







Nanoencapsulation of Polyphenolic-Rich Extract from Biloxi Blueberries (*Vaccinium Corymbosum* L.) by Electro spraying using Zein as Encapsulating Material

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Abstract: Zein prolamine nanocapsules encapsulating polyphenolic compounds extracted from Biloxi blueberries were produced by means of the electro spraying technique and characterized. Nanocapsules were prepared with different ratios zein:extract 10: 1, 5: 1, 3.3: 1, 2: 1, using glycerol as an adjuvant. The SEM analysis showed homogeneity in the morphology of the nanocapsules, being not completely spherical, with a smooth surface, without cracks or dents, and with sizes around 200 to 300 nm. The DSC analysis showed that there were no strong interactions between the core and the encapsulating materials used. The study of the photoprotective effect revealed that zein is capable of photostabilizing the phenolic compounds present in the extract. The TGA analysis showed that the encapsulated extract presented a displacement of 100 °C in the decomposition temperature in comparison with the unencapsulated extract, which indicates that the zein offers thermoprotection to the phenolic compounds.

Keywords: electro spraying; polyphenols; zein.

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1. Introduction

Blueberry is a fruit of high economic importance worldwide, which is well-known due to its high concentration of polyphenols, among which anthocyanins stand out [1]. Blueberry Biloxi variety showed a higher concentration of polyphenols and anthocyanins than others varieties such as blue crop, Elliot, Darrowii [2]. Anthocyanins have shown biological activity concretely to prevent chronic degenerative diseases [3]. However, phenolic compounds are susceptible to structural modification due to the effect of temperature, pH, exposure to oxygen, light, and the presence of acids, which causes the decrease or loss of their biological activity [4].

In this sense, the alternatives to protect photo and thermolabile compounds are varied. Nowadays, nanoencapsulation is the most popular process; it uses polymeric matrices to wrap compounds of interest positioned in the center. This technique provides greater stability and controlled release of the active compound associated with the nanometric size of the capsule.

In addition, it causes minimal changes in the sensory properties of food [5]. The production of nanocapsules has been carried out using a wide range of techniques, including spray drying, emulsion-crosslinking, and coacervation [6]. However, these techniques use high temperatures or organic solvents, which causes the loss of bioactivity and the decomposition of encapsulated compounds [7]. In this regard, electrospray is a simple, versatile, and non-thermal process used to produce capsules. This process applies an external electrostatic field between two electrodes to a polymer solution. As the electrostatic field increases, the electrical charges on the surface of the pendant drop overcome the surface tension, and the shape of the drop changes from partially spherical to conical (Taylor's cone effect). As the charged jet accelerates to lower potential regions, the solvent evaporates, and the capsules are obtained in the collector [6]. The electrospraying process has been widely used to encapsulate functional ingredients with applications in the food industry. This technology has been used to encapsulate bioactive compounds such as vitamins, probiotics, antioxidants, and omega-3 fatty acids [8, 9]. The encapsulates obtained through electrospraying exhibited higher bioavailability than traditional capsules since this process generates particles with small particle size distribution and high encapsulation efficiency, and the process is carried out at ambient temperature [6].

There is a growing interest of the food, pharmaceutical, and biomedical industry to use GRAS (Generally Recognized as Safe) encapsulating materials capable of protecting bioactive compounds, with physicochemical characteristics such as being biocompatible, biodegradable, resistant to moderate to high temperatures, mechanical resistance, modifiable, as well as having protective properties against oxygen, light, and humidity [10]. In this sense, zein is a protein extracted from corn belonging to the prolamines family. It is soluble in alcohol classified as safe for human consumption. In addition, zein has excellent mechanical properties, it is biocompatible, biodegradable, modifiable, and its hydrophobic nature gives it the ability to act as an oxygen barrier [11]. However, hydrophobic materials require the incorporation of surfactants or materials with adjuvant properties to improve the encapsulation efficiency of hydrophilic compounds [12].

The adjuvant most used in the pharmaceutical and food industries is glycerol, which belongs to the group of alcohols. Glycerol is a viscous liquid of low toxicity and sweet taste. It has been used in various biological applications to protect proteins, stabilize them or improve their solubility [13]. The hydroxyl groups in the glycerol molecule make it easily miscible in water, hygroscopic, similarly to sugars. It has recently been used as a vehicle for incorporating bioactive compounds in polymeric matrices [14].

The objective of this study was to evaluate the encapsulation capacity of zein in the protection of polyphenolic compounds extracted from blueberry, using glycerol as an adjuvant, by means of the electrospraying technique. The nanocapsules produced were characterized by means of morphology, particle size distribution, color, encapsulation efficiency, chemical structure, photostability, and thermal resistance.

2. Materials and Methods

The Biloxi blueberry fruits were obtained from crops in Tepic, Nayarit, Mexico (Latitude: 21.5039, Longitude: -104.895 21 ° 30 ' 14 " North, 104 ° 53 ' 42 " West); Later, they were frozen at -80 and lyophilized in a Freezone 4.5 (Labconco, Kansas City, MO, USA) and stored at 25 °C and 30% relative humidity (RH) until use. Zein prolamine from corn and glycerol, Folin-Ciocalteu 2N phenol reagent, potassium persulfate, 2,2-azinobis-3-

ethylbenzotiazoline-6-sulfonic acid (ABTS), methanol, and ethanol were purchased from Sigma-Aldrich (Madrid, Spain). Distilled water was used throughout the study.

2.1. Production of Biloxi blueberries extract.

The polyphenolic compounds from Biloxi blueberries were extracted according to the methodology of González-Cruz *et al.* [2]. Methanol acidified with citric acid at 1% (w/v) was used as an extraction solvent. One gram of lyophilized Biloxi blueberries was extracted in 10 mL of extraction solvent. The mixture was sonicated for 30 min in a Kendal CD-4820 (Pittsburgh, USA), at a constant frequency of 42 kHz at 25 °C. The extract was filtered using Whatman N°1 filter paper and concentrated on a rotary evaporator (IKA RV Basic S1, Staufen, Germany) at 45 °C.

2.2. Preparation of the polymer solution.

The 10% (w/w) zein solution was prepared in 70% (v/v) ethanolic solution with 1% glycerol (w/v). Solutions with different extract content were prepared with zein:extract ratios of 10:1, 5:1, 3.3:1, and 2:1. The solutions were homogenized under magnetic stirring for 10 min at 300 rpm. The extract concentration of total soluble polyphenols was determined, and the anthocyanins were previously identified [2].

2.3. Encapsulation by electrospraying.

The polymer solutions were subjected to electrospraying in Fluidnatek LE-10 equipment (Bioinicia, Valencia, Spain). The electrospraying device was equipped with a variable high-voltage power supply (0-30 kV). Solutions were introduced in a 5 mL plastic syringe and were electrospun under a steady flowrate using a stainless-steel needle of 700 µm diameter. The needle was connected through a polytetrafluoroethylene (PTFE) tube to the syringe. The syringe was lying on a digitally controlled syringe pump while the needle was horizontal towards the collector. Electrospraying conditions for obtaining capsules with the prepared solutions were optimized at fixed at 0.1 mL/h of flowrate, 17.4 kV of voltage in the needle, and a tip-to-collector distance of 18 cm. Collected capsules were stored in a desiccator and protected from light for subsequent analysis.

2.4. Scanning electron microscopy (SEM).

1 mg of sample was fixed on a double-sided adhesive tape and placed on a metal slide; subsequently, the samples were coated with gold-palladium for 3 min and were observed in a Hitachi S-4100 scanning electron microscope (Tokyo, Japan) with an acceleration voltage of 5 kV, to evaluate the morphology of the capsules. Particle diameters were determined using Image J Launcher v1.41 (National Institutes of Health, Bethesda, MD, USA). The medium particle size is based on measurements from a minimum of 100 microparticles.

2.5. Nanocapsules encapsulation efficiency.

This parameter determines the quantity of phenolic compounds adhered to the surface of the capsule in comparison to the total concentration of phenolic compounds in the capsule. 10 mg capsules were placed in a funnel with Whatman n°1 filter paper and washed with 5 mL of 1% (v/v) formic acid water solution in order to drag the phenolic compounds on the

nanocapsules surface. Finally, the concentration of total soluble phenols was determined by the Folin Ciocalteu method described in section 2.6. The samples were analyzed in triplicate, and the results were expressed according to equation 1.

$$EE\% = \frac{PT - PL}{PT} * 100 \quad \text{Eq. 1}$$

where EE is the encapsulation efficiency expressed in percentage, PL is the amount of polyphenol on the nanocapsules surface, and PT is the total amount of polyphenols in the nanocapsules

2.6. Determination of total soluble polyphenols in the nanocapsules.

Total soluble polyphenols (TSP) were analyzed via the Folin-Ciocalteu method. The PST content of the nanocapsules was determined by diluting 10 mg of nanocapsules in ethanol at 70% (v/v). After dissolution, 250 μ L of the nanocapsules solution were mixed with 1 mL of sodium carbonate 7.5% (w/v) in test tubes, and it was left to stand for 5 min. Subsequently, 1.25 mL of the Folin reagent solution 10% (v/v) were added. The tubes were placed in a water bath at 55 °C for 15 min. Finally, the absorbance was measured at 780 nm in a Varian Cary 50 UV-Vis spectrophotometer (Sydney, Australia). Nanocapsules without extract were used as control. Measurements were made in triplicate.

2.7. Determination of the color of the nanocapsules.

A Konica Minolta CR-300 colorimeter (Osaka, Japan) was used for color analysis. One gram of sample was placed in Petri dishes, and readings were done directly on the powder. Zein capsules without extract were used as a control. The equipment determines the parameters L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) on the Cie L*a*b* system scale (International Commission on lighting). The total color difference (ΔE) was determined according to equation 2.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad \text{Eq. 2}$$

where L, a, and b are the differences between the sample color parameter and the white plate color standard, which is used as the capsules background.

2.8. Determination of free radical inhibition (ABTS) of nanocapsules.

The free radical inhibition capacity of the nanocapsules was determined by the ABTS method. A 7 mM solution of ABTS reagent was prepared with potassium persulfate at 2.45 mM in distilled water, which was left stirring for 16 h in the dark. The absorbance at 734 nm was adjusted with 85% ethanol (v/v), up to 0.70 ± 0.02 . The sample was prepared by diluting 3.5 mg of nanocapsules in 1 mL of ethanol, homogenized for 5 min, and centrifuged at 13000 rpm for 5 min. 50 μ L of the nanocapsules solution in 85% ethanol (v/v) were added to 950 μ L of the ABTS solution, and it was left to react for 1 min. Subsequently, the absorbance was measured at 734 nm on a Varian Cary 50 UV-Vis spectrophotometer (Sydney, Australia). The samples were analyzed in triplicate.

2.9. Study of photoprotection of nanocapsules.

The protective effect of the capsules was evaluated by exposing them to ultraviolet light, with a 300 W lamp (Osram Ultra Vitalux, Munich, Germany). 1 g of nanocapsules were placed in Petri dishes, they were exposed to ultraviolet light (315-480 nm) at a distance of 15

cm from the irradiation source. The percentage of free radical inhibition was calculated according to the method proposed in section 2.5. Measurements were made for the encapsulates in periods of 24 h. The results were expressed as % ABTS inhibition according to equation (3).

$$\% \text{ ABTS inhibition} = \frac{(A - B)}{A} \times 100 \quad \text{Eq. 3}$$

where, A is the absorbance of ABTS control solution, and B is the absorbance of the sample.

2.10. Attenuated Total Reflectance Fourier transform infrared spectroscopy (ATR-FTIR).

The ATR-FTIR spectra of the capsules were obtained on a Bruker Tensor 37 FTIR spectrometer (Ettlingen, Germany). Approximately 50 mg of capsules were placed on the ATR accessory (Specac Ltd Golden Gate, Low-Temperature ATR Sampling Accessory, Orpington, U.K.). All spectra were recorded within the wave range of 4000-600 cm^{-1} averaging 10 scans at a resolution of 4 cm^{-1} . All measurements were made in triplicate. The spectral data analysis was carried out using the OPUS 4.0 Bruker program (Ettlingen, Germany).

2.11. Differential scanning calorimetry of nanocapsules.

Glass transition temperatures (T_g) were determined by differential scanning calorimetry (DSC) analysis. A DSC 250 (TA Instruments, New Castle, USA) was used. 5 mg of sample were placed in hermetically sealed aluminum crucibles. A heating ramp from 25 to 250 $^{\circ}\text{C}$ was used at a heating rate of 5 $^{\circ}\text{C}/\text{min}$, in a nitrogen atmosphere [15]. The DSC was calibrated using indium as standard (T_{onset} : 156.6 $^{\circ}\text{C}$). Measurements were made in triplicate and the data obtained were analyzed with the TRIOS software (TA Instruments).

2.12. Thermogravimetric analysis of the nanocapsules.

The thermogravimetric analysis of the nanocapsules with extract in a 3.3:1 ratio, was carried out on TGA 550 equipment (TA Instruments, New Castle, USA). Approximately 15 mg of sample were placed in a platinum crucible, using a heating ramp from 25 to 800 $^{\circ}\text{C}$, at a heating rate of 5 $^{\circ}\text{C}/\text{min}$, in a nitrogen atmosphere. Measurements were made in triplicate, and the data obtained were analyzed with the TRIOS software (TA Instruments).

3. Results and Discussion

3.1. Morphology of the nanocapsules.

The encapsulates presented a non-completely spherical morphology with smooth and continuous surfaces regardless of the extract concentration and with a slight tendency to form aggregates, as shown in Figure 1. This result is similar to that obtained by Gomez-Estaca *et al.* [15], who reported this type of structure when encapsulating curcumin into zein using the electrospraying technique. The agglomeration phenomenon is attributed both to the glycerol's hygroscopicity and the size of the particles [16]. In this sense, relative humidity is a factor to control to avoid water absorption and changes in the morphology of the capsules.

The nanocapsules made without glycerol (control) presented smooth surfaces (Figure 1f), without cracks or dents, without agglomerations, and in the shape of a plate. The morphologies of the nanocapsules with glycerol (Figure 1d) differ from the control nanocapsules (without glycerol), so the adjuvant negatively affects the encapsulates morphology, provoking a negative effect on the appearance of cracks or dents and increasing

the hygroscopicity and agglomeration of the capsules. Likewise, it has been shown that this polymer by itself does not offer spherical morphology, except for the presence of surfactants and oils due to their lipophilic character related to the hydrophobic character of the protein [17].

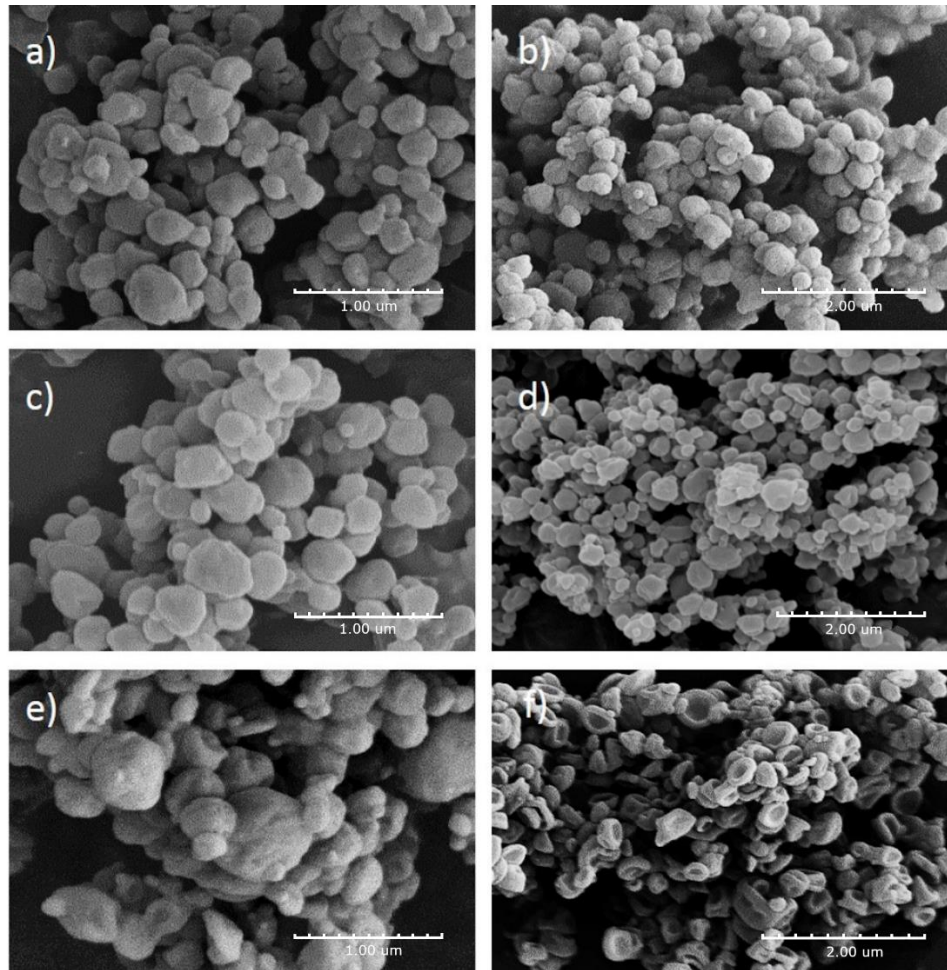


Figure 1. Scanning electron microscopy of zein capsules with different ratios (zein: extract). (a) Capsules 1: 0, (b) capsules 10: 1, (c) capsules 5: 1, (d) capsules 3.3: 1, (e) capsules 2: 1; and (f) control capsules 3.3: 1.

Those encapsulated with and without glycerol at different extract concentrations presented a similar particle size distribution (Figure 2), more frequently in diameters between 200 and 300 nm. These results are similar to those obtained by Gómez-Mascaraque *et al.* [18], who encapsulated epigallocatechin gallate with zein as encapsulating matrix by electrospaying, obtaining sizes between 200 and 500 nm.

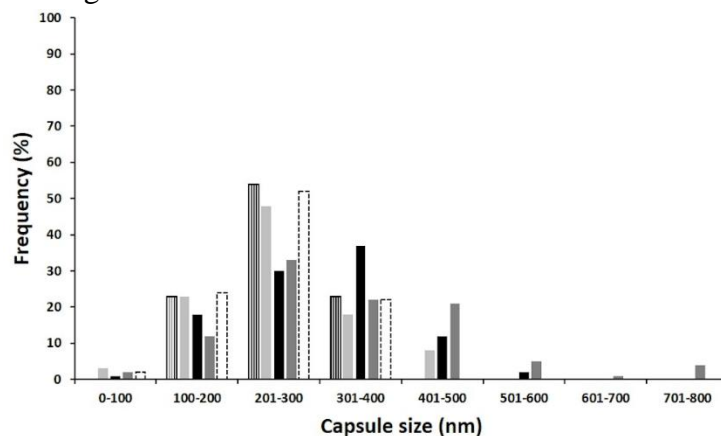


Figure 2. Particle size distribution of zein capsules with different ratios (zein: extract). Capsules 10:1 (□), capsules 5:1 (■), capsules 3.3:1 (■), capsules 2:1 (■) and capsules control (□).

3.2. Encapsulation efficiency.

The encapsulates presented an encapsulation efficiency higher than 50% in all the conditions studied. It was observed that in the 10:1, 5:1, and 3.3:1 ratios (polymer: extract), the behavior was upward, while in the 2:1 ratio, the encapsulation efficiency decreased (Figure 3). This decrease in encapsulation efficiency may be related to the coating limit of the zein. This property is conditioned by the concentration and molecular weight of the polymer in relation to the active compound to be encapsulated. The obtained results differed from Anu Bhushani and Anandharamakrishnan [19], who encapsulated epigallocatechin into zein by electro spraying, obtaining encapsulation efficiencies of 92.75% for the 10: 1 zein: bioactive compound ratio and 89.96% for the 5:1 ratio. However, the encapsulation efficiency obtained with the 3.3:1 ratio was 99.16% and was higher than the reported by these authors for the 50:1 ratio. The difference between these values may be associated with the type and chemical structure of the compound and the moderate operating conditions of the electro spraying technique [20, 21].

To evaluate the effect of glycerol on the encapsulation efficiency of phenolic compounds, capsules were made without this adjuvant, with the ratio of 3.3:1, which presented the highest encapsulation efficiency. It was observed that the capsules without glycerol presented a lower encapsulation efficiency of 86.7%. This result demonstrated that incorporating glycerol in the formulation for the encapsulates preparation helps maintain the phenolic compounds within the core of the capsule (Figure 3). This behavior may be related to the polarity of the glycerol and its amphiphilic character, which allows a better affinity of the action with the polymer. These results are similar to those obtained by López-Rubio and Lagaron [22], who evaluated the effect of incorporating glycerol in the preparation of whey protein capsules for the encapsulation of β -carotene, they concluded that using it as a vehicle favors the stability of the compound keeping it in the core.

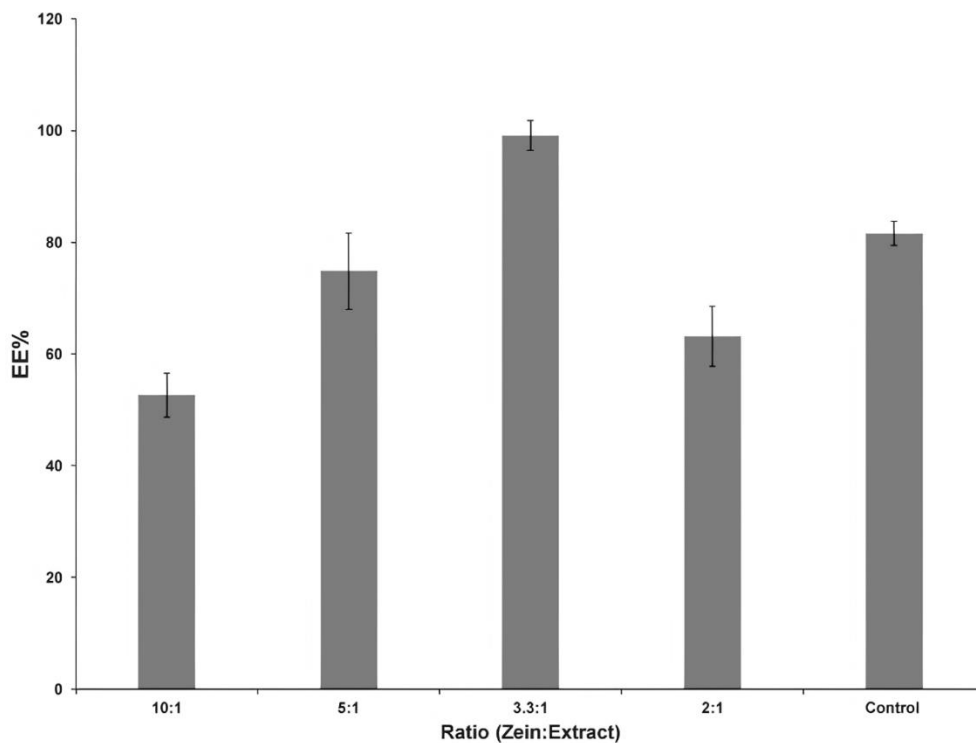


Figure 3. Comparison of the encapsulation efficiency of each sample at different zein:extract ratios. The control sample was produced with a ratio of 3.3:1 without glycerol.

3.3. Color evaluation of nanocapsules.

The color of the nanocapsules was evaluated as a control parameter of anthocyanins stability present in the extract of Biloxi blueberries. The nanocapsules presented total color differences (ΔE) in all cases greater than 3 (Table 1), which means that for all concentrations, the color difference was perceptible to the human eye. Likewise, color preservation showed that the conditions in which the encapsulation process was carried out did not cause structural change in anthocyanins. It was observed that the luminosity decreased, and the chromaticity increased with respect to the concentration of the extract. Likewise, the increase in hue saturation was associated with the concentration of chromophore compounds in the encapsulates. This behavior is similar to that observed by Prietto *et al.* [23]; these authors reported an increase in tone saturation by increasing the concentration of fruit extracts with anthocyanins to manufacture pH indicators.

The nanocapsules with the 3.3:1 ratio and the control (capsules without glycerol) were compared to verify the adjuvant's effect on color (Table 1). The nanocapsules showed no perceptible color difference between the two ($\Delta E = 0.91$). This can be explained because glycerol is colorless alcohol, which shows that its incorporation does not change the color of the nanocapsules. The positive values of Chroma (C^*) and H assign the pink color to the nanocapsules, with a hue saturation of 27% and 88% lightness. This hue suggests that the phenolic compounds, especially anthocyanins, remained stable, absent from structural modification.

Table 1. Color evaluation of nanocapsules with different concentrations of extract.

Ratio (Z:E)	L*	a*	b*	Chroma	h	ΔE
1:0	92.04	1.30	3.36	3.60	68.80	-----
10:1	92.04	1.30	3.36	3.60	68.80	3.62
5:1	90.60	4.12	1.51	4.39	20.13	7.02
3.3:1	87.41	7.14	-1.23	7.25	350.19	12.27
2:1	79.17	14.14	-0.98	14.18	356.00	22.28
Control	86.63	7.44	-0.87	7.49	353.30	12.67

3.4. Evaluation of the photoprotective effect of nanocapsules.

The encapsulates were exposed to UV light to evaluate the photoprotection capacity of the zein on the encapsulated extract. It was observed that UV light had no significant effect on the radical inhibition capacity of the phenolic compounds present in the extract within a period of 60 hours, as shown in Figure 4. Similar results were obtained by Gomez-Estaca *et al.* [24] for turmeric with zein as encapsulating material. The percentage of free radical inhibition of the phenolic compounds was correlated with the loss of color of the nanocapsules (Figure 4). It was observed that all concentrations showed loss of color, being more evident in the capsules obtained at 3.3:1 and 2:1 ratios. This can be explained because as the amount of extract in the capsules increases, the thickness of the shell decreases considerably, which causes the light to present a greater incidence on the phenolic compounds. This behavior can be related to the encapsulation efficiency values obtained for the 2:1 ratio. It was evidenced that most of the extract was on the surface of the capsule, explaining why the loss of color was more noticeable in this sample. Likewise, the encapsulates presented a color transition (pink to brown) associated with the chalcone structure resulting from the photooxidation of anthocyanins [25]. Similar results regarding the loss of color of the chromophore and photosensitive compounds were reported by Gomez-Estaca *et al.* [24] for turmeric.

The control sample presented a similar photooxidation behavior related to the encapsulated samples with glycerol. However, the loss of color was more evident in the nanocapsules with glycerol as an adjuvant. This phenomenon could be associated with the adjuvant incorporation into the formula. Because the glycerol provides greater elongation to the zein polymeric chains, a transparent space occupied by the plasticizer is associated between the polymer chains. This space allows light passage inside the capsule [26]. The correlation between the loss of color vs. the decrease in the percentage of free radical inhibition was 86%, so these two variables can be related and indicate the stability of the anthocyanins encapsulated with zein during storage.

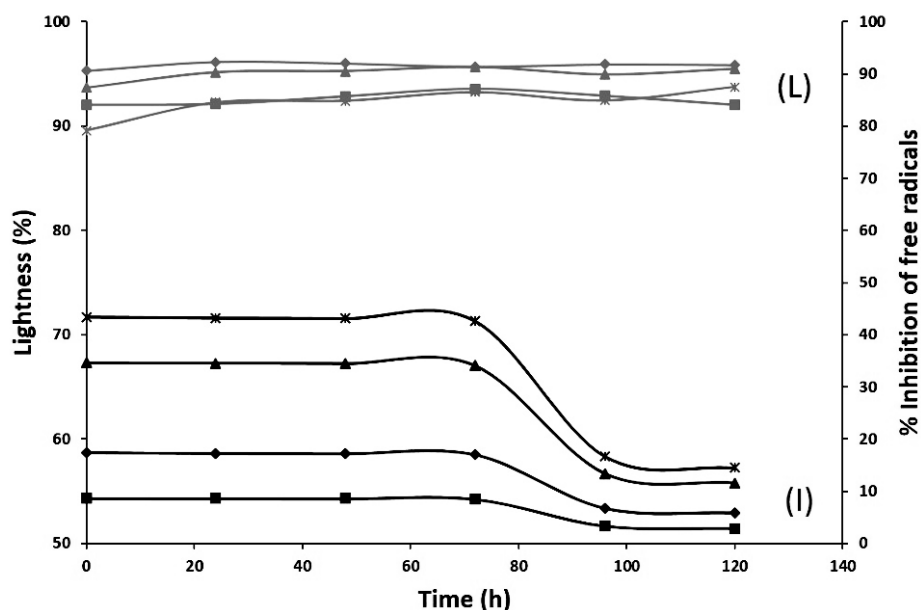


Figure 4. Comparison of the loss of lightness (L) and percentage of inhibition of free radicals (I) of zein capsules exposed to UV light. Capsules 10:1 (■), capsules 5:1 (◆), capsules 3.3:1 (▲) and capsules 2:1 (*).

3.5. Attenuated total reflectance Fourier transform infrared spectroscopy analysis (FTIR-ATR).

The presence of phenolic compounds in the extract was determined by ATR-FTIR. The ATR-FTIR spectra obtained for each component and the prepared samples are shown in Figure 5. The extract spectrum shows a broad signal in the band 3600 to 2987 cm^{-1} that corresponds to the stretching of the hydroxyl groups (-OH) associated with the humidity of the sample and the auxochromic groups of the phenolic compounds. The signal in the band 2987 to 2923 cm^{-1} corresponds to the stretching of the CH_2 bond group. The band between 1100 and 912 cm^{-1} is the characteristic signal of the aromatic groups ($\text{C} = \text{O}$), present in the phenolic compounds of the extract. These results coincide with those obtained by Cai *et al.* [27], who analyzed the anthocyanins cyanidin-3-glucoside and peonidin -3- glucoside.

The spectra of the encapsulate with a 3.3:1 ratio and capsules without extract were similar except for the signal shown in the range 1100 to 912 cm^{-1} , these differences are attributed to the deformation of the double bonds ($\text{C} = \text{O}$) of the ring A and B of the benzopyrillium ion, belonging to the phenolic structure. The spectra showed signals at 1645 and 1517 cm^{-1} corresponding to amides I and II present in the amino acids of the zein protein. The absorption band of the amide I is related to the axial stretching of the C-O bond during the absorption band of the amide II with an asymmetric angulation of the N-H bond. The absorption bands in the range 3000 to 2883 cm^{-1} correspond to the CH_2 and CH_3 radicals' C-H bonds

related to the zein's structure. The absorption band at 3600 to 3014 cm^{-1} belongs to the axial stretching of the hydroxyl functional group (-OH). These results coincide with those reported by Jayan *et al.* [28], who encapsulated catechins with zein using the electrospraying method.

The encapsulates of the 3.3:1 ratio were analyzed after being subjected to UV radiation to verify structural changes of the phenolic compounds (Figure 5). It was observed that the nanocapsules showed differences between samples with and without exposure to UV light, precisely in the bands 1100 to 912 cm^{-1} that corresponds to the C = O of the aromatic ring of the phenolic compounds. The decrease in signals was associated with the photooxidation of anthocyanins present in the polyphenolic extract of blueberry.

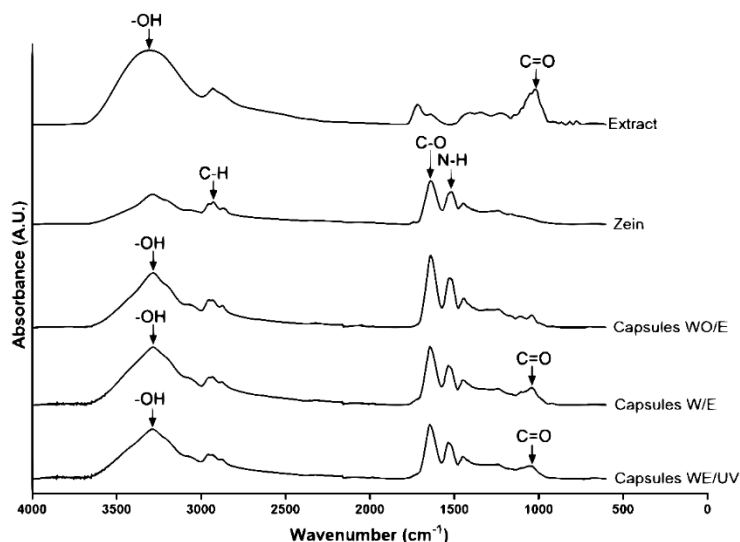


Figure 5. Comparison of the ATR-FTIR spectra the capsules obtained by electrospraying with extract, without extract, and capsules exposed to UV light.

3.6. Differential scanning calorimetry of nanocapsules.

The glass transition temperature (T_g) of both the polymer and the encapsulates was determined to demonstrate the absence of changes in the structural conformation of the zein due to the incorporation of the extract and the adjuvant as by the method used for encapsulation. The comparison of DSC thermograms for the pure compounds and the capsules is shown in Figure 6. Zein presented a T_g of 132.53 $^{\circ}\text{C}$. This result differs from that reported by Neo *et al.*[29], who obtained a value of 156.30 $^{\circ}\text{C}$. This may be mainly due to environmental conditions, humidity, and polymer purity. However, the nanocapsules with extract (3.3:1) compared to the neat polymer did not show changes in the T_g (133.12 $^{\circ}\text{C}$). This absence of changes demonstrates that the phenolic compounds are encapsulated without strong interactions with the polymer. The electrospraying process and adding the adjuvant to the formulation do not cause structural modifications in the zein. These results are in concordance with the obtained by ATR-FTIR. Likewise, endothermic peaks were observed in both thermograms, which correspond to the evaporation of humidity and volatile compounds, mainly glycerol due to its alcoholic nature, in a temperature range of 50 to 63 $^{\circ}\text{C}$. However, the nanocapsules without glycerol (control) showed a significant difference ($p = 0.05$) in T_g (138.56 $^{\circ}\text{C}$) compared to the polymer and the capsules with the adjuvant. This could be due to the union of phenolic compounds with zein induced by the application of voltage, causing an increase in the T_g of the polymer; this phenomenon differs from that reported by Neo *et al.* [29], who observed a decrease in T_g by adding 20% gallic acid to zein structures without the

presence of surfactant. This behavior indicated that incorporating glycerol into the formulation guarantees that the interactions between the core and the biopolymer are weak, ensuring the release of the active ingredient under defined conditions.

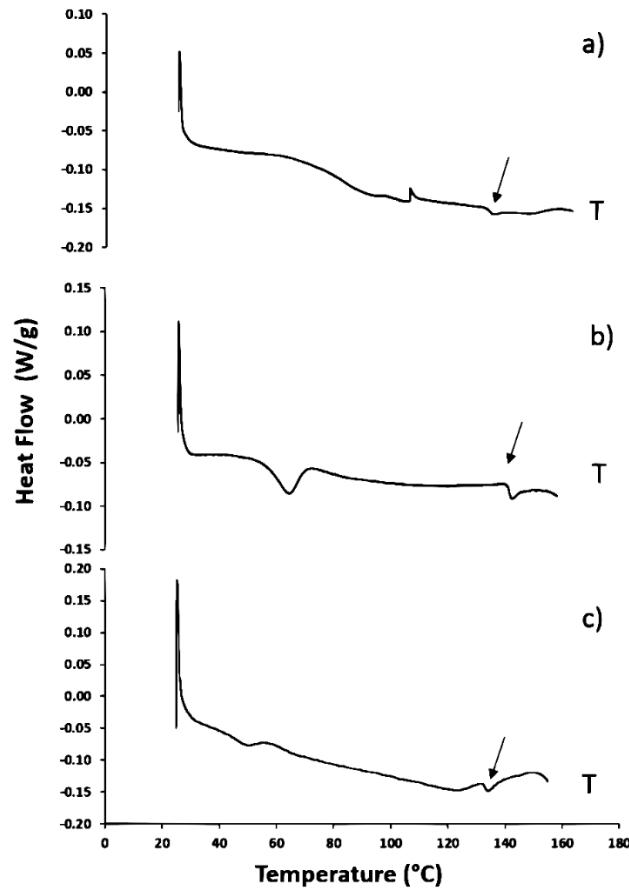


Figure 6. Determination of vitreous transition temperatures of (a) zein; (b) capsules with extract; (c) control.

3.7. Evaluation of thermal stability of nanocapsules.

The thermogram of the blueberry extract presented four mass variations as a function of temperature (Figure 7c); the first is related to the moisture of the extract, it is in a range of 70.2 to 100.1 °C, corresponding to 15.07% of the total mass of the sample. The second variation (120.2 to 204.6 °C) was related to the presence of disaccharides and simple sugars and the breakdown of the β -glucosidic bond of the sugars attached to phenolic compounds (anthocyanidin). The third variation is associated with the decomposition of phenolic acids and anthocyanidin, with a maximum decomposition temperature of 197.05 °C. The fourth variation (263 to 380 °C) could be attributed to the flavillium ion since it corresponds to the structural base of the anthocyanins present in the extract. These results are similar to those obtained by Wang *et al.* [17], who reported a decomposition temperature of 300 to 390 °C for cyanin-3-glucoside. The difference between the previously reported temperatures with those obtained in this study could be due to the degree of purification of the extract. The presence of different compounds in the sample makes the mass variation of a compound due to the effect of temperature less perceptible [30].

The analysis of the encapsulates (Figure 7d) showed that they contained 4.5% humidity, associated with the first mass variation (50 to 100.1 °C), which guarantees the stability of the phenolic compounds during storage. The second variation is attributed to glycerol

decomposition 162.32 to 194.03 °C (Figure 7 a). The nature of this adjuvant belonging to the group of alcohols explains why it presents low decomposition temperatures. Zein presented a decomposition temperature corresponding to the third mass variation (Figure 7b). This value is similar to that reported by Karim *et al.* [31], who obtained a decomposition temperature of 240 to 400 °C for zein as carrier material. The polymer showed no increase or decrease in temperature compared to capsules without extract. A displacement of the decomposition initiation temperature of 100 °C was observed for the encapsulated extract, which makes it evident that the zein is capable of providing thermoprotection to the phenolic compounds. Additionally, low humidity was observed in the samples. This is related to the effectiveness of the encapsulation method used, which positions the electro spraying process as an effective technique for obtaining stable polyphenol-rich capsules.

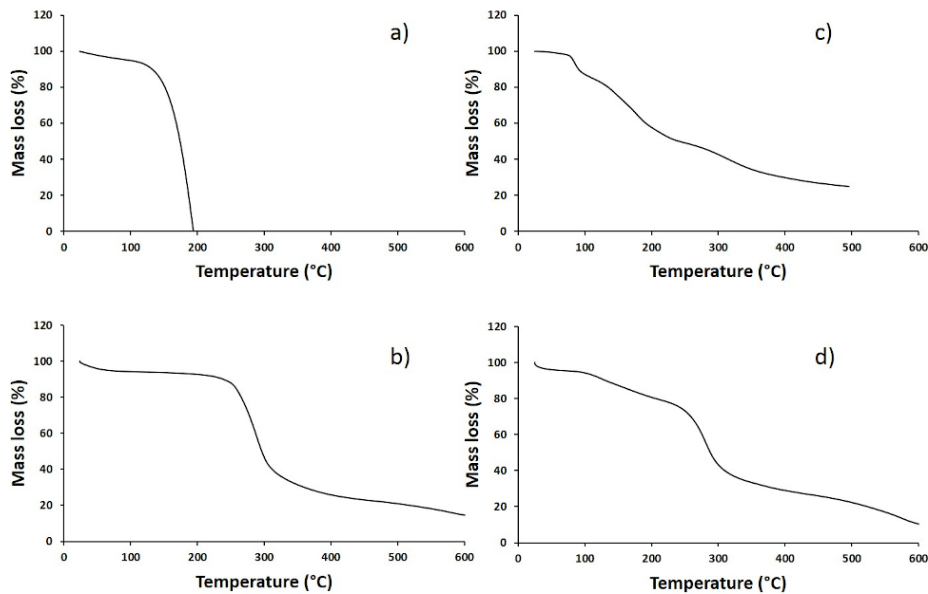


Figure 7. Comparison of the thermal stability of capsules and components. (a) Glycerol; (b) zein; (c) extract; (d) capsules with extract.

4. Conclusions

This study demonstrated the feasibility of using zein to nanoencapsulation bioactive compounds via the electro spraying process, managing to photo and thermo stabilize the polyphenolic compounds present in the extract of Biloxi blueberries. Nanocapsules with homogeneous morphology, without cracks or dents and without modifications in their thermal properties were obtained using this technique. The importance of adding an adjuvant such as glycerol to increase the encapsulation efficiency of hydrophilic compounds in partially hydrophobic matrices was also demonstrated. Additionally, the presence of glycerol did not affect the color of the samples. Finally, a correlation between the loss of color with the percentage of inhibition of free radicals in the encapsulates was established. Therefore, the color parameter could serve as an indicator of the stability of phenolic compounds during storage.

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Conflicts of Interest

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

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