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Determination of catechins in wines [1])

by

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Introduction

The determination of catechins (flavan-3-ols) is based on the reaction of the phloroglucinol ring with vanillin (8) that produces a red colour with a maximum absorption around 500 mμ and is relatively stable at high concentrations of sulfuric or hydrochloric acid.

The method currently used for the dosage of catechins in wines has been set up by Burkhardt (1) and modified by Rebelein (6). It gives fairly reproducible results with a degree of sensibility adequate to the range of concentrations found in wines.

Rebelein (6) has clearly demonstrated that the most critical condition of the reaction is represented by the hydrogenionic concentration in the medium. Good reproducibility results from a very careful control of the HCl normality. Increasing the HCl concentration produces higher yields of the reaction.

In the operative conditions suggested by Rebelein (6), 1-10 ml of wine are mixed to 10 ml of 11.5 N HCl and 5 ml of a solution of vanillin 1% in ethanol 96%. The mixture is brought to a volume of 25 ml with a solution of 10 % ethanol in water. The Optical Density (O. D.) readings are carried out at 490 mμ after 30 min against a blank prepared by mixing the corresponding amount of the wine with 10 ml of the HCl solution and bringing to volume with 10 % ethanol in water.

The standard obtained in these conditions with pure (+)-catechin is a curved line that strongly flattens at O. D. higher than 0.600. It is therefore suitable not to exceed this limit in the dosage.

Another limitation of the method suggested by Rebelein is that the use of this standard curve is limited to the dosage of catechins in solutions having the same ethanol concentration. It cannot be used for reference when catechins are in aqueous solution or when the ethanol concentration is different from 10 %, because the actual H^+ concentration strongly depends on the water content of the solvent medium.

Materials and Methods

Reagents

10% and 96% ethanol in distilled water; absolute ethanol (purum, C. Erba).
Solution of vanillin (purum, C. Erba) 1 % (w/v) in 96 % ethanol.
(+)-catechin (puriss., Fluka), solutions in water and in 10 % and 96 % ethanol.
HCl 11.5 N.

Thin-layer chromatography

TLC was carried out on ethyl acetate extracts of two white wines, obtained by fermenting two musts of high and low phenolic content, obtained respecti-

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vely from pressed and free-run musts of destemmed white Trebbiano grapes.

The extracts were prepared by mixing equal volumes of ethyl acetate and wine, saturated with NaCl. The EtOAc phase was decanted, concentrated under reduced pressure, in N_2-stream, at 25° C, and quantitatively applied to the TLC plates [silica-gel G (Merck), 0.5 mm thickness]. The elution solvent was the mixture toluene:ethyl formate:formic acid (5:4:1 v/v) (TEF) (9).

The chromatograms were sprayed with vanillin-HCl (3), and tested for phenolics with the Folin-Ciocalteu reagent.

Standard curves

Three standard curves of pure (+)-catechin were prepared as follows:

	test (ml)	blank (ml)
Standard n. 1		
solution of (+)-catechin in 96 % ethanol	1-10	—
HCl 11.5 N	10	10
Solution of vanillin	5	5
96 % ethanol to a volume of	25	25
Standard n. 2		
solution of (+)-catechin in 10 % ethanol	1-10	—
HCl 11.5 N	10	10
Solution of vanillin	5	5
10 % ethanol to a volume of	25	25
Standard n. 3		
solution of (+)-catechin in water	1-10	—
HCl 11.5 N	10	10
Solution of vanillin	5	5
Water to a volume of	25	25

Optical Densities were read at the E_{max} of 500 mμ, after 20 min. The three standard curves are reported in Fig. 1.

Results and Discussion

As can be noted from Fig. 1 the sensibility of the method greatly increases in the more alcoholic solvents. With the 96 % ethanol solution the standard curve is a straight-line. We suppose that these differences are due to the different H^+ concentration in the solvent media.

For purpose of comparison we have calculated the "equivalent normality" of the solutions corresponding to the three standard curves, by applying the formulas set up by Levasseur (5) for the calculation of pH in non-aqueous solutions. The values are respectively:

Standard n. 1: 6.95 N
Standard n. 2: 5.05 N
Standard n. 3: 4.93 N

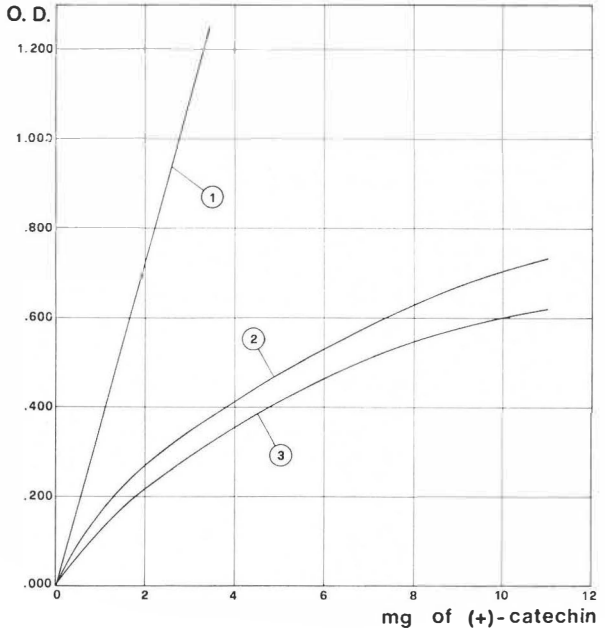

Fig. 1: Standard curves of (+)-catechin in different solvent media.
1. 96 % ethanol, 2. 10 % ethanol, 3. water. The values represent mg of (+)-catechin in the sample taken for the analyses.

The 96% ethanol solution clearly gives the best results as for both reproducibility and sensibility. With the wines this standard may be used by carrying the analyses on 1 ml samples and bringing to the 25 ml volume by addition of absolute ethanol.

The blank prepared according to BURKHARDT (1) gives a small error due to the absorption of the vanillin at 500 mμ. If the dosage is carried out on white wines it is preferable to prepare the blank, as suggested, with vanillin and without wine.

The O. D. of the products of the reaction varies with the time. After an initial increase the colour remains sensibly stable between 10 and 30 min, after which it increases again, progressively turning to violet. As a good limit the readings should be carried out between 20 and 25 min.

Interferences

It is well known (4) that the vanillin reacts with all phenolics having a phloroglucinol or a resorcinol ring; therefore the reaction is not specific for catechins. In the conditions described, different compounds are cumulatively dosed: the phloroglucinol, the pyrogallol and other, more complex phenolics, such as catechins, leucoanthocyanins, anthocyanins and ester derivatives of gallic acid and catechin. Among the flavonoids the 4-keto derivatives such as flavonols, flavones, aurones, chalcones etc. do not react (7). The yield of the reaction of catechins and leucoanthocyanins rapidly drops as the degree of polymerization increases (4).

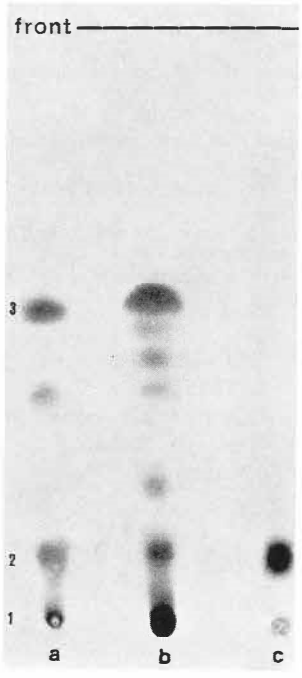

Fig. 2: TLC of ethyl acetate extracts of:
a. White wine from free-run must (white Trebbiano grapes). — b. White wine from a must obtained by pressing destemmed Trebbiano grapes. — c. (+)-catechin. Identification: 1. catechin polymers, 2. catechins, 3. unknown Rf 0.55.

Fig. 2 reports the picture of a TLC plate prepared with the ethyl acetate extracts of two white wines, obtained respectively by straining (a) and by pressing (b) white Trebbiano grapes. For comparison the third spot was pure (+)-catechin (c). It can be noted that in the wine of high phenolic content, catechin and catechin polymers, immobile to the start, are abundantly present. In the wine obtained from the free-run must there was very little catechin and a major spot at Rf 0.55 reactive to the vanillin and to the formaldehyde, characterized by a phloroglucinol ring, actually studied for identification in our laboratory. This compound has been quantitatively isolated from the plates and tested with the vanillin-HCl reagent in alcoholic solution: it represents a minimum of 10% of the total catechins dosed in the wine. The reaction of this compound with vanillin produces a violet colour with E_{max} at 550 mμ, with strong interference in the 500 mμ region.

In the wines of medium and high phenolic content the catechin monomers and polymers by far represent the largest fraction of the compounds reactive to the vanillin and therefore this method of analysis remains valid. On the contrary in the wines of low phenolic content, deriving from free-run musts or detannized with selective absorbing agents such as PVP and Nylon (2), the interference of simple non-catechin phenolics may be very important.

Summary

The method by Rebelein for the dosage of catechins in white wines has been revised. A straight-line standard of (+)-catechin and higher sensibilities may be obtained by carrying out the vanillin-HCl reaction in alcoholic media.

Interference of non-catechin low molecular weight compounds is negligible when the dosage is carried out in wines of medium and high phenolic content, but causes significant errors in wines of low phenolic content, obtained by selective detannization with PVP or Nylon or by fermentation of free-run musts.

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