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Jambolão extract and potassium sorbate as antimicrobial components in active packaging

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Abstract

This study aimed to use the raw extract of jambolão (*Syzygium cumini* L.) and potassium sorbate as antimicrobial components in cellulose acetate films and evaluate their potential for application as active packaging for sugar-free banana preserve. The films were prepared using the casting method and evaluated for thickness and inhibition potential. The efficiency of the films as active packaging, *in situ*, was evaluated through the enumeration of aerobic mesophiles and mold and yeast over 36 days of storage of banana preserves. Furthermore, in order to analyze the fungicidal effect of the films against banana preserve with an initial load of microorganisms, mold and yeast were counted for 25 days of storage. The results showed that the incorporation of crude jambolão extract increased the film thickness, and its inhibition potential was similar to the film with incorporated potassium sorbate. Furthermore, the film with crude jambolão extract reduced mold and yeast growth by up to 1 log cycle over 36 days of storage. Furthermore, the use of the film with potassium sorbate promoted the same effect as this preservative added directly to the fruit preserve and proved to be efficient even with relevant initial contamination. Thus, the use of these active packagings presents an opportunity for industries as they reduce health risks and improve the safety and quality of preserves.

Keywords: antimicrobial packaging, inhibition potential, healthiness, *Syzygium cumini* L

Introduction

Packages containing antimicrobial agents are active systems that suppress the growth cycle of microorganisms on the surface of the food in contact with the package (Thanakkasaranee et al., 2020). Antimicrobial packaging can take various forms, such as the incorporation of volatile and non-volatile antimicrobial agents directly into the polymers and coating of antimicrobials on the polymer surfaces (Wong et al., 2020). During the storage period the antimicrobial agent is released slowly from the packaging material, thus inhibiting microorganisms present on the surface of the food, increasing its shelf life (Thanakkasaranee et al., 2020; Wong et al., 2020).

Numerous polysaccharides have been used as matrices for antimicrobial active packaging (Akhtar et al., 2018; Zhong et al., 2020). Among them, cellulose acetate stands out due to its excellent sharpness and optical stiffness (Dannenberg et al., 2017).

There are several studies evaluating the incorporation of natural extracts in polymeric matrices formed by polysaccharides for the preparation of antimicrobial active packaging (Saberi et al., 2017; Jafarzadeh et al., 2020). These natural extracts have significant amounts of bioactive compounds, mainly phenolic compounds, which are compounds with strong antimicrobial activity (Wang et al., 2020).

Syzygium cumini L., known as jambolão in Brazil is native to India and has high amounts of flavonoids, phenolic acids, tannins and terpenes (Nahid et al., 2017).

Banana preserve is a very common product in Brazil, with high energy content and long shelf life, as it contains significant amounts of sugar (Pereira et al., 2011). In the last decade, changes in eating habits in Brazil have resulted in a decrease in the consumption of fruit preserves, such as the case of banana preserve

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(Pereira et al., 2013). Therefore, to maintain the historical importance of this product and to meet market demands, it was necessary to prepare sugar-free banana preserve (Pereira et al., 2011). However, removing sugar from this product increases its water activity, thus decreasing its shelf life (Sandrou & Arvanitoyannis, 2000). As a result, it is essential to add preservatives, such as potassium sorbate, in order to guarantee the safety of this food (Pylypiw Jr. & Grether, 2000), but its use, in addition to decreasing the quality of the final product (Pereira et al., 2011), is under the scrutiny of consumers as it can trigger allergic reactions (Hugo & Hugo, 2015). Therefore, the use of antimicrobial active packaging as a way to exclude synthetic preservatives from the food matrix is extremely important (Requena et al., 2019).

The objective of this study was to use the raw extract of jambolão and potassium sorbate as antimicrobial components in films and to evaluate its potential for application as an active packaging for sugar-free banana preserve.

Materials and Methods

The experiment was conducted in the Sensory Analysis and Food Microbiology laboratories of the School of Nutrition at the Federal University of Ouro Preto, MG.
To evaluate the application potential of cellulose acetate films added with raw extract of jambolão or potassium sorbate, two sugar-free banana preserve formulations were prepared, one with the addition of sorbate (F1) and the other without the addition of sorbate (F2).

Bananas of the Caturra cultivar, purchased in the local market, were satinized in chlorinated water (2.5%), manually peeled and homogenized in an industrial blender (Tron, Tron Master 2L, Catanduva, Brazil) for one minute to obtain the whole pulp.

For the preparation of preserves, initially, banana pulp (60%) and polydextrose (Nutramax, Catanduva, Brazil) (36.64% in F1 and 36.69% in F2) were added in an open stainless steel pan. Polydextrose was used as a body agent, helping to replace sucrose. The mixture went through the cooking process up to 80 °C and then low methoxylation pectin (Rica Nata, Piracema, Brazil) (1.64%), carrageenan gum (Gastronomy Lab, Distrito Federal, Brazil) (1.04%) and $CaCl_2$ (Rica Nata, Piracema, Brazil) (0.61%) were added. The mixture was kept under cooking up to 65 °Brix, measured with the aid of a manual model RT-82 refractometer (Higmed, Tatuapé, Brazil). At the end of the cooking process, sweeteners (Nutramax, Catanduva, Brazil) (0.01875% of acesulfame-k and 0.00625% of sucralose) and potassium sorbate (Rica Nata, Piracema, Brazil) were added (0.05%) only in the F1 formulation. Subsequently, the preserves were packaged in polypropylene pots and stored in an incubator chamber at 25 °C.

Jambolão was collected during the fruiting season (January to May) in the rural area of the city of Viçosa, Minas Gerais (latitude: 20°45’14'' S and longitude: 42°52’55'' W), at the ripe stage. After washing, the fruits were immersed in chlorinated water (2.5%) for 15 minutes (Oliveira et al., 2016). Afterwards, the pulp and seed were manually separated and stored at -18 °C.

The crude extract was obtained through the liquid-liquid extraction method according to the methodology described by Oliveira et al. (2016), with adaptations for the plant material in question. Jambolão pulp was crushed and homogenized with ethanol (Alphatec, São Paulo, Brazil), methanol (Neon, Suzano, Brazil) and acetone (Alphatec, São Paulo, Brazil) in a ratio of 1:1:1 (v/v/ v). The mixture was vacuum filtered on Whatman paper (n° 1). The solvents were evaporated in a rotaevaporator (Buchi, Rio de Janeiro, Brazil) at 40 °C, thus obtaining the crude extract. This extract was stored in sterile falcon tubes lined with aluminum foil, for protection from light, and frozen in a freezer at -20 °C.

The films were prepared by the “casting” method, according to the methodology proposed by Soares et al. (2008), using cellulose acetate (Sigma-Aldrich, São Paulo, Brazil) as polymer matrix, crude extract of jambolão pulp and potassium sorbate. The filmogenic solution was formed by cellulose acetate in acetone (1% m/v), and after complete solubilization of the polymer, 1 mL of crude extract of jambolão pulp or 0.29 g of potassium sorbate was added. The amount of crude extract incorporated into the filmogenic solution was determined by previous tests, which showed that this is the maximum amount of extract for film formation, since at higher concentrations it did not promote film formation. The concentration of potassium sorbate added to the film was obtained through previous calculations using the amount added directly to the food (0.05%).

After homogenizing, the solutions were placed on a flat and sterile surface for film formation and solvent evaporation (acetone) at room temperature. Then, the films were cut into an area of 9 cm² and stored in sterile Petri dishes protected from light. The control film was prepared only from the filmogenic solution, without adding extracts.

Film thickness (μm) was obtained through the average of ten measurements taken in random regions of the film, with the aid of a micrometer (Digimess, São Paulo, Brazil) (Lopes et al., 2014). Analyzes were performed at room temperature (25 °C).

The antimicrobial activity of the films was evaluated in liquid medium by calculating the Inhibition Potential (IP) according to the methodology proposed by Alvarez et al. (2012), with adaptations. In 3 mL of BHI broth (Brain Heart Infusion – Himedia, Sumaré, Brazil) an 18 cm^2 film (considering both sides) was inoculated or not (control) with crude extract of jambolão pulp or potassium sorbate and 3 µL of *Penicillium chrysogenum*, previously activated in BHI broth. Afterwards, the tubes were incubated at 25 °C/4 days, and plated (surface) on potato dextrose agar (BDA - Sigma-Aldrich, São Paulo, Brazil), acidified with 10% tartaric acid (Alphatec, São Paulo, Brazil). The plates were incubated under the same conditions described above, counting the colony forming units (CFU). The results were expressed through the IP, calculated according to equation (1).

$$IP = \log (No/N) \quad \text{Eq. (1)}$$

Where No is the count in CFU/mL of the control sample (0% extract) and N is the count of CFU/mL of the sample at the concentration under test. An IP equal to 1 indicates 10 fold inhibition, due to logarithmic scale.

The antimicrobial activity of the films was evaluated using the methodology proposed by Lee et al. (2015), with adaptations. 10 g of the banana preserve were weighed (about 3 cm x 3 cm x 1 cm in width x length x height) and then the films were added on one of the sides of the preserve, according to the treatments described in Table 1.

Table 1. Treatments applied to evaluate the antimicrobial activity of films incorporated with crude jambolão extract and potassium sorbate as to the stability of sugar free banana preserve.

Treatments	
T1	Banana preserve without potassium sorbate with unincorporated film of crude jambolão extract or potassium sorbate
T2	Banana preserve without potassium sorbate with film incorporated with crude jambolão extract
T3	Banana preserve without potassium sorbate with film incorporated with potassium sorbate
T4	Banana preserve with potassium sorbate without films

The samples were stored for 36 days in an incubator chamber at 25 °C, and plating was performed on the surface for enumeration of aerobic mesophiles in PCA (Plate Count Agar - Himedia, Sumaré, Brazil) and fungi and yeasts in PDA medium at times 0, 6, 25 and 36 days. The plates were incubated at 32 °C for 48 hours to count aerobic mesophiles, and at 25 °C for 4 days to count fungi and yeasts. The count was expressed in Log CFU/g banana preserve.

In order to analyze the fungicidal effect of the films against a food with an initial load of microorganisms (challenge mode), the antimicrobial activity was evaluated according to Lee et al. (2015), with adaptations. 10 g of the banana preserve was weighed (about 3 cm x 3 cm x 1 cm in width x length x height). One of the faces was then contaminated with approximately 10^6 CFU/mL of *Penicillium chrysogenum*. After drying the inoculum (10 minutes), the films were added on one side of the banana preserve, from each of the treatments described in Table 1.

The samples were stored for 25 days in an incubator chamber at 25 °C, counting molds and yeasts at times 0, 6 and 25 days. Appropriate dilutions were made in peptone water (0.1%) (Himedia, Sumaré, Brazil) and plating (surface) on PDA for the quantification of molds and yeasts. The plates were incubated at 25 °C for 4 days and the count was expressed in Log CFU/g banana preserve.

The experiment was carried out according to a completely randomized design (CRD) with 2 replications.

Thickness and inhibition potential results were submitted to analysis of variance (ANOVA) and means test (Tukey) at 5.0% probability in Sisvar software (Ferreira, 2014).

The results of the antimicrobial activity were evaluated by calculating the means and standard deviation of two repetitions and subsequently, a descriptive analysis of the data was carried out.

Results and Discussion

Table 2 shows the mean thickness and Inhibition Potential (IP) results of the control films with crude jambolão extract and potassium sorbate.

Table 2. Thickness and Inhibition Potential (IP) of the control films, with crude jambolão extract and potassium sorbate.

Films	Thickness (µm)	IP
Control	0.032 ± 0.001 c	0.38 ± 0.006 b
Potassium sorbate	0.047 ± 0.005 b	0.49 ± 0.06 a
Jambolão	0.058 ± 0.002 a	0.55 ± 0.04 a

Means value ± standard deviation. Means followed by the same letter, in the column, do not differ from each other by the Tukey test at 5% significance.

In relation to the thickness, there was a difference between all the evaluated films ($p \leq 0.05$), and the addition of potassium sorbate and the crude extract of jambolão contributed to the increase in thickness compared to the control (Table 2). The film incorporated with the jambolão extract was thicker ($p \leq 0.05$). The incorporation of substances modifies interactions in the polymer matrix (Espitia et al., 2011), due to interactions between the incorporated substances and cellulose acetate caused by hydrogen bonds and hydrophobic forces (Akhtar et al., 2018).

Campos et al. (2014) found that the higher the percentage of antimicrobial agents in the film, the more its thickness, since the extract solution is denser than water. According to Saberi et al. (2017) the phenolic compounds present in plant extracts can be distributed in the film matrix, interacting with hydroxyl groups in the molecular structure of the film through the formation of hydrogen bonds leading to an increase in film thickness.

According to the American Society for Testing and Materials (1985), as the thicknesses ranged from 0.032 μm to 0.058 μm they are thin enough to be classified as films.

Regarding the Inhibition Potential, the films added with potassium sorbate and crude jambolão extract presented higher ($p \leq 0.05$) IP when compared to the control (Table 2). There were also no differences between the inhibition potentials of the films with potassium sorbate and with crude jambolão extract ($p > 0.05$). Potassium sorbate is one of the most used synthetic preservatives in the processing industries of sweets and fruit jellies due to its proven effect in inhibiting the growth of mold and yeast (Pereira et al., 2011; Campoli et al., 2018). However, consumers are increasingly demanding the removal of these synthetic preservatives in food, as they can trigger allergic reactions (Wang, 2006; Hugo & Hugo, 2015), in addition to interacting with food components and decreasing the quality of the final product. (Pereira et al., 2011). Thus, the results of the present study are extremely important, since the crude extract of jambolão inhibited the growth of *Penicillium chrysogenum* in a way similar to that potassium sorbate, suggesting that jambolão is a good source of antibacterial compounds and can be used in development of natural preservatives and applied in cellulose acetate films as active packaging.

Table 3 shows the mean results of aerobic mesophiles and mold and yeast counts in sugar-free banana preserves as a function of storage time.

Table 3. Count (log CFU/g) of aerobic mesophiles and mold and yeast in sugar-free banana preserves as a function of storage time.

Treatments	*Storage time (days)*			
	0	6	25	36
Aerobic mesophiles				
T1	< 2 ± 0.21	< 2 ± 0.00	< 2 ± 0.00	< 2 ± 0.00
T2	< 2 ± 0.21	< 2 ± 0.00	< 2 ± 0.00	< 2 ± 0.00
T3	< 2 ± 0.21	< 2 ± 0.00	< 2 ±0.21	< 2 ± 0.00
T4	< 2 ± 0.21	< 2 ± 0.00	< 2 ± 0.00	< 2 ± 0.00
Mold and yeast				
T1	2.17 ± 0.36	< 2 ± 0.00	< 2 ± 0.00	4 ± 1.41
T2	2.17 ± 0.36	< 2 ± 0.00	< 2 ± 0.00	3.20 ± 0.85
T3	2.17 ± 0.36	< 2 ± 0.21	< 2 ± 0.00	< 2 ± 0.00
T4	2.39 ± 0.28	< 2 ± 0.00	< 2 ± 0.00	< 2 ± 0.00

Note: n = 2. Means value ± standard deviation. T1 = Banana preserve without potassium sorbate with unincorporated film of crude jambolão extract or potassium sorbate; T2 = Banana preserve without potassium sorbate with film incorporated with crude jambolão extract; T3 = Banana preserve without potassium sorbate with film incorporated with potassium sorbate; T4 = Banana preserve with potassium sorbate without films.

In addition to sweetening and helping to form the gel, sugar plays an important role in conservation, as it reduces water activity (Morris, 2006). Therefore, low sugar preserves are more vulnerable to the growth of bacteria, mold and yeast. Despite the low sugar content present in banana preserves, a low aerobic mesophilic count (< 2 CFU/g of banana preserve) can be seen over 36 days for all analyzed treatments (Table 3). These results are related to the satisfactory quality resulting from the processing and/or storage techniques of the product (Castro et al., 2015). However, from the results obtained, it can be inferred that the total count of aerobic mesophiles is not an indicator for assessing the potential of the films for application as active packaging for sugar free banana preserves, it is only a necessary assessment to gain knowledge about the preserves processing conditions.

The cellulose acetate film incorporated with crude extract of jambolão pulp (T2) exerted antimicrobial activity (Table 3), reducing the mold and yeast counts by almost a log cycle when compared to the control film, without incorporation of the extract (T1) after 36 days of storage. It is known that jambolão pulp has a relevant amount of phenolic compounds (Nahid et al., 2017), and several studies have shown an antimicrobial effect of these compounds (Daglia, 2012; Singh et al., 2016).

Nascimento et al. (2000) evaluated the antimicrobial activity of alcoholic plant extracts and phytopharmaceuticals in relation to sensitive and resistant microorganisms to antibiotics. It was observed that, among the analyzed extracts, jambolão presented one of the highest antimicrobial potentials, inhibiting about 57% of the microorganisms. According to Xu et al. (2014) the antimicrobial action is associated with the partially hydrophobic nature of these compounds, which allows interaction with the lipid layer of the cytoplasmic membrane of microorganisms, reducing their stability. This interaction can cause the extravasation of cell content and dissipation of the promoting force, interfering with cell transport or even with metabolic enzymes (Negi, 2012).

Even in small concentrations, phenolic compounds can cause a restructuring of the membrane architecture, decreasing the infective capacity of bacteria (Xu et al., 2014). Furthermore, the chelation of metals by phenolic acids can also cause the death of microorganisms (Negi, 2012). Heipieper et al. (1991) demonstrated that polyphenols cause an efflux of potassium ions from the membranes of microorganisms that altered their activity, influencing the protein/lipid ratio. Furthermore, it is also observed that the film incorporated with potassium sorbate (T3) had the same antimicrobial effect as potassium sorbate added to the banana preserves (T4) (Table 3), showing that there was no difference in adding the sorbate to the film or directly to the preserves.

One of the advantages of using packaging containing antimicrobial agents is the diffusion of these substances from the packaging to the surface of the food in a controlled manner. Thus, these compounds are present in food in smaller quantities, meeting a current demand by consumers who seek foods with a low content of preservatives and where their presence is most required, that is, mainly on the surface of the food, where most of the deterioration takes place. As the antimicrobial is released from the package over time, the microorganism development kinetics and the microbial activity on the product surface can be balanced. Thus, antimicrobial activity can be expanded, ensuring safety during food distribution (Quintavalla & Vicini, 2002).

As mentioned above, there are also case reports of seizures, allergies and inflammation in people who are sensitive to potassium sorbate (Wang, 2006; Hugo & Hugo, 2015). Thus, as the active packaging provides a gradual migration of the antimicrobial present in its structure to the surface of the food, this will result in a lower intake of this preservative, reducing the chance of potassium sorbate side effects in the consumer.

Although the jambolão extract promoted a lower antimicrobial effect than the film incorporated with potassium sorbate, the mold and yeast counts after 36 days of storage remained within the standards established by RDC nº 12 (Brasil, 2001), whose maximum allowed value is 10^4 CFU/g. Thus, the application of natural antimicrobials, such as plant extracts or spices, is a promising alternative with regard to research on active packaging due to its natural product appeal and consumer preference.

Table 4 shows the mean values of the counts (log CFU/g) of mold and yeast, in challenge mode, over 25 days of storage.

Table 4. Count (log CFU/g) of mold and yeast in sugar-free banana preserves as a function of storage time in challenge mode.

Treatments	*Storage time (days)*		
	0	6	25
T1	2.81 ± 0.08	4.20 ± 0.34	7.4 ± 0.34
T2	2.81 ± 0.08	4.43 ± 0.18	6.7 ± 0.00
T3	2.81 ± 0.08	< 2 ± 0.21	< 2 ± 0.00
T4	2.69 ± 0.15	< 2 ± 0.00	< 2 ±0.61

Note: n = 2. Means value ± standard deviation. T1 = Banana preserve without potassium sorbate with unincorporated film of crude jambolão extract or potassium sorbate; T2 = Banana preserve without potassium sorbate with film incorporated with crude jambolão extract; T3 = Banana preserve without potassium sorbate with film incorporated with potassium sorbate; T4 = Banana preserve with potassium sorbate without films.

It was verified that the cellulose acetate film incorporated with crude extract of jambolão pulp (T2) exerted antimicrobial activity, reducing the *Penicillium chrysogenum* count by almost a log cycle when compared to the control film (Table 4), without the incorporation of the extract (T1) after 25 days of storage. However, jambolão extract was not able to reduce the levels of mold and yeast to within the standards of current legislation (Brazil, 2001). Furthermore, the sorbate had a greater antimicrobial effect than jambolão extract and the cellulose acetate film incorporated with potassium sorbate (T3) had the same antimicrobial effect as the jam directly added with potassium sorbate (T4), reducing the population of *Penicillium chrysogenum* to <2 log CFU/g of banana preserve (Table 4) after 25 days of storage.

Potassium sorbate incorporated directly into the banana preserve or the film was efficient in controlling the population of molds in the banana preserve, even when the product had relevant initial contamination.

Conclusion

The present work studied films of cellulose acetate added with potassium sorbate or raw extract of jambolão and potential application as antimicrobial active packaging in sugar-free banana preserves. The incorporation of crude jambolão extract increased the film thickness (control-0.032 μm and jambolão-0.058 μm), and its inhibition potential against *Penicillium chrysogenum* was similar to the potassium sorbate incorporated film.

The use of these antimicrobial films as active packaging for sugar-free banana preserves proved to be a promising alternative for the packaging of this product, since the film with crude jambolão extract reduced the growth of mold and yeast by up to 1 log cycle over 36 days of storage. Furthermore, the use of the film with potassium sorbate promoted the same effect as this preservative added directly to the banana preserve. Moreover, the films with potassium sorbate proved to be efficient even with relevant initial contamination. Thus, the use of these active packagings presents an opportunity for industries, as they reduce health risks and improve the safety and quality of preserves.

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