COMPARISON ON DIFFERENT METHODS OF DEPOLYMERIZATION TO THE CHARACTERIZATION AND QUANTIFICATION OF PROANTHOCYANIDINS IN GRAPE SEED, GRAPE SKIN AND WINE

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Three different nucleophilic compounds are usually used for acid-catalyzed depolymerization of proanthocyanidins, namely toluene-α-thiol (Prieur *et al*, 1994), phloroglucinol (Kennedy *et al*., 2001) and cysteamine (Torres *et al*., 2001). Each nucleophile has advantages and disadvantages: nucleophilic power, smell, toxicity, stability of the adducts. It would be also of interest to have different possibilities to analyse proanthocyanidins depending of raw materials. Phloroglucinol and cysteamine adducts are more polar than toluene-α-thiol ones and may be more easily separated from other polyphenols such as anthocyanins, pyranoanthocyanins (Atanasova *et al.*, 2002) and anthocyanin-flavonol adducts (Remy *et al.*, 2000) in complex samples like wine, for example. We compared efficiency, reproducibility, accuracy of the reaction with these nucleophiles for analysis of proanthocyanidins from grape seed , grape skin, red wine polyphenol extract and finally red wine.

Experimentation

Depolymerization was followed by HPLC analysis on C18 column that exhibits enhanced retention of polar compounds (Atlantis dc18, Waters). LC-MS analyses in positive mode were performed on the same column to identify components of the reaction media. The stability of the reaction products was studied under different conditions and percentage of epimerization was calculated for terminal units. Proanthocyanidin composition (i.e: mean degree of depolymerization (DPm), % of galloylated and trihydroxylated units, concentration) was determined using the three reagents, after isolating and calibrating each of the reaction products.

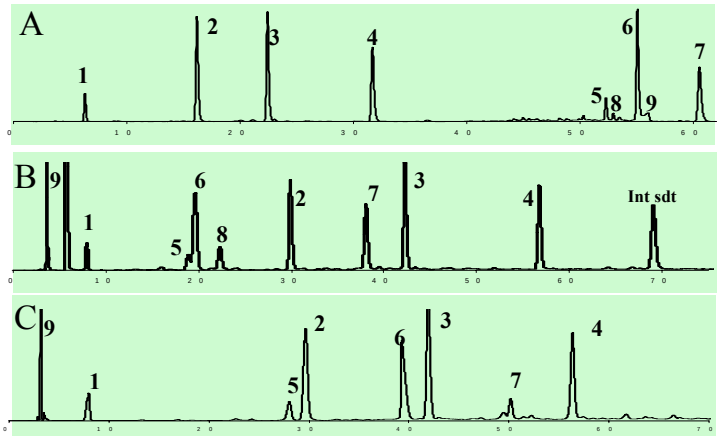


Figure 1: examples of HPLC analysis (seed): **A:** with toluene-thiol, **B**: with phloroglucinol, **C**: with cysteamine **1**: gallic acid, **2**: catechin, **3**: epicatechin, **4**: epicatechingallate, **5**: cat-nucleophile, **6**: epi-nucleophile **7**: epicatechingallat-nucleophile, **8**: nucleophile adduct in position 2, **9**: nucleophile in excess.

Results

Depolymerization with phloroglucinol and toluene-α-thiol gave higher reaction yields on isolated dimers, trimers, and tetramers (Table 1). Epimerization was very important with cysteamine, clearly present with toluene-α-thiol and practically non existent with phloroglucinol .

All the adducts expected with flavanol monomers in position 4 were present but LC-MS analysis after reaction with phloroglucinol showed additional adduct. This may result from reaction of the nucleophile in position 2 of a terminal unit (catechin or epicatechin) as already mentioned by Gu *et al,2002* for the reaction with toluene-α-thiol.

Table 1: Comparison on depolymerization. Epimerization= % epimerization on terminal units; new adduct= % additional adduct / terminal units, ND: not detected.

	Toluene thiol 2 min 90°C				Phloroglucinol 20 min 50°C				Cysteamine 15 min 60°C			
	DPm	Recovery %	Epimerization	New adduct	DPm	Recovery %	Epimerization	New adduct	DPm	Recovery %	Epimerization	New adduct
Cat	1	92	6	8	1	98.2	0	10.8	1	85	20	ND
Epi	1	100	19	7.2	1	104	3	9.75	1	89	50	ND
E-E	2.27	108	14	5	2.3	105	2	12	2.3	71	30	ND
E-C	2.14	88	4	5	2.3	93	0	10	2.3	70	23	ND
Eg-E	2.24	90	10	6	2.2	92	3	9	1.6	68	25	ND
E-Eg	2.52	83	0	-	2	96		0	2.2	72	-	ND
E-E-E	3.18	95	-	-	2.98	75	-	8.3	2.7	86	-	ND
E-E-E-E	4.6	94	-	-	4.2	91	-	4	-			ND

Epimerization decreased when depolymerization was performed at room temperature during 24 hours but the percentage of the additional adducts increased. Proanthocyanidin analysis in different matrices (grape seed, grape skin, and wine extracts) gave similar results with all three reagents but reproducibility of DPm and concentrations values as higher with phloroglucinol and toluene-α-thiol (Table 2).

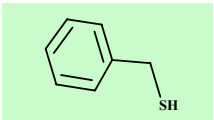
Table 2: **A**: toluene-α-thiol, **B**: phloroglucinol, **C**: cysteamine, * mg/g, ° mg/l.

	DPm			%galloylated units			Concentration			%trihydroxylated units		
	A	B	C	A	B	C	A	B	C	A	B	C
Grape seed 1	2.6±0.1	2.5±0.2	2.9±0.5	16.3±0.9	15.0±0.6	17.2±1.2	460*±15	420*±30	330*±30	0	0	0
Grape seed 2	3.9±0.1	4.4±0.1	4.9±0.5	22.6±0.5	18.9±0.7	17.7±1.0	430*±20	390*±30	310*±50	0	0	0
Grape skin	2.0±0.2	2.1±0.2	2.5±0.8	2.4±0.3	2.4±0.3	1.7±0.3	270*±20	300*±30	270*±50	6.3±0.4	7.1±0.4	6.9±0.5
Wine powder	2.6±0.1	3.1±0.3	3.4±0.8	4.0±0.5	4.2±0.6	3.1±0.3	120*±20	126*±40	180*±40	9.1±0.2	11.4±0,3	9.6±0.4
Red wine	3.1±0.3	3.4±0.4		5.1±0.7	6.0±0.5		1021±80	965±36		7.52±0.5	7.46 ±0.5	

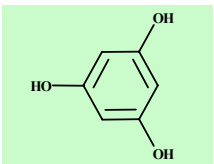
Conclusion

Advantages and limits of the three nucleophiles used in proanthocyanidin analysis are summarized below. Controlling reaction conditions is essential to ensure accurate quantification.

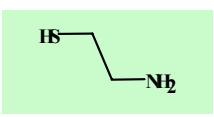
Toluene-α-thiol
- excellent nucleophile
- epimerization
- low olfactory threshold 0.0005ppm
- adducts coeluted with anthocyanins



Phloroglucinol
- odorless
- low epimerization rate
- polarity of adducts
- unstable reagent



Cysteamine
- high olfactory threshold
- high epimerization rate
- lower yield
- formation of by-products



Acknowledgements

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It does not reflect their views and in no way anticipates the Commission's future policy in this area.

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