SCAVENGING ACTIVITY OF RUTIN ENCAPSULATED IN LOW METHOXYL PECTIN BEADS

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This study investigated the scavenging activity of rutin encapsulated in non-amidated low methoxyl pectin beads in various formulations with or without sorbitol and/or sodium bicarbonate ($NaHCO_3$). The beads were made and the releases of rutin from various bead formulations were investigated. Also, the DPPH radical scavenging activity of all bead formulations was determined. The rutin loaded in non-amidated pectinate beads containing 15% sorbitol showed the best release efficiency, whereas the beads containing 15% sorbitol with 1% $NaHCO_3$ (3Na1Bica15Sor) showed burst release with less lag time. At 0.05 mg/mL, 3Na1Bica15Sor exhibited the highest DPPH radical scavenging activity among the beads with the %scavenging of 84.00 ± 4.47. These results can be applied to increase the release, decrease the lag time, as well as enhance the activities of the active compounds encapsulated in calcium pectinate beads by adding the suitable proportion of sorbitol and $NaHCO_3$ in the bead formulation.

Keywords: low methoxyl pectin, rutin, scavenging activity, DPPH, *in vitro* release

INTRODUCTION

Pectin, a well-known plant polymer, is one of the main structural water-soluble polysaccharides of plant cell walls. Pectins, in which the degree of esterification (DE) of the galacturonic acid residues is higher than 50%, are known as high methoxyl pectins (HMP), and those in which the DE is less than 50% are regarded as low methoxyl pectins (LMP).[1] Amidated and non-amidated LMP are used to prepare gels by cross-linking with divalent cations such as calcium. The gel formation process involves the simultaneous bonding of calcium ions to carbonyl groups of two adjacent pectin molecules and two hydroxyl groups from one of the molecules.[2] An eggbox-like model was proposed to explain the structure of pectin molecules bound by the calcium ions.[3] Moreover, the texture of the gel could be changed by adding compounds such as sugars, especially sorbitol.[4,5]

Plant flavonoids are responsible for the intrinsic antioxidant properties found in botanicals. Rutin (quercetin-3-*O*-rutinoside), the flavonol glycoside of quercetin, is abundantly found and distributed in plants such as buckwheat seed, fruits and fruit rinds, especially citrus fruits. It presents important properties for human health, like its significant scavenging properties on oxidizing species such as hydroxyl radical, superoxide radical and peroxyl radical,[6] as shown by many *in vitro* and *in vivo* experiments.[7,8] The aim of this study was to investigate the scavenging activity of rutin encapsulated in non-amidated low methoxyl pectin beads in various formulations with or without sorbitol and/or sodium bicarbonate.

EXPERIMENTAL

Preparation of low methoxyl pectin (LMP) beads by encapsulation

The ionotropic gelation technique of using a drug encapsulated in the beads[9] was modified as follows: LMP aqueous formulations, including 3NA (3% non-amidated LMP), 3NA15Sor (3% non-amidated LMP with 15% Sorbitol), 3NA1Bica (3% non-amidated LMP with 1% $NaHCO_3$) and 3NA15Sor1Bica (3% non-amidated LMP with 15% sorbitol and 1% $NaHCO_3$), were prepared. Then, 2% w/v of rutin was dispersed in the solution and stirred until a uniform dispersion was obtained. The beads were made using a UNIT VAR1 Encapsulator (Nisco Engineering Inc., Zurich, Switzerland) with a nozzle of 0.7 mm inner

diameter. The slurry was dropped into 50 mL of a gently agitated solution of cross-linking agent (2% w/v $CaCl_2$) at a flow rate of 100 mL/h with a falling distance of 4 cm. Gelled beads were formed immediately and were allowed to stand in 2% $CaCl_2$ (cross-linking solution) for 10 min. Then, the beads were separated by filtration, washed with deionized water and dried at 37 ± 2 °C for 24 h in a drying room.

Characterization of microparticles

Morphological study

Surface morphology examination of the LMP microbeads was conducted by scanning electron microscopy (SEM), using a JEOL scanning electron microscope (JSM-6400F) at 20 kV.

Encapsulation efficiency

The amount of encapsulated rutin was determined by adding 100 mg to each bead into 1L of phosphate buffer (PB) pH 7.4 for 3 h until disintegration. The solution was filtered and the absorbance was measured at 267 nm by UV spectroscopy (Biochrom Libra S22, Cambridge, England). The percentages of encapsulation efficiency were calculated according to the following equation:

$$\text{Encapsulation efficiency } (\%) = AQ/TQ \times 100$$

where AQ is the actual quantity of rutin present in the matrices and TQ is the theoretical quantity of rutin.

***In vitro* rutin release**

The release of rutin from various bead formulations was investigated using an *in vitro* rotating paddle dissolution apparatus (Sotax AT7, Binningerstrasse, Allschwil). The dissolution study was performed in PB buffer pH 7.4 at a rotation speed of 50 rpm and a temperature of 37 ± 0.2 °C. A precise quantity of beads (100 mg) was added to 1 L of dissolution medium.

Samples were withdrawn at various time intervals up to 900 min by an automatic pump and were analyzed spectrophotometrically (Cary 50Bio UV-Visible Spectrophotometer, France) at 267 nm.

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined by a modified method, as previously described.[10] Briefly, five serial concentrations of beads [0.05-0.4 µg/mL of encapsulated rutin] and 50 µL of an ethanolic solution of DPPH were put into each well of a 24-well microplate (Nalge Nunc International, NY, USA). The reaction mixture was allowed to stand for 30 min at 27 ± 2 °C, and the absorbance was measured at 515 nm by a well reader (Bio-Rad, model 680 microplate reader, USA) against a blank (ethanol). Ascorbic acid, BHT and non-encapsulated rutin were used as positive controls. The experiments were done in triplicate. The percentages of DPPH radical scavenging activity were calculated:

$$\text{Scavenging } (\%) = (Abs_{control} - Abs_{sample})/Abs_{control} \times 100\%$$

RESULTS AND DISCUSSION

Microbead preparation and characterization

When the pectin droplets came in contact with the calcium chloride solution, ionic interaction occurred and gelled spheres were formed. These interactions in non-amidated LMP allowed the formation of a compact pectinate network, in which rutin particles were encapsulated.

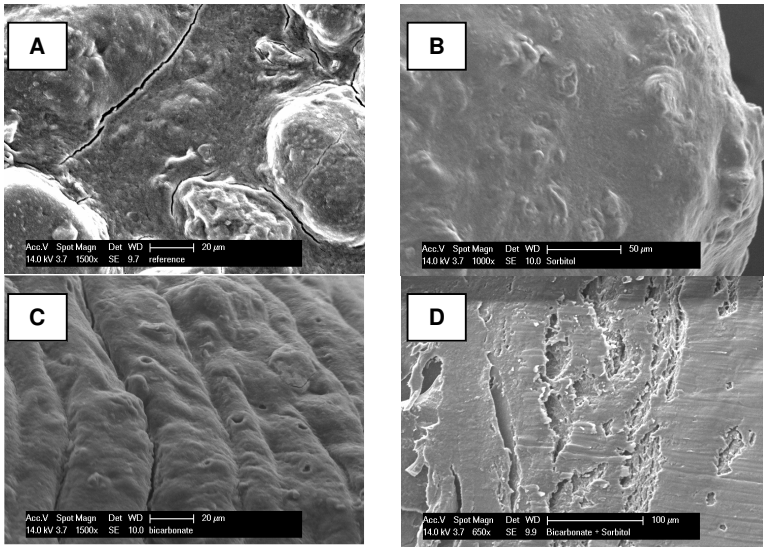

Figure 1: SEM of surface morphology of 3NA (A), 3NA15Sor (B), 3NA1Bica (C) and 3NA15Sor1Bica (D)

All beads were of oblong or spherical shape and of around 600 µm size. Scanning electron micrographs of their surfaces are presented in Figure 1. High encapsulation efficiency was observed for 3NA and 3NA15Sor beads (95.58±1.36 and 94.43±0.79%, respectively). Low solubility in the cross-linked solution of rutin was related to its high encapsulation in the beads. Adding sorbitol to the 3NA solution did not affect the encapsulation efficiency. However, the formulation containing $NaHCO_3$ (3NA1Bica and 3NA15Sor1Bica) revealed lower rutin encapsulation efficiency (80.40±0.84 and 79.77±1.45%, respectively). This is probably due to the fact that $NaHCO_3$ in the formulations dissolved and increased the pH of the $CaCl_2$ solution, which was the cross-linking solution, and this may have increased the diffusion and solubility of rutin into $CaCl_2$.[11]

***In vitro* rutin release**

Rutin release from the formulations occurred in the following order: 3NA15Sor > 3NA15Sor1Bica > 3NA > 3NA1Bica in PB (Figure 2). Adding 15% of sorbitol (3NA15Sor) into the pectinate bead matrix led to higher release of rutin. Adding 15% of sorbitol together with 1% $NaHCO_3$ (3NA15Sor1Bica) determined a faster release of rutin. This may be due to the lower Young's modulus value of the 15% sorbitol gel and the water adsorption ability of sorbitol, resulting in the softness of the beads, followed by the high release efficiency of rutin.[5]

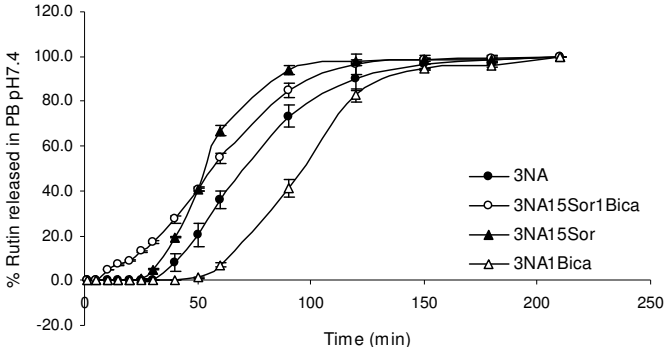

Figure 2: Dissolution profiles of beads in PB (pH 7.4)

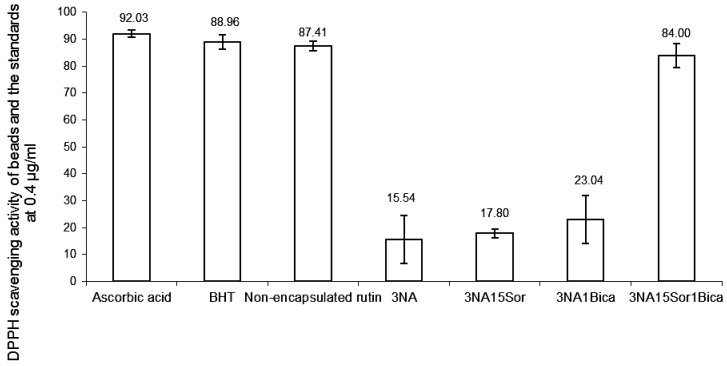

Figure 3: Comparison of percentages of DPPH radical scavenging activity of beads and standard antioxidants at 0.4 µg/mL

DPPH radical scavenging activity

Figure 3 illustrates the percentages of DPPH radical scavenging activity of the beads at 0.4 µg/mL. The standard antioxidants (ascorbic acid, BHT and non-encapsulated rutin at 0.4 µg/mL) gave scavenging activities of 92.03±1.33, 88.96±2.77 and 87.41±0.17%, respectively. The beads composed of 15% sorbitol and 1% $NaHCO_3$ exhibited higher DPPH radical scavenging activity (84.00±4.47%) than those of other beads. The bead softness and its cracked surface (Figure 1D) may be synergistic and responsible for this activity.

CONCLUSION

The rutin loaded in non-amidated pectinate beads containing 15% sorbitol showed the best release profile, whereas the beads containing 15% sorbitol with 1% $NaHCO_3$ (3Na1Bica15Sor) exhibited burst release with less lag time. At 0.05 mg/mL, 3Na1Bica15Sor presented the highest DPPH radical scavenging activity. The results from this study can be applied for the further development of the bead formulation to increase the release, decrease the lag time, as well as enhance the activities of the active compounds encapsulated in calcium pectinate beads by adding a suitable proportion of sorbitol and $NaHCO_3$ in the bead formulation.

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