RESOLUTION OIV/OENO 352/2009

DIFFERENTIATION METHOD FOR COMMERCIAL OENOLOGICAL TANNINS - AMENDMENT TO THE MONOGRAPH

THE GENERAL ASSEMBLY

IN VIEW of Article 2 paragraph 2 iii of the Agreement establishing the International Organisation of Vine and Wine

HAVING STUDIED the research by the “Methods of Analysis” Sub-commission and the group of experts “Specification of Oenological products”

CONSIDERING resolution OENO 12/2002 concerning the oenological tannins monograph

DECIDES, upon the proposal of Commission II "Oenology" to amend and complete the existing monograph with the addition of an annex related to the following method

DIFFERENTIATION OF COMMERCIAL ENOLOGICAL TANNINS BY GC-MS ANALYSIS OF MONOSACCHARIDES AND POLYALCOHOLS

1. Introduction

According to the International Enological Codex of the O.I.V., the enological tannins should be extracted from gall nuts (of Quercus, such as Aleppo galls, and of Tara, also called Caesalpina Spinosa), oak wood (Quercus sp.), grape seeds and skins (Vitis vinifera) and the wood of certain trees such as quebracho (Schinopsis balansae) and chestnut (Castanea sp.).

2. Scope

The method described here is suitable for the differentiation of commercial enological tannins from different origins (plant galls, seed and skin grape, oak wood, chestnut and quebracho).

3. Principle

The concentration of monosaccharides (arabinose, xylose, fructose and glucose) and polyalcohols (arabitol, quercitol, pinitol, chiro-inositol, muco-inosotol, scyllo-inositol

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and meso-inositol) in tannin samples was determined by gas chromatography-mass spectrometry (GC-MS) after their previous derivatization into their trimethylsilyl ethers.

4. Reagent and materials

Reagents

Trimethylsilylimidazole (TMSI) 97 % pure

Trimethylchlorosilane (TMCS)

Dried pyridine 99.5 % pure

High purity water produced in a Milli-Q synthesis A10 system

Standards

Phenyl-β-glucoside (internal standard): 1 mg/mL prepared in 70 % methanol

Preparation of the standard solutions (of monosaccharides and polyalcohols)

Standard solutions of glucose, fructose, arabinose, xylose, arabitol, pinitol, meso-inositol, scyllo-inositol, muco-inositol and chiro-inositol were disolved in methanol: water 30:70 at concentrations varying between 0.05 and 0.5 mg/mL of each standard. As quercitol and bornesitol are not commercially available, aqueous extracts were prepared from oak acorns of Quercus sp. and from leaves of Echium vulgare. The extracts were evaporated at low temperature under vacuum, silylated and injected as described below. Carbohydrate composition (in triplicate, RSD $\leq$ 5 %) of oak extract was 68 % quercitol, 20 % fructose and 18 % glucose and 20 % fructose, 33 % glucose, 27 % bornesitol, 2 % meso-inositol and 19 % saccharose for the Echium extract.

Note: All standard solutions have to be prepared working daily and preferably stored cold in a refrigerator prior to injection. All samples have to be derivatised and analysed in the day.

5. Samples

Twenty eight samples of different commercial tannins, including oak wood (O; n=4), grape seed (S; n=6), grape skin (H; n=2), plant galls (G; n=6), chestnut (Ch; n=3), quebracho (Q; n=3), gambier (GMB; n=1) and mixtures of grape+quebracho (GQ; n=1), quebracho+chestnut+plant gall (QChG; n=1) and chestnut+quebracho (ChQ; n=1) tannins, were directly purchased in the market or supplied by the manufacturers.

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6. Apparatus

- Fume cupboard
- Laboratory glassware: beakers, vessels, etc.
- Micropipets
- Rotaevaporator
- Vortex
- Domestic mill
- Centrifuge
- Gas chromatograph equipped with a flame ionisation detector (FID)
- Gas chromatograph coupled to a quadrupole mass spectrometry detector operating in electronic impact (EI) mode at 70 eV. MS data were registered from 40 to 700 m/z.
- Column: 25 m x 0.25 mm i.d. x 0.25 µm film thickness fused silica column coated with crosslinked methyl silicone.

7. Procedure

Derivatization procedure

50 mg of tannins are dissolved in 5 mL of deionized water and filtered through Whatman No. 1 or similar filter paper. 1 mL of the sample is mixed with 1 mL of phenyl-β-glucoside, as internal standard. This mixture is evaporated under vacuum and trimethylsilyl derivates were formed by addition of 100 μL of anhydrous pyridine, 100 μL of TMSI and 100 µL of TMCS, shaking after each addition. Extraction of the trimethylsilyl (TMS) derivatives is carried out using 100 µL of hexane and 200 µL of water.

GC analysis

1µL of the hexane upper layer is injected on the GC. Identity of each compound is confirmed by comparison of their retention times and mass spectra using GC-MS

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method with those of standards. The typical chromatographic profile of each tannin origin is shown in Figure 1.

GC-FID analysis: chromatographic conditions

Injections are made in splitless mode. Injector and detector temperature are 300 °C. Oven temperature is maintained at 100 °C for 1 min, then programmed with a heating rate of 30 °C/min up to 200 °C kept for 15 min and finally programmedat a heating rate of 15 °C/min up to 270 °C maintained for 20 min. Carrier gas is nitrogen.

GC-MS analysis: chromatographic conditions

Injections are made in splitless mode. The injector is at 300°C and the oven temperature is maintained at 100 °C for 1 min, then programmed with a heating rate of 30 °C/min up to 200 °C kept for 15 min and finally programmed at a heating rate of 15 °C/min up to 270 °C maintained for 20 min. Carrier gas is He at 1 mL/min.

8. Calculation (Results)

Quantitative analysis is carried out using the response factor (RF) of each standard relative to phenyl-□-D-glucoside (internal standard) over the expected range. Reproducibility of the method is evaluated analyzing one sample on five different days. However this method does not allow to distinguish quebracho tannins from those of skin grape.

For example the limits of detection (LOD) and quantification (LOQ) (Tables 1 and 2) are calculated for each compound according to Foley and Dorsey (1984). Mean values of 0.42 ng and 1.41 ng injected were obtained for LOD and LOQ, respectively. Concentrations of polyols and monosaccharides in tannins analysed are respectively in tables 3 and 4.

This method allows the classification of tannins according to the scheme suggested in Figure 2. The presence of quercitol is indicative of tannins from oak wood, whereas pinitol is mainly indicator of tannins from tara galls and bornesitol of tannins from gambier. The absence of arabinose and xylose in gall tannins can also help to the characterization of these samples. Therefore, bornesitol, quercitol, pinitol, arabinose and xylose could be used to unequivocally differentiate these products, and furthermore, to distinguish these tannins from the rest of the products analyzed. Tannins from galls and grapes can be easily differentiated from tannins of other origins due to the absence of arabinose and xylose in their monosaccharide composition. Referring to grape tannin samples, fructose could be observed in seed

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grape tannins, whereas it was absent in skin grape tannin. The presence of muco- and chiro-inositol could be useful to distinguish tannins from chestnuts from those of quebracho or grape skin.

9. Bibliography

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Table 1. Repeatability of the GC method for the determination of carbohydrates in tannins (sample Q3).

	Mean value	Standard deviation
Xylose	0.17	0.01
Arabinose	0.43	0.03
Arabitol	0.04	0.00
Quercitol	0.00	0.00
Fructose	0.32	0.04
Glucose	0.60	0.02
Muco-inositol	0.02	0.00
Chiro-inositol	0.00	0.00
Scyllo-inositol	0.00	0.00

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Meso-inositol	0.05	0.00

Table 2.

Table 2. Limit of detection (LOD) and of quantification (LOQ) of the GC method for the determination of carbohydratesand of plyols in oenological tannins samples by means of gas-chromatography (expressed in injected ng)

	LOD (ng)	LOQ (ng)
Xylose	0.50	1.66
Arabinose	0.66	2.21
Arabitol	0.21	0.70
Fructose	1.11	3.70
Glucose	0.51	1.70
Muco-inositol	0.16	0.52
Chiro-inositol	0.22	0.74
Scyllo-inositol	0.20	0.68
Meso-inositol	0.24	0.80

Table 3. Concentration of polyols (mg/100g, tr=traces) in commercial tannins

		mg/100g							
		Arabitol	Quercitol	Pinitol	Bornesitol	*Muco*-inositol	*Chiro*-inositol	*Scyllo*-inositol	Meso-inositol
Oak wood	O1	0.06	6.92	-	-	0.10	0.10	0.52	0.49
	O2	0.06	4.49	-	-	0.11	0.11	0.57	0.55
	O3	0.05	1.57	-	-	0.04	0.02	0.13	0.12
	O4	0.09	3.14	-	-	0.14	0.17	0.17	0.30
Gall plant	G1	-	-	0.73	-	-	-	-	-

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	G2	-	-	0.26	-	-	-	-	tr
	G3	-	0.03	0.07	-	-	-	0.03	tr
	G4	-	0.06	0.06	-	-	-	0.04	-
	G5	-	-	1.35	-	-	-	-	0.02
	G6	-	-	-	-	-	-	-	-
Seed grape	S1	-	-	-	-	-	-	tr	0.16
	S2	-	-	-	-	-	-	tr	0.01
	S3	-	-	-	-	-	-	0.38	2.34
	S4	-	-	-	-	-	-	tr	0.01
	S5	-	-	-	-	-	-	-	0.01
	S6	0.64	-	-	-	-	-	tr	0.25
Skin grape	H1	-	-	-	-	-	-	-	-
	H2	-	-	-	-	-	-	-	tr
Chestnut	Ch1	0.08	-	-	-	0.14	0.55	-	0.62
	Ch2	0.04	-	0.49	-	0.03	0.33	-	0.05
	Ch3	0.07	-	-	-	0.19	0.52	-	0.49
Quebracho	Q1	tr	-	-	-	-	-	-	0.01
	Q2	0.02	0.05	0.09	-	-	-	-	tr
	Q3	0.03	-	-	-	0.02	-	-	0.05
Gambier	GMB	0.01	-	tr	0.02	-	-	-	0.03
Grape+quebracho	GQ	0.10	-	0.19	-	0.02	0.06	-	0.07
Quebracho+chestnut+gall	QChG	0.03	-	0.19	-	0.03	0.12	-	0.12
Chestnut+quebracho	ChQ	0.05	-	-	-	0.13	0.56	-	0.53

Table 4. Concentration of monosaccharides (mg/100g, tr= traces) in commercial tannins

		mg/100g			
		Xylose	Arabinose	Fructose	Glucose
Oak wood	O1	0.29	1.18	-	0.22

	O2	0.57	2.53	-	0.07
	O3	0.37	0.85	0.12	0.58
	O4	0.41	1.84	1.82	2.69
Gall plant	G1	-	-	0.26	0.42
	G2	-	-	0.07	0.17
	G3	-	-	0.05	0.05
	G4	-	-	0.11	0.16
	G5	-	-	0.50	0.63
	G6	-	-	-	-
Seed grape	S1	-	-	10.01	9.59
	S2	-	-	0.64	0.50
	S3	-	-	45.23	32.46
	S4	-	-	0.61	0.46
	S5	0.13	-	-	0.03
	S6	-	-	1.22	tr
Skin grape	H1	-	-	-	0.07
	H2	0.31	0.48	0.30	0.67
Chestnut	Ch1	0.50	1.46	1.15	0.78
	Ch2	0.41	1.04	0.95	0.91
	Ch3	0.65	1.55	0.28	0.69
Quebracho	Q1	0.30	0.44	0.22	0.20

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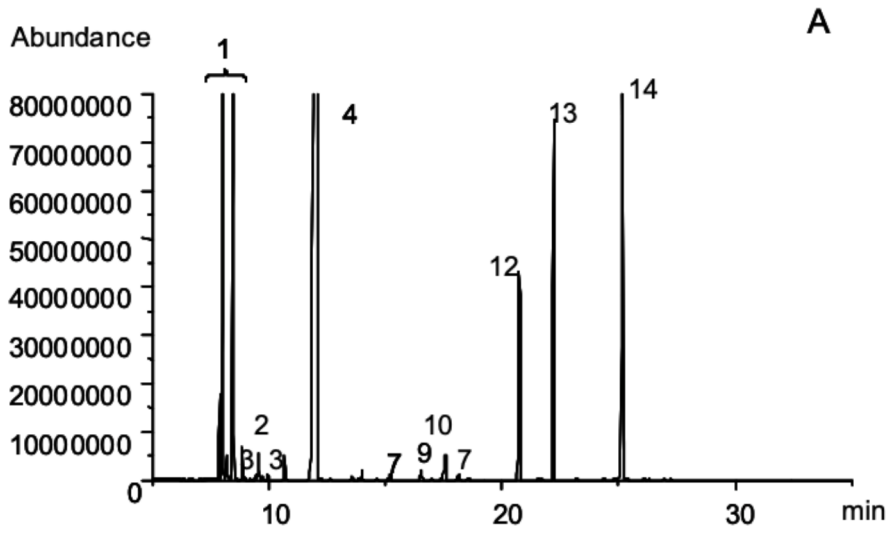
	Q2	0.07	0.10	0.05	0.10
	Q3	0.16	0.42	0.32	0.59
Gambier	GMB	0.02	-	0.42	0.12
Grape+quebracho	GQ	0.07	0.11	0.25	0.28
Quebracho+chestnut+gall	QChG	0.04	0.07	0.17	0.30
Chestnut+quebracho	ChQ	0.29	1.29	1.34	1.46



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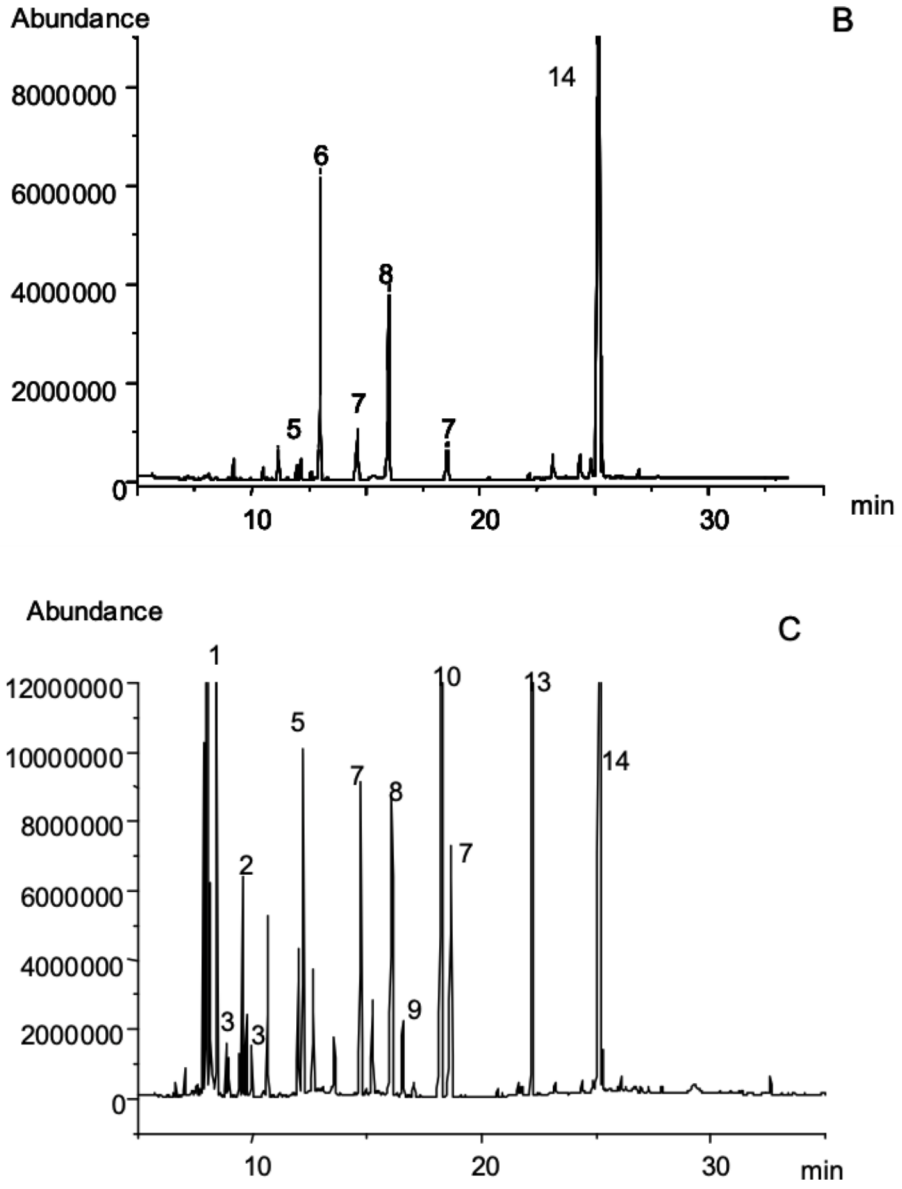

Figure 1. Gas chromatographic profiles of polyalcohols and carbohydrates in commercial tannins of A) oak wood, B) plant gall, C) chestnut wood, D) seed grape, E) skin grape, F) quebracho wood, G) Gambier. 1-Arabinose, 2-Arabitol, 3-Xylose, 4-Quercitol, 5-Fructose, 6-Pinitol, 7-Glucose, 8-Gallic acid, 9-Muco-inositol, 10-Chiro-inositol, 11-Bornesitol, 12- Scyllo-inositol, 13-Meso-inositol, 14-Phenyl-□-D-glucoside (i.s.)

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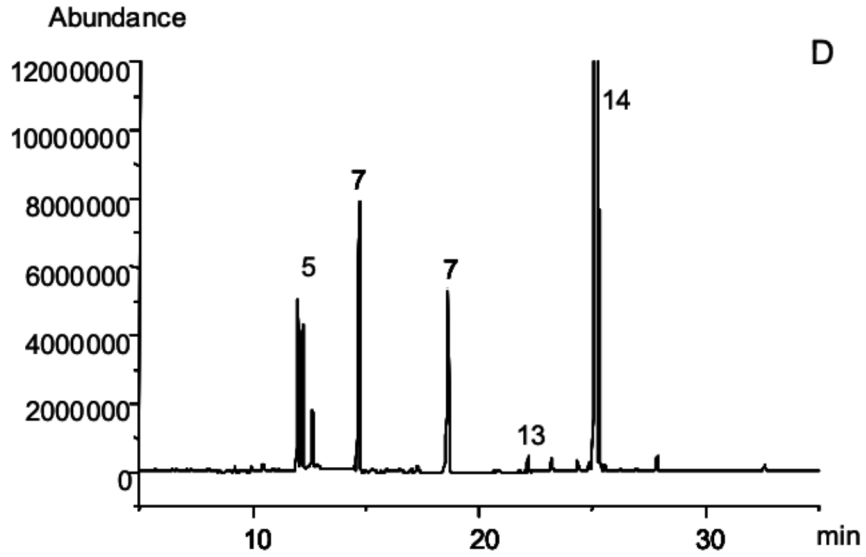
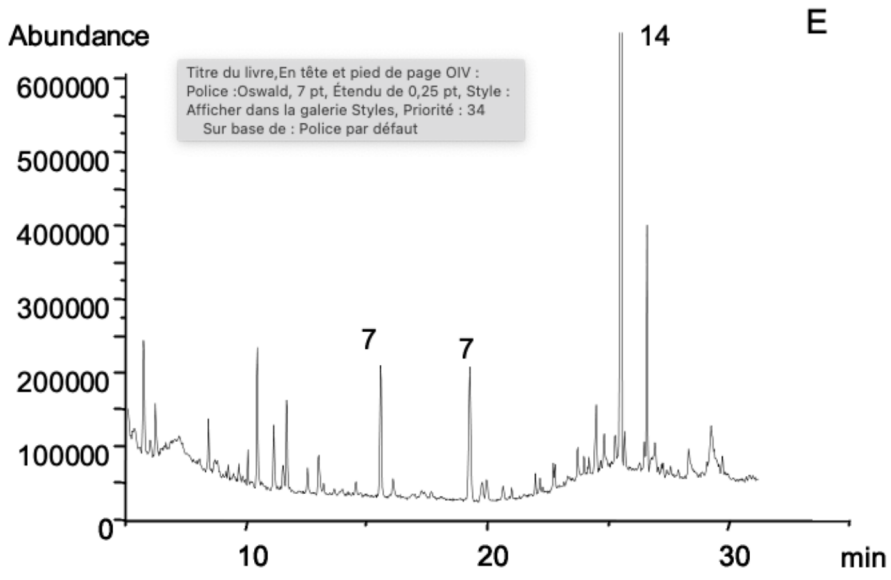
Figure 1 . Continuation





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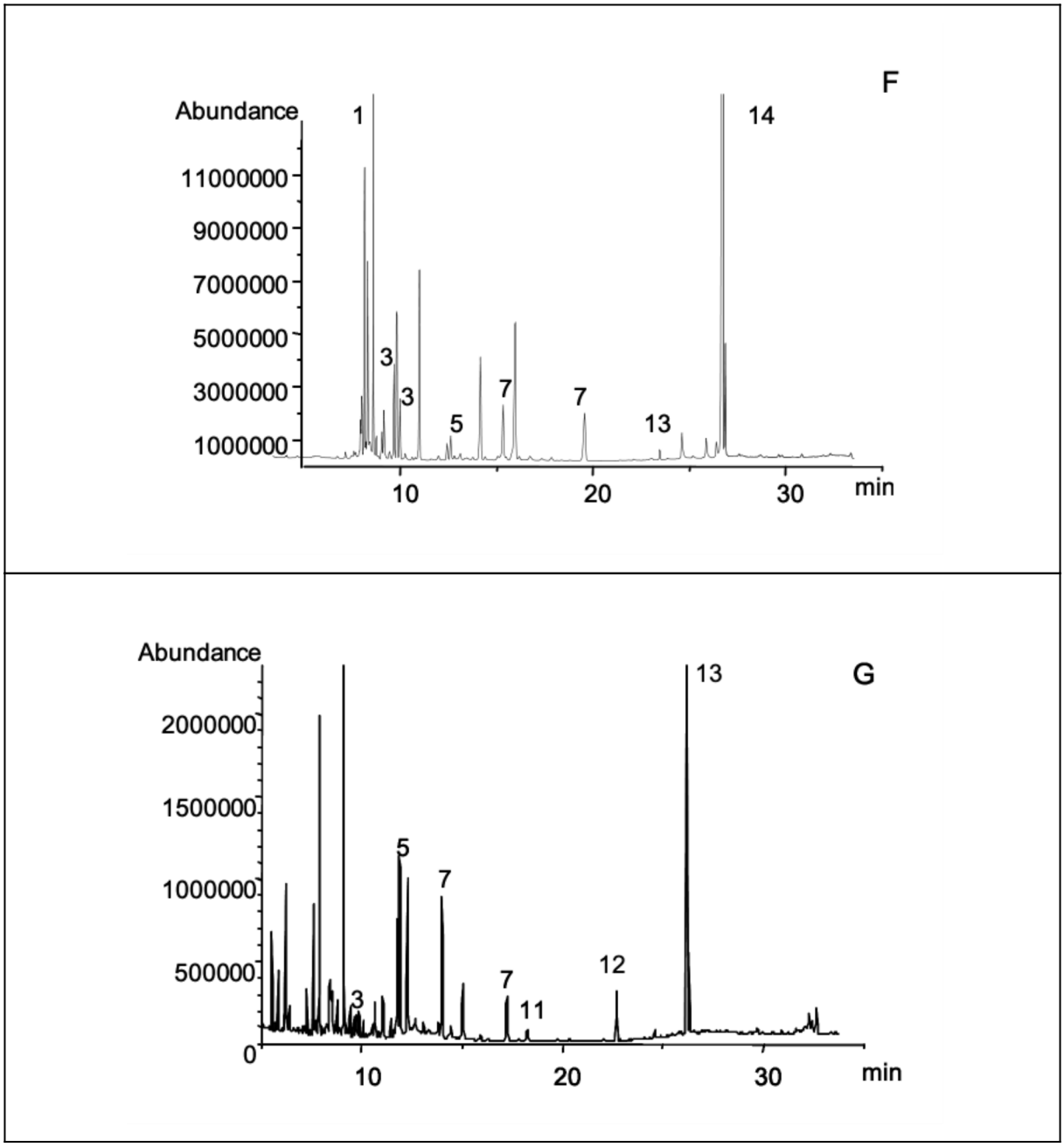

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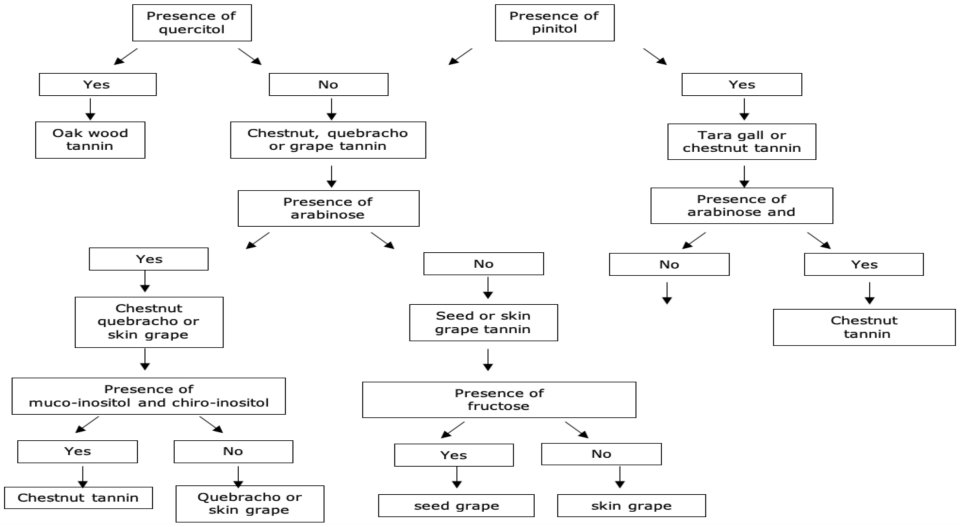

Figure 2: Scheme of tannins classified according to their monosaccharide and polyalcohol composition

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