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FOOD TECHNOLOGY

Brown propolis bioactive compounds as a natural antimicrobial in alginate films applied to *Piper nigrum* L.

Milena da Cruz Costa[1] Alexsandra Iarlen Cabral Cruz[1] Mariza Alves Ferreira[1] Aline Simões da Rocha Bispo[1] Paulo Roberto Ribeiro[3] João Albany Costa[2] Floricéa Magalhães Araújo[3] Norma Suely Evangelista-Barreto[1*]

[1]Programa de Pós-graduação em Microbiologia Agrícola, Centro de Ciências Agrárias, Ambiental e Biológicas (CCAAB), Universidade Federal do Recôncavo da Bahia (UFRB), 44380-000, Cruz das Almas, BA, Brasil. E-mail: nsevangelista@ufrb.edu.br. *Corresponding author.
[2]Centro de Ciências Exatas e Tecnológicas (CETEC), Universidade Federal do Recôncavo da Bahia (UFRB), Cruz das Almas, BA, Brasil.
[3]Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia (UFBA), Salvador, BA, Brasil.

ABSTRACT: *An edible coating of sodium alginate incorporated with brown propolis (2.5%, 5%, 10%, and 15%) was applied to black pepper grains to improve microbiological quality over 30 days. Gas chromatography coupled with mass spectrometry identified 29 metabolites in the extract, mainly terpene compounds (51.74%), phenolic compounds (25.83%), and flavonoids (14.48%). Brown propolis showed greater antibacterial activity for Gram-positive bacteria (MIC from 0.1 to 0.5 mg.mL^{-1}) and lower activity for* Escherichia coli *(MIC 18 mg.mL^{-1}). A 5% increase in propolis in the coating reduced* Bacillus cereus *counts by 7-fold, 9.4% for* Staphylococcus aureus*, and 5.4% for mesophilic bacteria. The edible sodium alginate coating containing brown propolis was effective in reducing microbes on black pepper, with a concentration of 15% propolis assuring the microbiological quality of the spice after 20 days.*
Key words: *natural antimicrobial,* Bacillus cereus*, microbiological contamination, bioactive compounds, black pepper.*

Potencial dos compostos bioativos da própolis marrom como antimicrobiano natural em filmes de alginato aplicados na *Piper nigrum* L.

RESUMO: *Revestimento comestível de alginato de sódio incorporado com própolis marrom (2,5%, 5%, 10% e 15%) foi aplicado em grãos de pimenta-do-reino para melhorar a qualidade microbiológica ao longo de 30 dias. Análise de cromatografia gasosa associada à espectrometria de massa identificou 29 metabólitos no extrato, principalmente compostos terpênicos (51,74%), compostos fenólicos (25,83%) e flavonóides (14,48%). A própolis marrom apresentou maior atividade antibacteriana para bactérias Gram-positivas (CIM de 0,1 a 0,5 mg.mL^{-1}) e menor atividade para* Escherichia coli *(CIM 18 mg.mL^{-1}). Um aumento em 5% no revestimento da própolis reduziu a contagem de* Bacillus cereus *em sete vezes, 9,4% para* Staphylococcus aureus *e 5,4% para bactérias mesófilas. O revestimento comestível de alginato de sódio e própolis marrom foi eficaz na redução microbiana da pimenta-do-reino, em que a concentração de 15% de própolis garantiu a qualidade microbiológica da especiaria até 20 dias.*
Palavras-chave: *antimicrobiano natural,* Bacillus cereus*, contaminação microbiológica, compostos bioativos, pimenta preta.*

INTRODUCTION

Black pepper (*Piper nigrum* L.) has a spicy taste and aroma and is widely used in food preparation, including in fish and meat (GAFAR, 2022). During the process of harvesting and drying herbs and spices, lack of good agricultural practices, storage, and transport are factors that contribute to the contamination and proliferation of microorganisms, such as coliforms at 45 °C, *Salmonella* spp., *Staphylococcus aureus*, and microorganisms that form spores such as *Bacillus cereus* (WEIL et al., 2020). However, microbial contamination in black pepper is not restricted to pathogens; mesophilic bacteria are detected in the 8 log CFU.g^{-1} range. The presence of these bacteria in contaminated condiments can cause illness in consumers, as they act as a vehicle for transferring pathogens and toxin production (THANH et al., 2018), in addition to reducing the product's useful life.

Current decontamination technologies used in spices are thermal or chemical, such as steam treatments, irradiation, and fumigation with ethylene oxide (GOLDEN et al., 2019), but the high cost

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has been a barrier for small-scale spice processors, mainly traders who sell spices in markets and fairs. A safe, simple, and attractive alternative has been the application of edible coatings using natural compounds with antimicrobial and antioxidant activities (PASTOR et al., 2011).

Edible coatings act as a barrier to oxygen and water permeability, slowing down oxidation reactions and retaining moisture to improve food quality and extend storage life. Among the biopolymers used in the coating, sodium alginate is a salt of alginic acid, a polysaccharide obtained from brown seaweed, and is widely used in the food industry (MOSKALEWICZ et al., 2022). Its popularity is due to its non-toxicity, biodegradability, and edibility, in addition to being a good carrier of bioactive compounds that have antioxidant and antimicrobial properties (HEYDARI et al., 2015).

Propolis is a natural resinous product collected by bees from plant secretions and used to cover and protect hives (PASSOS et al., 2016). It stands out for its antimicrobial and antioxidant activities (ANDRADE et al., 2017) and is generally recognized as a safe substance.

Although, the most well-known propolis in Brazil is green propolis, due to great Brazilian biodiversity, there are more than 13 types of propolis, including red and brown propolis, which are less common and classified based on production site (ANDRADE et al., 2017). The brown propolis used in this study is a product of beekeeping in the Recôncavo Baiano region, Bahia, Brazil. Although it has great potential because of its bioactive compounds, it has not been extensively explored by the local community.

In recent years, propolis from tropical regions such as Brazil has become an object of growing interest in research, leading to an increase in the identification of new metabolites with biological activity, stimulating new research into their chemical composition, biological activities, and food quality. Given that propolis is rich in phenolic compounds, and that thus far, no study has been carried out using it for the conservation of spices, this research evaluated the efficiency of the edible coating using sodium alginate incorporated with brown propolis extract (BPE) in controlling bacterial growth on black pepper.

MATERIALS AND METHODS

Obtaining the brown propolis extract and black pepper

The ethanol extract of brown propolis (EEBP) at 30% was purchased commercially from a certified producer (Abmel) located in the city Cruz das Almas, from the municipality of Cabaceiras do Paraguaçu (12º 32 '08 "S 39º 11' 27" W), Bahia, Brazil, with the following quality control characteristics indicated by Brazilian legislation (BRASIL, 2001): moisture (5.53%), ash (1.95%), wax (20.7%), mechanical mass (34.4%), solids soluble in ethanol (65.6%), total flavonoids (1.38 mg EAG.g^{-1}), total phenols (12.35 mg EQ.g^{-1}), and DDPH (2,2-diphenyl-1-picrilhidrazil) (38.12%).

Black pepper (2.5 kg) was purchased from street markets in the municipalities of Cruz das Almas, Cachoeira, and Santo Antônio de Jesus in Recôncavo Baiano, Bahia, Brazil.

Gas chromatography-mass spectrometry (GC-MS)

The chemical composition of BPE was identified through the analysis of mass spectra obtained in a spectrometer (model GCMS-QP2020) coupled to a gas chromatograph (model GC2010), both brand Shimadzu, using a capillary column DB-5MS (30 m × 0.25 mm and 0.25 µm), with a mobile phase flow rate (He) set to 1.8 mL.min^{-1}, inlet temperature of 280 °C, total flow of 13.8 mL.min^{-1}, column flow 1.80 mL.min^{-1}, linear velocity 48.9 cm.sec^{-1}, purge flow of 3.0 mL.min^{-1}, split ratio 5.0 and oven programmed from 60 ºC to 280 ºC with a heating rate of 10 ºC.min^{-1}, then kept at 280 ºC for 35 min. Sample injection was performed in pulsed mode without division (111.5 kPa), and source and interface temperatures were maintained at 280 °C. The full scan spectra were recorded from 37 to 660 m/z (mass/load), with two scans per second (CRUZ et al., 2021).

Identification of components in brown propolis extract

The BPE compounds were identified using standards contained in the NIST08, Mainlib, and Wiley7 databases and by comparison with data in the literature (MARCUCCI, 1996; BANKOVA et al., 2000; MOHAMMADZADEH et al., 2007; ISHIDA et al., 2011; CZYZEWSKA et al., 2014; JERZ et al., 2014, SOLTANI et al., 2017; OLEGÁRIO et al., 2019). In addition, to comparison of retention indices obtained through the linear curve using the homologous series of hydrocarbons C_9-C_{40} as standards, data on the linear retention index obtained in the scientific literature for columns of the same polarity were also used (EL-SAYED, 2019). The relative quantity of the individual components was expressed as a percentage area of the peak relative to the total area of the identified compounds.

Antibacterial activity of brown propolis extract

The Gram-positive bacteria *Staphylococcus aureus* ATCC 43300 and *Bacillus cereus* ATCC 14579, as well as Gram-negative *Escherichia coli* ATCC 25922 were used. Initially, 100 µL of Muller-Hinton broth was placed into each well of the 96-well plate. Then, 100 µL of the BPE extract was added to the wells in the first line and, after homogenization, microdilution was performed to obtain the concentrations 300, 150, 75, 37, 18, 9, 4, 2, 1, 0.5, 0.25, and 0.1 $mg.mL^{-1}$. After that, an aliquot of 10 µL of the inoculum ($1.5x10^7$ $CFU.mL^{-1}$) (CLSI, 2016), of each bacteria was added to the wells. Chloramphenicol was used as positive control in concentrations ranging from 1.0 to 30 $\mu g.mL^{-1}$. The plates were incubated in a bacteriological oven at 37 ºC for 24 h. After this period, 20 µL of the sodium resazurin (Sigma-Aldrich) dye (0.01%) was added to the wells and the plate was incubated for 3 h. The minimum bactericidal concentration (MBC) corresponded to the lowest concentration of the extract that did not show visible bacterial growth (WIEGAND et al., 2008).

Preparation of coating suspension

For coating suspension, the methodology proposed by OUSSALAH et al. (2006) and PASSOS et al. (2016) with modifications was used. The coating solution (300 mL) was prepared using a sodium alginate suspension (Êxodo Científica, Sumaré, SP, Brazil) (3 g: final concentration of 1% v/v) which was solubilized in sterile water (262.5 mL), at room temperature under stirring for 2 h. Then glycerol (Sigma-Aldrich, St. Louis, MO, USA) (3 mL: final concentration of 1% v/v). BPE was added at concentrations of 2.5% (25 $mg.mL^{-1}$), 5% (50 $mg.mL^{-1}$), 10% (100 $mg.mL^{-1}$) and 15% (150 $mg.mL^{-1}$). Each suspension was shaken for 10 min to mix.

Edible coating of black pepper

The black pepper samples (150 g) were immersed for 1 min (PASTOR et al., 2011) in sodium alginate (SA) and propolis extract (PE) treatments T1 (SA1% + PE2.5%), T2 (SA1% + PE5%), T3 (SA1% + PE10%), and T4 (SA1% + PE15%). The control sample was not coated. Coated samples were drained in a nylon mesh at 25 °C for 1 h to eliminate excess liquid and dried in paper bags in an air circulation oven at 40 °C for 72 h. All tests were repeated three times, and microbiological analyses were performed at 0-, 10-, 20-, and 30-day intervals for analysis of mesophilic bacteria, coliforms at 45 °C, *S. aureus*, and *B. cereus* (APHA, 2015).

The quantification of mesophilic bacteria was performed using the pour plate technique in Plate Count Agar (PCA) medium, after incubation at 36 °C for 48 h. The estimate of the most probable number (MPN) of coliforms at 45 ºC was performed by the multiple tube fermentation technique, in Lauryl Sulfate Tryptose Broth (LST) and the results expressed in $NMP.g^{-1}$. The *S. aureus* count was performed using the surface seeding technique (spread plate) in Baird Parker Agar (BPA) selective medium supplemented with egg yolk solution, and the plates were incubated inverted at 35 ºC for 48 h. The *B. cereus* count was also performed by surface seeding on Mannitol Egg Yolk Polymyxin (MYP) agar, and the plates were incubated at 30 ºC for 24 h.

Statistical analysis

The data were subjected to an analysis of variance (F-test) and multilinear regression. The most representative regression model was selected based on the significance of the F test ($P < 0.05$), the linear regression coefficients using Student's t-test ($P < 0.05$), and according to the most adjusted determination coefficient of the model. For the analysis, regression analysis was performed using SPSS Statistics for Windows, version 25.0 (IBM CORP, 2017).

RESULTS

Chemical composition of propolis

Through GC/MS analysis, it was possible to identify in BPE 29 secondary metabolites belonging to different classes, including acids, esters, sugars, phenols, flavonoids, steroids, and triterpenes, in addition to the 9-methylthio-Androst-4-en-3 diterpene, 11,17-trione (Table 1).

Until now, there have been no reports of 11 of the constituents identified from propolis, including steroids: cholestan-3-ol,2-methylene-(3β,5α), cholestane,4,5-epoxy-(4.alpha.,5.alpha.), and beta-amyrin; flavonoids: 5-hydroxy-4',7-dimethoxyflavanone, and 6-O-methylapigenin; phenols: 3-(4Z,7Z)-4,7-heptadecadienyl-, (Z)-3-(pentadec-8-en-1-yl), Z)-3-(heptadec-10-en-1-yl; diphenol: (Z)-5-(pentadec-8-en-1-yl) benzene-1,3-diol; triphenol: 1,2,4-benzenetriol; and diterperno: androst-4-en-3,11,17-trione,9-methylthio.

Terpenes represented the main class, with 51.74% of the relative area of the constituents identified in the brown propolis extract, followed by phenolic compounds (25.83%) and flavonoids (14.48%). Among the terpenes, triterpenes, cycloartenol (28.64%), and cycloeucalenol (12.94%)

Table 1 - Chemical composition of brown propolis extract identified by gas chromatography-mass spectrometry.

Class	Metabolites	TR (min.)	%relative area
Acidic	Hexadecanoic acid	17.20	0.25
	Cis-9, cis-12-octadecadienoic acid	19.10	0.46
	Octadecanoic acid, ethyl ester	19.40	0.72
Sugars	Alfa-L-galactopyranoside, methyl 6-deoxy-	6.77	0.35
	Ethyl α-d-glucopyranoside	13.59	0.59
Esters	Butanoic acid, 3-oxo-, methyl ester	3.25	0.25
	Ethyl palmitate	17.51	1.19
	Ethyl oleate	19.16	1.81
	Docosanoic acid, ethyl ester	24.42	1.63
Steroids	Cholestan-3-ol, 2-methylene-(3β,5α)	20.27	0.33
	Cholestane, 4,5-epoxy-(4.alpha.,5.alpha.)	22.14	0.37
Phenols	Phenol, 3-(4Z,7Z)-4,7-heptadecadienyl-	23.40	0.50
	(Z)-3-(pentadec-8-en-1-yl) phenol	23.61	3.84
	3-pentadecylphenol	23.66	1.68
	(Z)-3-(Heptadec-10-en-1-yl) phenol	25.73	9.23
Phenols(Di-)	Hydroquinone	9.19	0.51
	5-Pentadecylresorcisol	24.55	1.30
	(Z)-5-(Pentadec-8-en-1-yl) benzene-1,3-diol	27.02	8.00
Phenol(Tri-)	1,2,4-Benzenetriol	12.18	0.77
Flavonoids	5-hydroxy-4',7-dimethoxyflavanone	23.15	1.49
	Sakuranetin	24.35	6.17
	6-O-methylapigenin	28.35	2.46
	4',5,7-trihydroxy-3'6-dimetoxyflavona	28.58	4.36
Terpenes	Beta-amyrin	33.79	5.53
	Cycloeucalenol	34.37	12.94
	Cycloartenol	35.08	28.64
	Cycloaudenol	35.44	4.28
	Lupeol	37.76	0.35

RT - Retention time; min. – minutes.

were the major terpenes, and among the phenols, (Z)-3-(heptadec-10-en-1-yl) phenol (9.23%).

Antimicrobial activity of brown propolis extract

MIC values ranged between 0.1 and 18 $mg.mL^{-1}$, while the range for MBC ranged from 18 to 150 $mg.mL^{-1}$. Gram-positive bacteria (*B. cereus* and *S. aureus*) were more susceptible to BPE, with lower MIC values than *E. coli* (18 $mg.mL^{-1}$) (Table 2). The highest efficiency of the extract was observed for *S. aureus*, both for MIC and MBC, with values of 0.1 and 18 $mg.mL^{-1}$, respectively. This contrasted with *B. cereus*, which had a low MIC, but high MBC (Table 2).

Effect of edible coating on black pepper

Based on the results of antimicrobial activity assays, different concentrations of propolis were chosen for the coating step. When samples of pepper treated with the T1 (SA1% + PE2.5%) and T2 (SA1% + PE5%) coatings were analyzed, there was a 100% reduction in the microbial load of coliforms at 45 ºC during the storage period, compared to the control group (2.66 log $NMP.g^{-1}$ to > 3.04 log $NMP.g^{-1}$).

Figure 1 (A) shows that increases in the percentage of propolis concentration in the coating matrix caused a linear average reduction of 0.170 logarithm units in the number of *B. cereus*, regardless

Table 2 - Antimicrobial activity of brown propolis extract against Gram-positive and Gram-negative bacteria.

Microorganisms	-------Antimicrobial activity------	
	MIC (mg.mL^{-1})	MBC (mg.mL^{-1})
Escherichia coli	18	28
Bacillus cereus	0.5	150
Staphylococcus aureus	0.1	18

of the number of days evaluated. With this, it can be estimated that the 5% increase in the concentration of BPE caused an average reduction of approximately 7-fold in microorganism count.

On day T0, on samples with no coating (Control), the microbial load was 5.77 log CFU.g^{-1}. In pepper samples coated with 15% BPE (T4), the microbial count was 3.22 log CFU.g^{-1}, a 355-fold reduction in the number of *B. cereus*. Conversely, after 30 days 30 days, Control samples showed a microbial load of 7.96 log CFU.g^{-1} in T4, microbial load decreased 347-fold (5.42 log CFU.g^{-1}).

Over time, statistical analysis showed that, regardless of the concentrations tested, there was an increase of 0.73 units in the log of the number of *B. cereus* corresponding to each 10 days of product exposure, representing an estimated multiplication rate 5.4-fold the number of *B. cereus* (Figure 1A). In figure 1B, an increase of 1% in the concentration of BPE in the coating creates an estimated reduction of 0.195 log units in the *S. aureus* count, regardless of the storage time of the samples. Similarly, the increase to 5% BPE concentration caused a 9.4-fold reduction in the microbial count of *S. aureus*.

When analyzing the effect every 10 days, there was an average increase of 0.481 log units in the number of *S. aureus*, corresponding to a 3-fold increase in the growth of the microorganism. In the analysis of mesophiles (Figure 1C), the regression coefficients of the model indicate that the addition of 1% BPE to the coating reduces 0.147 log units in the number of mesophiles. Additionally, for every 5% increase in the BPE concentration, there is an average decrease of 5.4-fold in the microbial count. To gauge the effect of storage time, an average increase of

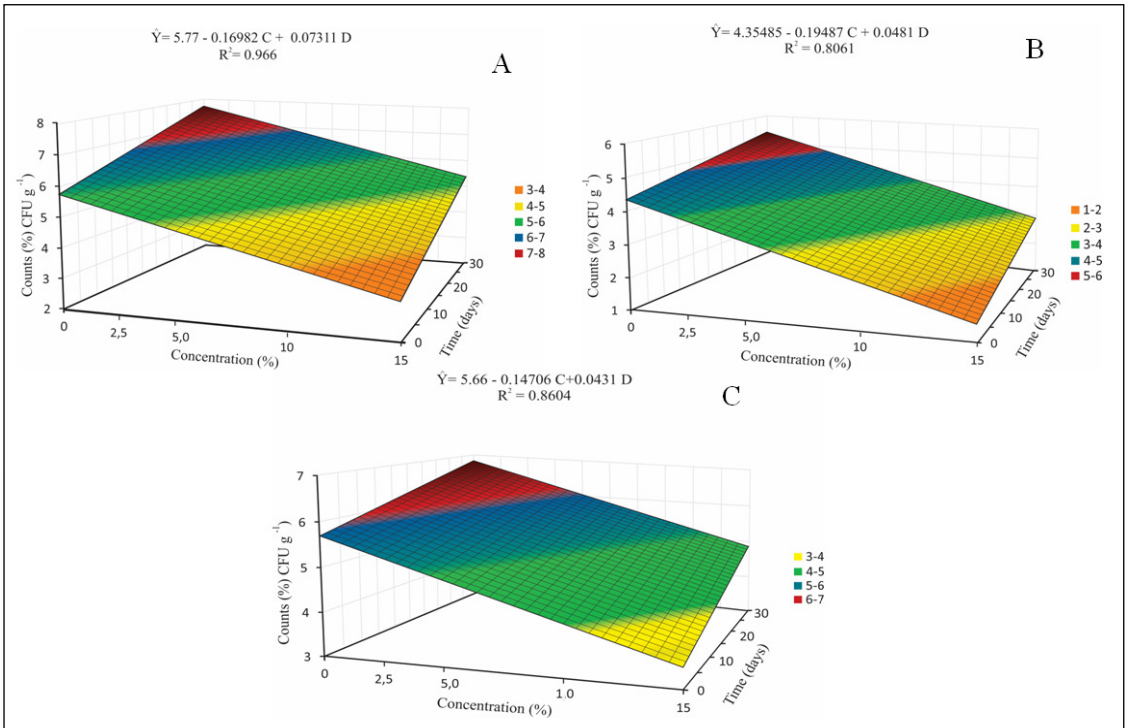

Figure 1 - Effect of the coating of sodium alginate incorporated with brown propolis in black pepper for *Bacillus cereus* (A); *Staphylococcus aureus* (B) and mesophilic bacteria (C) during 30 days of storage during.

0.432 log units in the count of mesophilic bacteria was verified every 10 days, indicating a 2.7-fold increase in the number of microorganisms, which was lower than that observed for *S. aureus*.

The analysis of the model determination coefficients shows that 96.65%, 80.61%, and 86.04% of the variations that occurred in the counts of *B. cereus, S. aureus*, and mesophilic bacteria, respectively, are explained by differences in the concentration and time variables, demonstrating that the model is highly representative and reliable for the researched data.

DISCUSSION

Chemical composition of propolis

The chemical composition of propolis varies depending on factors related to the geographical region of its production, mainly the vegetation that grows around the hives, climatic conditions, and time of collection (OLEGÁRIO et al., 2019). A wide range of bioactive substances, resulting from different plant sources, exhibiting high therapeutic potential, characterizes brown colored propolis extracts. With a favorable climate and diverse relief, the Recôncavo Baiano region expresses favorable conditions for the development of plant species. According to RODRIGUES et al. (2021), the most representative species in Cabaceiras do Paraguaçu, place of origin of the analyzed propolis, are *Cymbopogon citratus* Stapf., *Lippia alba* L., *Plectranthus barbatus* Andrews and *Mentha spicata* L.

Among the triterpenoids reported in brown propolis, pentacyclics such as lupeol and beta-amyrins, and tetracyclics such as cycloartenol, have been reported to be beneficial for human health due to their anti-inflammatory activity (SILVA et al., 2005). Phenolic and flavonoid compounds are also important active constituents of propolis because they act as eliminators of free radicals or prevent their formation. This property contributes to the ability of propolis to prevent the lipid oxidation of foods (ANDRADE et al., 2017).

Cycloartenol, $C_{30}H_{50}O$ (PubChem CID: 92110) is a pentacyclic triterpenoid, a 3beta-sterol and a member of phytosterols. It derives from a hydride of a lanostane and is found as a plant metabolite (NCBI, 2021a). There are reports of its occurrence in several plant species, such as *Morinda lucida* Benth. (*Rubiaceae*) (ISHOLA & ADEWOLE, 2019) and *Mercurialis* spp. (*Euphorbiaceae*) (BLANCO-SALAS et al., 2019).

We highlighted cycloeucalenol (PubChem CID: 101690) as the second major component in the analyzed samples of BPE, it is an isomer of cycloartenol; however, it derives from a hydride of a 5alpha-ergostane (NCBI, 2021b). The similarity in most of the chemical structure is what justifies the proximity of the retention time. Substance reported in several plant species such as *Olea europaea* L. (*Oleaceae*) (GHANBARI et al., 2012); in the pollen of *Brassica rapa* L. (*Brassicaceae*) (LI et al., 2009) among others, it exhibits bioactive properties of commercial interest, potentially beneficial to health.

As the third largest chemical compound identified in BPE, 3-(10-heptadecenyl) phenol, $C_{23}H_{38}O$ (PubChem CID: 44575468), also known as cardanol C17:1, belongs to the class of organic compounds known as 1-hydroxy-4-unsubstituted benzenoids. These are phenols that are unsubstituted at the 4-position (NCBI, 2021c). NEGRI et al. (2019), reported this compound among the main constituents identified as unsaturated cardanols, in hexane extract from the *Scaptotrigona aff. postica* geopropolis. Among the main activities mentioned by the authors the phenolic lipids exhibit antioxidant, anticarcinogenic, antimicrobial, antileishmanial and larvicidal properties, recommending their use in the food industry and in the pharmaceutical.

Recently published article describes the importance of pentacyclic triterpenoid as potential antiviral agents for the treatment of COVID-19. The authors used seeds and oil from *Nigella sativa* (*Ranunculaceae*), popularly known as black seed or black cumin, rich in terpenoids and flavonoids (SIDDIQUI et al., 2020).

In the propolis under study, the eight types of phenols identified, together with the five types of flavonoids, demonstrate the important role of brown propolis in terms of antibacterial and antioxidant activity (YANG et al., 2015). BITTENCOURT et al. (2015), who reported the presence of flavonoids and other phenolic compounds and their antibacterial and antioxidant properties.

Antimicrobial activity of brown propolis extract

BPE displays antimicrobial activity (TAKAISI-KIKUNI & SCHILCHER, 1994) and kinetic components that destabilize the cytoplasmic membrane and inhibit bacterial motility (MIRZOEVA et al., 1997). The antimicrobial activity of BPE against *E. coli*, *S. aureus*, and *B. cereus* is mainly attributed to phenolic compounds (flavonoids and phenolic acids) that are recognized to use mechanisms, such as interference with cell division, cytoplasmic changes, and inhibition of proteins, that are responsible for cell death (TAKAISI-KIKUNI & SCHILCHER, 1994)

and components that destabilize the cytoplasmic membrane and inhibit bacterial motility (MIRZOEVA et al., 1997).

Against *S. aureus*, brown propolis showed strong inhibitory and moderate-to-weak action [MIC values ranging from 0.25 to 0.50 $mg.mL^{-1}$ (SILVA et al., 2017)] against *B. cereus*. Similar results have been described by TIVERON et al. (2016) for organic propolis when reporting MIC values for *S. aureus* ranging from 0.05 to 0.2 $mg.mL^{-1}$ and MBC from 0.8 to > 1.8 $mg.mL^{-1}$. Conversely, this differed from the results reported by SILVA et al. (2017) when studying brown propolis from the states of Paraná and Rio Grande do Sul, in which researchers did not observe antimicrobial activity against *S. aureus* and *E. coli*.

The lower susceptibility observed for *E. coli;* and consequently, the high MIC obtained (18 $mg.mL^{-1}$) is due to the structural differences of the bacterial cells (CHEN et al., 2018). TIVERON et al. (2016), when studying extracts of propolis from different regions of Brazil, did not inhibit *E. coli* at the highest concentration tested (> 1.6 $mg.mL^{-1}$). DEMIRKOL (2013), studying turkey propolis, also showed no inhibition of *E. coli* at concentrations > 10 $mg.mL^{-1}$.

The mechanism of antimicrobial activity against microorganisms is a complex characteristic that involves the synergy of metabolites present in the extracts. BITTENCOURT et al. (2015) studied Brazilian green and brown propolis and reported that only triterpenes, steroids, sesquiterpenes, and hydrocarbons correlated with antibacterial activity, demonstrating a different action on different bacteria.

Edible coating of black pepper containing brown propolis extract

In the pepper-coating stage, despite the efficiency of treatment T1 (2.5%) for the coliform group, the antimicrobial concentration of propolis that most efficiently reduced the microbial count of the other bioindicators was T4 (15%), for a period of 20 days (ICMSF, 1974; EUROPEAN COMMISSION, 2004), due to the increase in the microbial load of *B. cereus* at 30 days.

The microbial resistance of *B. cereus* has also been reported by THANH et al. (2018), who intentionally contaminated different spices and did not observe a significant reduction in the count of *B. cereus* for a period of 50 weeks when compared to *S. aureus*.

An alternative to minimize the resistance of *B. cereus* endospores would be to reapply the coating every 20 days. Although, sodium alginate acts on the adhesion of the coating, promoting the oxygen barrier due to the compact and ordered structure of the hydrogen-bonded network, the propolis contains hydrophobic compounds that constitute a biodegradable film on the surface of the fruit, forming a semipermeable layer, reducing moisture (PASSOS et al., 2016) and providing a microbiological barrier. After 20 days of treatment, volatilization of the propolis compounds (PELLATI et al., 2016) reduces the microbiological action, allowing the growth of *B. cereus*.

The application of brown propolis as an edible coating is viable and may be an alternative for the food industry since propolis, a product of beekeeping, has been rarely used in the region of Recôncavo Baiano due to the lack of information on the part of beekeepers who direct their production only to honey. In this sense, the brown propolis can be used by the local community for the conservation of food products because of its richness in bioactive compounds, in addition to adding value to beekeeping activity. Extending the useful life of black pepper during storage also increases the microbiological quality for consumers, generating benefits for marketers, since this treatment improves the sanitary hygienic conditions of the product that is sold in open markets in the region.

CONCLUSION

The edible coating of sodium alginate incorporated with 15% brown propolis extract was effective in reducing the microbial counts of *B. cereus, S. aureus*, and mesophilic bacteria in black pepper, increasing the microbiological quality of black pepper after 20 days of storage. The good antimicrobial results presented in this work are due to the synergistic association of metabolites present in the propolis extract, which is rich in terpenic and phenolic metabolites.

The use of brown propolis in other types of condiments can be tested at different concentrations and diversifying the techniques for extracting bioactive compounds from propolis for proper incorporation into products. New studies can be developed involving brown propolis, sodium alginate or other plasticizing agents in foods, enhancing or adding nutritional value, in addition to prolonging the storage time in the formulations.

The identification of 12 new substances in brown propolis obtained in the region of Recôncavo, Bahia, Brazil, contributes to the knowledge of the chemistry of this propolis, particularly in the discovery of new plant sources of propolis in different regions of Brazil.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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