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Bixin, a performing natural antioxidant in active food packaging for the protection of oxidation sensitive food

Liana Stoll [a], Marie-Noelle Maillard [b], Even Le Roux [b], Simone Hickmann Flôres [a], Sonia Marli B. Nachtigall [c], Alessandro Rios [a], Sandra Domenek [b,*]

[a] Institute of Food Sciences and Technology, Federal University of Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, Prédio 43.212, Campus do Vale, Porto Alegre, RS, CEP 91501-970, Brazil
[b] Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 91120, Palaiseau, France
[c] Institute of Chemistry, Federal University of Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, Prédio 43.131, Campus do Vale, Porto Alegre, RS, CEP 91501-970, Brazil

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ABSTRACT

Natural antioxidants used in active packaging are an effective food technology to preserve oxygen sensitive foodstuff. Bixin, a carotenoid with coloring and antioxidant properties extracted from annatto seeds, was included in plasticized or not poly(lactide) (PLA) films using melt processing at 1 g/kg to increase the light barrier of the packaging and to deliver antioxidants to the food matrix. The melt processing caused degradation of bixin to more than 75%. The degradation products were analyzed using liquid chromatography coupled to mass spectrometry. The principal degradation process was *cis/trans*-isomerization. Despite this important structural change satisfactory barrier properties to UVA and UVB light were conserved in PLA films. Analysis of the protective performance of the packaging materials showed that the shelf-life of the vitamin riboflavin could be increased, and the augmentation of the peroxide values in sunflower oil slowed down. Bixin is therefore a very promising natural antioxidant, which could be used in packaging materials requiring melt processing.

1. Introduction

Packaging facilitates the handling, preservation, and distribution of food. It is a connecting link between production and consumption in urbanized societies, because most food products are consumed far away from their production sites and the moment of consumption is shifted in time with respect to the moment of harvesting. The packaging industry accounts for around 40% of the annual volume of plastics production, with approximately 60% applied in the food and beverages sector. The sustainable management of plastics waste is a challenge for producers and users of packaging. Recycling is one of the main strategies to reduce the environmental impact of plastics, but it requires an important investment in the waste treatment infrastructure, which cannot be afforded everywhere. Moreover, the use of recycled materials in food packaging is a challenge for food safety, because open recycling streams can be a source of contamination of packed products (Geueke, Groh, & Muncke, 2018). The use of biodegradable materials for food packaging represents still a promising alternative to reduce the investment and treatment costs of waste management. Biodegradable and biobased polymers are also an opportunity to include by-products of the agriculture and food industry in the production of packaging materials. This could increase on the one hand the part of renewable materials and decrease on the other hand the waste generation of the sector (Gutierrez-del-Rio et al., 2021; Nemes, Szabo, & Vodnar, 2020). Active packaging materials, which can contain substances such as antimicrobials, antioxidants, light blockers, or oxygen barriers, can help to increase the shelf-life of food products. These substances can be delivered or not to the food (Khezerlou et al., 2021; Sani, Azizi-Lalabadi, Tavassoli, Mohammadi, & McClements, 2021). They are regulated by specific regulations, such as EC 450/2009 on active and intelligent food packaging (European Community). Poly(lactide) (PLA) is a biopolymer produced from renewable sources such as corn starch. It is currently the second most produced biodegradable material (European_Bioplastics, April 2022). PLA can be blended with other resins and compounded with different fillers, such as fibers, or micro- and nanoparticles to optimize its properties for a given application (Faraj et al., 2021; Sadeghi et al., 2022). Phytochemicals such as terpenoids (for example monoterpenes and carotenoids) or polyphenols (quercetin, other flavonoids) are plant

* Corresponding author.
E-mail address: sandra.domenek@agroparistech.fr (S. Domenek).

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antioxidants largely found in fruits and vegetables. They are able to scavenge reactive oxygen and nitrogen species, such as superoxide, hydroxyl or peroxyl radicals. This reactivity can be used to preserve meat and other products by the prevention of the oxidation of lipids (Gutierrez-del-Rio et al., 2021). The development of active packaging using PLA is an active field of research and innovation (Garcia-Arroyo et al., 2020; Rojas et al., 2021; Tambawala, Batra, Shirapure, & More, 2022). The incorporation of carotenoids into PLA was shown to improve the light and oxygen barrier properties of films, which increased the protection of light or oxygen-sensitive foods (Stoll, Rech, Flôres, Nachtigall, & de Oliveira Rios, 2018). In particular, PLA films including the carotenoid bixin presented the best performance in the protection of sunflower oil from oxidation in comparison to beta-carotene and lycopene (Stoll et al., 2018). Bixin is a carotenoid abundant in annatto seeds (*Bixa orellana* L.), which are cultivated in tropical regions and mainly used as a natural colorant in the food and cosmetics industry (Rahmalia, Fabre, & Mouloungui, 2015). Bixin could be a promising substance for the development of active packaging, where its migration to the packed food could extend food shelf life by reducing oxidative reactions (Pagno, de Farias, Costa, Rios, & Flôres, 2016; Stoll, Rech, Flôres, Nachtigall, & de Oliveira Rios, 2019).

Polymer packaging materials are generally produced by melt processing, which implies the heating above the melting temperature of the polymer and the mixing of the additives with the polymer in the molten state. Therefore, natural antioxidants will be necessarily exposed to high temperatures and shear forces. Typical processes temperatures of PLA lie between 160 and 220 °C (Lim, Auras, & Rubino, 2008). As a consequence, there is a need to investigate the stability of additives during PLA processing in the aim to design performing systems able to integrate conventional processes. Bixin undergoes thermal degradation at temperatures higher than 70 °C (Scotter, 2009). A series of complex reactions leads to the oxidation and *cis/trans* isomerization. The main substance 9-*cis*-bixin can be transformed to *trans*-isomers and mono -and di-*cis* isomers (Scotter, Castle, & Appleton, 2001). The rapid degradation of bixin in vegetable oil at 160 °C was reported after only 2 min, while in biscuit dough bixin was stable at 150 °C for 30 min (Prabhakara Rao, Jyothirmayi, Balaswamy, Satyanarayana, & Rao, 2005). Encapsulation of bixin also increased the thermal stability (Khezerlou et al., 2021). This shows that matrix of inclusion is important for avoiding thermal degradation of bixin. In our previous paper, we produced PLA/bixin films plasticized or not with acetyl tributyl citrate (ATBC) using a thermal treatment mimicking the polymer processing temperature. As expected, the heat treatment led to the degradation of bixin. The bixin loss was characterized using UV-VIS spectrophotometry. ATBC did not help to stabilize the bixin in PLA. Surprisingly, despite that more than 74% of bixin degraded during the heat treatment, the films still blocked the transmission of up to 95% of UVA and 90% of UVB light (Stoll, Domenek, Flores, Nachtigall, & Rios, 2021). The isomerization of the bixin might therefore not be, in itself, detrimental for the use properties. The measurement of the bixin concentration by UV-VIS spectrophotometry gives however only a global information about the decrease of the adsorption linked to 9-*cis*-bixin. Liquid chromatography linked to mass spectroscopy is a specific method allowing to separate the different derivatives of bixin and to analyze their structure. In the present paper, we used this technology in the aim to better understand the relationship between the degradation of bixin caused by heat treatment and its anti-UV and antioxidant activity in PLA films. The performance of heat-treated PLA/bixin films in the preservation of food was investigated using riboflavin and sun flower oil.

2. Materials and methods

2.1. Materials

Polylactide (PLA) of extrusion grade "PLE001" containing at least 96% L-lactic acid was supplied from NaturePlast (France). We name our material polylactide because PLA is generally obtained by the polymerization of lactide. In some papers the same polymer is named poly (lactic acid), because the inital building block of lactide is lactic acid. Acetyl tributyl citrate (ATBC) was supplied by Sigma-Aldrich (France). Annatto seeds were acquired from the Ivory Coast. All chemicals used were of analytical grade. Formic acid and acetonitrile were supplied from Carlo Erba (France) in HPLC quality.

2.2. Bixin extraction

Annatto seeds were washed with hexane and methanol, and bixin was extracted from the seeds by mechanical stirring in CH_2Cl_2 for 30 min at ambient temperature in the dark. The purity of the bixin extracts (>97%) was confirmed by Ultra High Performance Liquid Chromatography (UHPLC) and the quantity of bixin in solution was measured with a UV-VIS spectrophotometer (see below).

2.3. Fabrication of PLA/bixin films

Film fabrication by casting. PLA pellets were dissolved in CH_2Cl_2 (50 g/L) at room temperature (RT). The bixin extract (10 g/kg or 1 g/kg in dry weight of film) and ATBC (130 g/kg in dry weight) were added to the PLA solution and the mixture was poured into Teflon Petri dishes at a leveled surface for evaporation of the solvent under the hood overnight. The resulting films were placed in an oven with forced air circulation at 40 °C for 6 h. The formulations of all samples are summarized in Table 1. The samples were stored in a desiccator over P_2O_5 in the dark.

Melt-mixing procedure. The melt mixing of the PLA formulations was performed in a HAAKE™ Rheomix Internal Mixer at 160 °C with 50 rpm speed. The dry PLA pellets were manually mixed with the plasticizer ATBC and then introduced in the internal mixer. After 2 min processing, cut pieces of PLA/bixin films (10 g/kg bixin), which were produced by casting, were added to the molten PLA and the formulation was mixed 2 more min. The final theoretical bixin content was 1 g/kg. The material was ground under liquid nitrogen to a coarse powder.

Production of PLA/bixin films. Films were fabricated by thermal pressing using the films obtained by the casting procedure or the powder obtained by the melt mixing. The materials were sandwiched between two Teflon sheets and two steel plates and pressed at 160 °C and 5 tons for 4 min (Table 1) with the help of a thermal press Darragon (France). The thermal treatment allowed cancelling out the morphological differences which were induced by the different processing methods. The films were stored in a desiccator at RT in the dark. The thermal treatments are summarized in Table 1.

2.4. Thermal analysis of PLA films

The analyses of the crystallinity degree (χ_c) and the glass transition

Table 1
Overview of PLA/ATBC/bixin film formulations and film fabrication methods.

Sample name	Processing	Film formulation			Bixin heat treatment	
		PLA (g)	ATBC (g)	Bixin (g)	Melt mixing (min)	Heating press (min)
Cast.	Cast	100	–	–	–	6
Cast. P	Cast	87	13	–	–	6
Cast.Bix	Cast	100	–	0.1	–	6
Cast.Bix. P	Cast	87	13	0.1	–	6
MP	MP	100	–	–	2	4
MP.P	MP	87	13	–	2	4
MP.Bix	MP	100	–	0.1	2	4
MP.Bix.P	MP	87	13	0.1	2	4

Abbreviations: Cast - film produced by casting; MP - film produced by melt processing; P – film with plasticizer; Bix - films with bixin.

temperature (T_g) were carried out using a Q100 DSC (TA Instruments, France) equipped with an intercooler. An indium standard was used for equipment calibration. The film samples (3–5 mg, loaded in TZero aluminum crucibles) were analyzed using a heat-cool-heat cycle from 10 °C–180 °C with a rate of 10 °C.min^{-1} under N_2 flow (50 mL min^{-1}). The glass transition temperature (T_g) was measured at the second heating scan and χ_c calculated from the data of the first heating scan:

$$\chi_c = \frac{\Delta H_{m-} \Delta H_{cc}}{\Delta H_f (1-x)} \times 100, \quad (1)$$

where ΔH_m is the enthalpy of melting, ΔH_{cc} is the enthalpy of cold crystallization, ΔH_f is the melting enthalpy of 100% crystalline PLA (93.7 J g^{-1}), and x is the concentration of carotenoids and plasticizer in the film. All analyses were carried out in duplicate.

2.5. Oxygen permeability of PLA films

The oxygen permeability of the films was determined using the manometric method thanks to a permeability testing apparatus (GDP-C permeameter, Brugger Feinmechanik GmbH, Munich, Germany) at 23 °C and 0% relative humidity and a pressure gradient of 1 atm. The films were outgassed *in situ* for 15 h under vacuum. The oxygen Permeability (P) was computed from the O_2 flow at steady state by correcting the value with the film thickness. The film thickness was measured at minimum 5 random points with a Digimatic micrometer (Mitutoyo, Japan). All permeability measurements were done in duplicate.

2.6. UV-light transmission of PLA films

The UV-A and UV-B light barrier properties of the films were measured against air in triplicate by placing the samples in a quartz test cell. The light transmission was measured during the scan from 200 to 800 nm in the UV–Vis spectrophotometer (Jasco V-750, Japan). The results were expressed as the percentage of transmittance.

2.7. Bixin release kinetics from PLA films

The bixin release to the food simulant was evaluated following the methodology of (Stoll et al., 2018, 2019). The solvent was ethanol/H_2O 95/5 (v/v), which is approved as a simulant for fatty foods by the EC regulation No 10/2011.20 mg (~1 cm^2) of film were immersed in 6 mL in 12 mL amber flasks. The flasks were sealed with plastic caps and stored at 40 °C for 400 h (16 days) in the dark. The flasks were shaken regularly to minimize the mass transfer resistance. The overall mass transfer coefficient (K) was obtained through the following equation (Stoll et al., 2021):

$$\frac{dC}{dt} = K\frac{A}{V}(C^* - C) - k_d C, \quad (2)$$

where C is the carotenoid concentration in the food simulant (μg.mL^{-1}), A is the surface area of the film in contact with the liquid (0.003 m^2), V is the volume of the food simulant solution (10^{-5}.m^3), C^* is the carotenoid concentration (μg.mL^{-1}) in the inner part of the film, and k_d is the first-order kinetic degradation coefficient of bixin in ethanol 95 vol% (2.155.10^{-7} s^{-1}).

2.8. Bixin quantification by spectrophotometry

The bixin content was evaluated following the methodology of (Stoll et al., 2021). The absorbance of annatto seed extracts or PLA samples (dissolved in CH_2Cl_2) was measured using a spectrophotometer (Jasco V-750, Japan) at λ_{max} = 470 nm (extinction coefficient = 2826) with CH_2Cl_2 as a blank. The analysis of the release kinetics was done using ethanol 95 vol% as a blank. The calibration curve of bixin in ethanol 95 vol% was established from 0.3 to 5.3 μg mL^{-1} (R^2 = 0.996). The limits of detection (LOD) and quantification (LOQ) were 0.002 and 0.008 μg mL^{-1}, respectively. The sampled volume was replaced in the flask by ethanol 95 vol%.

2.9. UHPLC- mass spectroscopy analysis of bixin and its degradation products

The identification of the compounds in the initial sample and the degradation products after heat treatment was carried out by UHPLC (Waters Acquity, France) coupled to a PDA detector and a mass spectrometer (Waters, Xevo G2-S-QTof). The analysis was carried out on the initial bixin extract in CH_2Cl_2 and on the samples of the release study. The mobile phases were phase A (0.1 vol% formic acid in water), and phase B (0.1 vol% formic acid in acetonitrile). An isocratic elution with phases A/B 25/75 (v/v) was used at a 0.6 mL min^{-1} flow rate, using an Ethylene Bridged Hybrid (BEH) C18 column (100 × 2.1 mm, 1.7 μm particle diameter, 130 Å) and a pre-column Vanguard BEH C18 (5 × 2.1 mm; 1.7 μm, Waters, St Quentin en Yvelines, France). The injection volume was 2 μL, and the auto-sampler temperature was 10 °C. The detection and quantification was done using the PDA detector (220–500 nm, resolution of 1.2 nm). The settings of the mass spectrometer were: ESI + ionisation, source parameters being a capillary 0.5 kV, sampling cone 40 V, source offset 80 V, 120 °C, desolvation 500 °C. The gas flow in the cone was 50 L h^{-1}, the desolvation gas flow 1000 L h^{-1}. The collision energy was 6 eV. The registered m/z ranged from 50 to 900 with a scan time of 0.5 s in centroid and an acquisition time of 10 min.

2.10. Protection of food and food simulants from oxidative degradation by PLA/bixin films

Protection of Riboflavin. The riboflavin stability protected or not by PLA/bixin films was measured using a validated methodology (Stancik et al., 2017). 8 mL of riboflavin solution (30 mg mL^{-1} in potassium phosphate buffer at pH 6.4) were put in open glass vials (12 mL). The glass vials were entirely coated with the PLA films. The samples were stored at 5 °C and positioned 15 cm away from a light source comprised of three white lamps (4700 ± 200 lux). Every 90 min, 0.5 mL of riboflavin solution was collected. The riboflavin content was quantified by HPLC. The test was interrupted when >95% of riboflavin was degraded.

The quantification of riboflavin was performed on an Agilent Chromatograph, 1100 series (Santa Clara, CA, USA), using a C18 ODS Phenomenex HyperCloneTM, Allcrom (120 A, 250 × 4.6 mm, 5 μm) column. A mixture of a potassium phosphate buffer pH 6.4 with dimethylformamide 85:15 (v/v) was used as mobile phase at isocratic flow (1 mL min^{-1}) at 25 °C. The injection volume was 10 μL. The detection was done by fluorescence with an excitation wavelength of 450 nm and an emission wavelength of 530 nm. The quantification was performed using a riboflavin calibration curve (R^2 = 0.99) (Schmidt et al., 2019).

Protection of the oxidative stability of sunflower oil. The capacity of MP films to protect sunflower oil from oxidation was evaluated at 40 °C in an oven in the light or in the dark. The experimental set-up is shown in Fig. 1. It consisted in a glass flask covered by printing paper with a lid formed by the PLA film. The flask contained 7 mL of sunflower oil and an immersed film of 3 cm^2. The vials were placed under white lamps (4700 ± 200 lux) at RT. The peroxide value (PV) of the oil samples was periodically determined according to the IUPAC titration method for oils, fats and derivatives oils, and the results were expressed in milli-equivalents of active oxygen per kilogram of oil (m_{eq}/kg).

3. Results and discussion

3.1. Characterization of formulated PLA films containing ATBC and bixin

Table 2 presents the main characteristics of the heat-treated PLA films. The formulation of the films was based on our previous study

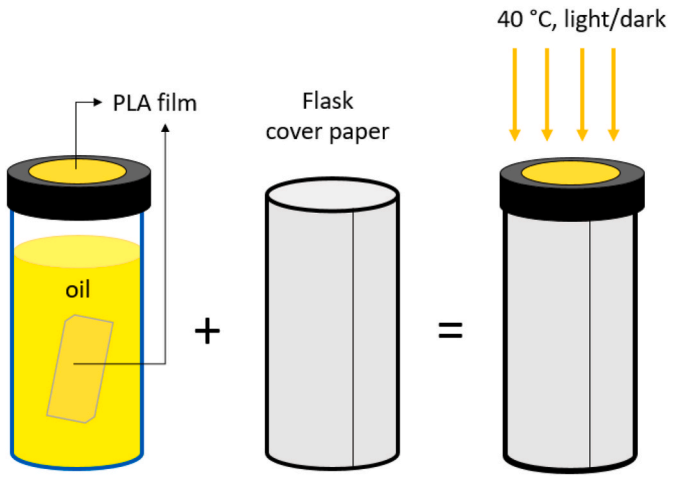

Fig. 1. Evaluation of the performance of MP films to protect the oxidative stability of sunflower oil.

Table 2
Characterization of PLA films: glass transition temperature (T_g), crystallinity degree (χ_c), oxygen permeability ($P(O_2)$), and light transmittance of UV-B (300 nm) and UV-A (380 nm) light.

	Thickness	T_g	χ_c	P (O_2)	Light transmittance	
	(μm)	(°C)	(%)	($m^3.m.m^{-2}.Pa^{-1}.s^{-1}$).$10^{-18}$	UV-B (%)	UV-A (%)
Cast	113	59 ± 1	2 ± 1	3.3 ± 0.3 ^{b}	63.1 ^{a}	73.8 ^{a}
Cast.P	128	32 ± 1	3 ± 1	5.1 ± 0.5 ^{a}	60.2 ^{a}	73.0 ^{a}
MP	200	60 ± 1	2 ± 1	3.1 ± 0.3 ^{b}	41.9 ^{b}	54.9 ^{b}
MP.P	170	38 ± 1	1 ± 1	5.0 ± 0.5 ^{a}	43.2 ^{b}	56.4 ^{b}
Cast. Bix	122	60 ± 1	2 ± 1	3.6 ± 0.3 ^{b}	10.0 ^{c}	4.4 ^{c}
Cast. Bix.P	113	37 ± 1	1 ± 1	5.0 ± 0.5 ^{a}	14.9 ^{c}	9.5 ^{c}
MP.Bix	217	59 ± 1	1 ± 1	3.0 ± 0.3 ^{b}	0.7 ^{d}	0.1 ^{d}
MP. Bix.P	166	38 ± 1	2 ± 1	3.8 ± 0.4 ^{a}	1.8 ^{d}	0.3 ^{d}

Different letters in the same column indicate significant differences ($p < 0.05$) among the films.
*Abbreviations: Cast - films produced by casting; MP - films produced by melt processing; P – film produced with plasticizer; Bix - films with bixin.

(Stoll et al., 2021) which showed that 130 g/kg of ATBC were sufficient to decrease the T_g of the polymer and yield ductile behavior. The observed T_g values (Table 2) were equal to the ones we observed before and coherent with other literature data. All samples were amorphous, which is also coherent with our previous results. The use of 1 g/kg bixin, did not impact the T_g or χ_c. The exclusion of O_2 from the packaging is an important mechanism for food preservation, because it decreases the concentration of the reactant inside the pack. The incorporation of bixin into the PLA matrix did not affect the oxygen permeability ($P(O_2)$). The $P(O_2)$ of the PLA films was in the upper bond of the literature values (Sonchaeng et al., 2018), because PLA was amorphous. The use of ATBC increased the $P(O_2)$ (Table 2), because plasticizers create new free volume in polymers and decrease the barrier properties (Ruellan, Ducruet, & Domenek, 2015). $P(O_2)$ was almost equal to the values of PLA with 170 g/kg ATBC published by (Courgneau et al., 2012). This increase is negative for the development of food packaging materials for oxygen-sensitive goods, because it increases the ingress of the reactant O_2 inside the packaging.

A second food preservation mechanism of packaging is the shielding of the food from UV light. The light barrier of bixin is due to the presence of conjugated C═C double bonds which serve as chromophore moieties and are responsible for its light absorption capacity (Morabito et al., 2012). The light barrier properties to UV-A and UV-B light are also reported in Table 2. PLA is relatively transparent to UV light and the incorporation of ATBC had no effect on the light barrier properties. The lower light transmission of MP films was caused by their higher thickness (Table 2). The UV-A and UV-B light transmittance of cast and heat-treated films formulated with 1 g/kg bixin decreased by 75% for UV-B and 95% for UV-A. The efficiency was a bit lower in the case of plasticized films, but bixin was still effective, even after a heat-treatment at 160 °C for 6 min. The efficiency was even increased when the films were produced by melt processing and thermo-compression. They blocked 98% of UV-B light and 99% of UV-A light with respect to the blank. These results are of great importance, demonstrating that the light absorbance capacity of the films was conserved, although the heat and shear forces during polymer processing caused a considerable loss of the carotenoid content.

3.2. Thermal degradation products of bixin caused by heat and shear treatment

The film production by casting aimed to evaluate the thermal stability of bixin stability, while the melt processing evaluated the stability to both, heat and shear forces. The initial bixin extracted from annatto seeds and the extract of the PLA/bixin films collected during the release study was analyzed with the help of UHPLC-MS in the aim to identify the main degradation products. The characteristics of the observed substances are detailed in Table 3 (compounds 1, 2, 3 and 4). The main degradation reaction was isomerization to *trans*-bixin. The identification of *trans*-bixin and 9′-*cis*-bixin was realized using literature data considering the major ions characteristic of bixin fragmentation, the peak retention times and absorbance maxima (Scotter, 1995). It is well known that the polyene chain is responsible for the instability of carotenoids

Table 3
UHPLC-MS annotation of the compounds found in the bixin extract and the film extracts in ethanol 95 vol%.

	Retention Time (min)	UV-VIS spectrum (nm)	MS Signal (m/z)	Identification
Compound 1	*1.22*	464 (M); 493 (m); 436 (s); 285 (m); 274(s)	[M+H]+ = 395.2211 (maj); [M•]+ = 394.2142; [M + H–H2O]+ = 377.2112; [M + H–CH3OH] = 363.1953	*trans*-bixin
Compound 2	*1.32*	455 (M); 481 (m); 430 (s); 404 (s); 285 (m); 274 (s)	small signal	bixin degradation product (tentative)
Compound 3	*1.38*	455 (M); 481 (m); 285 (m); 274 (s)	small signal	bixin degradation product (tentative)
Compound 4	*1.61*	460 (M); 485 (m); 433 (s)	[M+H]+ = 395.2207 (maj); [M•]+ = 394.2136; [M + H–H2O]+ = 377.2117; [M + H–CH3OH]+ = 363.1960	9′-*cis*-bixin

*UV-VIS peaks wavelenght information: M - maximum; m - minimum; s - shoulder.

and their susceptibility to oxidation. *Cis/trans* isomerization occurs due to various factors such as temperature and light (Scotter et al., 2001). A series of complex degradation reactions of 9′-*cis*-bixin happen at elevated temperatures which produce pale yellow to orange components (Levy & Rivadeneira, 2000). The thermal processing produced, in agreement to what is known of bixin degradation, a color change to orange or bright yellow films. Two supplementary bixin degradation products were observed, but could not be identified. Table 4 shows the estimated composition of the extracts. 9′-*cis*-bixin which is the principal coloring component of annatto was the only compound present in bixin extract in this study. Compound 1, i.e. *trans*-bixin was the main degradation product. The thermal processing of PLA films caused a substantial loss of bixin and its derivatives (Table 4). No statistical differences were found between MP films with and without plasticizer. However, the presence of ATBC increased 10% the bixin loss in cast films showing that ATBC destabilized bixin during heat-treaments, a result already observed before (Stoll et al., 2021). Shearing during melt processing had no supplementary effect on bixin degradation.

3.3. Release kinetics of bixin from heat-treated PLA/bixin films

The release of an antioxidant to the food matrix is a perservation technique used in active food packaging. The migration rate of bixin and its derivatives to the food simulant of fatty foods (ethanol/H_2O 95/5 v/v) was measured during 16 days in accelerated conditions (40 °C). The experimental results are shown in Fig. 2. The release kinetics were modelled with the model developed by (Stoll et al., 2019). The model parameters are given in Table 5. The cast films had the lowest mass transport rate. It increased in the plasticized films, because plasticizers accelerate generally the mass transport. The initial bixin release from melt processed films was 3 times faster than that from the cast non-plasticized films. Here, no difference was found between plasticized and not plasticized formulations, a result already observed in the analysis of the oxygen mass transport (Table 2). The pseudo-equilibrium value of all formulations was lower thant that of Cast.bix films. It was correlated to the relative quantity of 9-cis bixin in the film (Table 4). Diffusion is known to be the rate-controlling step in the release of active compounds. The faster release of bixin to ethanol (95 vol%) in MP films might be related to the PLA chain scission during the process, which may have facilitated bixin diffusion through the polymeric matrix. Melting processes, such as extrusion and melt mixing, result most probably in the thermo-mechanical degradation of PLA due to high temperature, presence of oxygen and shear (Oliveira et al., 2016). Furthermore, PLA is sensitive to thermo-hydrolysis (Salazar, Domenek, Plessis, & Ducruet, 2017), a degradation mechanism which could have happened during melt mixing.

3.4. Performance of PLA/bixin films in protection of food

3.4.1. Protection of the light sensitive vitamin riboflavin

The light barrier offered by the packaging material is a key factor for the preservation of food vitamins and other light-sensitive products such as fatty acids. Riboflavin is a light-sensitive micronutrient (vitamin B2)

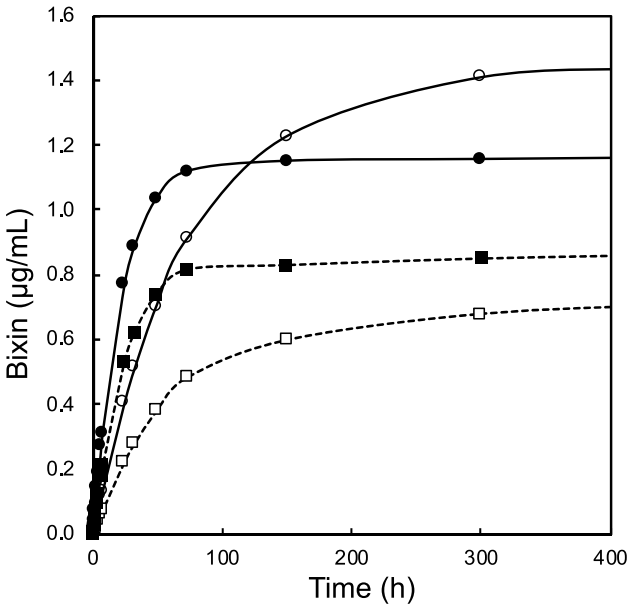

Fig. 2. Bixin migration from PLA films to ethanol 95 vol% at 40 °C. Lines are model curves using eq. (2). Symbols: ◯ Cast.Bix (film produced by casting with bixin), ● Cast.Bix.P (plasticized film produced by casting with bixin), □ MP.Bix (film produced by melt processing with bixin), ■ MP.Bix.P (plasticized film produced by melt processing with bixin).

Table 5
Bixin migration coefficients to the food simulant (ethanol 95% v/v).

	Bixin release coefficients[a]		
	K ($m.s^{-1} \times 10^{-7}$)	C* ($\mu g.mL^{-1}$)	R^2
Cast.Bix	1.8 ^{c}	1.64 a	0.98
Cast.Bix.P	3.7 ^{b}	1.03 b	0.99
MP.Bix	5.9 ^{a}	0.87 c	0.98
MP.Bix.P	5.8 ^{a}	0.99 b	0.99

[a] Bixin and bixin derivatives, quantified at 460 nm by spectrophotometry. K is the overall mass transfer coefficient (eq. (2)), C*. the bixin concentration in the inner part of the film adjusted from the model eq. (2). Cast - films produced by casting; MP - films produced by melt processing; P – film produced with plasticizer; Bix - films with bixin.

which acts as a photosensitizer. At specific wavelengths, the light energy leads to the photochemical excitation of riboflavin, promoting photochemical reactions that may lead to the degradation (Min & Boff, 2002). The photo-degradation of riboflavin protected by melt processed PLA films was recorded during 14 days (350 h). The results are shown in Fig. 3. The PLA films without bixin did not protect riboflavin against photodegradation, which was expected because of the high transparency of PLA to UV light. MP films containing bixin preserved efficiently the vitamin. After 350 h of storage under intense light exposure, the

Table 4
Composition of bixin and film extracts evaluated by UHPLC and evaluation of bixin degradation using spectrophotometry.

	Estimated composition of extract (%)				Bixin degradation[a]
	Compound 1	Compound 2	Compound 3	Compound 4	(%)
Bixin extract	0	0	0	100	–
Cast.Bix	49.9	5.0	8.0	37.2	74.9 ± 1.9 c
Cast.Bix.P	72.7	<loq	<loq	27.3	85.3 ± 0.3 a
MP.Bix	66.4	<loq	<loq	33.6	79.9 ± 1.1 b
MP.Bix.P	54.3	6.0	11.0	28.7	82.4 ± 4.5 ab

[a] Considering an initial bixin content of 1 g/kg and quantified by spectrophotometry; Different letters in the same column indicate significant differences ($p < 0.05$) among the films. Cast - films produced by casting; MP - films produced by melt processing; P – film produced with plasticizer; Bix - films with bixin.

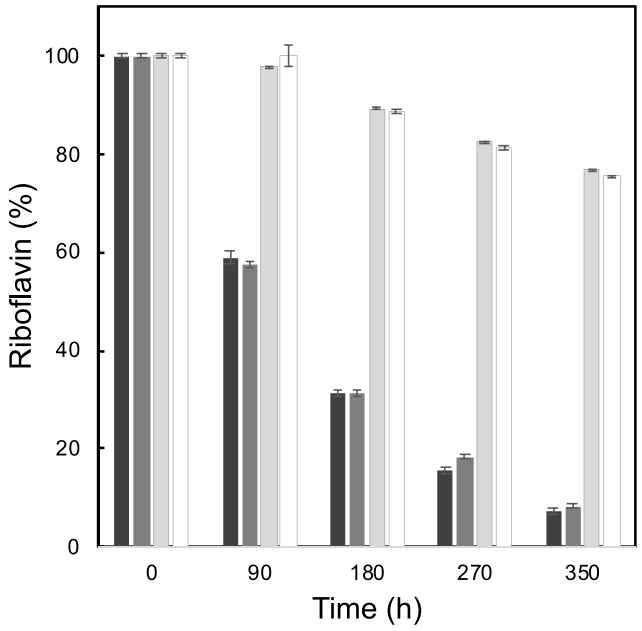

Fig. 3. Riboflavin stability under light exposure at 5 °C in a solution simulating milk prepared with 30 μg mL^{-1} riboflavin and covered by PLA/ATBC/bixin films. Symbols: from the left to the right: ■ MP (film produced by melt processing – control sample),■ MP.P (plasticized film produced by melt processing – control sample), MP.Bix (film produced by melt processing with bixin), MP.Bix.P (plasticized film produced by melt processing with bixin).

solutions covered by MP.Bix and MP.Bix.P preserved nearly 80% of the riboflavin (Fig. 3). This shows that the degradation products of bixin, and in particular *trans*-bixin (Table 3), remain efficient for the protection of photo-oxidation sensible goods.

3.4.2. *Preservation of oxidation sensitive sun flower oil*

The efficiency of the protection of food against oxidative damage was explored by combining the effects of light protection, oxygen barrier properties and release of bixin in the foodstuff using the experimental setup shown in Fig. 1. The bottles were stored either in the dark, to check the effect of bixin release, or in the light, to check the combined effect of light barrier and bixin release. According to the results shown in Fig. 4a, PLA films (MP) were not efficient in protecting sunflower oil during storage in the dark. The use of the plasticizer caused a supplementary increase of the peroxide concentration. The oil packed in PLA (MP and MP.P) presented levels of peroxides after 14 days which exceeded the limit of commercialization established by the Codex Alimentarius. The migration of bixin into the oil from MP.Bix and MP.Bix.P decreased the peroxide values, demonstrating that bixin had a protective action compared to the control films after 5 days.

The rapid increase of peroxides level in oil after 5 days stored under light (Fig. 4b) compared the slower increase during storage in the dark (Fig. 4a) confirmed the powerful lipid-oxidizing effect of light, where singlet oxygen ($^1O_2$) and triplet oxygen ($^3O_2$) react with the oil, respectively (Choe & Min, 2006). In our study, we observed that the films containing bixin reduced the oxidation of sunflower oil under light, since these kept the peroxides level under the limit of commercialization (10 m_{eq}/kg) for 5 days under accelerated storage conditions. In addition to the protection against autoxidation (release mechanism), bixin was also effective in protecting against photochemical degradation. These results were obtained, despite the degradation of the initial carotenoid 9-*cis*-bixin during melt processing.

4. Conclusion

PLA films containing bixin were prepared after melt mixing or solution casting to obtain new packaging materials for light-sensitive food products. The melt mixing caused around 85% of bixin loss, where the main degradation reaction was *cis/trans*-isomerization, as shown by UHPLC-MS. The light barrier properties of the PLA/bixin films remained however satisfying, although 9-*cis*-bixin was largely degraded. The analysis of the performance of the PLA/bixin films with an initial bixin content as low as 1 g/kg to shield oxygen sensitive food from degradation showed their ability to delay the degradation of riboflavin and decrease the level of peroxides in sunflower oil. The light shielding property was more performing than protection delivered by mass transfer of antioxidants to the oil. This shows that the degradation of this

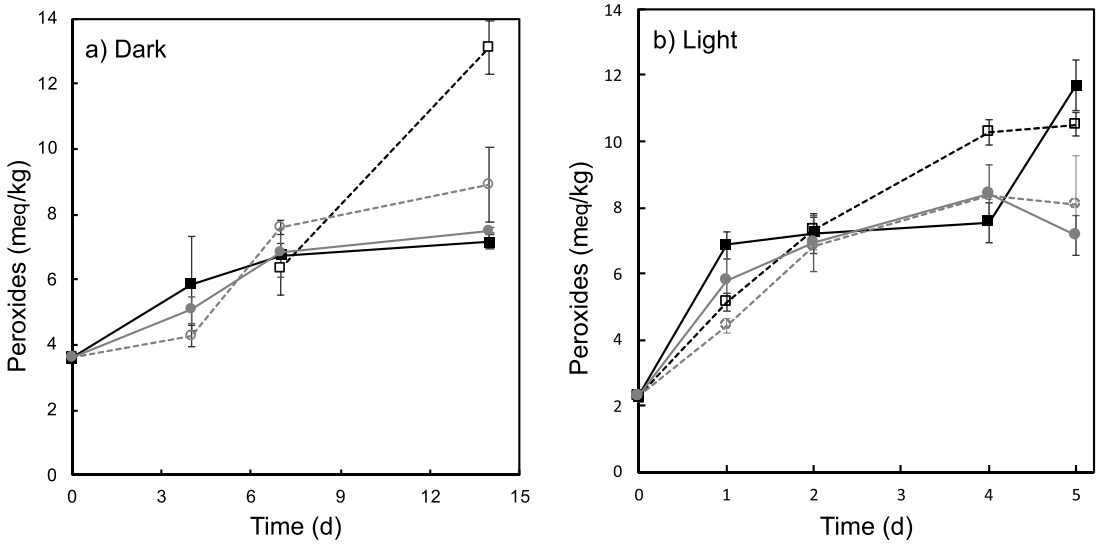

Fig. 4. Effect of MP films packaging performance on the oxidative stability (measured by peroxides formation) of sunflower oil stored at dark (a) and under light exposure (b) at 40 °C. Symbols: ■ MP (film produced by melt processing – control sample), MP.P (plasticized film produced by melt processing – control sample), ● MP.Bix (film produced by melt processing with bixin), ◯ MP.Bix.P (plasticized film produced by melt processing with bixin).

natural antioxidant during melt processing of polymers is not fatal for its performance. Bixin shows to be a very interesting natural additive to improve the performance of commercial packaging materials to preserve the quality of various food products.

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CRediT authorship contribution statement

Liana Stoll: Conceptualization, Investigation, Methodology, Writing – original draft. **Marie-Noelle Maillard:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Even Le Roux:** Investigation, Methodology. **Simone Hickmann Flôres:** Investigation, Methodology. **Sonia Marli B. Nachtigall:** Investigation, Methodology. **Alessandro Rios:** Conceptualization, Investigation, Project administration, Writing – review & editing. **Sandra Domenek:** Conceptualization, Investigation, Project administration, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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