BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Allspice (*Pimenta Dioica* Lindl) leaves essential oil as a potential antioxidant and antimicrobial source for use in mechanically deboned poultry meat

*Óleo essencial de pimenta-da-jamaica (*Pimenta Dioica *Lindl.) como potencial fonte antioxidante e antimicrobiana para uso em carne de frango desossada mecanicamente*

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Cite as: Andrade, B. F., Ferreira, V. R. F., Cardoso, G. P., Andrade, M. P. D., Ramos, A. L. S., Cardoso, M. G., & Ramos, E. M. (2023). Allspice (*Pimenta Dioica* Lindl) leaves essential oil as a potential antioxidant and antimicrobial source for use in mechanically deboned poultry meat. *Brazilian Journal of Food Technology*, *26*, e2022125. https://doi.org/10.1590/1981-6723.12522

Abstract

This work aimed to characterize the allspice leaves essential oil (EO) and evaluate its antimicrobial activity against specific food-borne pathogenic microorganisms as well as its *in vitro* antioxidant activity. The antioxidant activity of different concentrations (0, 500 and 1000 mg/kg) of allspice EO was also evaluated in mechanically deboned poultry meat (MDPM) during storage of up to 10 days at 2 °C. Allspice EO presented as major compounds eugenol (55.52%), myrcene (22.53%) and chavicol (5.12%), and was effective against Gram-negative (*P. aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria, having greater ($p < 0.05$) antimicrobial activity than its main component eugenol. In the *in vitro* antioxidant assays, the allspice EO had higher radical scavenging activity (90% *versus* 65%) than eugenol, presenting lower IC50 values (2.71 *versus* 9.49 µg/mL), but the antioxidant activity by the 2-thiobarbituric acid reactive substances (TBARS) did not differ ($p > 0.05$) from the synthetic antioxidant butyl-hydroxytoluene (BHT). Incorporating allspice EO in MDPM suppressed lipid oxidation during 8 days of cold storage, regardless of the amount used, exhibiting lower ($p < 0.05$) TBARS values during all storage periods than MDPM without EO. Allspice leaves EO had the high antioxidant potential to be used in MDPM and could also contribute an antimicrobial effect to the product in which the MDPM is used.

Keywords: Jamaican pepper; Natural preservative; Phenolic compounds; *P. aeruginosa*; Oxidative stability; Essential oil.

Resumo

O objetivo deste trabalho foi caracterizar o óleo essencial (OE) de folhas de pimenta-da-jamaica e avaliar sua atividade antimicrobiana contra microrganismos patogênicos específicos de alimentos e sua atividade antioxidante *in vitro*. A ação antioxidante de diferentes concentrações (0, 500 e 1.000 mg/kg) de OE de pimenta-da-jamaica também foi avaliada em carne de frango desossada mecanicamente (MDPM) durante o armazenamento até 10 dias a 2 °C. O OE de pimenta-da-jamaica apresentou como compostos majoritários eugenol (55,52%), mirceno (22,53%) e chavicol (5,12%), e foi eficaz contra bactérias Gram-negativas (*P. aeruginosa*) e Gram-positivas (*Staphylococcus aureus*), tendo maior (p < 0,05) ação antimicrobiana do que seu principal componente, o eugenol. Nos ensaios antioxidantes *in vitro*, o OE de pimenta-da-jamaica apresentou maior atividade sequestrante de radicais (90% *versus* 65%) do que o eugenol, apresentando valores menores de IC50 (2,71 *versus* 9,49 µg/mL), mas a atividade antioxidante pelas substâncias reativas ao ácido 2-tiobarbitúrico (TBARS) não diferiu (p > 0,05) do antioxidante sintético butil-hidroxitolueno (BHT). A incorporação de OE de pimenta-da-jamaica no MDPM suprimiu a oxidação lipídica durante oito dias de armazenamento refrigerado, independentemente da quantidade utilizada, exibindo valores de TBARS menores (p < 0,05) durante todo o período de armazenamento do que o MDPM sem OE. O OE de folhas de pimenta-da-jamaica apresentou alto potencial antioxidante para ser utilizado em MDPM e também pode contribuir com um efeito antimicrobiano para o produto em que o MDPM é utilizado.

Palavras-chave: Pimenta-da-jamaica; Conservante natural; Compostos fenólicos; *P. aeruginosa*; Estabilidade oxidativa; Óleo essencial.

Highlights

- There is a synergistic effect between the substances present in the allspice EO in its antimicrobial and antioxidant activities
- Allspice EO had significant activity against gram-negative and positive bacteria
- Allspice EO had a high potential antioxidant effect in mechanically deboned poultry meat, suppressing lipid oxidation during storage

1 Introduction

Today, Brazil occupies a prominent position as a producer and exporter of poultry meat, being the country with the third largest production (13.845 million tons) and the largest exporter, corresponding to 31% of its production (Empresa Brasileira de Pesquisa Agropecuária, 2021). However, a growth market of special cuts (66.99%), rather than the sale of whole poultry (25.26%) (Associação Brasileira de Proteína Animal, 2021), has provided considerable quantities of meat parts with low commercial value, particularly the back, neck and bones with flesh remaining, wherein the only cost-effective and rational process for its recovery is by mechanical deboning.

Mechanically Deboned Poultry Meat (MDPM) is a very important ingredient in food industries and is frequently used in comminuted meat products due to its fine consistency and relatively low cost (Jayathilakan et al., 2012). Due to its wide use in meat products sold in Brazil, especially in emulsified sausages (allowed addition of up to 60%), products with MDPM added have greater sensory acceptance by Brazilian consumers when compared to those not added (Pereira et al., 2016). However, due to its composition (bone marrow, heme group and lipids) and extensive stress and aeration during the machine-deboning process, the MDPM is highly susceptible to microbial growth and lipid oxidation, has limited shelf life, and is highly dependent on storage temperature (Akramzadeh et al., 2020; Pereira et al., 2011).

Although the shelf life of MDPM can be extended using synthetic preservatives, there is a worldwide interest in finding new and safe antioxidants and antimicrobials from natural sources to prevent oxidative and microbiological deterioration of foods (Bellucci et al., 2022). The exploration of natural preservatives can be an alternative to the

problem of selecting resistant microbial strains (Jugreet et al., 2020), and also a response to consumer demand for products of "natural" claim. The application of natural and synthetic preservatives is one of the simplest ways to reduce oxidation and microbiological contamination in foodstuffs, mainly meat products containing MDPM.

Essential Oils (EOs) can be used as an attractive natural preservative because they are generally recognized as safe (GRAS) and have wide acceptance from consumers (Falleh et al., 2020). The antioxidant and antimicrobial activities of essential oils have been investigated as an alternative to food additives added. An EO with great potential use in the meat industry is the allspice (*Pimenta dioica* L.) oil, also known as Jamaican pepper, since it can act as an antioxidant (Murali et al., 2021; Sarathambal et al., 2021) and antimicrobial (Murali et al., 2021; Marques et al., 2019; Chaudhari et al., 2020; Silva et al., 2021). The allspice EO unites the scent and flavor characteristic of cloves, cinnamon and nutmeg and its antioxidant and antimicrobial activity is attributed to the active compounds present in them, especially its major components eugenol, myrcene and limonene (Mérida-Reyes et al., 2020).

Although several studies have been conducted on the antioxidant and antimicrobial effectiveness of EO against oxidation and food pathogens in foods, few works (Oussalah et al., 2006; Oussalah et al., 2007) have been published on the effect of allspice leaves EO, especially in meat products. Due to its potential use in the meat industry, this work aimed to characterize the EO extracted from the leaves of allspice, evaluate its antioxidant activity and its antimicrobial effect against pathogenic bacteria and spoilages, which frequently contaminate meat products, and investigate the effect of allspice leaves EO on the inhibition of lipid oxidation and quality properties of MDPM during chilled storage.

2 Material and methods

2.1 Plant materials and EO extraction

The leaves from (*Pimenta dioica* L.) were collected in the municipality of Ribeirão Vermelho (elevation 1303 m, 21º11'27"S- 45º03'43"O), in the state of Minas Gerais, Brazil. The identification of plant materials was confirmed by plant taxonomists in the Biology Department at the Federal University of Lavras (UFLA), in the state of Minas Gerais, Brazil. The fresh leaves' moisture was determined according to the methodology described by Pimentel et al. (2006), and the EO was extracted by hydrodistillation (in three replicates) using a modified Clevenger apparatus according to a procedure described by Brazilian National Health Surveillance Agency (in Portuguese *Agência Nacional de Vigilância Sanitária* (ANVISA) (Agência Nacional de Vigilância Sanitária, 2010). The EO yield was obtained and expressed in the weight of oil per gram of material on a dry weight basis (DWB).

2.2 EO identification and characterization

The EO chemical components were identified in a Gas Chromatograph (GC) coupled to a Mass Spectrometer (MS) (GC-MS, model GC17A with QP5000 mass selective detector; Shimadzu®, Kyoto, Japan) operating under the following conditions: fused silica DB-5MS capillary column (5% phenyl- 95% dimethylsiloxane; 30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent J&W, Santa Clara, CA, USA); ion source temperature of 220 °C; column temperature programmed at an initial temperature of 40 °C, and increased by 3 °C/min. up to 240 °C; helium as carrier gas (at 1 mL/min.); initial column pressure of 100.2 kPa; the split ratio of 1:10; and volume injected of 1 µL (1% solution in dichloromethane). The following conditions were used for the MS: impact energy of 70 eV; decomposition velocity of 1000, decomposition interval of 0.50 and fragments of 45 Da and 450 Da decomposed. The mass spectra obtained were compared to those of the database (NIST107 and NIST21), and the Retention Index (RI) was calculated using the Van

Den Dool & Kratz (1963) equation (Equation 1) in relation to the homologous series of *n* alkanes (nC8-nC18) and compared to those determined by Adams (2007).

$$RI = 110n + \frac{100Rt(i) - Rt(n)}{Rt(n+1) + Rt(n)} \quad (1)$$

Where Rt = is the retention time of the unknown compound, i = unknown compound, n = number of carbons in the standard compound prior to the unknown compound, $n + 1$ = number of carbons in the standard compound immediately following the unknown compound.

The quantification of each constituent was achieved by means of area normalization (%) after chromatographic separation on the GC equipped with a Flame Ionization Detector (FID) under the same experimental conditions used to identify the constituents of the EO (Camargo et al., 2020).

2.3 In vitro EO antimicrobial activity

The allspice oils antimicrobial activity was examined using the disk diffusion method conducted according to the National Committee for Clinical Laboratory Standards (2003). Five bacterial strains were chosen for their pathogenicity and the high frequency for which they are able to contaminate meat products: *Pseudomonas aeruginosa* (ATCC 27853), *Listeria monocytogenes* (ATCC 19117), *Staphyolcoccus aureus* (ATCC 25923), *Salmonella enteric* Enteritidis (S64) and *Escherichia coli* (Incos181 CDC055). Stock cultures were grown in Trypticase Soy Broth (TSB) with 0.6% yeast extract at 37 °C for 24 h before the tests.

Aliquots of 0.1 mL from 10^8 CFU/mL of microbial suspensions were inoculated with a Drigalski handlers onto the entire surface of a Trypticase Soy Agar (TSA) plates. Sterile paper disks (6 mm in diameter; Whatman no. 1) were placed with the help of sterile forceps on the surface of inoculated plates (three discs per plate) and then impregnated with 5 µL diluted allspice essential oil solution (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5% in methanol). The plates were incubated aerobically at 37 ºC for 48 h. The halo of the inhibition zone was measured (in millimeters), edge to edge over the center of the disk, after 48 h incubation using a digital caliper (Digimess, São Paulo, Brazil). Antimicrobial activity was considered as the diameter of the inhibition halo minus the diameter of the paper discs (6 mm). As a reference, the major EO constituent eugenol was evaluated in the same manner. The tests were carried out in triplicates for eugenol and in quadruplicates for each microorganism evaluated.

2.4 In vitro EO antioxidant activities

2.4.1 DPPH free radical scavenging assay

The capacity of the allspice EO to scavenge the 'stable' free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·) was monitored according to the method described by Amarowicz et al. (2004) with modifications. An aliquot of 0.3 mL of the sample solutions (from 5 to 1000 mg/kg) in methanol was mixed with 2.7 mL of 0.004% DPPH· solution in ethanol. The mixture was vortexed for 15 s and then left to stand at room temperature for 60 min. The absorbance of the resulting solution was read in a spectrophotometer (Genesys 10 UV-Vis; Fisher Scientific Inc., Waltham, MA, USA) at 517 nm. The ethanolic DPPH· solution in the absence of the sample (replaced by ethanol) was used as a control and the sample aliquot in pure ethanol was used as a blank. As a reference, the major EO constituent eugenol (Sigma-Aldrich) was evaluated in the same manner. The radical-scavenging activities of samples (EO and eugenol), expressed as percentage inhibition of DPPH· (antioxidant activity, %AA), were calculated according to Equation 2.

$$\%AA = 100 - 100 \times \frac{A_S - A_B}{A_C} \quad (2)$$

Where A_S is the absorbance value of the test sample, A_B is the absorbance of the blank and A_C is the absorbance of the control.

The concentration needed to capture 50% of free radical DPPH· (IC_{50}) was also calculated using the dose inhibition curve, by plotting the EO concentration (µg/mL) *versus* the corresponding scavenging effect (%AA). The four-parameter logistic model was used for curve-fitting analysis using SigmaPlot for Windows, version 9.01 (Systat Software Inc., Chicago, IL).

2.4.2 TBARS assay

The antioxidant capacity of the allspice EO was measured by the 2-thiobarbituric acid reactive species (TBARS) assay described by Ruberto & Baratta (2000) using egg yolk homogenates as lipid-rich media and 3,5-di-tert-4-butylhydroxytoluene (BHT) as an antioxidant reference compound. Solutions (100, 500 and 1000 mg/kg in methanol) of the compounds to be tested (EO or BHT) were prepared immediately before use. In brief, 0.5 mL of 10% (w/v) egg yolk solution and 0.1 mL of sample solution were added to a test tube and made up to 1.0 mL with distilled water. Then, 0.05 mL of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) 0.07 M in water was added to induce lipid peroxidation, followed by 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% 2-thiobarbituric acid (TBA) in 1.1% sodium dodecyl sulphate (SDS) solution. The resulting mixture was vortexed and then heated in a water bath at 95 °C for 60 min. After cooling, 5.0 mL of 1-butanol was added to each tube and centrifuged (EBA21; Hettich Zentrifugen, Tuttlingen, Germany) at 1200 × *g* for 10 min. The absorbance of the organic upper layer was measured at a wavelength of 532 nm using a Genesys 10 UV-Vis spectrophotometer. To observe the complete lipid peroxidation, a control tube was prepared in which all reagents were added except for the sample tested (replaced by distilled water). The samples' antioxidant activities, based on the percentage Antioxidant Index (AI), were calculated according to Equation 3.

$$\%AI = \left(1 - \frac{A_S}{A_C}\right) \times 100 \quad (3)$$

where, A_S is the absorbance of the test sample and A_C is the absorbance of the fully oxidized control.

2.5 MDPM treatment with EO

To evaluate the practical application of allspice EO as a natural antioxidant in MDPM, a full-factorial design experiment with two factors (3 allspice EO concentrations × 5 storage times) and five repetitions were elaborated. A total of fifty broiler carcasses (4 °C) were obtained at the local market on five different days (batches) of ten carcasses per day, transported to the Laboratory of Meat and Meat Products (LabCarnes) of the Department of Food Science, in the Federal University of Lavras [*Universidade Federal de Lavras* (UFLA)], hand-deboned, and the backs, without skin and tail, were cut into three parts, packed and frozen (-18 °C) overnight for obtaining the MDPM. The broiler acquisition for the batches was always made in the first or third week of the month and in the same establishment, to obtain fresh carcasses (~ 24 h of slaughter), packed, from the same slaughterhouse located in the city of Lavras, MG.

For each batch (repetition), the frozen broiler backs were processed through an HT100-C separator (High Tech Ind., Chapecó, SC, Brazil), one day after obtaining the carcasses, and the MDPM obtained (with a yield of 59.5%) immediately blended with different concentrations (0, 500 and 1000 mg/kg) of allspice EO. Each blended batch was divided into smaller portions (~100 g), for sampling at the individual storage periods, and wrapped in plastic bags without vacuum. According to Brazilian legislation (Brasil, 2000), when not stored frozen, the MDPM can be stored for a maximum of 24 or 72 h at temperatures not exceeding 4 °C or 0 °C, respectively. Therefore, it was decided to refrigerate (climate chamber EL202; Eletrolab Indústria e Comércio Ltda., São Paulo, Brazil) the samples at 2 °C to speed up the lipid peroxidation reactions and allow the effects of the treatments to be seen. Samples were analyzed at 0, 2, 4, 8 and 10 days of storage (at 2 °C) for pH, by inserting a combined electrode coupled to a DM-20 potentiometer (Digimed, São Paulo, SP, Brazil), and for TBARS values, determined by the extraction method according to Raharjo et al. (1992) and with some adaptations described by Dutra et al. (2011). The TBARS values were expressed

as milligrams of malondialdehyde (MDA) per kilo of MDPM (mg MDA/kg), calculated from an analytical curve of 1,1,3,3-tetraethyoxypropane (TEP).

2.6 Statistical analysis

Mean values and standard deviations of the allspice EO antimicrobial and DPPH antioxidant assay were calculated from the experimental data obtained and a descriptive analysis was conducted. The treatments were arranged in a completely randomized design (CRD) with two factors for the TBARS assay of EO (antioxidant × concentration) and for the MDPM analysis (EO concentration × storage time). Effects of independent variables and interactions were evaluated by Analysis of Variance (ANOVA) using the SAS® v.9 software (SAS Institute Inc., Cary, NC) and considering the 5% level of probability. When necessary, Tukey's test was used to determine the significance ($p < 0.05$) of the mean values for multiple comparisons.

3 Results and discussion

3.1 Allspice EO characterization

The average extraction yield of the allspice EO was 0.75% in a Dry Weight Base (DWB). The GC-MS characterization of allspice essential oil allowed the identification of 14 compounds (Table 1), representing 100% of the EO. The components found in major proportions were eugenol (55.52%), myrcene (22.43%), limonene (7.79%) and chavicol (5.12%).

Table 1. Chemical constituents of allspice (*Pimenta Dioica* L.) essential oil identified by GC-MS.

Peak	Compounds	RI_{EXP}	RI_{LIT}	(%)
1	Tricyclene	919	AI 1921 KI 1926	0.74
2	Octen-3-ol <1>	973	AI 974 KI 979	2.68
3	3-Octanone	980	AI 979 KI 983	1.09
4	Myrcene	986	AI 988 KI 990	22.43
5	3-Octanol	994	AI 988 KI991	0.69
6	α-Phellandrene	1005	AI 1002 KI 1002	1.64
7	ρ-Cymene	1023	AI 1020 KI1024	0.32
8	Limonene	1027	AI 1024 KI 1029	7.79
9	γ-Terpinene	1056	AI 1054 KI 1059	0.19
10	Mentha-2,4-(8)-diene<ρ>	1083	AI 1085 KI 1088	0.2
11	Linalol	1098	AI 1095 KI 1096	1.07
12	Terpinen-4-ol	1179	AI 1174 KI 1177	0.52
13	Chavicol	1247	AI 1247 KI 1250	5.12
14	Eugenol	1351	AI 1356 KI 1359	55.52

RI_{EXP} = Experimental Retention Index; RI_{LIT} = Experimental literature index; AI = Arithmetic Van der Dool and Kratz Index; KI = Kovats Index.

The phytochemical profile of the allspice EO in this study agreed with the results of Sarathambal et al. (2021), who observed eugenol as the major component (54.0%), followed by myrcene (16.0%), but a greater amount of chavicol (12.5%) was found than limonene (4.6%). However, the extraction yield reported by these authors was higher (1.8%) than observed in our experiment. Similar results were found by Faria et al. (2014). These four compounds were also found in larger amounts in *Pimenta dioica* Lindl oil by Everton et al. (2021), however, with different percentages of eugenol (85.7%), chavicol (19.0%), myrcene (2.8%) and limonene (1.7%). Mérida-Reyes et al. (2020) found higher percentages of eugenol (71.4%), β-myrcene (10.0%), (E)-caryophyllene (5.25%), chavicol (3.1%), (E)-β-ocimene (1.8%) and 1,8-cineol (1.6%).

The variation in extraction yield and in the quality and quantity of chemical constituents present in the essential oil can be attributed to several factors, including geographical region, the climatic conditions of the plant collection site, the degree of terrain hydration, macronutrient and micronutrient levels and the plant material's drying conditions, extraction methods utilized and conditions used (solvent, temperature, time) (Jugreet et al., 2020; Contreras-Moreno, 2018). The variation in the constituents of our analyzed results could be attributed to these factors.

3.2 Antimicrobial assay

The antimicrobial activity of allspice EO was evaluated based on the halos of clear inhibition zone surrounding the paper disks. Figure 1 showed the antimicrobial activity of EO and eugenol with different concentrations against important pathogens (*S. enterica*, *L. monocytogenes*, *E. coli*, and *S. aureus*) and spoilage (*P. aeruginosa*) by disk diffusion. Both allspice EO and eugenol had inhibitory effects for all bacteria tested.

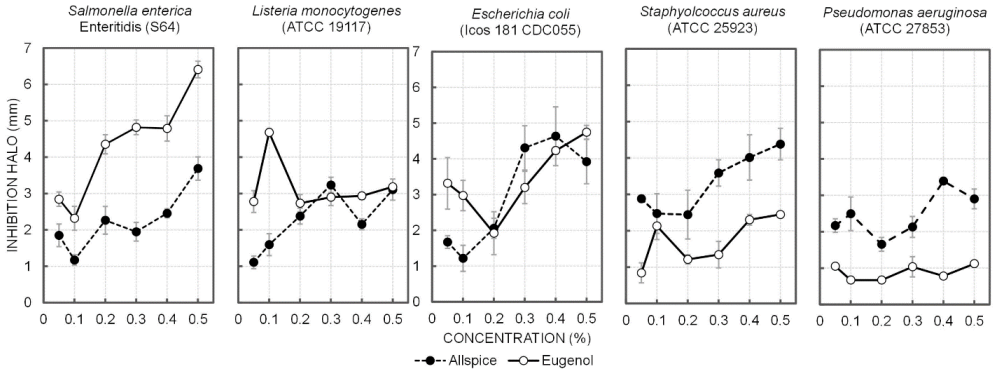

Figure 1. Antimicrobial activities (inhibition zone halo) of allspice essential oil and eugenol against food pathogens and spoilage bacteria using disc diffusion method. Bars represent the standard error of the means (SEM; n = 4 for allspice and n = 3 for eugenol).

Generally, EOs are slightly more active against Gram-positive (*B. cereus* and *S. aureus*) than Gram-negative (*E. coli* and *P. aeruginosa*) bacteria (Bharti et al., 2020), since they possess an outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Pateiro et al., 2021). However, the allspice oil inhibitory effect was observed for both Gram-positive and Gram-negative bacteria in the present study. Similar effects have already been found for cinnamon, clove and oregano essential oils (Pateiro et al., 2021), and in essential oil from *P. dioica* Lindl by Mérida-Reyes et al. (2020) and Everton et al. (2021).

In fact, it is expected that individual components of EO exhibit different degrees of activity against Gram-positives and Gram-negatives (Guo et al., 2021; Pateiro et al., 2021; Guimarães et al., 2019). In the present study, the essential oil was more effective in inhibiting *P. aeruginosa* and *S. aureus* than its major component (eugenol). Everton et al. (2021) also reported antibacterial actions against these bacteria. This may be due to the synergistic effect of one or more components present in the oil, not necessarily in larger quantities. Considering the large number of different groups of chemical compounds present in EO, it is likely that their antibacterial activity is not attributable to one specific mechanism but to several targets in the cell. Several authors suggested that phenolic compounds (such as eugenol, chavicol and p-cymene), and their precursors (such as ρ-cymene and ɤ-terpinene), are responsible for the inactivation of enzymes in charge of energy production and structure synthesis (Oliveira et al., 2011). Many terpenes are known to be active against a wide variety of microorganisms, including Gram-positive and Gram-negative bacteria (Silva et al., 2021; Falleh et al., 2020; Guimarães et al., 2019).

Salmonella and *Pseudomonas* are microorganisms of greater recurrence and concern in the poultry industry; once the meat is contaminated with them it can have its deterioration process accelerated and cause human

opportunistic infections (El-Ghany, 2021; Gomes Neto et al., 2012). *Pseudomonas* spp. are psychrotrophic bacteria that easily develop in foods stored aerobically and at low temperatures and are recognized as major food spoilers (Doulgeraki et al., 2012). Naturally present in poultry meat, including being associated with high mortality in young chickens and late death in the shell of embryos (El-Ghany, 2021), retail poultry products are incriminated as a primary source of *Pseudomonas* spp. for humans. Of the many different types of *Pseudomonas*, *P. aeruginosa* is the most common cause of infections in humans, being an opportunistic Gram-negative microorganism that can induce severe pulmonary infection and cystic lung fibrosis, especially in immunosuppressed persons (Bentzmann & Plésiat, 2011). Particularly, the effect of allspice EO observed against *P. aeruginosa* in this work was interesting since Barbut (2004) lists numerous studies that reported less sensitivity to the action of EOs for this genre. The antimicrobial effect of EO on *P. aeruginosa* was less effective only when compared to the pathogens *E. coli* and *S. aureus* in concentrations higher than 0.3%.

In the literature, an antimicrobial effect of allspice EO was also reported against *S. aureus* and *E. coli* (Oussalah et al., 2006; Oussalah et al., 2007; Lowe et al., 2017; Asha et al., 2013), *S. typhi*, and *P. aeruginosa* (Kumar et al., 2010) and *S. typhimurium* and *P. putida* (Oussalah et al., 2006; Oussalah et al., 2007).

3.3 Antioxidant activity

The dose-response curves for the radical-scavenging activity values of the allspice EO solutions and its major component (eugenol) were illustrated in Figure 2. The scavenging ability increased steadily with increasing of both compounds but was higher in the allspice EO than in the isolated eugenol. At a dosage of 20 µg/mL, for example, the allspice EO afforded greater than 90% scavenging ability on the stable DPPH· free radical, while eugenol showed only 65%. Moreover, the concentrations required for 50% inhibition (IC_{50}) of DPPH· radicals were 2.71 µg/mL for EO and 9.49 µg/mL for eugenol. These can be due to the high percentage of main phenolic constituents (eugenol and chavicol), but also to the presence of other constituents in small quantities and the synergy among them.

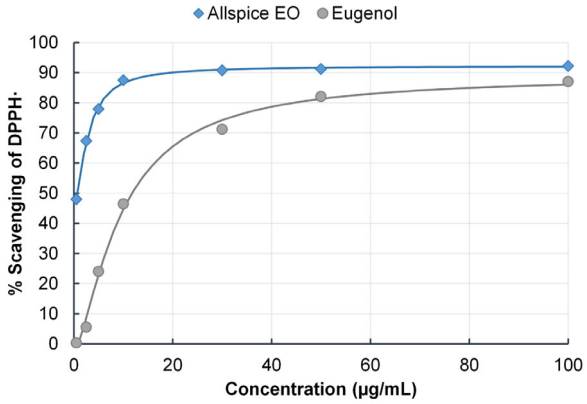

Figure 2. Scavenging effect of allspice essential oil (EO) and eugenol on 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical. The fitting curve was done by four logistic models: $p < 0.0001$ and $R^2 = 0.99$ for EO; and $p < 0.0003$ and $R^2 = 0.99$ for eugenol.

The stabilization of the DPPH· radical is directly related to the ability of a compound to donate electrons or hydrogen atoms to the radical. Other phenylpropanoids such as eugenol, myrcene and chavicol are among the phenolic compounds that have the greatest capacity to stabilize radicals because they act as direct antioxidants and have more than one site for donating hydrogen atoms (Amorati et al., 2013; Ferreira et al., 2019). The presence of two phenylpropanoids and other aromatic monoterpenes such as ρ-cymene in the EO (Table 1) justifies its greater activity compared to isolated eugenol.

The antioxidant activity of allspice EO was also determined by measuring the ability to delay the extent of lipid degradation through the TBARS assay, indirectly measuring the malondialdehyde (MDA) formation,

a secondary lipid peroxidation product. The Antioxidant Indexes (AI) of allspice EO were not different ($p > 0.05$) from that of reference (the synthetic antioxidant BHT), both being dose-dependent with the greatest activities observed at high concentrations (Figure 3). Similar results were obtained by Kumar et al. (2010). Again, the antioxidant activity of EOs is attributed to the active compounds present in them, especially their major components; in this case eugenol, myrcene and limonene. Ruberto & Baratta (2000) studied the antioxidant of these EO components activities using the TBARS method with pure compounds and showed that eugenol had higher AI (91.2%) than myrcene (32.1%) and limonene (27.4%) at 1000 ppm.

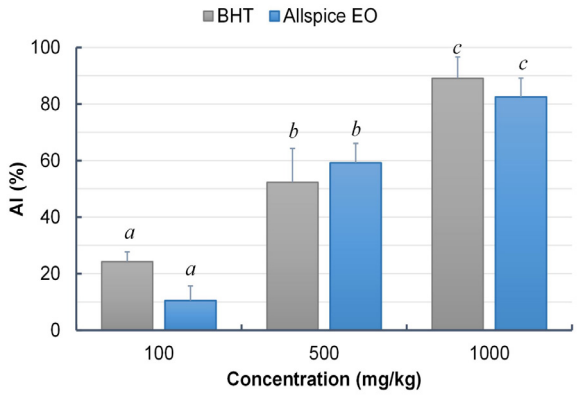

Figure 3. Antioxidant index (AI), according to the TBARS assay, of different concentrations of allspice leaves essential oil (EO) and butylhydroxytoluene (BHT) as the positive control. Bars represent the standard error of the means (SEM; n = 3) and means with different letters (a-c) differ ($p < 0.05$) by Tukey's test.

3.4 MDPM evaluation

For MDPM evaluation, there was an interaction ($p < 0.05$) between treatments (control, EO500 and EO1000) and storage times (0, 2, 4, 8 and 10 days) for pH and TBARS index.

The behavior of the pH values of the MDPM inoculated with EO was similar to the control, with a significant drop from the second day onwards (Figure 4). However, samples containing EO showed higher ($p < 0.05$) pH values than the control in the first four days of storage, not differing ($p > 0.05$) in the eighth and tenth days.

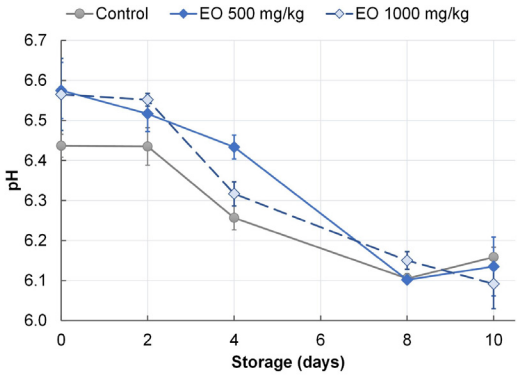

Figure 4. pH values of mechanically deboned poultry meat (MDPM) without (control) and with different concentrations of allspice leaves essential oil (EO) added during storage at 2 °C. Bars represent the standard error of the means (SEM; n = 5).

The pH value of control MDPM agrees with that found by Pereira et al. (2011) who evaluated the effects of the addition of MDPM and collagen fibers on the quality characteristics of sausages, and observed a pH

variation from 6.37 to 6.46, as higher amounts of MDPM were used. Higher pH values than poultry meat (5.6 to 5.8) are expected for MDPM, as there is an incorporation of bone marrow constituents and denaturation of proteins during the mechanical deboning process (Mohamed & Mansour, 2012).

The highest pH values recorded on day 0 storage for the treatments with essential oil added in this work can be attributed as a characteristic of the oil. Since essential oils have a complex chemical composition, this increase in pH for treatments containing allspice EO can be attributed to the action of one or more constituents present in the EO that interact with the MDPM matrix, resulting in a slight alkalization of the medium in relation to the control. Higher pH values for chicken MDPM with additives were also described by Bigolin et al. (2013) for the use of the antioxidants sodium erythorbate and ascorbic acid compared to that MDPM without additives. On the contrary, Ozer & Sariçoban (2010) found lower pH values in MDPM with the additives butylated hydroxyanisole (BHA), ascorbic acid and α-tocopherol added. In the work of Mohamed & Mansour (2012), beef hamburgers with MDPM with and without additions of rosemary or marjoram essential oil had no difference in pH values.

Different factors may be involved in the decrease in MDPM pH with storage time. The first to be considered is the production of organic acids from Lactic Acid Bacteria (LAB), which are common bacteria in refrigerated meats (Jayathilakan et al., 2012). Another possible factor involved in the drop in pH during storage is the fact that MDPM is a highly oxidizable matrix, with hydroperoxides being the primary product of this reaction. The increase in the hydroperoxide content can cause slight acidification of the medium, as they are weak acids (Pędziwiatr et al., 2018). Moreover, the more accentuated drop in pH of the control in relation to the treatments, from the first day, may be an indication of the formation of hydroperoxides. The formation of hydroperoxides and chemical reactions during the storage period can produce undesirable compounds, such as furfurals and heterocyclic compounds resulting from Maillard reactions. According to Lotfy et al. (2021), the consumption of amino groups of proteins and the production of acids by the Maillard reaction shifts the system to more acidic pH values. Bigolin et al. (2013) also observed a reduction in the pH of MDPM with the addition of different antioxidants during chilled storage.

During the chilled storage of MDPM, an increase in TBARS values was observed for all treatments (Figure 5). No differences ($p > 0.05$) were observed between the samples with EO added, but these differed from the control samples. In general, an increase in the TBARS index is expected with storage time due to the presence of pro-oxidant compounds naturally present in meat and meat products, especially in mechanically deboned meats. The mechanical process of removing meat from the bone causes cell breakage, protein denaturation, and an increase in polyunsaturated lipids from bone marrow and free iron from the heme group, which together with extensive aeration implies a lipid autoxidation with several disadvantages for its application in meat products, such as changes in color, flavor, and palatability (Pereira et al., 2011). However, the application of EO, a mixture rich in antioxidant compounds, acts on these foods in a protective way, that is, it tends to maintain lower levels of oxidation (Contreras-Moreno, 2018; Bigolin et al., 2013).

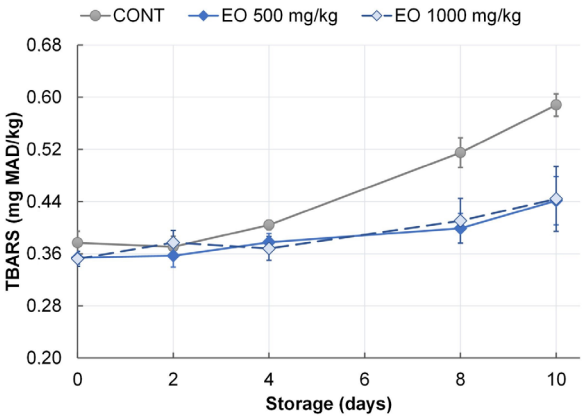

Figure 5. TBARS values of mechanically deboned poultry meat (MDPM) without (control: CONT) and with different concentrations of allspice leaves essential oil (EO) added during storage at 2 °C. Bars represent the standard error of the means (SEM; n = 5).

Since allspice is known to be a potent antioxidant (Mohamed & Mansour, 2012), it may have been able to inhibit the formation of early oxidation products, like hydroperoxides, for a longer period, delaying the formation of the secondary compounds from the lipid peroxidation. This effect was clearly observed in this work when evaluating the TBARS index compared to the control. During the first 4 days of storage, no differences ($p > 0.05$) were observed between treatments with EO and the control. However, from the fourth day onwards there was a greater increase ($p < 0.05$) in TBARS values in the control sample than in the samples with EO, and these differences (between control and EO samples) were significant on days 8 and 10. Furthermore, the increase in TBARS values from day 4 to day 8 was not significant in the samples containing EO. Therefore, the addition of EO suppressed the detection of lipid oxidation by the TBARS method for 4 days beyond what was observed in MDPM control samples during storage at 2 °C.

Mielnik et al. (2003) reported that the induction time for lipid oxidation in MDPM with antioxidants added will depend both on the type and level of antioxidant applied prior to storage. However, in this work, it was possible to perceive the antioxidant effect regardless of the concentration used. There are reports of some EO acting as pro-oxidants when added at high concentrations in meat products (Bellucci et al., 2022) but this effect was not observed, even when 1000 mg/kg allspice EO was added. The concentrations of EOs required to act as pro-oxidants depend on the type of EO and the medium, including species and breed. For example, Estévez & Cava (2006) observed that rosemary essential oil in low concentrations (150 mg/kg) reduced lipid and protein oxidation in pork frankfurters, but higher concentrations (300 and 600 mg/kg) favored the oxidation reaction.

The use of additives, whether synthetic or natural, tends to increase the MDPM oxidative stability (Chaudhari et al., 2020; Ferreira et al., 2019) and the antioxidant properties of *P. dioica* EO in this study as confirmed by the TBARS method. As observed in the present study with chilled storage, Mielnik et al. (2003) reported that at the end of the frozen storage period, TBARS values for all samples treated with antioxidants (commercial rosemary antioxidants, vitamin C and vitamin E) were significantly lower than the values of the control samples. The results also agree with those reported by Hać-Szymańczuk et al. (2017) who also found a decrease in lipid oxidation in MDPM treated with extracts and EO of rosemary under vacuum storage at -18 °C for 4 months.

4 Conclusion

Allspice leaves essential oil showed better antimicrobial and antioxidant activities compared to its main compound eugenol, suggesting that the perceived effect is the result of a synergistic effect between the components present in the oil. It can be concluded that essential oil was a good alternative for application in mechanically deboned poultry meat (MDPM) as a natural antioxidant. Both essential oil treatments (500 and 1000 mg/kg) acted positively as an antioxidant in MDPM and since there was no difference in antioxidant activity between them, the suggested amount for use is the lower concentration. However, additional studies are needed to establish whether this essential oil concentration in the MDPM could influence the technological and/or sensory characteristics of the meat products in which different amounts of treated MDPM could be used.

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Funding: Ministério da Ciência, Tecnologia e Inovação/Conselho Nacional de Desenvolvimento Científico e Tecnológico (430206/2016-0); Fundação de Amparo à Pesquisa do Estado de Minas Gerais (CVZ APQ-02904-17).

Received: Oct. 26, 2022; **Accepted:** Mar. 17, 2023

Associate Editor: Marco Antonio Trindade.