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Comparative study of the microencapsulation by complex coacervation of nisin in combination with an avocado antioxidant extract

Mariel Calderón-Oliver [a], Ruth Pedroza-Islas [b], Héctor B. Escalona-Buendía [a], José Pedraza-Chaverri [c], Edith Ponce-Alquicira [a, *]

[a] Departamento de Biotecnología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, 09340, México, D.F., Mexico
[b] Departamento de Ingeniería y Ciencias Químicas, Universidad Iberoamericana, 01219, México, D.F., Mexico
[c] Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México, 04510, México, D.F., Mexico

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ABSTRACT

The objective of this study was to evaluate several conditions for complex coacervation to encapsulate a mixture of nisin, an antimicrobial peptide, and an antioxidant avocado peel extract. We evaluated the effects of three factors: two matrix-wall systems (collagen-alginate and collagen-pectin), two drying methods (freeze and spray drying) and two core dispersion systems (water in oil emulsion or in suspension). The final characteristics measured for each microencapsulation treatment were: encapsulation efficiency, encapsulation loading, encapsulation yield, Aw, and moisture. The core dispersion method (emulsified or not) and drying method (freeze or spray), as well as their interactions, were important factors in the final characteristics of the microencapsulation produced by complex coacervation. The interaction of the three factors only had an effect on moisture. The core dispersion factor had an effect on the encapsulation loading of both bioactives, whereas the drying method had an effect on Aw and encapsulation yield. Higher loading was obtained with non-emulsified core; higher encapsulation efficiency and yield were obtained through the combination of spray drying with emulsified core. Higher Aw, and moisture were obtained with the freeze drying method and emulsified core. The drying methods modified the final morphology, shape and structure of the microcapsules.

The wide range of microcapsules produced by the combination of the three assessed factors may offer the food industry a wide range of option to deliver functional ingredients and meet various types of needs.

1. Introduction

Microencapsulation is a promising method in which various food ingredients can be protected from the environment; this process can also be used as a means to control their release to target specific sites or to improve their flow and organoleptic properties (Fang & Bhandari, 2010; Gouin, 2004; Sobel, Versic, & Gaonkar, 2014). Moreover, there are several microencapsulation methods, such as liposome formation, coextrusion, spray coating and fluid-bed coating, among others, but complex coacervation is one of the most important methods used currently. Complex coacervation is used to encapsulate different compounds, such as flavours, sweeteners, enzymes, vitamins, pre-probiotics, antioxidants, minerals, fatty acids, and others (Comunian et al., 2013; Eratte et al., 2015; Mendanha et al., 2009; Rocha-Selmi, Bozza, Thomazini, Bolini, & Fávaro-Trindade, 2013). This process involves the phase separation of one or more oppositely charged hydrocolloids (commonly a protein with a polysaccharide) that form a matrix-wall around the active compound, which had previously been suspended or emulsified in the same medium (Gouin, 2004; Koupantsis, Pavlidou, & Paraskevopoulou, 2014). Some hydrocolloids include gelatin, whey proteins, pea protein, lactoglobulin, alginate, carrageenan, pectin, and Arabic gum, among others.

Alginate is a polysaccharide obtained from brown seaweed and is composed of chains of D-mannuronic and L-guluronic acids

* Corresponding author. Departamento de Biotecnología, Universidad Autónoma Metropolitana Unidad Iztapalapa, 09340, México, D.F., Mexico.
E-mail address: pae@xanum.uam.mx (E. Ponce-Alquicira).

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(Xiao, Liu, Zhu, Zhou, & Niu, 2014). Pectin is a polysaccharide extracted from the cells and intercellular walls of plants and fruits that is composed of D-galacturonic units (Espitia, Du, Avena-Bustillos, Soares, & McHugh, 2014). Collagen is a fibrous protein present in bones, cartilage and skin tissues. Hydrolysed collagen consists of peptides from collagen type I and consists of 97% pure protein; it also contains glycine, proline and hydroxyproline.

Food additives have long been used to improve the sensory characteristics, lengthen the shelf life or increase the nutritional value of a given product. In recent years, there has been an increased tendency to use and study natural additives, such as natural antimicrobials and antioxidants.

Nisin is an antimicrobial peptide (34 amino acid residues) produced by *Lactococcus lactis* subsp. *lactis*. It has been used in a variety of products, such as cheese, meat, and canned foods, in the food industry since 1969 to prevent the growth of several bacteria, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and other pathogens (Balciunas et al., 2013). Unfortunately, some factors, such as pH (up to 8 and as low as 2), and the presence of endogenous proteases, divalent cations, fat, and glutathione in the food matrix can affect its activity (Balciunas et al., 2013; Davies et al., 1999; Liu & Hansen, 1990). The microencapsulation process has been proven effective in protecting its antimicrobial activity (Laridi et al., 2003; Xiao, Davidson, & Zhong, 2011), but the complex coacervation method has yet to be studied. This technique has enabled the highly efficient encapsulation of labile ingredients because it does not use high temperatures or organic solvents and has good controlled-release characteristics (Yan & Zhang, 2014).

Natural antioxidants are used in the food industry to prevent lipid and protein oxidation through different mechanisms. Most of these natural antioxidants include polyphenol components, and depending on the type of polyphenol, they are sensitive to temperature, light, pH or oxidation (Fang & Bhandari, 2010). They also possess an unpleasant flavour, as well as astringent and bitter notes. The encapsulation of these compounds can not only mask their undesirable sensory characteristics but also provide protection, lengthen their half-life, and control their release. The coacervation method is used to encapsulate essential oils containing polyphenols or antioxidants. For example, β-carotene has been encapsulated in a mixture of whey protein isolate & gum acacia, ascorbic acid has been encapsulated in gelatin-gum Arabic, and propolis has been encapsulated in isolated soy protein-pectin, among others (Comunian et al., 2013; Jain, Thakur, Ghoshal, Katare, & Shivhare, 2015; Nori et al., 2011). However, there are no studies where a mixture of an antioxidant and an antimicrobial compound was encapsulated by complex coacervation or by other techniques.

The aim of this study was to compare different factors involved in the complex coacervation microencapsulation process of a mixture containing the antimicrobial compound nisin and an avocado peel extract as antioxidant. The proportions of these compounds and their synergistic effects were optimized in a previous study (Calderón-Oliver et al., 2016). The following factors were evaluated: two matrix wall systems (collagen-alginate and collagen-pectin), two drying methods (freeze and spray drying) and two core dispersion systems (W/O emulsion or in suspension).

2. Materials and methods

2.1. Chemicals

An antioxidant avocado peel extract (ORAC value of 285.18 μg trolox equivalents/mg) was prepared according to the method reported in a previous study (Calderón-Oliver et al., 2016). The peel of fully ripened Hass avocado fruit was dried in an air flow oven and then boiled in hot water for 30 min, filtered and freeze dried. Nisin (2.5% w/w balanced with sodium chloride and denatured milk solids, 10^6 IU/g), regenerated benzoylated cellulose dialysis tubing with a 2000 NMWCO, and other reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Hydrolysed Collagen (Peptan 5000) was purchased from Rousselot (Eindhoven, Netherlands). Sodium alginate (Grinsted BC 171) was acquired from Danisco-Dupont (Copenhagen, Denmark), and partially amidated low methoxyl pectin (standardized with sucrose, Genu LM-104 AS) was purchased from CP Kelco (Atlanta, GA, USA).

2.2. Preparation of the matrix-wall stock solutions

Hydrolysed collagen (1 g; 1%, w/v; pH 6.42), pectin (1 g; 1%, w/v; pH 3.95) and alginate (0.5 g; 0.5%, w/v; pH 7.84) were prepared in Milli-Q water at room temperature at 24 h before each determination or microcapsule preparation. The solutions were maintained in constant agitation at room temperature to ensure complete hydration during the 24 h incubation.

2.3. Equivalence point of the biopolymer solutions

A potentiometric titration curve of each polymer stock solution was generated according to a previously reported method (Espinosa-Andrews, Sandoval-Castilla, Vázquez-Torres, Vernon-Carter, & Lobato-Calleros, 2010) to determine the proportion of protein and polysaccharide necessary to form the coacervates. The titrations were performed using 0.5 mL aliquots of a standard solution of 0.1 N NaOH. A one minute time lag was allowed between the two measurements to allow the solution to reach equilibrium. The pH of the solutions was measured with a pH meter (Orion, VersaStar 92, Thermo Fisher Scientific; Waltham, MA, USA) at 25 °C. The inflexion point in the titration, calculated as delta pH, was considered the equivalence point.

2.4. Zeta potential

The zeta potential of each stock solution prepared in section 2.2, was determined at different pH values using a Zetasizer Nano ZPS (Malvern Instruments, Ltd., Worcestershire, UK). The pH of the solutions was adjusted with 0.1 N HCl or NaOH. The mean zeta potential and standard deviations were obtained with the software included with the instrument.

2.5. Turbidity analysis of matrix-wall formation

A turbidimetric analysis was performed to identify the protein/polysaccharide ratio and pH conditions at which the phase separation required for coacervation occurred (Liu, Low, & Nickerson, 2009). The pH range was from 2 to 4.5, with changes of 0.5 units. The protein-polysaccharide combinations were mixtures from 0 to 100% of each biopolymer, at 10% intervals. The polysaccharide solution, pectin or alginate, was added to the collagen solution and the desired pH was achieved with 0.1 M HCl. The mixture was stirred for 30 min at room temperature and measured immediately in suspension, without allowing precipitation. The turbidity was measured at 600 nm using a Synergy HT spectrofluorometer (Biotek Instruments Inc., Winooski, VT, USA) with agitation during the measurement. The conditions in which the maximum turbidity was obtained, were used in the subsequent sections.

2.6. Preparation of the microcapsules

2.6.1. Non-emulsified microcapsules

Nisin (0.1 g/mL) was dialyzed to eliminate NaCl and other compounds that interfere with the microencapsulation process.