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Characterization and antioxidant activities of polysaccharide extracted from *Benincasa hispida* var. *chieh-qua* How

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Abstract

The water-soluble polysaccharide (BHCP) was isolated from the hot aqueous extract of Chieh qua (*Benincasa hispida* var. *chieh-qua* How) fruit. The polysaccharide was found to contain *D*-galactose and *D*-glucose in a molar ratio of 2.59:1 with both α- and β-glycosidic bond. The strong free radicals scavenging activity on DPPH, ABTS, and hydroxyl radicals of the BHCP was demonstrated, and showing the BHCP from Chieh qua has potential application value on the functional food.

Keywords: *Benincasa hispida* var. *chieh-qua* How; polysaccharide; antioxidant activity.

Practical Application: Antioxidant activity of polysaccharide from *Benincasa hispida* var. *chieh-qua* How.

1 Introduction

Benincasa hispida var. *chieh-qua* How (known as "Chieh qua" or "mini Donggua"), a variety of *Benincasa hispida* (Thunb.) Cogn. var. *hispida* (known as "Donggua or Winter melon"), is a popular vegetable in south of China, especially in Guangdong and Guangxi Province (Flora of China Editorial Committee, 1986). The fruit of Chieh qua is smaller than that of Donggua (Figure 1). Like Donggua, the fruit of Chieh qua is also a rich source of nutrients such as proteins, vitamins, and minerals (Flora of China Editorial Committee, 1986; Zaini et al., 2011). Many chemical components isolated from Donggua such as flavanoids, saponins, organic acids and polysaccharides have various biological activities and pharmacological functions (Bimakr et al., 2012; Du et al., 2005; Jayasree et al., 2011; Jiang et al., 2016; Zaini et al., 2011). However, no phytochemical research on Chieh qua was reported before. Natural polysaccharides have attracted more and more researchers' interest because of their various biological activities (Chen et al., 2021; He et al., 2020; Huang et al., 2015; Li et al., 2020, Liu & Li, 2021; Yu et al., 2021). Here we reported the antioxidant activities of water-soluble polysaccharide extracted from Chieh qua.

2 Materials and methods

2.1 Materials and chemicals

The fruit of *Benincasa hispida* var. *chieh-qua* How (Chieh qua) were provided by Institute of Vegetable, Zhejiang Academy of Agricultural Sciences. Dimethyl sulfoxide (DMSO), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ascorbic acid (Vc), 1,1-diphenyl-2-picryl- hydrazyl (DPPH), and monosaccharide standards (*D*-xylose (*D*-Xyl), *L*-arabinose (*L*-Ara), *D*-glucose (*D*-Glu), *D*-galactose (*D*-Gal), *D*-mannose (*D*-Man), *L*-rhamnose (*L*-Rha), and *L*-fucose (*L*-Fuc)), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemical reagents were of analytical grade.

2.2 Preparation of BHCP

Extraction of BHCP and protein removal

The powder of dried *B. hispida* var. *chieh-qua* How fruit was extracted with 95% EtOH refluxing 2 times (1 h for each time) to remove lipophilic compounds, and then successively extracted with 10 vol of distilled water at 90 °C for 3 times (2 h for each time). The aqueous extracts were combined and concentrated under reduced pressure and then precipitated with 75% ethanol at 4 °C overnight. The precipitate was centrifuged and re-dissolved in distilled water to remove the protein using Sevage method ($CHCl_3$: *n*-BuOH, 4: 1, v/v) (Li et al., 2021b). Finally, the samples were lyophilized, giving the polysaccharide (BHCP).

Monosaccharide composition

About 5 mg BHCP was hydrolyzed in 1 mL trifluoroacetic acid (TFA, 2 mol/L) at 120 °C for 3.0 h. The excess acid was completely removed by nitrogen. Neutral sugars and inositol were acetylated utilizing the method described as reported previously (Song et al., 2017; Zhang et al., 2010) and then simultaneously detected by GC. GC was performed on an Agilent 7890B instrument equipped with a capillary column of Agilent HP-5 (30 m × 0.32 mm × 0.25 μm). The temperature program was: 120 °C for 3 min, then to 210 °C at 3 °C/min and maintain for 15 min. The injection volume was 0.2 μL

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Figure 1. The fruit of *B. hispida* var. *chieh-qua*.

The sample of BHCP (30 mg) was dissolved in D_2O (99.9%) and lyophilized three times to substitute the exchangeable protons and finally dissolved in 0.6 mL D_2O. ^{13}C-NMR spectroscopy was analyzed using a Bruker AVANCE III 500 MHz spectrometer at 125 MHz.

2.3 Antioxidant activities in vitro

DPPH free radicals scavenging activity

DPPH free radicals scavenging activity of BHCP was evaluated using the method reported (Hu et al., 2021; Zhang et al., 2017) with some modifications. First, a series of BHCP solutions (50, 100, 200, 500, 1000, 2000 μg/mL) were prepared, respectively. Then, 2 mL of DPPH solution (0.1 mmol/L) was added into 2 mL of above prepared solution separately, blending, and then the mixture was placed in darkroom for 30 min at 37 °C. Vc was used as a positive control. The absorbance was determined at λ=517 nm. The scavenging rate was calculated according to the following equation.

$$Scavenging\ rate(\%) = \left[1-(A_1-A_2)/A_0\right] \times 100\% \quad (1)$$

A_0: Absorption (Abs) of the DPPH solution without BHCP.

A_1: Abs of the reaction mixture.

A_2: Abs of BHCP without the DPPH solution.

ABTS free radicals scavenging activity

ABTS free radicals scavenging ability of BHCP was determined in terms of the method conducted before (Chen et al., 2021; Hu et al., 2021) with some modifications. ABTS radicals cation was prepared by mixing 7 mmol /L ABTS with 2.45 mmol/L potassium persulphate in the ratio of 1:1 and incubated at room temperature in darkroom for 16 h. The solution was then diluted with phosphate buffer (10 mmol/L, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. 2 mL of BHCP solution with various concentrations (100, 200, 300, 400, 500 and 1000 μg/mL) were added to cationic ABTS radicals solution (2 mL), shaken and incubated at 37 °C for 30 min. The absorbance was measured at 734 nm. Vc was served as positive control.

$$Scavenging\ rate(\%) = \left[1-(A_1-A_2)/A_0\right] \times 100\% \quad (2)$$

A_0: Abs of the ABTS solution without BHCP.

A_1: Abs of the reaction mixture.

A_2: Abs of BHCP without the ABTS solution.

Determination of scavenging capacity of hydroxyl radicals

1.5 mL BHCP solution with different concentration of 100, 200, 500, 1500 and 2000 *u*g/mL was added to the test tube separately. 0.5 mL 6 mmol/L $FeSO_4$ solution and 0.5 mL 3% H_2O_2 solution were then added quickly. Ten minutes later, 0.5 mL 9 mmol/L salicylic acid-ethanol solution was added. The mixture was reacted in water bath at 37 °C for 30 min. The ultraviolet absorbance of the mixed solution was recorded at 517 nm (Chen & Huang, 2019; Zheng et al., 2020). Vc was used as a positive control.

$$Scavenging\ rate(\%) = \left[1-\left(A_1 - A_2\right)/A_0\right] \times 100\% \quad (3)$$

A_0: Absorption of BHCP replaced by distilled water.

A_1: Absorption of the reaction mixture.

A_2: Absorption of BHCP.

3 Results and discussion

3.1 Deproteinization and yield of BHCP

After the hot water extraction, ethanol precipitation, and deproteinization by Sevage method, the pale yellow polysaccharide (BHCP) was obtained (12.08% yield from the dry powder) from *B. hispida* var. *chieh-qua* How (Chieh qua). The weak ultraviolet absorbance of BHCP solution at 280 nm and 260 nm (Figure 2) indicated that it was almost free of protein and nucleic acid (Li et al., 2021a; Huang et al., 2015; Zhang et al., 2016).

3.2 GC and NMR analysis

Monosaccharide compositions of BHCP were analyzed by GC. BHCP consisted of *D*- galactose and *D*-glucose in the molar ration of 2.59: 1 (Figure 3).

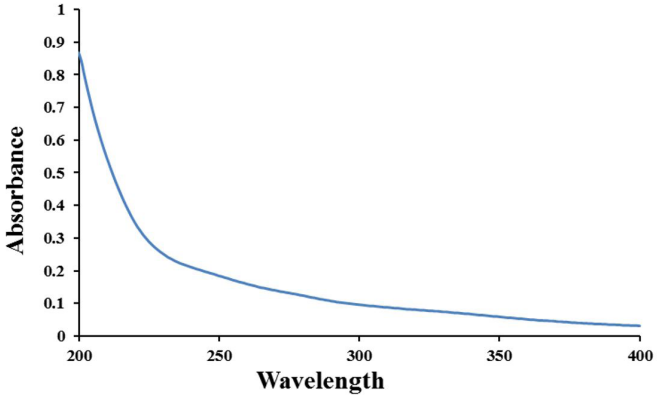

Figure 2. The UV spectrum of BHCP.

The ^{13}C-NMR spectrum of BHCP is shown in Figure 4. The chemical shifts of anomeric carbons indicated that there are both α- and β-glycosidic bond in BHCP. The chemical shifts from δ 99–101 ppm were attributed to α-galactose/α-glucoside anomeric carbons, while the chemical shift of δ 104 ppm was the characteristic resonance absorption peak of β-galactose/β-glucoside anomeric carbon (Liu et al., 2021; Pan et al., 2020). These results are consistent with the analytical results of GC.

3.3 Analysis of antioxidant activity results

Scavenging activity of BHCP on DPPH

The DPPH free radicals scavenging activity of BHCP was shown in Figure 5a. The BHCP showed preferable scavenging ability against DPPH free radicals. The scavenging activity increased from 4.30% to 73.00% in a concentration-dependent manner following the concentration of BHCP increased from 0.05 mg/mL to 2.0 mg/mL. As a control, the scavenging activity of Vc was higher than BHCP, reaching 98.80% at the concentration of 2.0 mg/mL.

Scavenging activity of BHCP on ABTS

Figure 5b showed the scavenging rate of ABTS free radicals. BHCP exhibited remarkably scavenging abilities on ABTS radicals cations in a dose-dependent pattern at the concentrations region of 0.1–0.5 mg/mL. The ABTS radicals cations scavenging ability of BHCP was 98.58% at 0.5 mg/mL, which was almost the same capacity as V_C (99.60%), suggesting that BHCP was a potential ABTS radical-scavenger.

Hydroxyl radicals scavenging activity of BHCP

The hydroxyl radicals scavenging activity of BHCP was shown in Figure 5c. The ability of scavenging hydroxyl radicals was enhanced with the increase of BHCP concentration. At a concentration of 2.0 mg/mL, BHCP displayed remarkable hydroxyl radicals scavenging rate of 82.30%, while Vc showed higher scavenging rate of 98.10% at the same concentration.

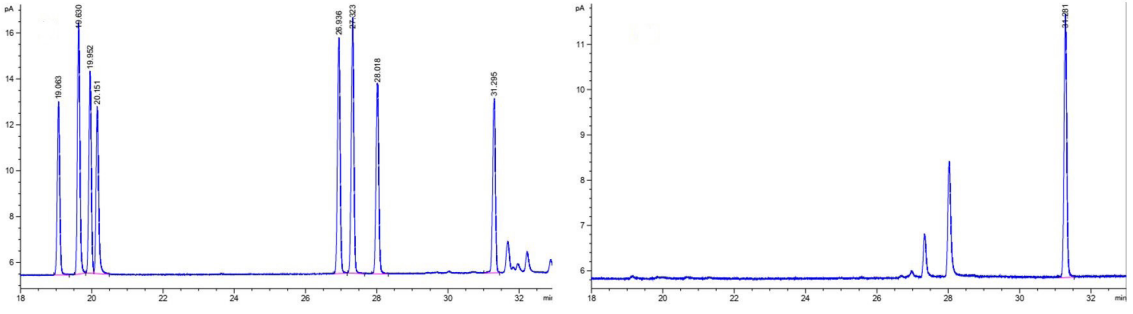

Figure 3. GC spectra of the acetylated derivative of standard monosaccharide mixture (a), BHCP (b). (R_t 19.063: *L*-Rha; R_t 19.630: *L*-Ara; R_t 19.952: *L*-Fuc; R_t 20.151: *D*-Xyl; R_t 26.936: *D*-Man; R_t 27.323: *D*-Glu; R_t 28.018: *D*-Gal; R_t 31.295: Inositol).

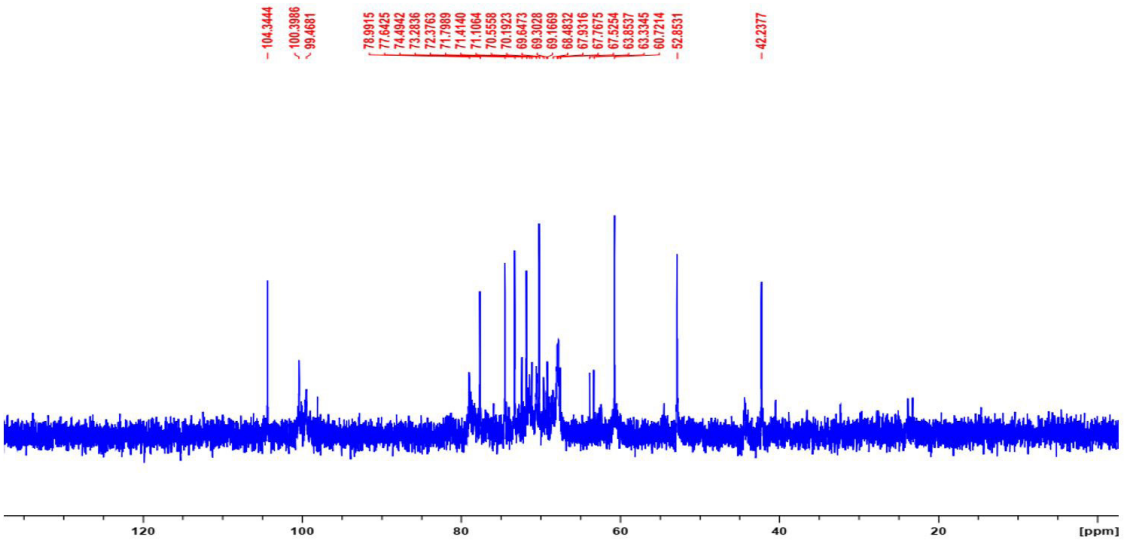

Figure 4. ^{13}C-NMR spectrum of BHCP.

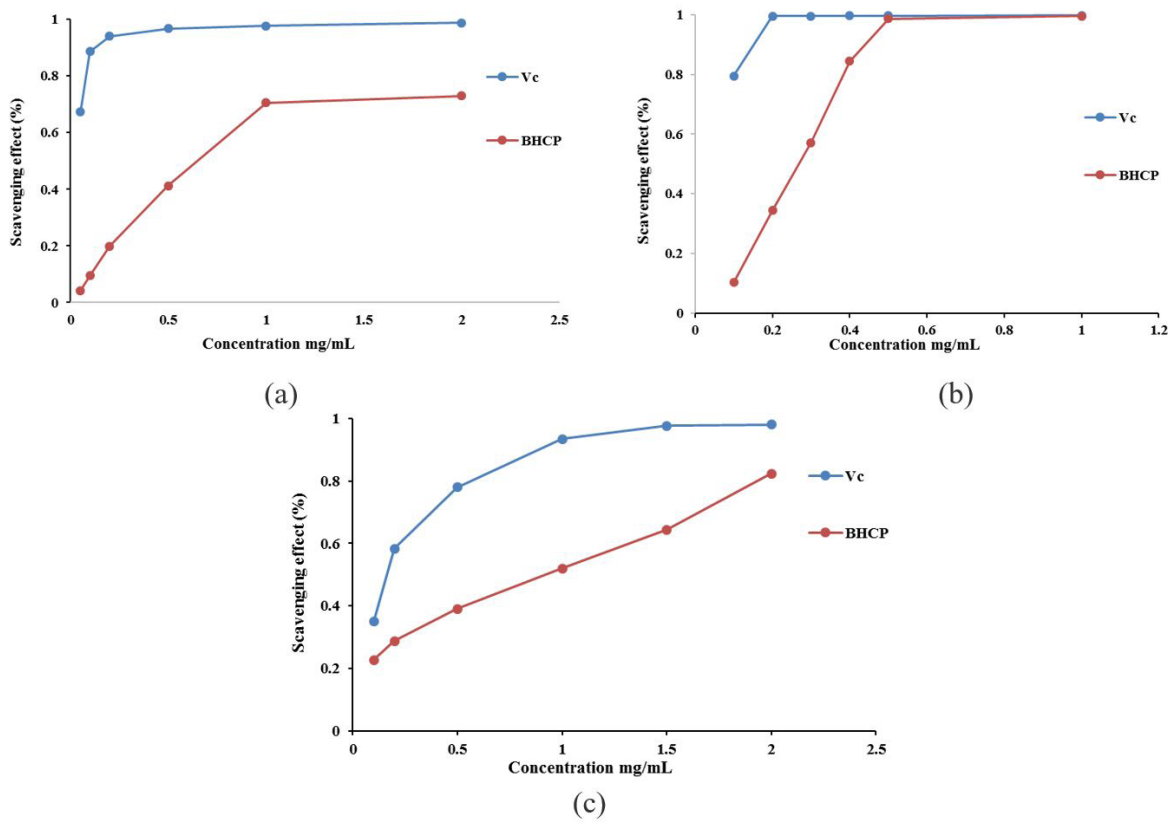

Figure 5. Antioxidant activities of BHCP. Scavenging activity of (a) DPPH radicals, (b) ABTS, (c) hydroxyl radicals.

4 Conclusion

In this study, the water-soluble polysaccharide (BHCP) was extract and isolated from Chieh qua (*B. hispida* var. *chieh-qua* How) fruit. Its structural characteristics, including the monosaccharide composition and glycosyl linkages, were elucidated. Furthermore, the antioxidant capacities of BHCP on DPPH, ABTS, and hydroxyl radicals were investigated *in vitro*. The results demonstrated that BHCP exhibited strong antioxidant activities, showing that the BHCP from Chieh qua has potential application value on the functional food.

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