



Physicochemical, microbiological and sensory evaluation of beef patties incorporated with destoned olive cake powder



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ARTICLE INFO

Article history:

Received 5 April 2016

Received in revised form 19 July 2016

Accepted 21 July 2016

Available online 22 July 2016

Keywords:

Antimicrobial

Beef patties

Natural antioxidant

Olive cake

Total phenolic

ABSTRACT

The biological efficacy of different concentrations (2%, 4%, and 6%) of destoned olive cake (DOC) as improvers of the quality, storability, and safety of beef patties was investigated. Increasing the percentage of DOC in the patties improved ($P \leq 0.05$) the protein and fat contents, cooking yield, moisture and fat retention, total phenolic, and DPPH radical scavenging activity, while the dimensional shrinkage and TBARS showed a progressive reduction. The pH of the patties decreased gradually with the storage time. DOC-incorporated patties showed significantly ($P \leq 0.05$) lower total plate count than untreated. Surface color values of raw beef patties were decreased gradually with the storage time. Throughout the storage period, all the sensory traits of non-formulated patties were significantly ($P \leq 0.05$) reduced, whereas the formulated patties revealed considerable stability of all characters. Overall, this study identified antioxidant and antimicrobial potentiality of DOC, which could pave the way for its use as an extender of the shelf life of the patties.

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1. Introduction

Beef and meat products, particularly beef patties, exhibited considerable increases in production and consumption throughout the world in recent years. This is due to the rapid growth in the fast food market beside the exceptional nutritional quality of meat products as it contains appreciable amounts of protein, vitamins and trace minerals with significant health benefits (National Health and Medical Research Council, 2006). However, mincing beef meat before patty formation disrupt the integrity of muscle membrane and increase the surface area that promotes lipid oxidation and microbial growth of stored meat products. Lipid oxidation and microbial activity are the primary causes of food deterioration, and they strongly affected the safety and the nutritional and sensory qualities of stored meat products (Aguirrezabal, Mateo, Domínguez, & Zumalzarregui, 2000; Jayawardana, Ruvini, Nirosh, Supeshala, & Pabodha, 2015). Thus, controlling microbial growth and lipid peroxidation in beef patties is a crucial strategy for sustaining the safety, nutritional and sensory potentials of these products. Previous researches have been directed toward discovering and developing natural or synthetic food additives for controlling microbial growth and lipid peroxidation in meat products (Hayes & Brunton, 2011; Mielnik, Aaby, & Skrede, 2003). Synthetic antioxidants such as ascorbate/ascorbic acid, isoascorbates, tocopherols, gallates, butylated hydroxyanisole (BHA), butylated hydroxytoluene, (BHT), and tertbutyl hydroquinone (TBHQ) are commonly used to retard oxidative reactions in minced meat products (Honikel, 2014). Other synthetic antimicrobials such as

nitrite, phosphate, potassium sorbate, propylparaben, lactic, citric, and acetic acids, sodium diacetate, acidified sodium chloride, acidified calcium sulfate, and cetylpyridinium chloride are used to inhibit the microbial growth in meat products (Mills, 2014). Nevertheless, the above synthetic additives are considered unsafe by both human health professionals and consumers (Tang, Kerry, Sheehan, Buckley, & Morrissey, 2001), which have prompted strict regulations for their use in food formulations. Consequently, interest in the development and use of naturally occurring safe alternatives has markedly increased in last decades (Jayawardana et al., 2015). In recent years, the addition of naturally occurring antimicrobial and antioxidant compounds derived from plant sources to meat products has increased because of their potential health benefits and safety (Hayes & Brunton, 2011).

The recent decades are witnessing a growing interest in olive oil production and consumption due to abundant health potentials of olive oil, as it believed to reduce the incidence of cardiovascular disease, certain types of cancer, and neurodegenerative disorders (Pérez-Jiménez, 2005). The protective effects of olives are predominantly attributed to its oleic acid and phenolic compound contents that possess free radical scavenging activity and protect organisms against oxidative damage (Covas et al., 2006). However, only 1–2% of the total phenolics of olive fruits are extracted in the oil and the majority (98–99%) remained in olive mill waste like alperujo and olive cake, which are obtainable in large amounts (approximately 3.5–6.0 million tons/year) (Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, & Fernández-Bolaños, 2013). These olive oil by-products are harmful to the environment due to their heavy load of phenolic compounds, lipids and organic acids (Dermeche, Nadour, Larroche, Mouliti-Mati, & Michaud, 2013). These compounds have adverse impacts on soil microbial communities

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(Paredes, Moreno, Ramos-Cormenzana, & Martinez, 1987), marine ecosystems (Della Greca et al., 2001) and air through emissions of phenol and sulfur dioxide (Rana, Rinaldi, & Introna, 2003). Therefore, numerous studies have intended to either decrease the environmental influence of olive cake or harness its potential economic value. Hence, it is frequently used as a natural fertilizer, substrate for fermentation and animal feeding, dying agent in biosorbing material, and for bioenergy utilization (Akar et al., 2008; Manios, 2004). Also, the olive cake is considered as a promising source of these phenolic compounds for the pharmaceutical, nutraceutical, cosmetic, and food industries (Rubio-Senent et al., 2013). Despite the fact that olive cake has massive amounts of phenolic compounds with antioxidant and antimicrobial activities, research on its application to extend the shelf-life of meat products is scarce (Dejong & Lanari, 2009). Therefore, in the present study, an attempt was made to investigate the effect of incorporation of olive cake on the physicochemical, microbiological and sensory quality of beef patties during cold storage ($4 \pm 1^\circ\text{C}$).

2. Materials and methods

2.1. Materials

Thirty-six kilograms of boneless beef rounds (*Musculus semimembranosus*) of young Holstein Friesians (*Bos taurus*) male and 4.5 kg of beef back fat from the same beef carcasses were obtained from a commercial market (Riyadh, King Saudi Arabia) within an hour of slaughter. About 6 kg of olive cake (Cultivar K18) was kindly donated by Dr. Dakhiel Allah Turki Al-Shamdain (Olive oil processing unit, Al-Jouf City, Saudi Arabia). Unless otherwise stated, all reagents used in this study were of analytical grade.

2.2. Sample preparations

The fat and visible connective tissues of the meat were removed manually and then lean meat and fat were separately ground for 2 min using a kitchen blender (Kenwood Manufacturing Co. Ltd., UK) under cold conditions to avoid an increase in temperature during blending. The olive cake was freeze-dried (Virtis Unitop 600SL, New York). Three patches for each beef burger formula were processed, and all treatments in each patch were replicated three times. For each patch, 12 kg beef meat was used, and four blends (3 kg each) were prepared by combining minced beef with 0, 2, 4 or 6% (w/w) destoned olive cake (DOC) and spices according to percentages specified in Table 1. All ingredients in each blend were mixed to homogeneity using a Stephan UM 12 (Stephan U. Sohner GmbH & Co., Germany) mixer. After mixing, 100 g portions of each blend were formed into patties (approx. 100 g) using a burger-forming device (Expro. Co., Shanghai, China). The patties were cooked for 20 min at 160°C in a Hobart CN85-19 convection oven (Hobart Corp., Troy, Ohio, USA) until the internal temperature attains 80°C as measured at the geometrical centre using a digital probe thermometer (Oakton, Eutech Instruments, China). At intervals of 10 min, the patties were turned upside down to ensure

uniformity of cooking. Triplicate samples from each batch of DOC-formulated and non-formulated patties were analyzed for the quality characteristics on the same day. For storage stability studies, raw DOC-formulated and non-formulated patties were separately placed in polyethylene bags and stored in a refrigerator ($4 \pm 1^\circ\text{C}$) for 0, 7, and 14 days. At the specified time intervals, raw patties were removed from the refrigerator and cooked as indicated above, and then both raw and cooked patties were assessed for quality characteristics.

2.3. Approximate composition

Approximate composition (moisture, protein, ash and fat contents) of freeze-dried samples of DOC, raw and cooked patties was determined using the official standard method (AOAC, 2003).

2.4. Cooking properties determination

The cooking yield, fat retention, moisture retention, and dimensional shrinkage were determined using the methods and equations described elsewhere (Al-Juhaimi, Ghafoor, Hawashin, Alsawmahi, & Babiker, 2016). Briefly, cooking yield was determined following the procedure of Murphy, Criner, and Grey (1975). The weight of the patties was recorded before and after cooking, and the cooking yield was calculated by dividing the weight of cooked patties by the weight of raw uncooked patties and expressed in percentage. The fat retention values represent the amount of fat retained in cooked patties, were determined as described by Murphy et al. (1975), and expressed as a percent. The moisture retention values represent the amount of moisture retained in cooked patties and were calculated according to the equation of El-Magoli, Laroia, and Hansen (1996) and expressed as a percent. The dimensional shrinkage was calculated following the formula of Murphy et al. (1975) as follow:

$$\text{Dimensional shrinkage (\%)} = \frac{(\text{Raw thickness} - \text{Cooked thickness}) + (\text{Raw diameter} - \text{Cooked diameter})}{\text{Raw thickness} + \text{Raw diameter}} \times 100 \quad (1)$$

2.5. Preparation of extracts of DOC and beef patties

The DOC and raw and cooked beef patties were freeze-dried, ground to fine powder and sieved through a 1 mm sieve. Nearly 3 g of powdered sample was weighed and extracted with 30 mL of distilled water followed by continuous stirring for overnight using a magnetic stirrer (Fisher, 14-511-1A, USA) at 4°C . Then the mixture was centrifuged (Hermle, Germany) at $4500 \times g$ for 30 min. The supernatant was collected and subsequently used for the determination of total phenolic content (TPC) and antioxidant activity.

2.6. Determination of total polyphenols

The total phenolic contents were analyzed using the Folin-Ciocalteu method with slight modifications (Singleton & Rossi, 1965). Briefly, 1 g of freeze-dried samples of raw beef patties with or without DOC was extracted with 1 mL distilled water. After that, 200 μL of appropriately diluted sample or a standard solution (gallic acid) of varying concentrations were mixed with 400 μL of Folin-Ciocalteu reagent and the volume rise to 4.6 mL with deionized water. After standing for 10 min at room temperature, 1 mL of 10% Na_2CO_3 solution was added, then immediately mixed, and allowed to stand for 2 h at room temperature. The absorbance was read at 765 nm on a UV-visible spectrophotometer (Apel, Saitama, PD-303UV, Japan). The total phenolic content was calculated using gallic acid (1 mg/mL) as standard, and the results were expressed in milligram gallic acid equivalent per gram samples (mg GAE/g sample).

Table 1
Formulation of beef patties with different concentration of destoned olive cake powder.

Ingredients (%)	Control	Olive cake powder level (%)		
		2	4	6
Lean meat	76.0	74.0	72.0	70.0
Added fat	9.0	9.0	9.0	9.0
Cold water	11.4	11.4	11.4	11.4
Salt	1.0	1.0	1.0	1.0
White pepper	0.2	0.2	0.2	0.2
Black pepper	0.2	0.2	0.2	0.2
Garlic powder	0.2	0.2	0.2	0.2
Onion powder	2.0	2.0	2.0	2.0
Olive cake powder	0.0	2.0	4.0	6.0

2.7. Determination of 1,1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH assay was carried out according to the method of Singh, Murthy, and Jayaprakasha (2002). Briefly, an aliquot of the extracts (200 µL), of DOC and raw beef patties with or without DOC, was diluted with 800 µL of 0.1 M Tris–HCl buffer (pH 7.4). To this mixture, 1 mL of DPPH (250 µM) was added, and the mixture was vortexed vigorously. After that, the tubes were stored in the dark at room temperature for 20 min, and then the absorbance was measured at 517 nm. The following equation was used to calculate the scavenging activity:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of the blank} - \text{Absorbance of the sample}}{\text{Absorbance of the blank}} \times 100 \quad (2)$$

2.8. Determination of 2-thiobarbituric acid reactive substances (TBARS)

Lipid oxidation of beef patties was evaluated by measuring the TBARS values according to the method described by Rosmini et al. (1996). Briefly, 5 g freeze-dried beef patties were homogenized with 20 mL distilled water, and the homogenates were filtered (Whatman no 1. Filter paper). To 1 mL of the filtrate in screw cap tubes, 4 mL of 20% thiobarbituric acid and 100 µL of 10% butylated hydroxytoluene were added. The tubes kept in boiling water bath (95–100 °C) for 10 min to develop a pink color, and then cooled under running tap water. The samples were centrifuged at 5500 × g (Mod. RC5C, Sorvall Instruments, Kendro LP, Hertfordshire) for 25 min and the absorbance of the supernatant was measured at 532 nm using a UV–visible spectrophotometer (Mod. 4050, Biochrom, Cambridge, UK). Results were expressed as mg malonaldehyde/kg beef patties from the standard curve using a 1,1,3,3-tetraethoxypropane. In each batch, triplicate samples were analyzed.

2.9. Determination of microbiological load and pH of beef patties

Total plate count of the raw beef patties at 0, 7 and 14 days of storage was determined according to the method described in International Commissions of Microbiological Specification for Foods (ICMSF, 1983). Briefly, 10 g of beef patties was homogenized with 90 mL of 0.1% sterile peptone water. Ten-fold serial dilutions were prepared by diluting 1.0 mL of homogenate in 9 mL of 0.1% peptone water. After that, appropriate serial dilutions were duplicate plated (Pour plate method) with plate count agar and the plates were incubated at 37 °C for 48 h. The microbial colonies were counted and expressed as log₁₀ cfu/g. For the determination of pH, 5 g of sample was homogenized with 50 mL

deionized distilled water, and the pH value was measured using a Corning 240 pH meter (Corning Scientific Products, New York, USA).

2.10. Color measurement

The instrumental color on the surface of formulated and non-formulated raw beef patties before and after storage was measured using Hunter Lab colourimeter (Model No. Miniscan® XE plus 4500 L, Hunter Associates Laboratory, Inc., VA, USA) as described previously (Al-Juhaimi et al., 2016).

2.11. Sensory evaluation

Sensory analysis of beef patties was performed immediately after manufacture and weekly during storage period. Twenty semi-trained panellists (male, age range 20–35 years old) evaluated the sensory attributes of cooked meat patties using a 9-point hedonic scale. About 3 g of samples were served to each panellist after 2 min of cooking and the panellists were asked to test appearance, juiciness, flavour, taste, tenderness, and overall acceptability of coded beef patties. All samples were coded with three-digit random numbers and served randomly to the assessors. The samples were recorded on a scale of 1–9 (1 = extremely dislike, 9 = extremely like). Sensory analysis was performed in three sessions at each storage time (0, 7 and 14 days) and the mean values of the scores of twenty panellists for each sample and session were calculated and used in data analysis. The scores of means from 5 to 9 were considered acceptable.

2.12. Statistical analysis

The experiments were designed using a completely randomized block design with four treatments (control, 2% DOC, 4% DOC, and 6% DOC) and the measurements were taken on three storage days (0, 7, and 14 days) and the entire blocks were independently replicated three times on three different days. A two-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (SAS 8.0 software, SAS Institute, Inc., Cary, NC, USA) were performed to analyze the effect of treatments, storage periods, and the interaction between them on the physicochemical, microbial, and sensory characteristics of beef patties. In the analysis models, the treatments, storage times, and their interaction were assigned as fixed effects, and the replications of the experiments as random effects. The scores of different sensory attributes were compared among the treatments and storage periods using General Linear Model (GLM) and Duncan's Multiple Range Test was used for means comparisons to show the effect of treatments and storage, in which the treatments, storage time, and assessors were considered as main effects, and the replications as random effects. Data was presented

Table 2
The chemical composition of raw and cooked beef burger formulated with different concentrations of destoned olive cake powder.

Olive cake concentration (%)	Chemical composition (%)			
	Moisture	Protein	Ash	Fat
Raw				
0	62.59 ± 0.70 ^a	17.22 ± 0.36 ^b	2.33 ± 0.47	14.55 ± 0.47 ^c
2	60.98 ± 0.42 ^b	18.14 ± 0.47 ^a	2.45 ± 0.12	15.53 ± 0.41 ^b
4	59.87 ± 1.11 ^b	18.87 ± 0.24 ^a	2.78 ± 0.14	15.58 ± 0.28 ^b
6	58.98 ± 0.76 ^b	18.93 ± 0.22 ^a	2.89 ± 0.15	16.09 ± 0.29 ^a
Cooked				
0	55.85 ± 0.53	18.54 ± 0.46 ^a	2.64 ± 0.19	14.75 ± 0.42 ^c
2	55.87 ± 0.50	18.97 ± 0.32 ^a	2.72 ± 0.18	15.59 ± 0.47 ^b
4	56.69 ± 0.62	19.49 ± 0.41 ^b	2.87 ± 0.27	16.37 ± 0.54 ^a
6	56.95 ± 0.65	19.86 ± 0.42 ^b	2.97 ± 0.33	16.89 ± 0.48 ^a
Freeze-dried destoned olive cake	5.83 ± 0.21	10.15 ± 0.22	3.69 ± 0.26	10.18 ± 0.25

Values are means of triplicate samples (± SE).

^{a–c} Means not sharing a common superscript in a column for each treatment are significantly different at $P \leq 0.05$ as assessed by Duncan's Multiple Range Test.

as mean and standard error (SE) and statistical significances of all experimental data were accepted at probability of $P \leq 0.05$.

3. Results and discussion

3.1. Chemical composition of raw and cooked beef patties extended with DOC

Table 2 shows the chemical composition (moisture, protein, fat, and ash) of the raw and cooked beef patties incorporated with different level of DOC. Incorporation of DOC significantly ($P \leq 0.05$) affected the moisture, protein, and fat content of raw beef patties whereas its effect on ash content was not significant. The moisture content of raw patties gradually decreased with DOC concentration, but the protein and fat were increased. The reduction in moisture content could be due to the increase in total solids contents following the addition of DOC. Similarly, Al-Juhaimi et al. (2016) reported a decrease in moisture content of beef patties formulated with *Moringa* seed flour. Also, Alakali, Irtwange, and Mzer (2010) indicated that an increase in Bambara groundnut seed flour decreases the moisture content of beef patties. The increase in protein and fat content could result from the considerable amounts of such constituents in DOC (Table 2). Al-Juhaimi et al. (2016) have observed a similar increasing trend in protein and fat in raw beef patties formulated with a different concentration of *Moringa* seed flour. Moreover, Alakali et al. (2010) observed a concomitant increase in protein and fat content in beef patties formulated with Bambara groundnut seed flour. Cooking of the patties slightly decreased moisture content but slightly increased the protein, ash and fat contents compared to raw patties. The incorporation of DOC alleviates the effect of heat treatment on such parameters. Increasing the concentration of DOC has led to a slight ($P \geq 0.05$) increase in moisture, protein, ash, and fat content of cooked beef patties. Recently, Al-Juhaimi et al. (2016) reported a similar trend of increase in moisture, protein, ash, and fat of *Moringa* seed flour incorporated beef patties concurrently with increasing the concentration of *Moringa* seed powder. The slight increase in protein, fat, and ash as the result of DOC addition is likely due to the loss of moisture, the rise in the

level of total solids, and the effect of DOC concentration (Alakali et al., 2010; Al-Juhaimi et al., 2016).

3.2. Cooking properties of beef meat patties incorporated with DOC

The cooking characteristics of beef patties extended with or without DOC are depicted in Fig. 1a–d. The addition of DOC to beef patties significantly ($P \leq 0.05$) affected cooking characteristics of the patties. Cooking yield, moisture retention and fat retention were significantly ($P \leq 0.05$) high in DOC containing patties compared to control samples (Fig. 1a–c). Progressive increase in the DOC concentration concurrently increased the cooking yield, moisture retention and fat retention of cooked patties. In agreement with our findings, a concentration-dependent increase in cooking yield, moisture retention, and fat retention of beef patties formulated with Bambara groundnut seed flour (Alakali et al., 2010) and *Moringa* seed powder (Al-Juhaimi et al., 2016) has recently been reported. In contrast, Das, Rajkumar, Verma, and Swarup (2012) and Hazra, Biswas, Bhattacharyya, Das, and Khan (2012) indicated that addition of crude extract of *Moringa* (1%) and curry leaf powder (0.2%) in cooked ground buffalo meat and raw goat meat patties respectively did not show any significant differences in the cooking characteristics compared to the control samples. The difference between these studies might be due to the lower concentration in the later studies compared to ones above. The improvement in cooking yield following DOC addition could be linked to the fat and water retention capability to retain moisture in the matrix of formulated patties (Alakali et al., 2010). However, the enhancement of moisture and fat retention of such patties may be accredited to rises in the water absorption capacity of denatured protein, the thermal dissociation of proteins, the gelatinization and swelling of starch and fibre (Alakali et al., 2010; Modi, Mahendrakar, Rao, & Sachindra, 2004). Incorporation of DOC in beef patties significantly affected the dimensional shrinkage of cooked patties (Fig. 1d). Increasing the concentration of DOC over 2% decreased ($P \leq 0.05$) the dimensional shrinkage of cooked beef patties, signifying that the DOC permitted the retention of the size and shape of the patties during cooking. Shrinkage in patties during cooking is mostly caused by denaturation of muscle

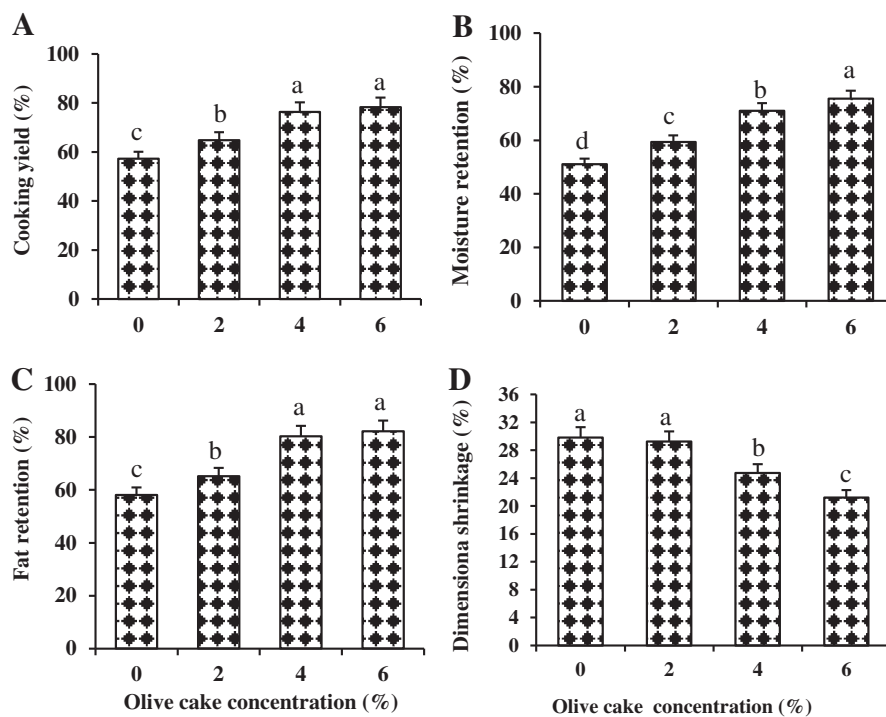


Fig. 1. Cooking properties (A, cooking yield; B, moisture retention; C, fat retention and D, dimensional shrinkage) of beef patties formulated with destoned olive cake powder at different concentrations (0, 2, 4 and 6%). Error bars show the standard error of three independent replicates. ^{a–d} Mean values with different letters are significantly different ($P \leq 0.05$).

protein that affects the textual quality of cooked patties. In addition, evaporation of water and drainage of melted fat and juices are other causes of shrinkage (Alakali et al., 2010). The lower shrinkage of DOC-formulated patties compared to the control could be due to the binding and stabilizing property of DOC, which held the meat particles together and prevented changes in product moisture, juice losses and consequently the shape and texture of the product (Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008).

3.3. Oxidative characteristics of raw beef patties formulated with DOC during storage

Table 3 shows the total phenolics, DPPH radical scavenging activity, and 2-thiobarbituric acid reactive substances (TBARS) of raw beef patties formulated with different concentrations of DOC during cold storage (4.0 ± 1.0 °C). The incorporation of DOC in beef patties gradually ($P \geq 0.05$) increased the total phenolics with DOC concentration. The enhancement of total phenolics following the addition of DOC could be attributed to the considerable amount of phenolics (6.49 mg GAE/g) in DOC used in the formulation of the patties. Previous studies have reported that olive cake extracts have high phenolic contents that ranged from 2.4 to 46.0 mg/g dry weight (Aludatt et al., 2010; Obied et al., 2005). The total phenolics for all DOC levels were significantly ($P \leq 0.05$) decreased during the first week of storage and after that fell gradually and recorded the minimum values at day 14. The reduction in total phenolics could be due to the utilization and hydrolysis of these compounds to control the oxidative rancidity during storage (Morello, Motilva, Tovar, & Romero, 2004). The DPPH radical scavenging activity increased ($P \leq 0.05$) concomitantly with an increase in DOC concentration and scoring the maximum values at 6% DOC (Table 3). This increment could be due to the substantially high DPPH activity of the DOC (87.65%). Similarly, previous reports have shown that increase in the concentration of the plant extract resulted in a progressive increase in the DPPH radical scavenging activity in meat products (Jayawardana et al., 2015; Muthukumar, Naveena, Vaithiyanathan, Sen, & Sureshkumar, 2014; Naveena et al., 2008). The DPPH radical scavenging activity was increased reaching the maximum scores at day 7 and then decreased when the storage prolonged to 14 days. Nevertheless, the DPPH values of DOC-formulated patties at a concentration above 2% are still higher compared to those of non-formulated ones even after 14 days of storage. These results specified that incorporation of DOC in beef patties diminish the adverse effect of storage and free radical formation and hence could prolong the shelf life of stored patties. Apparently, DOC was effective in enhancing the total phenolic contents of beef patties, and consequently, it improves its antioxidant activity as

compared to the non-formulated samples. The lipid peroxidation in terms of TBARS values in raw beef patties formulated with DOC showed a slight ($P \geq 0.05$) decrease compared to non-formulated patties (Table 3). Increasing the concentration of DOC in the patties gradually ($P \geq 0.05$) reduced the TBARS that scoring minimum values at 6% DOC. The TBARS values of treated and untreated patties increased with storage indicating the persistent formation of aldehydes in the products. However, the TBARS values for all DOC containing patties were consistently ($P \geq 0.05$) lower than that for untreated control showing that DOC impart antioxidant activity to the formulated beef patties. Thus, the addition of DOC to meat products could inhibit lipid peroxidation and extend the shelf life of these products under cold storage conditions. Antioxidant activity is mainly resulted from polyphenols due to their redox properties, through which it break free radical chains of oxidation by donation of hydrogen from the phenolic groups, and thereby terminate free radical chain reactions and forming a stable product (Sherwin, 1998). The findings of our study are in agreement with those obtained by Al-Juhaimi et al. (2016) and Jayawardana et al. (2015), who reported the effectiveness of *Moringa* seeds and leaves powders in controlling lipid oxidation in meat products. In addition, Hayes et al. (2010) indicated that minced beef patties treated with olive leaf extract at concentrations of 100 and 200 µg/g muscle showed a lower level of lipid oxidation as compared to control in modified atmosphere packaging conditions.

3.4. pH and microbiological characteristics of raw beef patties formulated with DOC during storage.

The changes in pH and microbial loads of raw beef patties formulated with DOC during refrigerated storage (4.0 ± 1.0 °C) are shown in Fig. 2. The pH was significantly ($P \leq 0.05$) varied between formulated and unformulated-beef patties throughout the storage period (Fig. 2a). The pH values decreased gradually during storage, and there were significant ($P \leq 0.05$) effects of DOC on the pH values. The reduction rate of pH in non-formulated beef patties was significantly higher ($P \leq 0.05$) than those of formulated patties. This was in line with the total plate count results in which the plate count of control patties was the highest among all treatments (Fig. 2b). Noticeably, the addition of DOC significantly enhances the pH stability of the formulated patties simultaneously with the level of DOC suggesting the protective role of DOC against spoilage microorganisms. Reduction in pH during storage may be attributed to the accumulation of lactic acid due to the growth of lactic acid bacteria in stored patties (Wang, Ren, Liu, Zhu, & Wang, 2013). Similarly, Jayawardana et al. (2015) reported a significant reduction in pH of chicken sausages with and without *Moringa* leave powders during the

Table 3
Oxidative characteristics of raw beef burger formulated with different concentrations of destoned olive cake powder during storage at 4 °C (± 1.0).

Storage period (days)	Olive cake powder concentration (%)			
	0	2	4	6
Total phenolics (mg Gallic acid/g sample)				
0	4.31 \pm 0.11 ^a	4.43 \pm 0.09 ^a	4.59 \pm 0.11 ^a	4.71 \pm 0.36 ^a
7	3.08 \pm 0.08 ^b	3.26 \pm 0.22 ^b	3.88 \pm 0.24 ^b	3.98 \pm 0.25 ^{ab}
14	3.05 \pm 0.55 ^b	3.18 \pm 0.52 ^b	3.42 \pm 0.24 ^b	3.82 \pm 0.01 ^b
Freeze-dried destoned olive cake	6.49 \pm 0.01			
DPPH (%)				
0	23.35 \pm 0.33 ^{ap}	25.39 \pm 0.28 ^{aq}	27.23 \pm 0.57 ^{ar}	29.22 \pm 0.50 ^{as}
7	24.02 \pm 0.26 ^{ap}	25.78 \pm 0.17 ^{aq}	27.53 \pm 0.42 ^{ar}	29.52 \pm 0.13 ^{as}
14	22.84 \pm 0.24 ^{bp}	23.36 \pm 0.32 ^{bp}	25.25 \pm 0.33 ^{bq}	27.79 \pm 0.42 ^{br}
Freeze-dried destoned olive cake	87.65 \pm 0.87			
Thiobarbituric acid reactive substances (TBARS) in mg malonaldehyde/kg samples				
0	1.91 \pm 0.07 ^b	1.89 \pm 0.05 ^b	1.87 \pm 0.006 ^b	1.85 \pm 0.06
7	2.26 \pm 0.15 ^a	1.98 \pm 0.10 ^b	1.97 \pm 0.03 ^{ab}	1.87 \pm 0.08
14	2.48 \pm 0.20 ^a	2.19 \pm 0.25 ^a	2.04 \pm 0.24 ^a	1.97 \pm 0.13

Values are means of triplicate samples (\pm SE). Means not sharing a common superscript(s) a or b in a column or p, q, r or s in a row are significantly different at $P \leq 0.05$ as assessed by Duncan's Multiple Range Test.

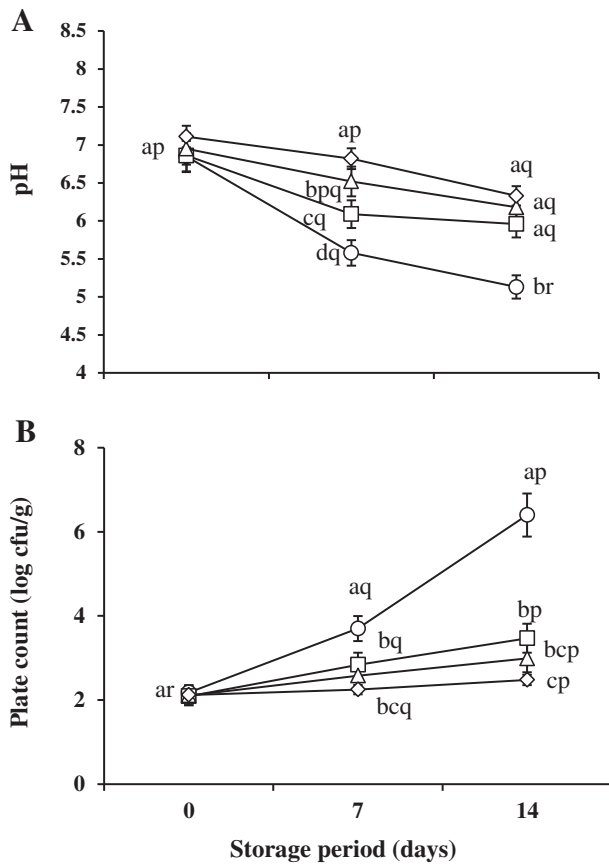


Fig. 2. pH (A) and microbiological characteristics (B) of raw beef burgers formulated with destoned olive cake powder at different levels (0%, \square ; 2%, \square ; 4%, Δ and 6%, \diamond) during storage. Error bars indicate the standard error of three independent replicates. ^{a–d} Mean values with different letters in the same treatments are significantly different ($P \leq 0.05$); ^{P–T} Mean values with different letters at the same day of storage are significantly different ($P \leq 0.05$).

storage period. Moreover, Wenjiao, Yunchuan, Junxiu, and Yongkui (2014) reported a decrease in the pH value in tea polyphenol treated sausage during the storage.

The microbial load of beef patties varied significantly ($P \leq 0.05$) among the treatments and between the days of storage. The total count of beef patties significantly ($P \leq 0.05$) increased with the progress of storage period but that of DOC-formulated samples showed least increase. All the samples incorporated with DOC showed significantly ($P \leq 0.05$) lower total plate than untreated indicating that DOC used in the present study exhibited significant ($P \leq 0.05$) antimicrobial property. The difference in the bacterial growth between the control and DOC-containing patties during the storage period may also cause the differences in pH, which were affected by the level of DOC (Fig. 1a). Apparently, the growth of microorganisms in beef patties decreased concomitantly with an increase in DOC concentration. This could be due to the fact that olive mill waste contain appreciable amounts of antimicrobial compounds mainly oleuropein and hydroxytyrosol, 4-hydroxybenzoic acid, vanillic acid, and *p*-coumaric acid that exhibit great inhibition activity against wide range of microorganisms (Obied et al., 2005). Therefore, our findings could suggest that incorporation of DOC in beef patties will extend the shelf life of the product up to 14 days under cold storage through the inhibition of the growth of spoilage microorganism. Similarly, other authors have documented the inhibitory effect of natural plant extract on microbial growth in meat products. *Moringa* seeds and leaves powders have been found to retard microbial growth in beef patties stored at 4 °C for 21 days (Al-Juhaimi et al., 2016) and herbal chicken sausage (Jayawardana et al., 2015) stored

at 4 °C for 5 weeks. Furthermore, Mariem et al. (2014) reported that *Nitraria retusa* fruits extract delayed bacterial growth in beef patties during cold storage (4 °C for 9 days).

3.5. Surface color characteristics of raw beef patties formulated with DOC during storage

The color of meat products is one of the most significant sensory attributes that affects the consumer acceptance and it is a sign of meat quality and freshness. The effect of the addition of DOC on the surface color of beef patties is depicted in Table 4. The lowest values of redness (a^*) were observed in control patties, and these values were concurrently ($P \geq 0.05$) increased with increasing DOC level reaching maximum values at 6% DOC. The a^* values decreased ($P \leq 0.05$) progressively with the storage time of both formulated and non-formulated patties. Shah, Bosco, and Mir (2014) reported a similar trend for fresh beef meat treated with different concentration (0.1, 0.2, and 0.3 g/L) of *Moringa* leaves extract and stored under modified atmospheric packing at 4 °C. Moreover, Muthukumar et al. (2014) observed a progressive reduction in a^* values of both non-formulated and *Moringa* leaves formulated pork patties during refrigerated storage. Higher a^* values of DOC containing patties compared to control suggests that the incorporation of DOC was responsible for the color stabilization as the reduction in a^* values is due to the oxidation of myoglobin and formation of metmyoglobin (Mancini & Hunt, 2005). Naveena et al. (2008) reported higher a^* values in chicken patties incorporated with pomegranate ring extract compared to untreated ones. However, Al-Juhaimi et al. (2016) indicated that addition of *Moringa* seed flour significantly reduced the a^* values of beef patties compared to untreated. The incorporation of DOC significantly altered yellowness (b^*) values of raw beef patties. The lowest b^* values were observed in non-formulated patties and concomitantly increased with the addition of DOC and reached the maximum at 6% DOC formulated patties. Similarly, Al-Juhaimi et al. (2016) reported that addition of *Moringa* seed flour simultaneously

Table 4

Color characteristics of raw beef burger formulated with various concentrations of destoned olive cake powder during storage at 4 °C (± 1.00).

Storage period (days)	Olive cake concentration (%)			
	0	2	4	6
a^*				
0	4.32 \pm 0.30 ^a	4.98 \pm 0.16 ^a	5.32 \pm 0.54 ^a	5.33 \pm 0.29 ^a
7	3.74 \pm 0.31 ^b	3.82 \pm 0.18 ^b	3.97 \pm 0.24 ^{ab}	5.21 \pm 0.19 ^a
14	3.34 \pm 0.28 ^b	3.44 \pm 0.22 ^b	3.65 \pm 0.25 ^b	4.04 \pm 0.28 ^b
b^*				
0	8.20 \pm 0.33 ^{ar}	8.42 \pm 0.29 ^{ar}	10.30 \pm 0.07 ^{aq}	13.32 \pm 0.31 ^{ap}
7	8.06 \pm 0.44 ^{aq}	8.31 \pm 0.02 ^{aq}	9.04 \pm 0.32 ^{aq}	12.93 \pm 0.44 ^{ap}
14	6.12 \pm 0.00 ^{br}	6.57 \pm 0.16 ^{br}	8.98 \pm 0.46 ^{bq}	11.19 \pm 0.24 ^{bp}
L^*				
0	42.58 \pm 0.43 ^{ap}	33.68 \pm 0.03 ^{aq}	30.84 \pm 0.05 ^{ar}	30.03 \pm 0.06 ^{ar}
7	41.77 \pm 0.34 ^{ap}	31.43 \pm 0.54 ^{bq}	30.77 \pm 0.20 ^{aq}	29.45 \pm 0.30 ^{aq}
14	40.46 \pm 0.57 ^{bp}	31.14 \pm 0.53 ^{bq}	26.98 \pm 0.35 ^{br}	26.60 \pm 0.40 ^{br}
a^*/b^*				
0	0.53 \pm 0.03	0.53 \pm 0.04 ^a	0.52 \pm 0.06 ^a	0.40 \pm 0.02 ^a
7	0.46 \pm 0.01	0.46 \pm 0.02 ^b	0.44 \pm 0.05 ^a	0.40 \pm 0.05 ^a
14	0.55 \pm 0.05 ^p	0.52 \pm 0.006 ^{ap}	0.41 \pm 0.05 ^{bp}	0.36 \pm 0.03 ^{bq}

Values are means of triplicate samples (\pm SE). Means not sharing a common superscript(s) a or b in a column or p, q or r in a row are significantly different at $P \leq 0.05$ as assessed by Duncan's Multiple Range Test. a^* : Redness, b^* : Yellowness, L^* : Lightness, a^*/b^* : Color quality.

increased the yellowness values of formulated beef patties compared to non-formulated patties. They attributed the enhancement of the yellowness values to the presence of carotenoids pigment in *Moringa* seed flour (Al-Juhaimi et al., 2016). The b^* values of both DOC-formulated and non-formulated beef patties were significantly ($P \leq 0.05$) decreased during the storage period reaching the minimum values at day 14. Muthukumar et al. (2014) and Shah et al. (2014) observed reductions in b^* values respectively, for raw pork patties and modified atmosphere packing raw beef meat incorporated with *Moringa* leaves extract during cold storage. Incorporation of different levels of DOC in beef patties progressively ($P \leq 0.05$) reduced the lightness (L^*) values of formulated patties compared to controls (Table 4), which might be due to the dilution of the patties color by the added powder of DOC. Muthukumar et al. (2014) reported a reduction in L^* value in raw beef patties incorporated with *Moringa* leave extract. However, Jayawardana et al. (2015) did not observe any alteration in L^* value following the incorporation of *Moringa* leaves in herbal chicken sausages. However, Al-Juhaimi et al. (2016) have reported an increase in L^* value of beef patties due to the addition of *Moringa* seed flour. Storage of raw patties significantly reduced the L^* values of both formulated and non-formulated patties. Similar observations on the reduction of L^* values during storage of beef patties incorporated with different levels of *Moringa* seeds were recently reported (Al-Juhaimi et al., 2016). The a^*/b^* value is generally used as an indicator of the color quality. The results showed that a^*/b^* values were decreased as the percentage of DOC increased; however, the reduction was not significant (Table 4). The result specified that the incorporation of patties with DOC had an insignificant effect on color quality of the patties as shown by low a^*/b^* values. The storage of beef patties without DOC and that with 2%DOC had no significant effect on the a^*/b^* value. However, the beef patties with high concentration (4% and 6%) showed a significant reduction in a^*/b^* values during storage. Al-Juhaimi et al. (2016) reported similar findings for beef patties formulated with different level of *Moringa* seed flour.

3.6. Sensory attributes of cooked beef patties formulated with DOC during storage

The sensory panel results of cooked DOC formulated and non-formulated beef patties stored at 4 °C for 14 days are presented in Table 5. Incorporation of DOC in beef patties at a concentration up to 4% did not affect the sensory attributes (appearance, juiciness, flavour, taste, tenderness, and overall acceptability) of formulated patties. However, at 6%DOC the sensory characteristics were significantly ($P \leq 0.05$) lower than non-formulated patties on day 0 of storage. Throughout the storage period, all the sensory traits of non-formulated patties were significantly ($P \leq 0.05$) reduced, whereas the formulated patties revealed excellent stability of all characters. Similarly, Al-Juhaimi et al. (2016) reported that the sensory attributes cooked beef patties insignificantly decreased with increasing *Moringa* seed flour and storage period. Also, Muthukumar et al. (2014) found no significant change in sensory attributes of cooked pork patties due to the incorporation of *Moringa* leave extract. Moreover, Kumar and Sharma (2004) reported a slight decrease in sensory values during storage of low-fat ground pork patties formulated with carrageenan. The stability of sensory attributes of DOC incorporated patties might be due to the protective role of DOC against deteriorating causes as it possesses antioxidant and antimicrobial activities. These results demonstrated that incorporation of DOC in beef patties could stabilize the sensory quality and prolong the shelf life of the patties.

4. Conclusions

This study concludes that DOC is a reliable source of polyphenols and free radical scavengers, and it provides considerable antioxidant and antibacterial benefits to beef meat patties during cold storage (4.0 ± 1.0 °

Table 5

Sensory characteristics of cooked beef burger formulated with the various concentrations of destoned olive cake powder during storage at 4 °C (± 1.00).

Storage period (days)	Olive cake concentration (%)			
	0	2	4	6
Appearance				
0	7.85 \pm 0.38 ^{ap}	6.35 \pm 1.05 ^p	5.80 \pm 0.44 ^{pq}	4.93 \pm 0.51 ^q
7	4.63 \pm 0.54 ^b	5.47 \pm 0.20	5.88 \pm 0.29	5.11 \pm 0.19
14	0.00 \pm 0.00 ^{cq}	5.18 \pm 0.19 ^p	5.15 \pm 0.24 ^p	4.60 \pm 0.28 ^p
Juiciness				
0	7.71 \pm 0.57 ^{ap}	6.85 \pm 0.22 ^{ap}	5.93 \pm 0.20 ^{pq}	5.46 \pm 0.29 ^q
7	4.47 \pm 0.44 ^b	6.31 \pm 0.02 ^a	6.11 \pm 0.50	5.88 \pm 0.50
14	0.00 \pm 0.00 ^{cq}	5.68 \pm 0.16 ^{bp}	5.90 \pm 0.41 ^p	5.10 \pm 0.27 ^p
Flavour				
0	7.64 \pm 0.53 ^{ap}	6.64 \pm 0.37 ^p	5.86 \pm 0.28 ^{pq}	5.40 \pm 0.32 ^q
7	5.21 \pm 0.56 ^b	5.52 \pm 0.26	6.23 \pm 0.29	5.70 \pm 0.37
14	0.00 \pm 0.00 ^{cq}	5.56 \pm 0.38 ^p	5.45 \pm 0.47 ^p	4.85 \pm 0.30 ^p
Taste				
0	7.14 \pm 0.74 ^{ap}	6.92 \pm 0.24 ^{ap}	6.06 \pm 0.32 ^p	5.26 \pm 0.41 ^q
7	4.10 \pm 0.54 ^b	5.78 \pm 0.34 ^b	6.41 \pm 0.37	5.35 \pm 0.36
14	0.00 \pm 0.00 ^{cq}	5.50 \pm 0.40 ^{bp}	5.70 \pm 0.25 ^p	5.20 \pm 0.15 ^p
Tenderness				
0	7.64 \pm 0.58 ^{ap}	6.71 \pm 0.36 ^p	5.80 \pm 0.22 ^{bpq}	5.40 \pm 0.36 ^q
7	5.47 \pm 0.59 ^{bq}	6.15 \pm 0.37 ^q	6.82 \pm 0.15 ^{ap}	6.08 \pm 0.32 ^q
14	0.00 \pm 0.00 ^{cq}	5.93 \pm 0.38 ^p	5.60 \pm 0.24 ^{bp}	5.10 \pm 0.36 ^p
Overall acceptability				
0	7.60 \pm 0.37 ^{ap}	6.70 \pm 0.19 ^p	5.88 \pm 0.43 ^{apq}	5.30 \pm 0.28 ^q
7	4.37 \pm 0.41 ^{bpq}	5.85 \pm 0.38 ^p	6.29 \pm 0.24 ^{ap}	5.63 \pm 0.28 ^q
14	0.00 \pm 0.00 ^{cq}	4.64 \pm 0.42 ^p	4.51 \pm 0.22 ^{bp}	3.98 \pm 0.47 ^{bp}

Values are means of triplicate samples (\pm SE). Means not sharing a common superscript(s) a, b or c in a column or p or q in a row are significantly different at $P \leq 0.05$ as assessed by Duncan's Multiple Range Test.

C) that the influences are concentration dependent. The addition of DOC significantly retarded lipid peroxidation and inhibited microbiological growth in beef patties compared to controls. Moreover, DOC improved the physicochemical properties, cooking characteristics, color properties, and storage stability without altering the sensory attributes of beef patties. Therefore, the use of DOC as a by-product of the olive oil industry to extend the shelf life of meat products could both satisfy the modern consumer's demands for natural, safe and healthy food ingredients and add commercial value to olive oil by-products. Additionally, the beneficial use of olive cake in food industry could diminish the environmental impacts of olive oil mill by-products.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgement

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research through the Research Group (RG-1435-049).

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