



## Rapid extraction of polyphenols from red grapes

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- It is widely accepted that the phenolic content of the grape can significantly influence the quality of the finished wine. Also, that it may be possible to predict wine quality from analysis of the phenolics present in the grape. However, we also know that assessment of phenolics in grapes is strongly dependent on the extraction method used. As a result, a prerequisite for obtaining a proper evaluation of grape phenolics is to define a robust and efficient extraction method.

- *Condensed tannins* and *anthocyanins* are the two most abundant classes of phenolics in grapes. *Anthocyanins* can be readily extracted from skins with several solvents. Extraction of *tannins* from seeds, on the other hand, is a bit more challenging. The problem here is that seeds are generally not crushed during winemaking, and extraction of tannins and smaller phenols - gallic acid, catechins - from seeds is only achieved slowly (5 to 12 days) and with the help of increasing alcohol levels during fermentation. On the other hand, during a rapid extraction in the lab, seeds are often left intact, and few of the phenols are extracted.

- Even though several rapid extraction methods have tried to overcome this problem, according to these authors, few studies have addressed the way important parameters - such as temperature, time of extraction, pH, or cultivar - affect the amounts of phenolic compounds extracted. The goal of the current study was to do just that.

- The authors carried their study in 2005, using a modified version of a simple extraction method published by Iland *et al* in 2004. Briefly, the grapes were crushed and the resulting puree –the homogenate- was extracted for 1 hour at room temperature (25°C) with an aqueous ethanol solution (50% ethanol, adjusted to pH 2) with constant stirring. The resulting solid extract was then used to read –by absorbance-the levels of anthocyanins and total phenols. The modification that the authors introduced here was that the solid grape residue from the first extraction was subjected to a **second extraction**. Thus, **the** final concentrations of anthocyanins and total phenols were calculated as the sum of the absorbances of the first and second extractions.

- Then, in a series of separate experiments with adequate random replications, they tested the effect on the yield of *total phenols* and *anthocyanins* of “everything under the sun”. Specifically, they tested the effect of the following parameters:

- 1) **variety** (Alicante, Merlot, Syrah, Cabernet Sauvignon, Mourvedre),
- 2) **extraction temperature** (20, 40, and 60°C),
- 3) **solvent composition** (0, 25, and 50% ethanol),
- 4) **hydrochloric acid concentration** (0 and 0.1 M),
- 5) **grape homogenization time** (0.5, 1, 3, and 4 minutes),
- 6) **solvent contact time** (0, 2, 5, 15, and 30 minutes),
- 7) **pre-heating the solvent solution** to reduce extraction time to a minimum (preheated solvent or non-preheated solvent), and,
- 8) **neutralization after acidification** (neutralization - with NaOH- or no neutralization).

- In all of the above experiments, the authors measured *anthocyanins* by absorbance at **520 nm** (mg of malvidin-3-glucoside equivalents per gram of grape). And they measured *total phenols* by absorbance at **280 nm** (absorbance units per gram of grape).

- **Results**. 1) Almost all of the anthocyanins were recovered during the first extraction, with the modification of adding a second extraction yielding only 3% more. In contrast, up to 14% more total phenols were removed in the second extraction - that's the reason why the authors decided to modify the published method.

- 2) Ethanol had the greatest influence on extraction: the higher the ethanol, the more extraction. 3) Regarding solvent contact time, 15 or 30 minutes made no difference – but 150 minutes, for example, yielded less extraction. In fact, most total phenols and anthocyanins were extracted within the first 5 minutes of contact time, and within the first minute of homogenization. 4) Extraction temperatures of 40°C or 60°C worked best. Acidification of the solvent also aided the extraction. 5) Neutralization after acidification – which the authors preferred because it avoids potential undesirable changes in the phenolic profile - rendered adequate extraction. Finally, 6) when the authors studied the phenolic composition by chromatography to see how stable these compounds remained during an extended extraction, they found that the main compounds remained significantly constant –only tannins decreased by 9% during long solvent contact periods.

In conclusion, the optimized extraction protocol was as follows: “5 minutes of solvent contact, using 50% aqueous ethanol with 0.1 M HCl at 40°C and a 1:1 ratio of solvent and grape homogenate, followed by acid neutralization”. This protocol gave efficiencies of 82% for total phenols and 92% for anthocyanins (highly efficient), and standard deviations of 6% for total phenols and 4% for anthocyanins (highly robust). Finally, the above was true across varieties. Even though many of us may not directly benefit from this optimized protocol, it's likely we will ultimately benefit from the improved chances of predicting wine quality based on grape phenolic profiles.

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