ESTIMATION OF THE PHENOLIC MATURITY OF RED GRAPES USING THE STANDARD ITV METHOD

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SUMMARY

For several years, I.T.V. France has been working on a method to estimate the polyphenolic potential of red grape harvests, called the Standard ITV Method. This method could be suitable to provide the viticulturist pertinent and complementary information to that which is currently provided from the following of sugar and total acidity levels during the course of maturation. An in depth study of the different steps of the Standard I.T.V. Method allowed for their optimization and rendered them more reliable.

Hence, the sampling, transport and stocking conditions of the samples were specified; the maceration phase of the grapes in the presence of an alcoholic solution was reduced to one hour; in the case of grape varieties with strong colorant potential this step become facultative; the dosage of the anthocyanins makes use of the Puissant-Léon method, which is simple to complete and is rapid, reliable and can be automated (can process up to 60 samples in 6 hours). The repeatability of the method, starting from the sampling of the grape berries up to the anthocyanin measurement, is very good with an average variation coefficient of lower than 5%.

The determination of an optimal harvest date, in other words, the moment where the grapes present the highest oenological potential remains, all factors combined, one of the major concerns of modern viticulture, since the quality of the wine is in part determined by a non negligible influence of the primary material quality. To help the producer in their choice of the appropriate moment, a number of tools were developed to determine the quality of the grapes based on the following of qualitative markers during the maturation period.

The classic analytical criteria, such as sugar content, total acidity and pH, have prevailed for a long time as favoured indicators of the maturity state of the harvest, because of their simple analytical determination. Their pertinence is however questionable in numerous situations, notably those where a grape variety is cultivated in conditions that do not allow it to express itself fully. One can note therefore a large off-set between the information given by these indicators and the real quality of the grape (colorant matter, tannins and aromas). In the case for red grape varieties, the observing the evolution of phenolic compounds during the maturation is implicated as an interesting complementary technique, so as not to say necessary.

ITV France, across a network of five regional ITV units, implanted in key French vineyards, has worked for some time on the adjustment and validation of a standardized evaluation method for the polyphenolic richness of French grape varieties, responding to criteria of reliability, simplicity and rapidity of functioning (treatment of a large number of samples), security and cost. Today, we propose to the profession a validated method which permits to encompass in a satisfactory manner the optimum maturity of the grape varieties studied and to harvest in respect to the realities of the vintage and terroir.

1- The anthocyanins and the polyphenolic maturity: some reminders

The anthocyanins and tannins are the principal components which represent the phenolic compounds present in grapes and red wines. They participate in an key manner on the organoleptic

characteristics. The anthocyanins belong to the the flavonoid family, characterized by a carbon backbone of the type 2-phenyl-benzopyrone (1,2); the anthocyanins present in red grapes of *Vitis vinifera* are monoglucosides of the flavylium center and are differentiated according to the degree of hydroxylation/methylation of the lateral center and also by the nature of the acid which esterificates the glucose (acetic, coumaric or caffeic acid). At present it is possible to enumerate 17 forms of anthocyanins, of which 5 are glycosylated, 5 acylated, 5 coumarated and 2 caffeoated (3). The aglycon forms of the anthocyanins are called anthocyanidins. The colour of these molecules is dependant on there chemical structure, the conditions of the medium (pH) and on their interactions with other constituents (4, 5, 6). These particular chromatic characteristics were put to good use during the development of the principal methods to measure the total anthocyanins. Hence, the Stonestreet method uses the capacity of sulfur dioxide to combine with anthocyanins to give a colourless product, and the Puissant-Léon method applies the principal where at very acid pH, the majority of the anthocyanins are present in the flavylium cation form, of red colour (7)

The anthocyanins are localized in the vacuoles of the skin cells, in the first cellular layers of the hypoderm (8). They appear in the grapes at the beginning of the veraison stage, and undergo an evolution during the maturation in three steps: an initial rapid accumulation phase, a stagnant phase, where the concentration passes by a maximum, and finally a decreasing phase. This theoretical model has some exceptions, notably in the cases of a poor adaptation of the vine to its environment (9). The repartition and the quality of the different anthocyanins in *Vitis vinifera* varies in accordance with the grape varieties, the pedoclimatic conditions and the cultivation methods. It hence appears that the 3-glucoside of malvidin is the major anthocyanin and that Pinot Noir possesses no acylated anthocyanins (10 and 11). The global quantities at maturity range from 500 to 3000mg/kg (12).

The following of the dynamic accumulation of anthocyanins and total polyphenols during the maturation phase of the grapes, has proven to be an interesting tool for the determination of the maturity of red grapes. We were able to observe that a satisfactory level of maturity is attained when we register a significant drop in the anthocyanin content. At this stage, the pigments are in a lower concentration, but the degradation of the walls of the cells of the skin favourizes their diffusion in the liquid phase. In addition, we record in the seeds a notable drop of the extractable anthocyanin quantities which have a prominent astringent character (13).

At present there are numerous methods for the qualitative or quantitative determination of the phenolic potential of red grapes (13, 14, 15, 16, 17, 18, 19). They differ in accordance with the objectives which they satisfy – the following of the total or partial phenolic potential during the maturation, aid for the determination of the optimal maturity, the estimation of the extractable phenolic potential and of the contribution of the seeds to this potential, aid for the management of the vinification…- but also by the techniques employed during the treatment of the grapes. The principal points on which these methods diverge are the following:

- The sampling of the grapes in the field : whole bunches, fractions of bunches, or only grape berries
- The treatment of the sample: grinding, centrifugation, samples treated when fresh or after lyophilization
- The extraction of the phenolic compounds: no extraction other than the grinding treatment or centrifugation or maceration in different conditions (length, temperature, nature of the extraction solution, agitation,…)
- The methods of measurement of the different phenolic fractions

These divergences in the choice of the principals and materials for the execution have an important impact on the simplicity and rapidity of execution of the method, its cost and certainly the information that it delivers.

2- OPTIMIZATION AND RELIABILITY OF THE STANDARD ITV METHOD

The principal steps of the standard ITV method- sampling, stocking and treatment of the grapes, maceration conditions, methods of anthocyanin measurement- have been the subject of an important study for their optimization and validation, by the five regional ITV units, on many regional grape varieties.

2.1- Choice of a sampling method for the grapes

The sampling of grapes in the field cannot be considered as marginal in the ITV method: it is a crucial point that merits to be discussed further.

There are many ways to collect a sample of grapes which are representative of a given "parcelle": whole bunches, portions of bunches or single grape berries.

The first method is relatively destructive if we desire to obtain a representative sample during the following of the maturation. Therefore, in 1995 and 1996, only the methods of sampling, by fractions of bunches and by grape berries were compared.

The comparison was completed using the following parameters: weight of 200 grape berries, levels of sugars (or Potential Alcohol), total acidity, pH, concentration of anthocyanins and total polyphenol index. The results are given in tables 1 and 2.

Measured Parameters	Determination Coefficient (r^2) between the methods « Portions of bunches » et « Single grape berries »
Weight of 200 grape berries (g)	0.94
Potential Alcohol	0.92
Total Acidity	0.88
Anthocyanins	0.76
Total phenolic compounds	0.72

Table 1 : correlation between the deux grape sampling methods in the field for 5 analytical parameters – 1995/1996

Measured parameters	Repeatability of the sampling methods (10 samplings)					
	« Portions of bunches »			« Single Berries »		
	Average	Standard Deviation	Variation Coefficient (%)	Average	Standard Deviation	Variation Coefficient (%)
Weight of 200 grape berries (g)	296.3	4.80	1.60	201.5	7.5	3.7
Potential Alcohol	193.3	0.88	0.45	218.8	2.32	1.1
Total Acidity	4.9	0.78	1.59	5.4	0.023	0.4
Anthocyanins	1.6	0.038	2.33	1.8	0.051	2.8
Total phenolic compounds	5.3	0.16	3.00	9.2	0.24	2.6

Table 2: measure of the repeatability of the two sampling methods for 5 analytical parameters- 1996

The results obtained for the 5 measured analytical parameters are highly correlated between the two sampling methods (table 1). The correlations are particularly high for the classic analytical

criteria (Potential Alcohol and Total Acidity) and the weight of the grape berries; they remain satisfactory for the anthocyanin content and the total polyphenolic compounds.
The repeatability of the sampling methods was tested on 10 lots of grapes collected consecutively by the same person. The difference observed between the samples for the 5 analytical parameters were low and the variation coefficients (CV) do not exceed 4%.

These results were very adequately confirmed by those registered in the Midi-Pyrenees by the regional I.T.V. Station for the « single grape berry » method. ($CV_{berry\ weight}$ = 3.2 % ; $CV_{anthocyanins}$ = 4 % ; $CV_{total\ polyphenol\ content}$ = 4.1 %).

These observations show that the two sampling methods tested give comparable content and reliability information (high correlation and low variability coefficients). In the end, for the standard I.T.V. method we retained the “single berry” sampling method, since it is simple and rapid to use.

We even attempted to measure the reproducibility of the “single berry” sampling method. When the sampling is completed while following the guidelines (§ 3.1) with a minimum of thoroughness, the variation coefficients for the main parameters (weight of berries, alcohol potential, anthocyanins, total polyphenol content) are below 5%.

On the other hand, a sampling completed by a person who did not take into consideration the guidelines, or who demonstrates bad will, yields results which are not very reproducible; thus we were able to observe variation coefficients of greater than 15%.

In conclusion, to obtain reliable results, it is desirable that all the people destined to complete the sampling, follow at the beginning of the campaign a repeatability test, and in all cases, it is indispensable that a single “parcelle” be sampled by the same person during the campaign.

2.2- The problem of stocking samples

The treatment of the samples in the shortest delay possible after their arrival in the laboratory is very highly recommended to assure a good degree of reliability for the method.

Conscious of the difficulty to follow this rule, we studied the effect of a prolonged stocking of samples on the quality of the analytical results – as berries or as filtrates after the maceration (see § 3.3) – at a controlled temperature (refrigeration, freezing), in order to propose a differed treatment without loss of information.

2.2.1 – 24 hour conservation of whole grape berries at 4°C

Ten lots of two hundred grape berries were consecutively collected by the same person. Five lots were subjected to immediate treatment and five lots were treated after twenty four hours of stocking in a refrigerator at 4°C in their collection box. In our conditions, we register a loss of weight of 12% and a drop in the anthocyanin concentration by 25%. The total phenolic compounds demonstrate a consistent comportment. It is to be noted that these modifications are relatively reproducible; the variation coefficients registered at a 24 hour interval were practically identical (table 3).

2.2.2 – Frozen grape berries

Forty-three lots of 400 grape berries coming from different « parcelles » were each split into two representative samples of 200 berries, the first being treated as soon as they arrived in the laboratory and the second after being frozen for a period. Figure 1 shows a not very close relationship between the results observed on the fresh grapes and their frozen replicates for the anthocyanin and total

polyphenol content parameters. The comportment of the samples is not stable; for certain lots there was a loss in the amounts of anthocyanins, sometimes even substantial, whereas on other lots the opposite evolution was observed.

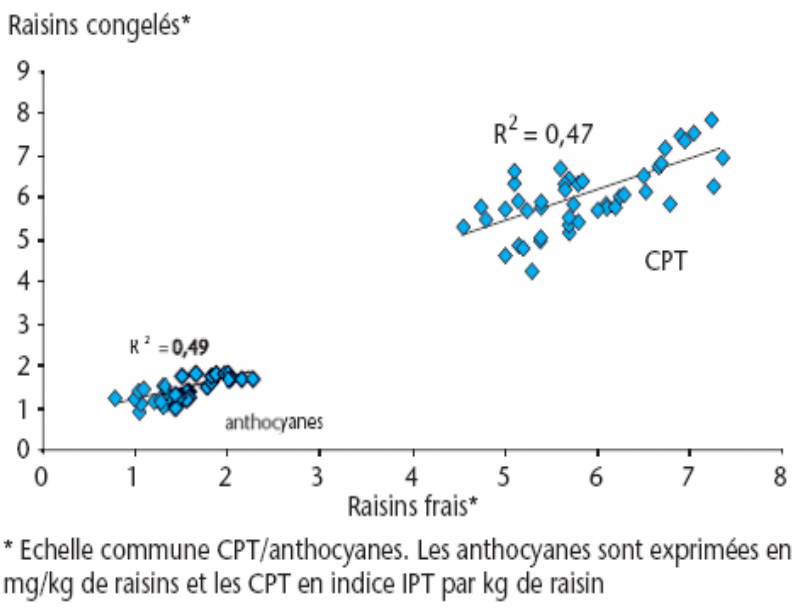


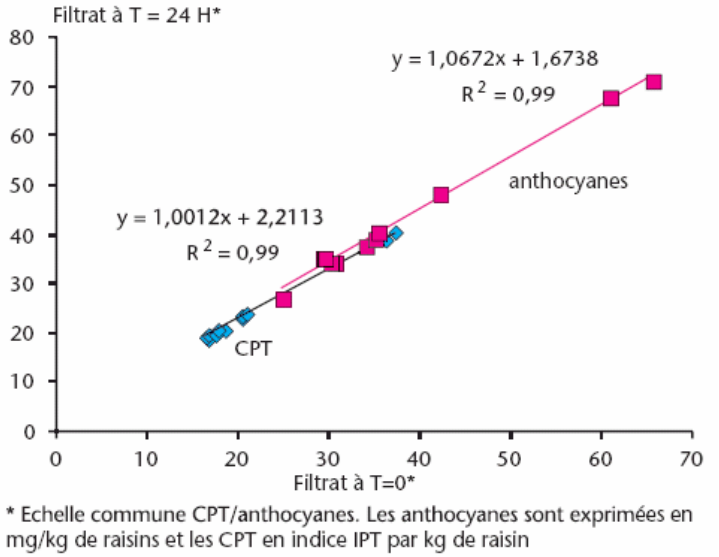
Figure 1- Relationship between the analytical results (anthocyanins and total polyphenol content) registered on fresh and frozen grape berries, 1994/1995

2.2.3- 24 hour conservation at 4°C of the filtrate after crushing, maceration and filtration

The standard I.T.V. method foresees a maceration step of the grape berries after grinding in a hydroalcoholic solution (see § 3.3.). The macerated pomace is subsequently filtered on glass cotton and there is the recuperation of the clear liquid phase destined for analysis. We studied the evolution of the analytical characteristics of this liquid phase (or filtrate) as we submitted it to a stocking at 4°C for 24 hours.

The results obtained for the measurement of anthocyanins and total polyphenolic compounds before and after stocking show a perfect linear correlation (figure 2). The slopes of the regression curves are close to unity, this means that the filtrate only undergoes a slight modification in its quantitative composition in anthocyanins and total polyphenol content.

Figure 2- Relationship between the analytical results (anthocyanins and total polyphenol content) registered on the fresh filtrate and after 24 hours of conservation at 4°C, 1999



2.2.4 – Conclusion on the stocking of samples

The stocking conditions of the samples are, as we have seen, very important. An unsuitable stocking can harm in a significant manner the pertinence of the analytical results. Thus, the technique consisting of freezing the grape berries before treatment is to be avoided, the results obtained on frozen grape berries not being very reproducible. The stocking of grapes or of the filtrate for 24 hours in refrigeration can be considered, keeping in mind that we register a quantitative loss, which is reproducible, in anthocyanins from grape berries, however the comportment of the filtrate is very stable in this interval of time but requires a prior preparation of samples. In all cases, the choice of the conservation method of the samples before analysis must be done at the beginning of the campaign and be applied in a similar manner up until the last control of maturity.

2.3 – Determination of the maceration time

2.3.1- Long or short maceration ?

To determine the minimal time necessary for the total diffusion of the anthocyanins in our operating conditions, we completed kinetic measurements of the extraction of anthocyanins during a two hour maceration. We can observe in figure 3 that the amounts of anthocyanins hardly evolve more after one hour of maceration; 95% of the diffusible anthocyanins are present in the liquid phase at this point. A supplemental maceration of one hour is therefore not justifiable. Measurements of repeatability of the maceration phase allowed us to register variation coefficients which do not surpass 5% for the measurements of anthocyanins and total polyphenol content. This technique is characterized by a significant extraction yield allows for a reliable estimation of the anthocyanin content of the grapes and this regardless of their potential.

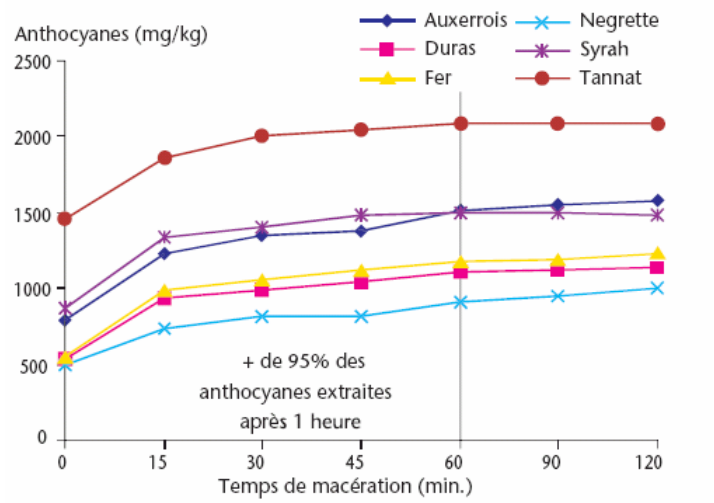


Figure 3- Evolution of the amounts of anthocyanins during the maceration of the pomace in a hydroalcoholic solution. Results on six grape varieties of the Midi-Pyrenees, 1999

2.3.2 – Is it possible without the maceration ?

The measurement of the anthocyanins and total phenolic compounds can be executed immediately after crushing. At this point the concentration of anthocyanins of the liquid phase represents, depending of the grape varieties, between 46 and 64% of the concentration which is observed after two hours of maceration. The repeatability is very good since it is situated above 5%. If we want to gain time on the completion of the method, such an extraction can be considered. However, due to the extraction yield being lower, it is highly recommended to use this method on grape varieties with high anthocyanin potentials.

2.4- Methods of anthocyanin measurement : discoloration with SO_2 or Puissant-Léon?

The optimization of this method requires the use of an anthocyanin measurement technique which is precise, pertinent and rapid. In this view, we compared the two principal methods of global anthocyanin measurement by visible spectrophotometry- the Stonestreet method (discoloration with sulfur dioxide) and the Puissant- Léon method (acidification of the medium) (20) – for the content and reliability of the information that they deliver.

2.4.1- Correlation between the two measurement methods

The pluriannual observations that were completed in the I.T.V. network France, show a strong relationship between the two methods (R2 > 0,95) for the anthocyanin measurements (figure 4). The given results are comparable but not identical in absolute value. The Puissant-Léon method underestimates the amounts of anthocyanins by around 20%. This observation has its explication not in the principals of the measurements, but in the values of the conversion coefficients for the absorbance values at 520nm of anthocyanin content expressed in mg/L, which are respective to each of the two methods. (Note: When the measurements are completed according to the Stonestreet method, in the calculation formula, the coefficient 870 must be replaced by 701 to obtain values at the scale of those given by the Puissant-Léon method; inversely, the results obtained by the Puissant-Léon method can be expressed according to the scale of the Stonestreet method by replacing the coefficient 22,76 by 28,25).

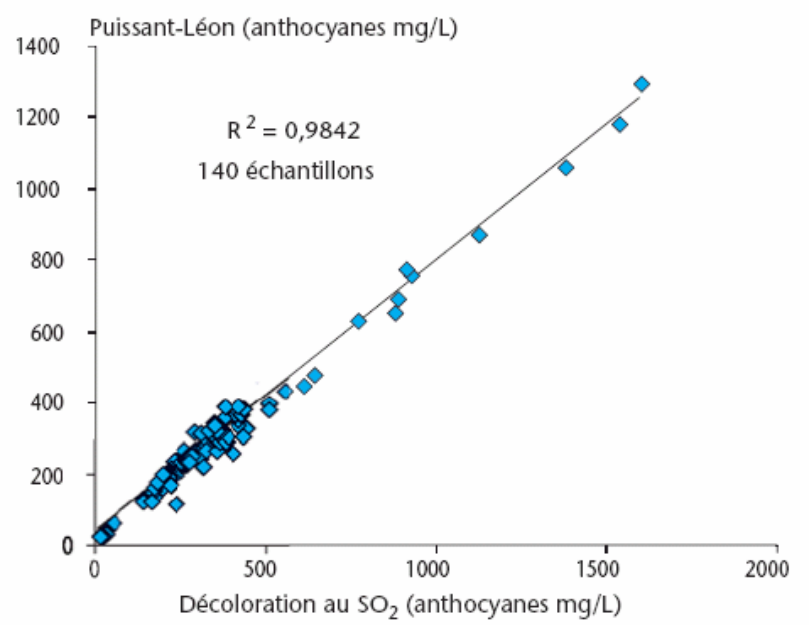


Figure 4- Example of the relationship between the anthocyanin content measured by the Stonestreet and Puissant-Léon methods, 1999

2.4.2- Repeatability of the measurement methods

Over the course of two consecutive campaigns, we have proved the reliability of each of the mentioned methods for the anthocyanin measurement. Starting from an extraction solution, we completed ten consecutive measurements. The treatment of the data allowed us to estimate the precision (confidence interval) for a probability of 95% and the variability (variation coefficient) of the two methods. We state in table 4 the results of the measurements executed on two different extraction solutions in 2000.

		Average (anthocyanins in mg/L)	Standard Deviation	Confidence Interval (in %)	Variation Coefficient (in %)
Series n°1	Puissant-Léon	321.9	2.98	2.2	1
10 repetitions	Stonestreet	438	9.4	4.9	2.3
Series n°2	Puissant-Léon	318	1.95	1.4	0.6
10 repetitions	Stonestreet	439	10.6	5.5	2.5

Table 4- repeatability of the anthocyanin measurement methods - 1999

The two methods present a very good degree of reliability with variation coefficients which do not surpass 3%. The Puissant- Léon method presents however more precision with a narrower confidence interval located between ± 2 % against ± 5 %. Despite equivalent pertinence and reliability, the Puissant-Léon method can be favoured due to the more simple and rapid execution.

2.5 – Automation of the anthocyanin and total polyphenolic compound measurements

In the setting of routine use of the estimation protocol for the polyphenolic richness, the management of manual analyses becomes troublesome. We have therefore adapted and optimized this method by using a Gilson sample preparer-passer. The automated Gilson carries out the preparation of the samples (dilution to 1/100th and preparation of the tubes for the anthocyanin measurement), and ensures the distribution in the spectrophotometer vat. An RS 232 series linked between the spectrophotometer and a program adapted on a micro-computer allows for the continuous acquisition of data. Transferred onto a spreadsheet, the results can very easily be utilized.

This automated method allows for an important gain of time (60 samples processed in 6 hours) and improves the reliability of the measurements. A technician must however supervise to ensure the link between the different steps (preparation of the samples, reading the absorbencies). Nevertheless, the costs implicated for such equipment (Automated Gilson: 80KF and spectrophotometer: 50KF) can only be considered in cases which require routine analyses.

2.6- Repeatability of the ITV method in its entirety

After the principal steps of the I.T.V. method were passed for review, we studied its global repeatability; this is to say all the steps starting from the treatment of the grapes at their arrival in the laboratory up to the measurement of the phenolic compounds. This was completed across two consecutive campaigns (1999 and 2000) according to the following protocol: one lot consisting of 2000 grape berries collected by the same person was divided into 10 representative samples of 200 grape berries. Each sample was processed independently on the same day by the same person. From the results of the anthocyanin measurements, we calculated the average of the series, the standard deviation, as well as the confidence interval and the variation coefficient (table 5). This manipulation was repeated twice in 2000.

	1999	2000	
		1st Series	2nd Series
Sample of 200 berries	10	10	10
Average (anthocyanins in mg/kg	315.7	299.1	302.9
Standard Deviation	11.7	8.6	7.9
Variation Coefficient %	3.7	3.0	2.7
Confidence Interval % (5%)	8.5	6.9	6.2

Table 5 – repeatability of the ITV method I.T.V. in 1999 and 2000 for the "anthocyanin" parameter

We note that for the principal parameter studied- anthocyanin content- that the reliability of the method is very good as it is lower than 5%. As for the global precision it is lower than 10%. It is to be noted that these values incorporate the variability due to the varying composition of the lots of 200 berries. In the end, for a given sampling, we can expect from this method, results which present a very good degree of reliability.

2.7- The I.T.V. method and current analyses

We compared, for the classic analytical parameters- sugar, total acidity and pH- the results obtained on the juice from the bottom of the tank (initial must before alcohol fermentation) with those obtained from one part from the grinded juice (I.T.V. method), and another part from the press juice (the grape berries are squashed with a mini-press of straw).

Table 6 gives an example of the results obtained for the Mourvèdre grape variety.

Sugars (g/L)			pH			Total Acidity (g H_2SO_4/L)		
Pressing	Grinding	Bottom of tank	Pressing	Grinding	Bottom of tank	Pressing	Grinding	Bottom of tank
225.2	226.4	232.3	3.59	3.97	3.57	4	3.6	4.6
240.3	235.8	246.4	3.5	4.05	3.69	3.6	4	3.6
202.2	204.5	209.1	3.57	3.99	3.60	4.2	3.8	4.4
217.2	224.1	206.8	3.36	3.82	3.51	4.8	3.9	3.8
174.9	180.5	172.6	3.29	3.67	3.28	5.3	4.2	5.8

Table 6: comparison of the sugar, pH and total acidity values for the pressed juice, crushed juice and juice from the bottom of the tank - 2000

The most noticeable effect is registered for the pH. The pressing allows for a good estimation of the pH of the must. The grinding extracts too much potassium, and this considerably increases the pH. The potassium present in the skins reacts very rapidly with the tartaric acid of the pulp, which leads to a bad estimation of the acidity, this corresponds to the resulting precipitations. A comparison of the grinding and pressing techniques on 370 samples of 200 grape berries shows that the grinding over-estimates the pH values by 14%, on average and under-estimated the total acidity values by 16%, on average. For the sugar content, the results of both techniques are comparable.

The I.T.V. method therefore does not permit to evaluate directly the pulp maturity, since the total acidity and pH values obtained on the grinding juice are too far from those noted in the musts when put in the tanks. To complete such an estimation, it is therefore necessary to execute the commonplace analyses on a juice coming from the pressing preliminary to the sample destined for the I.T.V. method.

3- Pertinence of the standard I.T.V. method for the determination of the phenolic maturity

The determination of an appropriate harvest date rests on the observation of the evolution of the anthocyanin content in the grapes during the maturation. We consider that the phenolic maturity is reached at the beginning of the over-maturation phase of the grapes, corresponding to a notable drop in the anthocyanin concentration after they initially reached a maximum. The construction of a curve representing the evolution of the anthocyanins in the grapes in function of time, facilitates the visualization of this phase.

We wanted to verify the pertinence of the I.T.V. method for the determination of the phenolic maturity by following on various sites, the evolution of the anthocyanins during the maturation and in

completing several successive harvests (5 dates) followed by standard minivinifications. The results that we provide were obtained in the Midi-Pyrenees for the Duras and Syrah grape varieties in 1998.

3.1- Evolution of anthocyanins during the maturation

The aspect of the curves is very close for the two grape varieties (figure 5); we note a rapid increase of the anthocyanin during the first twenty days of the maturation. This increase continues for several days but with lower amplitude. At this stage, the behaviour of the two grape varieties differ; for the Syrah it needs 15 days to reach a maximal concentration of anthocyanins, whereas for the Duras, 10 days are sufficient. Afterwards, the drop in anthocyanins for the Duras is more rapid than for the Syrah, showing a slower maturation for the last mentioned. The five harvest dates are positioned in the anthocyanin accumulation phase (D1 and D2), around the maximum (D3 and D4) and in the decline phase (D5). After the analysis of the curve, the phenolic maturity is judged to be optimal on the 22nd of September (D4) for the Duras and the 6th of October (D5) for the Syrah.

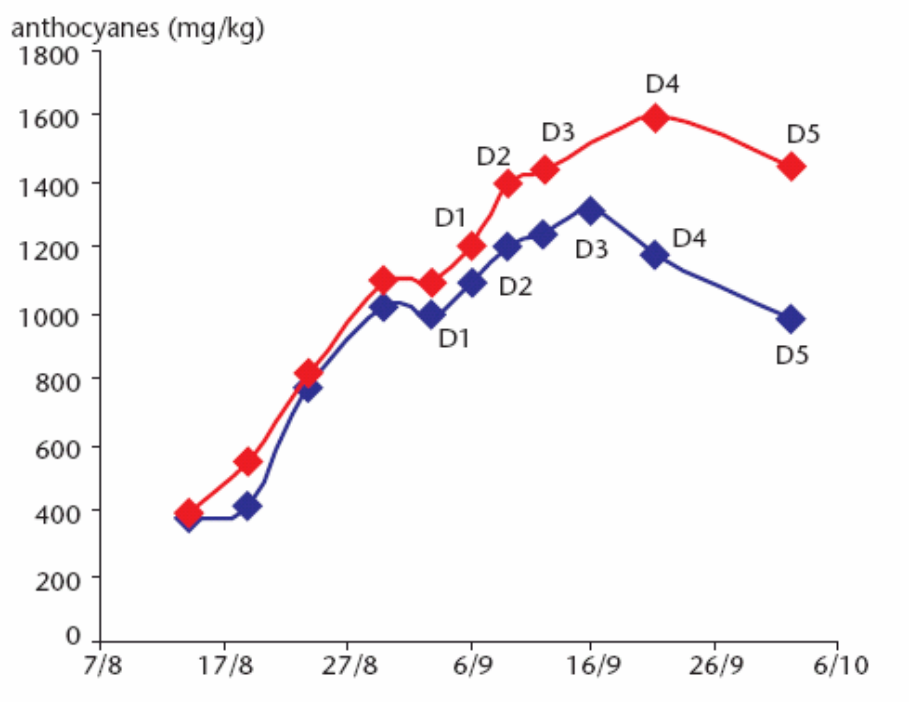


Figure 5- Anthocyanin evolution during the maturation of two red grape varieties of the Midi-Pyrenees (Red curve Syrah, Blue curve Duras), 1999

3.2 – Relationship between the harvest date and the analytic and organoleptic qualities of wines

A good relationship exists between the concentration of anthocyanins in the grape berries, estimated by the I.T.V. method and those that we find in the wine for early harvest dates. However, if we compare on one hand the harvest dates which correspond to the maximum anthocyanin accumulation, and on the other hand to the over-maturation phase, the correlation between grapes and wines is not longer verified. Thus, the wines elaborated from grapes harvested in slight over-maturation (D4 for the Duras and D5 for the Syrah) present anthocyanin contents superior to those measured on the wines from earlier harvesting which presented the highest anthocyanin potentials. This illustrates the notion of pigment extractability evolution during the maturation phase of the fruit. The tasting of wines demonstrates the interest and emphasis that the tasters give to the wines resulting from grapes harvested at optimal phenolic maturity (table 7).

Harvest Date	DURAS			SYRAH		
	Anthocyanins		Classement of wines according to the criteria « global quality »	Anthocyanins		Classement of wines according to the criteria « global quality »
	Grapes (mg/kg)	Wines (mg/L)		Grapes (mg/kg)	Wines (mg/L)	
D1	995	543	3	1090	690	4
D2	1200	607	2	1400	840	5
D3	1310	627	3	1430	836	3
D4	1180	685	1	1590	929	2
D5	980	601	4	1450	1058	1

Table 7 : relationship between the harvest date and the analytic and organoleptic qualities of the wines - 1998

These wines are judged as presenting nice and intense fruit aromas, having a dense mouthfeel and being well-balanced and having a notable colour. A too late harvest (Duras D5) seems to harm the aromatic expression with overly pronounced cooked and jammy fruit notes. The earlier harvests leaning towards a slight misbalance in mouth with the presence of rough tannins.

4- Description of the method (illustration 1)

The standard ITV method is based on a micro-maceration, in an acidic hydroalcoholic medium, at room temperature. This last is a “model” of the phenomena that intervene during a “standard” vinification. Also, in a few hours, we estimate the total quantity of phenolic compounds, and also their capacity to be extracted from the skins. A regular following of this polyphenolic evolution, via the completion of one sampling per week, and two per week closer to the harvest, permits for the determination of the optimal maturity phase. Theoretically, it corresponds to the moment where the anthocyanin concentration in the grapes drops significantly after having passed by the maximal value.

4.1- Grape sampling of grapes in the field

On two rows considered as representative of the parcel on which the maturation follow-up must be completed, we select and identify 100 vines which are exempt of wood diseases or viruses. We collect two grape berries per vine, one grape berry per side of the row, while alternating the sample collection object in order to respect the heterogeneity of the grape constitution within the grapevine- the position of the grape berry in the grape bunch, the position of the bunch on the branch, the insertion rank of the branch on the grapevine. The two hundred grape bunches thus collected are placed in a freezer box lined with an absorbent paper. The transport is completed in an ice-box in the presence of a refrigerant.

4.2 – Preparation of the samples for analysis
4.2.1- Numbering, weighing of grape berries and grinding

If the collection was completed after rain, we dry the grape berries using an absorbent paper and a hair dryer. The grape berries are then counted and weighed precisely in order to express the anthocyanin content as per grape berry or by a unit of mass. They are then crushed using a home appliance like a “blender” during two minutes at a reduced speed.

4.2.2 – Phenolic compound extraction by maceration

We collect a fraction of the crushed mass- about 50g – which we weight precisely and which we macerate in a hydroalcoholic solution (15mL of 95% ethanol, 85mL of 0.1% HCl) for 1 hour at room

temperature with an agitation every 15 minutes. The crushed and macerated mass is then filtered on glass cotton or is centrifuged. We then recuperate a limpid extraction solution, a condition which is necessary for the good execution of the further spectrophotometry measurements. Filtration with glass cotton does not cause any modification to the phenolic content of the extraction solution.

4.3 – Anthocyanin and total polyphenol index measurement

The total phenolic compounds are estimated by the measurement of absorbance at 280nm over a 1cm distance (quartz cuvette) of the extraction solution after a 1/100 dilution. The anthocyanin concentration is determined by the "Puissant-Léon" method, corresponding to the measurement of absorbance at 520nm over a 1cm distance of the extraction solution after acidification (dilution of the solution by 1/20 with 1% HCl in weight). The anthocyanin potential is expressed in milligrams per kilogram of grape.

5- Conclusion

The determination method for the anthocyanin richness of grapes adjusted by the I.T.V. is interesting on more than one level. It is the result of collaboration between several I.T.V. centers. The method was adjusted and validated from data taken from different vineyards representing different grape varieties, pedoclimatic conditions and cultivation methods. It is therefore not a tool which is limited to a single vegetal material or to a particular viticultural region. Its execution is simple and rapid. All manipulation that could be subjected to a gain of time were optimized and in this sense, assuring the preservation of the integrity of the information. Thus, the anthocyanin measurement times and the maceration times were halved – we can, further, consider the reasonable abandoning of the maceration step for grape varieties with strong anthocyanin potentials. The main steps of the method were studied in detail in order to determine their precision and repeatability. We thus have a method of which the global variability does not exceed 5% in normal execution conditions. It permits to obtain with a good degree of reliability the optimal zone of phenolic maturity, to gain information about the potential of a vintage by comparison with a past vintage (reference vintage) or a parcelle within the exploitation (parcelle reference). The pressing of the grapes before grinding gives access to a pertinent measurement of the acidity and pH of the must without having to multiply the samples during the sampling. The I.T.V. method does not in itself substitute the classic criteria for the determination of maturity, but rather brings complementary useful information.

Acknowledgement
The authors would like to thank all the people who participated in this programme :
C. Liadouze, E. Vinsonneau unité I.T.V. Bordeaux, J. Marsault, F. Etienne unité I.T.V. d'Angers, J. Beguin, A. Bruetchy, P. Poupault unité I.T.V. de Tours, S. Grivot, V. Bouckenooghe de l'unité de Nîmes, J.Y. Cahurel unité I.T.V. de Villefranche sur Saône, B. Barthelemy, F. Davaux, E. Serrano unité I.T.V. de Gaillac, B. Labarbe de la SICAREX Beaujolais.
As well as the financial partners:
ANDA, ONIVINS, Conseils Régionaux dans le cadre des contrats
Etat / Régions, Comités interprofessionnels et syndicats viticoles…

Keywords : viticulture, phenolic maturity, phenolic compounds, anthocyanins

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