



Ethanollic extract from *Capsicum chinense* Jacq. ripe fruits: phenolic compounds, antioxidant activity and development of biodegradable films

Larissa Sousa SANTOS¹, Cassia Cristina FERNANDES¹, Lauriane Sousa SANTOS¹, Isabella Pelosi Borges de DEUS¹, Tainara Leal de SOUSA¹, Mayker Lazaro Dantas MIRANDA^{2*} 

Abstract

Capsicum spp. pepper has great economic and social importance in agribusiness worldwide, mainly associated with its high performance in cookery as a spice. This study aimed at determining the chemical profile of the ethanollic extract from *Capsicum chinense* Jacq. (EECC) ripe fruits by liquid chromatography-mass spectrometry (LC-MS) and at evaluating its antioxidant activity by DPPH, ABTS and FRAP. Besides, biodegradable films were prepared by incorporating EECC into arrowroot biofilms. LC-MS identified 10 phenolic compounds, a fact that corroborates its high concentration of total phenolic compounds, i. e., 277.62 ± 12.06 mg gallic acid/100g crude extract. High antioxidant activity of EECC was expressed as IC_{50} values for reagents DPPH ($IC_{50} = 18.04$ μ g/mL), ABTS ($IC_{50} = 25.33$ μ g/mL) and FRAP ($IC_{50} = 128.58$ μ g/mL). Biodegradable films incorporating different doses of EECC (250-1000 μ L) were obtained by a casting technique. The higher the EECC concentration, the higher their thickness, color, variable moisture content and the lower their solubility. This study proposes a new use to the plant extract from *C. chinense*, a natural product that may be applied to the development of biofilms to coat food and retard its deterioration. In addition, antioxidant activity of this type of pepper is also shown.

Keywords: food packaging; arrowroot; habanero-type pepper; capsaicin; dihydrocapsaicin.

Practical Application: *Capsicum chinense* may be considered an alternative source of antioxidant agents for pharmacological applications and the food industry. Arrowroot is a promising raw material to develop biodegradable films (or biodegradable coatings). Incorporation of ethanollic extract from *C. chinense* into arrowroot film may be produced with good physico-chemical properties.

1 Introduction

Capsicum chinense, whose common name is habanero-type pepper (*pimenta biquinho* in Brazilian Portuguese), belongs to the genus *Capsicum* and to the family Solanaceae. It is native to Brazil and its fruits have sweet flavor and mild pungency. Peppers usually exhibit several nutrients, phenolic compounds, carotenoids, capsaicinoids and capsinoids in their chemical composition. Phenolic compounds play an important role as antioxidants in plant defense mechanisms against fungi, bacteria and predators. Due to their antioxidant activity, plant extracts with phenolic compounds may be considered potential additives to active films that aim at protecting food (Antonious et al., 2006; Alves et al., 2014; Aguiar et al., 2014, 2019).

Packaging, which has been used for helping food and goods transportation, storage and protection, is usually made from plastic material, even though this polymer may be associated with food contamination and issues related to environmental pollution (Kalpana et al., 2019; Piñeros-Hernandez et al., 2017).

In order to ensure food safety and decrease impacts on the environment as the result of high amount of waste generated by petroleum-based plastic packaging, new choices of biodegradable packaging have been proposed worldwide. Therefore, natural polymers, such as starch, have been considered the best substitutes for synthetic polymers (Jiménez et al., 2012).

Starch is an abundant plant polysaccharide which is available in 60% of cereal grains. It is a renewable, low-cost and biodegradable source. Since this polymer has excellent properties to form gels, it may be used for producing films and coatings (Jiménez et al., 2012; Thakur et al., 2019).

Among starchy plants, arrowroot (*Maranta arundinacea*) has high potential as raw material for starch extraction. Starch found in arrowroot rhizomes, unlike corn and manioc starch, has digestibility characteristics and high content of amylose, a polymer that has great ability to form films (Guilherme et al., 2019; Nogueira et al., 2018). However, it should be highlighted that there are few studies of arrowroot starch, despite its excellent physico-chemical characteristics.

Food packaging films made from natural polymers are biodegradable, non-toxic and use renewable raw material. In addition, they may be enriched by having antioxidant, antimicrobial and antifungal agents added to them. These active compounds may migrate from packaging to food and increase its service life (Piñeros-Hernandez et al., 2017; Rambabu et al., 2019).

Films that have antioxidant agents may be used for avoiding lipid oxidation and degradation of fat products, such as meats. Even though synthetic additives are often used in active packaging, they may cause adverse effects on human health. Thus, natural

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¹Instituto Federal de Educação, Ciência e Tecnologia Goiano, Campus Rio Verde, Rio Verde, GO, Brasil

²Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Campus Uberlândia Centro, Uberlândia, MG, Brasil

*Corresponding author: maykermiranda@ifm.edu.br

products with antioxidant activity, such as plant extracts and essential oils, may be alternatives to replace synthetic products (Domínguez et al., 2018; Vásconez et al., 2009).

Taking into account that arrowroot starch has excellent characteristics to produce biodegradable films and that peppers that belong to the genus *Capsicum* usually exhibit antioxidant capacity, this study aimed at determining the chemical profile of the ethanollic extract from *C. chinense* ripe fruits (Figure 1), at evaluating its in vitro antioxidant potential, at incorporating it into arrowroot starch biofilms and at evaluating some physico-chemical properties of resulting biofilms.

2 Materials and methods

2.1 Sample preparation

C. chinense ripe fruits (only red ones) were bought in fairs in Santa Helena de Goiás and in Rio Verde, two cities in Goiás (GO) state, Brazil. They were then taken to the Laboratory of Natural Product Chemistry at IF Goiano - Campus Rio Verde, located in Rio Verde, GO, where they were washed with distilled water. Afterwards, they were dried with paper towels and had their peduncles removed. Fruits were then weighed and dehydrated in an air circulation oven at 40 °C for 96 hours. Finally, they were ground, placed into a sealed container and stored in a refrigerator up to the preparation of crude ethanollic extract.

2.2 Preparation of ethanollic extract

Extraction was carried out with 5.0 g sample and 100 mL ethanol; it was kept under constant magnetic agitation for 2 hours. Contact between solvent and raw material was kept for four days at room temperature (26 °C), in the dark. It was manually agitated on a daily basis. The mixture that resulted from the extraction was separated by filtration, followed by solvent evaporation which was



Figure 1. *Capsicum chinense* red fruits.

carried out by a rotary evaporator at reduced pressure. The resulting ethanollic extract from *C. chinense* (EECC) ripe fruits had syrup-like consistency. The EECC content was determined by Equation 1:

$$\text{Yield (\%)} = \frac{\text{extract mass}}{\text{sample mass}} \times 100 \quad (1)$$

2.3 Characterization of phenolic compounds by LC-MS

The analysis of EECC was carried out at the Centro Regional para o Desenvolvimento Tecnológico e Inovação (CRTI) that belongs to the Universidade Federal de Goiás (UFG). An Ultimate 3000 liquid chromatographer, Thermo Scientific, with Agilent-C18 column (4.6 x 100 mm; 3 µm), coupled with a Thermo Scientific Q-Exactive high-resolution mass spectrometer, with H-ESI source, operating in both positive and negative modes, spray voltage 3.5 kV, sheath gas 30, auxiliary gas 10, capillary temperature 350 °C, auxiliary gas temperature 250 °C, tube lens 55 and mass range *m/z* 150-700 was used. HPLC analysis was carried out with deionized water which was acidified with 0.1% formic acid (mobile phase A, v/v) and methanol acidified with 0.1% formic acid (mobile phase B, v/v). Gradient programming started at 93:07 (A:B %), 70:30 (A:B %) for 10 minutes, 50:50 (A:B %) for 5 minutes, 30:70 (A:B %) for 3 minutes, 20:80 (A:B %) for 2 minutes, 100 (B %) for 3 minutes, kept for 3 minutes, 93:07 (A:B %) for 2 minutes, kept for 2 minutes. Runtime was 33 minutes at flow rate of 0.3 mL/min, injection volume 10 µL and column temperature 20 °C. In the study of fragmentation, Parallel Reaction Monitoring (PRM) was conducted with collision energies (NCE) of 15 and 30. In order to identify phenolic compounds, a stock solution with methanol standards at the concentration of 1 mg/mL was used. Stock solutions were used for preparing the solution of the mixture of standards at the concentration of 50 µg/mL. The analysis of standard mixture was carried out in the conditions used for samples. Standards of phenolic compounds were: gallic, protocatechuic, gentisic, caffeic, *p*-coumaric, vanillic, ferulic and ellagic acids, besides catechin, epicatechin, rutin, quercetin, naringenin, luteolin and kaempferol. Data were processed by the Xcalibur™ software program.

2.4 Total phenolic compounds

In order to quantify total phenolic compounds, 1.9 mL Follin-Ciocalteu reagent in distilled water (1:9) was added to 200 µL EECC. To neutralize the mixture, 1.9 mL aqueous solution of sodium carbonate (60 g/L) was used. The reaction was kept in the dark at room temperature for 120 minutes. Then, absorbance was measured at 725 nm. Calculation was carried out with the use of the standard curve and results were expressed as mg gallic acid/100 g EECC (Arbos et al., 2010).

2.5 Evaluation of antioxidant activity by DPPH, ABTS and FRAP

Antioxidant activity of EECC was investigated by three well-known methods, i. e., DPPH, ABTS and FRAP, following the methodology described by Mardigan et al. (2019).

2.6 Film preparation with EECC

Biodegradable films were obtained by a casting technique, with the use of the methodology proposed by Issa et al. (2017),

with modifications. In order to produce every film, 5 g commercial arrowroot starch was dissolved in 100 mL deionized water. The solution was then moderately agitated at room temperature (26 °C). Afterwards, it was heated at 70 °C, at constant agitation for 30 minutes. After starch gelatinization, glycerol was added as a plasticizer (30% p/p); this dispersion was agitated for five more minutes. When the filmogenic solutions reached 50 °C, one of them, which was called FAP1, did not have any EECC incorporated into it, while EECC doses of 250 µL (FAP2), 500 µL (FAP3), 750 µL (FAP4) and 1000 µL (FAP5) were added to the others under constant agitation for 15 minutes. It should be mentioned that EECC – in its syrup-like consistency – was better solubilized as 200 mg EECC per 1 mL Tween 80 at 5%. All filmogenic solutions were poured on polystyrene slabs and dried in an air circulation oven at 30 °C for about 48 hours.

2.7 Morphology by scanning electron microscopy (SEM)

Films were fixed on gold-plated brass sample holders. Images were captured by a JEOL JSM-IT300 scanning electron microscope (SEM) in high-vacuum mode to detect secondary electrons at electron accelerating voltage of 7 kV.

2.8 Characterization of films incorporating EECC

Film thickness

Film thickness was measured by a digital caliper, whose precision was 0.01 mm. Measurements were carried out in 10 spots on every film and the thickness mean was calculated.

Moisture content

Films were weighed and then dried in an oven at 105 °C for 24h. Three replicates per film treatment were used, in agreement with the methodology described by Rambabu et al. (2019).

Measurement of water solubility

Films which measured about 2 cm² were dried in an oven at 105 °C for 3 hours and then weighed so that initial mass (Mi) could be determined. They were immersed in 50 mL distilled water and kept under constant agitation at 26 °C for 24 hours. Afterwards, solutions with the films were filtered through filter paper which had been previously weighed. Sheets of filter papers with films were dried at 105 °C for 24 hours and weighed so that final mass (Mf) could be found, in agreement with the methodology described by Jahed et al. (2017). Every treatment was analyzed in triplicate. Film solubility (%) was calculated by Equation 2:

$$\text{Water solubility}(\%) = \frac{Mi - Mf}{Mi} \times 100 \quad (2)$$

Biodegradability

The analysis was carried out by the methodology described by Martucci & Ruseckaite (2009), with modifications. Film samples (2 x 2 cm) were dried up to constant weight so that initial mass (Mi) could be determined. Samples were then placed in open

polyethylene packages to enable microorganisms and moisture to gain access to them. After that, they were buried in organic soil, which had been previously prepared, at constant moisture and room temperature. Thirty days after the experiment installment, the packages with the samples were removed from the soil, washed with distilled water and dried up to constant weight (Mf). Biodegradability (%) was calculated by Equation 3:

$$\text{Biodegradability}(\%) = \frac{Mf - Mi}{Mi} \times 100 \quad (3)$$

Light transmittance rate (UV-VIS)

Ultraviolet (UV) and visible light transmittance of films was conducted by a LAMBDA 750 UV-Vis spectrophotometer (PerkinElmer). Film samples were cut and placed in cuvettes so that transmittance could be measured over a wavelength range between 250 and 850 nm (Hosseini et al., 2015a).

2.8.6 Analysis of color

Analysis of film color was carried out by a ColorQuest II colorimeter (HunterLab, Reston, USA). Parameters under evaluation were L* (luminosity) and chromaticity parameters (a* and b*). Measurements were conducted on nine randomly selected film spots. Difference in color (ΔE) was calculated by Equation 4:

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2 \right]^{1/2} \quad (4)$$

where ΔE is the mean of five measurements per film.

Statistical analysis

Analyses were carried out in triplicate and standard deviations were calculated. Means were compared by the Tukey's test at 5% significance with the use of the Sisvar 5.6 software program.

3 Results and discussion

EECC exhibited mean extraction yield of 8.42 ± 0.35%. Studies of extraction yield of habanero-type pepper by different methods and solvents, conducted by de Aguiar et al. (2014), showed that polar solvents exhibited higher extraction yield than nonpolar ones and led to the conclusion that this pepper must have high concentrations of compounds with high polarity, such as phenolic compounds, sugars and vitamins.

Ten out of 15 standard phenolic compounds – used for chemical characterization of plant extracts – were identified in EECC (Table 1). They are protocatechuic, gentisic, caffeic, vanillic, *p*-coumaric and ferulic acids, besides quercetin, naringenin, luteolin and kaempferol. Even though habanero-type pepper has low pungency, two probable capsaicinoids – capsaicin and dihydrocapsaicin – were also identified (Table 2); the reference was just their molecular mass, since there were no commercial standards when the analysis was carried out.

Alcaloids capsaicin and dihydrocapsaicin, besides flavonoids kaempferol, quercetin and luteolin, are the main compounds found in *Capsicum* peppers (Morales-Soto et al., 2013; Nascimento et al., 2014). The importance of capsaicin and dihydrocapsaicin is

related to several factors, but the main one is the fact that they are the active ingredients that not only represent organoleptic and pharmaceutical properties, but also bestow burning sensation to peppers (Domenico et al., 2012). In a recent study, Guillen et al. (2018) reported that chili fruits contain other capsaicinoid components, such as nordihydrocapsaicin, norcapsaicin, homocapsaicin I, homodihydrocapsaicin I, homocapsaicin II, homodihydrocapsaicin II and nonivamide.

Antioxidant capacity and phenolic content of samples of plant extracts may vary as the result of several factors, such as the polarity of the solvent used for extraction, the extraction method and the material concentration. In addition, to measure antioxidant capacity of samples, there are several methods which are based on parameters of electron and hydrogen atom transfer (Oldoni et al., 2019). Therefore, FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) were the methods used for evaluating the antioxidant potential of EECC.

Results of antioxidant activity are shown in Table 3. EECC exhibited antioxidant activity in all methods under evaluation; ABTS and DPPH were based on hydrogen atom transfer, while FRAP was based on electron transfer. According to Morales-Soto et al. (2013), most antioxidant activity of peppers that belong to the genus *Capsicum* is due to their total phenolic compounds (Tables 1 and 2). Their promising antioxidant activity is also

due to capsaicinoids, such as capsaicin and dihydrocapsaicin (Nascimento et al., 2014).

Starch films into which different EECC doses (250-1000 µL) had been incorporated were analyzed by scanning electron microscopy (SEM) so that their morphology, which is related to their physical properties, could be studied. Superficial film areas were about x1500, x500 and x300. On their superficial areas, all films exhibited a granular structure and light rugosity; both increased exponentially as EECC concentration increased. These characteristics are related to certain factors, such as the type of plasticizer, the starch and the relation between time and temperature throughout film development (Thakur et al., 2019).

A positive aspect is that films exhibited neither fractures nor phase separation, a fact that shows that EECC – added at different doses – was incorporated into the filmogenic solution in a uniform way. Film micrographs are shown in Figures 2, 3, 4, 5 and 6.

Film thickness depends on factors related to drying and to the method of preparation. Thickness should be measured because it affects mechanical properties of films, such as water vapor permeability (Adilah et al., 2018; Hosseini et al., 2015b). Table 4 shows values of film thickness. The control film (FAP1) exhibited the lowest values of thickness, while in the others, the higher the EECC doses added to the filmogenic solutions, the higher their thickness values.

Table 1. Compounds identified in EECC by LC-MS $[[M - H]^-]$ (negative mode)].

| Retention Time (RT) (min) | Standard RT (min) | Compounds | Molecular formula | Molecular mass | Detected mass | Calculated mass | Error (ppm) | Fragments <i>m/z</i> |
|--------------------------------------|-------------------|-------------------------|--|----------------|---------------|-----------------|-------------|-------------------------------------|
| [M - H] ⁻ (negative mode) | | | | | | | | |
| 16.23 | 16.23 | Protocatechuic acid | C ₇ H ₆ O ₄ | 154.02661 | 153.01830 | 153.01879 | 0.424 | 109.02827 |
| 19.84 | 19.84 | Gentisic acid | C ₇ H ₆ O ₄ | 154.02661 | 153.01828 | 153.01879 | 0.293 | 109.02824 |
| 20.80 | 20.66 | Caffeic acid | C ₉ H ₈ O ₄ | 180.04226 | 179.03407 | 179.03432 | 1.032 | 135.04399 152.01044 |
| 20.67 | 20.67 | Vanillic acid | C ₈ H ₈ O ₄ | 168.04226 | 167.03403 | 167.03444 | 0.867 | 123.04396 108.02041 |
| 22.82 | 22.82 | <i>p</i> -coumaric acid | C ₉ H ₈ O ₃ | 164.047345 | 163.03906 | 163.03896 | 0.548 | 119.04904 |
| 22.96 | 22.96 | Ferulic acid | C ₁₀ H ₁₀ O ₄ | 194.05791 | 193.04985 | 193.05009 | 1.630 | 178.02625 149.05972 134.03618 |
| 25.68 | 25.82 | Quercetin | C ₁₅ H ₁₀ O ₇ | 302.042655 | 301.03552 | 301.03483 | 3.524 | 273.04034 151.00262 |
| 25.97 | 25.97 | Naringenin | C ₁₅ H ₁₂ O ₅ | 272.068475 | 271.06113 | 271.06065 | 1.777 | 151.00259 |
| 26.24 | 26.24 | Luteolin | C ₁₅ H ₁₀ O ₆ | 286.04774 | 285.04037 | 285.03992 | 1.604 | 133.00544 |
| 26.78 | 26.78 | Kaempferol | C ₁₅ H ₁₀ O ₆ | 286.04774 | 285.04041 | 285.03992 | 1.744 | 187.03900 143.05045 |

Table 2. Compounds identified in EECC by LC-MS $[[M + H]^+]$ (positive mode)].

| Retention time (RT) (min) | Probable compound | Molecular formula | Molecular mass | Detected mass | Calculated mass | Error (ppm) | Fragments <i>m/z</i> |
|--------------------------------------|-------------------|---|----------------|---------------|-----------------|-------------|----------------------|
| [M + H] ⁺ (positive mode) | | | | | | | |
| 28.49 | Capsaicin | C ₁₈ H ₂₇ NO ₃ | 305.19909 | 306.20637 | 306.20692 | 3.658 | 137.05941 |
| 29.11 | Dihydrocapsaicin | C ₁₈ H ₂₉ NO ₃ | 307.21474 | 308.22202 | 308.22257 | 3.181 | 137.05951 |

Arrowroot starch has high amylose concentration in its rhizomes. This polymer induces high sensitivity to moisture, which may also affect mechanical properties of films (Thakur et al., 2019). Moisture contents of films depended directly on the amount of EECC that was added to them. However, it should be highlighted that the highest moisture percentage, i. e., 11.44% (FAP5), is still considered low, by comparison with the ones of other films (Hosseini et al., 2015b). Rambabu et al. (2019) applied different concentrations of extract from mango leaves to chitosan films and found that the lowest moisture content in films evaluated by them was 15.84%.

Table 3. Antioxidant activity of EECC evaluated by DPPH, ABTS and FRAP methods (in $\mu\text{g/mL}$) and content of total phenolics (TF).

| | TF | DPPH | ABTS | FRAP |
|------|----------------------|------------------|------------------|-------------------|
| EECC | $277.62 \pm 12.06^*$ | 18.04 ± 2.79 | 25.33 ± 2.84 | 128.58 ± 2.75 |

Positive control: gallic acid ($\text{IC}_{50} = 12.06 \mu\text{g/mL}$); *Milligram of gallic acid/100 g EECC.

Solubility is also an important parameter that should be analyzed in starch films. The ideal level of film solubility depends on their final use. Even though data on solubility shown in Table 4 do not differ statistically, incorporation of different EECC doses

Table 4. Thickness, moisture and solubility of arrowroot films incorporating EECC.

| Film | Thickness (mm) | Moisture (%) | Solubility (%) |
|------|---------------------------|--------------------------|---------------------------|
| FAP1 | $0.24 \pm 0.07\text{c}$ | $10.94 \pm 1.73\text{a}$ | $41.83 \pm 7.00\text{a}$ |
| FAP2 | $0.26 \pm 0.01\text{bc}$ | $7.69 \pm 1.50\text{b}$ | $34.11 \pm 6.24\text{a}$ |
| FAP3 | $0.29 \pm 0.02\text{abc}$ | $9.43 \pm 0.03\text{ab}$ | $33.27 \pm 10.02\text{a}$ |
| FAP4 | $0.30 \pm 0.02\text{ab}$ | $7.82 \pm 0.70\text{b}$ | $32.81 \pm 12.36\text{a}$ |
| FAP5 | $0.34 \pm 0.03\text{a}$ | $11.44 \pm 0.65\text{a}$ | $20.63 \pm 6.83\text{a}$ |

Different letters in a column show significant difference ($p < 0.05$) by the Tukey's test; FAP1: Arrowroot starch film with no EECC; FAP2: Arrowroot starch film with 0.25% EECC; FAP3: Arrowroot starch film with 0.50% EECC; FAP4: Arrowroot starch film with 0.75% EECC; FAP5: Arrowroot starch film with 1% EECC; \pm Mean standard deviation.

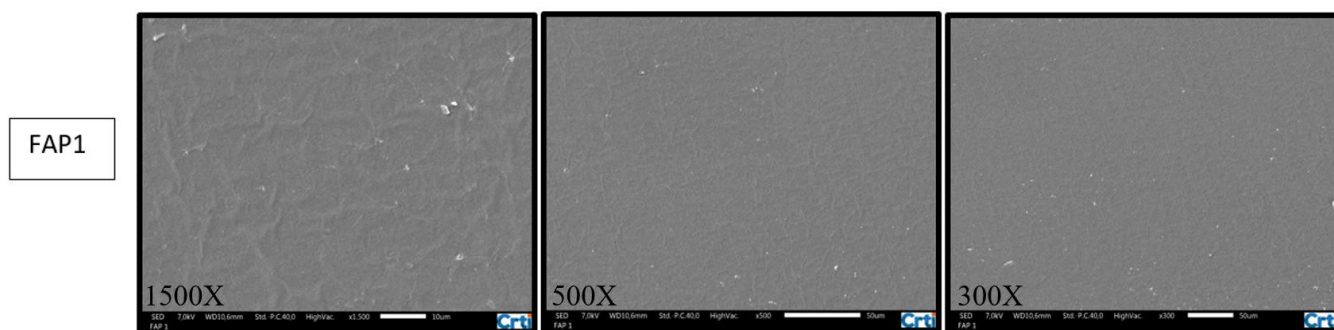


Figure 2. Micrographs of a superficial area of the starch film with no EECC addition (FAP1).

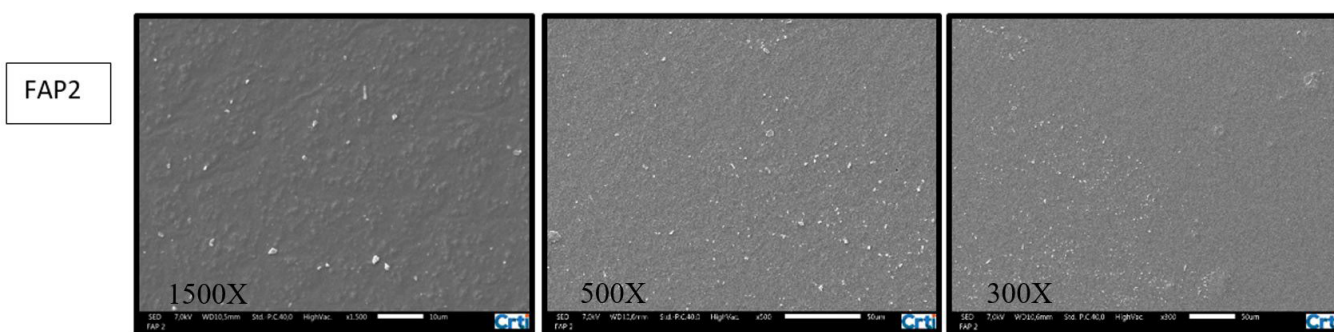


Figure 3. Micrographs of a superficial area of the starch film with 250 μL EECC (FAP2).

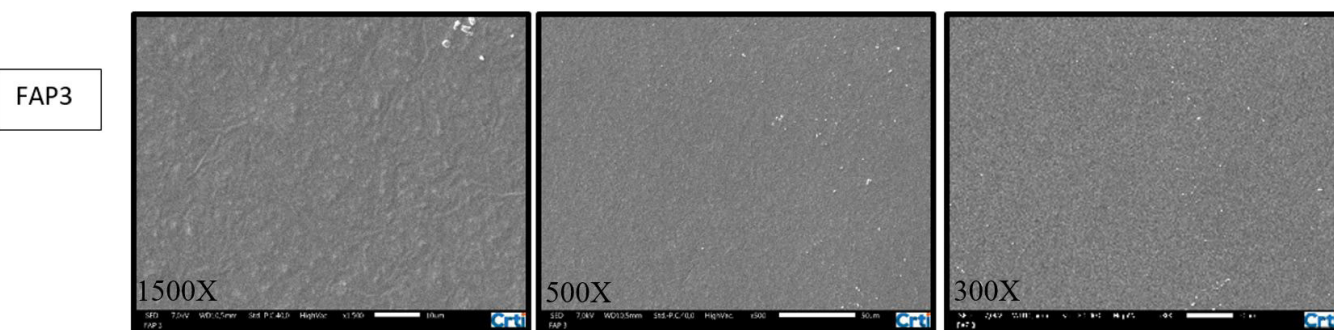


Figure 4. Micrographs of a superficial area of the starch film with 500 μL EECC (FAP3).

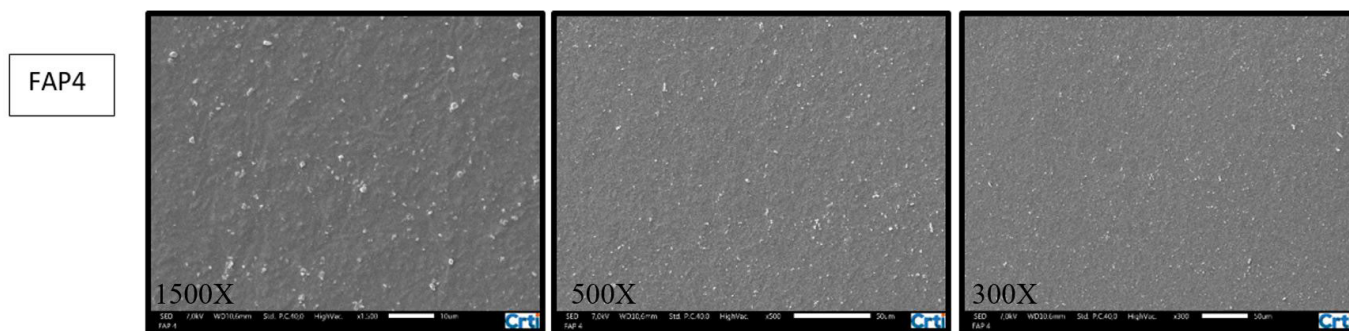


Figure 5. Micrographs of a superficial area of the starch film with 750 µL EECC (FAP4).

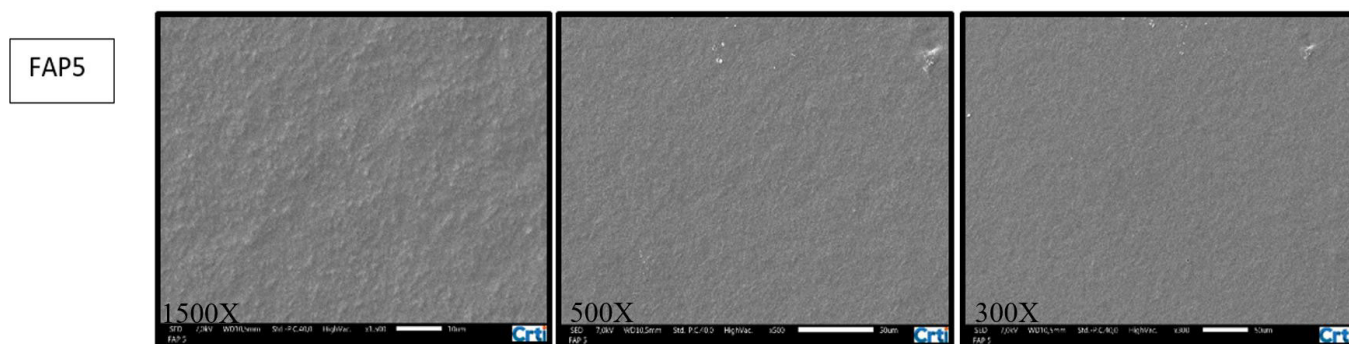


Figure 6. Micrographs of a superficial area of the starch film with 1000 µL EECC (FAP5).



Figure 7. Arrowroot starch films incorporating EECC. FAP1: Arrowroot starch film with no EECC; FAP2: Arrowroot starch film with 0.25% EECC; FAP3: Arrowroot starch film with 0.50% EECC; FAP4: Arrowroot starch film with 0.75% EECC; FAP5: Arrowroot starch film with 1% EECC.

into arrowroot films decreased film solubility. Solubility of the control film was 41.83%, while the film with the highest EECC dose exhibited solubility of 20.63%.

Starch is a hydrophilic material, thus, when a starch film is exposed to water, its polymeric molecules form hydrogen bonds with water and lead to film dissolution (Bertuzzi et al., 2007; Kim et al., 2015). Decrease in the hydrophilic nature of films with EECC is due to the fact that polymeric molecules interact with polyphenols in the crude extract and result in starch-polyphenol bonds, rather than in starch-water ones. Adilah et al. (2018) also observed decrease in solubility of jelly films when extracts from mango skin were added to them, because hydrogen bonds were

formed between molecules of the extract and the jelly, a fact that prevented bonds between hydrogen and water.

After the 30-day analysis, the hollow polyethylene packages that contained arrowroot starch films were removed from the soil. Since films had been thoroughly degraded, the material could not be weighed to quantify biodegradability. Thus, the conclusion may be the fact that incorporation of EECC into films does not decrease biodegradability of arrowroot starch, which may be considered a promising material for biodegradable and eco-friendly packaging.

Optical properties of five arrowroot starch films were evaluated on both matte and shiny sides. Films incorporating EECC got more colorful as its doses increased. Figure 7 shows images of every film under development.

Films were analyzed in agreement with color parameters L^* , a^* , b^* and Δe . L^* is the film luminosity (light/dark), a^* is the red/green coordinate (+/-); and b^* is the yellow/blue coordinate (+/-). Color parameters are shown in Table 5. Results showed that the use of EECC affected film color. Incorporation of EECC into FAP2 and FAP5 did not affect values of luminosity significantly, by comparison with values of films with no extract (FAP1). However, FAP3 and FAP4 were significantly different from the control film.

The control film exhibited negative values of a^* and b^* chromaticity and was green/blue. Films incorporating from 0.25 to 1% EECC exhibited positive values of a^* and b^* chromaticity and were red and yellow. Regarding total difference in colors (Δe), the control film (FAP1) exhibited

Table 5. Measurements of film colors.

| Film | L* | a* | b* | Δe |
|------|-------------|-------------|-------------|-------------|
| FAP1 | 15.51±2.10a | -0.3±0.04d | -0.58±0.11c | 4.00±0.23c |
| FAP2 | 15.00±1.00a | 1.44±0.85c | 5.88±3.68b | 7.61±0.14b |
| FAP3 | 10.57±1.00b | 2.26±1.16bc | 7.17±3.26ab | 7.03±0.14b |
| FAP4 | 10.79±1.11b | 4.20±0.71a | 9.14±1.33a | 3.53±0.11c |
| FAP5 | 15.37±3.26a | 3.02±1.94b | 8.30±5.85ab | 11.99±2.82a |

Different letters in a column show significant difference ($p < 0.05$) by the Tukey's test; parameters CIELab of color L* (luminosity), a* and b* (chromaticity) and Δe (total difference in color) of arrowroot starch films incorporating EECC; FAP1: Arrowroot starch film with no EECC; FAP2: Arrowroot starch film with 0.25% EECC; FAP3: Arrowroot starch film with 0.50% EECC; FAP4: Arrowroot starch film with 0.75% EECC; FAP5: Arrowroot starch film with 1% EECC; \pm Mean standard deviation.

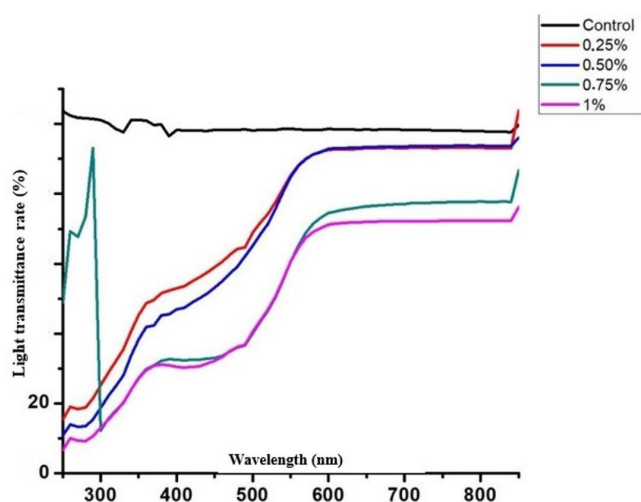


Figure 8. UV-VIS light transmittance rate in arrowroot films incorporating different doses of EECC.

the lowest difference and the film incorporating 1% EECC (FAP5) had the highest difference in color. These results were expected, since EECC was red/yellow, a fact that may be the result of both carotenoids capsanthin and capsorubine, which are exclusive of *Capsicum* species, as mentioned by Neitzke et al. (2015).

Concerning colors of films under development, Figure 8 shows decrease in light transmittance rates of films incorporating different doses of EECC in the visible region (from 400 to 800nm). Color is an important parameter to be evaluated since it influences consumers' acceptance. Films used as packaging are usually transparent so that the product can be seen. However, colorful and opaque films help to protect food exposed to visible and UV light, mainly in food with high fat content, such as meats, preventing them from undergoing oxidative degradation (Rambabu et al., 2019; Romani et al., 2018).

4 Conclusions

This study revealed that the ethanolic extract from *C. chinense* ripe fruits has great potential to be applied to the manufacturing of bioactive packaging. The promising antioxidant activity exhibited by EECC is mainly the result of its high concentration of phenolic compounds. On their superficial areas, all arrowroot films incorporating EECC exhibited a granular structure and light rugosity; however, in all experiments, EECC was incorporated

in a uniform way. Physico-chemical observations related to the incorporation of EECC into arrowroot films led to increase in thickness, decrease in solubility and change in color as its doses increased. In sum, these results disclose the biotechnological potential of *C. chinense* fruits and reinforce the need for new in vitro and in vivo microbiological studies in order to analyze bioactivity of films incorporating EECC against bacteria and fungi.

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