.00± 0.00bB	1.50 ± 0.58aB	1.75 ± 0.50aA	1.50 ± 0.58aA
A2	1.50 ± 0.58aB	1.50 ± 0.58aB	1.50 ± 0.58aA	1.25 ± 0.50aA
A3	1.00± 0.00bB	1.75 ± 0.50aB	1.75 ± 0.50aA	1.50± 0.82aA
Overall acceptability				
Control	3.00 ± 0.27aA	2.75 ± 0.50aA	3.09 ± 0.42bA	3.00 ± 0.47aA
A1	2.83 ± 0.34aB	3.00 ± 0.47aB	3.75 ± 0.17aA	3.42 ± 0.50aAB
A2	2.83 ± 0.43aB	3.00 ± 0.61aB	3.42 ± 0.50abA	3.58 ± 0.57aA
A3	3.17 ± 0.33aB	3.17 ± 0.43aB	3.59 ± 0.17abA	3.67 ± 0.61aA

a-c Means within a column with different letters are significantly different ($p<0.05$).
A-C Means within a row with different letters are significantly different ($p<0.05$).
± Standard deviations
A1: Valonia oak acorn extract A2: Ordinary oak acorn extract A3: Holy oak acorn extract
Sensory scores are measured on 5 point hedonic scale where 1 = extremely dislike and 5 = extremely like for color and overall acceptability, 1 = extremely dry and 5 = extremely juicy for juiciness and 1 = not all astringent and 5 = very astringency for astringency

For the sensory color evaluation, panelists are selected as semi-trained about the lightness and redness of the burgers. The highest and the lowest sensory color scores were determined for the control and A2 samples at the beginning of the storage, respectively ($p<0.05$). The sensory color scores of all samples were gradually decreased during storage periods. This trend is coherent with instrumental a* values of the color. Except for first day, the extract-treated samples had higher the sensory juiciness scores than the control samples on all sampling days ($p<0.05$). At the beginning of the storage period, the juiciness scores of the control, A1 and A2 were similar, but significantly lower than that of the A3 samples ($p<0.05$). The astringency scores were between 1.00 and 1.75. During the storage period, some fluctuations in the astringency scores of the samples were generally observed. It was expected that the astringency scores of extracts treated the burgers would be higher because of the presence of tannins in the acorns. Although the control samples had the lowest astringency score during storage, there were only significantly ($p<0.05$) differences among the samples (except that A2 samples) on day 1. Moreover, the control samples had lower than other samples in terms of the astringency score on day 7 ($p<0.05$). As a matter of fact, on the 4th, 7th and 14th days of storage, the astringency scores were higher than the control although the differences were not significant in some cases. Nevertheless, the maximum astringency score (1.75) was quite low in a 5 point hedonic scale. At the

beginning of the storage, all the samples got similar overall acceptability scores. The overall acceptability scores were generally stable for the control samples and increased for the all extract-treated samples throughout the storage (except that A1 samples). On the last sampling day, though the overall acceptability scores were higher for the extract-treated samples compared to the control samples, there were no significant differences among the samples ($p>0.05$). Gómez *et al*. (2016) evaluated that the effects of grape seed extract on sensory color scores of the raw ground beefs and they did not find any differences among the samples, contrary to our results. Turgut *et al*. (2017) stated that the effects of pomegranate peel extract did not negatively affect the sensory color scores of meatballs. However, the sensory rancid odor scores of meatballs increased during storage and this situation was indicated as a marker of the lipid oxidation of meatballs. Sharafati-Chaleshtori *et al*. (2015) reported that addition of essential oil of the basil (*Ocimum basilicum* L.) have considerable effect with regard to overall acceptance in beef burgers.

Conclusions

In conclusion, acorn, fruit of oak tree grown in the Mediterranean basin, is rarely used by the food industry for any specific purposes. The extracts of this fruit have been tested for the first time to prevent the oxidation in the meat burgers. The treating meat burgers with the extract of acorn fruit improved both instrumental and sensorial color values. The extract-treated samples had relatively higher than the total phenolic contents and the antiradical scavenging activity. Moreover, the acorn extracts were found to be effective in delaying the oxidation of the lipids and proteins in the meat burgers. Furthermore, the incorporation of these extracts did not negatively affect, on the contrary, improved the sensory properties and increased the acceptability of the product. Therefore, it can be concluded that promising results were obtained with the use of acorn extracts as natural antioxidants to contribute to solving the oxidation problem of the meat industry.

Acknowledgment

The authors would like to thank Dr. Hakan Karaca for reviewing the manuscript. Also, Orhan Özünlü is thankful to the Scientific and Technological Research Council of Turkey (TUBITAK-2211-A National PhD program) and YOK 100-2000 PhD scholarship program for financial support

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