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Pitanga and *grumixama* extracts: antioxidant and antimicrobial activities and incorporation into cellulosic films against *Staphylococcus aureus*

Extratos de pitanga e grumixama: atividade antioxidante, antimicrobiana e incorporação em filmes celulósicos contra *Staphylococcus aureus*

Extractos de *pitanga* y *grumixama*: actividad antioxidante, antimicrobiana y incorporación en películas celulósicas contra *Staphylococcus aureus*

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Abstract

There is great interest in developing alternatives to improve food safety, since food-borne diseases (FBD) are a major public health concern worldwide. Plant extracts have the potential to inhibit microbial growth due to the action of secondary metabolites, such as phenolic compounds. This study evaluated the antioxidant and antimicrobial activities of phenolic compounds extracted from *grumixama* (*Eugenia brasiliensis*) and *pitanga* (*Eugenia uniflora*) fruits and the antimicrobial potential of extract of *grumixama* upon incorporation into cellulosic films. Both fruits were rich in total phenolic compounds and their extracts showed antioxidant activity. The crude and phenolic extracts of *grumixama* showed higher activity than those isolated from *pitanga*. All extracts inhibited the growth of *Staphylococcus aureus*. After incorporation into cellulosic films, the crude extract of *grumixama* remained active, reducing the *S. aureus* population in 4 log cycles. The cellulosic films incorporated with grumixama extract were stable after seven days of storage under refrigeration at 7º C; but they partially lost antimicrobial activity when exposed to UV radiation. These phenolic compound-containing cellulosic films could be used as a complementary preservation method of foods that are prone to contamination with *S. aureus*.

Keywords: Antimicrobial activity; Brazilian fruits; Foodborne microorganisms; Phenolic compounds; *S. aureus.*

Resumo

Existe um grande interesse em desenvolver alternativas para melhorar a segurança dos alimentos, uma vez que as doenças transmitidas por alimentos representam um grande problema de saúde pública em todo o mundo. Os extratos vegetais possuem potencial de inibição do crescimento microbiano devido à ação de metabólitos secundários, como os compostos fenólicos. Dessa forma, o presente estudo avaliou as atividades antioxidante e antimicrobiana de compostos fenólicos extraídos de grumixama (*Eugenia brasiliensis*) e pitanga (*Eugenia uniflora*), bem como o potencial antimicrobiano do extrato de grumixama incorporado em filmes celulósicos. Foi demonstrado que ambas as frutas possuem elevado

teor de compostos fenólicos totais e seus extratos apresentaram atividade antioxidante. Os extratos bruto e fenólico de grumixama apresentaram maior atividade do que os extratos de pitanga. Todos os extratos inibiram o crescimento de *Staphylococcus aureus*. Após incorporação aos filmes celulósicos, o extrato bruto de grumixama permaneceu ativo, reduzindo a população de *S. aureus* em 4 ciclos logarítmicos. Os filmes celulósicos permaneceram estáveis após sete dias de armazenamento sob refrigeração a 7ºC; no entanto, perderam parcialmente a atividade antimicrobiana quando expostos à radiação UV. Conclui-se que esses filmes contendo compostos fenólicos podem ser usados como método complementar de preservação de alimentos propensos à contaminação com *S. aureus*.

Palavras-chave: Atividade antimicrobiana; Compostos fenólicos; Frutas brasileiras; Microrganismos de origem alimentar; *S. aureus*.

Resumen

Existe un gran interés en desarrollar alternativas para mejorar la seguridad alimentaria, ya que las enfermedades transmitidas por los alimentos son un importante problema de salud pública a nivel mundial. Los extractos de plantas tienen el potencial de inhibir el crecimiento microbiano debido a la acción de metabolitos secundarios, como los compuestos fenólicos. Este estudio evaluó las actividades antioxidantes y antimicrobianas de compuestos fenólicos extraídos de frutos de grumixama (*Eugenia brasiliensis*) y pitanga (*Eugenia uniflora*) y el potencial antimicrobiano del extracto de grumixama tras su incorporación a películas celulósicas. Ambas frutas eran ricas en compuestos fenólicos totales y sus extractos mostraron actividad antioxidante. Los extractos crudos y fenólicos de grumixama mostraron mayor actividad que los extractos de pitanga. Todos los extractos inhibieron el crecimiento de *Staphylococcus aureus*. Después de su incorporación a películas celulósicas, el extracto crudo de grumixama permaneció activo, reduciendo la población de *S. aureus* en 4 ciclos logarítmicos. Las películas celulósicas incorporadas con extracto de grumixama se mantuvieron estables tras siete días de almacenamiento en refrigeración a 7ºC; pero perdieron parcialmente la actividad antimicrobiana cuando se expusieron a la radiación UV. Estas películas celulósicas que contienen compuestos fenólicos podrían usarse como un método de conservación complementario de alimentos que son propensos a contaminarse con *S. aureus*.

Palabras clave: Actividad antimicrobiana; Compuestos fenólicos; Frutas brasileñas; Microorganismos alimentarios; *S. aureus*.

1. Introduction

Traditionally, food packaging has been designed to contain, protect and sell the products in them. Food protection includes the preservation of the product's quality by creating conditions that minimize chemical, biochemical, and microbiological changes that promote food spoilage (Han et al., 2018). One of the main requirements of food packaging is inertness; the packaging must not interact with the food and must provide a barrier between the food and the environment. However, in the last few decades, a new generation of packaging systems has been developed. These active packages interact with the food in a desirable way; they are designed to alter desired properties in the product, as well as to increase or monitor its shelf life (Dannenberg et al., 2017; Han et al., 2018; Silveira et al., 2007).

Active packaging has been used in a large number of food products (Silveira et al., 2007), and each of these products has different mechanisms that result in their deterioration and it is critical that these mechanisms are understood for an appropriate form of active packaging to be defined (Restrepo et al., 2018). There are several types of active packages available including those that incorporate enzymes such as lactase, lysozyme and naringinase in polymeric supports, the use of modified atmosphere packaging, oxygen and ethylene absorbers, CO_2 absorbers and generators, humidity regulators, release of additives, liberators and/or flavor and odor absorbers, temperature indicators, radiation absorbers, and antimicrobial films (Cunha et al., 2007; Han et al., 2018; Restrepo et al., 2018).

The antimicrobial food packaging can be classified into two types: packaging in direct contact with the food surface, where the antimicrobial agents can migrate to the food product; or packaging without making direct contact with the food surface, including technologies like modified atmosphere packaging (Han et al., 2018). In recent years, polymers with antimicrobial properties have generated significant interest because most solid and semi-solid foods can have microbial growth on their surfaces. In this way, the antimicrobial substance, when establishing an intense contact with the food, inhibits the growth of the microorganisms present in it (Dannenberg et al., 2017; Santos et al., 2020). The release of additives from the active packaging enhances consumer safety as these compounds are not directly added to the food but are released in a controlled manner. For this reason, they will be present at lower concentrations and only on the surface of the product where their presence is required to prevent food deterioration from occurring (Muñoz-Bonilla et al., 2019; Oliveira & Oliveira, 2004).

In fact, there is a great interest in developing alternatives to improve food safety, since food-borne diseases (FBD) continue to be a major public health concern worldwide, with an estimated 600 million people falling ill and 420,000 die annually after eating contaminated food (WHO, 2020). Among several pathogenic bacteria, *Staphylococcus aureus* is a significant cause of FBD (Finger et al., 2019), resulting from the contamination of food by preformed *S. aureus* enterotoxins (Machado et al., 2020). The presence of *S. aureus* has been reported in various types of foods, including meat products, salads, bakery products (Argudín et al., 2010), vegetables (Wu et al., 2018), pasteurized milk (Dai et al., 2019) as well in food establishment surfaces (Machado et al., 2020). The onset of the Staphylococcal food poisoning is abrupt and includes nausea, vomiting, abdominal cramping with or without diarrhea, due to the production of toxins during growth by bacteria at permissive temperatures (Silva et al., 2017; Tong et al., 2015). The pathogenicity is associated with the secretion of multiple virulence factors and the increasing resistance of *S. aureus* to various drugs is limiting treatment of infections (Wu et al., 2018). Hence, the development of alternative antimicrobial strategies involving new compounds has attracted great attention (Silva et al., 2017; Tayyarcan et al., 2019).

The antimicrobial potential of several natural and synthetic entities has been analyzed in line with this concept such as metal ions, organic acids, bacteriocins and fungicides including benzoates and sorbates (Oliveira & Oliveira, 2004). Importantly, phenolic extracts obtained from plants have also been analyzed recently for their antimicrobial activity (Garzón et al., 2020; Quecán et al., 2019; Rais et al., 2019; Santos et al., 2020).

Phenolic compounds are secondary metabolites that are normally involved in the adaptation of plants to environmental stressful conditions such as exposure to UV radiation, pathogen attack and injury (Vasco et al., 2008). They have one or more hydroxyl groups (OH) attached to a benzene ring but they may also have other substituent groups such as carbonyls, methoxy groups or non-aromatic cyclic structures. More than 8000 phenolic structures are currently known, divided into different classifications, such as phenolic acids, flavonoids, phenolic amides and other polyphenols (Tsao, 2010), and they have both antioxidant and antimicrobial activities (Chuah et al., 2020; Bouarab-Chibane et al., 2019).

Fruits and vegetables contain a wide variety of phenolic compounds in their tissues (Tsao, 2010). For this reason, they have been studied as sources of these compounds as natural preservatives (Quecán et al., 2019; Rodrigues et al., 2016; Santos et al., 2020).

Grumixama (*Eugenia brasiliensis*) grows on a tree in the Atlantic forest of the Brazilian rainforest producing fruits usually in December. It belongs to the family Myrtaceae,

and the fruit of this tree has a flavor that is similar to the fruit of the jabuticaba tree (*Myrciaria cauliflora*) (Silva et al., 2014; Zola et al., 2019). Several phenolic compounds have been identified in the pulp of this fruit including the anthocyanins delphinidin 3-glycoside, cyanidin 3-glycoside, cyanidin 3-galactoside and cyanidin 3-acetyl-hexoside; the first two compounds were detected in greater quantities (Silva et al., 2014).

Eugenia uniflora is a plant popularly known as *pitanga* that also belongs to the family Myrtaceae, also known as "Brazilian cherry". The plant produces edible fruits that are widely appreciated (Borges et al., 2016; Freitas et al., 2016). These fruits are also recognized for their medicinal properties. Extracts obtained from the *pitanga* leaf have been mentioned as efficient inhibitors of fungi including *Aspergillus* and *Candida*, and bacteria including *S. aureus*, *Mycobacterium*, *Salmonella* Choleraesuis, *Listeria monocytogenes* and *Pseudomonas aeruginosa* (Muñoz-Bonilla et al., 2019; Rodrigues *et al*., 2016).

The objective of this study was to evaluate the antioxidant and antimicrobial activity of phenolic compounds extracted from *pitanga* and *grumixama* pulps, and to examine the antimicrobial potential of *grumixama*'s extract after its incorporation into cellulosic films.

2. Material and Methods

2.1 Material

The fruits were collected in Ouro Preto, Minas Gerais (Brazil), during the fruiting season, between October and January (geographical coordinates 20°17′15″S 43°30′29″W). The species were identified and deposited as exsicata in *Herbário Professor José Badini*, from the Institute of Exact and Biological Sciences (ICEB), Federal University of Ouro Preto.

After cleaning and sanitizing with sodium hypochlorite solution at 50 ppm for 15 minutes, leaves, seeds, and twigs were removed and the pulp was separated manually. Subsequently, homogenization was carried out in a domestic multiprocessor and the pulp was frozen at -20 °C until use.

2.2 Preparation of phenolic extracts

The phenolic compounds were extracted using solid phase extraction (SPE), according to the methodology described by Rodrigues et al. (2016) and Zola et al. (2019). Minor adaptations were made to the protocol. The pulps were thawed, ground and homogenized with

1: 1: 1 (v / v / v) ethanol: methanol: acetone solution, respectively. After vacuum filtration through Whatman filter paper, the solvents were evaporated in rotavapor at 40 °C (Büchi, Switzerland) and a sample of the aqueous extracts (95 mL *grumixama* crude extract and 85 mL *pitanga* extract) was frozen at -80 °C. The remainder was applied to a C18 Sep-Pak Vac 35cc 10g 20 cm^3 column (Waters Corporation, Milford, MA) for the purification of the phenolic compounds. The polyphenols adsorbed on the cartridge were eluted with methanol (adsorbed fraction) and the non-adsorbed aqueous fractions were discarded. The extract was again evaporated to remove the added methanol, thus obtaining the phenolic extract (Zola et al., 2019).

The crude and phenolic extracts were used for evaluation of antioxidant and antimicrobial activities of the *grumixama* and *pitanga* fruit pulps.

2.3 Quantification of total phenolic compounds

The content of total phenolic compounds was determined using the Folin-Ciocalteu assay (Waterhouse, 2002), with modifications. One-hundred microliters of the crude and phenolic extracts were placed in test tubes with 2.5 mL of Folin-Ciocalteu reagent (10% v / v). Subsequently, 2.0 mL saturated Na_2CO_3 solution (4% w / v) was added. After 2 hours of incubation, the absorbance was determined at 750 nm by spectrophotometry in the UV-Visible region (UV-1601 PC Shimadzu, Tokyo, Japan). The total phenolic index was determined using a standard curve of gallic acid. The results were expressed as a gallic acid equivalent; mg of gallic acid equivalent (mg AGE) per 100 g of fruit. The whole experiment was carried out in the dark and in triplicate for each sample.

2.4 Quantification of antioxidant activity

The antioxidant capacity of the crude and phenolic extracts of each fruit was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, according to the methodology reported by Brand-Williams et al. (1995), with modifications.

To perform the analyses, 3.9 mL DPPH solution dissolved in methanol (100 mM) were added to test tubes containing 100 µL of pulp extracts. The mixture was homogenized and kept in the dark at room temperature. Methanol (100 mM) was used as a negative control. The absorbance of the blank was determined at 515 nm immediately after mixing at time zero. The samples were kept at rest and quantified every 1 minute. The absorbance values were

obtained from the different dilutions of the fruit extracts. A standard curve was generated with DPPH at different concentrations (10 μM, 20 μM, 30 μM, 40 μM, 50 μM and 60 μM). The absorbance values were plotted on the Y-axis and the concentration of the extracts (mg / L) on the X axis. A linear equation was determined and used to calculate the antioxidant activity. Antioxidant activity was expressed as EC_{50} (g fruit / g DPPH). This corresponds to the concentration of extract that results in half the maximum response, i.e., the concentration required to reduce the concentration of DPPH radicals by 50%.

2.5 *In vitro* antimicrobial activity of extracts

The antimicrobial activity of the crude and phenolic extracts of *grumixama* and *pitanga* was evaluated by the plate diffusion test according to the methodology described by Salawu et al. (2011) against *S. aureus*, with modifications. A 20 mL volume of BHI agar (Himedia) inoculated with 10^5 CFU / mL of the microorganism was poured into previously sterilized Petri dishes. After solidification, 5 mm holes were made with 1 mL pre-sterilized tips. Subsequently, aliquots of 20 μL of the crude extract or the phenolic extract were added to each well. The plates were refrigerated overnight and then incubated at 37ºC for 24 h. The inhibitory activity of the extract was verified by the formation of light zones around the punched holes in the solid medium, and these zones were compared to those present around the control (sterile water). The antimicrobial activity was expressed as the mean diameter of the inhibition halos, which was calculated as the average of three replicates.

The crude and phenolic extracts were tested against the Gram-positive bacterium *S. aureus* (ATCC 22923™) which was donated by FIOCRUZ (Rio de Janeiro - Brazil).

2.6 Cellulosic film development

The cellulosic films were produced by the casting method according to the methodology reported by Soares & Hotchkiss (1998), with some modifications. The polymer matrix was prepared by dissolving cellulose acetate (Aldrich Chemistry) in acetone (Kinetics, 99.5%) at a ratio of 1:10. A total of 2 mL of *grumixama* crude extract (which exhibited higher antimicrobial activity) was added to this solution; sufficient to result in a final concentration of 0.1242 mg AGE / cm^2 on each side of the film surface. After homogenization, this solution was deposited on a glass plate to allow the evaporation of the solvent and the formation of the film.

The thickness of each cellulosic film was determined by measuring the film at five positions using a digital micrometer (Digimess, Brazil).

2.7 Antimicrobial activity of the films

The antimicrobial activity was evaluated by the diffusion test (halo test) method according to the methodology proposed by Bawer et al. (1966) with some adaptations. One hundred microliters (100 μL) of the *S. aureus* ATCC 6538 microorganism (10^6 CFU / mL) were spread into petri dishes containing BHI agar. Subsequently, discs (2 cm in diameter) of the films incorporated with the crude *grumixama* extract, previously cut with a sterile slide, were added on the surface of the agar at equidistant points. The plates were kept under overnight refrigeration and subsequently incubated at 37ºC / 24h. The inhibitory activity was verified by the formation of clear zones around the films in comparison to the control (film without extract). The experiment was carried out with two replicates.

To assess antimicrobial activity in a liquid medium, four discs, each with a diameter of 2 cm, were cut from the film (containing approximately 0.8 mg AGE / mL of extract each). These discs were immersed in 2.5 mL of BHI broth that had previously been inoculated with 10^5 CFU / mL of *S. aureus*. Subsequently, the tubes were incubated at 37 ° C for 24h. Cellular viability was evaluated by surface plating at time zero (immediately after inoculation) and after 24 h. As a positive control, the extract was added directly to the BHI medium inoculated with *S. aureus*. The negative control experiment was performed with films that did not contain any plant extract.

2.8 Film stability under refrigeration and UV radiation

The stability of the cellulosic films was determined by storing them under refrigeration for 7 days and then evaluating their antimicrobial activity according to the methodology described in topic 2.7.

The effect of UV radiation on the activity of the films was evaluated according to the methodology proposed by Dannemberg et al. (2017), with modifications. The film was prepared and exposed to 5 min to UV radiation for sterilization. Subsequently they were analyzed by the diffusion test in liquid medium as described in topic 2.7.

2.9 Statistical analysis

Data on phenolic compounds, antioxidant activity and inhibition halo were evaluated by analysis of variance (ANOVA) and Tukey's test at 5% significance in Sisvar software (Ferreira, 2014), performed in triplicate. The figures were performed using GraphPad Prism software version 5.01.

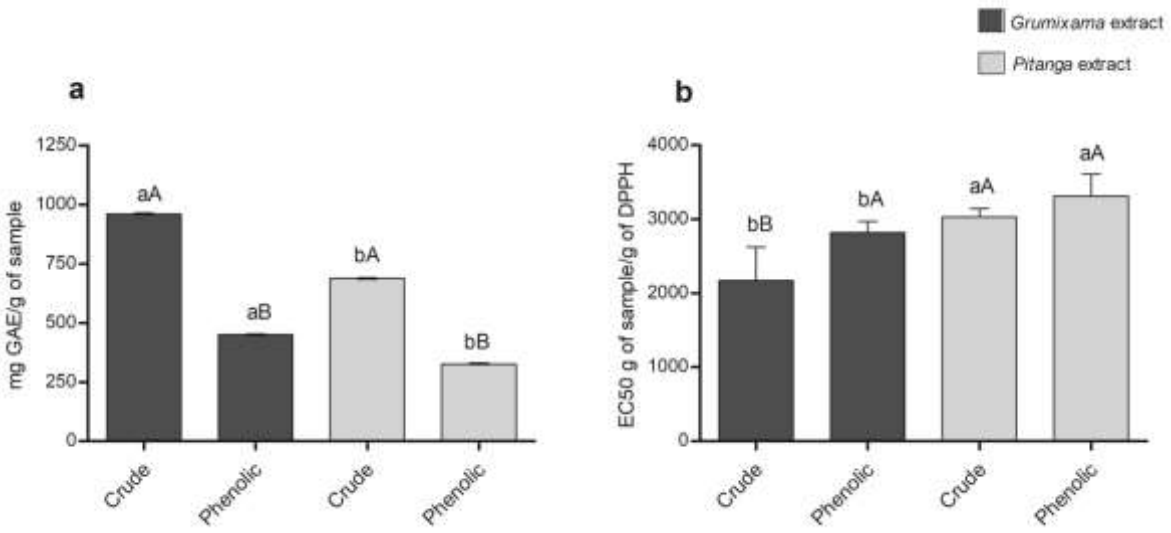
3. Results

3.1 Determination of total phenolic compounds and antioxidant activities of the crude and phenolic extracts of *grumixama* and *pitanga* pulp

Figure 1 shows the total phenolic compounds and antioxidant activities of the crude and phenolic extracts of the *grumixama* and *pitanga* pulps. The content of total phenolic compounds present in the crude and phenolic extracts of *grumixama* was higher ($p < 0.05$) than that found in both extracts of *pitanga* (Figure 1a). Our data also showed that the crude extract of both fruits had a higher concentration ($p > 0.05$) of phenolic compounds compared to the phenolic extract (Figure 1a).

The antioxidant activity (EC_{50}) of the phenolic extract of *grumixama* and *pitanga* was 2852.98 and 3309.82 (g of fruit / g of DPPH), respectively. The crude extracts showed EC_{50} values of 2204.44 and 3088.17 (g of fruit / g of DPPH), as demonstrated in Figure 1b. The crude extract of *grumixama* presented higher antioxidant activity than phenolic extract for the same fruit ($p < 0.05$), while both extracts of *pitanga* did not differ ($p > 0.05$), demonstrating the potential to use both extracts regarding the antioxidant activity (EC_{50}).

Figure 1: Total phenolic content (a) and antioxidant activities (b) for the crude and phenolic extracts of *grumixama* and *pitanga.*



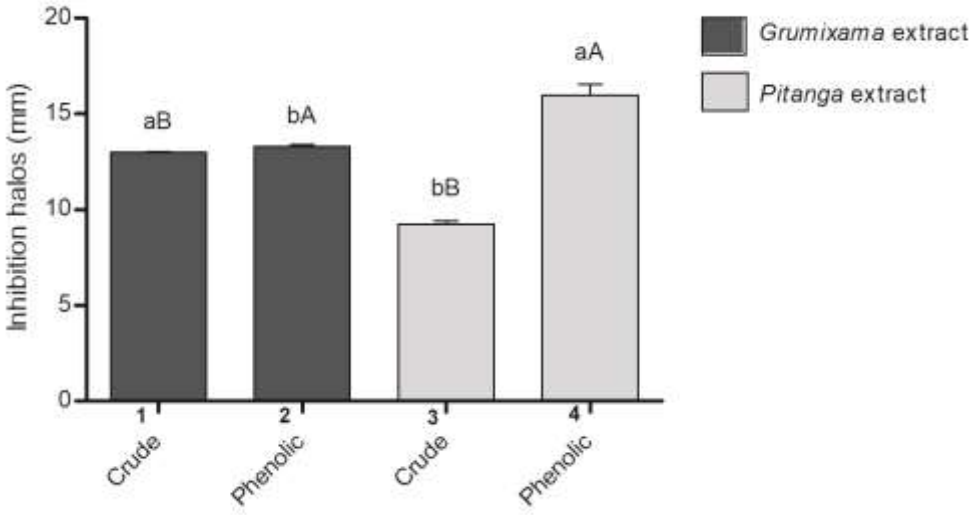
Data shown as mean values ± SD of three repetitions. Means followed by the same capital letters (A, B) within the same fruit (*grumixama* or *pitanga*) do not differ ($p > 0.05$); means followed by the same lower case letters (a, b) within the same extract type (crude or phenolic) do not differ by Tukey's test ($p > 0.05$). Source: The authors.

3.2 Antimicrobial activity of extracts

Staphylococcus aureus ATCC 6538 was tested in a plate diffusion assay and was inhibited by the crude and phenolic extracts from both the *grumixama* and *pitanga* pulps at the tested concentrations (Figure 2). Both crude and phenolic extracts were efficient at inhibiting the growth of *S. aureus*, promoting inhibition halos ranging from 9 to 18 mm.

We observed that the phenolic extracts of the pulps of both fruits promoted greater inhibition halos ($p < 0.05$) for *S. aureus* than the crude extracts (Figure 2). The phenolic compounds are purified in this extract, demonstrating that they are the compounds that actually have antimicrobial activity. The crude extract of *grumixama* pulp promoted larger halos ($p < 0.05$) of inhibition than the crude extract of *pitanga*. It is possible that the higher concentration of phenolic compounds (Figure 1a) present in the extract of this pulp contributed to the higher inhibitory effect.

Figure 2. Mean of the inhibition halos (mm), indicating inhibition of *S. aureus* growth, promoted by the crude and the phenolic extracts (20 μL) of the *grumixama* and *pitanga* fruit pulps.



Extracts concentrations: [1]0.251 mg GAE; [2]0.225 mg GAE; [3]0.166 mg GAE; [4]0.160 mg GAE. The mean of inhibition halos (in mm) were quantified by subtracting the diameter of the orifice (5 mm). Data shown as mean values ± SD of three repetitions. Means followed by the same capital letters (A, B) within the same fruit (*grumixama* or *pitanga*) do not differ ($p > 0.05$); means followed by the same lower case letters (a, b) within the same extract type (crude or phenolic) do not differ by Tukey's test ($p > 0.05$). Source: The authors.

3.3 Antimicrobial activity of films

The films that were developed showed variations in thicknesses, and the addition of antimicrobial agent contributed to an increase in the thickness of the film. The mean thickness values were 0.035 ± 0.003 for the control film and 0.158 ± 0.022 for the film containing 2 mL (0.1242 mg GAE / cm^2) *grumixama* crude extract.

The films containing the crude extract of *grumixama* exerted antagonistic effect on the growth of *S. aureus*, promoting a halo of inhibition of 2.0 mm ± 0.0. The lower inhibitory effect of the extracts when incorporated into the film may be due the fact that the compounds were unable to diffuse out of the matrix of the film into the solid medium. To evaluate if the films containing the antimicrobial compounds were effective in media with high moisture content, the stability of the cellulosic discs containing *grumixama* extract was evaluated in liquid medium.

Regarding the inhibition in liquid medium, the mean number of *S. aureus* (CFU / mL) in BHI after exposure to different treatments is shown in Table 1. The data demonstrate that

the crude extract of *grumixama* at a concentration of 1.4 mg GAE / mL of BHI exerted a bactericidal effect on *S. aureus*; no growth of this microorganism was detected at even the lowest dilution. The film with extract of *grumixama* also exerted a bactericidal effect on *S. aureus*, reducing the initial population of this microorganism (5.8 x 10^5 CFU / mL) in 4 log cycles (8.9 x 10^1 CFU / mL). This shows that the process involved in film production does not eliminate the antimicrobial compounds present in the extract. It is therefore possible to use this extract as an antimicrobial agent in foods with a high moisture content in order to control *S. aureus* growth. We also observed that the bactericidal effect on *S. aureus* was promoted exclusively by the crude extract of *grumixama* as the counts of this microorganism were not altered by exposure to the control film (4.6 x 10^8 CFU / mL) (Table 1).

Table 1. Counts (CFU/mL) of *S. aureus* in BHI broth after exposure to different treatments.

Treatments	*S. aureus* (CFU/mL)	
	Time 0h	Time 24h
BHI + *S. aureus*	5.8 x 10^5	6.8 x 10^8
BHI + Crude extract of *grumixama*[1]		<10
BHI + Film with extract[2]		8.9 x 10^1
BHI + Film without extract (control)		4.6 x 10^8

The values correspond to the means of two replicates. BHI: Brain Heart Infusion; [1] Concentration: 1.4 mg GAE / mL; [2] Film incorporated with crude extract of *grumixama* in the concentration of 3.14 mg of GAE. Source: The authors.

The results for the film stability test stored under refrigeration for seven days are described in Table 2. We observed that the *grumixama*-containing films remained active after seven days of storage under refrigeration. They were able to promote a reduction of three log cycles in the *S. aureus* population. We also noticed that the storage of the film promoted a small reduction in the inhibition capacity of the films since prior to storage they promoted reduction of four log cycles (8.9 x 10^1) in the *S. aureus* population (Table 1). The reduction in inhibitory capacity may have been promoted by the oxidation of the phenolic compounds during storage.

Table 2. Counts (CFU/mL) of *S. aureus* after exposure to a crude extract of *grumixama*-infused film that had been stored for seven days under refrigeration at 7 ºC

Treatments	*S. aureus* (CFU/mL)	
	Time 0h	Time 24h
BHI + *S.aureus*		4.4×10^8
BHI + Film with extract†	5.9×10^5	3.8×10^2
BHI + Film without extract (control)		7.1×10^8

† Film incorporated with crude extract of *grumixama* in the concentration of 3.14 mg of GAE. Source: The authors.

The films containing crude extracts of *grumixama* partially lost their antimicrobial activity when they were exposed to UV radiation. UV-treated films promoted a reduction of one log cycle in the *S. aureus* population (Table 3).

Table 3. Counts (CFU/mL) of *S. aureus* after exposure to a crude extract of *grumixama*-infused film, that had been exposed to UV radiation.

Treatments	*S. aureus* (CFU/mL)	
	Time 0h	Time 24h
BHI + *S.aureus*		8.7×10^8
BHI + Film with extract†	5.9×10^5	7.9×10^4
BHI + Film without extract (control)		7.1×10^8

† Film incorporated with crude extract of *grumixama* in the concentration of 3.14 mg of GAE. Source: The authors.

4. Discussion

The total phenolic compounds data corroborates previous reported results in the literature where higher values for total phenolic content were obtained for *grumixama* (Abe et al., 2012; Infante et al., 2016) compared to *pitanga* (Abe et al., 2012; Argudín et al., 2010; Rodrigues et al., 2016; Zola et al., 2019).

The content of total phenolic compounds for the crude extract of *grumixama* (958.67 mg GAE/ g of sample) was lower than those reported by Abe et al. (2012) and Infante et al. (2016) and higher than those obtained by Bagetti et al., (2011). In these studies, the phenolic content of fresh *pitanga* pulp was 95 to 201 mg GAE / 100 g. However, higher values (21.16

and 31.03 g GAE /100 g in dry weight of fresh red and purple *pitanga* pulps, respectively) were obtained by Borges et al. (2016). The final content of phenolic compounds in fruits can be influenced by several factors including fruit maturation, species, cultivation practices, geographical origin, growth stage, harvest conditions, and storage processes (Kim et al., 2003; Rais et al., 2019). These factors could explain the differences between the values obtained in this study and those reported in the literature for the same fruit.

Vasco et al. (2008) classified the content of total fruit phenolic compounds as low (<100 mg GAE / 100 g), medium (100-500 mg GAE / 100 g), and high (> 500 mg GAE / 100 g fresh weight). According to the scale proposed by these authors, the crude extract of *grumixama* and *pitanga* can both be classified as having a high phenolic compounds content.

The higher concentration of phenolic compounds in the crude extract compared to the phenolic extract may be due to other compounds present in the crude extract ended up being quantified as phenolic compounds. According Sánchez-Rangel et al. (2013), compounds other than phenolics present in plant extracts can react with the Folin Ciocalteu reagent, skewing the real phenolic compound determination. Thus, compounds that are not of phenolic origin with reductive activity, including organic acids and sugars could contribute to the phenolic compound activity. The elimination of these compounds during the purification process may result in the reduced phenolic extract activity that we have observed for the purified extract (Huang et al., 2005).

The antioxidant activity of phenolic compound is attributed to the capacity of quench free radicals, donating hydrogen atoms, electrons, or chelating metal cations. In this way, they are able to prevent the deleterious effects of oxidation, and when incorporated in the human diet, the phenolic compounds promote health benefits and have been linked to lowered risks of some chronic and cardiovascular diseases (Chuah et al., 2020; Minatel et al., 2017; Tsao, 2010).

Due to the importance of *S. aureus* to cause diseases, the study of alternative ways to inhibit the growth of this pathogen has become necessary (Silva et al., 2017; Tong et al., 2015). Plant-derived bioactive compounds are promising, due to antimicrobial activity which have been generally demonstrated (Bouarab-Chibane et al., 2019; Garzón et al., 2020; Tayyarcan et al., 2019; Zola et al., 2019).

The mechanisms of antimicrobial action of phenolic compounds are not yet fully understood, but it is known that they can alter the permeability of cell membranes, change some intracellular functions due to binding to cellular enzymes, and cause modifications to the cell wall (Bouarab-Chibane et al., 2019; Cushnie and Lamb, 2011; Garzón et al., 2020).

This causes interference with the synthesis of ATP and the transport of solutes into the cell (Cushnie & Lamb, 2011).

The results obtained in this work are consistent with data observed by Silva et al. (2012) who reported that *S. aureus* was the most sensitive among the tested pathogens to plant crude extracts derived from *pitanga* and Asteracea species *Bacharis dracunculifolia* D.C., *Vernonia polyanthes* Less., and *Matricaria chamomilla* L. Additionally, Gonçalves et al. (2005) demonstrated the antimicrobial effect of a 10% m / v crude extract derived from the dry pulp of *pitanga* on the growth of *S. aureus* (24 mm inhibition halo), and *Staphylococcus* spp. (14 mm inhibition halo). In a study performed by Pessini et al. (2003) with several types of plant extracts, more than 77% of the selected extracts, including *pitanga*, showed a degree of antibacterial activity, particularly against Gram-positive bacteria *S. aureus* and *Bacillus subtilis*.

Recently, the antimicrobial activity of phenolic compounds against *S. aureus* has being proven in other studies. Chen et al. (2016) observed a reduction of virulence in *S. aureus* in the presence of the flavonoid baicalein (5,6,7-trihydroxyflavone), including the reduction of staphylococcal enterotoxin production and inhibition of biofilm formation. According to the authors, baicalein has the potential to be a novel drug candidate and an effective treatment strategy with infections caused by this bacterium. Similarly, Silva et al. (Silva et al., 2017) concluded that myricetin - a flavonoid present in fruits, vegetables, tea, berries and red wine - has the potential to control *S. aureus* pathogenicity.

Although both extracts of *grumixama* and *pitanga* promoted greater inhibitory effects (Fig. 2), the crude extract of *gruximama* presented higher content of total phenolic compounds and antioxidant activity (Fig. 1), and the preparation crude extract is more straightforward. In this way, we decided to continue the studies of extract incorporation into cellulosic films with crude extract of *grumixama*.

Cellulose is a semicrystalline homopolymer of high molecular weight, formed by repeated units of cellobiose, a glucose dimer. This natural polymer is one of the most abundant and studied recently, besides being used in several industrial applications, such as packaging and textile production. The increase in interest in this material responds to the need for the replacement of synthetic polymers, aiming at the development of sustainable and ecologically functional materials (Muñoz-Bonilla et al., 2019).

The stability of phenolic compounds in films is related to the oxidation of these molecules, which can be influenced by different factors including temperature, light exposure and storage conditions (Han et al., 2018; Lima et al., 2005). In a study carried out by Lima et

al. (2005), the anthocyanin extract of the purple cherry showed that the extract isolated in the dark remained more stable than that isolated in the light. A total of 50% of the phenolic compounds was lost when exposed to light. However, the cellulosic films infused with *grumixama* extract still presented good stability, which was still active after seven days of storage under refrigeration.

The markedly reduced bactericidal effect of the film after UV treatment demonstrates that, under the conditions tested (laminar flow for 5 min according to Dannember et al. (2017), this would not be a good method for film sterilization, if incorporated with *grumixama* extract. The test performed was preliminary, so future studies are needed in order to optimize this sterilization process and simulate the real conditions in the food industry, with reduced exposure time.

Phenolic compounds, when subjected to the action of UV light and high temperatures can give rise to new radicals. This could compromise the efficiency of the antioxidant activity, which is determined by the presence, position and the chain length of the functional groups in the aromatic ring of the phenolic compounds (Angelo & Jorge, 2007; Minatel et al., 2017). This could explain the partial loss of the *grumixama* extract antimicrobial activity in this condition.

5. Conclusion

The *grumixama* and *pitanga* fruit pulps demonstrated the presence of high levels of antioxidant substances, particularly phenolic compounds. The crude and phenolic extracts of fruit pulps showed antioxidant activity and exerted an antimicrobial effect on the growth of *S. aureus*. The cellulosic films impregnated with *grumixama* extracts could be used as active antimicrobial packaging in foods with higher humidity in order to control *S. aureus* development. The advantage is that this product is biodegradable. This study provides the opportunity to develop and use natural antimicrobials that are found in the Brazilian flora in the food packaging industry, where there is a need for new natural and safe antimicrobial agents.

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