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Antioxidant and Antimicrobial Potential of Artichoke (*Cynara scolymus* L.) Extract in Beef Patties

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Abstract

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The antioxidant and antimicrobial potential of artichoke extract (AE) in raw beef patties (RBPs) was evaluated during the storage. The RBPs were prepared with the addition of 500 and 1000 ppm AE. Also, *Escherichia coli* ATCC25922 and *Listeria monocytogenes* ATCC19118 were inoculated to each RBP to follow the antimicrobial activity. An evaluation of the instrumental colour, pH, total phenolic content (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, TBARS and microbiological properties was carried out during storage. The evaluation revealed that the *a** value decreased significantly, so that the reduction level of the AE500 and AE1000 samples during storage was 35, 57, and 56%, respectively, in the control. AE addition slightly decreased the pH of RBPs, which varied between 5.72 and 5.96. The TPC and DPPH values of samples with added 500 and 1000 ppm AE were 2 to 3-fold and 3 to 7-fold higher than in the control, respectively. Also, the TBARS values were determined as 43 and 54% lower than in the control at the end of storage when 500 and 1000 ppm AE were used. The AE in RBP inhibited the viability of total aerobic psychrophilic bacteria, coliform bacteria and yeast-mould in a concentration-dependent manner. AE prevented the growth of *E. coli* ATCC25922 and *L. monocytogenes* ATCC19118 inoculated to the RBPs. In conclusion, 1000 ppm AE was sufficient for antioxidant and antimicrobial activity in RBP. To our knowledge, this is the first study that presents the antioxidant and antimicrobial activity of AE used in a food model system.

Keywords: *Escherichia coli*; *Listeria monocytogenes*; meat product; natural antioxidant; plant extract

Meat and meat products contain micro- and macro-nutrients that are essential for a well-balanced and healthy diet (Biesalski 2005). However, meat products spoil easily due to microbial growth or chemical reactions, such as oxidation, which may lead to health risks. In patty-like products that are produced from minced meat, the surface area, free water level, and pH of the product are increased, because the cellular integrity is disrupted as a result of size reduction. Consequently, as well as promoting the oxidative changes, these changes create a proper environment for the growth of spoilage and pathogenic microorganisms (Kim *et al.* 2013).

Several studies have shown that synthetic or natural preservatives could be used to control microbial spoilage and lipid oxidation (Jayasena & Jo 2013; Karre *et al.* 2013; Falowo *et al.* 2014). The level of interest that has been shown in natural antioxidants in meat products at utilization has increased significantly, especially since consumers became concerned about the use of synthetic antioxidants, due to their potential toxicological effects (Choe *et al.* 2011; Karre *et al.* 2013; Falowo *et al.* 2014). Several plant sources have shown natural antimicrobial and antioxidant properties, and different kinds of vegetables, fruits, aromatic plants and spices could be used for this purpose (Hygreeva *et al.* 2014). In particular, plants that contain phenolic compounds and OH groups in phenolic compounds are thought to be responsible for their antioxidant and antimicrobial actions (Kim *et al.* 2013). Phenolic compounds show their antimicrobial effects by impairing the function of the bacterial cell wall and inhibiting the function of bacterial enzymes (Van Dijck & Van de Voorde

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1976). Likewise, the antioxidant properties of these compounds are related to their H-donating activity (Falowo *et al.* 2014).

The artichoke (*Cynara scolymus* L.), which belongs to the family *Asteraceae*, grows up to 50–150 cm tall and has purple flowers. It is native to the Mediterranean region (Lattanzio *et al.* 2009). The artichoke possesses some remedial properties, and its benefits are mainly due to the high content of polyphenols and inulin. The major constituents of artichoke phenolics are the most important caffeic acid derivatives and caffeoylquinic acid (Cynarin), chlorogenic acid and flavonoids, such as luteolin and apigenin. The artichoke is used as a hepatoprotective, anticarcinogenic, antioxidative, antibacterial, anti-HIV and hypocholesterolaemic agent. Artichokes exhibit a wide range of pharmacological effects, which are provided by the synergistic roles of many compounds they contain (Lattanzio *et al.* 2005). Artichoke hearts are mostly used in the canned food industry and the remaining parts, such as the outer bracts and leaves, are referred to as by-products. These by-products (approximately 60–80% of the total plant) are used to produce herbal food supplements, dietary fibre and animal feed. The outer leaves of the artichoke could be used as a natural additive due to their rich phenolic content, which has antimicrobial and antioxidant effects (Lattanzio *et al.* 2009). However, to our knowledge, the use of artichoke by-products in food matrices has not been previously reported. To contribute to the literature, this study aims to evaluate the antioxidant and antimicrobial potential of artichoke extract (AE) in raw beef patties (RBPs) during storage by inoculating important pathogens like *Escherichia coli* ATCC25922 and *Listeria monocytogenes* ATCC19118.

MATERIAL AND METHODS

The fresh, boneless beef meat was purchased from a local meat-processing plant (Pinar a.s., Turkey). All the chemicals and reagents used in this study were purchased from Merck (Merck, Germany) and Sigma Chemicals (Sigma-Aldrich, Germany).

Preparation of the artichoke by-product extract. The artichoke by-products (external bracts) were supplied by a local canning plant (Feast, Turkey) and transported to the laboratory, where they were washed under running tap water. The bracts were dried in a laboratory tray dryer (Armfield UOP8; UK) at 40°C until moisture content reached 12% and then were ground in a hammer mill (Brook Crompton, UK) to a particle size of 0.65 mm. Twenty grams of the dried and ground residue were macerated with 100 ml of ethanol 80% (v/v) under constant mechanical agitation on a rotary shaker at 40°C for 4 hours. The extract was subsequently filtered (12.5 mm qualitative filter paper), and the filtrate was concentrated in a vacuum rotary evaporator (IKA RV8; Germany) at 45°C until all the ethanol was evaporated. The aqueous extract was stored in dark glass bottles under frozen storage (–40°C). The extract obtained by evaporation was analysed for total phenolic content, DPPH radical scavenging activity was also incorporated into the beef meat patties.

Preparation and storage of beef patties. Beef meat (boneless rib and round; moisture 60.83% ± 0.98, protein 16.80% ± 0.41, fat: 19.22% ± 0.86) 1–2 days *post mortem*, trimmed of all visible connective tissue. The beef was minced in a conventional meat grinder (Ari Makina PKM 12; Turkey) through a 3 mm matrix. The freshly minced beef was assigned to one of the following three treatments: control (no antioxidant additive), 500 and 1000 ppm artichoke extract (AE) and 1.5% NaCl were added to each formulation. The patties were formed with a circular stainless steel shaper (5 cm diameter and 1 cm thickness) and stored in polythene bags at 4 ± 1°C for seven days. For sampling (days 0, 1, 3, 5, and 7), the beef patties were removed from the refrigerator and analysed for colour, pH, total phenolics, DPPH radical scavenging activity, and TBARS.

To investigate the effect of AE on microbiological quality, *E. coli* ATCC25922 and *L. monocytogenes* ATCC19118 strains were inoculated, as 4- and 6-log, respectively, to the patties. For this purpose, seven separate experimental patty groups were prepared as follows: the first group CE (positive control) included only *E. coli* ATCC25922, the second group CL (positive control) included only *L. monocytogenes* ATCC19118, the third and fourth treatment groups AE500E and AE1000E included *E. coli* ATCC25922 and AE at 500 or 1000 ppm concentrations, the fifth and sixth treatment groups AE500L and AE1000L included *L. monocytogenes* ATCC19118 and AE at 500 or 1000 ppm concentrations and the seventh group NC (negative control) did not contain any AE or indicator microorganisms.

Instrumental colour and pH measurements. The instrumental colour analysis was performed using a HunterLab colorimeter (Miniscan XE Plus; USA).

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For determining the pH, 10 g of sample was homogenized with 50 ml distilled water and the pH value was measured using a digital pH meter (Crison Basic 20; Spain).

Total phenolic content (TPC). The RBPs were analysed for total phenolic content using the Folin-Ciocalteu (F-C) assay after 1 g of the patty was homogenized with 10 ml of methanol and kept overnight for extraction at refrigeration temperature (Li *et al*. 2006).

Antioxidant scavenging activity against DPPH. The ability of the patty extract (which was prepared for the F-C assay, above) to scavenge DPPH radicals was estimated using the method devised by Wang *et al.* (2003) and Fratianni *et al.* (2010), together with slight modifications. Aliquots of 0.1 ml patty extracts were mixed with 5 ml of 0.1 mM (prepared in methanol) DPPH radical in a test tube. The mixture was allowed to stand for 20 min at room temperature before the absorbance was measured at 517 nm spectrophotometrically. The scavenging activity was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \\ = [(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{blank}}] \times 100$$

The absorbance of blank is the absorbance of the control reaction (containing all reagents except the test compound), and the absorbance of the sample is the absorbance of the test compound read (517 nm).

TBARS values. TBARS were determined using the extraction method described by Witte *et al.* (1970). TBARS numbers were calculated as mg of malonaldehyde per kg of meat (mg malondialdehyde/kg).

Microbiological analysis. To evaluate the microbiological quality of the RBPs, and the antimicrobial effects of AE on *E.coli* ATCC25922 and *L. monocytogenes* ATCC19118 strains, 10 g of patty samples were used and serially diluted in MRD. Then, coliform bacteria (CB), total aerobic psychrophilic bacteria (TAPB) and yeast-mould (YM) were determined on Violet Red Bile agar (VRB; Merck, Germany), Plate Count Agar (PCA; Merck, Germany), and Dichloran Rose Bengal Chloramphenicol agar (DRBC; Merck, Germany) where Sorbitol MacConkey or PALCAM agar (Merck, Germany) were used for enumerations of *E. coli* ATCC25922 or *L. monocytogenes* ATCC19118 inoculated to the RBPs, respectively. The plates were incubated at 10°C for 48 h for TAPB; 37°C for 24 h for CB and *E. coli* ATCC25922 and 30°C for 48 h for YM and *L. monocytogenes* ATCC19118.

Statistical analysis. The RBPs were produced twice, and all the analyses were performed in duplicate. Analyses of variance (ANOVA) and Duncan's tests, using SPSS for Windows (v. 15.0), were carried out to study the effect of the addition of AE on the measured parameters. Differences were considered significant at $P < 0.05$. The average values were reported, along with standard deviation (± SD).

RESULTS AND DISCUSSION

Instrumental colour and pH values. Colour plays an important role in both the quality and the acceptance by consumers of meat and meat products. The instrumental colour values are given in Table 1. The L^* values of the samples were between 45.98 and 51.03, and there was a significant difference between storage days ($P < 0.05$). During the storage period, no significant differences were observed in the control and 1000 ppm AE samples ($P > 0.05$), but there was a significant difference in the 500 ppm AE samples. Also, we found higher L^* in the AE1000 samples compared to the control or AE500 samples, on all storage days. Although similar to our results, the powdered additives can bind free water and cause a decrease in L^*, it was reported that adding the liquid antioxidant increased L^* (Fernandez-Lopez *et al.* 2005). On the other hand, lipid oxidation leads to colour darkening of meat samples, which can lead to lower L^*. In our study, a comparison of the natural antioxidant group with the control group showed that natural antioxidants did not cause a decrease, but rather a small increase in the L^* value of the sample during storage on the seventh day, compared with the first day. Different L^* values have been observed in studies that use natural antioxidants in meat products (Fernandez-Lopez *et al.* 2005; Abdel-Aziz & Morsy 2015; de Almeida *et al.* 2015).

The most important criterion for the evaluation of oxidation is a^* (redness), and the decrement of redness in meat is considered as an indicator of oxidation. In this study, the a^* value of the samples was found to be between 4.38 and 15.43. There was a significant difference between storage days ($P < 0.05$) and the highest a^* value for all storage days was observed in the control groups. Similarly, the redness of all groups significantly decreased during storage ($P < 0.05$).

The redness of all the samples clearly decreased between day 1 and day 0, or between day 3 and day 1;

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Table 1. Colour parameters (L^*, a^*, and b^*) and pH values of raw beef patties

		Storage period (days)				
		0	1	3	5	7
	control	48.21 ± 0.51^{b}	47.72 ± 0.44^{b}	48.12 ± 0.75^{b}	48.34 ± 0.71^{b}	48.40 ± 0.75^{b}
L*	AE500	47.08 ± 1.49^{bAB}	47.29 ± 0.85^{bAB}	45.98 ± 0.59^{cB}	48.35 ± 0.88^{bA}	48.21 ± 0.33^{bA}
	AE1000	50.15 ± 1.36^{a}	49.55 ± 0.35^{a}	49.77 ± 1.29^{a}	50.97 ± 0.92^{a}	51.03 ± 0.82^{a}
	control	15.43 ± 1.12^{aA}	10.78 ± 0.69^{aB}	9.31 ± 1.13^{aB}	9.42 ± 1.54^{aB}	10.04 ± 0.81^{aB}
a*	AE500	13.60 ± 0.96^{bA}	9.12 ± 1.76^{aB}	7.47 ± 0.46^{bC}	6.25 ± 0.45^{bC}	5.85 ± 0.98^{bC}
	AE1000	9.92 ± 0.96^{cA}	6.89 ± 0.29^{bB}	4.85 ± 0.43^{cC}	4.47 ± 0.66^{cC}	4.38 ± 0.24^{cC}
	control	19.54 ± 0.56^{A}	16.91 ± 0.46^{bB}	15.96 ± 0.10^{bC}	16.73 ± 0.35^{bB}	17.36 ± 0.67^{aB}
b*	AE500	19.33 ± 0.66^{A}	17.48 ± 0.69^{abB}	16.74 ± 0.16^{aB}	15.69 ± 0.71^{abC}	15.66 ± 0.64^{bC}
	AE1000	18.54 ± 0.87^{A}	17.88 ± 0.32^{aAB}	17.05 ± 0.44^{aB}	16.96 ± 0.98^{aB}	16.86 ± 0.47^{aB}
	control	5.93 ± 0.05^{aA}	5.86 ± 0.04^{aB}	5.85 ± 0.01^{aB}	5.93 ± 0.01^{aA}	5.90 ± 0.05^{bAB}
pH	AE500	5.90 ± 0.01^{aB}	5.81 ± 0.02^{aC}	5.81 ± 0.01^{bC}	5.91 ± 0.01^{aB}	5.96 ± 0.02^{aA}
	AE1000	5.78 ± 0.01^{bC}	5.72 ± 0.01^{bD}	5.73 ± 0.01^{cD}	5.84 ± 0.02^{bB}	5.89 ± 0.01^{bA}

$^{a–c}$values in the column with different letters are significantly different ($P < 0.05$); $^{A–D}$values in the row with different letters are significantly different ($P < 0.05$); AE500 – 500 ppm artichoke by-product extract; AE1000 – 1000 ppm artichoke by-product extract; values are means ± standard deviations; samples were stored at 4 ± 1°C for 7 days

however, after the third day, the colour loss appeared to slow down in the patty samples. On day 7, lipid oxidation was successfully blocked; although a^* of the AE1000 samples reached the lowest value (Table 1). The AE used in this study contains greenish-brownish colour pigments, which may cause contradictory results by decreasing the redness of patty samples. The negative correlation between concentrations and the a^* value further supported this conclusion.

The desired redness of the AE added samples could not be maintained as expected. The AE used in this study contained greenish-brownish colour pigments, which may have caused the decreasing redness of the patty samples; however, the AE samples inhibited lipid oxidation to a greater extent in this study. In some studies, in which natural antioxidants were used, including tea catechins and pomegranate juice and rind extract, decreased a^* was found, similar to the results of our study (Kroll & Rawel 2001; Mitsumoto *et al.* 2005; McBride *et al.* 2007; Akarpat *et al.* 2008; Banerjee *et al.* 2012).

In this study, the b^* values of RBP were determined to be different in all groups (except on day 0) compared with the control groups ($P < 0.05$). In addition, the b^* value of the samples was found to be significantly different for all storage days ($P < 0.05$). In the control groups the yellowness of the samples showed fluctuations which decreased until day 3 and increased on the following days. In the AE500 and AE1000 groups, b^* continuously decreased during storage. The b^* value of the samples ranged between 15.66 and 19.54. Similar to the a^* values, the decreases in b^* could be affected by the colour pigments of AE.

The pH values were significantly different on all days and in all samples during storage ($P < 0.05$). Between the first day and the third day, the pH values decreased, while they increased on the fifth and the seventh day due to microbiological spoilage (Table 1). There was a negative correlation between the doses and pH values, which is related to the acidic pH of the AE. Similar to our results, acidic extracts, like pomegranate shell and seed extracts, were shown to decrease the pH of the samples (Devatkal *et al.* 2010).

Total phenolic content (TPC) and antioxidant scavenging activity. The total phenolic content and DPPH radical scavenging activity of the RBPs are given in Table 2. The radical scavenging activity and total phenolic content of artichoke are mostly ascribed to a wide range of caffeoylquinic acid derivatives with chlorogenic acid (5-*o*-caffeoylquinic acid) and other phenolics, such as the flavonoids apigenin and luteolin (Lattanzio *et al.* 2009). The total amount of phenolic compounds in the RBPs ranged between 8.34 and 117.99 mg gallic acid/100 g meat. On all the experimental days, the highest phenolic compounds were observed in the AE1000 group, which is related to the doses of the additive. At the end of day 7, the phenolic content of the AE1000 group was 53.69 mg gallic acid/100 g meat. Compared with the day 1 re-

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Table 2. DPPH radical scavenging activity, total phenolic content (TPC) and thiobarbituric acid reactive substances (TBARS) value of RBP

	Storage period (days)				
	0	1	3	5	7
DPPH (%)					
Control	39.12 ± 0.21^{cA}	30.81 ± 1.43^{cB}	22.17 ± 0.73^{cC}	16.63 ± 1.08^{cD}	9.74 ± 0.57^{cE}
AE500	45.61 ± 1.44^{bA}	38.17 ± 2.21^{bB}	30.91 ± 1.93^{bC}	23.03 ± 0.74^{bD}	16.66 ± 1.69^{bE}
AE1000	60.34 ± 1.13^{aA}	51.91 ± 0.42^{aB}	42.81 ± 1.46^{aC}	34.37 ± 2.20^{aD}	26.64 ± 1.08^{aE}
TPC (mg gallic acid/100 g meat)					
Control	22.69 ± 1.01^{cA}	17.60 ± 0.43^{cB}	14.43 ± 0.44^{cC}	12.25 ± 0.68^{cD}	8.34 ± 0.43^{cE}
AE500	64.77 ± 0.82^{bA}	51.77 ± 2.51^{bB}	42.85 ± 1.17^{bC}	30.42 ± 1.66^{bD}	23.25 ± 2.05^{bE}
AE1000	117.99 ± 3.99^{aA}	99.82 ± 0.80^{aB}	82.16 ± 1.55^{aC}	61.09 ± 1.52^{aD}	53.69 ± 2.36^{aE}
TBARS (mg MA/kg meat)					
Control	0.38 ± 0.01^{E}	0.56 ± 0.01^{aD}	0.64 ± 0.01^{aC}	0.98 ± 0.02^{aB}	1.26 ± 0.03^{aA}
AE500	0.38 ± 0.01^{E}	0.43 ± 0.01^{cD}	0.50 ± 0.01^{bC}	0.62 ± 0.01^{bB}	0.72 ± 0.03^{bA}
AE1000	0.38 ± 0.02^{D}	0.46 ± 0.01^{bC}	0.48 ± 0.01^{cC}	0.53 ± 0.01^{cB}	0.58 ± 0.01^{cA}

$^{a–c}$values in the column with different letters are significantly different ($P < 0.05$); $^{A–E}$values in the row with different letters are significantly different ($P < 0.05$); AE500 – 500 ppm artichoke by-product extract; AE1000 – 1000 ppm artichoke by-product extract; values are means ± standard deviations; samples were stored at 4 ± 1°C for 7 days

sult (117.99 mg/100 g), the AE1000 group preserved approximately 50% of their phenolic content. On the other hand, the control group and the AE500 group lost more than 60% of their phenolic content. In addition, the TBARS values of the AE1000 group were significantly lower at the end of day 7, and these results showed that 1000 ppm AE could prevent lipid oxidation (Table 2). TPC significantly decreased in all samples during storage, and the highest decrease was detected in the AE500 group ($P < 0.05$).

The by-products of artichoke, especially the outer bracts, contain rich phenolic compounds. However, to our knowledge, there has not been any research into the usage of artichoke as a natural antioxidant additive. In several studies, many natural antioxidant sources, such as pomegranate rind and beet (El-Gharably & Ashoush 2011), blueberry and dried plum purees (Leheska *et al.* 2006), pomegranate shell, pomegranate seed and mandarin shell (Devatkal *et al.* 2011) were added to different meat products, and these additives increased the antioxidant capacity of the related products.

In this study, the addition of AE to RBPs showed higher antiradical scavenging activity compared with the control group. At the same time, DPPH antiradical scavenging activity showed differences during storage ($P < 0.05$), and the highest level of antioxidant activity (26.64%) was determined in the AE1000 samples. Previous studies on the antioxidant properties of phenolic compounds have shown that the use of herbal additives that have a high phenolic compound content maintains the antioxidant activity at the highest levels during shelf life (Biswas *et al*. 2012; Gallo *et al.* 2012). AE extracts showed sustainable antioxidant activity throughout the storage period.

In this study, trace amounts of phenolic compounds were determined in the control group, and antiradical activity was determined in the samples. This is believed to result from the naturally occurring antioxidant system present in the meat, which protects it from oxidative damage.

The results of TBARS analysis are shown in Table 2. TBARS values in the patty samples showed significant differences between the groups. Similarly, the duration of storage had an effect on the TBA number ($P < 0.05$). On all storage days, the AE1000 samples had the lowest TBARS values. At the end of the storage period, the TBARS value in the control group was three times higher than its baseline value. On the other hand, the TBARS values in the extract-containing groups were lower than 1 mg MDA/kg meat, which is considered as the safe limit. At the end of day 7, the TBARS values in the control, AE500 and AE1000 samples were 1.26, 0.72, and 0.58 mg MDA/kg meat, respectively. The results showed that the phenolic compounds in the artichoke inhibited lipid oxidation, by showing antioxidant effects. Multiple studies have also shown that various herbal extracts have

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Table 3. Microbiological results of RBP prepared with or without AE

Day	Treatments	Microbiological results (log CFU/g)				
		E. coli ATCC25922	*L. monocytogenes* ATCC19118	CB	YM	TAPB
0	NC	3.54 ± 0.09^{Aa}		4.85 ± 0.14^{Ac}	3.62 ± 0.02^{Aa}	4.60 ± 0.01^{Aabc}
	CE	4.00 ± 0.01^{Ab}		4.01 ± 0.20^{Ae}	3.82 ± 0.01^{Ab}	4.48 ± 0.26^{Aad}
	AE500E	4.00 ± 0.01^{Ab}		4.24 ± 0.29^{Ad}	3.87 ± 0.06^{Ab}	4.64 ± 0.01^{Abc}
	AE1000E	4.24 ± 0.34^{Ab}		5.00 ± 0.01^{Ac}	3.60 ± 0.14^{Aa}	4.54 ± 0.03^{Aabd}
	CL		6.41 ± 0.01^{Aa}	4.15 ± 0.21^{Aa}	4.12 ± 0.01^{Ac}	4.72 ± 0.02^{Ac}
	AE500L		6.36 ± 0.03^{Aa}	4.80 ± 0.14^{Ab}	3.84 ± 0.01^{Ab}	4.54 ± 0.04^{Aabd}
	AE1000L		6.36 ± 0.15^{Aa}	4.00 ± 0.21^{Ab}	3.68 ± 0.18^{Aa}	4.45 ± 0.01^{Ad}
1	NC	3.87 ± 0.04^{Ba}		4.57 ± 0.07^{Bb}	4.07 ± 0.16^{Ba}	6.25 ± 0.01^{Ba}
	CE	4.48 ± 0.01^{Bb}		4.78 ± 0.01^{Bde}	4.26 ± 0.04^{Bb}	6.22 ± 0.03^{Ba}
	AE500E	4.30 ± 0.01^{Bc}		4.15 ± 0.21^{Bc}	3.75 ± 0.02^{Bc}	5.11 ± 0.04^{Bc}
	AE1000E	4.39 ± 0.12^{Ad}		4.90 ± 0.01^{Be}	3.52 ± 0.11^{Ad}	4.30 ± 0.09^{Be}
	CL		6.80 ± 0.01^{Ba}	4.71 ± 0.08^{Bd}	4.12 ± 0.04^{Aa}	6.76 ± 0.10^{Bb}
	AE500L		6.27 ± 0.04^{Bb}	3.68 ± 0.11^{Cb}	3.68 ± 0.06^{Bc}	5.04 ± 0.02^{Bd}
	AE1000L		6.31 ± 0.02^{Ab}	3.69 ± 0.02^{Ba}	3.69 ± 0.11^{Ac}	4.32 ± 0.01^{Be}
3	NC	4.64 ± 0.19^{Ca}		5.65 ± 0.04^{Ca}	5.95 ± 0.01^{Ca}	7.64 ± 0.01^{Ca}
	CE	5.36 ± 0.05^{Cb}		5.31 ± 0.01^{Cb}	5.33 ± 0.17^{Cb}	7.42 ± 0.03^{Cb}
	AE500E	3.68 ± 0.02^{Cc}		3.94 ± 0.09^{Bc}	4.65 ± 0.02^{Cc}	6.39 ± 0.02^{Cc}
	AE1000E	4.01 ± 0.01^{Bd}		4.16 ± 0.05^{Cd}	3.09 ± 0.27^{Bf}	4.39 ± 0.02^{Cd}
	CL		7.09 ± 0.01^{Ca}	6.30 ± 0.01^{Ce}	5.59 ± 0.02^{Bd}	7.66 ± 0.03^{Ca}
	AE500L		6.15 ± 0.03^{CDb}	2.74 ± 0.06^{Abf}	4.05 ± 0.12^{Ce}	6.21 ± 0.07^{Ce}
	AE1000L		5.97 ± 0.02^{Bc}	2.15 ± 0.21^{Dg}	3.22 ± 0.11^{Bf}	4.06 ± 0.02^{Cf}
5	NC	5.12 ± 0.09^{Da}		7.92 ± 0.02^{Da}	5.91 ± 0.09^{Ca}	8.61 ± 0.11^{Da}
	CE	6.20 ± 0.10^{Db}		7.74 ± 0.06^{Db}	6.10 ± 0.01^{Db}	8.34 ± 0.06^{Db}
	AE500E	3.69 ± 0.08^{Cc}		3.96 ± 0.05^{Bc}	4.99 ± 0.08^{Dc}	8.29 ± 0.01^{Db}
	AE1000E	3.87 ± 0.01^{Bd}		4.01 ± 0.01^{Dc}	2.80 ± 0.14^{Cd}	6.39 ± 0.01^{Dc}
	CL		7.08 ± 0.05^{Ca}	8.50 ± 0.04^{Dd}	6.20 ± 0.05^{Ce}	8.85 ± 0.06^{Dd}
	AE500L		6.18 ± 0.04^{Cb}	2.69 ± 0.12^{Abe}	3.74 ± 0.01^{Df}	8.29 ± 0.06^{Db}
	AE1000L		5.67 ± 0.02^{Cc}	2.45 ± 0.21^{BCf}	3.08 ± 0.05^{Bg}	5.22 ± 0.07^{De}
7	NC	6.06 ± 0.11^{Ea}		8.91 ± 0.03^{Ea}	5.94 ± 0.01^{Ca}	9.00 ± 0.01^{Ea}
	CE	6.87 ± 0.07^{Eb}		8.34 ± 0.05^{Eb}	6.27 ± 0.09^{Eb}	8.32 ± 0.09^{Db}
	AE500E	3.83 ± 0.06^{Dc}		3.98 ± 0.07^{Bc}	4.74 ± 0.09^{Ec}	8.36 ± 0.13^{Db}
	AE1000E	3.37 ± 0.10^{Cd}		3.98 ± 0.02^{Ec}	2.92 ± 0.11^{BCd}	6.43 ± 0.07^{Dd}
	CL		6.85 ± 0.04^{Da}	8.70 ± 0.08^{Ed}	6.09 ± 0.11^{De}	8.39 ± 0.02^{Eb}
	AE500L		6.12 ± 0.01^{Db}	2.59 ± 0.16^{Be}	3.86 ± 0.11^{Af}	8.32 ± 0.03^{Db}
	AE1000L		5.70 ± 0.07^{Cc}	2.39 ± 0.12^{Cf}	2.63 ± 0.21^{Cg}	6.04 ± 0.06^{Ed}

$^{a–c}$values in the column with different letters are significantly different ($P < 0.05$); $^{A–E}$values in the row with different letters are significantly different ($P < 0.05$); values are means ± standard deviations; samples were stored at 4 ± 1°C for 7 days; CB – coliform bacteria; YM – yeast and mould; TAPB – total aerobic psychrophilic bacteria; NC – negative control; CE and CL – patties including *E. coli* ATCC25922 and *L. monocytogenes* ATCC19118; AE500E and AE1000E – patties prepared with AE and including *E. coli* ATCC25922; AE500L and AE1000L – patties prepared with AE and including *L. monocytogenes* ATCC19118

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antioxidant effects on different meat products. For instance, pomegranate peel extract in beef meatballs during refrigerated storage (Turgut *et al.* 2016) and grape dietary fibres in chicken hamburgers (Sayago-Ayerdi *et al.* 2009) could inhibit lipid oxidation by maintaining low TBARS values during storage, when compared with control groups.

The microbial quality and the viability. The TAPB were 4.56 log CFU/g on average when the RBP samples were prepared. The TAPB increased accordingly with the storage of all the experimental groups (Table 3). However, the TAPB were lower in the samples, including AE, depending on the concentration. In fact, the TAPB rose rapidly in patties without AE. At the end of the storage, the TAPB were 2.5 log lower in the RBP, including 1000 ppm AE (AE1000E and AE1000L) than in the control RBP (CE and CL). These results indicate that the addition of AE to patties inhibits the growth of psychrophilic bacteria in a concentration-dependent manner.

The CB in the control RBP groups (NC, CE and CL) doubled at the end of storage ($P < 0.05$). As expected, the CB increased rapidly in the RBP (CE) harbouring *E.coli* ATCC25922 but not containing AE. Adversely, the CB in the RBPs, including AE (AE500E and AE1000E), slightly decreased during storage ($P < 0.05$). Accordingly, the CB content decreased by 6% and 20% after seven days storage of the AE500E and AE1000L patties, respectively. Also, the CB were found to be 46% and 40% lower in the AE500L and AE1000L patties (Table 3). These results clearly showed that the added AE inhibited the growth of the CB existing spontaneously in the patty flora.

The YM increased during storage in the patties (NC, CE and CL) that did not contain AE ($P < 0.05$). The average 3.85 log CFU/g YM content of these samples increased to 6.10 log CFU/g at the end of seven days storage (Table 3). A slight increase was observed in the patties (AE500E and AE500L) containing 500 ppm AE ($P < 0.05$), but when the AE concentration increased to 1000 ppm in the patties (AE1000E and AE1000L), a 1-log decrease in YM occurred ($P < 0.05$). These results indicated that AE could inhibit the growth of YM in RBPs in a concentration-dependent manner.

In this study, an analysis was also carried out of the antimicrobial activity of AE against pathogenic bacteria (Table 3). *E. coli* ATCC25922 and *L. monocytogenes* ATCC19118 strains were inoculated in patty doughs as 4.00 and 6.00 log CFU/g, respectively, and the viability changes of these strains were monitored during storage. The addition of AE inhibited the growth and decreased the viability of both *E. coli* ATCC25922 and *L. monocytogenes* ATCC19118. In particular, AE showed higher antimicrobial effects on *E.coli* ATCC25922 compared with *L. monocytogenes* ATCC19118. The viability of *E. coli* ATCC25922 and *L. monocytogenes* ATCC19118 was reduced by 4.25 and 3.77% in patties, including 500 ppm AE (AE500E and AE500L). When 1000 ppm AE was used in the patties (AE1000E and AE1000L), the inhibition rate increased to 20.5% for *E. coli* ATCC25922 and 10.37% for *L. monocytogenes* ATCC19118.

Despite the reports on the antimicrobial activity of AE *in vitro* (Zhu *et al*. 2004; Kukić *et al*. 2008; Emanue *et al.* 2011), to our knowledge, there is no data on its antimicrobial activity in model food systems. The minimum inhibitory concentration of water and many solvent extractions from artichoke on *E. coli* varies between 1.5–5 mg/ml (Zhu *et al.* 2004; Kukić *et al.* 2008).

According to the only study in which *Listeria* has been used, 5 mg/ml of AE was shown to inhibit *L. innocua* growth (Emanue *et al.* 2011). It may therefore be concluded that 1000 ppm concentration of AE in patties is not sufficient for complete inhibition; however, it can repress the cell growth even at low concentrations.

One of the interesting findings in this study relating to the antimicrobial effect of AE was that CB and *E. coli* were more sensitive to AE in patties compared with YM and *L. monocytogenes*. This is consistent with evidence showing that Gram-negative cells are more sensitive to herbal extracts that exert antimicrobial activity by damaging cell walls.

CONCLUSIONS

In this study, the phenolic compounds extracted from the by-products of canned artichoke processes were determined to be a natural antioxidant and antimicrobial additive for RBPs. The antimicrobial and antioxidative activities of AE in RBPs increased with the concentration, of which 1000 ppm was found to be sufficient. To our knowledge, this study is the first to have shown the antioxidant and antimicrobial activity of AE in the food system.

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