J Food Sci Technol (November 2018) 55(11):4413–4423
https://doi.org/10.1007/s13197-018-3351-3

ORIGINAL ARTICLE

Antioxidant and antimicrobial activity of *Agave angustifolia* extract on overall quality and shelf life of pork patties stored under refrigeration

Julio Cesar López-Romero[1] · Jesús Fernando Ayala-Zavala[1] · Etna Aida Peña-Ramos[1] · Javier Hernández[2] · Humberto González-Ríos[1]

Revised: 4 July 2018 / Accepted: 16 July 2018 / Published online: 14 September 2018

Abstract *Agave* plants contain different bioactive compounds that are related to different biological activities; however, the application of *Agave* as a food additive has rarely been evaluated. The objective of this study was to evaluate the antioxidant and antimicrobial potential of *Agave angustifolia* extract (AAE) on pork patties stored at 4 °C during 10 days. According to the spectrophotometric analysis, AAE contained phenolic compounds and saponins. In addition, AAE exhibited antioxidant activity based on DPPH, ABTS and FRAP assays (94.2, 239.1 and 148.8 µmol ET/g, respectively). Likewise, AAE showed bactericidal activity against *Staphylococcus epidermidis* (60 mg/mL) and *Escherichia coli* (60 mg/mL). AAE demonstrated a protective effect against oxidative processes (TBARS and metmyoglobin) in patties compared to the control group. Mesophilic and psychotropic counts showed that AAE exhibited a weak antimicrobial effect. AAE showed a protective effect on redness and lightness (at 3 and 10 days of storage, respectively). Sensory evaluation found that AAE had no effect on the analyzed parameters. AAE exhibited antioxidant activity that preserve quality and extended the shelf life of pork patties.

Keywords Antimicrobial · Antioxidants · *Agave angustifolia* · Pork patties

✉ Humberto González-Ríos
hugory@ciad.mx

[1] Research Center for Food and Development, (CIAD, A.C.), 83000 Hermosillo, Sonora, Mexico

[2] Unidad de Servicios de Apoyo en Resolución Analítica, Universidad Veracruzana, 575, Xalapa, Veracruz, Mexico

Introduction

Meat and meat products are highly susceptible to oxidative and microbiological damage, and these factors may occur during different elaboration stages such as reception, processing, storage and distribution (Sebranek et al. 2005). The high incidence of these factors is mainly related to polyunsaturated fatty acids, proteins, minerals, pH values and water activity (Dave and Ghaly 2011). These modes of damage cause alterations to the macromolecules in the meat (lipids and proteins); which can cause changes in color, texture, water holding capacity, flavor, odor and nutritional value, as well as affect the quality, safety and stability of meat and meat products (Lucera et al. 2012). The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 20% of meat and meat products are wasted worldwide every year (FAO 2015).

Taking into account the above, the meat industry still needs to provide high quality and safe food to consumers. Therefore, synthetic additives such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) and nitrites are used to prolong the shelf life of meat and meat products, delaying oxidative and microbiological degradation (Qin et al. 2013). However, the application of synthetic additives has been limited because the additives have the potential to be toxic or hazardous to human health. Therefore, consumer preferences for natural additives are increasing because they are perceived as healthy, and have been shown to possess different biological activities. Natural sources such as plants represent excellent sources from which to extract active compounds (antioxidants and antimicrobials), however, the extraction process increases the cost of single compounds compared to synthetic additives. In other

words, other natural options, such as industrial by-products, represent potential sources for obtaining natural additives.

The food industry generates large amounts of by-products that are normally discarded as waste; these are principally pomace, peels, pulps, seed and leaves (Balasundram et al. 2006). The plant by-products are estimated to constitute approximately 50% of the original plant material, and they show similar or higher quantities of secondary metabolites than edible food parts (Ayala-Zavala et al. 2011). In addition, the food industry generates approximately 0.3–67 metric tons of waste every year from plant products (Gyawali and Ibrahim 2014). Based on the above, the food by-products represent an economical and practical source of antioxidant and antimicrobial additives, which are a promising alternative to the food industry.

Agave plants are distributed throughout the American continent and have large and fibrous leaves. The *Agave* genus contains approximately 200 species, and Mexico is considered their center of origin, with 75% of the all *Agave* species (García-Mendoza 2007). In Mexico, *Agave* plants have been used for medicinal purposed and for production of Mexican alcoholic beverages such as tequila, sotol, mezcal and bacanora. Bacanora is a fermented and distilled beverage made from the head of *A. angustifolia* Haw, but the leaves are discarded as by-products (Gutiérrez-Coronado et al. 2007). In this way, these by-products represent a potential source of bioactive extracts with antioxidant and antimicrobial potential for the food industry. Therefore, to provide alternative uses for plant by-products, research is needed to understand the potential bioactive properties of different species of *Agave*.

Based on the above, the aim of this research was to evaluate the antioxidant and antimicrobial activity of *Agave angustifolia* Haw extracts (AAE) in pork patties stored under refrigeration for 10 days.

Materials and methods

Chemicals and reagents

Folin–Ciocalteu's phenol reagent, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, potassium hydroxide, potassium persulfate, ferric chloride, 2,4-dinitrophenylhydrazine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-striazine (TPTZ), sodium acetate trihydrate, hydrochloric acid, trichloroacetic acid, 2-thiobarbituric acid, 1,1,3,3-tetramethoxypropane, sodium phosphate dibasic, monosodium phosphate, diosgenin, anisaldehyde, gallic acid, rutin, cyanidin and BHT were purchased from Sigma-Aldrich (USA). Mueller–Hinton broth (MHB), plate count agar (PCA) and peptone were obtained from Becton–Dickinson (USA).

Agave angustifolia extract (AAE) preparation

Mature (7–9 years) *Agave angustifolia* plants were obtained from the Plants Biotechnology Laboratory of our research center. AAE was prepared as described by Ahumada-Santos et al. (2013) with several modifications. *Agave* leaves were cut and freeze dried (Labconco Corp., Kansas City, MO). Dried leaves were ground to pass through a 1 mm mesh. After this, 1 g of *Agave* powder was homogenized (Ultra-Turrax T25, Ika Works Inc., USA) with 10 mL ethanol for 1 min (13,000 rpm), sonicated for 15 min (Branson 2510 ultrasonic, Danbury, CT, USA), centrifuged for 15 min at 20 °C at 17,664 × g (Beckman Coulter Avanti J-25i, California, USA) and then filtered. The precipitate was extracted a second time using the same procedure. The supernatants obtained from extractions were combined. The ethanol was removed under vacuum at 40 °C (Yahamato, Japan) to obtained a concentrate, which was freeze dried and stored at − 30 °C. For the analysis of the bioactive compounds (phenolic compounds and saponins) and antioxidant evaluations (DPPH, ABTS and FRAP assays) the AAE was dissolved in methanol. All measurements were analyzed in triplicates.

Total phenols content

Total phenolic concentration was evaluated according to the method described by Singleton and Rossi (1965) with some modifications. The extract (15 µL) was mixed with 75 µL of Folin–Ciocalteau reagent diluted [1:10] with distilled water. Subsequently, 60 µL of sodium carbonate (7.5%) was added, and the mixture was incubated in darkness for 30 min. Finally, the absorbance was read at 765 nm (Omega FLUOstar BMG LABTECH). The concentration of total phenols was calculated using a standard curve of gallic acid, and the results were expressed as milligrams of gallic acid equivalents (GAE)/g of dry weight (d. w.).

Flavone and flavonol content

The flavone and flavonol contents of AAE were evaluated according to the method reported by Popova et al. (2004). A 10 µL sample of AAE extract was diluted with 10 µL of aluminum chloride (5% w/v) and 130 µL of methanol. The mixture was stored for 30 min, and the absorbance was read at 412 nm. The results were expressed as milligrams of rutin equivalents (RE)/g d.w.

Flavanone and dihydroflavonol content

Flavanone and dihydroflavonol content was determined using the method described by Popova et al. (2004). A 14 µL sample of AAE was blended with 80 µL of 2, 4-dinitrophenylhydrazine solution. The sample was heated to 50 °C in a water bath (50 min), after which, 280 µL of potassium hydroxide (10% in methanol, w/v) was added, and 13 µL of the mixture was diluted with 250 µL of methanol. The absorbance of the sample was measured at 490 nm. The results were expressed as milligrams of hesperidin equivalents (HE)/g d.w.

Hydrolyzable and condensed tannins content

Levels of hydrolyzable and condensed tannins were determined based on the method described by Hartzfeld et al. (2002), modified as required. For hydrolyzable tannins, 1 g of AAE was extracted with a solution of methanol-sulfuric acid (10:1 v/v) for 20 h at 85 °C, after which the Singleton and Rossi (1965) method was used to measure the concentration. The results were expressed as milligrams of GAE/g d.w. For condensed tannins, 1 g of AAE was extracted with a hydrochloric acid-butanol solution over 3 h at 100 °C. The absorbances of the samples were measured at 555 nm, and the results were expressed as milligrams of cyanidin equivalents (CE)/g of d.w.

Saponin concentrations

Saponin content was assessed by the method described by Uematsu et al. (2000). A 2 mL sample of AAE was mixed with 1 mL of sulfuric acid-distillated water solution (1:1) and 1 mL of an anisaldehyde-methanol solution (0.005:1). The sample was mixed and placed in a 100 °C water bath for 30 min, after which the sample was cooled in an ice-water bath for 10 min and the absorbance was measured at 430 nm. The results were expressed as milligrams of diosgenin equivalents (DE)/g d.w.

DPPH method

Measurement of the antioxidant activity by DPPH radical scavenging activity was carried out using the modified method of Brand-Williams et al. (1995). A 20 µL sample of AAE was mixed with 280 µL of DPPH solution in methanol (adjusted to 0.7 ± 0.02 at 515 nm). The sample was kept in darkness for 30 min, and the absorbances were read at 515 nm in a microplate reader. The results were reported as µmol of Trolox equivalents (TE)/g d.w.

TEAC method

Trolox equivalent antioxidant capacity was measured according to the method reported by Re et al. (1999). First, the radical cation ABTS• + was generated by addition of 88 µL of potassium persulfate (0.139 mM) to 5 mL of ABTS solution (7 mM), which was then left to react let for 16 h. Afterwards, the absorbance was adjusted to 0.7 ± 0.02 with ethanol. Second, 5 µL of extract or Trolox standard was added to 245 µL of the adjusted ABTS• + solution. Finally, the absorbances of the samples were measured 1 and 6 min after mixing at 734 nm. The results were expressed using a Trolox standard curve and expressed as µmol of Trolox equivalents (TE)/g d.w.

FRAP method

A modified FRAP assay was used to measure antioxidant capacity as described by Benzie and Strain (1996). The working FRAP reagent was prepared by reacting 300 mM acetate buffer (pH 3.6), with 40 mM TPTZ (dissolved in 40 mM HCl) and 20 mM of aqueous ferric chloride in a 10–1 ratio. The FRAP reagent (280 µL) was mixed with 20 µL of AAE and the absorbance was measured at 630 nm after 30 min of storage in the dark. The results were reported as µmol of TE/g d.w.

Minimal bactericidal concentration (MBC)

The microorganisms used in this research were *Listeria monocytogenes* ATCC 7644, *Listeria ivanovii* ATCC 19119, *Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 2228, *Escherichia coli* ATTC 25922, *Escherichia coli* O157:H7 ATCC 43890, *Salmonella typhimurium* ATCC 14028, *Salmonella choleraesuis* ATCC 14029, *Salmonella seftenberg* ATCC 8400 and *Pseudomona aeruginosa*. This assay was performed followed the methodology described by Lopez-Romero et al. (2015). Bacterial strains were grown in Mueller–Hinton broth (MHB) for 18 h at 37 °C, after which bacterial suspensions were adjusted (OD_{600nm}) with MHB to concentrations of 1.6×10^6 cells/mL (0.134 ± 0.02). Aliquots of AAE were dissolved in MHB to produce solutions of different concentrations (0.1–60 mg/mL). Sterile 96-well microtiter plates were used. The microplates were inoculated with 180 µL of cells and 20 µL of AAE and the microtiter plates were incubated for 24 h at 37 °C. Afterward, 10 µL from each well was inoculated on sterile plate count agar (PCA), and the inoculated plates were incubated for 24 h at 37 °C. MBC was defined as the lowest concentration of AAE that showed complete absence of bacterial growth.

Shelf life study of pork patties

Preparation of pork patties

Commercial fresh pork was purchased from a local meat market at 1–2 days post-slaughter. Pork meat was ground through a 4.5 mm plate (Hobart Dayton 4152, Troy, Ohio, USA). After mincing, pork samples were assigned to one of the following four treatments: treatment (1): control (without preservative); treatment (2): BHT at 100 ppm; treatment (3): gallic acid (GA) at 100 ppm; treatment (4): *Agave angustifolia* extract (AAE) at a concentration of 60 mg of total phenolic compounds/kg of meat (1400 ppm) (Selani et al. 2011). Each treatment was tested on 25 pork patties of 70 g each. Pork patties were placed in polypropylene trays, wrapped with PVC film and stored at 4 °C for 10 days. All measurements were made on days 0, 3, 6, 8 and 10 of storage. For sensory evaluation, pork patties were cooked in a classic electric grill until an internal temperature of 71 °C was sustained for 5 min. Finally, sensory evaluation was carried out.

Thiobarbituric acid reacting substances (TBARS)

Lipid oxidation of pork patties was determined using the 2-thiobarbituric acid (2-TBA) test (Pfalzgraf et al. 1995). A 5 g sample of pork patty was homogenized with 15 mL of trichloroacetic acid (10%, w/v). Samples were centrifuged at 2300×*g* (20 min, 4 °C), and the supernatants were filtered. The filtrate (2 mL) was mixed with 2 mL of 2-TBA (20 mM). The samples were vortexed and incubated in a water bath (97 °C, 20 min) to develop the color. The mixture was cooled in cold water (15 min), and the absorbance was measured at 531 nm. The results were expressed as mg malondialdehyde (MDA)/kg of patty.

Metmyoglobin (MetMb %) assay

Meat pigments were extracted following the method described by Warris (1979). A 2 g sample of pork patty was mixed with 20 mL of phosphate buffer (0.04 M, pH 6.8), and centrifuged at 2737 × g (10 min, 4 °C) and the supernatants were filtered. The absorbance (Abs) was measured at 525 (Abs_{525}), 572 (Abs_{572}) and 700 (Abs_{700}) nm to determinate the total MetMb %. Total MetMb % was calculated with following equation:

$$\%\,MetMb = \left\{1.395 - \left[\frac{(Abs_{572} - Abs_{700})}{(Abs_{525} - Abs_{700})}\right]\right\} * 100$$

Mesophilic and psychotropic bacteria count in pork patties

To evaluate the mesophilic and psychrophilic bacteria counts in the various treatments, 10 g of pork patty was mixed with 90 mL of peptone water (0.1%) and homogenized (1 min). Afterward, peptone water was used to dilute the solution which was then pour-plated (1 mL) in PCA. Inoculated PCA plates were incubated at 35 °C for 48 h and at 4 °C for 7 days to determined mesophilic and psychotropic counts, respectively. Colonies were counted, and the results were expressed as $\log_{10}$ colony forming units (CFU)/g of patty.

pH determination

The pH of the pork patties was determined by blending 3 g of patty with 27 mL of distilled water and measuring with a digital pH meter (model pH211, Hanna, Woonsocket, RI, USA).

CIE-Lab color

After allowing the meat to bloom for 30 min, the surface colors of the pork patties were measured using a colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc. Japan) with D65 illuminant, with 10° and 8 mm of aperture in the observer. The color parameters L* (lightness), a* (redness) and b* (yellowness) were evaluated. At least 5 different determinations were carried out per sample.

Sensory evaluation

Sensory evaluation was carried out by a trained 8-member panel, and evaluations were made under controlled conditions at 21 ± 1 °C and 55 ± 5% relative humidity in a room partitioned into booths. The loss of fresh odor and fresh flavor attributes of the pork patties were measured using a 5-point hedonic scale, where 1 indicated no loss of fresh odor and flavor and 5 indicated extreme loss of fresh odor and flavor.

Statistical analysis

Statistical analysis was performed using the commercial software NCSS, 2007. Data were analyzed by analysis of variance in a 5 × 4 factorial arrangement (storage time × treatment). Data for sensory evaluation were analyzed by repeated measures analysis. Means comparisons were done by Tukey–Kramer test. Statistical significance was considerate at $P < 0.05$ for type I error.

Result and discussion

Bioactive compounds and antioxidant activity

Secondary metabolites are bioactive compounds produced by plants during development and adaptation to the environment (Balasundram et al. 2006). Phenolic compounds and saponins are two of the main compounds produced by plants. These compounds play important roles as defense mechanisms against herbivores, competitors and microorganisms. In addition, phenolic compounds and saponins are strongly associated with different biological activities. The content of phenolic compounds by groups (flavones and flavonols, flavanones and dihydroflavonols, hidrolisated and condensed tanins, and total phenolics) and saponins of *Agave angustifolia* extract (AAE) evaluated by different spectrometrically methods are shown in Table 1. The obtained results exhibited that concentration of phenolic compounds varied according to the group, being flavanones and dihidroflavonols are the main group of phenolic compounds presents in AAE, followed by flavones and flavonols, and hidrolisated and condensed tanins, respectively. The amount of phenolic compounds identified in AAE is consistent with other *Agave* plants studies (0.01–29 mg GAE/g d.w.) (Ahumada-Santos et al. 2013; Hamissa et al. 2012; Rizwan et al. 2012). Added to this, other studies focused on the identification of specific phenolic compounds in *Agave* plants, which have shown that *Agave* extracts are a source of kaempferol and quercetin and their glycosides mainly, as well as homoisoflavonoids (López-Romero et al. 2017). Moreover, other important group of bioactive compounds identified in AAE was saponins, which have been shown to be one of the most abundant compounds in *Agave* plants. In this regard, different studies isolated and characterized a wide variety of saponins in *Agave* plants, such as steroidal, spirostanol and furastanol saponins (Hackman et al. 2006). On the other hand, it is known that concentration of bioactive compounds varies among plants. This behavior is associated to several factors such as genetic, ontogenetic, morphogenic, environmental, and solvent and extraction technique (Verma and Shukla 2015). These factors determine the profile and concentration of bioactive compounds in plants, directly influencing the biological potential of its extracts.

Table 1 Bioactive compounds of *Agave angustifolia* extract

Bioactive compounds	Content
Total phenols (mg GAE/g)	21.7 ± 0.8
Flavones and Flavonols (mg RE/g)	8.5 ± 0.4
Flavanones and dihydroflavonols (mg HE/g)	38 ± 1.2
Hydrolysate tannins (mg GAE/g)	0.2 ± 0.003
Condensed tannins (mg CE/g)	0.003 ± 0.0001
Saponins (mg DE/g)	7.2 ± 0.3

Data are represented as mean ± standard error

Bioactive compounds such as phenolic compounds and saponins are largely associated with different biological activities as antioxidant activity. In this regard, different assays have been performed to determine the antioxidant potential of plant extracts. Most of these assays are based on scavenging specific radicals such as DPPH and ABTS, or metal reducing potential such the FRAP assay. In this sense, in the present study, we evaluated the antioxidant potential of AAE using DPPH, ABTS and FRAP methods. Results demonstrated that AAE exhibited free-radical scavenging activity based on DPPH (94.2 ± 0.4 μmol ET/g) and ABTS (239.1 ± 11.2 μmol ET/g) assays, as well as ferric reducing potential (FRAP, 148.8 ± 5.3 μmol ET/g). This behavior can be associated to the bioactive compounds (presents in AAE) capacity to transfers electron and hydrogen to stabilize free radicals and reduce metals. This suggest that AAE present the ability to act as preventive and chain-breaking antioxidants against biological and synthetic radical and also inhibit the generation of reactive oxygen species. In this regard, AAE could represent a good option as antioxidant agent in different areas such as food industry and pharmaceuticals, where oxidative process play an important role on the development negative effect in foods and human health, respectively. By other hand, the antioxidant values obtained in the present research are consistent with those from other *Agave* studies (Ahumada-Santos et al. 2013; Hamissa et al. 2012).

The results showed that phenolic compound presents in AAE influenced antioxidant activity. In this regard, Hamissa et al. (2012) demonstrated that phenolic compounds from *Agave americana* showed a strong correlation with antioxidant activity. These results confirmed that phenolic compounds were the main responsible compounds for the antioxidant effect of *Agave* plants. Antioxidant mechanism of phenolic compounds is related due to their redox properties and the types of functional groups and the position where those groups are located in the molecule (Perron and Brumaghim 2009). The presence of a 3′, 4′-dihidroxy group in the B ring, a 3-hidroxy group in the C ring and double bond (C2–C3), in combination with a 4-keto group influence the antioxidant potential, increasing the ability to donate electrons and protons to stabilized free radicals and reduce metals. In this regard, it was previously mentioned that most of the phenolic compounds identified in *Agave* extracts showed these characteristics, suggesting that antioxidant potential of AAE could be associated with the presence of flavonols such as kaempferol and quercetin,

which have shown antioxidant effect by different in vitro methods.

Antimicrobial activity

Currently, the occurrences of multidrug resistant pathogens are increasing worldwide, which presents a challenge to the medical and food industries. Therefore, it is necessary to develop new alternatives to combat resistant pathogens. Accordingly, plants represent an interesting source for discovering new potential antimicrobials. In this way, to provide information about the antimicrobial effect of plants, we evaluated the bactericidal effects of AAE against ten pathogens. The results from this bactericidal activity study are summarized in Table 2. The results showed that AAE exhibited low antimicrobial potential against the evaluated microorganism. The *S. epidermidis* and *E. coli* were the most sensitive microorganisms, with the lowest MBC values. Added to this, our results showed that AAE not produced bactericidal effect against the other tested microorganism at the analyzed concentrations (0–60 mg/mL). In addition, was evidenced that AAE not present specificity against any group of bacteria, because was active against gram-positive (*S. epidermidis*) and gram-negative bacteria (*E. coli*). Our results were in agreement with the data observed by Ahumada-Santos et al. (2013) and Hammuel et al. (2011). These authors also observed that *Agave* extracts presented bactericidal effect against gram-negative and gram-positive bacteria.

Antimicrobial effects of AAE are related with the identified bioactive compounds such as phenolic compounds and saponins, which can interact with bacterial membrane proteins and lipids, causing viability cell damage. In this regard, Borges et al. (2013) and Monte et al. (2014) demonstrated that the bioactive compounds presents in natural extracts, such as phenolic compounds and saponins, can induce changes in hydrophobicity, surface charge and membrane integrity causing leakage of intracellular components of gram-positive and gram-negative bacteria, resulting in cell death. Added to this, Cushnie and Lamb (2005), reported that phenolic compounds could provoke inhibition of energy metabolism, inhibition of cytoplasmic membrane functions, inhibition of vital enzymes and inhibition of nucleic acid metabolism in different bacteria, which result in bacterial death.

Table 2 Bactericidal activity of *Agave angustifolia* extract

Bacteria	MBC (mg/mL)
Listeria monocytogenes	NP
Listeria ivanovvi	NP
Staphylococcus aureus	NP
Staphylococcus epidermidis	60
Escherichia coli	60
Escherichia coli O157:H7	NP
Salmonella Tiphy	NP
Salmonella choleraesuis	NP
Salmonella seftenberg	NP
Pseudomonas aeruginosa	NP

MBC Minimal bactericidal concentration, *NP* Not bactericidal activity was detected

Shelf life study of pork patties treated with *Agave* extract

Tbars

The effect of AAE on lipid oxidation over the shelf life of pork patties stored under refrigeration is presented in Fig. 1a. The levels of TBARS of all evaluated treatments increased as the storage time increased ($P < 0.05$). A significant difference between treatments ($P < 0.05$) was observed at day 8 of storage, where control treatment exhibited the highest TBARS values, in comparison with the other treatments. GA was the most effective treatment to decrease TBARS values followed by BHT and AAE, presenting 68, 46 and 42% (respectively) les oxidation vs control treatment. In addition, AAE showed similar effect ($P > 0.05$) that BHT at 8 day of sampling, demonstrating that AAE was effective to decrease the lipid oxidation process of pork patties stored at 4 °C. However, at day 10 of storage, the control and AAE treatments showed the higher TBARS values ($P < 0.05$).

The increase in TBARS values is associated with the formation of oxidative products during the advancement of oxidation such as aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones, which are related to the development of unacceptable flavor and aroma in meat products, affecting their acceptability (Nawar 1996). Wenjiao et al. (2014) mentioned that TBARS values of 2 mg MDA/kg in meat are considered the rancidity detection limit by consumer. In this regard, patties treated with AAE remained below 2 mg MDA/kg during 8 days of storage, while the control patties did not, demonstrating the antioxidant potential of AAE extending the shelf life of pork patties stored under refrigeration.

Based on the above, is evident that AAE is a good source of phenolic compounds with antioxidant potential able to reduce lipid oxidation process in pork patties stored under refrigeration. In this regard, it is possible to hypothesize that AAE delayed lipid oxidation by interfering in the initiation and propagation of oxidative processes. The initiation process can be delayed by reducing trace metals present in meat (iron and copper), which play

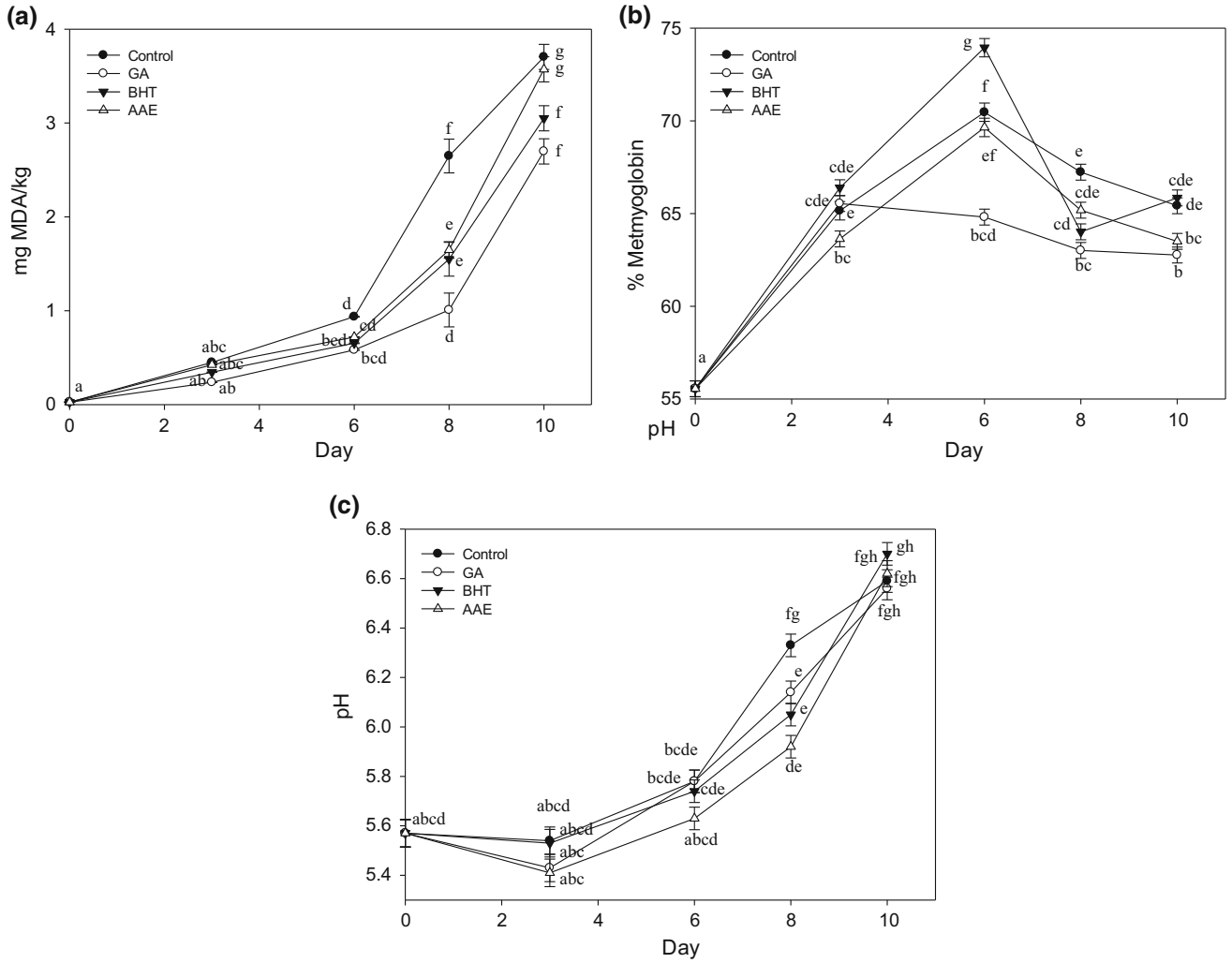

Fig. 1 Changes in **a** TBARS, **b** Metmyoglobin (MtMeb) and **c** pH values of pork patties added with *Agave angustifolia* extract under refrigeration. Treatments: Control, without preservative; *GA* gallic acid, *BHT* butylated hydroxytoluene, *AAE Agave angustifolia* extract. Axis X: days at 4 °C, Axis Y: **a** TBARS values (mg of MDA/kg), **b** MtMeb (%), **c** pH values. Data are presented as mean ± standard error. Means with different letters, are different ($P < 0.05$). Treatment × storage time interaction, was significant ($P < 0.05$)

important roles in the production of free radicals and the propagation process. In addition, AAE can stabilize free radicals produced during the propagation stage by promoting the reduction of oxidative rancidity products, which cause rancid odors and flavors in meat and meat products. These assumptions are based on the ability of AAE to stabilize free radicals and reduce metals in vitro tests.

MetMb

The AAE effects on MetMb formation of pork patties storage under refrigeration are presented in Fig. 1b. Significant difference between treatment was observed at 4 day of storage, where AAE treatment showed the lowest MetMb values ($P < 0.05$), followed by the other treatments. The highest values ($P < 0.05$) were observed on the 6th day of storage. Samples treated with BHT had a highest ($P < 0.05$) concentration of MetMb compared control, AAE and GA. At day 8 and 10 of storage GA and AAE significantly reduced ($P < 0.05$) MetMb formation, compared with control, showing that this treatment possess the ability to inhibit the MetMb formation in the storage pork patties. Generally, it was observed that MetMb values increased and then decreased during the storage time, which is related to oxygenation or oxidation of myoglobin during the evaluation period. Similar results were reported by Gallego et al. (2015) in beef patties added with *Caesalpinia decapetala*, in which delayed MetMb formation was observed compared to the control.

Meat color is one of the main characteristics consumers consider because is associated with freshness. Meat color is principally affected by the formation of MetMb as result of oxidation of ferrous ion to ferric ion, which results in the formation of brown color. In addition, myoglobin may be

oxidized by lipid oxidation products, such as hydroperoxides, and contribute to MetMb formation (Elroy et al. 2015). In this way, this study showed that treatment with AAE and GA resulted in lower MetMb values during storage ($P < 0.05$). This demonstrates that AAE can have a protective effect on myoglobin oxidation, suggesting that AAE may be a good option to decrease MetMb production in meat. The protective effect of AAE against the production of MetMb is related to the bioactive compounds present in its extract, which have the ability to stabilize free radicals and ferric reduction activity, delaying the oxidative process.

Mesophilic and psychotropic analysis

Microbial growth is mainly associated with meat products spoilage, since mesophilic and psychotropic microorganism are considered important indicators of meat quality deteriorations. The microbiological evaluation results for the pork patties stored under refrigeration are shown in Fig. 2. The mesophilic counts of the evaluated treatments increased during storage (Fig. 2a). Mesophilic counts were similar ($P > 0.05$) for all treatments during storage time. On day 8 of sampling, the evaluated treatments showed lower counts (10, 10 and 11% for AAE, BHT and GA, respectively) of mesophilic bacteria compared to the control; however, the difference was not significant ($P > 0.05$). On the other hand, it was observed that psychotropic count in analyzed pork patties increasing thought the storage time ($P < 0.05$) (Fig. 2b). Generally, AAE showed similar counts than control treatment during the shelf life ($P > 0.05$). These results evidenced that AAE was not effective to decrease the microbial count during storage, indicating that AAE extracts possess low antimicrobial effect. These results are consistent with the obtained results in the antimicrobial evaluation by in vitro test, which demonstrated the low antimicrobial effect of AAE against Gram-positive and Gram-negative bacteria.

On the other hand, psychotropic microorganism counts were greater than mesophilic accounts during the storage. This behavior may be associated with the storage temperatures (4 °C), where metabolic activity decreased with temperature reduction, reflected in lower microbial counts than phychotropic microorganism. Moreover, the microbial count for psychotropic was increased due to storage conditions, which could be associated with *Pseudomonas* spp., which has been identified as the main psychophilic microorganisms group associated with the spoilage of meat products (Addeen et al. 2017). Thus, this issue is in agreement with the results obtained by the in vitro microbiological tests where AAE did not present activity against these microorganisms.

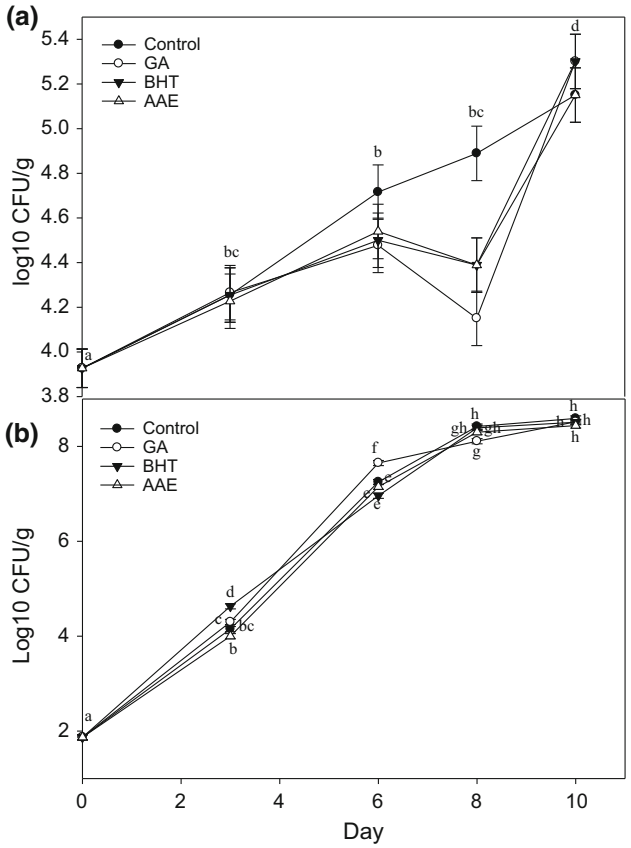

Fig. 2 Changes in **a** mesophilic count and **b** psychotropic count of pork patties added with *Agave angustifolia* extract under refrigeration. Treatments: Control, without preservative; *GA* gallic acid, *BHT* butylated hydroxytoluene, *AAE Agave angustifolia* extract. Axis X: days at 4 °C, Axis Y: mesophilic and psychotropic count (log CFU/g). Data are presented as mean ± standard error. Means with different letters, are different ($P < 0.05$). For mesophilic count, storage time effect; and a Treatment × storage time interaction effect to psychotropic count was observed ($P < 0.05$)

On the other hand, low antimicrobial effects by AAE may be related to the complex meat matrix because some components, such as fat, may provide a protective effect for bacteria. In addition, meat contains nutrients and optimal conditions for bacterial growth. These conditions could have an important effect on the success of antimicrobial treatments (Juneja et al. 2013).

pH

The changes in the pH values of pork patties over the course of the 10 days of storage are presented in Fig. 1c. pH values of pork patties stored under refrigeration increased when the storage time increased ($P < 0.05$). At day 8 of storage were observed differences between treatment, where control treatment ($P < 0.05$) exhibited the highest pH value followed by GA, BHT and AAE.

Moreover, the pH values increased further by day 10 of storage, where all treatments showed similar ($P > 0.05$) pH values.

In the present study, an increase in the pH values through of the storage period was observed. In this regard, several studies mentioned that the increase in pH values is associated with bacterial spoilage. This is related to the action of endogenous or microbial enzymes, such as proteases and lipases, which promote degradation of nitrogenous compounds during storage, causing an increase in pH values (Chaijan et al. 2005).

Based on the above, our findings agree with the obtained mesophilic and psychotropic count, because both parameters (pH values and microbial count) increased during the evaluation period, which suggests that microbial growth in the analyzed pork patties induce an increase in pH values.

CIE-Lab color

The lightness, redness and yellowness values of the pork patties are shown in Table 3. The evaluation of lightness showed that values decreased as a function of storage time; and in general, lower values were observed in the controls than in the other treatments. However, the differences were not significant ($P > 0.05$) for the first 8 days of storage. At day 10 of sampling, the control treatment exhibited lower lightness values compared to GA, BHT and AAE treatments ($P < 0.05$). These results suggest a protective activity by the additives on color lightness.

Table 3 Color evaluation of pork patties added with *Agave angustifolia* extracts during storage period

Parameter	Storage days				
Treatment[1]	0	3	6	8	10
L (lightness)*					
Control	58.9^{de}	56.6^{c}	57.4^{cd}	54.2^{ab}	53.5^{a}
GA	59.7^{e}	56.4^{c}	58.2^{d}	56^{bc}	55.4^{bc}
BHT	58.6^{de}	57.5^{cd}	58^{d}	55.1^{b}	54.8^{b}
AAE	57.8^{cd}	56.4^{c}	59^{de}	55.8^{bc}	55.6^{bc}
SEM[2]	0.226	0.243	0.188	0.196	0.306
a (redness)*					
Control	10.7^{cde}	7^{a}	7.7^{ab}	11.6^{e}	11.2^{de}
GA	10.4^{cd}	8.8^{b}	7.9^{ab}	10.8^{cde}	9.9^{cd}
BHT	11.4^{e}	8.4^{b}	8.3^{ab}	10.5^{cde}	11.3^{de}
AAE	11^{de}	9.5^{c}	7.2^{a}	11.5^{e}	11.2^{de}
SEM[2]	0.182	0.272	0.124	0.140	0.152
b (yellowness)*					
Control	11.7^{ab}	13^{bc}	12.4^{b}	13.7^{cd}	12.5^{b}
GA	11.5^{ab}	11.3^{a}	13.1^{bc}	13^{bc}	12.6^{b}
BHT	12.2^{ab}	11.5^{ab}	13.1^{bc}	11.7^{ab}	12.4^{b}
AAE	12.7^{b}	11.9^{ab}	12.9^{bc}	14.1^{e}	12^{ab}
SEM[2]	0.161	0.219	0.180	0.261	0.165

[1]Treatments: Control, without preservative; *GA* gallic acid, *BHT* butylated hydroxytoluene, *AAE Agave angustifolia* extract

[2]*SEM* Standard error of mean

[a–e] Means with different superscript within rows or column, are different ($P < 0.05$)

For redness evaluation, values ranged from 7 to 12.7 over the course of the storage period. A difference ($P < 0.05$) was observed by the 3rd day of storage, where the control treatment had a lower redness value than BHT, GA or AAE. This result showed that the additives imparted a protective effect against meat discoloration, which is related to the antioxidant and chelation potential of the bioactive compounds. Moreover, no differences ($P > 0.05$) among the evaluated treatments were observed on the other days of storage (6, 8 and 10). In contrast, the yellowness evaluation (b^*) was different ($P < 0.05$) on day 8 of storage, with BHT and AAE treatments showing higher values relative to the other treatments ($P < 0.05$).

Generally, color parameters were not steady during shelf life. These results are consistent with MetMb values, where a similar behavior was observed. In this way, O'Grady et al. (2001) described that MetMb formation interfered with the red color (a^*) in meat and meat products, resulting in a reduction in a^* values as products of meat discoloration. In addition, Bekhit et al. (2003) mentioned that oxidative degradation of several nitrous pigments and protein oxidation products may induce color changes in meat products during storage. Moreover, the incorporation of natural extracts may induce changes in the color parameters by their own characteristic color.

Sensory evaluation

The loss of fresh odor and flavor attributes of cooked pork patties are summarized in Table 4. Respect to the loss of fresh odor, it was observed that patties at day 0 had the least loss scores in this experiment ($P < 0.05$). At day 10, AAE and BHT exhibited the highest losses ($P < 0.05$), followed by the control and GA. These values agree with the TBARS values, because the trained panel detected higher loss after 10 days of storage, with all treatments exhibiting values higher than 2 mg MDA/g, which is considered the limit of detection for undesirable odors and flavors in meat and meat products. In contrast, scores of losses in fresh flavor were similar ($P > 0.05$) throughout the sampling days for all evaluated treatments, except AAE at days 6 and 8 of storage ($P < 0.05$), which presented higher loss scores. However, the obtained values mean that there were at most slight losses in fresh flavor during storage. At day 10 of storage, the loss of fresh flavor was not evaluated to avoid microbial risk to the panelist. The

Table 4 Sensory evaluation[1] of pork patties added with *Agave angustifolia* extract during storage period

Parameter	Storage days				
Treatment[2]	0	3	6	8	10
Loss of fresh odor					
Control	1^{a}	1.11^{bc}	1.21^{bc}	1.45^{bc}	1.78^{c}
GA	1^{a}	1.19^{bc}	1.19^{bc}	1.35^{bc}	1.45^{bc}
BHT	1^{a}	1.16^{cd}	1.24^{bc}	1.23^{bc}	3.06^{d}
AAE	1^{a}	1.3^{bc}	1.67^{bc}	1.44^{bc}	3.12^{d}
SEM[3]	0.0	0.046	0.102	0.109	0.210
Loss of fresh flavor					
Control	1^{a}	1.08^{abc}	1.14^{abc}	1.4^{abcd}	
GA	1^{a}	1.12^{abc}	1.17^{abc}	1.45^{abcd}	
BHT	1^{a}	1.09^{abc}	1.23^{abc}	1.14^{abc}	
AAE	1^{a}	1.21^{abc}	1.6^{bcd}	1.51^{bcd}	
SEM[3]	0.00	0.100	0.152	0.127	

[1]Sensory evaluation were measured using a 5 point hedonic scale, where 1 indicated without loss of fresh odor and flavor and 5 indicated extreme loss of fresh odor and flavor

[2]Treatments: Control, without preservative; *GA* gallic acid, *BHT* butylated hydroxytoluene, *AAE Agave angustifolia* extract

[3]*SEM* Standard error of mean

[a–d] Means with different superscript within rows or column, are different ($P < 0.05$)

results of our study demonstrated that AAE did not affect sensory parameters during the shelf life of pork patties.

Conclusion

The present study showed that AAE exhibited interesting free radical scavenging and ferric reducing activities in vitro tests. In addition, AAE was effective in reducing lipid and myoglobin oxidation and extending the shelf life of pork patties stored at 4 °C for 8 days. AAE did not affect the sensory characteristics of treated pork patties. Moreover, AAE exhibited low antimicrobial activity in pork patties. Based on the above, AAE represents an interesting natural and cost-effective alternative for the control of oxidative processes in meat and meat products.

Acknowledgements The authors thank to Thalia Yamileth Islava-Lagarda for the technical support provided in the laboratory evaluations.

References

Addeen A, Benjakul S, Prodpran T (2017) Slaughtering method affects lipid oxidation, volatile profile and overall quality of chicken patties during storage. Emir J Food Agric 29(5):387–395. https://doi.org/10.9755/ejfa.2016-09-1331

Ahumada-Santos YP, Montes-Avila J, De Jesús Uribe-Beltrán M, Díaz-Camacho SP, López-Angulo G, Vega-Aviña R, López-Valenzuela JA, Heredia JB, Delgado-Vargas F (2013) Chemical characterization, antioxidant and antibacterial activities of six *Agave* species from Sinaloa, Mexico. Ind Crop Prod 49:143–149. https://doi.org/10.1016/j.indcrop.2013.04.050

Ayala-Zavala J, Vega-Vega V, Rosas-Domínguez C, Palafox-Carlos H, Villa-Rodriguez J, Siddiqui MW, Dávila-Aviña J, González-Aguilar G (2011) Agro-industrial potential of exotic fruit byproducts as a source of food additives. Food Res Int 44:1866–1874. https://doi.org/10.1016/j.foodres.2011.02.021

Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 99:191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

Bekhit A, Geesink G, Ilian M, Morton J, Bickerstaffe R (2003) The effects of natural antioxidants on oxidative processes and metmyoglobin reducing activity in beef patties. Food Chem 81:175–187. https://doi.org/10.1016/S0308-8146(02)00410-7

Benzie IF, Strain J (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76. https://doi.org/10.1006/abio.1996.0292

Borges A, Ferreira C, Saavedra MJ, Simoes M (2013) Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microb Drug Resist 19:256–265. https://doi.org/10.1089/mdr.2012.0244

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Chaijan M, Benjakul S, Visessanguan W, Faustman C (2005) Changes of pigments and color in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. Food Chem 93:607–617. https://doi.org/10.1016/j.foodchem.2004.10.035

Cushnie TT, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26(5):343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Dave D, Ghaly AE (2011) Meat spoilage mechanisms and preservation techniques: a critical review. AJABS 6:486–510

Elroy N, Rogers J, Mafi G, Vanoverbeke D, Hartson S, Ramanathan R (2015) Species-specific effects on non-enzymatic metmyoglobin reduction *in vitro*. Meat Sci 105:108–113. https://doi.org/10.1016/j.meatsci.2015.03.010

FAO (Organización de Las Naciones Unidas) (2015). Iniciativa mundial sobre la reducción de la pérdida y el desperdicio de alimentos. http://www.fao.org/3/a-i4068s.pdf. Accessed 22 Mar 2015

Gallego MG, Gordon MH, Segovia FJ, Almajano MP (2015) *Caesalpinia decapetala* extracts as inhibitors of lipid oxidation in beef patties. Molecules 20:13913–13926. https://doi.org/10.3390/molecules200813913

García-Mendoza A (2007) Los Agaves de México. Ciencias 87:14–23

Gutiérrez-Coronado M, Acedo-Félix E, Valenzuela-Quintanar A (2007) Bacanora industry and its process of production. CYTA-J Food 5:394–404. https://doi.org/10.1080/11358120709487718

Gyawali R, Ibrahim SA (2014) Natural products as antimicrobial agents. Food Control 46:412–429. https://doi.org/10.1016/j.foodcont.2014.05.047

Hackman DA, Giese N, Markowitz JS, McLean A, Ottariano SG, Tonelli C, Ulbricht C (2006) *Agave* (*Agave americana*) an evidence-based systematic review by the natural standard

research collaboration. J Herb Pharmacother 6:101–122. https://doi.org/10.1080/J157v06n02_09

Hamissa AMB, Seffen M, Aliakbarian B, Casazza AA, Perego P, Converti A (2012) Phenolics extraction from *Agave americana* (L.) leaves using high-temperature, high-pressure reactor. Food Bioprod Process 90:17–21. https://doi.org/10.1016/j.fbp.2010.11.008

Hammuel C, Yebpella GG, Shallangwa GA, Magomya AM, Agbaji AS (2011) Phytochemical and antimicrobial screening of methanol and aqueous extracts of *Agave sisalana*. Acta Pol Pharm 68:535–539

Hartzfeld PW, Forkner R, Hunter MD, Hagerman AE (2002) Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. J Agr Food Chem 50:1785–1790. https://doi.org/10.1021/jf0111155

Juneja VK, Altuntaş EG, Ayhan K, Hwang CA, Sheen S, Friedman M (2013) Predictive model for the reduction of heat resistance of *Listeria monocytogenes* in ground beef by the combined effect of sodium chloride and apple polyphenols. Int J Food Microbiol 164:54–59. https://doi.org/10.1016/j.ijfoodmicro.2013.03.008

López-Romero JC, González-Ríos H, Borges A, Simões M (2015) Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. Evid Based Complement Alternat Med 2015:1–9. https://doi.org/10.1155/2015/795435

López-Romero JC, Ayala-Zavala JF, González-Aguilar GA, Peña-Ramos EA, González-Ríos H (2017) Biological activities of Agave by-products and their possible applications in food and pharmaceuticals. J Sci Food Agric. https://doi.org/10.1002/jsfa.8738

Lucera A, Costa C, Conte A, Del Nobile MA (2012) Food applications of natural antimicrobial compounds. Front Microbiol 3:1–13. https://doi.org/10.3389/fmicb.2012.00287

Monte J, Abreu AC, Borges A, Simões LC, Simões M (2014) Antimicrobial activity of selected phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. Pathogens 3:473–498. https://doi.org/10.3390/pathogens3020473

Nawar WW (1996) Lipids. In: Fennema O (ed) Food Chemistry, 3rd edn. Marcel Dekker, New York, pp 225–319

O'Grady M, Monahan F, Brunton N (2001) Oxymyoglobin oxidation and lipid oxidation in bovine muscle—mechanistic studies. J Food Sci 66:386–392. https://doi.org/10.1111/j.1365-2621.2001.tb16115.x

Perron NR, Brumaghim JL (2009) A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys 53:75–100. https://doi.org/10.1007/s12013-009-9043-x

Pfalzgraf A, Frigg M, Steinhart H (1995) Alpha-Tocopherol Contents and Lipid Oxidation in Pork Muscle and Adipose Tissue during Storage. J Agr Food Chem 43:1339–1342

Popova M, Bankova V, Butovska D, Petkov V, Nikolova-Damyanova B, Sabatini AG, Marcazzan GL, Bogdanov S (2004) Validated methods for the quantification of biologically active constituents of poplar-type propolis. Phytochem Anal 15:235–240. https://doi.org/10.1002/pca.777

Qin YY, Zhang ZH, Li L, Xiong W, Shi JY, Zhao TR, Fan J (2013) Antioxidant effect of pomegranate rind powder extract, pomegranate juice, and pomegranate seed powder extract as antioxidants in raw ground pork meat. Food Sci Biotecnol 22:1063–1069. https://doi.org/10.1007/s10068-013-0184-8

Rizwan K, Zubair M, Rasool N, Riaz M, Zia-Ul-Haq M, de Feo V (2012) Phytochemical and biological studies of Agave attenuata. Int J Mol Sci 13(5):6440–6451. https://doi.org/10.3390/ijms13056440

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 26:1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Sebranek JG, Sewalt VJH, Robbins K, Houser TA (2005) Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Sci 69:289–296. https://doi.org/10.1016/j.meatsci.2004.07.010

Selani M, Contreras-Castillo C, Shirahigue L, Gallo C, Plata-Oviedo M, Montes-Villanueva N (2011) Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. Meat Sci 88:397–403. https://doi.org/10.1016/j.meatsci.2011.01.017

Singleton V, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticut 16:144–158

Uematsu Y, Hirata K, Saito K, Kudo I (2000) Spectrophotometric determination of saponin in Yucca extract used as food additive. J AOAC Int 83:1451–1454

Verma N, Shukla S (2015) Impact of various factors responsible for fluctuation in plant secondary metabolites. JARMAR 2:105–113. https://doi.org/10.1016/j.jarmap.2015.09.002

Warris P (1979) The extraction of haem pigments from fresh meat. Int J Food Sci Tech 14:75–80. https://doi.org/10.1111/j.1365-2621.1979.tb00849.x

Wenjiao F, Yongkui Z, Yunchuan C, Junxiu S, Yuwen Y (2014) TBARS predictive models of pork sausages stored at different temperatures. Meat Sci 96:1–4. https://doi.org/10.1016/j.meatsci.2013.06.025