










Extract from *Bixa orellana* L. seed: development of biodegradable packaging and biological activities

Leonardo Almeida JATOBÁ¹ , Antonio Carlos Pereira de MENEZES FILHO^{2*} ,
Marconi Batista TEIXEIRA² , Frederico Antônio Loureiro SOARES² ,
Carlos Frederico de Souza CASTRO² , Porshia SHARMA³ , Matheus Vinícius Abadia VENTURA^{1,2} 

¹UniBRAS University Center of Southwest Goiás, Rio Verde, GO, Brazil

²Goiano Federal Institute, Rio Verde, GO, Brazil.

³Institute for Innovative Learning and Research (IILR), Madhya Pradesh, Indore, India.

*E-mail: astronomoamadorgoias@gmail.com

Submitted on 07/19/2023; Accepted on 08/14/2023; Published on 08/17/2023

ABSTRACT: Active biodegradable packaging with *Bixa orellana* extract has potential for producing new products that combine natural renewable use and food conservation. *B. orellana* seeds were collected and their extract was produced. The packages were produced and incorporated with different concentrations of *B. orellana* extract. Phytochemical parameters were tested in several groups of special metabolism; physicochemical for thickness, moisture, solubility, UV-Vis light transmission, color L*, a* and b*; biodegradability in soil and antifungal activity on *Sclerotinia sclerotiorum*, *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. The packaging showed potential for use as a new product for food, the large number of phytochemicals allows an active packaging, in addition to physicochemical characteristics of thickness, humidity and ideal solubility and use for foods that need protection at different wavelengths in the UV-Vis that deteriorate important characteristics of the product. Moreover, it demonstrated an effect with biodegradability of less than 40 days and antifungal activity on phytopathogenic fungi that cause deterioration in vegetables of agricultural interest.

Keywords: *Bixa* genus; bixin; carotenoids; natural Polymer; norbixin; polymer matrix.

Extrato das sementes de *Bixa orellana* L.: desenvolvimento de embalagens biodegradáveis e atividades biológicas

RESUMO: Embalagens biodegradáveis ativas com extrato de *Bixa orellana* apresentam potencial para a produção de novos produtos que aliam o uso renovável natural e a conservação de alimentos. Sementes de *B. orellana* foram coletadas e o extrato produzido. As embalagens foram produzidas e incorporadas com diferentes concentrações do extrato de *B. orellana*. Foram testados parâmetros fitoquímicos em diversos grupos do metabolismo especial; físico-químicos para espessura, umidade, solubilidade, transmissão de luz UV-Vis, cor L*, a* e b*; biodegradabilidade em solo e atividade antifúngica sobre *Sclerotinia sclerotiorum*, *Colletotrichum acutatum* e *Colletotrichum gloeosporioides*. As embalagens demonstraram potencial para uso como novo produto para alimentos, o grande número de fitocompostos admite uma embalagem ativa, além de características físico-químicas de espessura, umidade e solubilidade ideais e uso para alimentos que necessitam proteção em diferentes comprimentos de ondas no UV-Vis que deterioram características importantes do produto. Além disso, demonstrou efeito com biodegradabilidade inferior a 40 dias e atividade antifúngica sobre fungos fitopatogênicos que causam deterioração em vegetais de interesse agrícola.

Palavras-chave: gênero *Bixa*; bixina; carotenóides; matriz polimérica; norbixina; polímero natural.

1. INTRODUCTION

Bixa orellana is a South and Central American native plant that can also be grown in Peru, Mexico, Ecuador, Indonesia, India, Kenya, and East Africa. The seeds contained in it have natural dyes (bixin and norbixin) that are being used in the Pharmaceutical and Food industries as they contribute in several biological activities (HIRKO; GETU, 2022).

The usage of substances extracted from special metabolism of plants have been gaining popularity due to their various qualities that help development of active and intelligent packaging. It is observed that bixin and norbixin (carotenoids) are potentially able to work as antioxidant, antifungal, anticarcinogenic and among other biological

activities. Bixin is a linear apocarotenoid having double bonds and constitutes up to 80% of the total carotenoid content present in *B. orellana* (annatto or *urucum*) seeds (LUIGGI et al., 2020).

The incorporation of plant extracts in natural polymeric matrices is beneficial as it provides; reduction of synthetic packaging, easily degradable on discarding in atmosphere, has little to no pollution rate and also serves the purpose of preservation of food from harmful micro-organisms and oxidation (Shaikh et al., 2021). The usage of biodegradable materials in food packaging counts as a promising alternative as it reduces investment and also the treatment cost during waste management.

Some studies demonstrate the incorporation of bixin into a polymeric matrix; this results in formation of great barrier against oxygen, water vapour, thermal stability and to different wavelengths of light such as ultraviolet (UV) and visible (*Vis*) (STOLL et al., 2021a). Although, there is no experimental evidence of using polymeric matrix of starch obtained from *Maranta arundinacea* L. (arrowroot or *araruta*) with extract of *B. orellana*. Thus, there is no known proof of interaction between this polymer and bixin extract obtained from the seeds. Thus, this study aimed to evaluate the addition of ethanolic extract of *Bixa orellana* seeds regarding phytochemical constitution, formation of biodegradable packaging (BP), physicochemical characteristics, biodegradability and antifungal activity at different concentrations.

2. MATERIAL AND METHODS

2.1. Reagents, equipments and glasswares

Ethyl alcohol P.A - ACS (Neon, Brazil), glycerin P.A - ACS (Dinâmica, Brazil), fungicide Frownicide 500 SC (ISK, Brazil) Fluazinam concentration (500 g/L⁻¹), Tween 80 P.A - ACS (Dinâmica, Brazil), PDA culture medium (Himedia, India).

Digital analytical balance (Shimadzu, Mod. AY220, Brazil), digital colorimeter (HunterLab, Mod. ColorFlex EZ, U.S.A), digital caliper (Mitutoyo, Mod. 500-196-30, Japan), digital thermometer (Bmax, Mod. TP-101, Brazil), electric greenhouse with forced air circulation (Thoth, Mod. th-510-480, Brazil), lyophilizer (Terroni, Mod. LS6000 B, Brazil), magnetic stirrer with heating (Solab, Mod. SL 91, Brazil), UV-*Vis* spectrophotometer (Bel, Mod. M-51, Italy).

Beaker 500 mL (Deltex, Brazil), glass rod (Uniglas, Brazil), precipitation cup 250 and 500 mL (Uniglas, Brazil), polystyrene *Petri* dish (FirstLab, Mod. FL3-9015RI, China), qualitative filter paper (Unifil, Brazil), universal tip 1 mL (Olen, U.S.A), variable micropipette 10-1000 μ L (Pegupet, Brazil).

2.2. Species collection and identification

Bixa orellana seeds were collected in July 2022 in the municipality of Rio Verde, Goiás State, Brazil, with the following geographic coordinate (17°48'00.0"S and 50°54'41.5"W). The species was identified, exsiccate herborized and deposited in the Herbarium of the Laboratório de Sistemática Vegetal at Instituto Federal Goiano, Rio Verde, Goiás State, Brazil. Voucher (HRV: 17.786).

2.3. Seed processing

The seeds were grounded in a food processor for 3 min. After this, the obtained seed powder was stored in a plastic food packaging and was kept at -12 °C until further analysis.

2.4. Production of extract

Extraction was carried out with 50 g sample and 100 mL ethanol (*m/v*); it was kept under constant magnetic agitation for 4 h. Contact between solvent and raw material was kept for seven days at room temperature (25 °C), in the dark. It was manually agitated on a daily basis. The mixture that resulted from the extraction was separated through filtration (Whatman qualitative filter), followed by solvent evaporation which was carried out by a vacuum rotary evaporator at 70 °C. The lyophilized extract was kept under refrigeration at -

12 °C until further analysis. The extracts were lyophilized. The yield seed ethanolic (EEBO) extract from *B. orellana* content was determined by equation 1.

$$\text{Yield (\%)} = (\text{Em}/\text{Sm}) * 100 \quad (01)$$

where: Em = Extract mass; Sm = sample mass.

2.5 Phytochemical analysis

For phytochemical analysis, 50 mL of ethanol solution containing 5 g (*w/v*) of extract was prepared. Crude ethanolic extract of *B. orellana* seeds was subjected to number of analysis for phytochemical characterization according to Bessa et al. (2013) and Shaikh; Patil (2020) with adaptations. The positivity for each group of special metabolites and constituents analyzed was achieved from the development of color and/or precipitate, characteristic for analysis of each phytochemical class. saponins, alkaloids, anthraquinones, catechins, coumarins, steroids and triterpenoids, phenols, tannins, flavonoids, quinones, organic acids, carboxylic acids, polysaccharides, aromatic and aliphatic compounds, oxylates, bixin and norbixin, reducing sugars, non-reducing sugars and carbohydrates were evaluated. The detection was based on visual observations of a color change or precipitate formation after adding specific reagents.

2.6. Biodegradable packaging preparations

Biodegradable films were obtained by a casting technique, use of the methodology proposed by Issa et al. (2017), modified. To produce each film, 5 g arrowroot starch was dissolved in 100 mL deionized water. The solution was then moderately agitated at room temperature 25 °C. Afterward, it was heated at 70 °C, at constant agitation for 30 min.

After starch gelatinization, glycerol was added as a plasticizer (30% *w/w*); this dispersion was agitated for 5 more min. When the filmogenic solutions reached 50 °C, the control did not show any EEBO incorporated into it, while EEBO dose of (250 (EESBO), 500 (EESBO), 750 (EESBO) and 1000 (EESBO) μ L mL⁻¹) were added to the others under constant agitation for 15 min. The seeds extract from *B. orellana* was better solubilized as 200 mg EEBO 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 36 h.

2.7. Characterization of biodegradable packaging incorporating

2.7.1. Thickness

Biodegradable packaging thickness was measured by a digital caliper in millimeters (mm). Measurements were carried out in 10 spots on every packaging and the thickness mean was calculated.

2.7.2. Moisture content

The moisture content of packaging was weighed and then dried in an oven at 105 °C for 4 h. Three replicates per packaging treatment were used, in agreement described by Santos et al. (2021).

2.7.3. Measurement of water solubility

Biodegradable packaging which measured about (2 x 2 cm²) was dried in oven at 50 °C for 4 h and then weighed so that initial mass (*M_i*) could be determined. They were

immersed in 60 mL distilled water and kept under constant agitation (170 rpm) at 25 °C for 24 h.

Afterward, solutions with the packaging were filtered using filter paper (qualitative filter) with packaging had been previously weighed. Sheets of filter papers with biodegradable packaging were dried at 105 °C for 24 h and weighed so that final mass (*M_f*) could be found, in agreement with the methodology described by Jahed et al. (2017). Every treatment was analyzed in quadruplicate. Biodegradable packaging solubility in percentage (%) was calculated by equation 2.

$$\text{Water solubility (\%)} = \text{Mi} - (\text{Mf}/\text{Mi}) * 100 \quad (02)$$

2.7.3. Light transmittance (UV-Vis)

Ultraviolet (UV) and visible light (*V_{is}*) transmittance of film was conducted with the help of UV-*V_{is}* spectrophotometer. The BP samples were cut and placed in quartz cuvettes so that transmittance could be measured over a wavelength range between 900 at 200 nm as described by Hosseini et al. (2015).

2.7.4. Analysis of color

Analysis of BP color was carried out a ColorEZ digital colorimeter. Parameters under evaluation were L* (luminosity) (0- lower brightness and 100- higher brightness) and chromaticity parameters a* (green -180 and red +180) and b* (yellow +180 and blue -180). The equipment was calibrated with black crystal, green crystal and white crystal. Measurements were conducted on three randomly selected packaging spots.

2.8. Biodegradable activity

The soil biodegradability test was performed using by the methodology described by Martucci; Ruseckaite (2009). The BP samples (2 x 2 cm²) were dried up to constant weight so that initial mass (*M_i*) could be determined.

Samples were then placed in open polyethylene packaging to enable microorganisms and moisture to gain access to them. Now, these were buried in previously prepared organic soil (red dystroferric soil type) at constant moisture and room temperature. Thirty days after the experiment installment, the biodegradable packages with the samples were removed from the soil, cleaned with a fine brush and dried up to constant weight (*M_f*). Biodegradability in percentage (%) was calculated by equation 3.

$$\text{Biodegradability (\%)} = \text{Mf} - (\text{Mf}/\text{M}_i) * 100 \quad (03)$$

2.9. Antifungal assay

Antifungal activity of BP was analyzed against the phytopathogenic fungi *Sclerotinia sclerotiorum*, *Colletotrichum acutatum* and *C. gloeosporioides* by a disk diffusion assay. *Petri* dishes, half full with potato, dextrose and agar medium (PDA), were inoculated with 100 µL suspension with 10⁷ CFU mL⁻¹.

Then, four samples of packaging which had been cut in circles of about 7 mm were placed on every dish. *Petri* dishes were incubated at 25 °C for 15 days. As standard fungicide Frownicide 500 SC concentration 10 µL mL⁻¹ was used. Finally, diameters of the zone of inhibition were measured using a digital caliper and expressed in millimeters (mm),

described by Valadares et al. (2020) and Menezes Filho et al. (2022b) modified.

2.10. Statistical analysis

Analysis was carried out in triplicate and quadruplicate and the standard deviations (SD) among them were calculated. The data were collected and represented as the standard error of the mean. Following ANOVA, a Duncan test was used to examine the statistical relevance of the mean differences at *P* < 0.05, using SigmaPlot statistical software.

3. RESULTS

3.1. Phytochemical screening

A low number of groups of phytomolecules present in both extract from *B. orellana* seeds were observed (Table 1). Some phytomolecules were found in both extracts such as alkaloids, reducing sugars, flavonoids, phytosterols, carotenoids, coumarins, organic acids, aromatic compounds, proteins, hemolytic saponins, bixin and norbixin.

Table 1. Phytochemical prospection of ethanolic extract of *Bixa orellana* seeds.

Tabela 1. Prospecção fitoquímica do extrato etanólico das sementes de *Bixa orellana*.

Phytogroup	EEBO
Tannins	Blue
Alkaloids	+
Carbohydrates	-
Reducing sugars	+
No-reducing sugars	-
Glycosides	-
Cardiac glycosides	-
Amino acids	+
Flavonoids	+
Phenolics	+
Phlobatannins	+
Phytosterols	+
Cholesterol	-
Terpenoids	+
Triterpenoids	-
Diterpenes	-
Lignins	-
Carotenoids	+
Quinones	-
Anthraquinones	-
Anthocyanins	-
Carboxylic acids	-
Coumarins	+
Emodins	-
Gums & mucilages	-
Resins	-
Fixed oils & Fat	-
Volatile oils	-
Organic acids	+
Aliphatic & aromatic	Red
Polysaccharides	-
Proteins	+
Foamy saponin	-
Hemolytic saponin	+
Oxylates	-
Bixin	+
Norbixin	+

Note: EEBO: ethanolic extract of *Bixa orellana* seeds. (-) no detected. (+) positive. Blue: hydrolyzable or gallic tannins. Red: aromatic compounds. Source: Authors, 2023.

Nota: EEBO: extrato etanólico das sementes de *Bixa orellana*. (-) não detectado. (+) positivo. Azul: taninos hidrolisáveis ou gálico. Vermelho: compostos aromáticos. Fonte: Autores, 2023.

3.2. Physicochemical characteristics of packaging

The control BP exhibited the lowest values of thickness, while in the others, the higher the extract doses added to the BP, the higher their thickness values (Table 2). Moisture contents of BP depended directly on the amount of extract that was added to them (Table 2). Control BP had significantly higher in solubility due to the e -OH group presence in starch, and thereby affecting the starch BP by its hydrophilic nature. This consequently leads to higher interaction with water molecules. Increasing the extract from 250 to 1000 µL has lowered the solubility. Basically, low water solubility exhibits great water resistance (Table 2).

Concerning colors of BP under development, (Figure 1) shows decrease in light transmittance rates of BP incorporating different doses of extract in the visible and ultraviolet region (from 200 to 850nm).

Table 2. Thickness, humidity and solubility of biodegradable packages incorporated with ethanolic extract of *Bixa orellana* seeds. Tabela 2. Espessura, umidade e solubilidade das embalagens biodegradáveis incorporadas com extrato etanólico das sementes de *Bixa orellana*.

Film	Thickness (mm)	Moisture (%)	Solubility (%)
Control	0.24±0.01d	10.40±0.73c	50.43±3.30a
EESBO 250	0.26±0.01d	8,28±0.31d	40.88±3.82b
EESBO 500	0.32±0.01c	9.26±0.45cd	29.99±1.52c
EESBO 750	0.36±0.01b	12.92±1.02b	24.94±2.21c
EESBO 1000	0.43±0.01a	14.65±0.31a	22.95±4.48d

Note: Different letters in the column show statistical difference by the Duncan's test ($P < 0.05$) significance.

Nota: Letras diferentes na coluna apresentam diferença estatística pelo teste de Duncan com ($P < 0,05$) de significância.

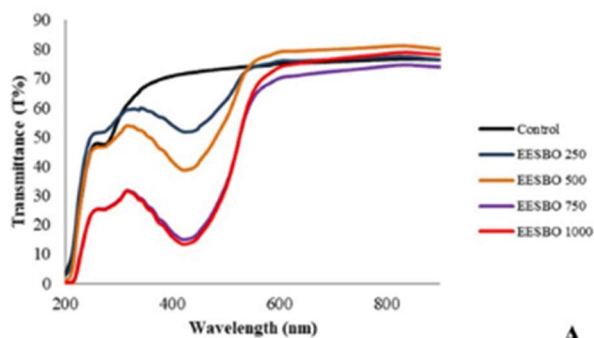


Figure 1. Transmission of UV-Vis light in biodegradable packaging incorporated with ethanolic extract from *Bixa orellana* seeds. Source: Authors, 2023.

Figura 1. Transmissão de luz UV-Vis em embalagens biodegradáveis incorporadas com extrato etanólico das sementes de *Bixa orellana*. Fonte: Autores, 2023.

Results showed that the use of extract increased BP luminosity. The highest extract concentrations showed the best luminosity results, being superior to the standard BP. The control BP exhibited negative values of a* and b* chromaticity and was green/blue. Biodegradable packaging incorporating from 250 to 1000 µL extract exhibited positive values of a* and b* chromaticity and were red and yellow (Table 2).

3.3. Biodegradable activity

The lowest concentrations 500 and 250 µL mL⁻¹ showed the highest biodegradation capacity, showing no statistical

difference with the control (Figure 2). Although they have shown potential, higher concentrations verified suggest that they are toxic because they reduce biodegradability and statistically longer time in the soil.

Table 2. Color parameters of biodegradable packages incorporated with ethanolic extract from *Bixa orellana* seeds.

Tabela 2. Parâmetros de cor das embalagens biodegradáveis incorporadas com extrato etanólico das sementes de *Bixa orellana*.

Film	L*	a*	b*
Control	14.59±0.24e	-0.71±0.09e	-0.91±0.05e
EESBO 250	17.41±0.11c	0.34±0.09d	9.03±0.13c
EESBO 500	15.48±0.02d	0.94±0.01c	10.19±0.05b
EESBO 750	19.67±0.03a	1.65±0.03b	8.29±0.03d
EESBO 1000	19.11±0.04b	3.53±0.01a	11.95±0.09a

Note: Different letters in the column show statistical difference by the Duncan's test with 5% significance. Source: Authors, 2023.

Nota: Letras diferentes na coluna apresentam diferença estatística pelo teste de Duncan com 5% de significância. Fonte: Autores, 2023.

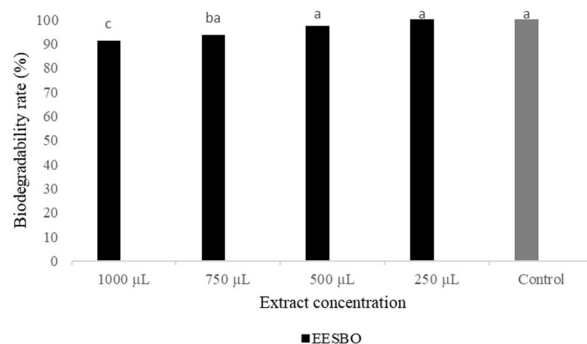


Figure 2. Biodegradability test in soil *in natura* in packages incorporated with ethanolic extract of *Bixa orellana* seeds. Note: Different letters in the column show statistical difference by the Duncan's test ($P < 0.05$) significance.

Figura 2. Ensaio de biodegradabilidade em solo *in natura* em embalagens incorporadas com extrato etanólico das sementes de *Bixa orellana*. Nota: Letras diferentes na coluna apresentam diferença pelo teste de Duncan ($P < 0,05$) de significância.

3.4. Antifungal activity

The *B. orellana* seed extract incorporated into the BP exhibited good antifungal potential on the three evaluated strains. For *S. sclerotiorum*, no significant difference ($P < 0.05$) was observed between the 1000 and 750 µL concentrations with 58% inhibition. The same was observed for *C. gloeosporioides* with inhibition above 25%. For *C. acutatum* growth inhibition activity was above 30%.

Table 4. Antifungal activity of biodegradable packaging incorporated with ethanolic extract of *Bixa orellana* seeds.

Tabela 4. Atividade antifúngica das embalagens biodegradáveis incorporadas com extrato etanólico das sementes de *Bixa orellana*.

Strain	Inhibition Zone (%)			
	1000	750	500	250
1	58.75b	55.95b	32.83c	19.82d
2	31.91b	29.67bc	27.93c	22.57d
3	26.31b	23.92b	19.54c	15.62d

1 = *Sclerotinia sclerotiorum*. 2 = *Colletotrichum acutatum*. 3 = *Colletotrichum gloeosporioides*. Standard 100a. Control 0.00e.

Note: Different letters in the line show statistical difference by the Duncan's test ($P < 0.05$) significance.

Nota: Letras diferentes na linha mostram diferença estatística pela significância do teste de Duncan ($P < 0,05$).

4. DISCUSSION

The diversity of plants with a formidable source of active compounds is extremely high (COSTA; FREIRE, 2018; MENEZES FILHO et al., 2023). Here, our plant *B. orellana* exhibit this potential through many of its group having important phytomolecules for several sectors such as pharmaceutical, agricultural and food. Our findings corroborate the study by Santos et al. (2022) where they found results similar to ours for the phytochemistry of *B. orellana* seed extract, although our results were more complex for other phytochemical groups. According to Gutierrez-del-Rio et al. (2021) and Stoll et al. (2023), some phytochemical groups such as terpenoids (monoterpenes or carotenoids) such as bixin and norbixin present in *B. orellana* seeds have antioxidant action. Carotenoids can eliminate reactive species of oxygen, nitrogen, superoxide, hydroxyl or peroxy radicals. In addition, when the extract is incorporated into the polymeric matrix or directly into the product, it conserves meat and other products by preventing lipid oxidation (PACHECO et al., 2018).

Polymeric matrices based on natural starch have high specificity, a characteristic of great importance in incorporating extracts, essential oils, resinous oil, fixed oil, among others. Among the various characteristics, we can mention the physicochemical parameters such as the intramolecular arrangement that undergoes changes, which are positive mainly in the physicochemical parameters. Increase in BP thickness were observed, and this may be related to conformational changes in the starch chain by the addition of *B. orellana* seed extract. Package thickness is an important parameter that affects mechanical properties and permeability to oxygen, carbon dioxide and water vapour. In addition, the drying conditions and preparation methods are factors that also influence the thickness of the packaging and that must be verified (HOSSEINI et al., 2015).

Furthermore, denser BP were observed in this study due to a greater distribution of carotenoids in the starch polymeric matrix, retaining more moisture. It was observed that *B. orellana* extract induces a high sensitivity to moisture absorption, which can also affect the mechanical and thermal properties of BP (THAKUR et al., 2019).

Although, we observed an increase in moisture content; the high concentration of carotenoids (bixin or norbixin) in the extract can form hydrogen bonds within the starch carbohydrate chain. Thus, this limits the bonding of the hydrogen group with water. This can result in BP starch with extract to not completely solubilized due to lack of affinity for water molecules. It was observed that with increasing concentration the solubility decreases. In the same sense Nor Adilah et al. (2018) found lower solubility with the addition of increasing concentrations of carotenoid-rich mango extract.

Regarding the colors of the biodegradable packaging developments, the transmission scan shows a decrease in the light transmission rates of BP incorporating different doses of EEBO in the ultraviolet and visible regions (from 200 to 850 nm). Our packages visually showed an increasing reddish tone as a higher concentration of extract was added. Additionally, luminosity increased as higher extract concentrations were added. Chroma a^* tended to reddish tones and chroma b^* to yellow. Similar results were observed

by Santos et al. (2020) for packaging incorporated with *Capsicum chinense* extract using arrowroot as a polymeric matrix.

Color is an important parameter to be evaluated, as it influences product acceptance (ROMANI et al., 2018; RAMBABU et al., 2019; SANTOS et al., 2022). The biodegradable packaging used is usually transparent so that the product can be seen. However, colored and opaque packaging helps to protect foods exposed to UV-Vis light, especially in foods with a high fat content, such as meat (beef, pork, fish), thus preventing them from suffering oxidative degradation (PRIYADARSHI et al., 2021; SANTOS et al., 2022).

Packaging must have a short degradation time, thus avoiding large volumes in landfills. That is why during the development of a new package the biodegradability time must be observed. In our study, the biodegradability of the packages in soil in nature was performed to determine their degradation time and confirm their potential as alternatives to replace synthetic polymers, which require a long time for degradation; greater than 500-1000 years. Biodegradation is defined as the loss of mechanical properties, fragmentation or chemical modifications due to the action of microorganisms and enzymes present in the natural microbiota of the soil or beach sand. According to Filipini et al. (2020) biodegradability is influenced by the presence of gases, humidity, light, temperature and natural biota.

Our biodegradability time is similar to studies that include plant extracts in starch polymeric matrix or similar (RIAZ et al., 2020). We noticed that higher concentrations of extract become toxic to soil microorganisms, as we found that there was a decrease in the percentage of biodegradability. Antifungal and antibacterial activity is reported for *B. orellana* extract in different extraction solvents, Jena; Bhatnagar (2021) found inhibition activity on *Aspergillus flavus* and *Aspergillus niger* for *B. orellana* extracts produced from *n*-hexane solvents, chloroform, ethyl acetate and methanol; Majolo et al. (2013) observed that the hydroethanolic extract of *B. orellana* showed intense antibacterial activity on *Salmonella* serovar *Enteritidis*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes*, and on *Candida albicans* by the ethanolic extract by Poma-Castillo et al. (2019).

The antifungal effect is one of the bioactive characteristics that enhance the use of a new active packaging capable of inhibiting the development of deteriorating microorganisms. Our results were encouraging and are similar to other studies with extracts incorporated into a natural polymeric matrix (FILIPINI et al., 2020; DEY et al., 2021).

5. CONCLUSIONS

The phytochemical study showed richness in several important groups that present biological activities, including antifungal. The interaction between the *Bixa orellana* seed extract and the arrowroot polymeric matrix showed potential for the development of new biodegradable active packaging, this statement was observed by the good results on the physicochemical characteristics, high biodegradability and antifungal potential on food phytopathogens.

Due to the satisfactory results obtained, new studies can evaluate mechanical characteristics, in vitro and in vivo, and

crystalline morphology of packages produced with the ethanolic extract of *Bixa orellana* seeds.

6. REFERENCES

- COSTA, R. M. C.; FREIRE, A. L. O. Efeito alelopático de extratos aquosos de *Prosopis juliflora* (SW.) DC. na emergência e no crescimento inicial de plântulas de *Mimosa tenuiflora* (Willd.) Piret. **Nativa**, v. 6, n. 2, p. 139-146, 2018. <https://doi.org/10.31413/nativa.v6i2.4768>
- DEY, D.; DHARINI, V.; SELVAM, S. P.; SADIKU, E. R.; KUMAR, M. M.; JAYARAMUDU, J.; GUPTA, U. N. Physical, antifungal, and biodegradable properties of cellulose nanocrystals and chitosan nanoparticles for food packaging application. **Material Today: Proceedings**, v. 38, part. 2, p. 860-869, 2021. <https://doi.org/10.1016/j.matpr.2020.04.885>
- FILIPINI, G. S.; ROMANI, V. P.; MARTINS, V. G. Biodegradable and active-intelligent films based on methylcellulose and jambolão (*Syzygium cumini*) skins extract for food packaging. **Food Hydrocolloids**, v. 109, e106139, 2020. <https://doi.org/10.1016/j.foodhyd.2020.106139>
- GUTIERREZ-DEL-RIO, I.; LOPEZ-IBANEZ, S.; MAGADAN-CORPAS, P.; FERNANDEZ-CALLEJA, L.; PEREZ-VALERO, A.; TUNON-GRANDA, M.; LOMBO, F. Terpenoids and polyphenols as natural antioxidant agents in food preservation. **Antioxidants**, v. 10, n. 8, e1264, 2021. <https://doi.org/10.3390/antiox10081264>
- HIRKO, B.; GETU, A. *Bixa orellana* (Annatto Bixa): a review on use, structure, extraction methods and analysis. **Journal of Agronomy, Technology and Engineering Management**, v. 5, n. 1, p. 687-696, 2022.
- JENA, S.; BHATNAGAR, S. Antifungal activity of *Bixa orellana* extract against *Aspergillus flavus* and *Aspergillus niger*. **World Journal of Pharmaceutical Research**, v. 10, n. 14, p. 1148-1154, 2021. <https://doi.org/10.20959/wjpr202114-22366>
- LUIGGI, F. G.; PACHECO, P. D. G.; RACANICCI, A. M. C.; MUYNARSK, E. S. M.; FASANARO, R.; SARTORI, J. R. Uso de bixina na dieta de frangos de corte e seus efeitos no desempenho zootécnico e qualidade da carne. **Archives of Veterinary Science**, v. 25, n. 1, p. 95-108, 2020.
- MAJOLO, C.; CARVALHO, H. H.; WIEST, J. M. Atividade antibacteriana “in vitro” de diferentes acessos de urucum (*Bixa orellana* L.) e sua relação com o teor de bixina presente nas sementes. **Boletim CEPPA**, v. 31, n. 1, p. 115-124, 2013.
- MENEZES FILHO, A. C. P.; VENTURA, M. V. A.; ALVES, I.; CASTRO, C. F. S.; SOARES, F. A. L.; TEIXEIRA, M. B. Extratos florais do domínio cerrado e inibição da acetilcolinesterase. **Nativa**, v. 11, n. 2, p. 207-211, 2023. <https://doi.org/10.31413/nat.v11i2.14555>
- NAGAMANI, S.; GNANASOUNDARI, A.; THANGAMATHI, P. Phytochemical analysis of *Bixa orellana* seed extract. **International Journal of Species**, v. 13, n. 41, p. 58-67, 2015.
- NOR ADILAH, A.; JAMILAH, B.; NORANIZAN, M. A.; NUR HANANI, Z. A. Utilization of mango peel extracts on the biodegradable films for active packaging. **Food Packaging and Shelf Life**, v. 16, p. 1-7, 2018. <https://doi.org/10.1016/j.fpsl.2018.01.006>
- PACHECO, S. D. G.; GARCIA, C. E. R.; ARLORIO, M.; WAGNER, R.; STRAPASSON, G.; MASSON, M. L.; OCAMPOS, F.; BARISON, A.; PASQUINI-NETTO, H.; DECASTELLI, L.; BELLIO, A.; MIGUEL, O. G. Antioxidant and effects of processing using bixin potassium salt as a nitrite replacement in restructured meat products. **Visão Acadêmica**, v. 19, n. 2, p. 4-23, 2018.
- POMA-CASTILLO, L.; ESPINOZA-POMA, M.; MAURICIO, F.; MAURICIO-VILCHEZ, C.; ALVÍTEZ-TEMOCHE, D.; MAYTA-TOVALINO, F. Antifungal Activity of ethanol-extracted *Bixa orellana* (L.) (Achiote) on *Candida albicans*, at six different concentrations. **The Journal of Contemporary Dental Practice**, v. 20, n. 10, p. 1159-1163, 2019. <https://doi.org/10.5005/jp-journals-10024-2672>
- PRIYADARSHI, R.; KIM, S.-M.; RHIM, J.-W. Carboxymethyl cellulose-based multifunctional film combined with zinc oxide nanoparticles and grape seed extract for the preservation of high-fat meat products. **Sustainable Material and Technologies**, v. 29, e00325, 2021. <https://doi.org/10.1016/j.susmat.2021.e00325>
- RAMBABU, K.; BHARATH, G.; BANAT, F.; SHOW, P. L.; COCOLETZI, H. H. Mango leaf extract incorporated chitosan antioxidant film for active food packaging. **International Journal of Biological Macromolecules**, v. 126, p. 1234-1243, 2019. <http://dx.doi.org/10.1016/j.ijbiomac.2018.12.196>
- ROMANI, V. P.; HERNÁNDEZ, C. P.; MARTINS, V. G. Pink pepper phenolic compounds incorporation in starch/protein blends and its potential to inhibit apple browning. **Food Packaging and Shelf Life**, v. 15, p. 151-158, 2018. <http://dx.doi.org/10.1016/j.fpsl.2018.01.003>
- RIAZ, A.; LAGNIKA, C.; LUO, H.; DAI, Z.; NIE, M.; HASHIM, M. M.; LIU, C.; SONG, J.; LI, D. Chitosan-based biodegradable active food packaging film containing chinese chive (*Allium tuberosum*) root extract for food application. **International Journal of Biological Macromolecules**, v. 150, p. 595-604, 2020. <https://doi.org/10.1016/j.ijbiomac.2020.02.078>
- SANTOS, R. S.; MENEZES FILHO, A. C. P.; BATISTA-VENTURA, H. R. F.; CASTRO, C. F. S.; VENTURA, M. V. A. Prospecção fitoquímica, teor de bixina e atividade alelopática de extratos de *Bixa orellana* L. **Brazilian Journal of Science**, v. 1, n. 12, p. 96-107, 2022. <https://doi.org/10.14295/bjs.v1i12.243>
- SANTOS, L. S.; FERNANDES, C. C.; SANTOS, L. S.; DEUS, I. P. B.; SOUSA, T. L.; MIRANDA, M. L. D. Ethanolic extract from *Capsicum chinense* Jacq. Ripe fruits: phenolic compounds, antioxidant activity and development of biodegradable films. **Food Science and Technology**, v. 41, n. 2, p. 497-504, 2021. <https://doi.org/10.1590/fst.08220>
- SHAIKH, S.; YAQOOB, M.; AGGARWAL, P. An overview of biodegradable packaging in food industry. **Current Research in Food Science**, v. 4, p. 503-520, 2021. <https://doi.org/10.1016/j.crfs.2021.07.005>
- STOLL, L.; MAILLARD, M.-N.; LE ROUX, E.; FLÔRES, S. H.; NACHTIGALL, S. M. B.; RIOS, A.; DOMENEK, S. Bixin, a performing natural antioxidant in active food packaging for the protection of oxidation sensitive food.

LWT Food Science and Technology, v. 180, e114730, 2023. <https://doi.org/10.1016/j.lwt.2023.114730>
TONGNUANCHAN, P.; BENJAKUL, S.; PRODPRAN, T. Structural, morphological and thermal behaviour characterisations of fish gelatin film incorporated with *basil* and *citronella essential oils* as affected by surfactants. **Food Hydrocolloids**, v. 41, p. 33-43, 2014. <https://doi.org/10.1016/j.foodhyd.2014.03.015>

Acknowledgments: We thank Goiano Federal Institute, Rio Verde, Goiás, Brazil; to the Technological Chemistry, Plant Products Post-Harvest and Water and Effluent Laboratories, and to the Master's and Doctorate in Agrochemistry.

Author Contributions: L.A.J. - oriented, writing (original draft), investigation and data collection and final writing; A.C.P.M.F. - co-advisor, conceptualization, study design, methodology, submission, final corrections and publication; P.S. - Experimental validation and translation; C.F.S.C., M.B.T. and F.A.L.S. - acquisition of funding; M.V.A.V. - advisor, conceptualization, study design, supervision, statistical analysis. All authors read and agreed to the published version of the manuscript.

Funding: Goiás Research Support Foundation (FAPEG) for the doctoral scholarship for the second author; National Council for Scientific and Technological Development (CNPq); Financier of Studies and Projects (FINEP), and Higher Education Personnel Improvement Coordination (CAPES).

Institutional Review Board Statement: *Not applicable.*

Informed Consent Statement (Ethics Committee of the area): *Not applicable.*

Data Availability Statement: Study data can be obtained by request to the corresponding the second author, via e-mail.

Conflicts of Interest: There are no conflicts of interest between the authors. Supporting entities had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.