

Vanillin/Acid Butanol Ratio for Degree of Polymerization

Ann E. Hagerman © 2002

Without pure proanthocyanidin, the modified vanillin method cannot be used to directly estimate degree of polymerization. In that case, the relative degree of polymerization for the crude extracts can be estimated by comparing the absorbance obtained with the modified vanillin assay (only terminal units react) with the absorbance obtained with the acid butanol assay (all units except the terminal unit react).

The best application of this method is probably monitoring changes in tannins in a single species. Butler (*J. Agric. Food Chem.* 1982, 30, 1090-1094) described that type of study, following tannins during maturation of Sorghum grain. This method is only useful for comparisons of structurally identical tannins, since the reactivity in the acid butanol assay is a function of the reactivity of the interflavan bond.

The assumption made for this method is that all of the flavanoid units are released and form anthocyanidin pigments in the acid butanol assay; if some interflavan bonds are not broken, then the color yield in the acid butanol assay will be low and the apparent degree of polymerization will be underestimated. The stability of the interflavan bond depends on the pattern of substitution of the flavanoids; for example 5-deoxy-proanthocyanidins give low color yields in the acid butanol assay (Hemingway in *The Chemistry and Significance of Condensed Tannins* (R. W. Hemingway and J. J. Karchesy, eds; Plenum Press) page 98 (1989).

The crude extracts are assayed with the acid butanol assay and with the modified vanillin (glacial acetic acid) assay. For each extract, the ratio of the absorbances (acid butanol assay/vanillin assay) describes the relative degree of polymerization. The absolute degree of polymerization cannot be determined using this method.