POTENTIAL ANTIOXIDANT AND ANTIMICROBIAL OF CHITOSAN-BASED FILMS INCORPORATING JATOBÁ (*Hymenaea courbaril L.*) PEEL EXTRACT

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ABSTRACT

Biodegradable chitosan-based films containing different concentrations of Jatobá fruit peel extract (0.04%, 0.07%, 0.1% and 0.3%) were prepared. The films were evaluated for antioxidant activity by the ABTS, DPPH and FRAP methods and antimicrobial activity in 96-well microplates. Films with lower extract concentrations were more effective to inhibit nine bacterial strains, with emphasis on *Escherichia coli* (95.0% inhibition), *Bacillus cereus* (92.5% inhibition), *Bacillus subtilis* (96.5% inhibition), *Pseudomonas aeruginosa* (94.4% inhibition), *Salmonella* Typhimurium (90.4% inhibition) and *Salmonella* Enteritidis (93.9% inhibition). The addition of increased concentrations of the extract increased the antioxidant activity (approximately 9 to 60 times in relation to the film without extract, being greater in films containing 0.3% extract. The films incorporated with Jatobá fruit peel extract demonstrated potential to be used as bioactive and biodegradable "green" packaging, being an alternative to petroleum-derived packaging.

Keywords: Chitosan. Food packaging. Polyphenol compounds. Biodegradable film. Fruit residue.

1 INTRODUCTION

Petroleum-based plastic packaging has been widely used, however, this type of material shows an ecological problem, due to its greater resistance and difficult biodegradation.[1] In the search for new materials to replace conventional packaging, chitosan has low toxicity, good film-forming properties and biodegradability.[2] Furthermore, the incorporation of natural extracts improve the properties of the films, as they serve as carriers of antioxidant, antimicrobial compounds, additives and vitamins, which are released during food storage.[3]

Jatobá (*Hymenaea courbaril*) belongs to the *Fabaceae* family, has a rigid, pod-shaped shell and is brown in color, and is found in the cerrado region.[4] In previous studies, extracts from Jatobá fruit peel showed antioxidant and antimicrobial potential,[5] making it attractive to be incorporated into biodegradable film formulations.

Based on this, in the present study the effect of incorporating different concentrations of Jatobá peel extract on the antioxidant and antimicrobial activity of chitosan films, was investigated.

2 MATERIAL & METHODS

The Jatobá fruit peel was manually separated from the pulp and seed. The peel was crushed in an industrial blender, sieved through a 32 *mesh*, to obtain flour. The extract was obtained using a 70% aqueous ethanol solution at a 1:5 ratio (flour:solvent), filtered and the supernatant obtained was used to prepare films. [5] The films were prepared in duplicate (BR1020210264349). The extract was added at concentrations of 0, 0.04, 0.07, 0.1 and 0.3% and the films were named CH (chitosan without extract), CHJE-0.04 (chitosan film + 0.04 % extract), CHJE-0.07 (chitosan film + 0.07% extract), CHJE- 0.1 (chitosan film + 0.1% extract) and CHJE-0.3 (chitosan film + 0.3% extract).

The antimicrobial activity of the films was carried out in 96-well microplates[6] against the bacteria *Enterococcus faecalis* (INCQS 00531), *Salmonella* enteritidis (INCQS 00258), *Pseudomonas aeruginosa* (CBAM 0679), *Staphylococcus aureus* (CBAM 0629), *Bacillus cereus* (CBAM 0353), *Bacillus subtilis* (CBAMd f 0441), *Serratia marcescens* (CBAM 051), *Escherichia coli* (CBAM 0002) and *Salmonella* typhimurium (CBAM 0018) were donated by the Oswaldo Cruz Foundation (Manguinhos, Rio de Janeiro, Brazil) and the Leônidas and Maria Deane Institute (Amazon Bacteria Collection). The percentage of inhibition of the films was calculated according to Equation 1.

For the antioxidant activity, 100 mg of film samples, in triplicate, was mixed with 2 mL of pure methanol and agitated for 3 h, filtered through filter paper, and the supernatant was analysed [7] in triplicate by ABTS [8], DPPH [9] and FRAP [10] methods. The results were expressed in µmol Trolox/g (For FRAP) and percentage of inhibition according to Equation 1 (For ABTS and DPPH).

$$\%\ de\ inhibition = \frac{(A0-A1)}{A0} x100 \quad (1)$$

Where A0 corresponds to the absorbance value of control and A1 corresponds to the absorbance of the samples containing films.

All data were analysed by one-way analysis of variance (ANOVA) and Duncan test to detect significant differences (p≤0.05) using the program Statistica 10.0.

3 RESULTS & DISCUSSION

The films inhibited bacteria between 6.6% and 96.5% (Table 1). The film with the lowest extract concentration (CHJE-0.04) was more effective to inhibit *E. coli, E. faecalis, S. marcensces* and *S.* Typhimurium (90.5, 86.5 and 49.2%, respectively) differing statistically from other films (p≤0.05). *B. subtilis* and *P. aeruginosa* were more inhibited by the films CHJE-0.04 and CHJE-0.07, which did not differ among them (p>0.05). *B. cereus* was inhibited by all films with extract and *S.* Enteritidis by CHJE-0.07 film (p≤0.05).

The antimicrobial activity of films with extract may be related to the presence of phytochemical compounds, such as condensed tannins, cinnamic derivatives, flavonoids, catechin, rutin, terpenes/steroids, saponins and the flavonoid astilbin present in the Jatobá bark [12, 13].

The lower antimicrobial potential of films with higher concentrations of extract, CHJE-0.1 and CHJE-0.3, may be due to less access of microorganisms to the antimicrobial phenolic compounds in the extract, since these tend to form hydrogen bonds with the polymeric matrix. [11] In contrast, Nadira *et al.*[6] obtainedgreater antimicrobial activity in chitosan films with the highest concentrations of cashew leaf extract. Despite CH film (without extract) showed antimicrobial activity against 8 bacteria, except *S. marcensces*, the % inhibition was lower than those obtained with films containing extract.

Table 1 Percentage of inhibition of films incorporated with Jatobá bark extract against pathogenic microorganisms.

	CH	CHJE-0.04	CHJE-0.07	CHJE-0.1	CHJE-0.3
B. subtilis	65.4±3.8e	95±0.39 a	96.5±0.3 a	77.7±1.5cd	85.4±3.1 b
B. cereus	44.1±1.1b	92.5±0.9 a	92.5±0.01a	93±0.4 a	92.5±0.01a
E. coli	27.2±3.2c	90.5±0.8a	84.1±0.4b	84.5±0.8b	85.4±0.8b
E. faecalis	37.9±0.5d	86.5±2 a	49.6±0.7 b	45±1.3 c	43.2±1.2c
P. aeruginosa	6.4±2.2 d	94.4±0.4 a	92.2±0.8 a	86.1±1.7 b	80±0.8 c
S. aureus	27.1±2.9c	47.7±0.8 a	28.3±0.8 c	46.4±0.4a	42±0.4 b
S. Typhimurium	63.8±3.3c	90.4±0.02a	78.8±0.9b	81.7±0.9 b	80.7±0.9 b
S. Enteritidis	31.2±2.2e	87.9±0.02b	93.9±0.5 a	59.1±0.6d	73.9±2.03c
S. marcensces	ND	49.2±0.6 a	ND	6.6±1.3 c	38.4±3.38b

Mean ± standard deviation. a-e Different lowercase letters on the same line indicate a significant difference between treatments (p≤0.05) by Duncan's test. (ND): Not detected.

The CHJE-0.3 film showed the highest antioxidant activity (59.49 % inhibition of DPPH), and the CH film (without extract) the lowest (4.27 % inhibition of DPPH), differing statistically from tother films (p≤0.05). The incorporation of 0.3% extract on film increased the antioxidant activity by around 15 times. Also, , the CHJE-0.3 film showed about 60- and 9-fold higher antioxidant activity by FRAP and ABTS methods, respectively, in relation to CH film (without extract), differing statistically from other films (p≤0.05).

Table 2 Evaluation of the antioxidant activity of chitosan films incorporated with Jatobá extract.

	DPPH (% inhibition)	FRAP (µmol Trolox/g)	ABTS (% inhibition)
CH	4.27 ± 0.8 e	0.055 ± 0.01 c	9.04 ± 0.12 e
CHJE-0.04	20.07 ± 1.09 d	0.34 ± 0.006 c	16.42 ± 0.07 d
CHJE-0.07	36.23 ± 1.01 c	1.01 ± 0.04 b	26.43 ± 0.92 c
CHJE-0.1	55.21 ± 1.01 b	1.42 ± 0.33 b	45.99 ± 2.97 b
CHJE-0.3	59.49 ± 0.36 a	3.32 ± 0.001 a	83.64 ± 0.87 a

Mean ± standard deviation. a-e Different lowercase letters in the same column indicate a significant difference between treatments (p≤0.05) by Duncan's test.

The antioxidant activity of the films was proportional to the increase in the amount of extract added. This result may be due to the antioxidant potential of 70% ethanol extract previously determined [5](276.70 µmol Trolox/g residue by ABTS, 415.77 µmol Trolox/g residue by DPPH and 368.91 µmol Trolox/g residue by FRAP). This potential can be attributed to the presence of antioxidant compounds, such as flavonoids, terpenes/steroids and saponins, catechin, rutin and the flavonoid astilbin present in Jatobá peel extracts.[14] Another study[15] also found an increase in AA in chitosan films enriched with aqueous sage and rosemary extracts, due to the presence of phenolic compounds in the extracts.

4 CONCLUSION

Films with chitosan and different concentrations of Jatobá bark extract were prepared. Films with lower extract concentrations (CHJE-0.04 and CHJE-0.07) were more effective as antimicrobials against the pathogenic bacteria tested and films with higher

extract concentrations (CHJE-0.3) were more effective as antioxidants. The films incorporated with Jatobá peel extract, showed potential to be used in future applications in food preservation.

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