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Antioxidant properties of polymeric proanthocyanidins from fruit stones and pericarps of *Litchi chinensis* Sonn

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ABSTRACT

Fruit stones and pericarps of *Litchi chinensis*, waste products of the food, were studied as a source of polymeric proanthocyanidins. The structures of the polymeric proanthocyanidins isolated from *Litchi chinensis* were characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) coupled with high performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS) analysis. The spectra obtained through MALDI-TOF MS analysis revealed that the dominant A-type procyanidin polymers occurred in each polymer length with one or more A-type linkages. The polymeric proanthocyanidins of litchi fruit stones exhibited longer polymer length than those of fruit pericarps, with polymerization degrees up to 20 and 11 for fruit stones and pericarps, respectively. After depolymerization with toluene-α-thiol, HPLC-ESI-MS analysis showed that epicatechin and A-type dimer were the major constituent units, and the mean polymerization degrees were 15.4 and 5.8 for polymeric proanthocyanidins of fruit stones and pericarps, respectively. The antioxidant properties investigated using DPPH, ABTS and FRAP methods showed that the higher polymerization degree of polymeric proanthocyanidins from litchi fruit stones exhibited higher antioxidant activities than those from litchi pericarps.

1. Introduction

Litchi chinensis (Sapindaceae) is a tropical and subtropical fruit with commercial significance native to Southern China (Wu, Pan, Qu & Duan, 2009). Besides the distinctive flavor of litchi fruit, it has also been employed in traditional Chinese medicine to promote human health for a long time (Liu, Xie, Cao, Yang, Xu & Guo, 2007; Liu, Lin, Wang, Chen & Yang, 2009).

Litchi pericarps (Le Roux, Doco, Sarni-Manchado, Lozano & Cheynier, 1998; Sarni-Manchado, Le Roux, Le Guerneve, Lozano & Cheynier, 2000), seeds (Prasad et al., 2009) and flowers (Liu et al., 2009) contain significant amounts of phenolic compounds. Phenolics have a wide range of pharmacological activities, such as anti-tumoral, anti-inflammatory, due to their antioxidant property (Wisman, Perkins, Jeffers & Hagerman, 2008). In recent years, some phenolic constituents isolated from litchi also exhibited potent antioxidant activity (Liu et al., 2007; Prasad et al., 2009; Sun et al., 2006; Zhao, Yang, Wang, Li & Jiang, 2006). These phenolic constituents mainly focused on the lower-molecular-weight phenolics, such as monomers (epicatechin, gallocatechin, and epicatechin-3-gallate), dimers (procyanidin A2, procyanidin B2, and procyanidin B4), and procyanidin trimers. However, there is little known about the antioxidant property of high-molecular-weight phenolics, such as polymeric proanthocyanidins from litchi.

Proanthocyanidins (condensed tannins) are formed of flavan-3-ol monomer units (Hagerman, 2002), which can differ in polymer length (degree of polymerization), monomer unit composition and type of linkage between monomer units (Fig. 1). As the polymer size increases, the possible number of isomers increases more rapidly. Recently, increasing evidence on the polymers in a wide range of active assays has attracted attention to elucidate their structural characteristics (Es-Safi, Guyot & Ducrot, 2006; Zhang, Lin, Zhou, Wei & Chen, 2010). However, condensed tannins are diverse compounds with great variations in structure and concentration within and among plant species. Due to the challenges of structural characterization (Monagas, Quintanilla-Lopez, Gomez-Cordoves, Bartolome & Lebron-Aguilar, 2010), proanthocyanidins were considered by Dixon, Xie, and Sharma (2005) to be a final frontier in flavonoid research.

Thiolytic degradation coupled with reversed-phase HPLC-ESI-MS analysis was applied to analysis of the proanthocyanidins in foods (Gu et al., 2003), which obtained information about monomer composition and mean degree of polymerization (mDP), but only that of the average composition of the bulk mixture have been analyzed. An alternative technique, matrix-assisted laser desorption/ionization

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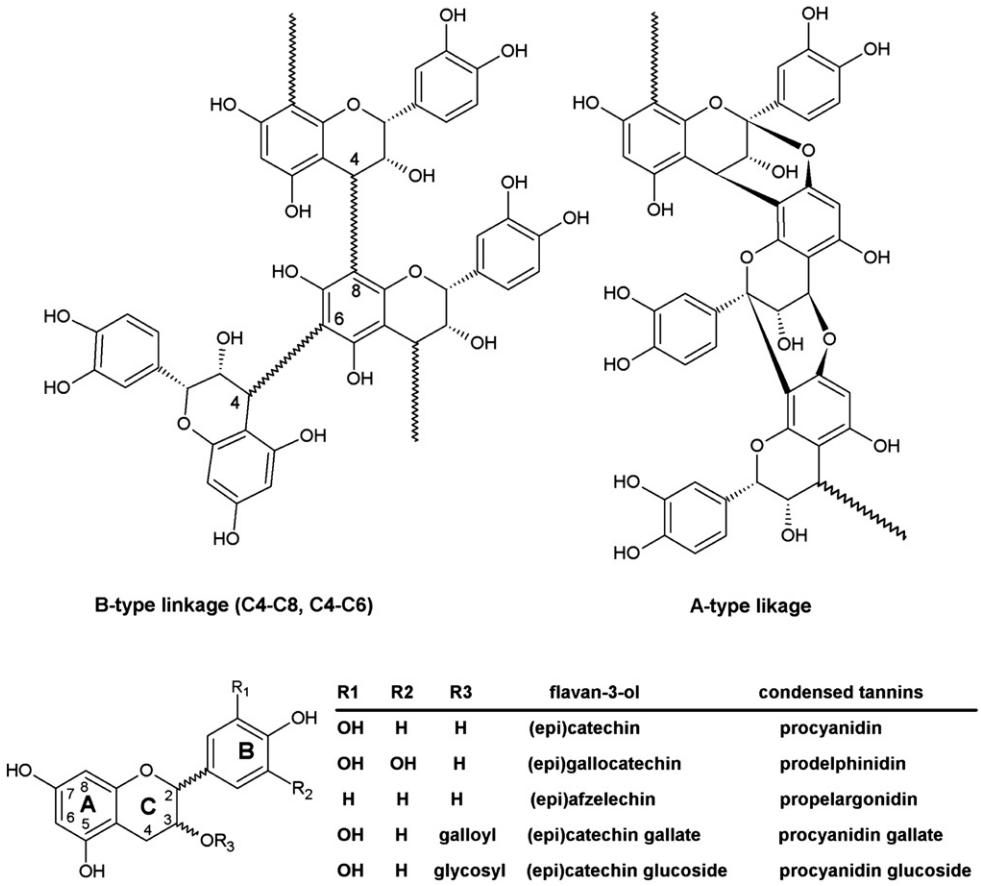

R1	R2	R3	flavan-3-ol	condensed tannins
OH	H	H	(epi)catechin	procyanidin
OH	OH	H	(epi)gallocatechin	prodelphinidin
H	H	H	(epi)afzelechin	propelargonidin
OH	H	galloyl	(epi)catechin gallate	procyanidin gallate
OH	H	glycosyl	(epi)catechin glucoside	procyanidin glucoside

Fig. 1. Chemical structure of flavan-3-ol monomer units and condensed tannins.

time-of-flight mass spectrometry (MALDI-TOF MS), is accepted as a powerful tool for the characterization of polymeric proanthocyanidins (Krueger, Vestling & Reed, 2003; Li et al., 2010; Monagas et al., 2010; Zhou, Wei, Li & Lin, 2010). Compared with another commonly used mass spectroscopy method, electrospray ionization mass spectrometry (ESI-MS), which is easy to generate multiple ions for the larger molecules inducing peak dispersion and frequent overlapping (Le Roux et al., 1998; Monagas et al., 2010), MALDI-TOF MS is ideally suited for characterizing polydispersed polymers. It allows the determination of the polymer chain length and the chemical constitution of individual chains. Moreover, the sequential succession of monomer units in individual chains can be elucidated (Behrens, Maie, Knicker & Kogel-Knabner, 2003). MALDI-TOF MS produces only a singly charged molecular ion for each parent molecule and allows detection of high mass with precision.

The present study therefore aims to characterize the structures and antioxidant properties of the polymeric proanthocyanidins extracted from litchi fruit stones and pericarps using MALDI-TOF MS coupled with reversed-phase HPLC-ESI-MS analysis after thiolysis. This technique is used for the first time with litchi polymeric condensed tannins to elucidate the monomer units, nature of the interflavan linkage, and distribution of polymerization degree.

2. Materials and methods

2.1. Plant materials

The fruits of *L. chinensis* cv. Heiye at commercial maturation were obtained from Zhangzhou City, China. The fruits were selected for uniformity of shape and colour without physical damage and injury from insects or fungal infection in the laboratory. These fruits were manually separated into stones and pericarps, which immediately freeze-dried for 48 h, ground and passed through a 40-mesh sieve. The samples were stored at −20 °C prior to analyses.

2.2. Chemicals

Water used in this experiment was purified on a Millipore Milli-Q apparatus (TGI Pure Water Systems, USA). HPLC grade acetonitrile (CH_3CN), trifluoroacetic acid (TFA) and all analytical grade solvents (acetone, methanol etc.) were obtained from Sinopharm (Shanghai, China). 2,5-dihydroxybenzoic acid (DHB), butylated hydroxyanisole (BHA), Amberlite IRP-64 cation-exchange resin, cesium chloride, toluene-α-thiol, ascorbic acid, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), and Folin-Ciocalteu reagents were obtained from Sigma (St. Louis, MO, USA), and Sephadex LH-20 was purchased from Amersham (USA). HPLC standards were purchased from Sigma (St. Louis, MO, USA).

2.3. Extraction and purification of condensed tannins

For extraction and purification of polymeric proanthocyanidins, 50 g freeze-dried fruit stones and pericarps were extracted with 70% aqueous acetone containing 0.1% ascorbic acid (3 × 500 mL) and the acetone was eliminated by evaporation under vacuum (at 38 °C). The remaining aqueous fraction was defatted with hexane (3 × 150 mL), followed by extraction with ethyl acetate (3 × 150 mL) in order to remove the low molecular phenolics, yielding dried crude tannin extract: 7.76 g of fruit stones and 8.39 g of fruit pericarps. The crude