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Encapsulation of *Beta vulgaris* extract by electrospraying and evaluation of antimicrobial potential

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ABSTRACT

Beets are rich in phenolic compounds and betalains that can be used as natural food colorings and have antimicrobial effects. However, these compounds are not very stable, and encapsulation can prolong their stability and action. The aim of this study was to investigate the antimicrobial activity of beetroot extract encapsulated by electrospraying with zein as wall material. The extract and capsules were characterized, as well as the encapsulation efficiency determined. Antibacterial activity was determined against gram-positive bacteria (*Listeria* monocytogenes, *Staphylococcus* aureus) and gram-negative bacteria (*Salmonella* Typhimurium, *Escherichia* coli) by the disk diffusion method using the minimum inhibitory concentration and the minimum bactericidal concentration. Encapsulation was efficient (92%) forming capsules with high thermal stability and spherical morphology with a regular surface. Beetroot extract and zein capsule with beetroot extract exhibited inhibition halos, inhibitory and bactericidal effects at a concentration of 200 mg mL^{-1}against *Salmonella* Typhimurium.

Keywords: betalains, antibacterial activity, stability.

INTRODUCTION

Beta vulgaris is known as beetroot, a root belonging to the Chenopodiaceae Family[1] rich in phenolic compounds and water-soluble nitrogenous pigments called betalains.[2,3] These pigments are divided into two groups: betacyanins, which are red-violet in color, and betaxanthins, which are yellow.[2] Both pigments are widely used as food colorings to replace synthetic ones,[2,4,5] which have been linked to toxicological effects, being potent carcinogens and related to food allergies or hypersensitivities.[6]

The food industry uses these pigments to restore the color lost in food processing, to enhance, color or standardize the color of food products.[7] Betalains are used in burgers, desserts, ice cream, jellies, soups, sauces, sweets, drinks, dairy products and yogurts.[8] In addition, they have antimicrobial and antiviral effects in foods and health benefits such as antioxidant, anticarcinogenic, anti-inflammatory, antilipidemic and hypotensive activities[9-11] with high potential for application in functional products.[12]

However, betalains are unstable, undergoing changes in the presence of sugars, light, oxygen, variations in water activity, pH and temperature.[4,13] In this sense, encapsulation can be an alternative for the preservation of these compounds. Among the various encapsulation methods, the electrospraying technique stands out because it promotes the formation of capsules with regular morphology, high encapsulation efficiency and good thermal stability, when compared to other methods such as spray drying and simple or complex coacervation.[14]

Maltodextrin, guar or arabic gum, pectin and xanthan have already been used as coating material for betalains,[15] but there are no studies using zein. Zein is formed by a group of prolamins from corn (*Zea mays*) with several non-polar amino acids (alanine, phenylalanine, leucine and proline) and is soluble in hydrophobic solutions[16,17] and can be used especially for controlled release of betalains in food or packaging.[18] Therefore, the objective of this study was to verify the encapsulation efficiency and antimicrobial activity of beetroot extract encapsulated by electrospraying using zein as wall material.

MATERIAL AND METHODS

Material and reagents

The beetroot samples were purchased from market in Pelotas, Brazil. To encapsulate the extract, corn zein (Sigma Aldrich) dissolved in a hydroalcoholic solution (ethanol:water, 70:30, *v/v*) (Sigma Aldrich) was used. To analyze the antimicrobial potential, the strains *Salmonella* Typhimurium (ATCC 13311), *Escherichia coli* O157:H7 (ATCC 43895), *Listeria monocytogenes* (ATCC 7644) and *Staphylococcus aureus* (ATCC 10832) were obtained from the Food Science and Molecular Biology Laboratory of Federal University of Pelotas, Brazil. The bacterial strains were cultivated in brain and heart infusion broth (BHI-Acumedia). For the reactivation of microorganisms, the following selective agars were used: Hektoen enteric agar (Sigma Aldrich), Baird-Parker agar (Sigma-Aldrich), methylene blue eosin agar (Sigma Aldrich) and Oxford agar (Sigma Aldrich). Muller-Hinton agar (Sigma Aldrich) was used for disk diffusion analysis.

Extraction and encapsulation of beetroot extract

The beets were sanitized under running water, freeze-dried (K108, Liotop, Brazil) for 48 hours and then ground in a ball mill (MA305, Marconi, Brazil). To prepare the extracts, 5 grams of freeze-dried and ground beetroot were weighed and then homogenized with 25 mL of a hydroalcoholic solution (ethanol:water, 70:30, v/v). The solution was then sonicated in ultrasound (Quimis, Brazil) for 15 min and centrifuged (Centrifuge 5430R, Eppendorf, Germany) at 8232 x g for 10 min at 4 °C. The supernatant was removed and stored in falcon centrifuge tubes at -76 ºC until use. The final extract was prepared at concentration of 250 mg mL^{-1} using solution and extracts.

The encapsulation of the extract was carried out using a horizontal electrospraying station containing a high voltage source with direct current (INSTOR, Brazil), infusion pump (KD Scientific, USA) and a metal collector coated with aluminum. For this step, a zein solution was first prepared at a concentration of 9% dissolved in ethanol:water (70:30; w/v) with a magnetic stirrer (Fisotom, model 752/6, Brazil). The beetroot extract was then homogenized with the zein solution in a 2 mL centrifuge tube at 30:70 (extract/zein, *v/v*). After homogenization, the solution was transferred to syringe contend a 1 mL and attached to an injector with a flow rate of 1 mL/h at 10 cm from a metal collector. The source conditions were + 16 KV and – 8 KV voltage.

Encapsulation efficiency

Encapsulation efficiency was determined by the content of anthocyanin compounds after encapsulation using espectrophotometry. For this, 1 mg of the beetroot extract capsule was washed with 200 µL of ultrapure water

to remove non-encapsulated compounds retained on the surface, and subsequently centrifuged (Centrifuge 5430R, Eppendorf, Germany) at 8232 x g and 4 °C for 5 min. Then, the sediment was resuspended in 200 µL of ethanol:water (70:30, *v/v*), and the mixture was centrifuged again under the same conditions, in order to determine the concentration of anthocyanins retained inside the capsule. An aliquot of the supernatant was analyzed in a spectrophotometer according to the method described previously.[19] The encapsulation efficiency was expressed as a percentage:

$$EE\ (\%) = (TABE) - (TABE\ capsule) \times 100\ \div TABE$$

Where: EE: Encapsulation efficiency; TABE: Total anthocyanins of beetroot extract; TABE capsule: Anthocyanins retained inside the beetroot extract capsule after removing the compounds present on the surface

Differential Scanning Calorimetry

This assay was realized by DSC Q20 TA Instruments. For this assay, 1 mg of sample (beetroot powder, zein capsule without extract, and zein capsule with addition of beetroot extract) was added in non-hermetic closed aluminum crucibles. Subsequently, the samples were heated at a rate of 0.5 ºC min^{-1}, in the range of 30 to 250 ºC under nitrogen flow (50 mL min^{-1}).[20]

Thermogravimetric analysis

Thermogravimetric analysis of the beetroot powder, zein capsule without extract, and zein capsule with addition of beetroot extract was carried out using thermogravimetric equipment (TGA, TA-60WS, Shimadzu, Japan). For this, 1 mg of sample was weighed in a platinum capsule and then subjected to heating up to 600°C with a heating rate of 50°C min^{-1} and nitrogen flow of 50mL min^{-1}. An empty platinum capsule was used for control.

Scanning electron microscope

The evaluation of the surface morphology of the zein capsule without extract and the zein capsule with the addition of beetroot extract occurred with the aid of scanning electron microscope (JSM6610LV, Jeol, Japan). The samples were metallized with gold using a current of 20 mA for 120 s, the acceleration voltage used was 10 keV, the images obtained were magnified 12.000 times. The average diameter of the capsules was determined by measuring 50 capsules using the ImageJ program.

Antimicrobial potential

The reactivation of the microorganisms used in the study was carried out as follows: one batch of each bacteria was transferred to individual tubes containing Soy Trypticasein broth and incubated in an oven for 24 h at 37 ºC. After each growth cycle was streaked in Petri dish with selective media, using Hektoen Enteric agar for *S. enteritidis*, Eosin Methylene Blue agar for *E. coli*, Oxford agar for *L. monocytogenes* and Baird-Paker agar for *S. aureus*, and incubated for 24 h at 37 ºC, to isolate the colonies. After growth, a portion of each bacteria was extracted and resuspended in saline solution (NaCl 0.85%), which was standardized at 0.5 on the McFarland scale (1.5 x 108 CFU mL^{-1}). All assays were performed in triplicates.

Disc diffusion

To determine the inhibition diameter of the beetroot extract, the zein capsule without extract and the zein capsule with beetroot extract against bacterial strains, disk diffusion analysis was performed according to a previously proposed protocol (Clinical and Laboratory Standards Institute 2018). For this, the standardized saline solution containing the inoculum was sown using a sterile swab on the surface of plates containing Muller-Hinton agar. Then, a sterile paper disk (Laborclin) of the 6 mm in diameter was placed in the center of each plate, on which were placed 10 µL of beetroot extract, 5 mg of the zein capsule without extract and 5 mg of the capsule of zein capsule with beetroot extract.

Then, a sterile paper disk (Laborclin®) of 6 mm in diameter was placed in the center of each plate, on which were placed 10 µL of beetroot extract, 5 mg of the zein capsule without extract and 5 mg of the zein capsule with beetroot extract. The plates were incubated at 37 °C and the diameters of the inhibition zones around the paper disk were measured after 24 hours with a digital caliper (King Tools) and expressed in millimeters. Sterile water was used as a negative control and all tests were performed in triplicate. The results were expressed in cm ± standard deviation after three equidistant measurements of the halos formed. To avoid possible interference from the solvent, the beetroot extract was lyophilized and resuspended in deionized water at the same concentration.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The assays were performed according to the method

described previously[21] with minor modifications. In a 96-well microtiter plate, 180 µL of Brain Heart Infusion (BHI) broth, 20 µL of inoculum and the samples (beetroot extract, zein capsule without added extract, zein capsule with added beetroot extract) were added to each in five different concentrations (200, 100, 50, 5 and 1 mg mL^{-1}). The absorbance of solutions containing the sample and bacteria was measured at 620 nm at the time of preparation and 24 h after incubation (37 °C) using a spectrophotometer (Biochrom EZ Read 400, UK). The MIC was considered as the lowest concentration at which there was no bacterial growth in the culture medium. From the wells that showed inhibition of bacterial growth in the MIC analysis, 15 µL were removed and streaked in Petri dishes with Brain Heart Infusion Agar (BHA) and incubated for 24 h at 37 °C. The lowest concentration at which no bacterial growth occurred was considered MBC. MIC and MBC were expressed in mg mL^{-1}.

Statistical analysis of encapsulation efficiency, antioxidant activity and antimicrobial activity was performed using analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) in the Statistica 7.0 (StatSoft, Inc.).

RESULTS AND DISCUSSION

Known edible sources of betalains are beets and chard.[9] Red beets are rich in betacyanins, while they have less betaxanthins such as vulgaxanthin I, which are predominantly yellow,[2] both pigments could replace synthetic dyes.[11] In this sense, encapsulation is a way of maintaining the color and stability of the anthocyanins with great possibility of incorporation into foods. Furthermore, the habitual consumption of beets, because they contain high concentrations of dietary nitrate, is absorbed and reduced to nitrite by digestive bacteria, bringing benefits to cardiovascular health (Miller & Collins, 2020).

Encapsulation characteristics

The beetroot extract capsule showed an encapsulation efficiency of 92.0%, this percentage was lower than that found by researchers that used maltodextrin and gum arabic to encapsulate beet extracts with efficiency between 96.8 and 99.6%.[22] Betalains extracted from *Salicornia fruticosa* encapsulated with gum arabic or maltodextrin showed lower efficiency than the current study (86.50–92.30%).[23]

In polyphenols, encapsulation is used to stabilize, improve bioavailability and facilitate administration of the compounds in foods[11] Authors suggest encapsulation as an efficient method to stabilize and facilitate the administration of betalain into the human body, increasing its bioavailability after oral ingestion, whether via foods containing capsules or nutraceuticals.[11]

The table 1 shows the thermal and thermogravimetric properties of the beetroot powder, zein capsule without extract, the and zein capsule with addition of beetroot extract. The beetroot powder and the zein capsule with the addition of beetroot extract showed endothermic events close to 100 °C, characteristic of the sample dehydration process (Table 1A). The zein capsule without added extract showed a single endothermic event at a temperature of 140 °C, this event is also characteristic of the sample dehydration process. To encapsulate betalains, temperatures between 140 at 150 °C are ideal, as above 180 °C there is a loss of compound due to drying of the compound and a reduction in storage stability.[13,14] It was not possible to observe other events because the thermal degradation of the zein structure occurs at temperatures above 300 °C.[25] So much so that the zein capsule without added extract showed a single mass loss of 52% at 318°C corresponding to thermal degradation of its structure (Table 1B).

Table 1. Thermal (A) and thermogravimetric (B) properties of zein capsule without extract, beetroot powder or zein capsule with addition of beetroot extract

	Thermal properties (A)			Thermogravimetric properties (B)				
Sample	**ΔT (°C)**	**Temp (°C)**	**ΔH (J/g)**	**Δm (mg)**	**Temp (°C)**	**ΔT (°C)**	**WL (%)**	**Res (%)**
B	60.0	80.1	110.7	34.6 70.6 58.6	180.3 246.2 348.2	0.05 1.82 0.42	1.7 60.6 14	23.7
Z	29.5	140.2	126.0	0.6	318.2	155.8	52.5	47.5
ZB	71.1 20.5	116.3 202.2	202.0 7.7	0.14 0.30	245.1 344.9	59.7 79.3	23.3 50.0	26.7

B – beetroot powder; Z –Zein capsule without extract; ZB - Zein capsule with addition of beetroot extract (30:70 extract:zein, *v/v*). Thermal properties: ΔT - temperature range for degradation; Tp (°C) -peak degradation temperature; ΔH - energy needed for degradation. Thermogravimetric properties: Δm – mass loss in mg; Tp (°C) –average temperature where mass loss occurred; ΔT – temperature range where mass loss occurred; ML (%) – mass loss; Res (%) – residue.

The morphologies of the zein capsule without the addition of extract and the zein capsule with the addition of beetroot extract can be seen in the figure 1. The zein capsule without the addition of extract had an average diameter of 750 nm and a flat shape (A), demonstrating the absence of extract, while the zein capsule with the addition of beetroot extract had an average diameter of 139 nm and a spherical morphology with a smooth surface, demonstrating the retention of the extract by zein (B). Encapsulation is an economical, flexible technique and produces good quality capsules by isolating the internal contents from the environment and releasing them at controlled rates for prolonged periods of time according to the action of the external environment such as pH, enzymes and temperature. [13] The barrier formed by the encapsulating agent, in this case zein, protects the beetroot extract, making the final product more stable.[26]

Antimicrobial potential

The presence and diameter of the inhibition halos of an extract against pathogenic bacteria indicate the susceptibility of the microorganism to the compound. When the halos are less than 0.7 cm they are considered non-active, between 0.7 and 1.2 cm they are active and when they are more than 1.2 cm they have a satisfactory inhibitory effect.[27]

The results of the action of the beetroot extract and the beetroot capsule against pathogenic bacteria based on the inhibition zones are presented in Table 2. Both the beetroot extract and the zein capsule with the addition of beetroot extract showed active halos against gram-negative bacterium *S.* Typhimurium (Table 2). This bacterium contaminates a wide range of hosts from ingestion of contaminated food or water and predominantly causes gastroenteritis, especially in immunocompromised individuals.[28-30]

Table 2. Disk diffusion analysis of beetroot extract, zein capsule without added extract and zein capsule with added beetroot extract against pathogenic bacteria *Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*

Bacterium	Inhibition zone (cm)		
	B	Z	CB
Salmonella Typhimurium	0.83	nd	0.67
Listeria monocytogenes	nd	nd	nd
Staphylococcus aureus	nd	nd	nd
Escherichia coli	nd	nd	nd

nd – not detected; B – beetroot powder; Z –Zein capsule without extract; ZB - Zein capsule with addition of beetroot extract (30:70 extract:zein, v/v).

A study that evaluated extract and essential oil from red beet leaves also did not observe antimicrobial activity against the other species strains studied.[27]

Beta vulgaris L. pomace extract was active against *S.* Typhimurium with inhibition halos similar to the present study.[31]

The table 3 and 4 shows Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). When an extract has an MIC value of up to 0.5 mg mL^{-1}, it is considered a strong antimicrobial agent; between 0.6 and 1.5 mg mL^{-1} had a moderate effect and above 1.6 mg mL^{-1} it showed weak activity.[32] Weak antimicrobial activity against *S.* Typhimurium was observed in

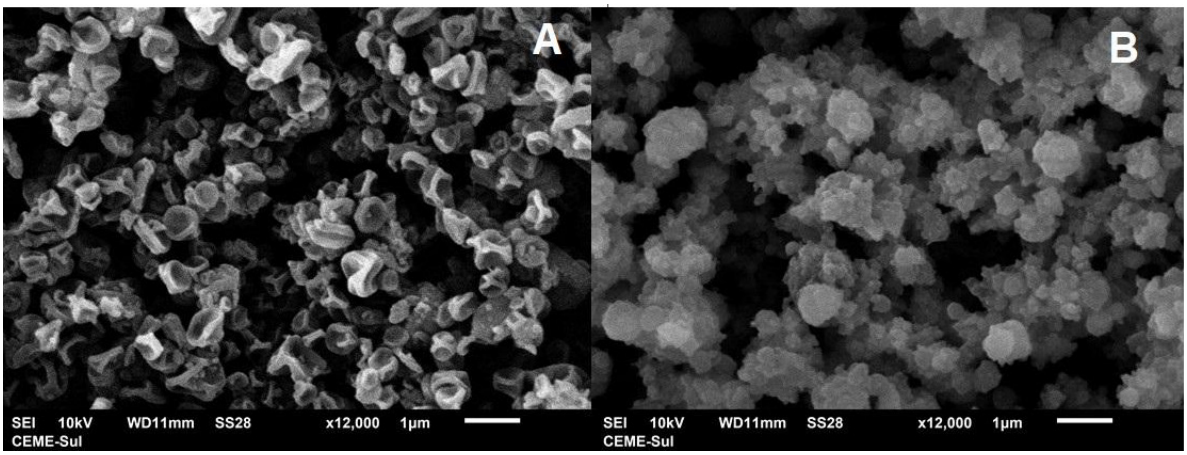

Figure 1. Scanning electron microscopy of the zein capsule without added extract (A) and the zein capsule with the addition of beetroot extract in a 30:70 ratio (extract:zein, v/v) (B). Magnified 12,000 times.

both the beetroot extract and the zein capsule with added extract using the MIC technique (Table 3), as well as bactericidal effects against *S.* Typhimurium (Table 4).

Table 3. Minimum Inhibitory Concentration of beetroot extract, zein capsule without added extract and zein capsule with added beetroot extract against pathogenic bacteria *Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*

Bacterium	Concentration (mg mL^{-1})		
	B	Z	ZB
Salmonella Typhimurium	200	nd	200
Listeria monocytogenes	Nd	nd	nd
Staphylococcus aureus	Nd	nd	nd
Escherichia coli	Nd	nd	nd

nd – not detected; B – beetroot powder; Z –Zein capsule without extract; ZB - Zein capsule with addition of beetroot extract (30:70 extract:zein, v/v).

Table 4. Minimum Bactericidal Concentration of beetroot extract, zein capsule without added extract and zein capsule with added beetroot extract against pathogenic bacteria *Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*

Bacterium	Concentration (mg mL^{-1})		
	B	Z	ZB
Salmonella Typhimurium	200	nd	200
Listeria monocytogenes	nd	nd	nd
Staphylococcus aureus	nd	nd	nd
Escherichia coli	nd	nd	nd

nd – not detected; B – beetroot powder; Z –Zein capsule without extract; ZB - Zein capsule with addition of beetroot extract (30:70 extract:zein, v/v).

CONCLUSIONS

The encapsulation technique can prolong the stability and action of anthocyanins in food products. This study demonstrated that the zein capsule with the addition of beetroot extract produced by the electrospraying technique presented high thermal stability and spherical morphology.

Both the beetroot extract and the zein capsule with beetroot extract exhibited inhibition halos, inhibitory and bactericidal effects at a concentration of 200 mg mL^{-1} against *Salmonella* Typhimurium, demonstrating stability for the phenolic compounds present in the extracts.

Thus, the encapsulation of beetroot extract is an alternative for the use of dyes in foods with good stability.

DATA AVAILABILITY

The entire dataset supporting the results of this study was used in this article.

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AUTHOR CONTRIBUTIONS

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