Sampling strategy and minimum sample size to judge correct determination of grape maturity

Stichprobenstrategien und minimaler Musterprobenumfang zur Bestimmung des Reifheitsgrad der Trauben

Stratégies d'échantillonnage et dimension minime de l'échantillon pour l'évaluation du niveau de maturité du raisin

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ABSTRACT
The key to a good estimate of fruit maturity is to collect a sample that is truly representative of the entire harvested unit (i.e., block of one variety, plot). It is important to recognize the high level of variability in fruit composition that exists within a vineyard and even within a single fruit cluster. The target of this research was to determine grape quality selecting a correct sampling strategy (part of cluster, bunch and whole vine) and minimum sample size according to vineyard variability. The results show less variability on part of bunches than whole vine and single bunch samples. Less amount of grape samples, over estimated primary and secondary metabolite levels. Finally the keys to accurate fruit assessment are to use appropriate sample collection methods, proper analytical equipment and careful sensory evaluation.

AUSZUG
Die Darstellungsfähigkeit der Musterproben zur Einschätzung des Reifheitsgrades der Trauben spielt eine entscheidende Rolle sowohl für die Weinbaupraxis (zur Bestimmung des günstigsten Zeitpunkts für die Weinlese) als auch für die Untersuchungen der Weinbauforschung (für die Bewertung der Ergebnisse). Einige Forscher haben sich mit diesem Problem beschäftigt und verschiedene Lösungen vorgeschlagen, um die minimale Menge nach spezifischen Kriterien geernteter Trauben zu bestimmen, die Qualität der Trauben und die Charakteristiken des Weinguts darstellen kann. Es gibt aber noch zahlreiche offene Fragen zu dieser Problematik. Die vorliegende Studie hat das Ziel, verschiedene Typologien der Traubenmusterentnahme zu vergleichen und den geringsten Musterprobenumfang zu bestimmen, der, unter Berücksichtigung der Variabilität der Weingüter, zur Qualitätsbestimmung der Trauben notwendig.

RÉSUMÉ
C'est essentiel pour une bonne évaluation du niveau de maturation du raisin prélever un échantillon représentatif de la vendange entière (ou du raisin d'une parcella). C'est importante repérer le niveau de variabilité qui existe dans la composition du raisin d'un vignoble ou des baies d'une grappe. Le but de cette recherche a été de déterminer une stratégie correcte de prélèvement de l'échantillon (partie de la grappe, grappe entière ou plante entière) et la dimension minimal de l'échantillon pou évaluer la qualité du raisin compte-tenu de la variabilité qui existe dans le vignoble ou dans la parcelle. Les résultats montrent que la variabilité entre les échantillons est plus basse quand on prélève parties de grappes en comparaison de grappes entières ou plantes entières. On a une surestimation des métabolites primaires et secondaires quand on prélève un échantillon très petit. C'est essentiel, enfin, pour l'évaluation précise de la qualité du

raisin employer des méthodes appropriés d'échantillonnage des équipements analytiques conformes et faire une analyse sensorielle soigneuse.

INTRODUCTION

To know the ripeness level of grape is an important component on wine quality production, but obtaining a field assessment of maturity, crucial to the proper timing of harvest, is hindered by variability in the field (Wolpert *et al*, 1984). This variability may result from vineyard heterogeneity (Baker et al, 1965), grapes variability within vines (Shaulis, 1956; Smart, 1985, Carbonneau *et al*., 1991) and berries variability within bunches (Smart, 1985). Nevertheless, sampling schemes can be used to minimize random variability and obtain reliable estimates of fruit quality (Wolpert *et al*., 1980). Several studies have investigated optimal sampling procedures for winegrapes. Shaulis (1956) find that samples of apical berries of Concord accurately estimated sugar content of cluster and were more precise than samples of berries randomly selected from the cluster. Rossler *&* Armerine (1963) concluded that the values of juice obtained by sampling of 100-200 berries randomly taken from 1000 different vines and the entire sampled field generally agreed to within ±0.6 °Brix, and berry and bunch samples did not consistently overestimate maturity. Moreover brix values had more variability in the whole vine samples than the bunches and berries ones. Rankine *et al*., (1962), recommended berry sampling because its precision was equal to that of cluster sampling in estimating whole vine soluble solids of three *Vitis vinifera* L. cultivars. A large number of grapes must be sampled in order to obtain reasonably accurate results because of these large variations. A sample consisting of 100 individual berries is considered the minimum size sample for a small vineyard. Large operations often collect 500 to 1000 berry samples. Most winemakers consider a few hundred berries to be an adequate sample size. Uniform collection necessary collecting sample grapes in a consistent way and from all parts of the vineyard is important for accurate results. For example, a large and uniform sampling of the entire vineyard would be collected if two grapes were picked from each vine in a vineyard containing 100 vines. Alternatively, taking one grape from every fourth vines would produce a uniform sample of 250 grapes in a vineyard containing 1000 vines (Eisenman, 1998). The most important consideration is to attempt to collect a reasonably large sized sample from the entire block that will be picked. All previous studies gave us sample number and mass information to have sugar and acidity amount assessment close to real field values. Insufficient information can be found concerning secondary metabolites assess as phenol compounds. The target of this research is to give more information concerning sample methodologies.

MATERIALS AND METHODS

Measurements were conducted in 2005 vegetative season on Cabernet Sauvignon grafted onto 1103P. Experimental block (approximately 1 Hectare) was located in western Sicily, Alcamo D.O.C. area (37° 55' 11,66'' N; 13° 04' 10,03'' E) at 300 m a.s.l. Vines were trained to a vertical trellis system, spur pruned and 2.4 m x 0.95 m spaced for a density of 4385 vines per hectare. During the summer season normally canopy management practices (as topping, vertical shoot position, suckering) were done. Vineyard was drip irrigated (800 m^3/ha) maintaining predawn water potential values less than –5.0 MPa.

The study was focused on a) vineyard variability, b) sampling strategy and c) minimum sample size to judge correct determination of grape maturity.

a) Multispectral hight resolution image (0.70 m in panchromatic and 2.70 m in blue, green, red and NIR) captured by Quickbird satellite was used. Image was georefenced (UTM ED 50), and setup with radiance and reflectance calibration (Santangelo, 2006). Vegetative, qualitative and reproductive parameters were measured on three vines within different parcel according to difference of vigour existing on the experimental block. Regression between agronomic data and Vegetation Index was calculated to obtain different agronomic parameters maps. Map calculator was applied on sugar map to obtain mean and standard deviation.
b) Three sampling strategies were compared: 1) berry (taken as part of bunches), 2) cluster and 3) whole vines. At harvest were collected 10 kg of grapes, coming from 72 bunches and 9 vines respectively from 2 and 3 typology (fig.a). Soluble solids (°Brix), extractable anthocyanins (mg/kg) and flavonoids (mg/kg) were determined in each sub sample.
Each sampling strategy was divided by 9 sub samples then, singularly analyzed. A sample with an amount of juice left from the 9 samples was also collected, three times replicated and analysed too (fig.b). Nine sub-sample results were used to study sample variability within strategies moreover total results were used to compare different sampling strategies. All results were compared to cellar reference (analysis that coming through the total experimental block). The must coming to the experimental block was also three times replicated and analysed.
c) Minimum sample size to judge correct determination of grape maturity was investigated on whole vine concerning soluble solids and extractable anthocyanins.
The methodology consisted on a re-sampling of the nine plant's values by a combinatory calculation which took into account any possible simple combination between all samples and using this formula:

$$C_{n,k} = \binom{n}{k}$$

with $n \geq k$ and $k>0$; n= single vine, k= class. In this particular case n=9. Table 1 shows all possible combinations derived from the data. An average of soluble solids and extractable anthocyanins values was calculated for each combination and compared by using an accuracy of ± 5 %, ± 10 % and ± 15 % to the average derived from the nine plants (class 9) which has been considered representative of the entire vineyard.

RESULTS
Soluble solids map within experimental block is shown in a figure 1. The figure shows the vineyard variability albeit error caused by regression with NDVI is preserved.
Soluble solids map was divided intro three classes: 1) green <20° Brix; 2) yellow between 20°Brix and 21°Brix and 3) red>21°Brix, according to different black grape enological destinations, to know soluble solids variability in a different area. Moreover, evaluating all the absolute values of pixel frequencies of experimental block it is possible to estimate block variability in terms of mean and standard deviation and so on (fig. 2). The three classes of soluble solids have a different percentage of incidence in the vineyard. The three classes of soluble solids shown values of 19.20°Brix, 20.07°Brix and 22.07°Brix (green, yellow and red respectively) and represent the 29.13%, 30.62% and 41.27% respectively of the total plot area which have an weighted average of 22.01°Brix (tab.2). Soluble solids values per green and yellow classes underestimated weighted average of 13.6% and 8.8% respectively while overestimation

of 3% was reported for red class. Moreover if during grapes sampling, percentage influence of three classes is not take into account (and so equal amount of grape will sampled per all three classes), a 7.3% of error can be done (20.40° Brix) compare to real value obtained by weighted average (22.01° Brix) of classes percentage influences on vineyard surface (tab.2).
Sampling strategy and not only plot variability, must be also taken into account in sampling grapes. Differences not significant, in terms of soluble solids, were found between part of grapes, cluster and whole vine and compare to the cellar reference value. Secondary metabolites as extractable anthocyanins and flavonoids shown significant differences between sampling strategy and compare to cellar reference value. Particularly extractable anthocyanins shown similar values in whole vine compare to cellar reference one (540 mg/kg vs 565 mg/kg respectively) and extractable flavonoids too (2814 mg/kg vs 2654 mg/kg respectively), moreover part of bunch and cluster overestimated cellar reference value. Whole vine sampling method, had higher soluble solids, extractable anthocyanins and flavonoids coefficient of variations (7.9%, 20% and 38% respectively) while part of bunch had the lowest (0.9%, 10% and 13% respectively).
Cusp trend was shown combining the nine vines, decreasing in variability when class increase in number (figg. 3&4). Particularly, sampling 6 vines was enough to reach values of soluble solids ±5% of the 9 vines average value. Reducing in vine numbers increase in error probability more than ±5% of the 9 vine average (from 0.8% at class 5 to 55% at class 1). Logically ±10% accuracy of 9 vines average value can be obtained sampling less number of vines (three vines in this particular case). Fifteen percent accuracy of 9 vines average values can be obtained sampling 2 vines (fig. 5). Higher variability was found for extractable anthocyanins. In this case accuracy of ±5% than 9 vines average value can be obtained sampling 8 vines while 17%, 24%, 39%, 48%, 62%, 67% and 100% of error probability is reach sampling 7, 6, 5, 4, 3, 2 and 1 vines respectively; ±10% accuracy can be obtained sampling 7 vines while ±15% accuracy with 5 vines. In this last case 3%, 8%, 30% and 44% of error probability is obtained sampling 4, 3, 2 and one vines respectively (fig.6).

CONCLUSIONS

Sampling strategy and minimum sample size in this study judge easier correct determination of primary metabolites than secondary ones. Knowledge vineyard variability is propaedeutic to reach a correct sampling strategy. To not consider in sampling practices a real vineyard variability incidence give more than 7% of error in estimating soluble solids values. Whole vine sampling strategy is the better way to judge a correct primary and secondary metabolites determination even than shows the higher coefficient of variation (Wolpert *et al*., 1984). Finally minimum sample size change according to accuracy level to obtain.
These results are unspecific to all vineyard cases but only in this particular case and for this specific variability; therefore is not easy to assess a single sampling strategy.
Finally the keys to accurate fruit assessment are to use appropriate sample collection methods, appropriate vineyard variability evaluation, proper analytical equipment and careful sensory evaluation.

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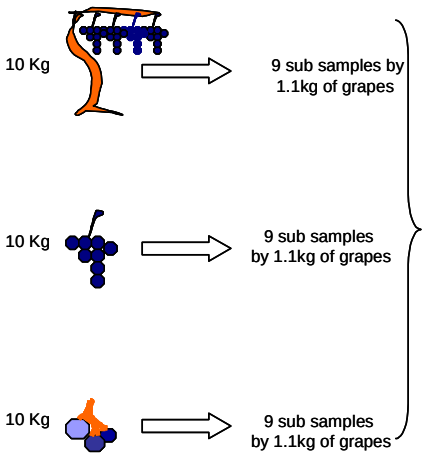

Fig. a – Samples collection

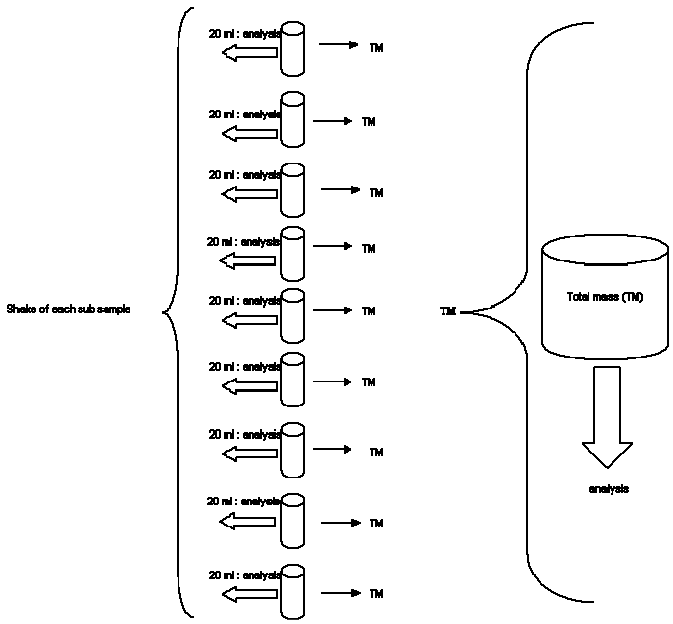

Fig.b – Design of samples analysis

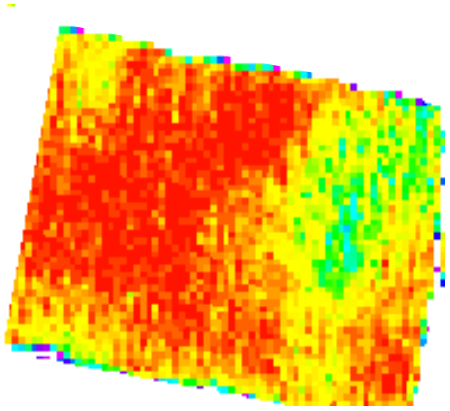

Fig. 1 - Soluble solids map.

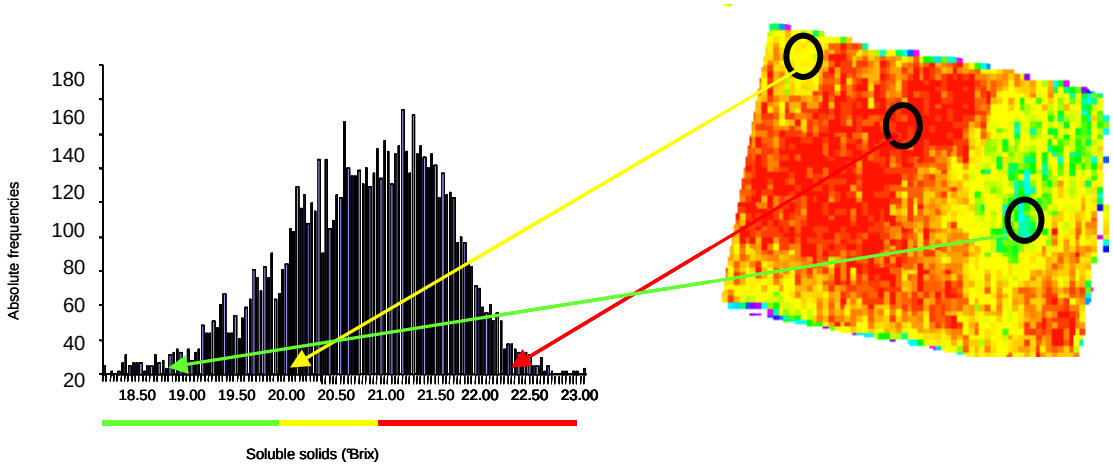

Fig. 2 - Soluble solids map distribution.

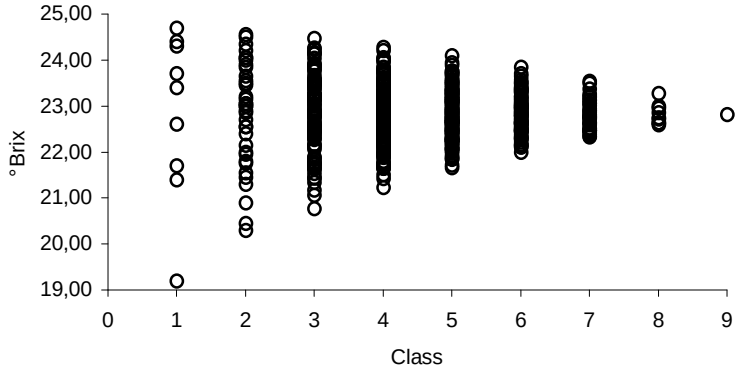

Fig. 3 – Soluble solids: distribution of combinations at different classes

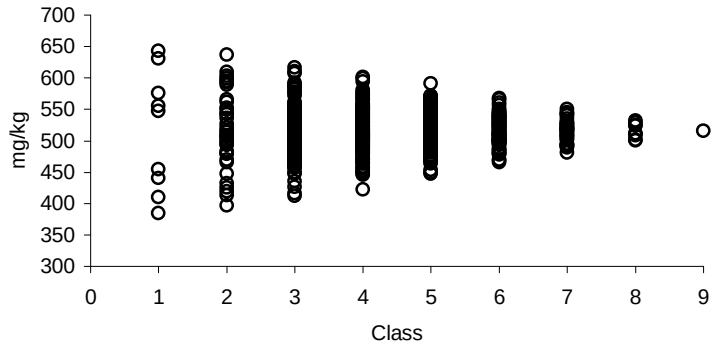

Fig. 4 – Extractable anthocyanin: distribution of combinations at different classes

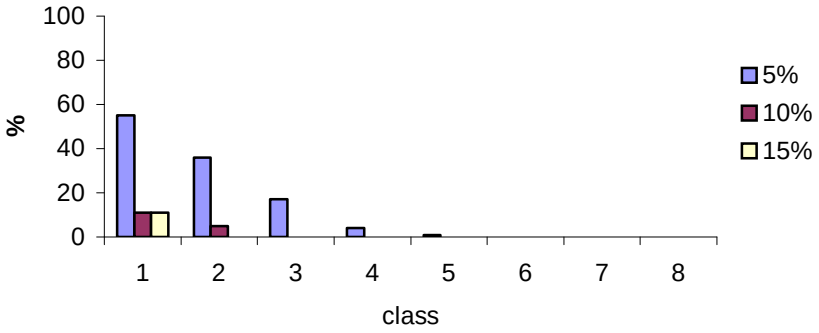

Fig. 5 – Soluble solids: probability of error of ±5%, ±10%and ±15% of accuracy

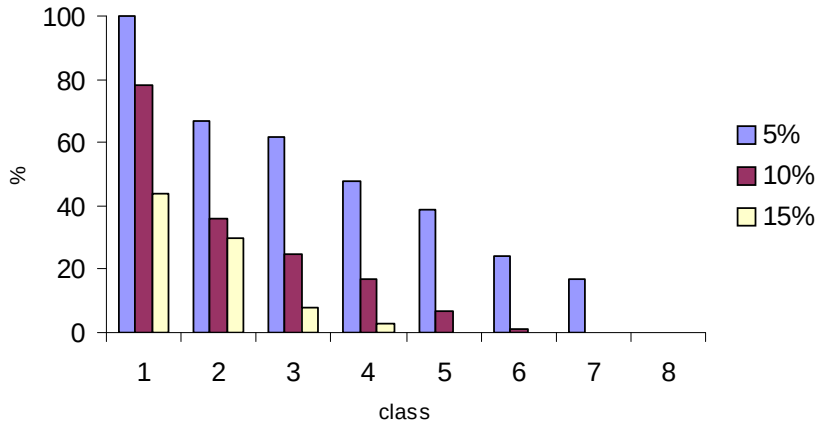

Fig. 6- Extractable anthocyanin: probability of error of ±5%, ±10%and ±15% of accuracy

Class	n°
1	9
2	36
3	84
4	126
5	126
6	84
7	36
8	9
9	1

Tab. 1 – Combinatory calculation: number of cases of all 9 classes obtained from 9 vines

Class		Incidence on total surface (%)	Average value per class	Weighted average value discard (%)
A	S.S. < 20	29.13	19.02	-13
B	20≤ S.S. <21	30.62	20.07	-6
C	S.S. ≥21	41.27	22.07	3
Mean			**20.40**	
Weighted average				**22.00**

Tab. 2 – Soluble solids (S.S.) average values per class and weighted average values

Simple typology	Part of bunch			Cluster			Whole Vine			Cellar reference	
	Mean		C.V. %	Mean		C.V. %	Mean		C.V. %	Mean	
Soluble solids (°Brix)	22.5		0.9	23.1		7.1	22.8		7 .9	22.7	n.s.
Extractable anthocyanin index (mg/kg)	766	a	10	767	a	15	540	b	20	565	b
Extractable flavonoid index (mg/kg)	3492	a	13	3172	b	15	2814	c	38	2654	c

Tab. 3 – Mean and coefficient of variation of soluble solids, extractable anthocyanin and flavonoid index, on three sample typologies and cellar reference
Values with the same letters on each lines do not differ significantly from each other at the 5% significance level