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Research Article

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Polysaccharides in jujube (*Ziziphus Jujuba* Mill.) fruit: Extraction, antioxidant properties and inhibitory potential against *a*-amylase *in vitro*

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ABSTRACT

The effects of different extraction methods on the content and bioactivity of polysaccharides from jujube dried with two methods were investigated. The antioxidant activities of Ziziphus jujube polysaccharides (ZJP) were investigated with DPPH radical scavenging activity and reducing power. Inhibitory effects of ZJP on a-amylase were also investigated. The most appropriate deproteinization method was sevag method. ZJP yields were affected differently. The highest DPPH radical scavenging capacity observed in USJPS for the oven-dried jujube and MSJPS for the freeze-dried. ZJP from oven-dried jujube had better reducing power than the freeze-dried. The higher a-amylase inhibition activity observed in MTJPS.

Keywords: Jujube, Polysaccharides, Extraction, Antioxidant activity, Drying methods, Inhibitory potential.

INTRODUCTION

Jujube, the most important economic fruit for edible-medicinal use, is a key member of the Chinese herbs. It belongs to the Rhamnaceae family, and is widely distributed in the temperate and subtropical areas of the North Hemisphere, especially the inland region of North China.[1] Traditionally, it is widely consumed as fruit for humans in many eastern Asian countries. Moreover, it has been used in Traditional Chinese Medicine as a home remedy for anorexia, lassitude, and loose stools in deficiency syndromes of the spleen and of hysteria in women for 4,000 years.[2] Recently, many reports on jujubes have been published concerning the health-promoting effects, including anticancer effects,[3-5] immune stimulating activity,[6] hepatoprotective effects,[7,8] gastrointestinal protective effects,[9] anti-inflammatory action,[10] and antioxidant properties.[11,1,12] Polysaccharides are one of the main components of jujube and have been shown to exhibit many biological activities including anti-proliferation capability on melanoma cell,[13] immune stimulating effects[14] and antioxidant activity.[15]

Drying is one of the most important preservation methods employed in storage of jujube and dried jujubes are valuable ingredients in a variety of sauces and soups. Gao et al.[16] reported that the amounts of epicatechin and catechin were significantly increased after the microwave-drying treatment of the jujube fruits. Up to now, there was no information about the effects of drying methods on biological activities of polysaccharides from jujube fruits. And the increasing interest in plant bioactive components is accompanied by a need to expand the application of plant extraction protocols.[17] Development of an economical and efficient extraction technique for jujube polysaccharides is of an urgent necessity.

As is known to all, oxidation is imperative to many organisms for the energy production. However, uncontrolled production of oxygen-derived free radicals can damage cellular components such as lipids and DNA,[18,19] which brings about some diseases such as cancer, rheumatoid arthritis and atherosclerosis, etc.[20] It has been reported that many plant polysaccharides have strong antioxidant abilities and should be paid more attention to exploring them as

novel potential antioxidants.[21-23]

Diabetes mellitus (DM), also simply referred to as diabetes, is a metabolic diseases characterized with a high blood glucose level that can cause serious damage to body system, such as blood vessels and nerves.[24] There are three main types of diabetes, type I, type II and gestational diabetes. Type II diabetes is the most common, which is affecting 90-95% of the U.S. diabetes population.[25] One of the therapeutic approaches to treat the diabetes is to decrease the postprandial hyperglycemia by retarding absorption of glucose. Inhibition of carbohydrate-hydrolyzing enzymes, such as a-glucosidase and a-amylase, is considered a possible pathway. Because the enzymes play a key role in digesting carbohydrates.[26] Thus, the retardation of the action of a-glucosidase and a-amylase by inhibitors might be one of the most effective approaches to control type 2 DM. So far, to control post-prandial hyperglycaemia after meals, acarbose and voglibose are used either alone or in combination with insulin. However, side effects of these compounds, such as liver disorders, flatulence, abdominal pain, renal tumours, hepatic injury, acute hepatitis, abdominal fullness and diarrhoea, have been reported.[27] Therefore, there is an increasing need for development of a natural, safe product without side effects.

Jujube polysaccharides are a sort of composite polysaccharides with complicated structures which are possible to be altered or lose its activities during extractions. Therefore, different extractions and the antioxidant activity and their inhibitory potential against *a*-amylase of jujube polysaccharides were investigated in this paper.

EXPERIMENTAL SECTION

2.1 Materials and Methods

2.1.1 Chemicals

2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) and D-glucose were purchased from Sigma–Aldrich (St. Louis, USA). Bovine serum albumin (BSA) was obtained from Biosharp (Göttingen, Germany). 4-Nitrophenyl-a -D-glucopyranoside (*p*NPG), and a-amylase (E.C.3.2.1.1) were obtained from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid (TCA) and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals and solvents were analytical grade.

2.1.2 Dried Jujube Preparation

A local cultivar of *Ziziphus jujuba* Mill., called 'muzao' is planted in Yulin (110°17´E, 37°36´N; average elevation, 1049 m), Loess plateau region of China. Jujube fruits obtained were carefully washed, halved and stoned. The retaining edible portions (flesh and skin) were thinly sliced (3 mm thickness) and processed as follows: (a) being dried in an oven (Gallenkamp, UK) at 70 ^{o}C for 8 h; (b) being freeze-dried in a freeze-drier, model No. G5200H at a temperature of -50 ^{o}C for 48 h. Then the dried jujube fruits were grind with a mill (FW-80, Taisite Co., Tianjin, China) and sealed in air-tight plastic bags stored under dry and dark conditions until used.

2.1.3 Preparation of Crude Polysaccharides

UAE of polysaccharides from dried jujube was performed using an ultrasonic clearer (KQ-700DE, Kunshan, Zhejiang, China) with thermostatic temperature control. Five grams of dried jujube powders were extracted with distilled water in a 250-mL beaker held in the ultrasonic clearer and extracted experimentally at the optimal extraction condition: extraction temperature of 60 ^{o}C, extraction time of 30 min, ultrasonic powers of 230 w, and W/M ratio of 30:1. After filtration to remove debris fragments, the filtrate was concentrated using a speed vacuum concentrator (BUCHI 409, Buchi Corp., New Castle, DE, USA). The protein was removed as described as follows, and the solution was then precipitated with a three fold of volume of 95% ethanol overnight at 4 ^{o}C. The resulting precipitate was collected by centrifugation at 3000 rpm for 10 min (PM180R, ALC International, Milan, Italy), washed with ethanol and acetone in turns, centrifuged, then vacuum-dried. Then the crude polysaccharide was obtained (named as CJPS). The CJPS were weighted with a balance (AUY 220, Shimadzu, Japan).

HWE was carried out in a water bath (HH-6 Guohua Wiring Company, Shanghai, China) at the optimal extraction condition: extraction temperature of 60 ^{o}C, extraction time of 4 h, and W/M ratio of 30:1.

MAE was carried out in a ME1-3 L microwave extraction apparatus (Wuxi Pulaima Instrument Co., Ltd., Jiangsu, China) with a power of 300 W, extraction time of 2 min, and W/M ratio of 30:1 based on the preliminary optimal experiment. After extraction, the post-treatment of the water extraction solutions was the same as that mentioned in UAE. In this study, all the experiments were conducted in triplicate.

The total sugars content was examined by phenol–sulfuric acid colorimetric method using glucose as standard reference material.[28]

2.1.4 Removal of Proteins

Sevag Method

The crude polysaccharide extracts were transferred into a 250 mL separatory funnel. After addition of sevag reagent (chloroform: *n*-butanol = 4:1, 10 mL), the solution was shaken vigorously for 20 min at room temperature and centrifuged at 12,000 rpm for 20 min. The supernatant were collected and the protein content was determined by the Coomassie brilliant blue G-250 method with bovine serumalbumin as a standard.[29]

TCA Method

Five milliliters TCA aqueous solution were further added separately to the crude polysaccharide extracts and stirred at 100 rpm and at 4 ^{o}C for 12 h using a stable temperature magnetic stirrer. After centrifugation at 12,000 rpm for 15 min, the supernatant was collected for protein and polysaccharide analysis.

2.1.5 Assay of DPPH Radical Scavenging Activity

The DPPH radical scavenging activity test of ZJP was carried out according to the method of Shimada et al.[30] with some modification. Briefly, 2 mL of DPPH solution (0.4 mmol/L DPPH in methanol) was added with 2 mL ZJP and reacted at room temperature. The mixture was shaken and the absorbance was measured at 517 nm. The percent DPPH radical scavenging effect was calculated according to the following equation:

DPPH scavenging effect (%) = $(1- A_1/A_0) \times 100$

where A_0 is the absorbance of DPPH solution without ZJP and A_1 is with ZJP.

2.1.6 Reducing Power

The reducing power was determined according to the method of Oyaizu[31] with some modification. ZJP samples in phosphate buffer (2 mL, 0.2 M, pH 6.6) were mixed with potassium ferricyanide (2 mL, 1%) and were incubated at 50 ^{o}C for 20 min. Trichloroacetic acid (2 mL, 10%) was added, and the mixture was centrifuged at 3000 r/min for 10 min. The supernatant (2 mL) was with distilled water (2 mL) and ferric chloride (0.4 mL, 0.1%) and the absorbance was read spectrophotometrically at 700 nm after 30 min. A higher absorbance of the reaction mixture indicated greater reducing power.

2.1.7 α-Amylase Inhibitory Assay

The *α*-amylase inhibitory activity of ZJP was determined according to the method described by Kim et al.[32] with slight modifications. Starch azure (2 mg, Sigma Chemical Co.) which was used as a substrate, was suspended in 0.2 mL of a 0.05 mol/L Tris-HCl buffer (pH 6.9) containing 0.01 mol/L $CaCl_2$ and boiled for 5 min. The starch solution was then pre-incubated at 37 °C for 5 min. The sample was dissolved in ultra pure water (the final concentration was 10 mg/mL) and 0.1 mL of PPA solution (2.11 U/mL) (Sigma Chemical Co.) in the above bufer was applied for each assay. The reaction was carried out at 37 ^{o}C for 10 min and stopped by adding 0.5 mL of 50% acetic acid. The reaction mixture was then centrifuged at 3000 rpm for 5 min. The absorbance of the resulting supernatant at 595 nm was recorded. The *α*-amylase inhibitory activity was calculated as follows:

$$\text{PPA inhibitory (\%)} = \left[\left(A_{c+} - A_{c-} \right) - \left(A_s - A_b \right) \right] / \left(A_{c+} - A_{c-} \right) \times 100$$

where A_{c+}, A_{c-}, A_s and A_b are defined as the absorbance of 100% enzyme activity (only the solvent with the enzyme), 0% enzyme activity (only the solvent without the enzyme), a test sample (with the enzyme), and a blank (a test sample without the enzyme), respectively.

2.1.8 Statistical Analysis

Statistical analysis was performed by using PASW Statistics 18 software (Somers, NY, USA). The results were presented as means of three determinations ± SD (standard deviation). The results obtained were analyzed using one-way analysis of variance (ANOVA) for mean differences among the samples. *p*-Values of < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

3.1 Polysaccharides Content

The selective dehydration of jujube for polysaccharides extraction by an appropriate method is very important. Both yield and activity of polysaccharides are strongly dependent on the type of dehydration employed. The polysaccharides yields of oven-dried samples were higher than that of freeze-dried ones except for UCJPS and SSJPS (Figure 1). Clearly, the application of oven-drying positively affected the polysaccharides yield. When

compared with sevag and TCA methods for the removal of proteins, the yields of polysaccharides with TCA method decreased sharply for freeze-dried samples. This is probably because of the tempestuous reaction between TCA and proteins, which can lead to the degradation of polysaccharides, and thus a higher loss of polysaccharides was observed in this way.[33] Based on the above results, in order to remove most proteins and avoid the loss of polysaccharide, the most appropriate deproteinization method was sevag method. In addition, compared with MWE, UAE and HWE, polysaccharides yields were affected differently.

3.2 Protein Content

Some polysaccharides contain neutral sugar, and they are usually conjugated with other components such as protein to exhibit various activities. So it was necessary to analyse the protein content in these polysaccharides samples. The protein contents of freeze-dried samples were higher than that of oven-dried ones except for USJPS and STJPS (Figure 2). TCA method was an effective for the removal of proteins of MTJPS for the freeze-dried sample and the sevag method was appropriate for MSJPS of the oven-dried sample.

3.3 Scavenging Effect on DPPH Radical

Methanol solutions of DPPH have a characteristic absorption maximum at 517 nm. The method of scavenging DPPH is based on the reduction of DPPH methanol solution in the presence of a hydrogen donating antioxidant, resulting in the formation of the non-radical form DPPH-H by the reaction.[34] It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrationsgh.[35] On the basis of this principle, the scavenging effects of polysaccharides samples on the DPPH radical are measured and shown in Figure 3.

The DPPH radical scavenging capacity of water extract from jujube was found to falls between sevag and TCA methods with the highest values observed in USJPS for the oven-dried jujube and MSJPS for the freeze-dried sample. For oven-dried samples with sevag method, the antioxidant activities of polysaccharides extracted by UAE were mostly higher than those extracted by HWE and MAE from the three samples except for SSJPS (Figure 3). The DPPH radical scavenging abilities were influenced by the different drying method because of the changes of the physicochemical properties of the polysaccharide in jujube samples. These findings provide useful considerations in the application of UAE for extraction of polysaccharides from jujubes as components of health food and medicinal products. TCA caused a significant decrease ($P < 0.05$) in the radical scavenging ability of freeze-dried jujubes compared with the sevag method.

The polysaccharides have been proved to be able to reduce the stable DPPH radical to yellow diphenylpicrylhydrazine, and the antioxidant activity of polysaccharides is highly related to their chemical structure and need to be done in the futrure work.[36]

3.4 Reducing Power

It has been reported that there was a direct correlation between antioxidant activity and reducing capacity.[37] The reducing properties are generally associated with the presence of reductones, which could donate a hydrogen atom and exert antioxidant action by breaking the free radical chain.[38] Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The antioxidant activity was concomitant with the reducing power.[39,40] The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In order to elucidate the relationship between the antioxidant activity and the reducing power of jujube polysaccharides, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of polysaccharides samples. The reducing powers of all samples are shown in Figure 4.

Results indicated that the reducing power of polysaccharides was highly increased with MAE, except for STJPS for oven-dried samples. However, it has been reported that microwave is an electromagnetic radiation with a wavelength from 0.001 m to 1 m (frequency from 3×10^{11} Hz to 3×10^{8} Hz), and may have the possibility influence to the characterization and biological activity of biopolymers.[41] When compared with sevag and TCA methods for the removal of proteins, the reducing power of polysaccharides with sevag method kept better antioxidant activity for oven-dried samples. For most of the samples, polysaccharides from oven-dried jujube powder had better reducing power than the freeze-dried ones.

3.5 Inhibition on the a-Amylase Activity

The low level of *a*-amylase inhibition in natural fruits, vegetables and legume grains is reported to offer a good strategy to control postprandial hyperglycaemia.[42,43] In this connection, the high *a*-amylase inhibition activity observed in MTJPS samples of the present study seems to be suitable for implementing in the dietary practice of type II diabetes.

The effects of the WSPs prepared from jujubes on the a-amylase activity were shown in Figure 5. MTJPS had the highest *a*-amylase inhibitory activity while UTJPS of the freezing powder had the least. Ultrasonic extraction significantly affected the *a*-amylase inhibition property (but not freezing powder) of presently investigated jujube samples. It was speculated that the WSP from jujube might contain some amylase inhibitory groups or components, leading to a direct effect in reducing the *a*-amylase activity. Gourgue et al. [44] have reported that the polysaccharides having a large amount of free carboxylic groups isolated from fruit would inhibit the enzyme activity. Some minor compounds such as tannins and phytic acid on the polysaccharides might also inhibit the activity of *a*-amylase.[45,46] These results suggested that the WSP from jujube might help in prolonging blood glucose response and hence control the postprandial glucose concentration.

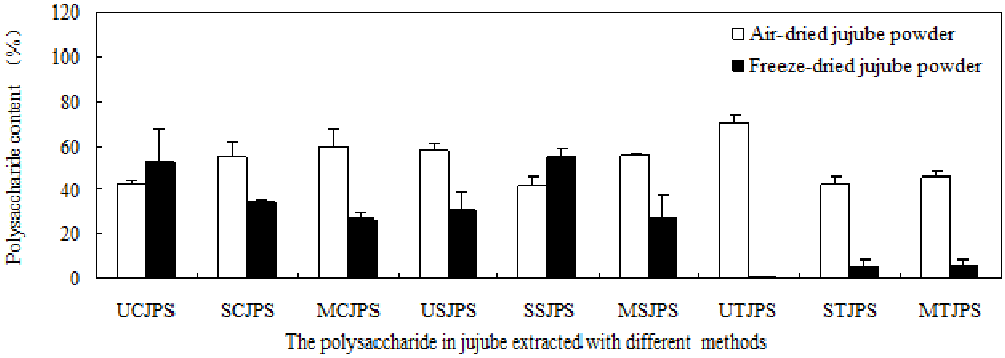

Figure 1: Polysaccharides content in jujube extracted with different methods

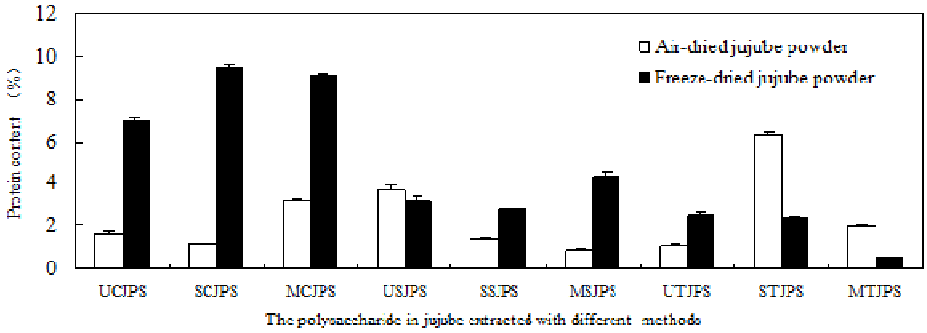

Figure 2: Prptein content in the polysaccharides from jujube extracted with different methods

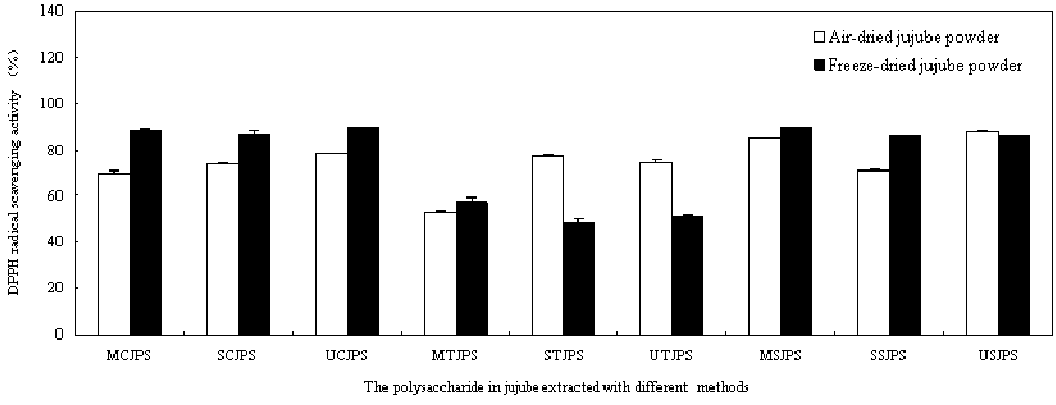

Figure 3: DPPH radical scavenging activity of polysaccharides in jujube extracted with different methods

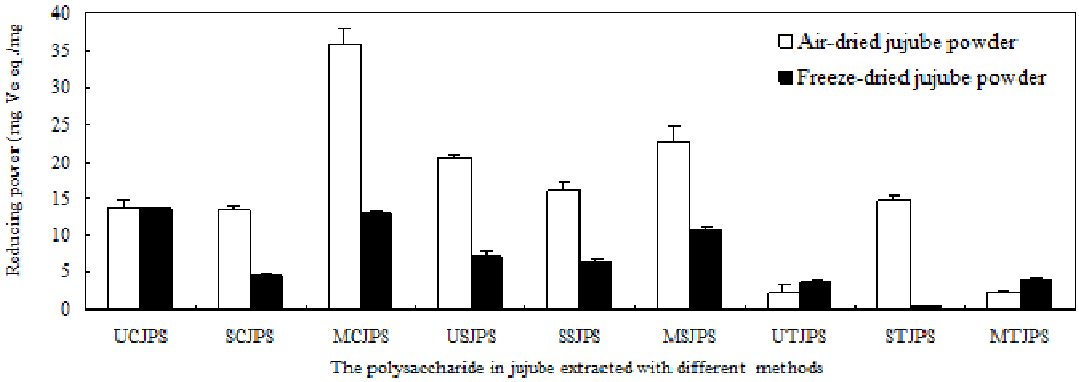

Figure 4: Reducing power of polysaccharides in jujube extracted with different methods

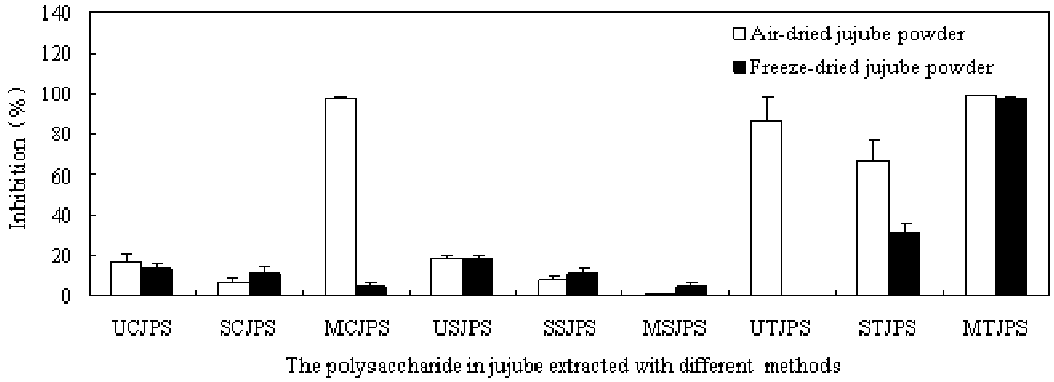

Figure 5: Inhibition against *a*-amylase of polysaccharides in jujube extracted with different methods

CONCLUSION

In the past decades, it has been found that the previous polysaccharides in plants are not only energy resources but they play key biological roles in many life processes as well.[47] In the present study, we obtained the optimum extraction method for production of the crude polysaccharides from jujubes for special biological activities. Anti-oxidation tests in vitro indicated that ZJP had activities of DPPH radical and reducing power. Applying to different methods would obtain different effective polysaccharides. One of the reasons is different polysaccharides with different molecular weight and the low molecular weight products are more effective than high molecular weight products.[48] Crude and different polysaccharide fractions extracted from the jujube fruits revealed strong antioxidant capacities however, fractions had the highest antioxidant activities and should be considered as a source of prospective antioxidants. To investigate the main radical scavenging polysaccharides fractions, further works on purify and functions evaluation are in progress.

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