Assessment of grape skin hardness by a puncture test

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Abstract

BACKGROUND: The release of grape components during wine making might be related to the mechanical properties of the skin, in particular its hardness. Samples from three varieties collected during the 2005 vintage season in Piedmont, Italy, were tested for their skin hardness using a texture analyser. The goal was to understand the statistical interactions between three factors – variety, cluster position and puncture point – and their influence on the grape skin hardness. A discussion on the relationship between the size of the sample used and the confidence level is also provided.

RESULTS: Results of the ANOVA test showed that there is an interaction between the variety and the puncture point when measuring the skin hardness with the break energy. The position of the berry on the cluster does not affect the berry skin break energy. We also show that a sample size depend on the variety tested.

CONCLUSION: The break energy is more useful in understanding the effect of the three factors on the skin hardness. Other factors that might affect the puncture test applied to grapes need to be studied in the future and the usefulness of the test in winemaking will need to be further developed.

Keywords: grape; texture analysis; berry skin hardness; break force; break energy; sample size

INTRODUCTION

Many factors are involved in identifying vines and, to date, a number of different approaches have been suggested for the characterisation and classification of grape varieties and for the definition of grape quality at ripeness.

Ampelographic characterisation according to morphological features has been useful in the past for the identification of grape varieties.[1] Unfortunately, morphological characterisation is a time-consuming process, and is based on properties which are often affected by the environment.[2] Further, it does not generally help to predict genetic identity with a high probability. Other methods, based on the use of genetic variability at the level of proteins or nucleic acids, have been used for these purposes.[3,4]

In wine production, the composition of the grapes at the moment of picking is an important determinant of their quality.[5] However, there is no single set of parameters that define ripeness for a particular grape variety under all circumstances and for all purposes. The evaluation of the technological ripeness, expressed in analytical parameters such as sugar and acids, is, by itself, not sufficient to completely predict grape oenological potential.[6] Various pieces of research have shown phenolic content as a key defining factor of grape maturity[7] and of the grape oenological potential.[8–10] The accumulation of anthocyanins and tannins in grape skins reaches maximum values close to technological maturity, and coincides with the degradation of the cell membranes, which facilitates the extraction of anthocyanins.[8]

Texture is one of the most important quality characteristics of edible fruits and vegetables. Texture includes all physical characteristics sensed by touch which are related to deformation under an applied force and can be measured objectively in terms of force, distance and time.[11] It is known that plant structure plays a key role in determining texture, which arises from the arrangement of various chemical species by physical forces into distinct micro- and macrostructures, texture being the external manifestation of these structures.[12]

In the case of grapevine, the literature contains numerous scientific contributions that analysed the modifications of some grape textural properties.[13–16] Many of these studies focused on 'table' varieties, with the pulp compactness and the berry skin consistency as main treated parameters, since they are related to customer acceptance of the product.[17–22] It is also known that grape mechanical properties can influence

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the winemaking process, though little attention has focussed on wine grape texture analysis.[23–25]

While, in some cases, such as quality control, it might be appropriate to search for a single parameter that reflects the overall texture, this approach frequently fails. Texture is a complex attribute that is influenced by numerous factors, not the least of which is the complexity and dynamics of the plant material itself. Only as a result of the application of a number of objective methods, which are based on different principles, can a food's texture be fully characterised. However, a complete texture evaluation may be impossible. Not all these data may be required because some measurements are probably redundant or more or less sensitive than others. On the other hand, with only partial characterisation there is a real risk of false conclusions being drawn or of results being misinterpreted.[26]

To date, however, few systematic texture studies have been carried out using these techniques. An example of the application of texture measurements made on wine grape varieties grown in the north-west of Italy is presented in this work. The purpose was to define the berry skin hardness, measured by a puncture test, through two different parameters: the break force and the break energy. This work is intended to be a guide for performing the puncture test measurement. Some of the factors that may or may not affect the test will be discussed.

MATERIALS AND METHODS

Sampling

During the 2005 vintage season, in the region of Piedmont, north-west Italy, three international red grape varieties, Cabernet sauvignon (CN), Pinot noir (PN) and Nebbiolo (NE), were collected at harvest and analysed.

Four hundred berries were randomly hand-picked from designated vines, according to the sampling method described by Carbonneau *et al*.[27] It consisted of randomly picking bunch fragments in the medium part of the cane or from the cordon excluding those in the first rank of the parcel.

For each variety, out of the 400 berries, three sub-samples of 60 berries each were randomly picked from three different positions on the cluster: the shoulder G1 (front and back), the middle G2 (front and back), and the bottom G3 (Fig. 1). Each berry was detached by cutting its pedicel, and then visually inspected for any skin damage. In order to avoid alterations on standing, the hardness test was performed on the same day as the berries were picked. As far as possible, berries of identical size were selected.

Chemico-physical determinations in grapes

The grapes were sampled at their technological ripeness or optimal ripeness level for wine production. This corresponds to the harvest time and varies depending upon the style of wine being made from each grape variety in its area of cultivation. The total solids (Brix), the titratable acidity and the pH were selected as characteristic parameters.[28]

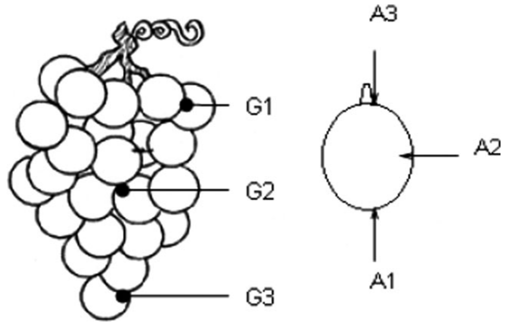

Figure 1. Left: Berry sampling positions in the cluster (G1 shoulder, G2 middle, G3 bottom). Right: Berry skin puncture test positions (A1 bottom, A2 side, A3 top).

Berry skin hardness test

In our previous work on table and wine grape, berries used for skin analysis were placed on the horizontal metal plate of the analyser, with the pedicel in a horizontal plane.[23–25] The same method was followed to determine the skin hardness of the berries.

A skin hardness test was performed on each berry and the results were used to compare three possible puncture positions on the berry (top, side and bottom). For this purpose, each sub-sample of 60 berries corresponding to the three cluster positions, was divided into three groups of 20 berries each and analysed by a puncture test applied to the three puncture points A1, A2 and A3 (Figs 1 and 2). A Universal Testing Machine TAxT2i Texture Analyser (Stable Micro System, Godalming, Surrey, UK) was used to measure the resistance of the berries' skins to puncture. Each berry was placed on a HDP/90 perforated platform.

Table 1 shows the operational parameters and the values used for the test.[29] Because of the nature of the analysed material (near-solid), a uni-axial force was applied.[11] The puncture test was performed with a 25 kg load cell and using a 2 mm needle probe.

A force calibration was required so that the system can calculate the relationship between the signal from the load cell and the force. The load cell measures the electrical resistance that is proportional to the force. The resistance is then converted to numbers by an analogue-to-digital converter. The first calibration

Table 1. Operational parameters for the execution of the berry skin hardness test

	Parameter
Test	Berry skin hardness
Probe	Needle P/2N
Test speed	1 mm s^{-1}
Compression	3 mm
Mechanical properties	F_{sk}, berry skin break force (N)
	W_{sk}, berry skin break energy (mJ)

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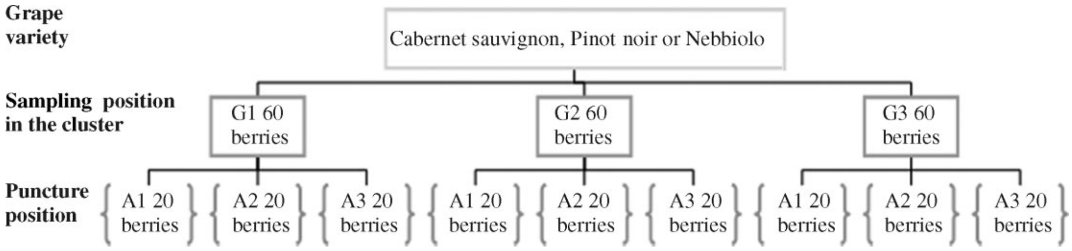

Figure 2. Experimental design. A1, A2 and A3 are different puncture points, and G1, G2 and G3 are the sampling positions in the cluster.

was achieved without the use of any weight and was based on what the software recognised as the zero force. The second step involved a 5 kg weight placed on the instrument platform and the result was stored as loaded level.

A probe calibration was also necessary in order for the instrument to register movements of the probe, as well as the position of the tip relative to another surface. A 1 g contact force and 10 μm return distance were used.

In order to avoid any damage to the probe by the berry seed, the penetration of the needle into the berry was limited to 3 mm. The acquisitions were made at 400 Hz. The force–deformation curve was acquired as a graph and elaborated using Texture Expert Exceed software (version 2.54 under Microsoft Windows 2000; Stable Micro Systems) As in Braga *et al.*,[30] the berry skin hardness was assessed by the maximum break force F_{sk} and by the break energy W_{sk}. F_{sk} corresponds to the resistance of the berry skin to the penetration of the probe and W_{sk} measures the area underneath the deformation curve between force values 0 and F_{sk}. Figure 3 shows a typical force–time (deformation) curve, obtained from the berry skin puncture test.

Statistical analysis

The effect of variety, cluster position and puncture point on the hardness of the berry skin was investigated. As discussed in the previous section, for each grape variety, the subset of 60 berries, taken from different positions in the cluster, was divided into three minisets of 20 berries each. Each miniset was punctured at a different point on the berry. A multi-factorial ANOVA test was used to explore the effect of the three factors on the skin hardness and verify the existence of any interaction between them.

The SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis.

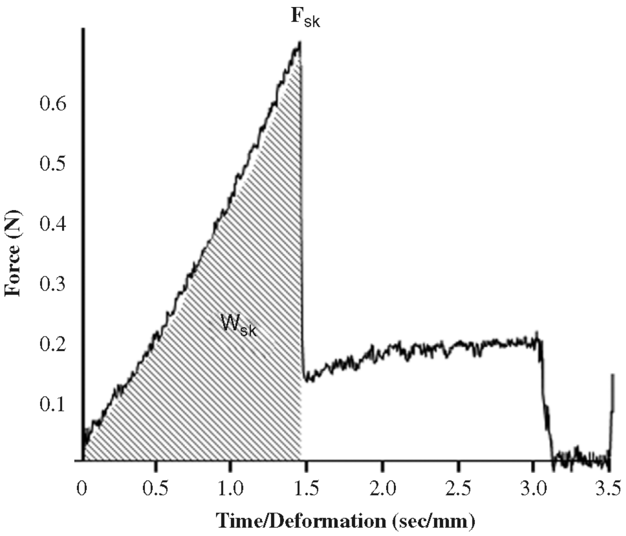

Figure 3. Force–time (deformation) curve corresponding to the berry skin puncture test.

RESULTS

Table 2 includes the technological ripeness parameters of Cabernet sauvignon, Pinot noir and Nebbiolo at harvest time. In this case, the three varieties do not have the same levels of Brix, titratable acidity and pH. Note that all three varieties were collected at the optimal ripeness time for the production of wines, in accordance with the Piedmont style of production.

Berry skin hardness test

Tables 3 and 4 list the means and standard deviations of the berry skin break force and energy, calculated for each variety (Cabernet sauvignon, Pinot noir and Nebbiolo), each sampling position (G1, G2 and G3), and each puncture point (A1, A2 and A3).

Tables 5 and 6 summarise the multi-factorial ANOVA test results for the three factors, variety referred to as V, sampling position G, and puncture point A, on the berry skin hardness.

Results in Table 5 show that there is evidence of an interaction between V, A and G. The presence of this interaction effect implies that it is not important to investigate the two way interactions $V \times G$, $V \times A$, and $G \times A$. We can then conclude that the skin break force is affected by the variety, the cluster position, and the puncture point.

Table 6 shows that there is no evidence of a three-way interaction $V \times A \times G$. We then examine the

Table 2. Technological ripeness of Cabernet sauvignon, Pinot noir and Nebbiolo at the 2005 harvest season

	Brix	Total acidity ($g\ L^{-1}$ tartaric acid)	pH
Cabernet sauvignon	22.4	7.5	3.18
Pinot noir	21.0	9.9	3.03
Nebbiolo	24.6	6.7	3.12

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Table 3. Mean and standard deviation (SD) of 20 values corresponding to the berry skin break force (Fsk) of Cabernet sauvignon, Pinot noir and Nebbiolo, sampled from different cluster positions (G1, G2 and G3) and measured at different puncture points (A1, A2 and A3)

		A1		A2		A3	
		F_{sk} (N)	SD	F_{sk} (N)	SD	F_{sk} (N)	SD
Cabernet sauvignon	G1	0.369	0.055	0.443	0.063	0.295	0.050
	G2	0.375	0.076	0.411	0.056	0.259	0.046
	G3	0.368	0.058	0.406	0.053	0.256	0.049
Pinot noir	G1	0.397	0.097	0.528	0.076	0.417	0.058
	G2	0.437	0.082	0.566	0.050	0.383	0.046
	G3	0.428	0.073	0.562	0.078	0.354	0.074
Nebbiol	G1	0.293	0.055	0.423	0.049	0.286	0.038
	G2	0.293	0.053	0.366	0.084	0.263	0.057
	G3	0.249	0.065	0.407	0.080	0.278	0.054

Table 4. Mean and standard deviation (SD) of 20 values corresponding to the berry skin break energy (W_{sk}) of Cabernet sauvignon, Pinot noir and Nebbiolo, sampled in different cluster positions (G1, G2 and G3) and measured in different puncture points (A1, A2 and A3)

		A1		A2		A3	
		W_{sk} (mJ)	SD	W_{sk} (mJ)	SD	W_{sk} (mJ)	SD
Cabernet sauvignon	G1	0.188	0.069	0.246	0.057	0.114	0.043
	G2	0.179	0.064	0.231	0.064	0.094	0.026
	G3	0.183	0.061	0.219	0.048	0.091	0.033
Pinot noir	G1	0.191	0.085	0.306	0.074	0.183	0.045
	G2	0.220	0.073	0.307	0.050	0.189	0.056
	G3	0.217	0.058	0.293	0.079	0.188	0.060
Nebbiolo	G1	0.131	0.055	0.252	0.054	0.111	0.034
	G2	0.140	0.053	0.201	0.079	0.089	0.039
	G3	0.101	0.054	0.245	0.083	0.106	0.041

Table 5. Effect of the factors variety, cluster position, puncture point and their interactions on the berry skin hardness expressed as the break force. Tests of between-subjects effects

Source	Significance
V	***
G	NS
A	***
$V \times G$	NS
$V \times A$	***
$G \times A$	*
$V \times G \times A$	*

V = variety; G = cluster positions; A = berry puncture points; *** $P < 0.001$; * $P < 0.05$; NS, not significant

Table 6. Effect of the factors variety, cluster position, puncture point and their interactions on the berry skin hardness expressed as the break energy

Source	Significance
V	***
G	NS
A	***
$V \times G$	NS
$V \times A$	***
$G \times A$	NS
$V \times G \times A$	NS

V = variety; G = cluster positions; A = berry puncture points; *** $P < 0.001$; NS, not significant.

three two-way interactions $V \times G$, $V \times A$, and $G \times A$. Only the interaction between V and A is significant, which means that the effect of the variety on the skin break energy depends on the puncture position or equivalently the effect of the puncture position on the break energy differs between varieties. Meanwhile, the effect of the G position on the break energy is not affected either by the variety factor or by the puncture position. Equivalently, the effect of the A position does not depend on the G position. In addition, the variety effect does not influence the effect of the G position on the skin break energy. It is therefore possible to assert that berry skin hardness expressed by the break energy is not affected by the original sub-sampling position on the cluster.

Thus, the break energy could be better than the break force for understanding the effect of the cluster position.

Since there is evidence of a two-way interaction $V \times A$, it is then not important to investigate the main effects of the variety and the puncture position.

In what follows, the impact of the sample size on the confidence intervals of the true population means of the force F_{sk} and the energy W_{sk} is investigated. The analysis is restricted to those berries that were punctured at position A2.

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Determination of sample size

As reported by Dell *et al.*,[31] in a pilot experiment similar to ours, the number of berries to be tested is based on experience and guesswork because no prior data exist to help estimate the correct value.

The calculation of an appropriate sample size relies on a subjective choice of certain factors and the sometimes crude estimates of others, and may, as a result, seem rather artificial. However, it is at worst a well educated guess, and is considered more useful than a completely arbitrary choice. Nonetheless, the value of the sample size needs to be carefully chosen to guarantee a desired degree of confidence on the results and conclusions. Typically, a trade-off exists between the confidence level and the availability of time and resources to study the gathered sample. In this section, guidelines on how to choose an appropriate sample size for the berry skin hardness test are provided.

For each variety, F_{sk} and W_{sk} results of the three minisets, punctured at point A2 and sampled from the three cluster positions were used. In the following, we designate these three minisets as R1, R2 and R3.

In general, three variables must be known or estimated to calculate the sample size: (1) the confidence interval width (representing the maximum difference between the sample mean and the real population mean); (2) the population standard deviation (if not known it is estimated by the sample standard deviation); and (3) the desired confidence level (for example, a 95% confidence level indicates the range in which 95% of results would fall if a study is repeated an infinite number of times, with each repetition including the number of individuals specified by the sample size; the higher the confidence level, the more precise are the observed means). The sample size is calculated using the equation[32,33]

$$n = \left(\frac{t_{n-1,\alpha} s}{L}\right)^2$$

where $t_{n-1,\alpha}$ is the value from the t-distribution with $n-1$ degrees of freedom (Table 7) and $1-\alpha$ confidence level, s is the standard deviation of the sample, L is the width of the full expected confidence interval, and n is the sample size.

The sample means and standard deviations of the three replicates of Cabernet sauvignon, Pinot noir and Nebbiolo corresponding to the break force and energy are reported in Table 8.

For each variety, the difference between the upper and the lower mean value of the three replicates of 20 berries was calculated and the absolute value of this difference was used as the desired confidence interval width of the true population mean for the force and the energy. The width of the confidence interval was varied for both the force (between 0.01 and 0.06 N) and the energy (between 0.01 and 0.06 mJ) to try to understand its influence on the sample size. These intervals were chosen so as to contain the calculated absolute value of the difference between the upper and the lower mean value of the three replicates of 20 berries. In addition to this, the impact of three confidence levels, 90%, 95% and 99%, was explored.

Table 7. $t_{n-1,\alpha}$ value with $n-1 = 59$ degrees of freedom and corresponding to selected significance criteria

Confidence level	$t_{n-1,\alpha}$
90%	1.671
95%	2.001
99%	2.660

Table 8. Sample means and standard deviations of the break force and break energy of Cabernet sauvignon, Pinot noir and Nebbiolo

		F_{sk} (N)	SD	W_{sk}	SD
Cabernet sauvignon	R1 ($n=20$)	0.443	0.063	0.246	0.057
	R2 ($n=20$)	0.411	0.056	0.231	0.064
	R3 ($n=20$)	0.406	0.053	0.219	0.048
	Rmax-min	0.037		0.027	
	Average ($n=60$)	0.421	0.059	0.233	0.057
Pinot noir	R1 ($n=20$)	0.528	0.076	0.306	0.074
	R2 ($n=20$)	0.566	0.050	0.307	0.050
	R3 ($n=20$)	0.562	0.078	0.293	0.079
	Rmax-min	0.038		0.014	
	Average ($n=60$)	0.551	0.070	0.302	0.068
Nebbiolo	R1 ($n=20$)	0.423	0.049	0.252	0.054
	R2 ($n=20$)	0.366	0.084	0.201	0.079
	R3 ($n=20$)	0.407	0.080	0.245	0.083
	Rmax-min	0.057		0.051	
	Average ($n=60$)	0.398	0.075	0.232	0.075

F_{sk} = mean value of the break force; W_{sk} = mean value of the berry skin break energy; SD = standard deviation of the break force; R1 = replicate 1; R2 = replicate 2; R3 = replicate 3; Rmax-min = absolute value of the difference between the maximum mean value and the minimum mean value of the three replicates; Average is referred to the total sample of 60 berries represented by the averaged three replicates.

Figure 4 shows the sample size corresponding to the force and energy as a function of the confidence interval width and the confidence level for Cabernet sauvignon, Pinot noir and Nebbiolo. For a fixed interval width L, the sample size n increases when the confidence level $1-\alpha$ increases, whereas it decreases when L increases and $1-\alpha$ is constant. For instance, as reported in Tables 9 and 10, for a confidence level of 95% and a break force confidence interval width of 0.02 N, the sample size for Cabernet sauvignon is 35, while it is 49 for Pinot noir and Nebbiolo grapes.

By choosing a larger interval width of 0.03 N, the sample size becomes 15 for Cabernet sauvignon, 22 for Pinot noir and 25 for Nebbiolo. The same observation could be made in the case of the break energy. That is, with a confidence level of 95% and a confidence interval width of 0.02 mJ, the sample size is 33 for Cabernet sauvignon, 46 for Pinot noir and 56 for Nebbiolo. On the other hand, with a confidence interval of 0.03 mJ the sample size becomes 14 for Cabernet sauvignon, 21 for Pinot noir and 25 for Nebbiolo. In other words, the lower the

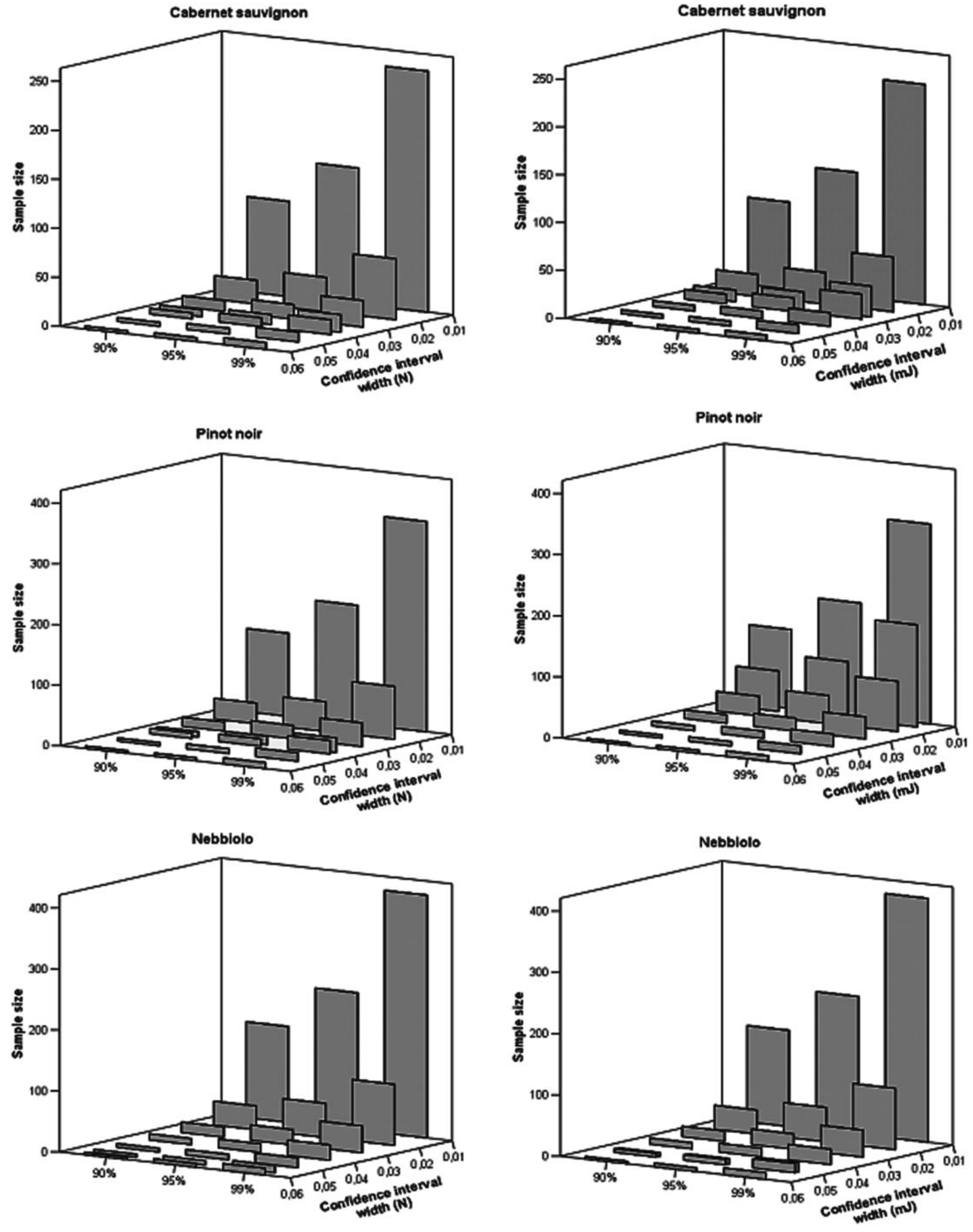

Figure 4. Influence of the confidence interval width (ranging between 0.01 and 0.06 N for the break force and between 0.01 and 0.06 mJ for the break energy) and the confidence level (90%, 95% and 99%) on the sample size for Cabernet sauvignon, Pinot noir and Nebbiolo.

number of analysed berries; the larger the width of the confidence interval of the true force and energy means.

Eng[34] reported that the sample size is important because it affects how precise the observed means are expected to be. In fact, with a confidence interval of 99% the sample means would be closer to the real population mean value, but this would require a larger sample size as indicated by Bourne[11] and verified in our case. A sample that is too large would require excessive time and resources to be analysed. On the other hand, a sample that is too small would not guarantee significance of the statistical results.

By choosing a mean difference of 0.03 N for the break force and 0.03 mJ for the break energy as the confidence interval width, and a confidence level of 95%, a sample size of 15 for Cabernet sauvignon, 22 for Pinot noir and 25 for Nebbiolo are sufficient for the break force confidence interval determination. To determine the break energy confidence interval, sample sizes of 14 for Cabernet sauvignon, 21 for Pinot noir and 25 for Nebbiolo, will be required. Since the difference between the above sample sizes is not large, a sample size ranging between 15 and 25 berries for the berry skin hardness test and for the three varieties can be considered sufficient for a skin hardness test.

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Table 9. Sample size estimation related to the berry skin break force (F_{sk}) at different confidence levels and different margins of error

Grape variety	Sample SD ($n = 60$)	Confidence interval width (L)	Confidence level (%)	Sample size
Cabernet sauvignon	0.059	0.037 (Rmax-min)	90	7
			95	10
			99	18
		0.030	90	11
			95	15
			99	27
		0.020	90	24
			95	35
			99	62
Pinot noir	0.070	0.038 (Rmax-min)	90	9
			95	14
			99	24
		0.030	90	15
			95	22
			99	39
		0.020	90	34
			95	49
			99	87
Nebbiolo	0.075	0.057 (Rmax-min)	90	5
			95	7
			99	12
		0.030	90	17
			95	25
			99	44
		0.020	90	34
			95	49
			99	87

SD = Standard deviation of the break force ($n = 20$); R1 = replicate 1; R2 = replicate 2; R3 = replicate 3

Table 10. Sample size estimation related to the berry skin break energy (W_{sk}) at different confidence levels and different confidence interval widths

Grapevine variety	Sample SD ($n = 60$)	Confidence interval width (L)	Confidence level (%)	Sample size
			90	10
Cabernet sauvignon	0.057	0.030	95	14
			99	26
			90	12
		0.027 (Rmax-min)	95	18
			99	32
			90	23
		0.020	95	33
			99	57
			90	14
Pinot noir	0.068	0.030	95	21
			99	36
			90	32
		0.020	95	46
			99	82
			90	66
		0.014 (Rmax-min)	95	94
			99	167
			90	6
Nebbiolo	0.075	0.051 (Rmax-min)	95	9
			99	15
			90	17
		0.030	95	25
			99	44
			90	39
		0.020	95	56
			99	100

SD = Standard deviation of the break energy ($n = 20$); R1 = replicate 1; R2 = replicate 2; R3 = replicate 3.

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With a higher confidence level (99%) and L equal to 0.03 N for the break force and 0.03 mJ for the break energy, the sample size range becomes 26 to 44 berries.

Browner *et al.*[35] listed different methods for minimising the sample size. One method consisted in expanding the confidence interval. When it is unnecessarily small, a larger one could be justified. For example, an L equal to 0.014 mJ for the determination of the break energy of Pinot noir gives high sample sizes even for a confidence level of 90% (66 berries). It is then more practical to increase the confidence interval width to 0.02 mJ or 0.03 mJ to reduce the sample size. An interval width L equal to 0.057 N for the assessment of the break force of Nebbiolo leads to low samples sizes even with a confidence level of 99% (12 berries); in this case it is better to reduce the confidence interval width to 0.03 N or 0.02 N.

When compared to 0.02, expressed in N or mJ, the confidence interval width of 0.03, also expressed in N or mJ, decreases the sample size. With L equal to 0.02 we note a difference between the sample sizes of Cabernet sauvignon, Pinot noir and Nebbiolo whilst 0.03 allows a generalisation of the sample size to 15–25 for the three grape varieties.

It is also noted that the sample size depends on the factor being studied; it is indeed higher in the case of the break energy than in that of the break force, and this was verified for all three grape varieties. For a fixed confidence level and confidence interval length, the sample size is different between varieties. This suggests that skin hardness may be different from one variety to another.

CONCLUSION

This work was intended to be a guide for performing the puncture test on grapes. The purpose was to define the berry skin hardness through two different parameters: the break force and the break energy.

The effect of the variety, the cluster position and the puncture point and their interactions were taken into consideration. Moreover, the size of the sample analysed could depend on the variety and the confidence interval.

The break energy is better than the break force for understanding the effect of the cluster position.

Further studies on additional grape texture parameters during different harvest seasons would be interesting in future researches.

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