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Structural and physicochemical properties of bio-chemical chitosan and its performing in an active film with quercetin and *Phaseolus polyanthus* starch

Propiedades estructurales y fisicoquímicas de quitosano bio-químico y su desempeño en una película activa con quercetina y almidón de *Phaseolus polyanthus*

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Abstract

Chitosan is a mucoadhesive and natural biodegradable polysaccharide derived by the deacetylation of chitin, such as an interesting biopolymer to form films and coatings for food preservation. The objective of this work was to isolate and characterize the structural and physicochemical properties of biochemical chitosan (BC) from shrimp (*Litopenaeus vannamei*) exoskeletons such as to develop an active film based on BC, native bean (*Phaseolus polyanthus*) starch (BS), and quercetin (Q). Further, the antioxidant activity and *in vitro* release tests were evaluated and compared with commercial chitosan (CC)- commercial tapioca starch (TS)-Q control film. X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy of BC were used to calculate the crystallinity index (58.91%) and deacetylation degree (88.45%), respectively. Also, the zeta (ζ) potential and solubility values of the stock solution of BC were 45.72 mV and 99.86% at pH 4.0, respectively. The $BC_{BS\,Q}$ film presented the highest release of Q in simulated foods in ethanol solutions at 50% and 95% concentrations, which could be used as an active packaging film both in oil-in-water emulsions and fatty foods with potential antioxidant activity.

Keywords: biodegradable polysaccharide, biopolymers, *in vitro* release, shrimp exoskeletons.

Resumen

El quitosano es un mucoadhesivo y polisacárido natural biodegradable derivado por la deacetilación de la quitina, así como un biopolímero interesante para formar películas y recubrimientos para conservación de alimentos. El objetivo de este trabajo fue aislar y caracterizar las propiedades fisicoquímicas y estructurales de quitosano bioquímico (QB) de exoesqueletos de camarón (*Litopenaeus vannamei*), así como desarrollar una película activa basada de QB, almidón nativo de frijol (*Phaseolus polyanthus*) (AF) y quercetina (Q). Además, la actividad antioxidante y las pruebas de liberación *in vitro* fueron evaluadas y comparadas con una película control de quitosano comercial (QC)-almidón de tapioca comercial (AT)-Q. La difracción de rayos X (DRX) y la espectroscopía infrarroja por transformada de Fourier (IRTF) de QB fueron usadas para calcular el índice de cristalinidad (58.91%) y el grado de desacetilación (88.45%), respectivamente. También, los valores de potencial zeta (ζ) y solubilidad de la solución patrón del QB fueron de 45.72 mV y 99.86% a pH de 4.0, respectivamente. La película QB_{AFQ} presentó la mayor liberación de Q en alimentos simulados en soluciones de etanol a concentraciones de 50% y 95%, lo cual puede ser usada como una película de empaque activa tanto en emulsiones aceite-en-agua y alimentos grasosos con potencial actividad antioxidante.

Palabras clave: biopolímeros, exoesqueletos de camarón, liberación *in vitro*, polisacárido biodegradable.

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1 Introduction

Chitosan is a cationic natural polymer composed of β-(1→4)-2-amino-2-deoxy-D-glucopyranosyl units, which is obtained by partial deacetylation from chitin under strong bases and acids to deproteinize and demineralize it (Pacheco *et al*., 2019; Pérez *et al*., 2022). This biopolymer is produced commercially and large amounts of the by-products are disposed directly into the environment without prior treatment (Jakubowska *et al*., 2023), so finding alternatives that promote the use of green and eco-friendly extraction methods have been explored to obtain chitosan by biological methods (Mohan *et al*., 2022). Due to the wide use of petroleum-based plastics, several types of research on chitosan have been focused on in the field of coatings and biodegradable films (Balderas-Gutiérrez *et al*., 2020; Rodríguez-Guzmán *et al*., 2022; Romero-Bastida *et al*., 2020), such as in the development of novel active and intelligent packaging systems (Jakubowska *et al*., 2023; Jamróz *et al*., 2023; Lee *et al*., 2023; Rong *et al*., 2023).

Starch is the most popular plant polysaccharide, which is composed of linked 1,4-linked D-glucose units and has been widely used in edible films because of its abundance, cost-effectiveness, and excellent film-forming abilities (Calderón-Castro *et al*., 2022; Piñeros-Guerrero *et al*., 2020; Thakur *et al*., 2019). Unconventional starches could be interesting sources of biopolymers to develop biodegradable films (Pech-Cohuo *et al*., 2021). In this sense, *Phaseolus polyanthus* starch presented in previous research works better physicochemical, structural, and rheological properties than other native bean starches (Zapata-Luna *et al*., 2021). However, the alone starch-based films have some limitations such as high-water vapor transmission rate and solubility, low tensile strength, which could restrict their uses in food applications and the researchers are concentrating their works on starch films utilizing different co-polymers to improve various properties (Chavan *et al*., 2022). Biopolymer blends such as starch and chitosan could be sustainable alternatives for the generation of this kind of biodegradable film with good properties to ensure the quality of food applications (Díaz-Cruz *et al*., 2022).

Q is a flavonoid commonly found in most fruits and vegetables tissues and it possesses several biological and pharmaceutical properties (Cuevas-Bernardino *et al*., 2018). Utilization of Q into different biopolymers has improved the antioxidant and antimicrobial activities, such as the oxygen/water barrier and mechanical parameters of functional packaging films (de Barros Vinhal *et al*., 2021; Ezati and Rhim, 2021). Also, the development of chitosan films incorporated with Q has presented effects on the surface morphology, tensile strength, and opacity properties and they could also represent a promising strategy for the design of bioactive-releasing films (Wiggers *et al*., 2022). Sani *et al*. (2023) mentioned that the development of polysaccharide blends loaded with Q could improve the solubility, water vapor permeability, water contact angle, and mechanical, antimicrobial, and antioxidant properties of active packaging films.

These innovative packaging systems could refer to the addition of additives that prevent deterioration or degradation of food quality and increase the shelf life of foods from environmental and intrinsic factors (Roopa *et al*., 2023); also, food packaging materials with biodegradable nonconventional biopolymers such as starch, which could be alternative sources to increase the techno-functional properties and valorization them (Westlake *et al*., 2023). Currently, the development of active films based on novel chitosan and starch biopolymers incorporated with bioactive compounds has been increased in this field (Kowalczyk *et al*., 2023; Pech-Cohuo *et al*., 2022; Rong *et al*., 2023; Venkatachalam *et al*., 2023). In order to get an understanding of the migration of active films components, many works have been focused on the release rate of bioactive compounds into food simulants such as water, isooctane, and ethanol:water solution (Agustinelli *et al*., 2021; Assis *et al*., 2021).

To the best of our knowledge, the biochemical chitosan and native bean (*Phaseolus polyanthus*) starch films added with quercetin as an antioxidant agent have not been studied in the context of food simulants to identify specific potential applications. The objective of this study was to characterize the physicochemical and structural properties of chitosan isolated from biological chitin such as to compare the quercetin release of active films into different food simulants (ethanol 50% and ethanol 95%) for six days and their chemical stability under UV light.

2 Materials and methods

2.1 Materials and chemicals

A biological chitin batch from shrimp (*Litopenaeus vannamei*) shells was obtained by a biological method as reported by Pacheco *et al*. (2011). Native BS from "Ibes" (*Phaseolus polyanthus*) bean was used in previous work (Zapata-Luna *et al*., 2021). CC (419419 product number; high molecular weight 310-375kDa; degree of deacetylation≥75%) was purchased from Sigma-Aldrich Mexico (Toluca, State of Mexico, Mexico). Commercial TS (1st F®, Oriental Foodbank, Inc., CA, USA) was purchased at a local market in Pomona, USA. Q was donated from Novel Ingredients (East Hanover, NJ, USA; purity ≥ 98%). Glycerol, acetic acid, citric acid, dimethyl sulfoxide (DMSO), 2,2-diphenyL^{-1}-picrylhydrazyl radical (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Toluca, State of México, Mexico). The water used for all the experiments was deionized.

2.2 *Extraction method of Biochemical chitosan (BC)*

The BC isolation from biological chitin was carried out according to the methodology described by Pacheco *et al*. (2011) with some modifications. A sample (100 g) of biological chitin was washed with 1,500 mL of 0.5 M HCl to initiate the demineralization process. This mixture was kept under constant stirring for 2 h. Subsequently, the sample was neutralized with NaOH (40%). Regarding the deacetylation process, the biological chitin was placed in 3,000 mL of NaOH (60%) and stirred for 96 h. The sample was neutralized with 0.5 M HCl and after that, the sample was sifted and rinsed with distilled water. Finally, the BC was dried in an oven (OV-12, Jeio Tech Co., Ltd., South Korea) at 50 °C for 48 h. The yield was calculated as follows:

$$\text{Yield } (\%) = \frac{\text{Recovered biological chitosan (g)}}{\text{100 g Biological chitin}} \times 100 \quad (1)$$

2.3 *Physicochemical characterization*

The moisture, ash, and total nitrogen contents were evaluated according to the AOAC methods. The ζ-potential was determined according to the method described by Espinosa-Andrews *et al*. (2013) with slight modifications. The stock solution of BC was diluted to a concentration of 5.0 mg mL^{-1} using deionized water. Diluted dispersions were adjusted at a 4.0 value using either 0.1 M NaOH or 0.1 M HCl. The ζ-potential was measured using the Zetasizer nano (Malvern Instruments, Worcestershire, UK). The refractive index of BC diluted dispersions was 1.530. Solubility was measured according to Martín-López *et al*. (2020).

2.4 *XRD and energy-dispersive X-ray spectroscopy (EDS)*

The XRD and EDS analysis of the BC sample was determined using the methodology proposed by Zapata-Luna *et al*. (2021). Crystallinity index percentage (CI%) was calculated as follows:

$$CI = \left[\frac{I_{110} - I_{am}}{I_{110}}\right] \times 100 \quad (2)$$

where I_{am} is the diffraction intensity in the amorphous area at 16°, and I_{110} is the maximum intensity in the crystallinity region at 20° (Martín-López *et al*., 2020).

2.5 *FTIR spectroscopy*

The spectra were obtained using a Spectrum GX FTIR spectrometer (Perkin Elmer, Branford, CT, USA) equipped with a crystal diamond universal sampling device according to Zapata-Luna *et al*. (2021)Zapata-Luna *et al*. (2021). The N-acetylation and deacetylation degrees (DAD) were determined and calculated according to the methodology reported by Brugnerotto *et al*. (2001) as follows:

$$\text{N acetylation degree } (\%) = 31.92\left(\frac{A_{1309}}{A_{1418}}\right) - 12.2 \quad (3)$$

$$\text{DDA}(\%) = 100 - \text{N acetylation degree} \quad (4)$$

2.6 *Active film formation*

2.6.1 *Stock solutions*

The BC and CC stock solutions were prepared by dissolving chitosan (2% w/w) in acetic acid (1% v/v) and keeping it under constant stirring for 24 h. Stock solutions (4% w/w) of BS and commercial TS samples were heated at 90 °C for 30 min with magnetic stirring at 250 rpm. Q solutions (0.071 mg/mL) in absolute ethanol were freshly prepared as needed.

2.6.2 *Film-forming solutions (FFS)*

Three FFS formulations were prepared according to the methodology described by Daudt *et al*. (2016) with slight modifications. First, BS and BC stock solutions were mixed in a ratio of 1:1 to form 100 g of FFS with a total biopolymer concentration of 3 g total biopolymer/100 g, then Q stock solution was added and stirred for 30 min. Second, TS and CC stock solutions were prepared similarly at first FFS. Third, TS and CC stock solutions were formulated with the same ratio of 1:1 but without Q, the Q stock solution was substituted by deionized water in the same amount. Finally, glycerol (0.75 g/g total biopolymer) and citric acid (0.15 g/g total biopolymer) were added to FFS as plasticizers with gentle stirring for 30 min at room temperature (25 °C). FFS samples were degassed with an ultrasonic bath (Bransonic 3510R-MT, Branson Ultrasonics, Co., Danbury, CT, USA) for 10 min to remove air bubbles.

2.6.3 *Active films formation by casting*

Active films were elaborated according to Daudt *et al*. (2016). Films were obtained by casting FFS (0.3 g/cm^2) onto plastic Petri dishes (diameter = 4.90 cm); after that, they were dried in an oven (OV-12, Jeio Tech Co., Ltd., South Korea) at 50 °C for 48 h. Then, the dried active films were peeled off and preprocessed at 25 °C and 50% relative humidity (RH) for 48 h using NaBr saturated solution before their characterization.

2.7 *Antioxidant activity by DPPH assay*

The antioxidant activity of films was evaluated according to Davidov-Pardo *et al*. (2012). Briefly, 36 mg of each film was added into 20 mL of DMSO under magnetic stirring for 4 h, and then a 60 μL of each film sample was mixed with 2940 μL of DPPH solution (60 μM). The mixture was left at incubation at room temperature in the dark for 30 min. The absorbance was measured at 515 nm using a UV-vis

spectrophotometer (GENESYS 10, Thermo Fisher Scientific Inc., USA). The absorbance of the control was obtained by replacing the sample with DMSO. The radical scavenging activity (RSC) was calculated as follows:

$$\text{RSC}\ (\%) = \left(\frac{A_c - A_m}{A_c}\right) \times 100 \tag{5}$$

where A_c is the absorbance of the DPPH solution without the addition of the film and A_m is the absorbance of the sample solution.

2.8 *Release tests*

The *in vitro* release of Q from films was assessed in two different food simulants according to the methodology described by Liang *et al*. (2017) with some modifications. 50% ethanol and 95% ethanol solutions were used for the release study. Food simulants with 50% ethanol and 95% ethanol are assigned for oil-in-water emulsions and high-fat foods, respectively. Q release of each film was performed with film pieces (2 × 2 cm) and 10 mL of food simulants into amber vials. Q release was determined at predetermined times (0, 1, 2, 4, 6, 8, 9, 24, 96, 120, and 144 h). All measurements were conducted in triplicate.

2.9 *Chemical stability*

The chemical stability against the degradation of Q was evaluated according to the methodology described by Cuevas-Bernardino *et al*. (2018) with some modifications. Films were placed in plastic petri dishes under direct ultraviolet light at 366 nm using a UV lamp (Black-Ray lamp, Model-UVL-56, San Gabriel, Ca, USA) for 4 h. A piece (2 × 2 cm) of each film was soaked with 10 mL of DMSO with constant magnetic stirring for 2 h. Then, 2 mL was measured at 415nm using a spectrophotometer (GENESYS 10, Thermo Fisher Scientific Inc., USA). Q solution was used as a control.

2.10 *Statistical analysis*

Analyses were conducted by triplicates using a randomized experimental design. Data were processed using Analysis of Variance (ANOVA) and Tukey's means comparison test. Significance was established at p≤0.05. Data analysis was conducted using the Statgraphics Centurion XVI software (Statistical Graphics Corp., Manugistics, Inc., Cambridge, MA, USA).

Table 1. Yield and physicochemical parameters of biochemical chitosan (BC) from shrimp (*L. vannamei*) shells.

Parameter	BC
Yield (%)	49.58 ± 0.21
Moisture (%)	8.95 ± 0.10
Ash content (%)	11.15 ± 0.03
Total nitrogen (%)	5.34 ± 0.20
ζ potential (mV)	45.72 ± 3.70
Solubility (%)	99.86 ± 0.11
CI (%)	58.91
DAD (%)	88.45

CI = crystallinity index; DDA = degree of deacetylation. Mean ± standard deviation.

3 Results and discussions

3.1 *Physicochemical properties of biochemical chitosan*

The yield and physicochemical parameters of BC are presented in Table 1.

Chemical isolation of chitosan from biological chitin was performed with a yield of 49.58% (Table 1), which presented a similar value to shrimp chitosan extracted by Cocoletzi *et al*. (2009) with a chemical method (51.94%). Nevertheless, Suresh *et al*. (2023) in their study of biological extraction of chitin from fish scale and alkali-based chemical deacetylation showed a lower chitosan yield of 8% (w/w), while Martín-López *et al*. (2020) reached a higher yield of chitosan with an ultrasonic-assisted (86.96%). The BC moisture, ash, and total nitrogen values were 8.95%, 11.15%, and 5.34 %, respectively. Jiang *et al*. (2023) reported similar moisture content (8.01%) of commercially available pharmaceutical chitosan such as ash values lower (0.55-0.81%) in both insect chitosan and commercial chitosan. The ash content in chitosan could be an important parameter that affects its solubility, viscosity, and also other important characteristics (Kumari *et al*., 2017). The high ash content of isolated chitosan could be related to the raw material and extraction conditions (Cocoletzi *et al*., 2009). Table 1 showed that the ζ-potential value of the BC solution was 45.72 mV at pH 4.0 which was the pH value of the active film-forming solution. This positive charge of BC was consistent with the solubility (99.86%) where chitosan groups were soluble at pH 4.0 by protonation of amino groups. The positive charge of chitosan is obtained after its solubilization in acetic acid by protonation of amino groups in the form NH_3^+ if the pH is increased the amine groups become deprotonated and chitosan loses their charge to become insoluble from pH 6.5 and this insolubility is manifested by the change in the appearance of the chitosan solution that becomes turbid (Saïed and Aïder, 2014).

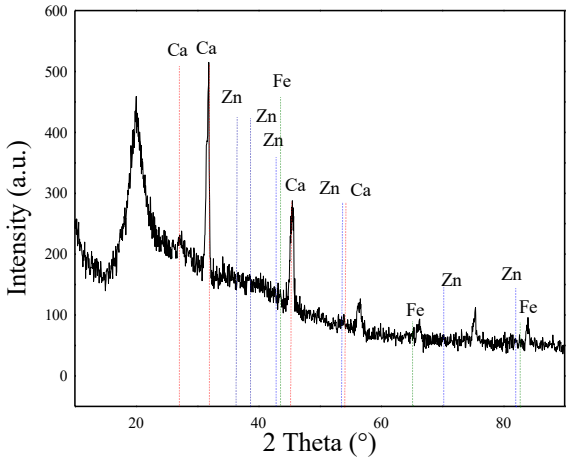

Fig. 1. XRD pattern and EDS spectra of biochemical chitosan from shrimp (*L. vannamei*) shells.

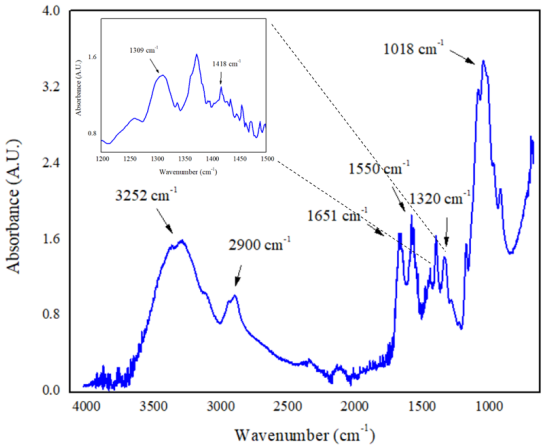

Fig. 2. FTIR spectra of biochemical chitosan from shrimp (*L. vannamei*) shells.

3.2 XRD and EDS analysis

The XRD patterns of the BC powder are presented in Fig. 1. In the XRD results of this research, eight peaks of BC were observed at 10.56, 19.96, 31.78, 45.49, 56.66, 66.32, 75.43, and 83.97°. Seven peaks have been reported in chitosan from six aquatic invertebrates by Kaya *et al.* (2014). However, several research works have reported the two characteristic peaks of chitosan at 10 and 20° that are typical crystal patterns of α-chitin. In this sense, two peaks of BC at 2θ = 10.56° and 19.96° were similar to those reported by Acosta-Ferreira *et al.* (2020) and they are related to the crystallographic planes (020) and (110), respectively. The biopolymer obtained showed 58.91% of CI (Table 1). This CI value was similar (50-65%) to the one reported by El Knidri *et al.* (2016). In this sense, it is important to mention that the solid chitosan molecules could be organized into ordered and crystalline regions and coexist with amorphous regions (Acosta-Ferreira *et al.*, 2020). Also, the intermolecular bonds present after the deacetylation process could explain the difference in the CI values found in the different research works (Martín-López *et al.*, 2020). EDS spectra for minerals of BC are presented in Fig. 1.

EDS results showed that the surface of BC mainly consisted of Ca (26.77, 31.93, 45.42, and 54.23° at 2θ), Zn (36.63, 38.63, 42.73, 53.63, 70.06, and 82.02° at 2θ), and Fe (43.42, 65.25, and 82.61° at 2θ). The relative abundance of minerals in isolated biopolymers could be an important parameter during the purification process. Rahman *et al.* (2023) reported that the surface of shrimp-based chitin and chitosan mainly consisted of C, N, and O; however, other inorganic elements as Ca, Al, Mg, P, and Si, could be in the biopolymer surface by their high acidic affinities forming strong stabilities.

3.3 ATR-FTIR spectroscopy

The FTIR-ATR spectra of the BC are presented in Fig. 2.

The absorption bands at 3252 cm^{-1} (O-H and N-H), 1651 cm^{-1} (N-H), 1147 cm^{-1} (-O-), and 1018 cm^{-1} (C_6-OH) are the infrared characteristic peaks of BC. Li *et al.* (2023) reported similar characteristic absorption bands for commercial chitosan. The bands at 1651, 1550, and 1320 cm^{-1} correspond to the C=O links of the primary amide, the secondary amide N-H links, and the band of the tertiary amide, respectively (Sixto-Berrocal *et al.*, 2023). The band at 2900 cm^{-1} corresponded to the CH2 and CH_3 groups, attributed to C-H asymmetric and symmetric stretching (Affes *et al.*, 2020). A strong band in the 3338-3252 cm^{-1} region in BC corresponds to N-H and O-H stretching as well as intramolecular hydrogen bonds (Hurtado-Fernández *et al.*, 2014).

The value of DDA depends on various factors such as the source and preparation, and also on the type of analytical methods employed, sample preparation, type of instruments used, and other conditions (Mohanasrinivasan *et al.*, 2014). The 1309/1418 cm^{-1} absorbance ratio was used to calculate the DAD (Brugnerotto *et al.*, 2001). The relative 1309/1418 cm^{-1} absorbance of BC was 88.45 which corresponds to the DAD value (Table 1). In another study, Yarnpakdee *et al.* (2022) reported DAD values from 73.56 % to 75.56 % from chitosan of mantis shrimp prepared using various deacetylation times. He *et al.* (2016) mentioned that chitosan may be classified according to its DAD as low (55-70%), medium (70-85%), and high (95-100%). In this study, BC was classified as medium DAD. It is known that a higher degree of deacetylation leads to additional amino groups in the C_2 position of chitosan, with a higher positive charge and stronger antimicrobial activity (Jiang *et al.*, 2023).

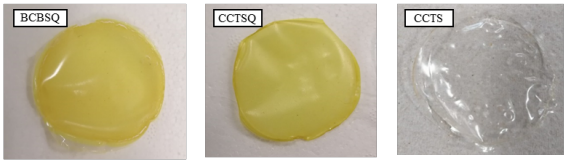

Fig. 3. Active BC_{BSQ} and CCTSQ films with Q concentrations capable of decreasing to initial concentration of DPPH by 50% and control CCTS film without Q.

3.4 *Antioxidant activity*

The antioxidant activities of the active films of BC_{BSQ} and CCTSQ were 56.44% and 62.43%, respectively, whereas the control CCTS presented minimum antioxidant activity (0.92%). In the pre-experimental phase of the study, the antioxidant activity of films with different Q concentrations was preliminarily tested. Therefore, based on preliminary experiments, active films with Q concentrations capable of decreasing the initial concentration of DPPH by 50% were chosen for conducting the Q release tests with food simulants (Fig. 3).

Similar results of the radical scavenging activity (4.96% - 95.15%) have also been reported by Pacheco *et al*. (2019) on active films based on biochemical chitosan, commercial starch, and gallic acid. The presences of flavonoids generally possess higher antioxidant activity because of double bonds existing in the C-ring and this biological activity depends on their structure and hydroxyl group arrangement as the quercetin (Loganayaki *et al*., 2013). In a previous study, Yadav *et al*. (2020) discussed that the quercetin added to starch and chitosan gelatinized film showed better antioxidant activity in comparison to the control film without quercetin. Also, they reported a value of 81.45% of radical inhibition of the quercetin. Other authors, Pech-Cohuo *et al*. (2022) evaluated the antioxidant activity in chitosan and Ramon (*Brosimum alicastrum*) starch films loaded with quercetin and their results did not show radical scavenging activity even when the antioxidant activity was caused by the amino groups in the chitosan structure which react with free radicals resulting in the formation of macromolecular free radicals and highly stable ammonium groups.

3.5 *Release tests*

Fig. 4 displays the Q release profile from active films into food simulants. The results reported in Fig. 4a show that the Q release initially increases with time during the first 9 h in both active films but reaches a steady state from 24 h to 144 h. Rubini *et al*. (2020) reported similar behavior of Q to release into gelatin films, which initially increases with time, but reaches a steady state in a few hours. On the other hand, the Q release in the food simulants at 50% ethanol generally increases during all evaluated times (Fig. 4b). The active BC_{BSQ} film suggested that the Q release (6.2%) into 95% was higher ($p \leq 0.05$) than Q release

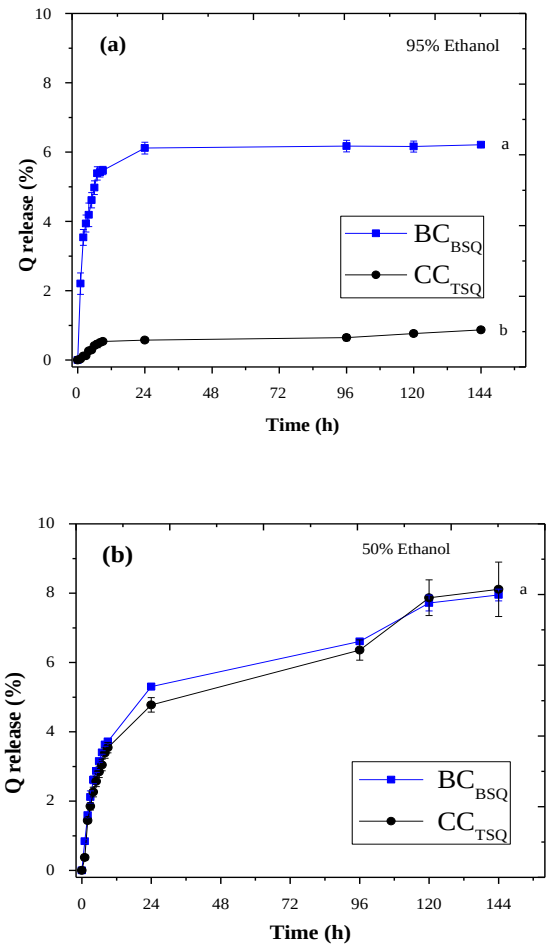

Fig. 4. Kinetic release of quercetin in BC_{BSQ} and CCTSQ films into food simulants: (a) 95% ethanol and (b) 50% ethanol. Different letters indicate a significant difference between means ($p \leq 0.05$).

(0.8%) in CC_{TSQ} film during the evaluated time (144 h). Similar results of carotenoids released into active films in ethanol 95% as food simulant was reported by Assis *et al*. (2021). They mentioned that the release of natural antioxidants was affected by the temperature and time of contact with the food simulant and that all films showed rapid release in the first few hours, followed by a controlled release profile over 10 days of the test. Also, Roy and Rhim (2021) showed that although Q is soluble in alcohol, its release in 95% ethanol was slower than in 50% ethanol, which is most likely due to the low solubility and swelling of chitosan-based films into concentrated ethanol solutions.

Liang *et al*. (2017) reported that the controlled release profile of epigallocatechin gallate into antioxidant edible films based on chitosan hydrochloride was better in a 95% ethanol solution than in 50% ethanol. In the case of the BC_{BSQ} and CC_{TSQ} films in 50 % ethanol, the Q release behavior was very similar to ≈8%. This similar release

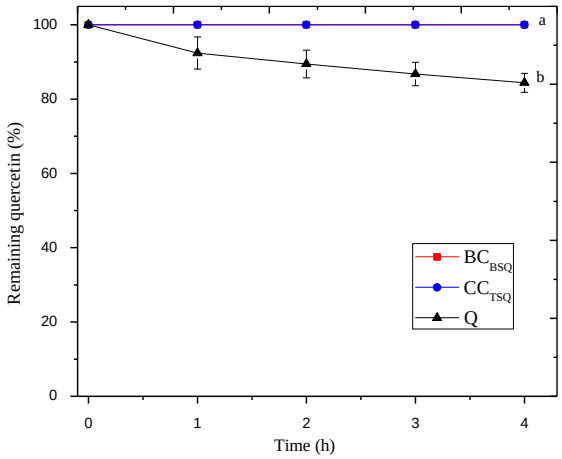

Fig. 5. Remaining percentage of free Q and Q-loaded active films during exposure to UV light for 4 h. Different letters indicate a significant difference between means ($p \leq 0.05$).

profile may be associated with the affinity of Q-biopolymers matrix interaction with 50% ethanol. Q is not soluble in aqueous solutions; however, this flavonoid could enhance its solubility when it is anchored to biopolymeric nanoparticles (Rubini *et al*., 2020). Ezati and Rhim (2021) reported that Q release kinetics in CMC, gelatin, and PLA films into different ethanol solutions as food simulant solutions could be affected by various factors such as food simulant solution, temperature, and type of polymer. They mentioned that Q release showed the highest release in 50% ethanol of gelatin/Q film at 25 °C. In this context, the results suggested that the active BC_{BSQ} film could be a good alternative to release specific bioactive compounds both in high-fat foods (95% ethanol) and foods that simulate oil-in-water emulsions (50% ethanol).

3.6 Q stability in films against UV-light

Among barrier properties, UV-shielding films have gained enormous attention in recent years, since UV light can adversely affect the quality of foods by the generation of free radicals (Yadav *et al*., 2020). Changes in the concentration of free Q and Q-loaded films after exposure to UV light are presented in Fig. 5.

The results showed that Q in both active films was constant (100%) during all evaluated periods (4 h). These results suggest that the polymeric matrix formed by the chitosan/starch protects the flavonoid from UV radiation at least during exposure. In this sense, Yadav *et al*. (2020) reported that the Q-starch complex (H-bonding interactions) could facilitate the interactions between positively charged ammonium groups of chitosan and functional groups present in Q via hydrogen bonds and electrostatic attractions. However, the rapid degradation of free Q (16%) to the UV light during 4 h of exposition may be mainly due to the oxidative decarboxylation of ring C. Similar results were presented by Cuevas-Bernardino *et al*., (2018).

Conclusions

In this study, it was found that BC and BS polysaccharides could be interesting alternatives as biodegradable materials for food packaging applications. Isolated BC from shrimp (*L. vannamei*) exoskeletons had yield, physicochemical, and structural parameters similar to commercial chitosan. XRD and EDS results reveal that the BC had eight peaks and three minerals on the surface, respectively. FTIR measurement confirmed the chemical structure of BC. This research has relevance in the food packaging industry as the first report on active films based on BC, native *Phaseolus polyanthus* starch, and Q; however, the quality parameters of active films such as water vapor permeability, color, thickness, and mechanical properties may be conducted to reinforce the complete characterization of them. The findings of BC_{BSQ} film in food simulants could be showed promising results for future application on high-fat foods and oil-in-water emulsions compared with active films based on commercial biopolymers. Future research should also be conducted to analyze the impact of the longer exposure time to the UV light on the Q-loaded BCBS films.

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