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Influence of Degree-of-Polymerization and Linkage on the Quantification of Proanthocyanidins using 4-Dimethylaminocinnamaldehyde (DMAC) Assay

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ABSTRACT: Proanthocyanidins (PACs) are naturally occurring flavonoids possessing health beneficial bioactivities. Their quantification often utilizes the 4-dimethylaminocinnamaldehyde (DMAC) spectrophotometric assay with the assumption that molar absorption coefficients (MACs) are similar across the various PAC species. To assess the validity of this assumption, individual PAC monomers and oligomers were examined for their absorbance response with DMAC. Our results have shown that PAC dimers and trimers with interflavan linkage variations exhibited differential absorbance response. Absence of A-type linkage between the terminal and second units in PAC molecule not only impacts absorbance intensity at 640 nm but also elicits a prominent secondary 440 nm absorbance peak. Cranberry (A-type) and cocoa (B-type) oligomeric PACs exhibited differential absorbance (MACs) relationship with degree-of-polymerization. Thus, PAC structural variations have considerable impact on the resulting MAC. The use of DMAC assay in PAC quantification, especially in comparing across specific oligomers and compositions, should not assume MACs are similar.

KEYWORDS: *4-dimethylaminocinnamaldehyde, DMAC, proanthocyanidin, procyanidin, spectrophotometry*

1. INTRODUCTION

Proanthocyanidins (PACs) are oligomeric and polymeric flavan-3-ols, which are members of naturally occurring flavonoid compounds present in plants and plant-derived foods. Dietary sources include fruit, vegetables, cereals, and beverages such as wine and tea.[1] PACs are considered to be most consumed from the dietary flavonol class[2] and have been associated with a number of bioactivities beneficial toward human health. PACs have been reported to have antioxidant and anti-inflammatory activities which may ameliorate degenerative diseases, have anticancer properties, and improve cardiovascular health.[3−9] PAC oligomers of specific degree-of-polymerization (DP), e.g. DP-4 and 9, from cranberries exhibited high bioactivities against pathogenic oral biofilms[10−12] and ovarian cancer cell lines.[13]

The polymeric nature of PACs provides for numerous structural variations, including DP, linkage type, and position between constituent units. Catechin and epicatechin (Figure 1A, B) are the two most common flavan-3-ol units present in PAC oligomers and polymers. The most common linkage between two linked flavan-3-ol units is a single C—C bond (B-type), between the C4 of one (referred to as upper) flavan-3-ol unit and the C8 or C6 of the second (lower) unit (Figure 1E, F, H). In PACs of cranberry and a few other plant sources, e.g., peanut skin[14,15] and cinnamon bark,[16,17] in addition to the C—C linkage, there is also an ether linkage between the C2 of upper unit and the oxygen at C7 or C5 of the lower unit forming a double linkage, referred to as A-type, between two subunits of the polymer (Figure 1C, D, G, I−M).[1] In addition, PACs of higher molecular weights, DP > 4, have been identified and isolated from cranberry and cocoa.

Because of their polymeric nature and variations in stereochemistry, the quantification of PACs has been challenging and problematic with suboptimal analytical methods and lack of available individual PAC standards beyond epicatechin and catechin monomers and certain dimers. PACs are also found to form complexes with other compounds such as anthocyanins during processing of PAC containing fruits,[18−20] which further contributes to the intricacy of their quantification analysis. HPLC methods are available for PAC quantification in various plant and food materials,[21−26] however, due to utility and reduced costs, previously developed spectrophotometric methods have been continuously optimized to facilitate quantification of PACs in plant tissues, food stuffs, and nutraceuticals.[27−30]

Two traditionally often used spectrophotometric methods for PAC quantification include the *n*-butanol hydrochloride assay and the vanillin-acid assay. In *n*-butanol hydrochloride

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