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SHORT COMMUNICATION

# ESTIMATION OF DIFFERENT PHENOLIC GROUPS IN VEGETABLE EXTRACTS

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**Abstract**—Simple phenolics, non-tannin flavans, hydrolysable and condensed tannins may be separately determined in vegetable extracts. The suggested procedure involves the application of the Folin-Ciocalteu method to the fractions obtained by selective precipitation of tannins with cinchonine sulphate and of flavonoids with formaldehyde.

## INTRODUCTION

THE MOST commonly used method for the quantitative estimation of phenolics in vegetable extracts is based on their ability of reducing the phosphomolybdic–phosphotungstic reagent of Folin–Ciocalteu,[1] with formation of a blue complex, that can be determined colorimetrically. The Folin–Ciocalteu procedure gives a measure of the total phenolic hydroxyl groups.

Useful informations may be obtained from specific evaluation of individual groups of phenolics, such as the colorimetric determination of catechins (5,7,3′,4′-tetrahydroxyflavan-3-ols) and leucoanthocyanins (5,7,3′,4′-tetrahydroxyflavan-3,4-diols).[2]

These methods have been often combined in order to obtain indications on the relative distribution of different phenolics in vegetable extracts.[2,3] The reliability of this procedure is limited by the fact that the data obtained by applying the above mentioned methods are not directly comparable. Also, the close structural relationship between catechins and leucoanthocyanins leads to strong reciprocal interference in their determination. Therefore, considerable interest is attached to procedures in which the same analytical method is applied to different groups of phenolics previously separated with partition or precipitation techniques.

Hillis and Urbach[4] have demonstrated that formaldehyde selectively reacts with phloroglucinol and compounds containing an undeactivated phloroglucinol nucleus, at room temperature and in the presence of high concentrations of HCl, producing insoluble condensation derivatives. The combined application of Folin–Ciocalteu method and formaldehyde precipitation, has allowed the evaluation of non-flavonoid and flavonoid phenolics in wines.[5] In fact, in this product, the flavonoid fraction is almost exclusively represented by flavans and anthocyanins, which readily react with formaldehyde.

[1] V. L. SINGLETON and J. A. ROSSI, JR., *Am. J. Enol. Viticult.* **16**, 144 (1965).
[2] T. SWAIN and W. E. HILLIS, *J. Sci. Food Agr.* **10**, 63 (1959).
[3] M. A. JOSLYN and J. L. GOLDSTEIN, *Agr. Food Chem.* **12**, 511 (1964).
[4] W. E. HILLIS and G. URBACH, *J. Appl. Chem.* **9**, 474 (1959).
[5] T. E. KRAMLING and V. L. SINGLETON, *Am. J. Enol. Viticult.* **20**, 86 (1969).

Methods have also been established for the selective precipitation of tannin phenolics, based on their property of forming insoluble complexes with proteins[6] and alkaloids.[7] Brugirard and Tavernier[8] have obtained a quantitative precipitation of tannins by treating apple and pear ciders with cinchonine sulphate at room temperature and pH 7·0–8·0.

## RESULTS

We have obtained a well differentiated distribution picture by applying the Folin–Ciocalteu method to the phenolic groups resulting from combined precipitations with both formaldehyde and cinchonine.

According to this procedure the 'total phenolics' value results from the sum of four groups of phenolic compounds.

*Simple phenolics.* Derivatives of hydroxybenzoic and hydroxycinnamic acids. This group also includes flavonols and other 'A' ring deactivated flavonoids.

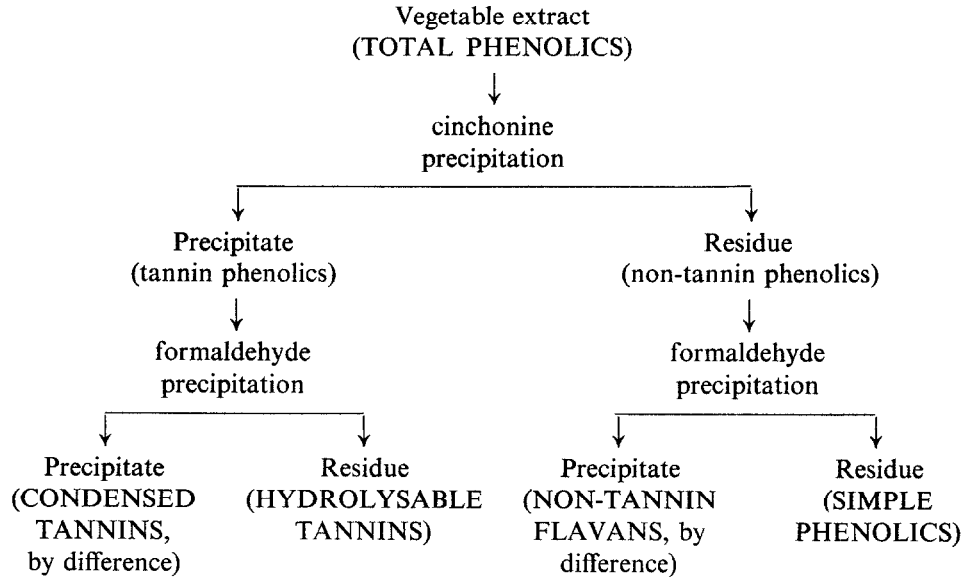
*Non-tannin flavans.* These include the monomeric anthocyanins, catechins and leucoanthocyanins.

*Hydrolysable tannins.* Polyesters of a sugar or related polyhydric alcohols and a phenolic carboxylic acid, usually gallic or ellagic acid.

*Condensed tannins.* Polymers and copolymers of catechins and leucoanthocyanins.

The first, second and fourth group are usually present in fruit juices, while the hydrolysable tannins are normally found in leaves and woody tissues.

These groups may be separately determined by applying the following partition procedure:




The Folin–Ciocalteu method is applied to the residues of formaldehyde and cinchonine precipitations and to the cinchonine–tannin precipitate, previously dissolved in an aqueous

[6] K. FREUDENBERG and K. WEINGES, *The Chemistry of Flavonoid Compounds* (edited by GEISSMAN), p.2 11, Pergamon Press, Oxford (1962).
[7] C. M. FEAR, *Analyst* **54**, 316 (1929).
[8] A. BRUGIRARD and J. TAVERNIER, *Ann. Technol. Agr.* **3**, 311 (1952).

TABLE 1. ESTIMATION OF DIFFERENT PHENOLIC GROUPS IN SIMPLE AND COMPLEX SOLUTIONS*

| Solutions | | Total phenolics | Simple phenolics | Non-tannin flavans | Hydrolysable tannins | Condensed tannins |
|---|---|---|---|---|---|---|
| 1 Gallic acid 3 mM | | 53·1 | 53·1 | 0·0 | 0·0 | 0·0 |
| 2 (+)-catechin 2 mM | | 58·5 | 2·9 | 55·6 | 0·0 | 0·0 |
| 3 Grape-seeds tannins† 0·2% | | 50·5 | 0·2 | 4·3 | 0·0 | 46·1 |
| 4 Tannic acid 0·05% | | 44·4 | 5·0 | 0·0 | 39·4 | 0·0 |
| 5 Solutions 1 + 2 + 3 (v/v) | calculated | 54·0 | 18·7 | 20·0 | 0·0 | 15·3 |
| | determined | 54·2 | 17·0 | 21·7 | 0·0 | 15·5 |
| 6 Solutions 1 + 2 + 3 + 4 (v/v) | calculated | 51·6 | 15·3 | 15·0 | 9·8 | 11·5 |
| | determined | 51·4 | 16·0 | 14·9 | 7·5 | 13·0 |

* Folin-Ciocalteu method. Results are expressed as mg of gallic acid per 100 ml of solution.

† This fraction was extracted and purified from grape seeds. It includes tannins of medium molecular weight, insoluble in ethyl acetate, soluble in ethanol 10% in water.

solution of ethanol containing 10% of HCl.[9] The Folin–Ciocalteu determination cannot be carried out on the insoluble formaldehyde precipitates. Therefore, the concentrations of condensed tannins and non-tannin flavans are obtained by subtraction.

The validity of this partition procedure has been tested on model solutions as shown in Table 1. The solutions were prepared in 10% aqueous ethanol and adjusted at pH 3·5 with HCl. The results in Table 1 are expressed as mg of gallic acid per 100 ml of solution. For solution 5 and 6, the 'calculated values' were obtained as arithmetic average of the concentrations determined on the simple solutions. Formaldehyde gave an incomplete precipitation of catechin, accounting for a 5% error.

Both tannic acid and grape-seeds phenolics contained about 10% of non-tannic constituents. This was probably due to partial hydrolysis in the first case and to imperfect purification in the second.

The close agreement between calculated and determined values shown in Table 1, demonstrates that the non tannin phenolics don't affect the cinchonine sulphate precipitate and the non-flavan phenolics are not involved in the formaldehyde precipitate. It also appears that the precipitations with formaldehyde and cinchonine can be applied to complex mixtures.

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[9] C. PERI, C. POMPEI, G. MONTEDORO and C. CANTARELLI, *J. Sci. Food Agr.* **22**, 24 (1971).