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Effect of Argel (Solenostemma argel) leaf extract on quality attributes of chicken meatballs during cold storage

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Abstract This study aimed to evaluate the antioxidant activity of Argel leaf water extract (ALWE) and its effect at different concentrations (0, 5, 10, and 20 mg/100 mL) on the antioxidant, antimicrobial, physicochemical, and sensory attributes of chicken meatballs during cold storage. ALWE contained substantial quantities of total phenolic content (TPC), anthocyanin, and exhibited high DPPH scavenging activity. ALWE incorporation in chicken meatballs had a varying effect on the chemical composition and sensory attributes of the product. However, ALWE incorporation at high concentration decreased the protein content of cooked meatballs and reduced fat content in both raw and cooked balls. Increased ALWE concentration in chicken meatballs lowered the pH, microbial load, and thiobarbituric acid reactive substances. Furthermore, ALWE raised the TPC and DPPH scavenging activity of chicken meatballs. Throughout the storage period, chicken meatballs formulated with ALWE showed better quality attributes than non-formulated chicken meatballs. In conclusion, ALWE can be employed as a functional ingredient for improved health benefits and shelf-life extension of chicken meatballs.

Keywords Antimicrobial · Antioxidant · Argel leaf water extract · Chicken meatballs · Quality attributes

Introduction

Chicken meat and products contain several desirable nutritional characteristics such as low amounts of lipid and greater polyunsaturated fatty acid content (Petracci et al. 2014) compared to most types of red meat. One such chicken product is chicken meatballs, which are popular worldwide. Traditionally, meatballs are produced by mixing all the required ingredients by hand and forming a dough by smashing movements until the required texture is obtained (Elmali and Yaman 2005). Despite the growing interest and high nutritional quality in chicken products, essential factors such as microbial spoilage, lipid oxidation, and enzymatic changes influence the keeping quality of such products. Among muscle foods, chicken products are more vulnerable to oxidation compared to beef or lamb due to their relatively high amount of mono- and poly-unsaturated fatty acids (Tang et al. 2001). Moreover, mincing and thermal processes such as cooking disrupt the integrity of muscle membranes and expose lipid membranes to metal ions, thus accelerating the interaction of pro-oxidants with unsaturated fatty acids, leading to the production of free radicals and propagation of oxidative reaction (Asghar et al. 1988).

Muscle lipids and proteins are modified by oxidation, which affects the sensorial and nutritional properties of the meat and meat products and thus may reduce the health and economic potentials of these important foods. Control of oxidation problems in meat products has been successfully achieved by the use of synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA). However, these synthetic antioxidants are considered unsafe by consumers and healthcare workers (Tang et al. 2001). Consequently, meat industries now search for natural antioxidants, mostly from plants to

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replace synthetic antioxidants, which are major concerns for consumer health. Plants such as herbs and spices are potential sources of natural antioxidants and antimicrobial compounds, as they contain varieties of phytochemicals such as polyphenols, flavonoids, tannins, and phenolic acids (Devatkal et al. 2010). Many research reports have demonstrated antioxidant effects of grape seed extracts in poultry meat (Brannan 2008), tea catechins in chicken patties (Mitsumoto et al. 2005), rosemary and sage in chicken nuggets (O'Sullivan et al. 2004), and extracts of pomegranate and kinnow peels in chicken meat products (Devatkal et al. 2011).

Argel (Solenostemma argel Hayne) is a wild herbal plant found in broad areas of Egypt, Saudi Arabia, and Sudan (Awad et al. 2006). The leaves, bark, and stems of Argel have diverse medicinal applications and are traditionally being used for the treatment of numerous diseases such as pain, diabetes, respiratory tract infections, cardiovascular disorders, gastrointestinal problems, urinary tract infections, and kidney and liver diseases (Awad et al. 2006). Previous studies have shown that Argel leaves, bark, and stems have antispasmodic, anti-inflammatory, antinociceptive, antipyretic, anticancer, anti-oxidant, and antimicrobial activities (Awad et al. 2006). Phytochemical investigation of argel demonstrated the presence of several phytochemicals, including various phenolic acids (gallic acid, protocatechuic acid, syringic acid, caffeic acid, p-coumaric acid, trans-ferulic acid, and trans-cinnamic acid), flavones (quercetin, (1)-catechin, naringenin, isorhamnetin, and kaempferol), glycosylated flavonoids apigenin-(quercetin-3-rutinoside and 7-glucoside), polyphenols (catechol and resveratrol), β-carotene, β-sitosterol, monoterpenes, pregnenes, and pregnane (Al-Juhaimi et al. 2017a; Hassan et al. 2001; Ounaissia et al. 2016; Plaza et al. 2005). Despite the fact that Argel leaves have high quantities of phenolics with antioxidant and antimicrobial potentials, limited information is available on their use in meat and meat products for extending shelf-life and preventing lipid oxidation (Al-Juhaimi et al. 2017a). Hence, this study was conducted to determine the effect of Argel leaf water extract on the microbiological characteristics and the physicochemical and sensory attributes of minced chicken meatballs during cold storage.

Materials and methods

Materials

Frozen minced chicken, chickpea powder, onion powder, table salt, white pepper, black pepper, garlic powder, and vinegar were purchased from a supermarket in Riyadh, Saudi Arabia. Argel leaves were collected from a farm in Sudan. Unless otherwise stated, all reagents used in the study were of analytical grade.

Preparation of Argel leaf water extracts (ALWE)

Argel leaves were cleaned by hand, washed thoroughly with water, dried at room temperature and then ground to a fine powder. The extracts were prepared by mixing 1.5 kg powder with distilled water (500 mL), stirred for 3 h using a magnetic stirrer (Fisher, 14-511-1A, USA), autoclaved at 121 °C for 21 min, cooled and then filtered through Whatman No. 1 filter paper. The filtrates (extract) were collected, freeze-dried and stored at -20 °C until further use. Three different concentrations (5, 10, and 20 mg/100 mL) of the extract were used.

Preparation of raw and cooked chicken meatballs formulated with ALWE

Triplicates chicken meatballs formulations were prepared by mixing minced chicken with 0, 5, 10, and 20 mg/ 100 mL ALWE and spices according to the percentages specified in Table 1. The ingredients were homogeneously mixed with the meat using a Stephan UM 12 mixer (Stephan U. Sohner GmbH and Co., Germany). About 75-g portions from each blended formulation were molded into a ball form by hand. The chicken meatballs were separately placed in low-density polyethylene bags and stored at $4 \pm 1^{\circ}$ C for 20 days. Samples were collected at 5-day intervals during the storage period and analyzed for the physicochemical, microbial, and sensory properties. As described by Al-Juhaimi et al. (2016), cooking of the chicken meatballs was carried out in a convection oven (Hobart Corp., Troy, Ohio, USA) at 180 °C till the geometric center of the meatballs reached a temperature of

 Table 1 Ingredients (%) of four blends of chicken meatballs formulated with Argel leaf water extract (ALWE)

Ingredients (%)	Control	ALWE level (mg/100 mL)		
		5	10	20
Lean meat	72	72	72	72
Vinegar	0.4	0.4	0.4	0.4
Chickpea powder	2.0	2.0	2.0	2.0
Salt	2.0	2.0	2.0	2.0
White pepper	0.4	0.4	0.4	0.4
Black pepper	0.2	0.2	0.2	0.2
Garlic powder	1.0	1.0	1.0	1.0
Onion powder	2.0	2.0	2.0	2.0
ddH ₂ O	20.0	15.0	10.0	0.0
ALWE	0.0	5.0	10.0	20.0

80 °C, as measured by a digital probe thermometer (Oakton, Eutech Instruments, China). At every 10-min interval, the balls were rotated to ensure uniform cooking. The raw and cooked ALWE-formulated and un-formulated meatballs were analyzed for various quality attributes during the storage period.

Chemical composition

The chemical composition (moisture, fat, protein, and ash content) of freeze-dried samples of raw and cooked chicken meatballs were analyzed as described by AOAC (2003).

Assessment of microbiological characteristics and pH of chicken meatballs

The method described by Harrigan and McCance (1976) was employed for the determination of total plate (TPC), yeast and mold counts of raw meatballs stored at 4 °C for 0, 5, 10, 15, and 20 days. About 1 g of the sample was homogenized in 9 mL of sterile peptone water (0.1 g/ 100 mL, w/v), serially diluted (ten-folds) with the same solution, and pour plated in duplicates on plate count and nutrient agar for the total plate, yeast, and mold counts, respectively. The plates were incubated at 35 °C for 1-2 days for bacteria and at 30 °C for 4-5 days for yeasts and molds, and the numbers of colonies were expressed as log10 cfu/g. The change in pH of the raw chicken meatballs during storage was determined by blending about 5-g samples with 45 mL of distilled water followed by filtration, and pH measurement (Corning 240 pH meter, Corning Scientific Products, New York, USA).

Preparation of chicken meatball and antioxidant extract

Raw chicken meatballs were freeze-dried (12525, Virtis Company, Gardner, New York), ground, and passed through a 1-mm sieve. About 2.5 g of meatball powder was mixed with distilled water (20 mL), stirred overnight using a magnetic stirrer (Fisher, 14-511-1A, USA) at 4 °C, centrifuged at $4500 \times g$ for 30 min (Hermle 66110068, Germany), and the supernatant was collected and used for the analysis of total phenolic content.

Determination of total phenolic content (TPC)

The TPC of the extracts was determined as described by Singleton and Rossi (1965) with a slight modification. Briefly, 200 μ L of the extracts were mixed with 400 μ L of Folin Ciocalteu reagents and 4.0 mL distilled water. The mixture was incubated for 10 min at 25 °C followed by

addition of Na_2CO_3 solution (20 g/100 mL, w/v), mixed thoroughly, and incubated at the same temperature for 2 h. The absorbance was checked at 765 nm (PD-303UV spectrophotometer, Apel, Saitama, Japan) and TPC was expressed as mg gallic acid equivalent per 100 grams (GAE/100 g) of extracts.

Determination of anthocyanin

The method of Ticconi, et al. (2001) was used for measuring total anthocyanins in ALWE which included homogenization of 0.5 g sample in a propanol, chlorhydric acid, and water (18:1:81) solution, followed by 3 min boiling of homogenates, and then incubation at room temperature for 24 h. After centrifugation at $6500 \times$ for 40 min, 3 mL of the supernatant was analyzed spectrophotometrically at 535 and 650 nm, and the absorbance was recorded. The final absorbance value was corrected and calculated using a formula: A = A₅₃₅ - A₆₅₀.

Determination of free radical scavenging activity

Free radical activity was determined using 1, 1-diphenyl-2picrylhydrazyl (DPPH) (Lee et al. 1998). Briefly, 1 mL extract at a concentration of 100 μ L/mL in methanol was mixed with 2 mL of 10 mg/L methanolic solution of DPPH. An equal amount of methanol and DPPH served as a control. The mixtures were shaken vigorously and allowed to stand at room temperature for 5 min, and optical density (OD) was recorded at 517 nm. The percentage inhibition of free radicals was calculated as follows:

$$RSA\% = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extract.

Determination of thiobarbituric acid reactive substances (TBARS)

The TBARS values of raw chicken meatballs at 0, 5, 10, 15 and 20 days of storage were determined as described by Rosmini et al. (1996). Briefly, 5 g of raw chicken meatballs were homogenized in 20 mL distilled water and filtered through Whatman No. 1 filter paper. Then, 1 mL of the filtrate was taken in a screw cap tube followed by addition of 4 mL of 20 g/mL thiobarbituric acid and 100 μ L of 10 g/100 mL butylated hydroxytoluene. The tubes were left to develop a pink color in a boiling water bath (95–100 °C) for 10 min and then allowed to cool. The mixture was centrifuged (5500×g) for 25 min and the absorbance of the supernatant obtained was measured at 532 nm. TBARS values were expressed as mg malonaldehyde/kg using a standard curve obtained using 1,1,3,3-tetraethoxypropane.

Sensory evaluation

Cooked chicken balls were evaluated for sensory traits by 10 semi-trained members (male, 20-35 years of age) selected from the staff of the Department of Food Science and Nutrition, College of Food and Agriculture, King Saud University. Before the sensory evaluation, the panel received preliminary training sessions (n = 3) to focus attention on the sensory attributes and accordingly, each panelist could carefully discuss and clarify each attribute. Panelists were instructed to evaluate color, texture, taste, flavor, juiciness, and overall acceptability using a 9-point hedonic scale. The test was conducted early in the morning in a sensory evaluation room at 20 ± 1.0 °C. Sensory attributes were scored as 'like extremely' = 9 to 'dislike extremely' = 1. The testing sessions were carried out three times at each storage time (0, 5, 10, 15 and 20 days) and the mean values of the scores from ten panelists for each sample and session were calculated and used for data analysis.

Statistical analysis Three meatball batches were produced, and all measurements were repeated three times. In each batch, four treatments (control, 5, 10, and 20% ALWE) were performed and measurements (n = 3) were carried out at four storage periods (0, 5, 10, 15, and 20 days). The effect of treatments and storage periods on the physicochemical, microbial, and sensory characteristics of chicken meatballs was statistically analyzed using the SAS program (SAS, v. 8.1; SAS Institute Inc., Cary, NC). The data of physicochemical properties, microbiological, and sensory attributes from different treatments, storage times and interaction between them were subjected to analysis of variance (two-way ANOVA) using the general linear model (GLM) procedure and Duncan's multiple range tests. In the analysis models, the treatments, storage times, and assessors (for sensory data) were considered as a fixed effect, and the replications of experiments were set as random terms. The mean separation procedure of LSD was employed, data were reported as mean \pm standard error (SE), and significances were accepted at $P \leq 0.05$.

Results and discussion

Total phenolic content, antioxidant activity and anthocyanin content of ALWE

Water extract of Argel leaf powder has a high TPC (1262.50 \pm 3.67 mg GAE/100 g), antioxidant activity (86.85 \pm 1.52% inhibition), and anthocyanin

 $(60.11 \pm 1.18 \,\mu\text{mol/g})$ which signifies the richness of phenolic components and antioxidants in the extract (Al-Juhaimi et al. 2017a). The TPC value obtained in this study was lower than that reported by Muddathir et al. (2017) for the TPC of the methanolic extract of Argel leaves (3290.00 mg GAE/100 g). These differences could be due to location, varieties, growth stage, and harvesting time, as well as the extraction method. However, these values were within the range of TPC (19.00-10133.00 mg GAE/100 g) in several medicinal plants (Li et al. 2013) and oilseeds (28.0–1887.0 mg GAE/100 g) (Alu'datt et al. 2017). Studies have shown that different parts such as leaves and hairy seeds of Argel plants are rich in phytochemicals including various phenolic acids, flavones, glycosylated flavonoids, polyphenols, β-carotene, β-sitosterol, monoterpenes, pregnenes, and pregnane (Al-Juhaimi et al. 2017a; Hassan et al. 2001; Ounaissia et al. 2016). Some of these phytochemicals are known to exhibit antioxidant and antimicrobial activities comparable to those of artificial antioxidants and antimicrobials (Bhattacharya et al. 2016; Santos et al. 2016). Therefore, the high TPC, antioxidant activity, and anthocyanin content of Argel leaves indicate the suitability of this plant material for several uses such as prolonging the shelf life of meat products (Al-Juhaimi et al. 2017a).

Chemical composition of chicken meatballs incorporated with ALWE

The chemical composition (moisture, protein, fat, and ash) of raw and cooked chicken meatballs formulated with different concentrations of Argel leaf water extract (ALWE) is presented in Table 2. Incorporation of ALWE had no significant effect on the chemical composition of the raw and cooked chicken meatballs except the protein and fat contents. Addition of ALWE to raw meatballs did not affect protein content but significantly $(P \le 0.05)$ decreased that of cooked samples. However, fat content was significantly (P < 0.05) decreased in both raw and cooked meatballs after addition of ALWE. Our findings were concomitant with those reported by Baldin et al. (2016) where incorporation of microencapsulated jabuticaba (Myrciaria cauliflora) extract had no significant effect on the proximate composition of chicken sausages. A similar finding was also reported in the proximate composition of aloe vera-containing low-beef patties (Soltanizadeh and Ghiasi-Esfahani 2015). Cooking of the chicken meatballs decreased the moisture and ash contents but slightly increased the protein contents as compared to raw chicken meatballs. During cooking, leaching of water and water-soluble constituents occurs, which may result in an increase in dry matter content with a concomitant rise in protein and fat content as reported by Al-Juhaimi et al. Table 2 Chemicalcompositions (%) of freeze-dried chicken meatballsformulated with differentconcentrations of Argel leafextracts

Extract Conc. %	Moisture	Ash	Protein	Fat
Raw				
0	11.54 ± 0.10^{a}	$5.11\pm0.16^{\rm a}$	58.05 ± 0.01^{a}	24.33 ± 0.89^a
5	11.28 ± 0.13^{a}	$5.14\pm0.14^{\rm a}$	56.60 ± 2.35^{b}	22.85 ± 0.52^{b}
10	11.57 ± 0.19^{a}	$5.09\pm0.12^{\rm a}$	57.95 ± 0.81^{a}	22.82 ± 0.19^{b}
20	11.14 ± 0.09^{a}	$5.14\pm0.09^{\rm a}$	58.22 ± 2.67^a	22.44 ± 1.30^{b}
Cooked				
0	6.75 ± 0.19^{a}	$4.28\pm0.22^{\rm a}$	62.22 ± 2.66^{a}	23.15 ± 0.80^a
5	6.29 ± 0.12^a	$4.42\pm0.16^{\rm a}$	62.31 ± 0.06^{a}	21.87 ± 1.14^{b}
10	6.66 ± 0.29^{a}	4.43 ± 0.08^a	61.13 ± 0.74^{b}	$22.37 \pm 0.57^{\rm b}$
20	$6.35 \pm 0.24^{\rm a}$	4.41 ± 0.26^{a}	61.45 ± 0.17^{b}	$22.53 \pm 2.05^{\rm b}$

Values are means (\pm SD). ^{a,b}Mean values with different superscripts within the same row are significantly ($P \le 0.05$) different

(2017b) for chicken patties formulated with pistachio hull water extract. Furthermore, Hawashin et al. (2016) demonstrated that cooking significantly increased the protein and fat content of beef patties formulated with destoned olive cake powder.

Microbiological characteristics and pH of raw chicken meatballs extended with ALWE during cold storage

Table 3 shows the pH profiles and the microbial loads of the formulated and unformulated raw chicken meatballs. Argel leaf extract had an acidic pH of 5.2 and reduced the pH of chicken meatballs. Chicken meatballs formulated with 20 mg/100 mL ALWE had the lowest pH value. This is because the addition of ALWE to chicken meatballs lowered their pH value such that it declined to 5.67 in chicken meatballs containing 20 mg/100 mL ALWE. Meat products are stable against most pathogenic bacteria like *Clostridium botulinum* at pH levels lower than that reported in this study (Soltanizadeh and Ghiasi-Esfahani 2015). The pH of chicken meatballs gradually decreased with advancement of storage period. However, the rate of decline was lower in those formulated with ALWE.

The low pH during storage may be due to the growth of acid producing microorganisms such as lactic acid bacteria in the stored balls (Wang et al. 2013). The higher microbial growth rate in unformulated chicken meatballs may lead to

Table 3 pH, total plate countand yeast and mold count of rawchicken meatballs formulatedwith different concentration ofArgel leaf extracts during coldstorage

Extract Conc. %	Storage period (days)					
	0	5	10	15	20	
pН						
0	5.96 ± 0.01	5.89 ± 0.02	5.68 ± 0.00	5.55 ± 0.02	5.41 ± 0.00	
5	5.79 ± 0.00	5.69 ± 0.01	5.56 ± 0.02	5.47 ± 0.02	5.32 ± 0.02	
10	5.75 ± 0.01	5.64 ± 0.02	5.49 ± 0.00	5.37 ± 0.02	5.24 ± 0.01	
20	5.67 ± 0.02	5.60 ± 0.00	5.40 ± 0.01	5.25 ± 0.01	5.18 ± 0.00	
Total plate count (log cfu/g)						
0	$2.14\pm0.04t$	3.81 ± 0.05^{as}	5.09 ± 0.02^{ar}	6.18 ± 0.06^{aq}	8.47 ± 0.05^{ap}	
5	$2.11\pm0.01^{\rm r}$	2.70 ± 0.06^{br}	3.06 ± 0.02^{br}	4.15 ± 0.02^{bq}	$6.41\pm0.08^{\rm bp}$	
10	$2.10\pm0.02^{\rm r}$	2.15 ± 0.04^{bcr}	2.53 ± 0.04^{cr}	$4.09\pm0.02^{\mathrm{bq}}$	5.35 ± 0.06^{cp}	
20	$2.07\pm0.04q$	2.07 ± 0.01^{cq}	2.68 ± 0.01^{cq}	4.06 \pm 0.08 $^{\rm bp}$	4.31 ± 0.04^{dp}	
Yeast and Mold count (log cfu/g)						
0	ND	ND	3.21 ± 0.06^{ar}	4.40 ± 0.01^{aq}	5.72 ± 0.04^{ap}	
5	ND	ND	2.08 ± 0.01^{bq}	2.72 ± 0.09^{bq}	3.48 ± 0.03^{bp}	
10	ND	ND	$1.93 \pm 0.04^{\rm bc}$	$2.06\pm0.08^{\rm b}$	$2.32\pm0.01^{\rm c}$	
20	ND	ND	1.05 ± 0.28^c	$1.14 \pm 0.09^{\circ}$	$1.87\pm0.08^{\rm d}$	

Values are means of triplicate samples (\pm SD). Means not sharing a common superscript (s) a, b or c in a column or p, q or r in a row are significantly different at $P \le 0.05$ as assessed by Duncan's multiple range test. ND = not detected

higher acid production resulting in a sharp decline in the pH of the controls. The antimicrobial effect of ALWE may have attributed to the lower acid production and less pH reduction with increasing ALWE concentration. Similarly, Al-Juhaimi et al. (2017b) reported a significant decrease in the pH of chicken patties with and without pistachio hull water extract during the storage period. Moreover, Jayawardana et al. (2015) reported a reduction in the pH value in Moringa leaf powder-treated sausages during storage.

The microbial load of both formulated and unformulated chicken meatballs was significantly ($P \le 0.05$) increased with increase in storage period but decreased gradually with an increase in ALWE concentration. At day 0 of storage, no significant difference was observed in the total plate counts of the control and treated chicken meatballs. As storage progressed, the total plate count in all chicken meatballs significantly ($P \le 0.05$) decreased with the incorporation of ALWE and the effect was higher as the extract concentration increased. The low total plate count in ALWE-containing chicken balls during storage might be due to the lower pH of ALWE-containing chicken balls compared to the controls. Addition of ALWE at a concentration of 10 and 20 mg/100 mL to the chicken balls resulted in total plate counts that were lower than 6 log cfu/ g at the end of the storage period. The value was still within the acceptable range of less than 6 log cfu/g for meat products as recommended by the International Commission on Microbiology Specification for Food, (ICMSF 1983) and thus deemed safe for human consumption. Similarly, incorporation of Argel leaf water extract in camel patties (Al-Juhaimi et al. 2017a), pistachio hull water extract in chicken patties (Al-Juhaimi et al. 2017b), and Moringa leaf extracts in meat patties (Falowo et al. 2016) significantly lowered the total plate count and were thus assumed to prolong the shelf life of these products. Furthermore, Hawashin et al. (2016) reported that the total plate count of beef patties formulated with the destoned olive cake was significantly lower than that of the control during storage.

Yeast and mold growth were first observed after 10 days of storage with the ALWE-formulated chicken meatballs exhibiting significantly ($P \le 0.05$) lower values compared to the unformulated chicken meatballs. Increase in ALWE concentration significantly ($P \le 0.05$) lowered the yeast and mold counts of the chicken meatballs throughout the remaining storage period. This antimicrobial action might be due to the presence of considerable amounts of phenolic compounds with antimicrobial properties in the Argel leaves (Al-Juhaimi et al. 2017a; Hassan et al. 2001; Ounaissia et al. 2016). Sousa et al. (2006) reported that foods rich in polyphenols correlate with a broad range of physiological properties like antimicrobial characteristics. Incorporation of plant extracts rich in antioxidant and total phenolic contents, in the formulation of meat products has been reported to prolong the shelf life of the products (Al-Juhaimi et al. 2017a, b; Hawashin et al. 2016).

TPC, DPPH radical scavenging activity and TBARS of chicken meatballs extended with ALWE

The effect of incorporating ALWE at different concentrations into chicken meatballs on the total phenols, DPPH radical scavenging activity, and TBARS during refrigerated storage are shown in Table 4. Increase in concentration of ALWE in chicken balls concomitantly raised $(P \le 0.05)$ TPC and DPPH scavenging activity, showing the highest values (427.50 mg GAE/100 g sample and 32.35% inhibition, respectively) in products containing 20 mg/100 mL ALWE concentration. The increase in TPC and DPPH scavenging activity following incorporation of ALWE could be due to the substantially high amount of TPC (1262.50 mg GAE/g sample) and DPPH scavenging activity (86.85% inhibition) of ALWE. Similarly, studies have shown that an increase in the concentration of plant antioxidants incorporated into meat products caused a progressive increase in the TPC and DPPH scavenging activity of the products (Al-Juhaimi et al. 2017a; Elhadi et al. 2017; Jayawardana et al. 2015).

The TPC and DPPH scavenging activity of the control and treated chicken meatballs gradually decreased with advancement of storage and recorded the minimum value at day 20. This reduction during storage could be attributed to the utilization of antioxidant compounds and hydrolysis of the compounds to prevent oxidative rancidity of the product. Likewise, previous reports have shown a decrease in the TPC and DPPH radical scavenging activity of chicken (Al-Juhaimi et al. 2017b) and pork (Muthukumar et al. 2014) products during storage. The high TPC and DPPH scavenging activity of ALWE formulated chicken meatballs throughout the entire storage period indicated that incorporation of the extract countered free radicals and overcame the negative impact of storage, thus prolonging the shelf life of chicken meatballs during storage.

Incorporation of ALWE in chicken meatballs significantly ($P \le 0.05$) reduced lipid peroxidation (in terms of TBARS values) compared to that in unformulated chicken meatballs. Increasing the ALWE concentration in chicken meatballs concomitantly lowered ($P \le 0.05$) the TBARS, with the lowest value recorded in those treated with 20% ALWE concentration. These observations showed that ALWE confers stability of lipid oxidation to formulated chicken meatballs compared to the non-formulated ones. The TBARS of formulated and unformulated chicken meatballs increased with storage showing continuous aldehyde production in the products. However, meatballs formulated at 20 mg/100 mL ALWE concentration

Table 4 Oxidative characteristics of raw chicken meatballs formulated with different concentrations of Argel leaves extract during storage at 4 $^{\circ}$ C (± 1)

Extract Conc. %	Storage period (days)					
	0	5	10	15	20	
Total phenolic (mg C	Gallic acid/100 g sample)					
0	$177.08 \pm 0.71^{\mathrm{ap}}$	170.23 ± 0.12^{dq}	161.13 ± 0.21^{dr}	$155.34 \pm 0.65^{\rm ds}$	153.36 ± 0.71^{dt}	
5	238.34 ± 0.95^{ap}	230.19 ± 0.92^{cq}	$221.23 \pm 0.95^{\rm cr}$	203.62 ± 0.73^{cs}	$197.56 \pm 0.88^{\rm ct}$	
10	301.33 ± 0.11^{ap}	298.29 ± 0.86^{bq}	$290.59 \pm 0.74^{\rm br}$	281.76 ± 0.74^{bs}	274.38 ± 0.87^{bt}	
20	427.50 ± 0.13^{ap}	412.23 ± 1.09^{aq}	$401.46 \pm 2.12^{\rm ar}$	391.72 ± 0.88^{as}	379.79 ± 1.92^{at}	
DPPH (% inhibition)					
0	10.89 ± 0.25^{dp}	10.32 ± 0.75^{dp}	9.85 ± 0.50^{dp}	9.14 ± 0.09^{dpq}	8.82 ± 0.75^{dq}	
5	$14.21 \pm 0.50^{\rm cp}$	13.28 ± 0.50^{cq}	$12.46 \pm 0.25^{\rm cr}$	$11.92 \pm 0.50^{\rm cr}$	10.85 ± 0.50^{cs}	
10	18.28 ± 0.50 $^{\rm bp}$	16.64 ± 0.50^{bq}	$15.87 \pm 0.25^{\rm br}$	$14.82 \pm 0.25^{\rm bs}$	$14.28 \pm 0.50^{\rm bs}$	
20	32.35 ± 0.50^{ap}	28.89 ± 0.25^{aq}	$26.42 \pm 0.13^{\rm ar}$	21.89 ± 0.75^{as}	$19.71 \pm 0.50^{\rm at}$	
Thiobarbituric acid r	eactive substances (TBA	RS) in mg malonaldehyde	e/kg sample			
0	5.23 ± 0.14^{aq}	5.70 ± 0.37^{aq}	6.01 ± 0.19^{apq}	$6.63\pm0.11^{\rm ap}$	6.89 ± 0.31^{ap}	
5	5.01 ± 0.07^{aq}	5.46 ± 0.18^{apq}	6.00 ± 0.18^{ap}	$6.09\pm0.24b^{ap}$	$6.16\pm0.08^{\rm bp}$	
10	4.69 ± 0.06^a	$5.01\pm0.11^{\rm a}$	5.14 ± 0.06^{b}	$5.54\pm0.11^{\rm b}$	$5.28\pm0.10^{\rm c}$	
20	4.51 ± 0.15^{a}	$4.18\pm0.25^{\mathrm{b}}$	$4.43 \pm 0.15^{\circ}$	$4.63\pm0.15^{\rm c}$	$4.80\pm0.22^{\rm c}$	

Values are means of triplicate samples (\pm SD). Means not sharing a common superscript (s) a, b or c in a column or p, q or r in a row are significantly different at $P \le 0.05$ as assessed by Duncan's multiple range test

exhibited the lowest TBARS values (4.80 mg malonaldehyde/kg sample) at the end of the storage period (20 days). Addition of ALWE to chicken meatballs reduced the rate of lipid oxidation and could thus extend the shelf life of the product during storage. Similar findings of reduction of the rate of lipid oxidation following the incorporation of Argel leaf powder in camel patties (Al-Juhaimi et al. 2017a) and destoned olive cake powder in beef patties (Hawashin et al. 2016) have been reported. Furthermore, Al-Juhaimi et al. (2016) observed that Moringa seed powder could efficiently control lipid oxidation in beef patties during cold storage.

Sensory attributes of chicken meatballs extended with ALWE during cold storage

Table 5 shows the sensory panel results of cooked ALWE formulated and non-formulated chicken balls during cold storage. No change was observed in the sensory attributes of cooked chicken balls due to incorporation of ALWE. The addition of ALWE did not produce appreciable changes in the color, odor, flavor or texture and all the products were equally acceptable as evidenced by the overall acceptability scores. These results are in agreement with those obtained by Devatkal et al. (2010) who reported no significant change in the sensory traits of cooked goat meat patties due to the incorporation of pomegranate rind

and seed powder extracts. Despite the green color of the ALWE extract, no significant effect on the meatball color was observed; however, certain grey discolorations in cooked beef and chicken patties have been reported after incorporation of tea catechins in the patties (Mitsumoto et al. 2005). These differences could be due to the type and amount of plant powder or extracts, and the procedure of incorporation of the plant material into the formulated meat products. However, there is variation in all sensory traits for both formulated and unformulated chicken meatballs during storage. Reduction in sensory attributes was observed during subsequent storage days compared to the first day of storage. Hashawin et al. (2016) reported a slight reduction in sensory values during the storage of beef patties formulated with destoned olive cake. Our findings revealed that addition of ALWE in chicken meatballs could extend the shelf life of the product without affecting its sensory properties (Al-Juhaimi et al. 2017a).

Conclusion

This study revealed that ALWE has a high total phenolic content, anthocyanin levels, and DPPH scavenging activity. Addition of ALWE to chicken meatballs at higher concentrations (10 and 20 g/100 g) improved the lipid oxidation, antioxidant activity, and microbiological Table 5 Sensory characteristics of cooked chicken meatballs formulated with different concentrations of Argel leaf extracts during storage at 4 °C (\pm 1)

Storage period (days)	ALWE (%)					
	0	5	10	20		
Color						
0	$6.4 \pm 1.07^{\rm q}$	$7.0\pm0.94^{\mathrm{apq}}$	$6.9 \pm 1.19^{\mathrm{apq}}$	7.7 ± 1.15^{ap}		
5	7.0 ± 1.05	$7.1 \pm 0.99^{\mathrm{a}}$	$7.0\pm0.81^{\rm a}$	7.2 ± 0.78^{a}		
10	6.4 ± 1.07	$6.0 \pm 1.24^{\rm b}$	$5.7 \pm 1.33^{\mathrm{b}}$	$6.4\pm1.42^{\rm b}$		
20	6.2 ± 1.13	6.1 ± 1.37^{b}	$5.9 \pm 1.10^{\rm b}$	$5.6\pm0.96^{\rm c}$		
Flavor						
0	7.0 ± 1.15^{a}	$7.3 \pm 1.05^{\rm a}$	7.1 ± 1.10^{a}	$6.9\pm0.87^{\rm a}$		
5	$5.6\pm1.57^{\rm c}$	$5.5\pm1.58^{\rm b}$	$5.4 \pm 1.42^{\rm b}$	$5.4 \pm 1.89^{\mathrm{b}}$		
10	$5.8 \pm 1.22^{\circ}$	$5.6 \pm 1.17^{\mathrm{b}}$	$5.8\pm1.03^{\rm b}$	$5.5\pm1.08^{\rm b}$		
20	$6.0 \pm 1.33^{\rm bc}$	$6.1 \pm 1.28^{\mathrm{a}}$	6.6 ± 1.07^{a}	6.3 ± 1.15^a		
Taste						
0	6.6 ± 1.26^a	$7.2 \pm 1.13^{\mathrm{a}}$	$6.9 \pm 1.52^{\rm a}$	6.7 ± 1.15^a		
5	$5.7 \pm 1.05^{\mathrm{b}}$	$5.8 \pm 1.22^{\mathrm{b}}$	6.1 ± 1.44^{a}	$5.4 \pm 1.64^{\rm b}$		
10	5.5 ± 1.26^{b}	$4.8\pm1.13^{\rm b}$	$5.3 \pm 1.25^{\mathrm{b}}$	$5.2\pm1.61^{\rm b}$		
20	6.5 ± 1.58^{a}	$6.4 \pm 1.34^{\mathrm{a}}$	6.4 ± 1.64^{a}	$5.7\pm1.05^{\rm b}$		
Texture						
0	$6.8\pm0.91^{\rm a}$	7.0 ± 0.81^{a}	7.2 ± 1.03^{a}	$7.1 \pm 1.44^{\rm a}$		
5	$6.4\pm0.96^{\rm a}$	$6.2 \pm 1.39^{\mathrm{ab}}$	$5.6 \pm 1.57^{\mathrm{b}}$	$5.6\pm1.71^{\rm b}$		
10	$5.7 \pm 1.49^{\rm b}$	$5.8\pm1.47^{\rm b}$	$5.5\pm1.17^{\rm b}$	5.5 ± 1.43^{b}		
20	6.0 ± 2.21^{ab}	6.3 ± 1.41^{ab}	$5.9 \pm 1.85^{\mathrm{b}}$	5.8 ± 1.75^{b}		
Juiciness						
0	$7.2\pm1.31^{\rm a}$	7.1 ± 1.10^{a}	7.0 ± 1.05^a	$7.1\pm0.99^{\rm a}$		
5	$5.3\pm1.25^{\rm b}$	$5.1 \pm 1.37^{\mathrm{b}}$	$5.3 \pm 1.56^{\mathrm{b}}$	5.5 ± 1.58^{b}		
10	$6.1\pm1.37^{\rm a}$	6.4 ± 1.64^{a}	6.5 ± 1.58^a	$5.9 \pm 1.66^{\rm b}$		
20	6.6 ± 1.42^{a}	$6.2\pm1.54^{\rm a}$	6.9 ± 1.19^{a}	6.3 ± 1.05^a		
Overall acceptability						
0	6.8 ± 0.78^{a}	$7.0\pm0.66^{\rm a}$	7.1 ± 1.19^{a}	7.2 ± 1.03^a		
5	5.7 ± 0.82^{ab}	$5.8\pm1.03^{\rm b}$	5.7 ± 1.49^{b}	5.8 ± 1.68^{b}		
10	6.1 ± 0.99^{ab}	6.2 ± 0.91^{ab}	6.4 ± 0.69^{ab}	$6.3\pm1.15^{\rm b}$		
20	6.0 ± 1.15^{ab}	6.4 ± 1.07^{ab}	6.6 ± 1.34^{ab}	$6.1\pm0.99^{\rm b}$		

Values are means of 20 samples (\pm SD). Means not sharing a common superscript(s) a, b, or c in a column or p, q, or r in a row are significantly different at $P \le 0.05$ as assessed by Duncan's multiple range test

stability without adversely affecting the sensory attributes of the product. Therefore, aqueous extracts from Argel leaves can be applied as functional ingredients to prolong the shelf-life and likely to improve health beneficial properties of chicken meatballs.

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