Note

Antioxidative Activity of Water Soluble Polysaccharide in Pumpkin Fruits (Cucurbita maxima Duchesne)

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We evaluated the antioxidative activity of a water soluble polysaccharide fraction (WSP) from pumpkin fruits (Cucurbita maxima Duchesne). In the WSP, DPPH radical scavenging and superoxide dismutase-like activity increased depending on the total sugar content. Furthermore, the WSP can serve as an inhibitor of ascorbic acid oxidation. The efficacy was also affected by the total sugar content.

Key words: pumpkin; water soluble polysaccharides; DPPH radical scavenging activity; SOD-like activity; ascorbic acid oxidation

Pumpkin (Cucurbita maxima Duchesne) generally contains large amounts of starch, free sugars, and vitamins such as B1, B2, and C, as well as several active ingredients, including β-carotene and γ-amino-butyric acid, all of which confer on the fruit various health-promoting functions, including antioxidative activity and a blood pressure-lowering effect.1–4) Pumpkin is also known as a rich source of fiber. The fiber is mainly present as cell-wall polysaccharides, of which the structure and composition are related to changes in firmness and texture during ripening, storage, and processing.5–8) Suggesting that these polysaccharides play important roles in the plants. Besides their cholesterol-lowering properties,9) pectins, components of cell-wall polysaccharides, have recently been shown to exert an antioxidative effect10–12) and inhibitory activity against ascorbic acid (AsA) oxidation.13–16) Although several studies have reported the antioxidative effect of pumpkin (Cucurbita moschata) polysaccharides, further details are available. To gain basic insights into the active components of pumpkin (Cucurbita maxima Duchesne), we fractionated water-soluble polysaccharides and evaluated their antioxidative activity.

Pumpkin was purchased from a market. After removing seeds and stringy pulp, a portion of approximately 100 g of pumpkin fruit was added to four volumes of ethanol and blended in an electric blender. The resulting mixture was vacuum-filtrated to separate soluble and insoluble fractions. The ethanol insoluble fraction was then transferred to the same blender, thoroughly mixed with 80% ethanol, and vacuum-filtered (this step was subsequently repeated twice). The precipitate was washed with acetone and air-dried to obtain an alcohol-insoluble fraction. A portion of the alcohol-insoluble fraction was weighed, added to Milli-Q water, and boiled (100 ℃, 1 h, 3 times) to extract water-soluble components. After centrifugation, the supernatant was concentrated, mixed with 3 volumes of ethanol, and again centrifuged (10,000 rpm, 10 min). The resulting precipitate was collected, washed with acetone, and air-dried to obtain a water soluble polysaccharide fraction (WSP). A total of 100 g of pumpkin fruit provided 18.1 g of an alcohol-insoluble fraction, which ultimately yielded 10.5 g of WSP. The WSP was then thoroughly extracted with 50% ethanol and centrifuged, and the supernatant collected. The supernatant was adequately diluted and subjected to total sugar and acidic sugar analyses. The total sugar content (measured by the phenol-sulfuric acid method) were estimated to be 19.1 and 14.4 mg/g WSP respectively. Using these values, the DPPH radical scavenging and SOD-like activities of the WSP were evaluated in terms of its sugar content.

The DPPH radical scavenging activity was determined as previously described.13 It was estimated in terms of the percentage inhibition of the formation of DPPH radicals by the WSP. The 50% inhibitory concentration (IC50) was defined as the concentration of WSP that inhibited radical formation by 50%.

The SOD-like activity was measured using a commercial kit (SOD Assay Kit-WST, Dojindo Laboratories, Kumamoto, Japan). In a microplate, an appropriately diluted WSP solution (20 μl) was added to the WST-working solution (200 μl) and the enzyme-working solution (20 μl), and incubated at 40 ℃ for 20 min. The inhibition rate (%) of radical formation was quantified by measuring the absorbance at 450 nm using a microplate reader (Model 680, Bio-Rad Laboratories Ltd., Mississauga, Ontario, CA). The IC50 was defined as the concentration of WSP that inhibited radical formation by 50%.

Figure 1 shows the DPPH radical scavenging activity and SOD-like activity of the WSP. The DPPH radical scavenging activity increased depending on the concentration, with an IC50 value of 8.0 mg/ml (11.2 μmol of Trolox equivalent/g of WSP). Previous reports indicate that vitamin C and polyphenols exhibit potent DPPH...
radical scavenging activity, and among cell-wall polysaccharides, those conjugated with polyphenols, such as ferulic acid, have been shown to possess such activity.\textsuperscript{12)}

The WSP used in this study contained no polyphenols, suggesting that the DPPH radical scavenging activity was caused by polysaccharides in the WSP rather than antioxidative polyphenols. The results of Rao et al.\textsuperscript{19)} that showed acidic sugars (galacturonic and glucuronic acids) and a hydroxyl group of acidic polysaccharides possessed radical scavenging activity. Therefore, the effect observed in this study is likely to be related to the acidic sugars present in the WSP. SOD-like activity also increased in a concentration-dependent manner, with an IC\textsubscript{50} value of 0.05 mg/ml (expressed in terms of the total sugar content). The efficacy of WSP was enhanced in proportion to the total sugar content, by indicating that polysaccharides contribute to SOD-like as well as DPPH radical scavenging activity. The SOD-like activities of extracts of buckwheat sprouts were almost equivalent to that of rutin, isoorientin, and orientin.\textsuperscript{20)} In addition, extracts of buckwheat sprouts were almost equivalent to antioxidative activity. The results of Rao et al.\textsuperscript{19)} suggest that with its SOD-like activity, the WSP from pumpkin can be a beneficial component that provides comparable biological effect such as those of rutin and sulforaphane.

Additionally, we examined the inhibitory effect of WSP on AsA oxidation. In aqueous solution, AsA is generally exposed to autoxidation, by which it is converted to the hydrated form from its reduced form, leading to a decrease in UV absorbance at 259 nm. Utilizing this autoxidation response, we evaluated the inhibitory effect of WSP on AsA oxidation by measuring AsA retention in an AsA-containing WSP solution. To determine the effects of temperature on AsA retention, a 2-mm AsA solution (0.2 ml) or a 2-mm AsA solution mixed with WSP solution (0.5 ml) was added to 3.3 ml phosphate buffer (pH 5.0) for 3 h at various temperatures. A reduction in the absorbance at each time point was expressed as a percentage compared to the baseline (0 h) value, which was considered to be AsA retention. As shown in Fig. 2A, when AsA was incubated for 3 h at 30 °C in phosphate buffer (pH 5.0), AsA retention was approximately 60%. In contrast, AsA retention in the presence of WSP was almost the same that at 0 h. It was found that the addition of WSP significantly improved AsA retention at under 50 °C. The effects of various pH levels on the AsA retention were also observed Fig. 2B. At pH 5.0, the AsA retention of AsA plus WSP was found to be significantly higher than that of AsA only. Further, the AsA solution was incubated with WSP solutions with different total sugar contents (0, 5, 10, or 25 mg/ml total sugar contents), and the absorbance at 259 nm was measured every 1.5 h from 0 to 6 h after start of incubation (Fig. 3). The value markedly decreased with time in 0 μg/ml, retaining approximately 10% of the baseline value at the 3 h time point. In the AsA + WSP (5, 10, or 25 mg/ml), the reduction of AsA retention was inhibited, and a stronger inhibitory activity was observed in the AsA + WSP containing higher amounts of total sugars. At the 3 h time point, stronger DPPH radical scavenging activity was exhibited in proportion to the total sugar content in the WSP solutions, with a similar tendency to that shown for AsA retention (data not shown). This was consistent with the finding that AsA was retained by the
addition of WSP, suggesting that WSP can serve as an inhibitor of AsA oxidation. In food processing, a reduction of AsA retention is inhibited by starch. Our results suggested that WSP from pumpkin contribute to AsA retention in food processing. Using pectin showing various degrees of esterification, a previous study demonstrated that the level of esterification of polygalacturonic acid, a component of the pectin main chain, affected the inhibitory activity of AsA oxidation.\textsuperscript{15,16} Therefore, the structural profile of polysaccharides in WSP, such as the degree of esterification, is likely to play a role in the inhibition of AsA oxidation.

In conclusion, the present study indicates that the WSP from pumpkin is an antioxidative component. This finding is of significance for the discovery of new functional foods and development of processed pumpkin products. Further studies are required to determine the precise structure of WSP, and because such polysaccharides undergo structural changes through the ripening and processing of raw materials. The involvement of the polysaccharide composition and structure in antioxidative properties also needs to be sufficiently investigated for effective and nutritionally appropriate food processing.

References