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Physicochemical, structural, mechanical and antioxidant properties of zein films incorporated with no-ultrafiltered and ultrafiltered betalains extract from the beetroot (*Beta vulgaris*) bagasse with potential application as active food packaging

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ABSTRACT

The development of active food packaging based on natural polymers and pigments is of interest to the food industry. Pigments to make this type of packaging can be extracted from waste and by-products, giving added value and reducing production costs. Therefore, the objective of this work was to study the physicochemical, structural, mechanical and antioxidant properties of zein films with non-ultrafiltered (*Z*-NUBE for films or NUBE for extract) and ultrafiltered betalain extract (*Z*-UBE for films and UBE for extract) from beet bagasse as active food packaging. SEM analysis showed that the films with *Z*-UBE showed a more uniform and smoother surface compared to *Z*-NUBE. FTIR analysis showed that the possible interaction of zein with betalains was due to physical forces, mains by hydrogen bonds. TGA analysis showed that the average degradation temperature for all films was around 313 °C, giving a greater weight loss due to the interaction between zein and betalains. Analysis by contact angle determined that films with *Z*-UBE were the most hydrophobic that films with *Z*-NUBE. Also, as the concentration of betalains increases, their hydrophobicity increases. Regarding antioxidant properties, *Z*-NUBE showed better antioxidant activity than films with NUBE, mainly by the FRAP method, *Z*-NUBE1 and *Z*-UBE1 (both with 1% extract) were 251.161 ± 16.45 and 523.49 ± 26.04 μM T/g, respectively. For the phytochemical content, *Z*-UBE showed a higher amount of betalains (specifically betacyanins) with increasing concentration between 56.05 and 76.43%, but similar content of phenolic compounds, less than 0.1 mEq GA/g. Therefore, it is concluded that the addition of UBE to zein films has a potential application in the food industry as active food packaging in refrigerated meats and products stored at room temperature.

1. Introduction

Over time, the export of food has grown and is important for the economy of the countries. Therefore, the storage of food and its arrival in supermarkets has received a greater demand. During the time and conditions that food goes through in handling, transportation and storage, the products begin to dehydrate, deteriorate, lose their color and appearance, taste, and more importantly their nutritional value (Montes Hernández et al., 2017; da Silva Filipini e al., 2020). On the other hand, food and beverage industry generate large amounts of non-ecological

Abbreviations: NUBE, Non-Ultrafiltered Betalain Extract; UBE, Ultrafiltered Betalain Extract; Z-C, Zein control film; Z-NUBE, Zein film with Non-Ultrafiltered Betalain Extract; Z-UBE, Zein film with Ultrafiltered Betalain Extract; FTIR, Fourier-transform infrared spectroscopy; SEM, Scanning electron microscope; TGA, Thermogravimetric analysis; FRAP, Ferric reducing antioxidant power; KGM, Konjac Glucomannan.

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food and plastic waste with a high impact on the environment (Arancibia et al., 2014; Stoica et al., 2020).

Therefore, the need of designing bio based food materials has increased (Mendes and Pedersen, 2021; Qamar et al., 2020). In this purpose, the use of biodegradable biopolymers for food packaging has gained interest given the inconvenience to sustainability that conventional food packaging has (Zhang et al., 2020; Torres-Giner et al., 2021). In addition, renewable polymers are currently the ones that receive the most attention for the manufacture of biodegradable films and be used as packaging in food, and their elaboration is exemplified as an environmentally friendly practice (Ketelsen et al., 2020; Verma et al., 2021). These biodegradable films can be manufactured from various sources such as polysaccharides (Jamróz and Kopel, 2020; Rajeswari et al., 2020), lipids (Zibaei et al., 2021) and proteins (Calva-Estrada et al., 2019; Hassan et al., 2018).

Among the proteins that have been used to make films such as food packaging are corn zein, wheat gluten and milk casein (Chen et al., 2019; Mihalca et al., 2021). Corn zein is defined as the major storage prolamin of the corn-grain endosperm (Kasaai, 2018) and used for obtaining micro- and nanomaterials with application in the food and pharmaceutical industry (Rodríguez-Félix et al., 2020; Tapia-Hernández et al., 2019). The total protein content of zein in corn grain is 8–12 (Tapia-Hernández et al., 2019). Also, zein is soluble in 70% ethanol, and it is a biodegradable, biocompatible and low toxicity biopolymer, as well, generally recognized as safe by the US Food Drug Administration (Rodríguez-Félix et al., 2019; Tapia-Hernández et al., 2018). This protein has been widely used due to its abundance and importance in consumption, which has made it a widely used protein in the manufacture of films (Gaona-Sánchez et al., 2015), nanoparticles (Zhang et al., 2021) and nanofiber (Jiang et al., 2021; 2021b).

Also, currently food packaging no longer has the only objective of containing and protecting food, but rather that the packaging exerts a positive and beneficial effect on the food, that is has an active effect, incorporating bioactive compounds to increase its useful life (Nogueira et al., 2020). This new type of container has allowed the incorporation of new materials to exert positive interactions between the container and the food (Halonen et al., 2020; Montes Hernández et al., 2017) named active packaging.

Active food packaging can be classified according to the function of the additive it contains, among which those that contain antioxidant and antimicrobial activity are the main ones, where those that possess antioxidant activity delay and prevent the appearance of free radicals, acting in oxidation reactions of the molecules present, while those that contain antimicrobial activity inhibit the action of microorganisms that cause contamination and deterioration of products (Umaraw et al., 2020). De Freitas et al. (2018) studied zein films with *Araucaria angustifolia* (Bertol.) Kuntze seed extract and concluded that the extract improved resistance to tensile strength, increased elongation at break, and added antioxidant activity. Similar results were presented by Mushtaq et al. (2018), they studied zein films with pomegranate peel extract and obtained that as the concentration of pomegranate increased, the tensile strength, elongation at break, total phenols and antioxidant activity increased. The incorporation of bioactive antimicrobial and antioxidant compounds to edible films generate great interest because they can be extracted from plants, currently it is taking a boom in obtaining from by-products, or agri-food waste, which can help reduce waste of food, conferring it an added value (Bhargava et al., 2020; Ko et al., 2021; Rodríguez-Félix at al., 2021).

Beetroot (*Beta Vulgaris*) is an important crop in Mexico and its production in 2011 exceeded 14 670 tons (Gómez-Aldapa et al., 2014). The primary processing of beetroot at a global level is through the extraction of its juice with high therapeutic properties, preventing chronic degenerative diseases. In addition, to be used for the extraction of pigments for use in food industry (Aztatzi-Rugerio et al., 2019; Rodríguez-Mena et al., 2021). From its processing, waste rich in batalains known as beet bagasse is obtained.

Betalains are pigments found in plants and foods that are characterized by their high antioxidant activity (Hadipour et al., 2020; Zin et al., 2020). Betalains are water-soluble pigments containing nitrogen considered as powerful antioxidants located in leaves, roots and fruits of some plants related to the order Caryophyllales, where the genera *Beta*, *Amaranthus*, *Opuntia* and *Hylocereus* are found (Gengatharan et al., 2015; Castro-Enríquez et al., 2020). Fig. 1 shows betalains derived from the betalamic acid and comprise two structural groups: red-violet betacyanins with a maximum absorption at 583 nm (including betanin, isobetanin and neobetanin) and yellow-orange betaxanthins with maximum absorption at 480 nm (includes indicaxanthin and vulgaxanthin I and II) (Coy-Barrera, 2020; Sadowska-Bartosz, and Bartosz, 2021; Strack et al., 2003).

The advantage of using materials from the agri-food industry (waste or by-products) as raw material for the production of active food packaging decreases the cost of production when carried on an industrial scale. This is due to the fact that the waste does not have a potential use, production costs can be lowered. Maraveas (2020) mention that despite the tendencies to take care of the ecological aspects of a product, it is not based directly on these parameters, but instead seeks economic aspects for its final decision. However, in the production of films as intelligent packaging, the added value of the product is influenced. Despite this, further studies of economic analysis of the cost of production of obtaining a film based on materials from waste and agri-food by-products should be carried out.

The present study is based on developing an active food packaging's of interest to the food industry. These can be made from natural pigments such as betalains and more recently from betalains obtained from beet bagasse, giving added value. Therefore, el objective was the use the ultrafiltration process to concentrate betalains from the extract and compare the addition UBE and NUBE to zein films. For which, the physicochemical (contact angle), structural (SEM, FTIR, TGA), mechanical (Young's Modulus, Tensile Strength, Fracture Strain) and antioxidant (FRAP, DPPH, FRAP) properties of the films obtained were evaluated. Also, quantification of betalains and phenols were analyzed in the films.

2. Materials and methods

2.1. Chemical reagents

Zein, with a protein content of 86.06% w/w (Sigma Z 3625), glycerol with a purity of ≥99% (Sigma G 5516), gallic acid (3,4,5-trihydroxybenzoic acid), 2 N Folin–Ciocalteu phenol reagent solution, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,20-azino-bis-[3ethylbenzothiazoline-6-sulfonic acid]), TPTZ (2,4,6-tripyridyl-s-triazine), were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethanol was purchased from Fagalab (Sinaloa, Mexico), and distilled water was also used.

2.2. Preparation of beetroot bagasse

The beetroots were purchased in a commercial establishment in the city of Hermosillo, Sonora, Mexico and transported to the agri-food bioprocesses laboratory of the University of Sonora for further processing. For the extraction of the juice and preparation of the bagasse, the methodology described by Janiszewska-Turak et al. (2022) with some modifications. Influence of Fermentation Beetroot Juice Process on the Physico-Chemical Properties of Spray Dried Powder. Molecules, 27 (3), 1008. Firstly, beetroot was washed with water and dried to remove its shell and cut into small pieces. Then, these pieces were incorporated into a juice extractor (Black and Deker, JE2400BD model, 400 W, Townson, Maryland, USA), where the juice and beetroot bagasse were obtained separately. This bagasse was lyophilized (Fig. 2a) in a lyohilizer (LABCONCO FreeZone 18 L Console Freeze Dry System, Kansas, MI, USA) and froze (−20 °C), until its use.

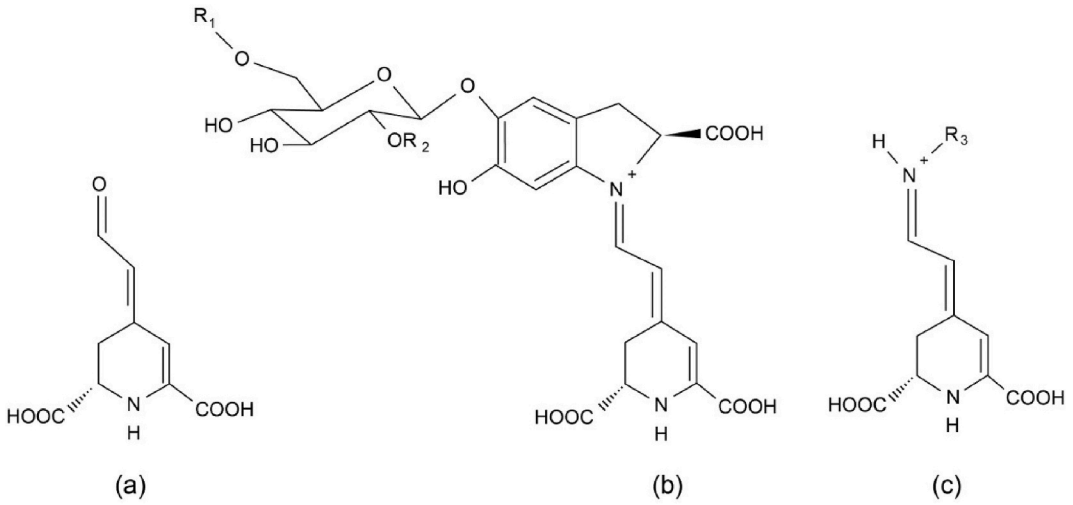

Fig. 1. Chemical structure of betalains: a) bethalamic acid, b) betaxanthins and c) betacyanins structures.

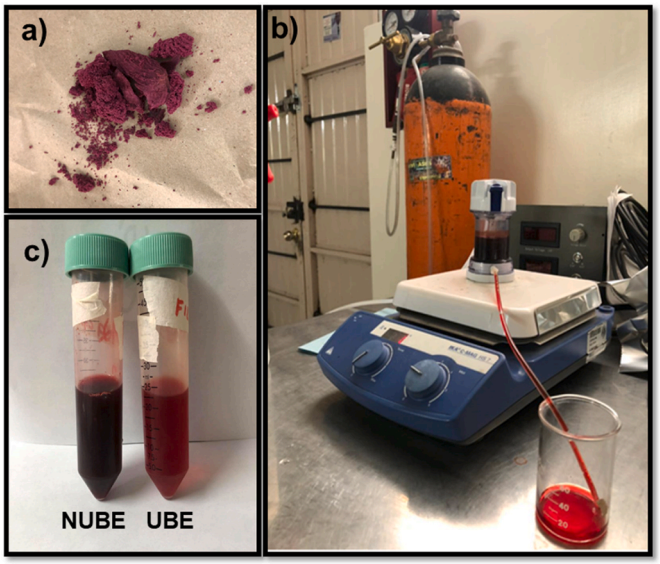

Fig. 2. Bagasse Processing: a) beetroot bagasse, b) ultrafiltration process to obtain the ultrafiltered betalain extract and c) physical comparison no-ultrafiltered betalain extract (NUBE) and ultrafiltered betalain extract (UBE) from beetroot bagasse.

2.3. Preparation of beetroot bagasse extracts

2.3.1. Non-ultrafiltered extract

Following the methodology of Castro-Enríquez et al., 2020) with modifications. Firstly, 2 g of lyophilized bagasse was added to 30 mL of distilled water. After, the sample was sonicated at 40% amplitude for 10 min in a sonicator (BRANSON digital sonifier 102C CE, St. Louis, MO, USA) and then centrifuged at 7500 rpm, 4 °C for 10 min in a centrifuge (OHAUS FRONTIER 5718R, New Jersey, USA). The supernatant obtained was lyophilized and stored at 4 °C, until later analysis. This sample corresponds to the non-ultrafiltered extract.

2.3.2. Ultrafiltered extract

From the liquid extract of the previous process NUBE, the ultrafiltered extract was obtained. The liquid was placed into a 50 mL ultrafiltration cell (Millipore 8050, Darmstadt, DE, Darmstadt, Germany) using 1 kDa membrane at 25 °C and 50 psi nitrogen. The system was left until all the liquid passed through the membrane and was collected (Fig. 2b). This liquid was named as ultrafiltered extract. Then, was lyophilized and stored until its analysis. The procedure was made by triplicated, and membranes were clean after their use with 10% ethanol and water.

2.4. Zein film preparation

According to the methodology of Mushtaq et al. (2018) with modifications, 2 g of zein were added into 10 mL ethanol 95% (v/v) and then 0.3 mL glycerol. The sample were stirred for 10 min using magnetic stirrer and placed on a heating plate (IKA-C-MAG HS 7, Staufen, Germany). After this, (1–3%) of non-ultrafiltered and ultrafiltered betalains extract were added to perform the different treatments (Table 1). The sample was poured on polypropylene petri dishes and stored in a 40 °C oven for 1 h. After this, the samples were brought to room temperature until they took off from the petri dishes. One zein film without betalain extract was made as control.

2.5. Contact angle

The contact angles of zein films with non-ultrafiltered and ultrafiltered betalain extract measured using Milli-Q water at room temperature and following the methodology described by Lenzuni et al. (2021) with some modifications. The method used was by sessile drop using a ChemInstruments CAM-PLUS apparatus, Fairfield, USA. The angle (θ) data reported is the result of 10 measurements for each zein film treatment.

2.6. Scanning electron microscope (SEM)

Surface morphology of zein films with non-ultrafiltered and

Table 1
Abbreviation and conditions for obtaining zein films with beetroot bagasse betalain extract.

Sample	Films Abbreviation	Zein % (p/v)	Glycerol mL	Extract % (p/v)
1	Z-C*	20	0.3	0
2	Z-NUBE1**	20	0.3	1
3	Z-NUBE2	20	0.3	2
4	Z-NUBE3	20	0.3	3
5	Z-UBE1***	20	0.3	1
6	Z-UBE2	20	0.3	2
7	Z-UBE3	20	0.3	3

*zein control film, ** zein film with no-ultrafiltered betalain extract, *** zein film with ultrafiltered betalain extract.

ultrafiltered betalains extract were analyzed in an equipment SEM (JEOL 7800F, Pleasanton, CA, USA). The determination was made at 10 kV and magnification of 1000x. The sample were fixed in the sample holder and covered themselves with a layer of gold for analysis.

2.7. Fourier-transform infrared spectroscopy (FTIR)

To observe the interaction between zein and betalains extract in the different treatments, FTIR was used. Analysis was carried out in a FTIR spectrophotometer (Frontier, PerkinElmer, Waltham, USA). A spectrum scan from 4000 to 500 cm^{-1} was used and an average of 16 scans was recorded.

2.8. Thermogravimetric analysis (TGA)

To determinate the stability and thermal degradation of films TGA was used. TGA analysis was carried out in a PerkinElmer, Pyris 1 model, Shelton, CT, USA. 4 mg of sample were heated from 25 °C to 900 °C at a heating and cooling rate of 10 °C· min^{-1} under a flow of 20 mL min^{-1} of nitrogen gas.

2.9. Mechanical properties analysis

Tensile mechanical properties were measured from a mechanical testing machine Electroforce 5110 (TA Instruments, Minnesota, USA), using a 200N load cell and a cross-head rate of 1 mm s^{-1}. The methodology used was described by Mathew et al. (2022) with modifications and the determinations analyzed were obtained from Chan-Chan et al. (2017). Samples were cut in rectangles of 20 mm length, 5 mm width and 0.2 mm tick approximately. Young's modulus from the linear section, tensile strength and fracture strain were reported.

Statistical analysis was performed by OriginPro 2021b) software (Origin Lab Co.). Normal distribution of the data was confirmed by Kolmogorov-Smirnov test and the equal variance by Levene's test. ANOVA One-Way was used to determine statistically significant differences and TUKEY (for equal variances) or Scheffe (if variances are different) post-hoc test were used for determining differences between formulations.

2.10. Antioxidant activity

2.10.1. ABTS radical scavenging assay

Following the methodology of Del-Toro-Sánchez et al. (2021). Firstly, 19.3 mg of ABTS radical was added to 5 mL distilled water. Separately, 0.0378 g of potassium persulfate and 1 mL distilled water were mixed. 88 μL persulfate solution were taken and added to ABTS solution, where it was left in the dark for 12 h. After, a solution adjusted to an absorbance of 0.7 ± 0.01 of 734 nm. Subsequently, 270 μL of the radical solution were taken and 20 μL of sample was added in a 96-well microplate. Then, left to rest for 30 min and the absorbance was read at 734 nm. A calibration curve of 0–0.1 mg/L of μMEqT/g sample was performed. Measurements were made in quadruplicate.

2.10.2. Radical scavenging capacity using the DPPH method

It was carried out based on the methodology proposed by Del-Toro-Sánchez et al. (2021). First, a solution of the DPPH radical was prepared, from 1.5 g of the DPPH radical in 50 mL methanol, which was adjusted to an absorbance of 0.7 ± 0.01 at 515 nm. From this mixture, 200 μL of the radical was added to 20 μL of sample in a 96-well microplate. Then, left to rest for 30 min in the dark and the absorbance was read at 515 nm. A calibration curve of 0–0.1 mg/L μMEqT/g sample was performed. Measurements were made in quadruplicate.

2.10.3. Ferric reducing antioxidant power (FRAP) assay

The FRAP analysis was carried out following the methodology of Pérez-Perez et al. (2020). First, a stock solution was made at acidic conditions (pH 3.6) that includes a 300 mmol/L sodium acetate buffer solution. The iron complex was prepared with 20 mmol $FeCl_3{*}6H_2O$ in a TPTZ 40 mmol HCl solution. For the FRAP solution, the solution was added in a ratio of 10: 1: 1 buffer, $FeCl_3{*}6H_2O$, TPTZ HCl, respectively. 20 μL of sample was added to 280 μL of the FRAP solution. Readings were taken at 30 min on a microplate reader at 638 nm. A calibration curve of 0–0.1 mg/L μMEqT/g sample was performed. Measurements were made in quadruplicate.

2.11. Quantification of betalains

The quantification of total betalains, betaxanthins, and betacyanins was based on the methodology described by Castro-Enríquez et al. (2020). The determinations were carried out by measuring the absorbance at 538 nm (for betacyanins) and 483 nm (for betaxanthins), using a UV–vis Spectrophotometer (Varian Co. Ltd., California, USA). The amount of betalains was calculated by the following equation

$$B\left(\frac{mg}{g}\right) = \frac{A{*}DF{*}MW{*}V}{\varepsilon * W * L}$$

where [B] means the betacyanins or betaxanthins concentration, A is absorbance of the sample, DF is dilution factor, MW is molecular weight (betanin 550 g/mol) (indicaxanthin 308 g/mol), V is volume, ε is molar extinction coefficient, for betanin it is 60 000 mol/L × cm and for indicaxanthin 48, 000 mol/L × cm, W is the weight of the sample (g) and L is the length of the cuvette (1 cm). The total betalains concentration was obtained with the sum of betacyanins and betaxanthins content.

2.12. Quantification of total phenols

Determination of total phenols was carried out with the Folin–Ciocalteu (FC) method (Del-Toro-Sánchez et al., 2021). Firstly, 10 μL of sample was mixed with 25 μL of FC 1 N were added in a 96-well microplate. The samples were left in the dark for 5 min at 25 °C. Then, 25 μL of 20% Na_2CO_3 and 140 μL of distilled water were added. It was allowed to stand for 30 min and the absorbance at 760 nm was determined. The results were expressed as milligrams of gallic acid equivalents for gram of dry sample (mg GAE/g). Measurements were made in quadruplicate.

2.13. Experimental design and statistical analysis

To determine the best film, different proportions of beet bagasse extract were added without ultrafiltration and ultrafiltered at three levels (1, 2 and 3%). To compare total phenols, antioxidant activity and betalains concentration in extracts and films, in addition to contact angle, the Tukey test was used with a confidence level of 95% ($p < 0.05$) using the statistical program Infostat 2008.

3. Results and discussion

3.1. Comparison of no-ultrafiltered and ultrafiltered betalain extracts

Fig. 2c shows the non-ultrafiltered and ultrafiltered betalains extract obtained from beet bagasse. First, the extract without ultrafiltration presented a dark red coloration due to the amount of bioactive compounds present, mainly phenolic compounds and betalains. This type of compounds presents in the crude extract (without ultrafiltration) have already been previously reported in beet samples. According to Kumar and Brooks (2018), they reported that the crude extract is rich in betalains, mainly betacyanins (betanin, isobetanin, neobetanin and their derivatives) and betaxanthins (miraxanthin, vulgaxanthin, isovulgaxanthin, isoindicaxanthin and their derivatives). Betanin is the main betalain in beetroot with y 300–600 mg/kg (Aztatzi-Rugerio et al., 2019), therefore, it is the main in beetroot bagasse, and can be used in

foods as E−162 (UE) and 73.40 (FDA, USA) color additives (Carreón--Hidalgo et al., 2022). In addition, Kumar and Brooks report that the phenolic compounds present are phenolic acid type (caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, syringic acid, vanillic acid) and flavonoids (Betagarin, Betavulgarin, Catechin, Dihydroisorhamnetin, Epicatechin, Kampferol, Myricetin, Rutin and Quercetin).

On the other hand, the ultrafiltered betalains extract (Fig. 2c) presented a light red coloration, because, once the extract was placed by the ultrafiltration process, most of the phenolic compounds present are retained in the membrane and only betalains and phenolic compounds with molecular weight similar to betalains. Castro-Enríquez et al. (2020) mention that, in the ultrafiltered pitaya extract, the ultrafiltration process helps to remove the phenolic compounds present in the extract but retains the concentration of betalains (Betacyanins and Betaxanthins). In addition, it mentions that the compounds that can pass through the membrane are mainly phenolic acids (ferulic acid, caffeic acid, p-coumaric acid) and very rarely flavonoids (quercetin). dos Santos et al. (2016) studied the effect of microfiltration and ultrafiltration processes and concluded that these processes clarify the extract, eliminating compounds such as peroxidases, showing a less turbid liquid with a more intense color than the extract without ultrafiltration, a similar result was obtained for the present study.

3.2. Physical characteristics of Zein films

Fig. 3 show the films developed in the present study, which have an average thickness of 0.2245 ± 0.09697 mm. The addition of extract did not show an increase in thickness, due to the low concentration of added extract. This has been previously reported by other researchers. Moradi et al. (2016) prepared zein films containing *Zataria multiflora* Boiss extract and observed that all the films had a very similar thickness (0.095 mm), with and without the addition of extract. These films exhibited a dull yellowish appearance and a waxy surface. According to Pena-Serna and Lopes-Filho (2013) this is a consequence of the poor interaction between glycerol and zein, causing an excess of glycerol that migrates from within the film to the surface. On the other hand, the yellow color of the films is due to the high concentration of zein used and there is no color influence due to the addition of betalains at the three added concentrations (1, 2 and 3%). This color effect with no change compared to the control film was for both non-ultrafiltered betalains extract and ultrafiltered betalains extract zein films.

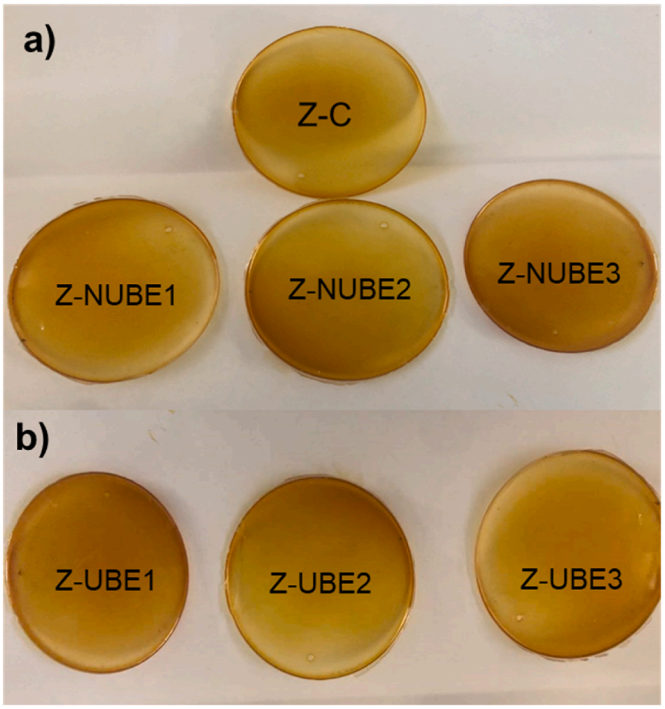

Fig. 3. Physical appearance of zein films with a) no-ultrafiltered betalain extract (NUBE) and b) ultrafiltered betalain extract (UBE) from beetroot bagasse.

3.3. Chemical-structural analysis of films by FTIR

In Fig. 4 the infrared spectra of non-ultrafiltered and ultrafiltered betalains extract are shown. In both bands they have the O–H bond at 3200 cm^{-1}, they also have the vibration of the aromatic ring around 1000 cm^{-1} which corresponds to the structure of betalains, or it could be the tension vibration of the C–O link (Oliveira et al., 2016). The ultrafiltration process does not show any displacement of any band, so there is no difference between the bands, which can be attributed to the fact that they are the same compound.

Fig. 5 shows the comparison of infrared spectra of zein films with 1% of non-ultrafiltered extract and ultrafiltered betalain extract. First, a similar pattern can be observed in the bands of the zein control film, and in *Z*-NUBE1% and *Z*-UBE1%. Analyzing the bands in a general way, a broad band is observed in the near infrared region at 3400-3000 cm^{-1} and more acute bands in the region of 1700–1000 cm^{-1}. In these bands, the 3273 cm^{-1} band can be identified, which corresponds to the tension vibration of the N–H bond. At 1645 cm^{-1}, 1537 cm^{-1} and 1415 cm^{-1} the presence of amides I, II and III, respectively, are identified. It was also possible to identify the 711 cm^{-1} band that corresponds to the C–S bond, which may be indicative of covalent interactions between the phenolic compounds of betalains and zein proteins. When analyzing the values of the bands, no differences were found, so there is no band shift, this means that the influence of ultrafiltration did not significantly change the interaction with the films.

Fig. 6 shows the infrared spectra of *Z*-NUBE1%, *Z*-NUBE2% and *Z*-NUBE3%. From the bands obtained, in the region around 3400 cm^{-1} compared to the bands of the extract and ultrafiltrate. Firstly, the zein films present the appearance of a characteristic band of the proteins corresponding to a vibration by tension of the N–H bond shown at 3273 cm^{-1}. Other important bands are observed at 1645 cm^{-1}, 1537 cm^{-1} and 1415 cm^{-1} which indicate the presence of amide I, amide II, and amide III, respectively. These bands are characteristic of polymers with a protein structure where amide I corresponds to a vibrational stretching of the C═O bond, amide II is due to the bending of the N–H bond and

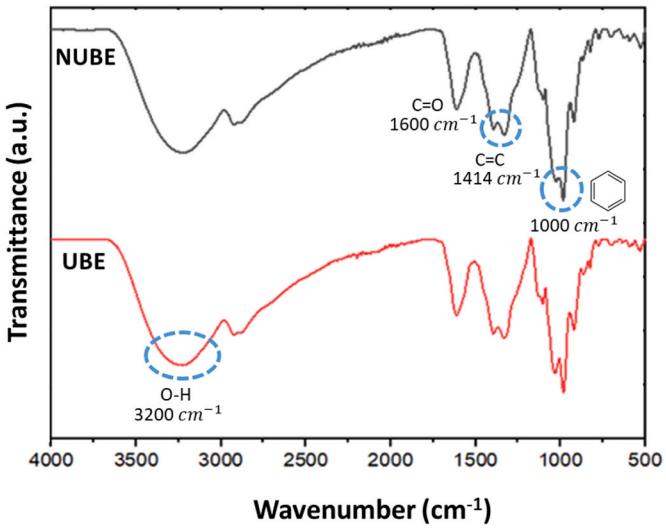

Fig. 4. FTIR spectrum of extracts: no-ultrafiltered betalain extract (NUBE) and ultrafiltered betalain extract (UBE) of beetroot bagasse.