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Extraction optimization and nanoencapsulation of jujube pulp and seed for enhancing antioxidant activity

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ABSTRACT

The aim of this study was to optimize extraction conditions for jujube pulp and seed in order to obtain maximum active ingredient yield and antioxidant activity, as well as to prepare chitosan nanoparticles loaded with jujube pulp and seed extracts for enhancing stability. The extraction conditions, i.e. temperature, time, and ethanol concentration, were optimized at the following respective values: 61.2 °C, 38 h, and 60.4% for pulp, and 58 °C, 34 h, and 59.2% for seed. The jujube nanoparticle size significantly increased with a higher chitosan/sodium tripolyphosphate ratio and extract concentration. Entrapment efficiency was greater than 80% regardless of preparation conditions. The stabilities of jujube pulp and seed extract in terms of total phenolic content and antioxidant activity were effectively enhanced by nanoencapsulation. In conclusion, jujube pulp and seed extracts prepared using optimal conditions could be useful as a natural functional food ingredient with antioxidant activity, and nanoencapsulation can be used to improve the stability of jujube extract. Therefore, these results could be used to promote the utilization of not only jujube pulp but also seed, by product.

1. Introduction

Jujube (*Zizyphus jujuba*) belongs to the Rhamnaceae family and is widely distributed from southwest Europe to Southeast Asia [1]. It is grown for its edible orange-brown fruits. Jujube pulp has been consumed in both fresh and dried form for thousands of years. However, the fresh jujube pulps cannot be stored for a long period, because it is highly perishable and susceptible to decay under ambient conditions [2]. Thus, dried jujubes have been predominantly used due to their long shelf life. Jujube pulps are also commonly processed into many food products, including teas, breads, cakes, jellies, and candy [3]. In addition, jujube pulp and seed have both been used in many traditional medicines, such as analeptics, antitussives, and antidiabetics [4,5]. However, only a tiny percentage of the jujube seed is used in preparing these medications; most is discarded as waste.

Recent studies have demonstrated the diverse health-promoting properties of jujube. Studies on its bioactivity showed that oral administration of jujube ethanol extract to rats fed high-cholesterol diets reduced their hepatic HMG-CoA reductase activity and plasma cholesterol and triglyceride concentrations [6]. Moreover, jujube extract exerted a protective effect on CCl_4-induced hepatic injury in mice by modulating oxidative stress [7]. Jujube extract was also found to have antioxidant activity by scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radicals, reducing ferric iron to its ferrous form, inhibiting lipid peroxidation, and protecting against DNA damage [1,8,9].

These health benefits of jujube have mainly been attributed to the presence of bioactive compounds, such as phenolic contents, carbohydrate, ascorbic acid, thiamine, and riboflavin [5]. Particularly, the many studies on the antioxidant activity of jujube showed that the various antioxidant activities such as DPPH and ABTS radical-scavenging capacity and ferric reducing antioxidant power (FRAP) was remarkably correlated to their total phenolic contents [10–12]. Although those studies focused mainly on jujube pulp, it was recently found that phenolics, which are responsible for much of the bioactivity of jujube, are contained in not only the plant pulp but also the seed [1,10,11,13]. The total phenolic content in seed was higher than that in the pulp. In addition, the insoluble-bound forms of phenolic acids are mainly found in jujube seed and peel, whereas the glycosided form in the pulp. Therefore, research into jujube seed bioactivity and promotion of its use is appropriate.

When developing a functional food from natural sources, the extraction, which is the main step for the isolation of bioactive phytochemicals, should be well characterized and optimized to increase production efficiency and the biological quality of the extracts [4]. Although the extraction conditions for jujube pulp have

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been optimized for maximal yield [14], relatively little is known about the conditions necessary to extract both maximum antioxidant activity and maximum active ingredient yield from jujube.

Research to enhance the stability of natural active ingredients is also needed for functional foods derived from medicinal plant sources. Phenolics (including catechin, chlorogenic acid, anthocyanin, and gallic acid) the primary bioactive compounds in medicinal plants are unstable in oxidative environments, which can limit their applications in functional foods [15,16]. Natural active ingredient stabilization during storage and food processing might be improved using encapsulation technology [17]. Encapsulation is an economizing approach wherein a bioactive ingredient is enclosed by a biopolymer matrix, thereby protecting it from oxygen, water, pH changes, light, and other conditions, to increase its shelf life [18].

Several studies have focused on the stability of jujube fruit to prolong its preservation [19,20]. To enhance stability in terms of physiological index, biochemical parameters, and nutritional indicators, whole jujube fruit was coated with chitosan and nano-silicon dioxide [19] and packed with nano-packing synthesized by blending polyethylene with nano-powder (nano-Ag, kaolin, anatase TiO_2, rutile TiO_2) [20]. Few studies have reported the effects of encapsulation on the active ingredient extracted from jujube. Sun et al. [21] suggested that when betulinic acid from jujube fruits was microencapsulated using beta-cyclodextrin, the inclusion complex improved the solubility of the betulinic acid and induced apoptosis through a mitochondrion-dependent pathway.

Biocompatible capsules can be easily produced by the ionic gelation of a biopolymer under mild conditions without the use of harmful organic solvents. Among biopolymers, chitosan is the most abundant polysaccharide in the world after cellulose and has been one of the most promising coating materials for many bioactive agents due to its nontoxic, biocompatible, and biodegradable characteristics [22,23]. Chitosan has unique properties that arise from the presence of positively charged ions from free amino groups in its deacetylated glucosamine residues. Thus, chitosan nanoparticles can be obtained spontaneously by ionic gelation with negatively charged multivalent ions such as tripolyphosphate. In addition, chitosan-based nanoparticles can interact with negatively charged mucosal surfaces, resulting in enhanced drug absorption and bioavailability because of a much longer residence time in the gastrointestinal tract and the opening of tight junctions between mucosal cells [24,25]. Therefore, it seems reasonable to consider a chitosan nanoparticle system as a potential oral carrier for natural bioactive substances such as jujube extracts.

The goal of the present study was to optimize extraction conditions for jujube pulp and seed to obtain not only maximum active ingredient yield but also maximum antioxidant activity, to prepare chitosan nanoparticles loaded with jujube pulp and seed extracts for enhanced stability, and to investigate their particle characteristics and effects on storage. We investigated antioxidant activity of the resulting jujube pulp and seed extracts by assessing their ability to scavenge α,α-DPPH, and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) radicals. Characteristics of these nanoparticles, including particle size, zeta potential, entrapment efficiency, and loading efficiency, were investigated with different preparation conditions. The stability of jujube pulp- and seed-extract-loaded chitosan nanoparticles was estimated by measuring antioxidant activity and total phenolic content during storage.

2. Materials and methods

2.1. Materials

The jujube fruits were grown in Boeun-gun, Chungcheongbuk-do, Korea, and harvested in October 2010. Chitosan with a deacetylation degree of 86.6% and low molecular weight; tripolyphosphate; DPPH; ABTS; potassium persulfate; Folin-Ciocalteu reagent; sodium carbonate; and gallic acid were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were analytical grade reagents.

2.2. Preparation of jujube extracts

The jujube fruits were cleaned with tap water and carefully divided with a knife into pulp and seeds. The pulp and seeds were lyophilized, ground to a fine powder, and stored at −20 °C until analyzed. The ground pulp and seed samples (1 g) were extracted in a shaking water bath with 100 mL of 40–100% ethanol for 2–42 h at 30–70 °C. The resulting extracts were filtered through Whatman No. 41 filter paper (Whatman Inc., Clifton, NJ, USA). All extracts were freshly prepared prior to experimentation.

2.3. Experimental design and data analysis for optimization

A three-factor, five-level central composite design was used to optimize the extraction conditions for jujube pulp and seed to obtain maximal polyphenolic content and antioxidant activity. The design consisted of 16 experimental points that included fractional 2^3 factorial points, six star points, and two replicates at the center point. In preliminary experiments, extraction temperature, time and ethanol concentration were considered as factors influencing the antioxidant activity and total phenolic content of jujube pulp and seed. The three preparation conditions examined as independent variables were X_1 = extraction temperature (°C), X_2 = extraction time (h), and X_3 = ethanol concentration (%). The actual values of each variable were coded at five levels for statistical analysis based on preliminary experiments. The quadratic model for predicting the optimal point was expressed using the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j>i}^{3}\beta_{ij} X_i X_j \quad (1)$$

where the dependent responses (Y) were the total phenolic content and DPPH radical-scavenging activity. β_0, β_i, β_{ii}, and β_{ij} are the constant, linear, quadratic, and cross-product regression coefficients, respectively. The Statistical Analysis System (SAS) program version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to analyze the data.

2.4. Total phenolic content

The total phenolic content in the extracts was determined using the method of Sun et al. [26] with some modification. Briefly, 0.1 mL of each extract was mixed with 0.5 mL of Folin-Ciocalteu reagent. After 3 min, 0.4 mL of sodium carbonate (7.5%, w/v) was added and incubated in a water bath at 45 °C for 15 min. Absorbance was measured at 765 nm using a spectrophotometer (DU 650, Beckman Coulter Inc., Fullerton, CA, USA). The total phenolic content was calculated using gallic acid as a standard and expressed as milligrams of gallic acid equivalent (mg GAE).

2.5. Determination of antioxidant activity

2.5.1. DPPH radical-scavenging activity

The scavenging capacity of jujube extract on DPPH free radicals was measured by the method of Brand-Williams, Cuvelier, and Berset [27] with minor modification. Briefly, the extract was mixed with 0.1 M DPPH solution (1:9, v/v) and shaken vigorously. The mixture was incubated for 30 min at room temperature. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. The DPPH radical-scavenging effect was