Improvement of Bixin Carotenoid Stability through the Formation of M–bixin (M = Mg^{2+}, Ca^{2+}, Zn^{2+}, and Se^{4+}) Complex Compounds Based on Photodegradation Kinetic Studies

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Abstract: *Bixin is used in wide-ranging food applications but is susceptible to degradation by many factors, including light. This research aims to increase bixin photostability through metal complexation. Bixin was complexed with Mg^{2+}, Ca^{2+}, Zn^{2+}, and Se^{4+}. The synthesis process was carried out in deep eutectic solvent media. Metal-bixin complexes have been successfully synthesized, indicated by the change in retention factor and %III/II ratio values resulting from thin-layer chromatography and UV-vis spectrophotometer analysis, respectively. The Fourier transform infrared analysis showed the interaction between central metal ion with bixin ligand has occurred by hydroxyl and carbonyl groups of bixin, which was characterized by a decrease in the intensity of the absorption peak in wavenumber 3000–4000 cm^{-1} (for the –OH carboxylic group vibration) and the peak shift of metal-bixin complexes towards the larger compared to bixin in the range wavenumber 1600–1700 cm^{-1} (for C=O ester vibrations). First-order photodegradation kinetics studies showed that the metal-bixin complexes were more stable than pure bixin. Se-bixin was four times more stable than pure bixin. Complexation of bixin with metals was shown to be a potential method to increase the stability of bixin, especially against light exposure.*

Keywords: *bixin; carotenoid; metal complex; kinetic; photostability*

■ INTRODUCTION

Color can be the main attraction of a product. Advances in technology can create a variety of synthetic colorants. Synthetic colorants have several advantages compared to natural ones, including being more stable, resistant to various environmental conditions, substantial coloring power, and a comprehensive range of colors that do not fade quickly and are bright. However, because of their toxicity, synthetic colorants can cause health and environmental problems [1]. Natural colorants become an alternative that is non-toxic, renewable, and easily degraded in nature, so they are environmentally friendly.

Carotenoids are an important group of chemical compounds that can act as natural colorants [2-3]. Among all types of carotenoids, achiote seeds (annatto) extract ranks second in the world trade of natural colorants. It is widely used in industry as a food coloring agent, textile dye, cosmetic, and medical ingredient. About 80% of the annatto extract contains bixin (Fig. 1) [4-5]. Bixin is a unique compound because it is only produced by the *Bixa orellana* (achiote) plant. Based on its structure, bixin contains nine conjugated double bonds with a carboxylic group at one end of the chain and an ester group at the other.

Bixin is safe to be consumed and even has health impacts [6]. Like other carotenoids such as β-carotene and astaxanthin, bixin has been reported to have good free radical scavenging activity due to its long system of

conjugated double bonds [7-8]. Tay-Agbozo et al. [9] have reported that bixin has an oxidation potential of 0.94 V, higher than β-carotene (0.64 V) due to its higher polarity. The high oxidation potential can increase the ability to capture free radicals, so the bixin compound promises to extend the shelf life of the food it adds to.

A chromophore in the form of nine conjugated double-bond systems in its structure causes bixin to produce the red color and many potential health benefits. However, the conjugated double bonds in the bixin structure also cause low stability of the bixin molecule during processing and storage. Bixin stability is influenced by several factors, including light, heat, and changes in pH. Najar et al. [10] reported that light had the most damaging effect on annatto extract, followed by the pro-oxidant benzoyl peroxide. Research conducted by de O Rios et al. [11] reported that treatment at high temperatures and in light caused a change from bixin to a degradation product with different colors and properties. Photochemical degradation is a significant transformation pathway of atmospheric organic matter. Light is the leading cause of the decomposition of organic materials, including bixin, into its degradation products [12].

The low *in vitro* photostability of bixin is one of the problems in forming functional materials from this bixin. Many researchers have published several methods that can improve the stability of bixin pigments, such as impregnation, complexation, encapsulation, and mixing bixin molecules with other materials [13-17]. Encapsulation was able to protect bixin from the heat effects, which was reflected in its crystal structure, which had a high melting point; however, it can remove the color of bixin [18]. Cortez et al. [19] reported that the metal ions used in the complex process were proven to stabilize the color of pigment extracts.

Rahmalia et al. [20] have performed bixin complexation with divalent metal ions (Zn^{2+} and Cu^{2+}) to increase the stability of bixin when used as a sensitizer in solar cells. The Zn-bixin complex showed better stability when compared to bixin and Cu-bixin, with the highest energy conversion efficiency and stable up to 700 W/m^2 light intensity. The interaction between bixin and metal precursors is predicted to originate from metal-to-ligand charge transfer (MLCT). In this paper, we present data on increasing the stability of bixin through the formation of Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin complex compounds based on studies of their photodegradation kinetics.

In addition to stabilizing the bixin molecule, the use of Ca^{2+} and Mg^{2+} also has several health benefits. Calcium (Ca) plays a vital role in forming and maintaining bone strength; apart from that, Ca also plays a role in the heart by helping the muscles in the walls of blood vessels contract [21-22]. Magnesium (Mg) also plays a role in the heart by helping the heart muscle relax so that both can simultaneously maintain a normal heart rhythm. Mg also increases the effectiveness of all classes of antihypertensive drugs [23].

The choice of zinc (Zn) and selenium (Se) is based on these two metals' crucial roles in developing various physiological processes, including the immune response. Zn is an essential micronutrient for human metabolism that catalysis more than 100 enzymes, facilitates protein folding, and helps regulate gene expression. Zn deficiency during growth periods results in growth failure. The organs most affected clinically by Zn deficiency are the epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems [24-25]. Recently, Oyagbemi et al. [26] reported that Zn possesses potential health benefits against the COVID-19 pandemic by improving immune response, minimizing infection and inflammation, preventing lung injury, inhibiting viral replication through the interference of the viral genome transcription, protein translation, attachment, and host infectivity.

■ EXPERIMENTAL SECTION

Materials

Achiote (*Bixa orellana* L.) seeds were obtained from Pontianak, West Kalimantan, Indonesia. Choline chloride and glycerol with a 1:3 molar ratio based on deep eutectic solvent (DES) was obtained from previous work by Rahmalia et al. [27]. The properties of DES: pH 7.13; freezing point < 20 °C, density of 1.207 g/cm^3, viscosity of 148.99 cP, and conductivity of 1.8 mS/cm.

Ethyl acetate ($C_4H_8O_2$, ≥ 99.5%), dimethyl sulfoxide ($(CH_3)_2SO$, ≥ 99.7%), hexane (C_4H_{14}, ≥ 99.0%), thin layer chromatography (TLC) plate F_{254}, and zinc chloride ($ZnCl_2$, 98%) were purchased by Supelco, Merck.

Instrumentation

Instruments used for the research included glassware sets, oven (Memmert), hotplate stirrer (IKA C-MAG HS 7), analytical balance (Bel Engineering M164A), UV-vis spectrophotometer (Shimadzu 1240), FTIR spectrometer (Thermo Scientific Nicolet Is10), and ^{1}H-NMR.

Procedure

Bixin extraction of achiote seeds

Achiote seeds (250 g) were immersed in 500 mL of ethyl acetate in an Erlenmeyer flask, stirred for 30 min, and filtered. The process was repeated until all the colors were extracted into the ethyl acetate solution, and it was then evaporated at 40 °C. The crude residue was successively washed with *n*-hexane, methanol, ethanol, and acetone. Each fraction dissolved in *n*-hexane, methanol, ethanol, and acetone was then analyzed using TLC. The fraction containing pure bixin was further characterized using ^{1}H-NMR.

Synthesis of M-bixin (M = Ca^{2+}, Mg^{2+}, Zn^{2+}, and Se^{4+}) complexes

M-bixin complexes were synthesized by modifying the method of Rahmalia et al. [27]. The complex Ca-bixin was prepared as follows. Much of 0.0140 g of $CaCl_2$ was mechanically mixed in a mortar with 0.0500 g bixin (Ca^{2+}:bixin 1:1 mol) for 15 min until homogenous powder mixtures were obtained. Then, 2 mL DES was added to the mixture, followed by magnetic stirring at room temperature for 24 h until the pasty combination was obtained. Then, 5 mL of demineralized water was added to the pasty product until the precipitate was obtained and filtered, and the residue was dried using N_2 gas. A powder complex of Ca-bixin was obtained. Mg-bixin, Zn-bixin, and Se-bixin were prepared using the same method, with weights of 0.0121, 0.1710, and 0.0173 g, respectively. The pure bixin, Ca-bixin, Mg-bixin, and Se-bixin complexes were analyzed by TLC, UV-vis spectrophotometer, FTIR, and ^{1}H-NMR.

Photostability test

Bixin, Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin photostability tests were carried out by modifying the method of Rahmalia et al. [28]. The test solution was prepared by separately dissolving 100 mL of pure bixin, Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin in dimethyl sulfoxide. The absorbance of each solution was measured using a UV-vis spectrophotometer at 300–600 nm, with the desired initial absorbance of one. If the test solution had an absorbance of more than one, then it was diluted with the addition of acetone until an absorbance of one was obtained.

The test solution was added to as much as 5 mL into different vials. The test solution was irradiated using a light source from a 500 W halogen lamp at a light intensity of 300 W/m^2 for 10 h with an irradiation interval of 1 h, while the test solution used as a control was stored in the dark and covered with aluminum foil, to avoid contact with light. Furthermore, an analysis was carried out using a UV-vis spectrophotometer in the wavelength range of 300–600 nm to analyze the decrease in absorbance of each test solution before and after irradiation. Analysis of the rate of degradation of bixin and M-bixin was carried out using first-order kinetic models by plotting ln A_0/A_t vs. t and determining the value of R^2, as well as the degradation rate constant.

■ RESULTS AND DISCUSSION

Characteristics of Bixin, and M-Bixin (M = Ca^{2+}, Mg^{2+}, Zn^{2+}, and Se^{4+}) Complexes

In this study, bixin was obtained from the extraction of achiote seeds using ethyl acetate solvent, followed by multilevel washing utilizing a variety of solvents. The purpose of washing is to separate bixin from other components in achiote seed extract. Several studies have found a method for extracting pigment from achiote seeds using vegetable oil; however, separating the compounds was not easy [29-31]. The extraction can also be done using ethanol solvent, but the result obtained was a crude pigment extract [32-33]. In this study, we used purified bixin, which had to undergo several purification steps. The aim was to study the formation mechanism of complex compounds with

metals. Because the solvents used are volatile for its food application future, evaporation is carried out until the solvent is completely gone or below the regulatory threshold.

The results of the TLC analysis, as presented in Fig. 1, show that the methanol and acetone fractions produce one spot with the same elution distance as standard bixin (Rf = 0.32). Meanwhile, the *n*-hexane and ethanol fractions produced more than one spot, indicating the complexity of the compound, so the purity of the bixin in these fractions is certain to be very low.

The methanol and acetone fractions were then analyzed using a UV-vis spectrophotometer. The spectra of the analysis results in acetone solvent are presented in Fig. 2. It shows the similarity of the spectra of the acetone fraction with standard bixin, which produced three peaks at wavelengths of 488, 453, and 434 nm. At the same time, the methanol fraction showed a different spectrum from standard bixin. These results are in accordance with previous studies reported by Rahmalia et al. [34]. Bixin is a semipolar compound that dissolves well in acetone.

The conjugated double bond system constitutes the light-absorbing chromophore of bixin, resulting in a strong absorption band above 400 nm. This transition is mediated by the $S_0(^1 1Ag^-) \rightarrow S_2(^1 1Bu^+)$ electronic dipole allowed and is oriented along the long axis of the molecule. There are three typical peaks in the absorption band of bixin in aprotic solvents, arising from the level of vibration (v_0, v_1, v_2, v_3, etc.) associated with the initial and final electronic states [34]. Based on the results of analysis using TLC and UV-vis spectrophotometer, the acetone fraction was proven to be a bixin compound and was subsequently used as a ligand in the synthesis of Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin complexes.

The synthesis of Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin complex compounds in this study was carried out using $CaCl_2$, $MgCl_2$, $ZnCl_2$, and SeO_2 precursors. The research we conducted previously on Rahmalia et al. [27] has synthesized M-bixin using metal sulfate precursors ($CaSO_4$, $MgSO_4$, $ZnSO_4$, and $CuSO_4$), while this research uses metal chlorides and oxides (MCl_2 and MO_2). This research aims to study the effect of counter ions on metal precursors on the complex formation and the characteristics of the resulting complexes. All precursors are in anhydrous form.

The DES used is a mixture of choline chloride and glycerol with a molar ratio of 1:3. The use of DES as a medium for complexation reactions is an alternative to conventional petroleum-based solvents. They show more accessible preparation methods and lower prices. They are also biodegradable and compatible with biological systems. In addition, they have interesting physicochemical properties, high thermal stability, and low volatility, and they are compatible with water. This way, an environmentally friendly and nontoxic production process could be achieved.

The results of TLC analysis (Fig. 3) with the same eluent as the previous stage showed that the Rf values of bixin, Mg-bixin, Ca-bixin, Zn-bixin, and Se-bixin were 0.32, 0.45, 0.53, 0.47, and 0.44, respectively. The shift in elution distance indicates the formation of Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin complexes. The difference

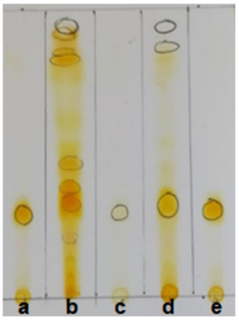


Fig 1. TLC analysis results of (a) standard bixin, (b) *n*-hexane fraction, (c) methanol fraction, (d) ethanol fraction, and (e) acetone fraction

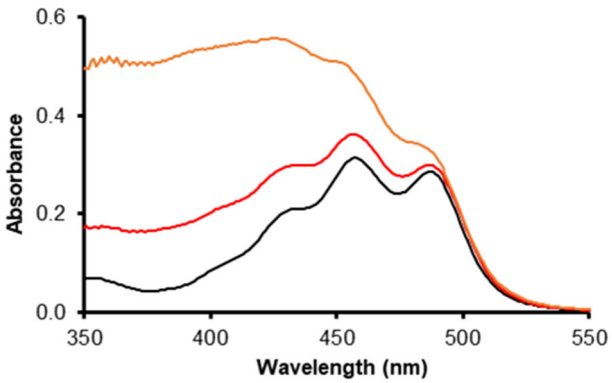


Fig 2. UV-vis spectra of standard bixin (black), methanol fraction (orange), and acetone fraction (red)

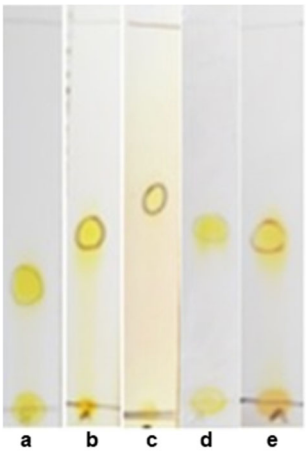


Fig 3. TLC analysis results of (a) bixin, (b) Mg-bixin, (c) Ca-bixin, (d) Zn-bixin, and (e) Se-bixin

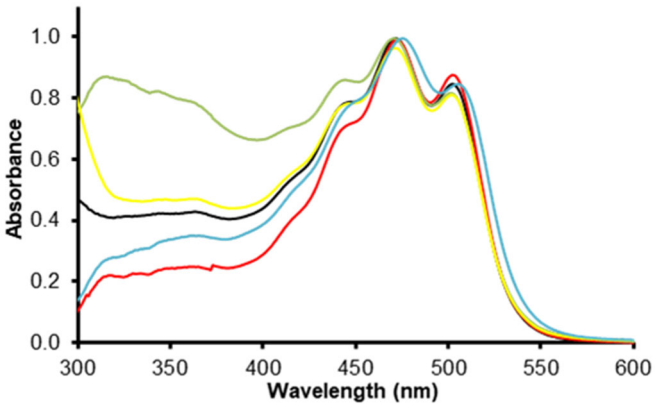


Fig 4. Normalized UV-vis absorption spectra of bixin (black), Mg-bixin (red), Ca-bixin (green), Zn-bixin (yellow), and Se-bixin (blue)

in Rf values also shows the difference in polarity between bixin, Zn-bixin, and Se-bixin, where bixin > Se-bixin > Mg-bixin > Zn-bixin > Ca-bixin. The difference in polarity of the complex compounds produced may be due to different ionic radii. Se^{4+}, Mg^{2+}, Zn^{2+}, and Ca^{2+} have 64, 86, 88, and 114 pm ionic radii, respectively. Increasing the value of the metal ion radius causes the polarity of the complex compound produced to be lower [35-36].

Fig. 4 shows that bixin in dimethyl sulfoxide solvent produces three absorption peaks, i.e., 502, 472, and 447 nm. This is influenced by the presence of conjugated double bonds, which play a role in the process of absorbing light by the chromophore group so that it can produce color. Bixin has a conjugated double bond (C=C) and a carbonyl group (C=O), which causes absorption in the visible light wavelength range. Similar to the use of MSO_4 as a precursor that we previously reported by Rahmalia et al. [27], the absorption spectra of the metal-bixin complex in Fig. 4 also show relatively overlapping spectra, indicating that there was no significant shift in the bixin absorption peak either before or after complexed with metal. However, at wavelengths in the range of 300–400 nm, differences in the shape of the absorption peaks were observed, indicating that new bonds were formed.

Another absorption spectrum characteristic that can be analyzed to determine the interaction between bixin and metal is the peak ratio (%III/II). The peak ratio (%III/II) is the ratio of the height of the absorption peak with the longest wavelength (III) and the middle absorption peak (II), taking the minimum between the two peaks as the baseline, multiplied by 100 [37]. The spectra of most carotenoids have a peak ratio (%III/II), which shows not only a single absorption band but three more or less distinct peaks. The %III/II value will indicate the spectrum's characteristic shape or fine structure [38]. Bixin, Mg-bixin, Ca-bixin, Zn-bixin, and Se-bixin have a %III/II value of 26.78, 23.28, 25.71, 29.41, and 21.21, respectively. The difference in %III/II values in the metal-bixin complex compared to pure bixin indicates an interaction between the carbonyl functional or chromophore groups in bixin and the metal [37].

The results of FTIR analysis, as presented in Fig. 5 show that bixin has an absorption at a wavenumber of 3394 cm^{-1} for the O–H stretching vibration of the carboxylic group. The bands at wavenumbers 2862 and 2931 cm^{-1} are vibrations from bending CH_2, at 1705 cm^{-1} is the C=O ester vibrations, at 1419 cm^{-1} is the C–C stretching vibrations of alkenes, at 1357 cm^{-1} is the bending vibrations C–H from the methyl group, at 1226 cm^{-1} is the absorption for C=O stretching, 1087 and 1041 cm^{-1} are the symmetric and asymmetric C–O–C vibrations of the ester group. The results of the FTIR analysis of bixin follows research by Rahmalia et al. [39]. The absorption band at 879 cm^{-1} shows the C–H vibration wagging out of the plane of 15-cis and 7-cis isomers [39].

The absorption band for stretching O–H carboxylate from bixin becomes wider with decreasing

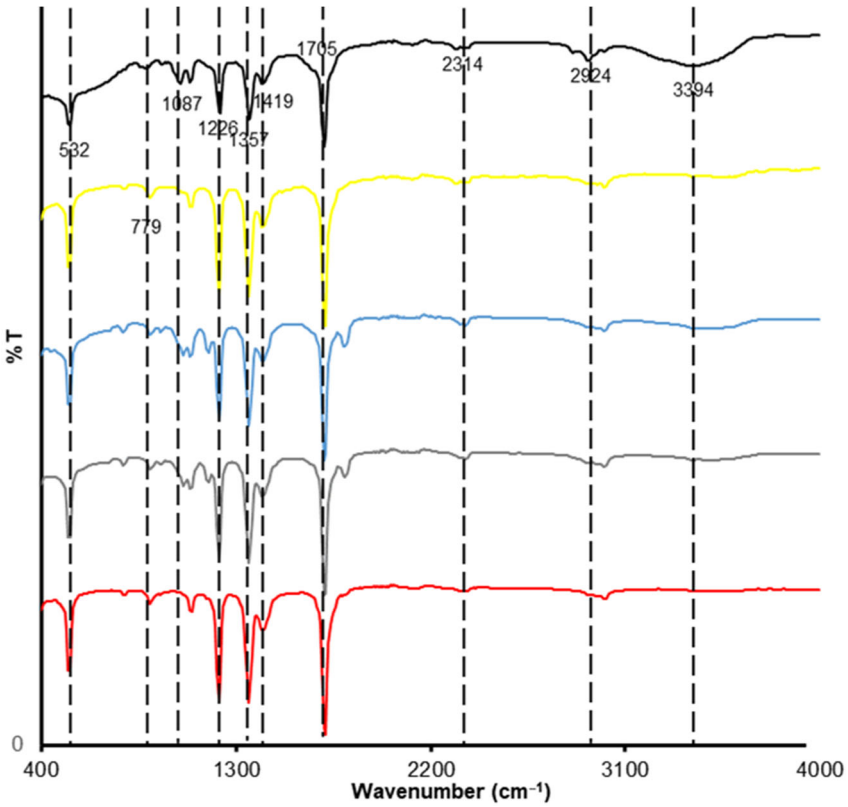


Fig 5. Infrared spectra of bixin (black), Mg-bixin (red), Ca-bixin (grey), Zn-bixin (yellow), and Se-bixin (blue)

absorption peak intensity, and there is a shift towards a larger wavenumber for Mg-bixin, Ca-bixin, Zn-bixin, and Se-bixin each of 3417, 3394, 3402, and 3495 cm^{-1} from bixin of 3394 cm^{-1}. The decrease in intensity and widening of the O–H absorption band depends on hydrogen bonds, which indicates that the stronger the bond, the wider the band shape will be with decreasing absorption peak intensity [40]. The characteristic absorption peaks of M–O appear at 779 cm^{-1}, where this absorption peak is not observed in the bixin spectrum. Zheng et al. [41] also reported that the characteristic absorption peak for M–O in metal-organic frameworks (MOF) is in the wavenumber of 773 cm^{-1}. The M-bixin complex was formed based on the UV-vis and FTIR analysis results. This formation is possible because, in the bixin structure, there is a carboxylate group that can act as a ligand for metal ions.

According to Abe et al. [42], differences in counter anions from the precursors used to form complex compounds will affect the final product yield. Although quantitative analysis regarding yield was not carried out in this study, the results of FTIR analysis showed a relatively large decrease in intensity in the O–H carboxylate characteristic peak of the resulting complex compounds. Meanwhile, in previous research, when we used MSO_4 as a precursor, there was no change in intensity in the 3300–3500 cm^{-1} wave number range; only a slight shift occurred. This could be an initial indication that the percentage of products produced in this research is greater than in previous research by Rahmalia et al. [27].

The NMR spectrometer is a chemical instrument used to obtain information regarding the structure and conformation of chemical compounds. NMR spectroscopy is an excellent explanatory method for determining the structure of organic compounds. Techniques can be used to determine the number of protons, relative stereo-atomic, and structural conformation in the structure of chemical compounds. In this study, ^{1}H-NMR was used to prove the successful formation of the Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin complex compounds. One important information

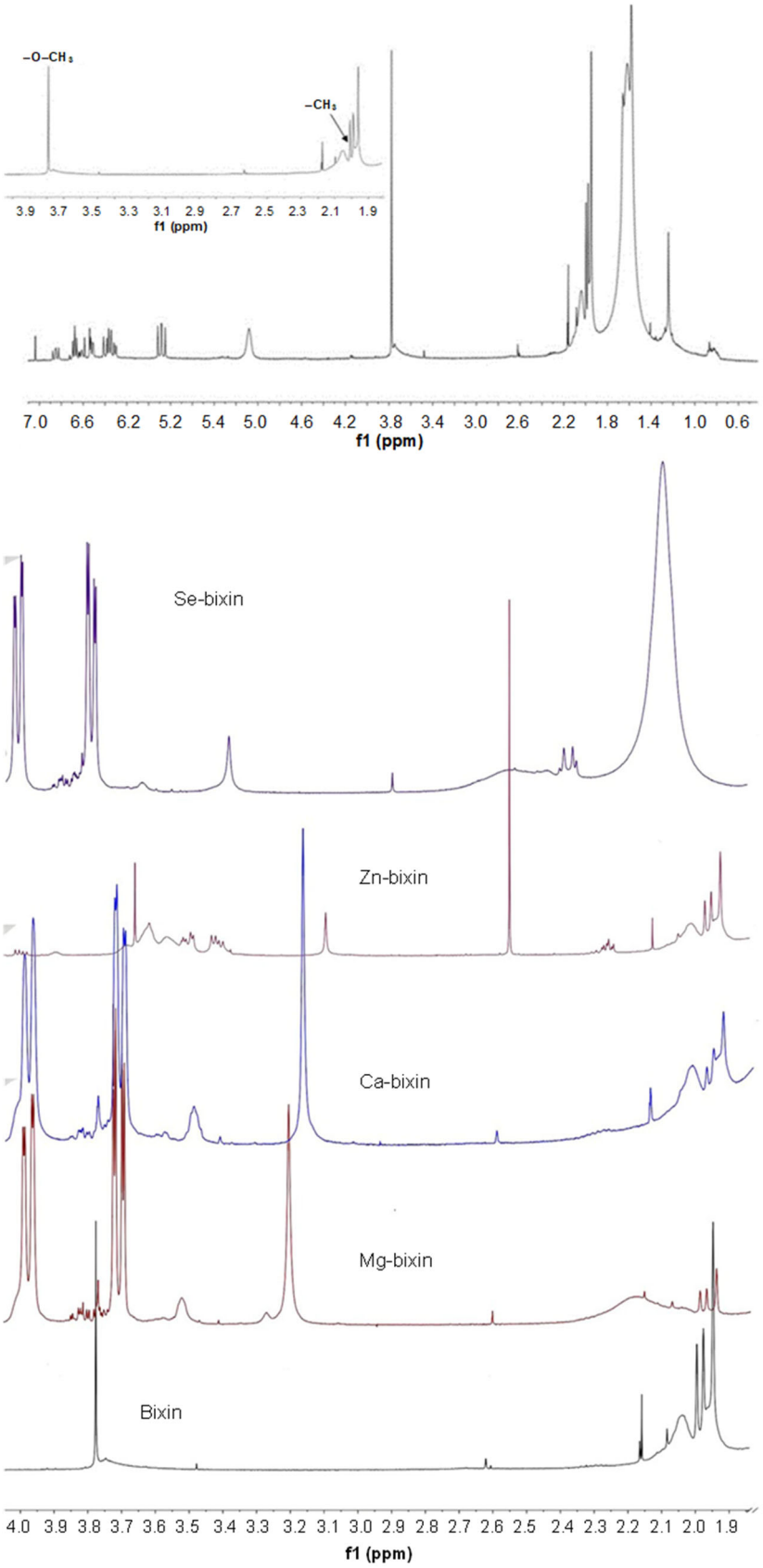


Fig 6. ^{1}H-NMR spectra of bixin, Mg-bixin, Ca-bixin, Zn-bixin, and Se-bixin

shown by the ^{1}H-NMR spectrum is the chemical shift of the different types of protons in the sample.

^{1}H-NMR (500 MHz, chloroform-d) of bixin (Fig. 6) shows δ 6.85 (*dd*, *J* = 14.9, 11.7 Hz, 0H), 6.74–6.57 (*m*, 1H), 6.52 (*dd*, *J* = 12.9, 4.4 Hz, 1H), 6.43–6.35 (*m*, 1H), 6.35–6.28 (*m*, 1H), 5.88 (*dd*, *J* = 18.1, 15.5 Hz, 1H), 5.08 (*s*, 3H), 3.78 (*s*, 1H), 3.74 (*s*, 2H), 2.04 (*s*, 9H), 2.00 (*s*, 1H), 1.96 (*s*, 4H), 1.24 (*s*, 1H). The chemical shift in the 5.08 to 6.85 ppm range indicates a conjugated double-bond system in the bixin molecule. According to the ^{1}H-NMR spectrum, it is evident that in the molecule, there are four –CH_3 groups having δ values 2.04, 2.00, 1.96, and 1.24 ppm. The presence of –OCH_3 groups is shown by δ values of 3.78. These results follow the report by Singha and Bag [43].

Fig. 6 also displays the chemical shift characteristics of the M-bixin complex compound (M = Zn^{2+}, Se^{4+}, Mg^{2+}, Ca^{2+}). It can be observed that there is a chemical shift towards the upfield in the protons of the methoxy group, and the –CH_3 proton for all the resulting complexes. The proton shift from the bixin methoxy group was 3.78 ppm to 3.18, 3.20, 3.49; 3.53 ppm for complexes with metal ions Zn^{2+}, Se^{4+}, Mg^{2+}, and Ca^{2+}. Meanwhile, the chemical shift of the –CH_3 bixin proton (1.98 ppm) shifted to 1.95; 1.95; 1.94 for complexes with metal ions Zn^{2+}, Se^{4+}, Mg^{2+}, the chemical shift of proton –CH_3 is not observed in the Ca-bixin complex. However, the chemical shift that occurred was insignificant, indicating no damage to the bixin structure after the completion process. The proposed bonding between metal ions and bixin is presented in Fig. 7.

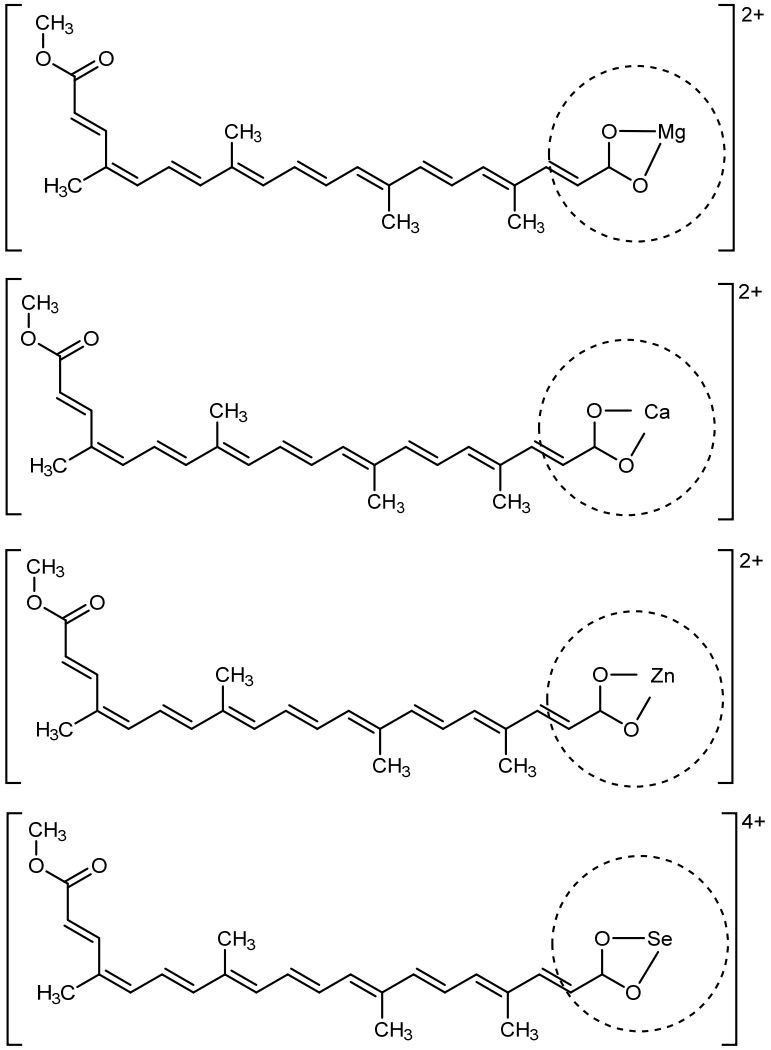
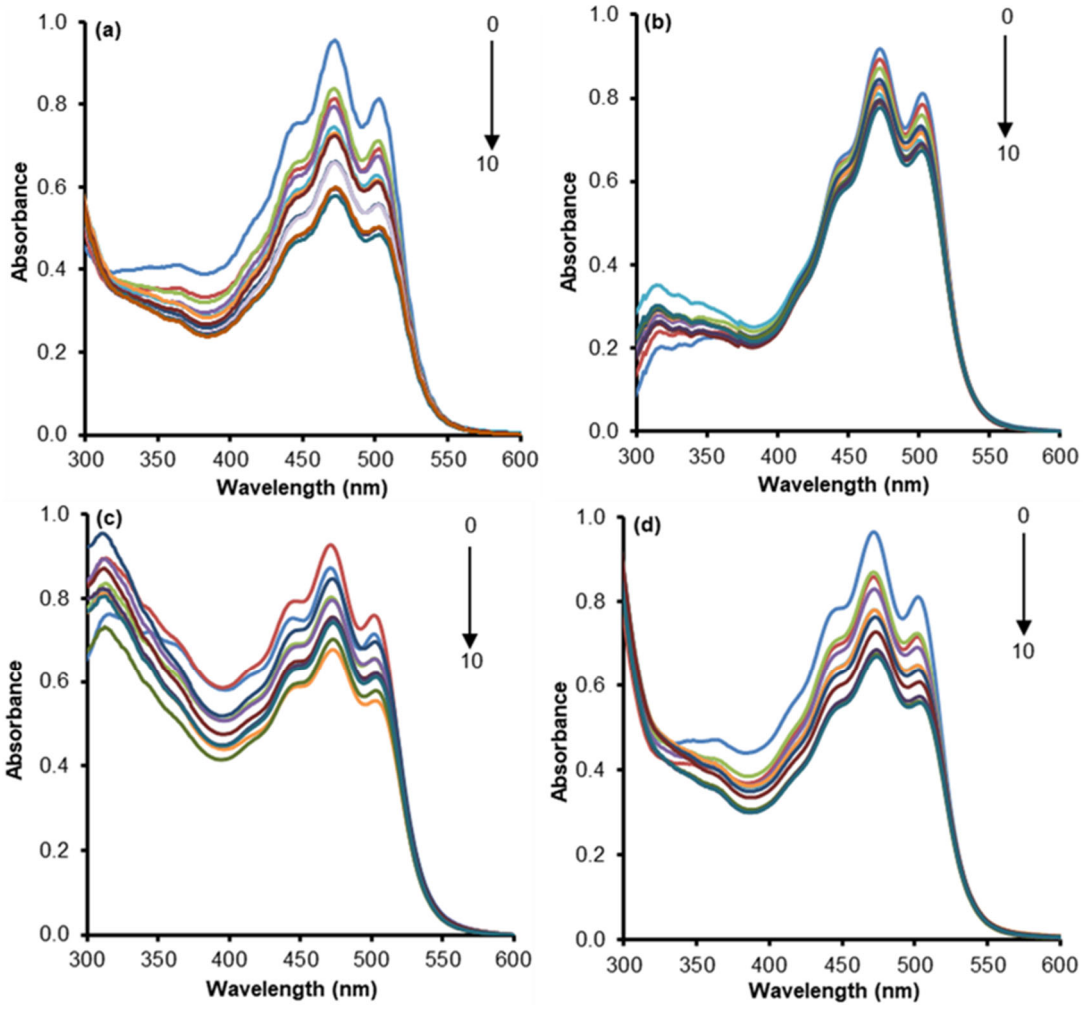


Fig 7. The proposed bonding between metal ions and bixin

Effect of Light on Stability of Bixin, Mg-bixin, Ca-bixin, Zn-bixin, and Se-bixin

The photostability of a substance may be defined as the response of the substance to exposure to solar, UV, and visible light that leads to a physical or chemical change. Photosensitized reactions may lead to the degradation of the substances by one or more pathways to form different products. The response of the substance to light absorption and excitation can be considered in terms of photodegradation (photolysis) reactions through the formation of free radicals or photosensitization reactions by intermolecular energy transfer. These reactions involve primary (photochemical) and secondary (chemical) reactions that give the final products [44]. Stability means that a complex may be stored for a long time under suitable conditions, or this compound may exist under appropriate conditions [43]. The photostability tests of bixin, Mg-bixin, Ca-bixin, Zn-bixin, and Se-bixin are important for long-term applications.

The UV-vis spectra profile of bixin, Mg-bixin, Ca-bixin, Zn-bixin, and Se-bixin for 10 h of irradiation is presented in Fig. 8. Overall, irradiation causes a decrease in sample absorbance but does not cause a shift in wavelength. This indicates that irradiation at an intensityof 300 W/m^2 causes some molecules, both bixin and its complex compounds, to experience degradation. The decrease in absorbance of bixin, Mg-bixin, and Zn-bixin was relatively more remarkable than that of Ca-bixin and Se-bixin. The C=C bond (oxidation/isomerization), a weak C–H bond (photoinduced fragmentation via hydrogen atom transfer or electron-proton transfer), and C=O group (reduction/fragmentation) are chemical functional groups



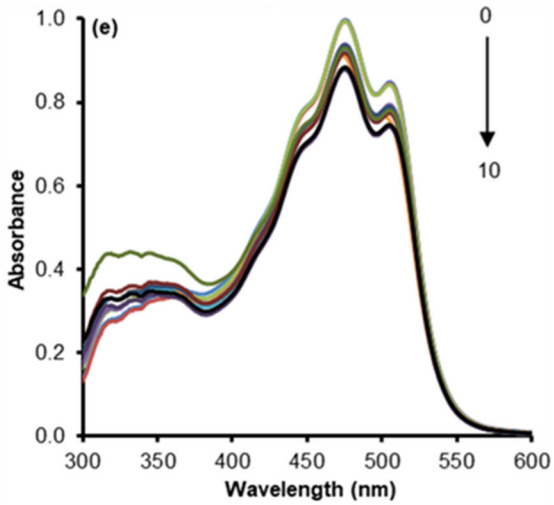


Fig 8. UV-vis spectra profile of (a) bixin, (b) Mg-bixin, (c) Ca-bixin, (d) Zn-bixin, and (e) Se-bixin for 10 h irradiation

in the organic molecules that are usually necessary for the occurrence of photochemical reactions [45].

The photodegradation efficiency of organic colorants increases with an increased exposure time [46]. The decrease in absorbance that occurs with increasing exposure time is due to the photosensitive nature of bixin, which causes photo-destruction and leads to the formation of a variety of lower molecular weight compounds. Breaking of double bonds is very likely to occur so that the degradation products formed do not show absorption in the visible area, causing the absorbance to decrease. The mechanism of carotenoid degradation due to the presence of light has been reported by Montenegro et al. [38]. According to their research results, photons are absorbed when light hits the bixin molecules, resulting in a long-lived energy-rich state(s) (typically triplet states), which can undergo reactions producing chemical alteration of another molecule. In another study, Semitsoglou-Tsiapou et al. [45] reported the main products observed due to photodegradation of carotenoids were apo-aldehydes and apo-ketones.

The photodegradation rate was determined quantitatively, and the results are presented in Fig. 9 and Table 1. The kinetic study of the photodegradation process of bixin, Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin was investigated using the first-order kinetic model presented in Eq. (1) and (2). The first order rate

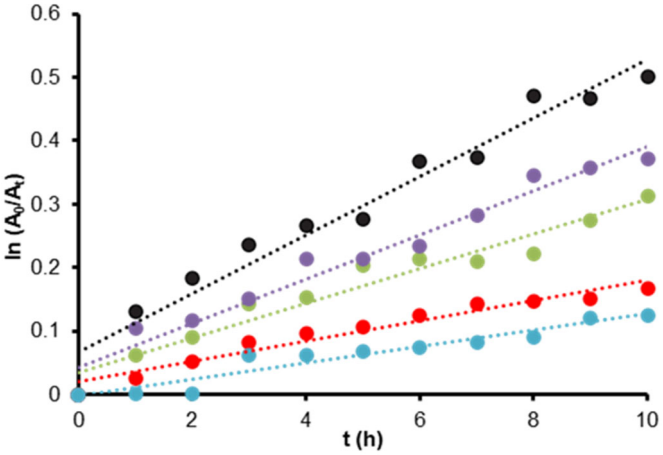


Fig 9. Photodegradation rate of bixin (black), Mg-bixin (red), Ca-bixin (green), Zn-bixin (purple), and Se-bixin (blue) for 10 h irradiation

Table 1. Results of stability test of bixin and M-bixin complexes against light exposure

Compound	Linear equation	R^2	k (h^{-1})	$t_{1/2}$
Bixin	Y= 0.0462x + 0.0664	0.9582	0.0462	15.0000
Mg-bixin	Y= 0.0160x + 0.0196	0.9488	0.0160	43.3120
Ca-bixin	Y= 0.0273x + 0.0347	0.9472	0.0273	25.3850
Zn-bixin	Y= 0.0349x + 0.0432	0.9648	0.0349	19.8570
Se-bixin	Y= 0.0129x – 0.0024	0.9238	0.0129	53.7210

constant, k (h^{-1}), was calculated from the slope of ln A_0/A_t versus irradiation time, t. A_0 is the initial absorbance at the maximum wavelength of bixin, Ca-bixin, Mg-bixin, Zn-bixin, and Se-bixin; A_t is the absorbance at any irradiation time. A series of apparent rate constant k are given in Fig. 9 and Table 1. The first-order half-life ($t_{1/2}$) was then determined using Eq. (3).

$$v = d_A/d_t = k.A \quad (1)$$

$$\ln A_0/A_t = k.t \quad (2)$$

$$t_{1/2} = \ln 2/k \quad (3)$$

It has been shown that bixin has poor light stability. About a 50% decrease in absorbance will occur after 15 h of irradiation time. Based on the photodegradation constant values given in Table 1, complexation succeeded in increasing the stability of the complex due to light exposure. The degradation rate of the Se-bixin and Mg-bixin complex compounds was almost four times slower than that of pure bixin. Se-bixin and Mg-bixin showed $t_{1/2}$ values of 53.721 and 43.312 h, respectively.

The order of stability of the complex produced based on first-order photodegradation kinetics studies is Se-bixin > Ca-bixin > Mg-bixin > Zn-bixin. In this case, the stability of M-bixin may be affected by the nature of central metal ions, including their ionic size and charge. The stability of metal complexes decreases with the increase in the size of central metal ions [47]. In the case of alkali metal as a central metal ion, the stability of Mg-bixin was greater than Ca-bixin due to the ionic radius of Mg^{2+} being smaller than Ca^{2+}. Zn^{2+} has an ionic radius almost the same as Mg^{2+} for bivalent metal ions of the first transition series, so its stability is also reduced. In contrast, the stability of metal complexes with identical ligands and similar coordinative environments increases with the increase of the charge on the central metal ion. As a result of this, Se-bixin shows the best stability.

■ CONCLUSION

An environmentally friendly and nontoxic production process of metal-bixin complexes has successfully been achieved using $MgCl_2$, $CaCl_2$, $ZnCl_2$, and SeO_2 precursors in a deep eutectic solvent. First-order photodegradation kinetics studies have proven that the metal-bixin complex is more stable than pure bixin. Se-bixin has a degradation rate constant of 0.0129 h^{-1} with a half-life of 53 h, which is four times more stable than pure bixin (0.0462 h^{-1}, 15 h). The information presented in this paper is crucial for planning the further processing, storage, and packaging of bixin molecules and their complex compounds. Furthermore, the M-bixin product has the potential to be tested for application as an active additive compound in food.

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■ CONFLICT OF INTEREST

The authors declare no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Winda Rahmalia: conceptualization, methodology, formal analysis, writing-original draft, project leader. Anis Shofiyani and Winda Rahmalia: supervision, resources, project administration, writing-review, editing, and validation. Fani Indriani, Alintiani Yolla Putri, and Septiani: formal analysis, research assistant. All authors have read and agreed to the published version of the manuscript.

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