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Development and characterization of nano biopolymer containing cumin oil as a new approach to enhance antioxidant properties of button mushroom

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ABSTRACT

The aim of present study was to design a controlled release system using *Cuminum cyminum* essential oil loaded chitosan nanoparticles (CEO-CSNPs) and evaluate its effect on catalase (CAT), glutathione reductase (GR), peroxidase (POD) activity and ascorbic acid content of *Agaricus bisporus* fruit bodies during 20 days of storage at 4 °C. The success of encapsulation was evaluated through TEM, DLS, FT-IR and spectrophotometry and its release behavior was studied in buffer solutions with different pH. The CEO-CSNPs exhibited an average size of 30 to 80 nm with a spherical shape. Encapsulation efficiency (EE) and loading capacity (LC) were 4.46 to 17.89% and 2.47 to 6.68%, respectively. The highest CAT and GR activity was observed in samples packed with CEO-CSNPs after 15 days of storage. In contrast, POD activity reached a peak at the end of storage in control samples. Interestingly, after 20 days the level of POD increased 17.13% in CEO-CSNPs treatment, as compared with the initial level of the mentioned enzyme. At the end of storage, ascorbic acid content in samples treated with CEO-CSNPs was significantly higher than that detected in the control samples. In brief, application of CEO loaded chitosan nanoparticles in packages effectively increased the antioxidant activity in white button mushroom and showed promising results for extending the shelf life of treated samples.

1. Introduction

Agaricus bisporus recognized as an important horticultural crop due to its nutritional value [1] and therapeutic activities such as anti-flammatory, cardiovascular, anticancer, antiviral, antibacterial and hepatoprotective properties [2,3]. The shelf life of this crop is short and it quickly loses its marketability due to water loss, tendency of fruit bodies to turn brown [4], and microbiological spoilage during storage [5]. The food preservation techniques are restricted due to limited availability of suitable bio-based polymers, regularity concerns and natural antimicrobial compounds in food industry. Chitosan, a linear polysaccharide, because of its non-toxic [6], bacteriostatic, fungistatic, anti-cancer and anti-chlosteremic properties [7], its good biodegradability and biocompatibility characteristics as well as natural occurring [8] and quick gelling ability [9] is considered to be an interesting material in food packaging.

Essential oils (EOs) extracted from different parts of plants show antimicrobial and antioxidant activities. Cumin seed is one of the most common spices in cooking. The active compounds of its essential oil including γ-terpinene, p-cymene, pinene, cumin aledehyde, safranal and cuminal [10] exhibit antioxidant [11] and antimicrobial [12] activities. It is suggested that the major compounds occurring in cumin seed oil such as cuminal and cuminic alcohol show very strong antioxidant activity with a broad spectrum [13]. This property is assigned to their ability to prominently quench hydroxyl radicals, 1,1-diphenyl-2-picryhydrazyl (DPPH) radicals and lipid peroxidase [14], as in previous studies, the promising effect of cumin essential oil in control of *Aspergillus flavus* [15] and meat protection [16] have been reported. However, these compounds decompose when exposed to light, heat, and pressure [17]. Controlled release may be defined as a technique which allows controlling time of material release at a specific rate and it is a new method to protect EO from the direct contact with crop. Technology of nanoencapsulation has been recently applied in food packaging systems to overcome the problem of susceptibility, better targeting of nanoencapsulated essential oil and controlled release of the bioactive compounds during processing and storage in comparison to micro-size carriers [18]. Therefore, encapsulation of EOs with subcellular size of chitosan tripolyphosphates (CS-TPPs) as controlled release systems can help to protect EOs against thermal or photo degradation. This assures preservation of antimicrobial activity against bacteria, fungi and yeast and leads to extended shelf life of crop [19].

Antioxidant activity is the important factor that prevents oxidation of products and has the ability to neutralize the damaging effects of

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reactive oxygen species (ROS) in tissues and subsequently leads to preserve foods quality. Polyphenolic compounds and antioxidant enzymes such as glutathione reductase (GR) and catalase (CAT) are considered as main antioxidants that protect the cells against reactive oxygen species (ROS) [20].

In previous literatures, the positive effect of different treatments such as essential oils fumigation [21,22], chitosan-based coating [23], microencapsulated essential oil [4] and coating with chitosan containing thyme oil [24] on postharvest quality of mushrooms was reported. However, in the coating method, the fruit bodies should be immersed in chitosan solution and immediately dried, as the delay in drying process leads to a rapid deterioration of crop. To our knowledge, there has been no report on chemical properties of cumin essential oil loaded chitosan nanoparticles with ionic gelation technique. Therefore, the purpose of this study was to evaluate the effect of *Cuminum cyminum* essential oil loaded chitosan nanoparticles (CEO-CSNPs) on enzymes associated with nutritional quality of white button mushroom stored for 20 days at 4 °C. In addition, the physicochemical properties of CEO-CSNPs including chemical structure, size and morphology were investigated.

2. Materials and methods

2.1. Material and regents

Cumin (*Cuminum cyminum*) seeds were prepared from a valid local market. Pentasodium triphosphate (TPP), glacial acetic acid, chitosan (degree of deacetylation: 85%; medium molecular weight) and Tween-80 were purchased from Sigma-Aldrich (Germany).

2.2. Isolation of the essential oil and chemical composition analysis

The dried cumin seeds ground to fine powder using electrical mill. Then 50 g of sample was added to 500 ml deionized water and EO extraction was performed by Clevenger apparatus for 2.5 h. The collected EO was stored in dark glass bottle in refrigerator until use. Chemical composition of essential oil was identified using GC–MS. A gas chromatograph (Agilent-7890B) equipped with HP-5MS capillary column (length: 30 m; diameter: 0.25 mm; film thickness: 0.25 μm) and helium carrier gas at a flow of 1 ml min^{-1} connected to a mass spectrometer (Agilent-MSD5975C) was used. The injector temperature was set at 250 °C and 1 μl of cumin EO was injected. Column temperature was kept at 50 °C for 3 min, and then increased to 180 °C for 2 min. EO components were identified by using retention time, comparison of the mass spectra with those of standards, Wiley 7NL library data of the GC–MS system.

2.3. Preparation of CEO-CSNPs

Cumin essential oil was used as an antioxidant agent in chitosan solution. *Cuminum cyminum* essential oil loaded chitosan nanoparticles (CEO-CSNPs) were synthesized by ionic gelation technique (Fig.1) [25]. Chitosan solution (0.5% w/v, 40 ml) was prepared by dissolving in 1% glacial acetic acid at room temperature. To prepare CEO-CSNPs, various contents of antioxidant agent were used to obtain different weight ratios of chitosan to cumin EO (1:0.25, 1:0.5, 1:0.75, 1:1 and 1:1.25). After dissolving completely, Tween-80 (HLB, 15) is slowly added as a surfactant. TPP solution (0.5% w/v, 40 ml) is dropped into emulsion under magnetic stirring. The final pH of above solution was ~5. After crosslinking, nanoparticles were isolated by centrifugation at 8944*g* for 10 min. The obtained wet particles were dispersed in 25 ml of distilled water.

2.4. Characterization of CEO-CSNPs

The particle size of the synthesized nanoparticles was measured using DLS (dynamic light scattering; Vasco™, nanoparticle size analyzer, Cordouan Technologies, France) with a wavelength of 657 nm at 25 °C with a fixed scattering angle of 135°. All DLS measurements were carried out in dilute aqueous nanoparticles suspensions. The results were reported as the mean of three measurements. The surface morphology of nanoparticles was evaluated by transmission electron microscopy (TEM). For TEM, the nanoparticles solution was dropped on copper grids and dried overnight at room temperature. The chemical structure of chitosan, CEO and CEO-CSNPs were analyzed by Fourier transform infrared spectrometer (FT-IR) operating between 400 and 4000 cm^{-1}.

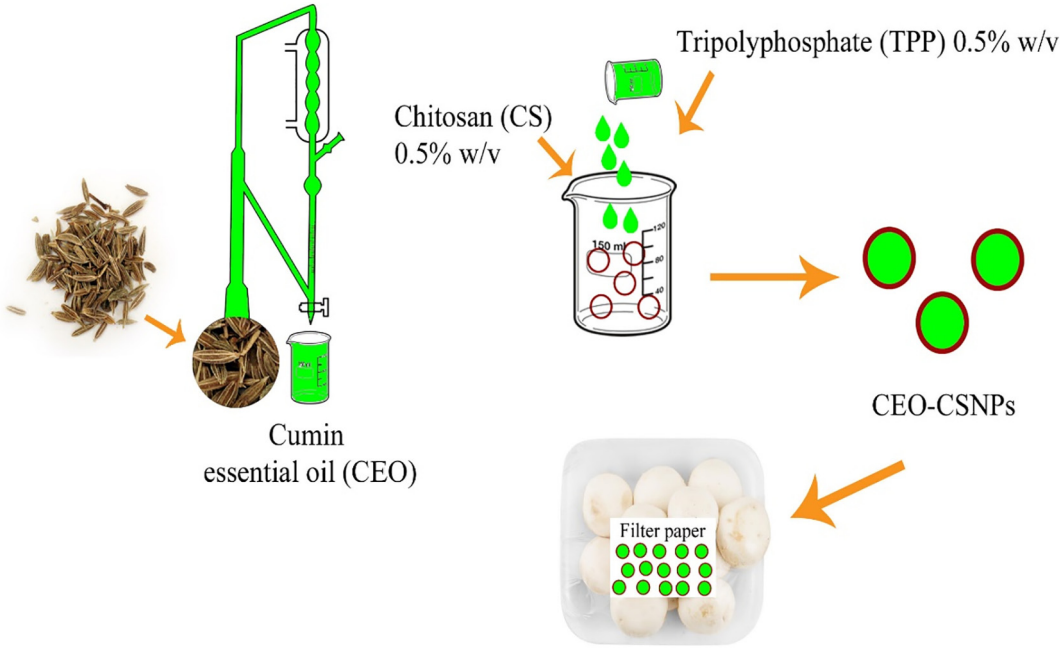

Fig. 1. The scheme of the synthesis of chitosan nanoparticles containing cumin seed essential oil.