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Influence of grape juice extraction methods on basic analytical parameters

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Summary

Currently, for monitoring the ripening of grape berries, different devices are used to produce the juices to be analysed. Crushing the berries is a key step that determines the quantity of juice extracted and may impact it composition. The effect of different devices on analytical parameters of the musts produced were compared in this study. Samples from four grape varieties ('Cabernet-Sauvignon', 'Ekigaïna', 'Marselan' and 'Vermentino'), showing a variability of berry size and precocity, were crushed using six different devices (ASieves, Bag mixer®, Crusher, Manual, TPress and Blender). Whatever the pressing equipment, sugar concentrations of the must were not modified by the extraction method, unlike other parameters. pH and titratable acidity were slightly impacted by the crushing method without changing the ranking of the varieties. However, potassium concentrations were more impacted by the pressing method. Differences in mechanical forces applied to skins and seeds according to the pressing equipment used may release more or less potassium. This study clearly discarded a complete grinding of the samples for grape ripening monitoring: this method strongly modified the potassium content and, consequently, the pH and the titratable acidity of the musts.

Key words: grapevine; berry; maturation; must.

Introduction

Grape maturation monitoring is frequently included in viticultural research and experimentation studies and the methodology for berry sampling has been addressed in several studies (Huglin and Juillard 1955 and 1959, Carbonneau *et al.* 1991). A sample of 200 berries, taken from different vines, is supposed to give a representative estimate of grape composition (Huglin and Juillard 1959). This technique was improved by harvesting small bunches of 3-5 berries in the middle part of each cane of guyot pruned vines (Carbonneau 1991). This method has the advantage of reducing the "operator" effect on the representativeness of the samples. Once the sample is collected, different methods exist to squeeze the grape berries to produce a sample of juice that will subsequently be analyzed. Juice production should be reproducible and representative of the grape juice obtained by an industrial wine press in cellars. However, few studies compared the influence of different pressing methods on the composition of the juice produced.

Aerny (Aerny *et al.* 2000) showed that results of juice analysis depended on the grape pressing technique used and was related to the rate of juice extraction. When the extraction rate increased from 55 to 85 %, density, pH, sugar content, and potassium concentration of the must increased while the tartaric and malic acid concentrations decreased. However, without any clear explanation, these results were not always reproducible.

In an other experiment, Cayla (Cayla *et al.* 2002) showed, using a crusher for pressing berry samples, that juices had similar pH, titratable acidity and sugar content compared to those obtained in the cellar. However, grinding entire berries induced an overestimation of the pH values of 14% on average compared to crushed grapes and conversely an underestimation of the total acidity values of 16 % on average however, they recommended this method, followed by a maceration phase, to analyze anthocyanin and tannin contents, after the extraction of the phenolic compounds.

According to Amerine and Roessler (Amerine and Roessler 1958), the use of a screw press, grinding the whole fruit, produces grape juice with higher pH and sugar values and lower total acidity by comparison to the use of a press consisting of two wooden rollers used in the cellar.

These results highlight the impact of extraction methods on must composition. Potassium is a major element in the quality of the berry (Rogiers *et al.* 2017) and [K^+] variations have direct effects on the pH and acidity of the grape juice. It is therefore important to quantify the variations in juice composition according to the pressing methods used for maturity monitoring. The purpose of this study was to compare the impact of different pressing methods on the composition of the musts, as well as their reproducibility. Moreover, the choice of the method should take into account additional experimental requirements such as : i) recovering the seeds for a complementary study, ii) recovering the complete sample and iii) estimating the extraction rate.

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Material and Methods

Five commonly used pressing methods, as well as the whole grinding of the samples, were compared in this study (Tab. 1 and Fig. 4):

Tamis Automatic, C80 Robot Coupe® (ASieves): the berries fall inside a sieve where two rubber blades rotating at 1,500 rpm produce centrifugation and extract the juice. The juice is automatically separated from the waste constituted by skins, seed and stalks. This device recovers almost all the juice present in the initial berry sample in less than a minute. There is no need to clean the machine between samples.

Bag mixer® from Interscience (BMixer): Two blades squish the sample in a plastic bag. In less than one minute, the grapes are crushed, the integrity of the sample is preserved and the juice is taken directly from the bag. No cleaning is necessary between each sample because the bag is changed between samples.

MiniFouloir Oeno System by Transtherm Technologies (Crusher): This device was developed by the French Institute of Vine and Wine (IFV) specifically to crush grape samples and to reproduce the conditions of pressing in real cellars. It is designed as a crusher. Two rubber rollers are manually operated and crush berries. The juice flows by gravity, together with some portion of skins and seeds. Remaining parts are removed from the rollers and integrated into the sample. Between each sample, the hopper and the two rollers are wiped with paper towels to prevent pollution of the next sample. The average processing time of a sample is 2 min.

Tomato press (TPress): This device is used to produce tomato paste without skins and seeds. Berries are crushed against a sieve using a metal wheel with a retractable finger. The juice obtained is free from skins and seeds. This method requires cleaning and wiping the parts of the device between each sample to ensure the next sample is not contaminated by the previous one. The average processing time of a sample is 3 min.

Manual method (Manual): berries are crushed in a sieve with a pestle. This method produces a clear juice that can be analyzed directly. However, it requires time to properly grind the berries and especially to clean the utensils. For few samples, this simple method is very easy to implement. The average processing time for a sample is 3 min, without considering cleaning time.

Blender, by Laboratory Blender (Blender): This sixth method consists in grinding a whole sample of grape berries with a blender. This laboratory equipment is not used for maturation monitoring because it is too far from the conditions of wine pressing (CAYLA *et al.* 2002). Indeed, all the constitutive elements of the berries (skins and seeds) are crushed and are thus analyzed.

All these methods, except the Blender, are currently in use in different laboratories. The Blender was integrated into this study to provide an extreme method, which was not integrated in statistical analyses.

Berry samples were collected in a vineyard located at Gruissan (France) on the INRA Domain of Pech Rouge (43°08'36.2"N3°08'05.9"). Four varieties were chosen according to the berry size and maturity parameters observed in 2016, in order to cover a range of possible features: 'Marselan', small black berries and early maturity, 'Vermentino', large white berries and early maturity, 'Cabernet-Sauvignon', small black berries and late maturity and 'Ekigaïna', large black berries and late maturity.

Bunches were harvested on the same day for all the varieties. The following day, after manual destemming, grapes of the four varieties were divided into 18 homogeneous samples of approximately 300 mL (3 replicates for the 6 modalities tested for each of the four varieties). The batches consisted of 103 to 323 berries with weights ranging from 184 to 395 g.

Measurements and analyses: For each sample, berries were counted and weighed. The 18 samples were then pressed with one of the six devices. For three equipments (ASieves, TPress and Manual), the juice produced was weighed in order to estimate the extraction rate. For the other equipments, the juice volume was not measured because i) it was mixed with skins and seeds, or ii) the amount of juice harvested was partial. The extraction rate was calculated as the ratio of the weight of juice produced to the weight of the initial sample.

40 mL of juice produced were collected and centrifuged at 3214 g for 5 minutes at 20 °C. The total soluble solids (TSS) was measured using a refractometer (Digital refractometer 0-32 °Brix Euromex ref. 5532). The titratable acidity (TA in eq. $g \cdot L^{-1}$ of H_2SO_4) and the pH were measured on a Crison automatic titrator (TitroMatic CRISON ref. 12203, CRISON pH probe type 50 11 T). The concentration of potassium [K^+] was determined by high performance liquid chromatography (HPLC). A HPLC Waters system (Waters

Table 1

Principal technical characteristics of the methods used for grape juice extraction

Pressing method	Sample processing time	Cleaning between two samples	Sample size	Pressing rate	Manufacturer, approximate cost
ASieves	< 1 mn	not applicable	no limit	yes	Robot Coupe, < 3000 €
BMixer	< 1 mn	not necessary	400 mL bag	no	Interscience, < 2000 €
Blender	< 1 mn	not applicable	limited to the bowl	purposeless	Conair Waring, < 1000 €
Crusher	2 mn	necessary	limited to the bowl	no	Prototype IFV, < 2000 €
Manual	3 mn	necessary	limited to the bowl	yes	-, < 100 €
TPress	3 mn	necessary	limited to the bowl	yes	OMCAN Society, < 100 €

Corporation, Milford, MA, USA) was used, equipped with a Waters 717 autosampler, a Waters 600 HPLC pump, and a Shimadzu CDD-10A conductivity detector (Shimadzu, Japan). The chromatographic separations were performed on a Waters IC-Pak cation M/D column (150 x 3.9 mm) maintained at ambient temperature. The mobile phase consisted of 0.004 N solution of nitric acid at a flow rate of 1 mL·min^{-1}, using isiocratic elution. The sample volume injected was 10 µL. Empower Pro 3 software (Waters Corp., Milford, MA) was used to control the HPLC system.

Statistical analyses were performed using the R version R 3.3.3 software (© R Foundation for Statistical Computing 2017).

In order to determine the statistical significance of the effects of the grape variety and of the pressing method on the composition of the grape juices, two-way analyses of variance (ANOVA) were used. The Tukey's honestly significant difference test (HSD) was used to identify differences significant at $P = 0.05$. The coefficient of variation was calculated as the ratio between the residual standard error extracted from the ANOVA to the overall mean of the studied variable.

One-way analyses of variance were performed for each method separately in order to compare their ability to classify the varieties.

Results

The variations in berry size and maturity between the four grape varieties were in line with the expected rankings. All the parameters measured on the juices were significantly different between varieties at $P = 0.001$ (Tab. 2).

The mean berry weights for 'Marselan' and 'Cabernet-Sauvignon' were half the mean berry weights for 'Ekigaïna' and 'Vermentino' (Fig. 1a). Sugar accumulation varied from 20.3 °Brix ('Ekigaina'), to 24.37 °Brix ('Marselan') *i.e.* a 4.3 °Brix difference (Fig. 1b). 'Ekigaina' had the lowest level of maturity of the four varieties with a titratable acidity higher by 3.1 eq. g·L^{-1} H_2SO_4 and pH lower of 0.27 units, when compared to Vermentino and Marselan (Fig. 1d and e). The average juice yields obtained with three pressing methods varied according to the variety: 67 % for 'Cabernet Sauvignon' and 'Marselan', while it was 72 % and 74 % respectively for 'Vermentino' and 'Ekigaina', which had larger berries. (Fig. 1c). Extraction rates were significantly different for the TPress compared to the Manual and ASieves modalities, with 63 %, 71 % and 76 % respectively (Fig. 2e).

There was no significant effect of the pressing method on TSS. (Fig. 2a). but pressing equipment had a significant effect ($P < 0.001$) on all the other parameters measured (Tab. 2). For pH, three significantly different groups were

Table 2

Results of analyses of variance for the parameters measured

	Df	F-Value	*p*-value	Significance[1]
Extraction rate				
Equipment	2	35.879	1.68E-07	***
Cultivar	3	8.29	8.225 E -04	***
Equipment x Cultivar interaction	6	1.342	1.36E-05	n.s.
TSS				
Cultivar	3	1406.515	< 2.2E-16	***
Equipment	4	1.1545	0.3452	n.s.
Equipment x Cultivar interaction	12	1.53	0.1537	n.s.
pH				
Cultivar	3	172.227	< 2.2E-16	***
Equipment	4	20.16	3.71E-09	***
Equipment x Cultivar interaction	12	0.9508	0.509	n.s.
Titratable acidity				
Cultivar	3	927.972	< 2.2E-16	***
Equipment	4	16.782	3.76E-08	***
Equipment x Cultivar interaction	12	1.422	0.1964	n.s.
$[K^+]$				
Cultivar	3	133.7143	< 2.2E-16	***
Equipment	4	65.4319	< 2.2E-16	***
Equipment x Cultivar interaction	12	5.6928	1.36E-05	***
pH				
Cultivar	3	196.42	< 2.2E-16	***
K	1	74.08	7.783E-10	***
Equipment	4	5.60	1.56E-03	**
K x Equipment interaction	4	3.05	3.09E-02	*
Cultivar x K interaction	3	0.44	7.23E-01	n.s.
Cultivar x Equipment interaction	12	0.72	7.26E-01	n.s.

[1] n.s. not significant, ** significant at $p < 0.01$, *** significant at $p < 0.001$.

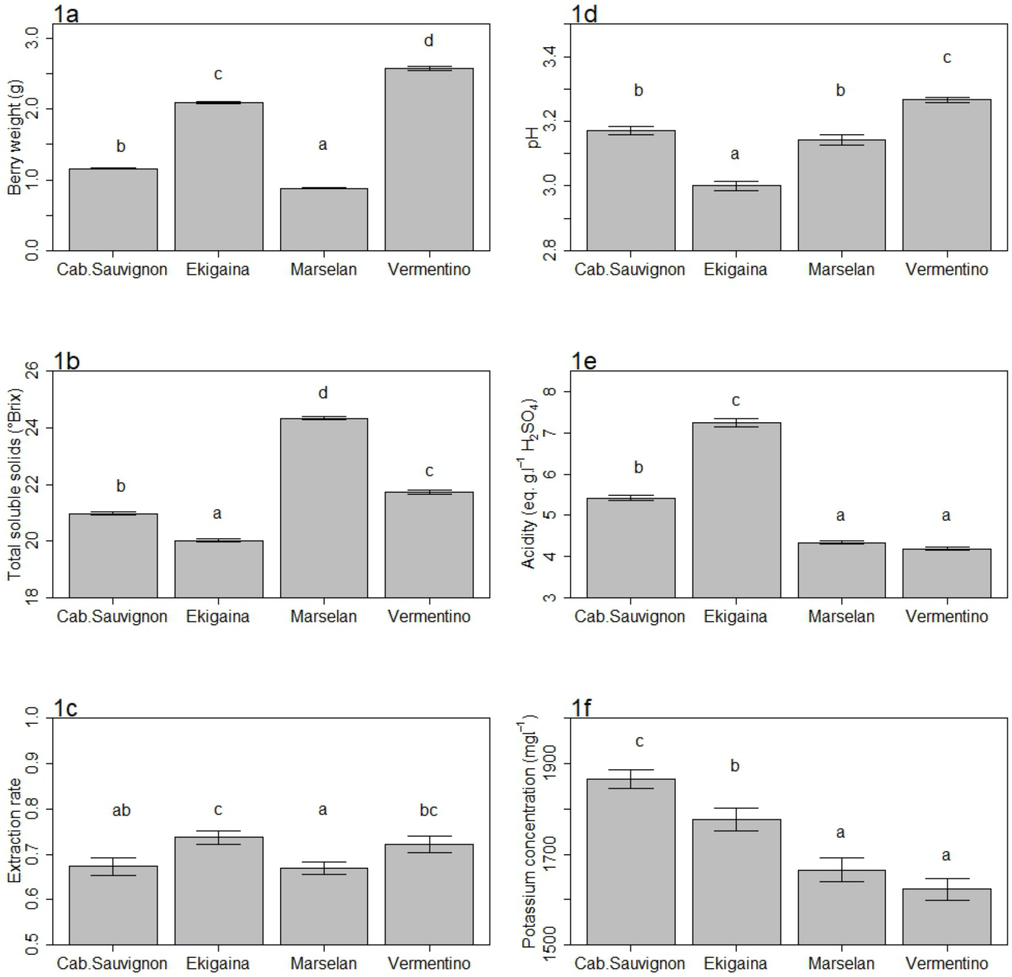

Fig. 1: Variability between varieties for **a**) berry weight (g), **b**) total soluble solids (°Brix), **c**) extraction rate, **d**) pH, **e**) titratable acidity (eq·g·L^{-1} H_2SO_4), and **f**) potassium concentration (mg·L^{-1}). Bars represent standard errors. Varieties with the same letter were not different at $p = 0.05$ according to Tukey's HSD test.

distinguished with the Tukey range test (Fig. 2b). The lowest pH (pH = 3.08) was measured with the Crusher. An intermediate group, significantly different, included the BMixer and Manual, followed by a third group consisting of ASieves and TPress for which the pH was the highest (pH = 3.19). The pH obtained using the Blender differed from the other modalities with values higher by 0.35 pH unit (11 % higher).

The mean TA values for each pressing method varied from 5.06 eq. g·L^{-1} of H_2SO_4 for ASieves to 5.57 eq. g·l^{-1} of H_2SO_4 for the BMixer. According to the Tukey's test, the TA obtained with the ASieves, the Manual and the TPress were significantly lower than those obtained with the Crusher and the BMixer (Fig. 2c). As for pH, the Blender differed from other materials with TA values on average 6 % lower.

For pH and TA, the varietal x equipment interaction was not significant: the comparisons between pressing method were valid regardless of the grape variety considered.

This was not the case for [K^+], which varied significantly with grape varieties and equipment, but with a significant interaction ($P < 0.001$) between these two factors. The range of values recorded between 'Cabernet-Sauvignon' and 'Vermentino' grapes of 242 mg·L^{-1} was the same as that observed between the Crusher and TPress. Potassium concentrations obtained with these two pressing methods were significantly different, $P < 0.001$ according to the Tukey's test, from the Manual, the BMixer and the ASieves (Fig. 2d). Even more marked than for the pH, the Blender standed out very clearly with an average $[K]^+$ of 2,672 mg·L^{-1} compared to an average of 1,733 mg·L^{-1} for the five equipments tested, *i.e.* a 58 % increase.

Discussion

The calculated values of the extraction rates, between 0.63 and 0.76, were in the range of rates observed in cellars. This rate increased slightly for large-berry varieties, which can be explained by a higher proportion of pulp in the berry. For the TPress, a fraction of the juice is lost and immobilized in the mechanism, this indirectly induced a lower extraction yield.

Being able to estimate extraction rates is important, but is not always directly accessible with all pressing methods. For some devices indeed, such as the BMixer, for which the sample is crushed in a bag, this measure is not possible. For the other materials, such as the TPress or the Crusher, it is not possible to calculate extraction rates because all the juice cannot be recovered from the initial sample.

TSS was a very stable parameter that was not influenced by the type of equipment tested. The residual error was very

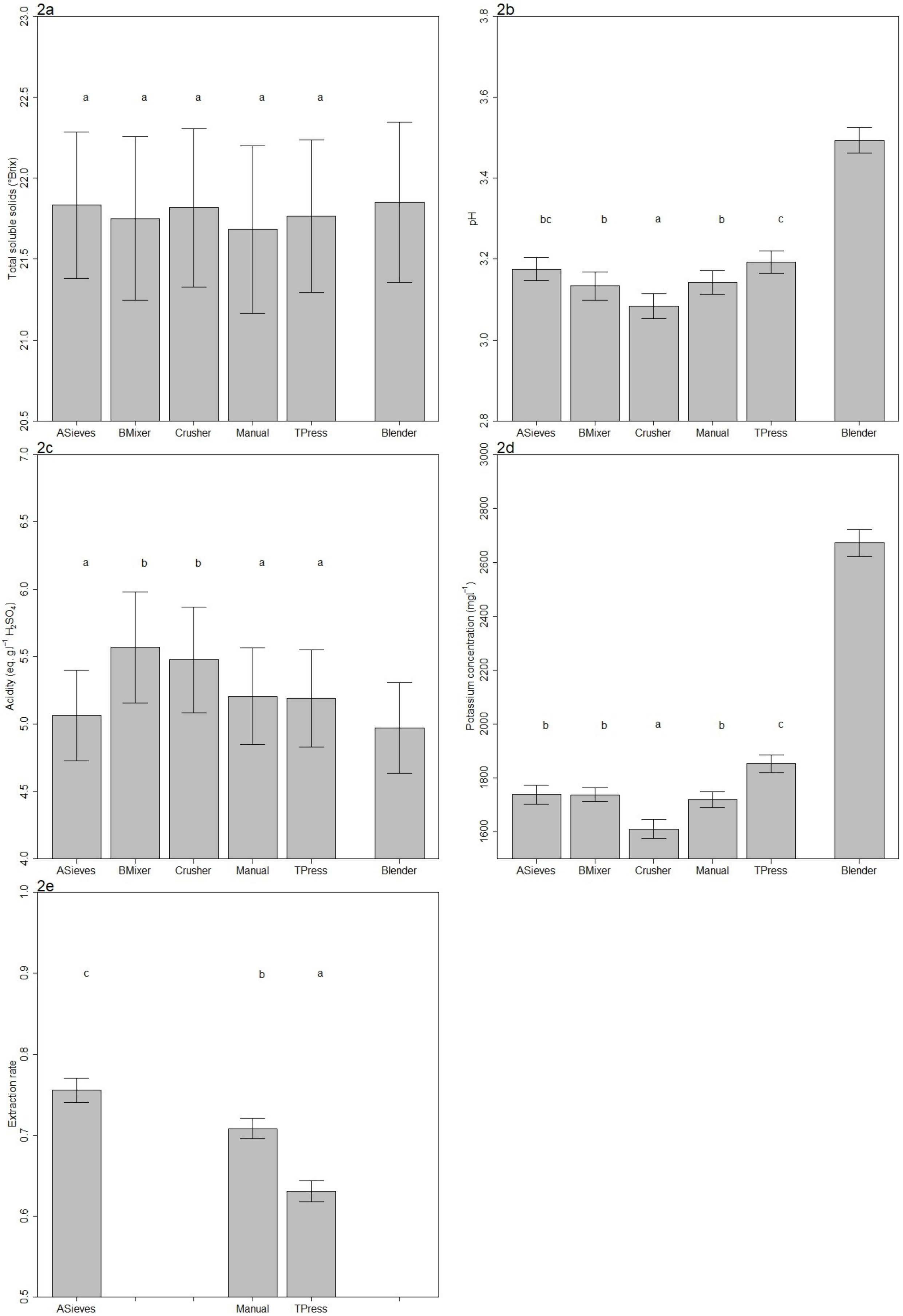

Fig. 2: Variability between crushing materials observed for **a**) total soluble solids (°Brix), **b**) pH, **c**) titratable acidity (eq·g·L^{-1} H_2SO_4), **d**) potassium concentration (mg·L^{-1}) and **e**) juice extraction rate. Bars represent standard errors. Methods with the same letter were not different at p = 0.05 according to Tukey's HSD test.

Table 3

Coefficient of variation (%) for each material, calculated as the ratio between the residual standard error (one-way analysis of variance with "variety" as tested effect) and the mean

	TSS	pH	TA	[K+]	Extraction rate
Crusher	0.84	1.11	4.15	2.44	-
Bmixer	0.99	1.47	3.67	1.53	-
Manual	1.30	0.77	3.25	2.17	2.25
Tpress	0.65	0.93	3.17	2.24	4.48
Asieves	0.26	0.72	2.16	2.15	6.72
Blender	0.65	0.61	2.24	1.99	-

small, whatever the material used, as illustrated by the low coefficient of variation, around one percent (Tab. 3). The variety ranking is identical regardless of the material used. However, the results of the one-way analyses of variance carried out for each method separately show (results not shown) that the Manual and the Crusher were slightly less discriminating, with three groups of grape varieties according to the Tukey test ($P < 0.05$), whereas for the three other materials (ASieves, BMixer and TPress) all the four grape varieties were significantly different according to the same test. These minor differences are to be put into perspective and do not call into question the great stability of the TSS whatever the pressing method used.

Although significant differences among methods were detected for pH and TA, variety ranking was identical for each method. Analysis of the varietal effect allowed to differentiate three significantly different groups. Thus, pressing methods marginally affected the mean level of the measured values but did not modify the interpretation of the results. In contrast, when the whole sample was crushed with the Blender, the pH was strongly impacted. The precisions of the varietal effect were around one percent for pH, whatever the material used. For TA, they varied from 2 to 4% between ASieves and Crusher (Tab. 3).

The results were different with [K^+], which was the parameter the most affected by the type of pressing method used, as shown by the high F-value of the parameter "equipment" in the ANOVA (Tab. 2). Two pressing methods stood out: Crusher systematically showed the lowest levels of [K^+] as opposed to the TPress for which the levels were the highest. These differences can be related to the differences in operation of these two apparatus. The Crusher, with its rubber rollers, was presumably the equipment that imposed the lowest mechanical constraints on berries during pressing. Thus, at the end of pressing, the pulp is still present either around the seeds or attached to the skins. This device requires a high level of berry maturity to be efficient. On the other hand, the TPress, because of its metal mechanism, induced more constraints: some seeds were broken and the skins strongly damaged. The other three materials provided intermediate values.

Potassium is present in the berry at decreasing concentrations among skin, seed and pulp (Possner and Kliewer 1985, Mpelasoka *et al.* 2003). Hence, it can be assumed that some of the potassium contained in the skins and seeds is more or less released according to the pressing process used.

The significant interaction ($P < 0.001$) between the grape variety and the device on [K^+] that, unlike the other parameters, the ranking of the varieties depended on the device used (Fig. 3): Vermentino did not systematically have the lowest [K^+]. In addition, Crusher and TPress discriminated two groups of significantly different grape varieties according to the Tukey test ($P < 0.05$) while three groups were identified with the three other methods. Although the coefficient of variation, calculated from the 1-factor (grape variety) model residues was lower than 2.5% (Tab. 3), ranking differences between the methods were observed.

At the extreme of the tested devices, the Blender, by grinding all the sample, released a lot of potassium in the juices. In this case, the levels of potassium measured were out of all proportion to those measured with the other tested devices (+ 58 %). The [K^+] of the must strongly influences the pH (Rogiers *el al.* 2017). Potassium partly neutralizes the organic acids and increases the pH. These results are consistent with those of Cayla (Cayla *et al.* 2002): released potassium leads to a significant increase in pH and a significant decrease in TA. Thus, all varieties included, the classification of materials used for the pH is identical to that observed for [K^+] (Fig. 2b and d). When incorporating potassium, grape variety and equipment in a

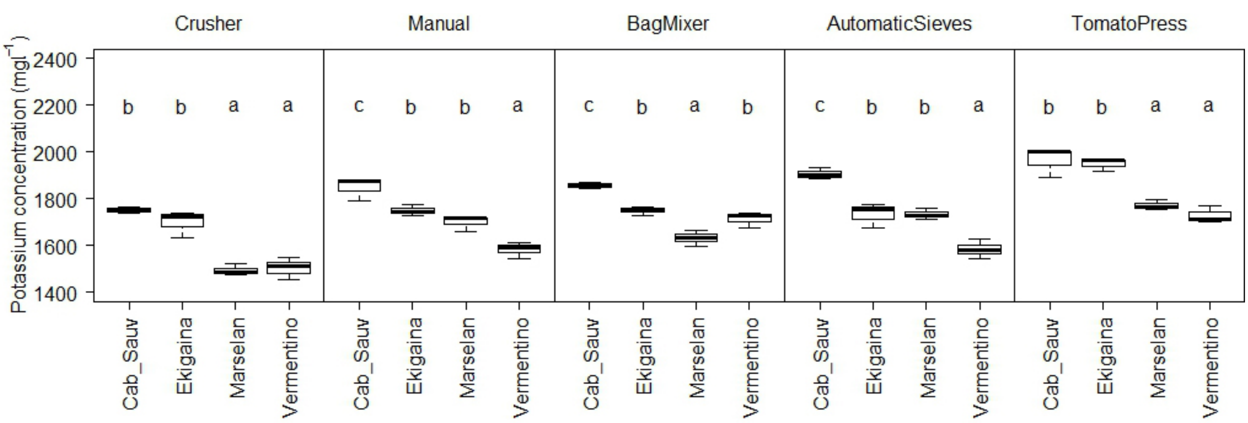

Fig. 3: Genetic variability for Potassium concentration (mg·L^{-1}) (n = 3) according to the materials use. Significance of differences were tested with Tukey HSD test ($P < 0.05$).

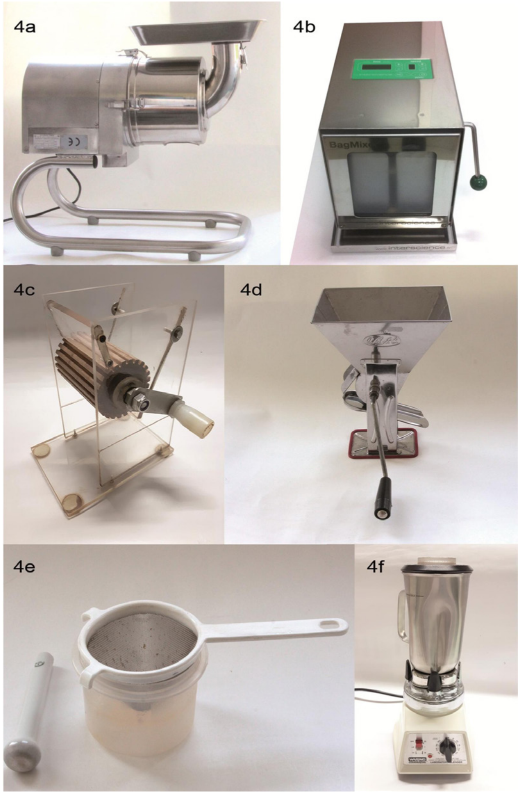

Fig. 4: Illustration of the different materials tested for grape juice extraction: **a**) ASieves, **b**) BMixer, **c**) Crusher, **d**) TPress, **e**) Manual and **f**) Blender.

covariance analysis model to explain pH, variety and [K^+] have significant effects ($P < 0.001$), followed by pressing equipment ($P < 0.01$) (Tab. 2). Nevertheless, an interaction ($P < 0.05$) between potassium and material is significant. These results are important and clearly discard the use of a Blender to extract grape juice for grape maturity monitoring. Also, knowing its use in the past, this requires being vigilant in the analysis of berry composition over long periods or for the comparison of different series, when this method of extraction is likely to have been used.

Conclusion

This study aimed at comparing different types of equipment currently used to press grape berries during the monitoring of the ripening process. The best method should produce a juice as representative as possible of winemaking practices, in a reproducible way. Although the techniques tested were very different, the main conclusion is that their effects on basic parameters such as TSS, pH and TA were low and did not provide different rankings of the varieties, without any bias due to berry size or maturity level. The methods compared had similar accuracy and were reproducible. Only [K^+] proved more sensitive to the pressing technique: if the pressing has strong mechanical constraints, the partial crushing of seeds and skins can lead to an overestimation of [K^+].

The best compromise is the method that does not extract potassium present in the skins and seeds, while maximising the extraction rate. We recommend to discard any method that crushes the berries entirely. Three of the tested equipments (Manual, BMixer and ASieves) fully fulfilled the requested specifications whereas the Crusher slightly minimized [K^+] and conversely the TPress slightly overestimated them compared to the other methods.

References

Aerny, J.; Dupraz, P.; Brückner, D.; 2000: Influence du mode d'extraction du moût sur sa composition lors des contrôles de maturité du raisin. Rev. Suisse Vitic. Arboric. **32**, 239-243.

Amerine, M. A.; Roessler, E. B.; 1958: Filed testing of grape maturity. Hilgardia **28**, 93-114.

Carbonneau, A.; Mouex, A.; Leclair, N.; Renoux, J. L.; 1991: Proposition d'une méthode de prélèvement de raisins à partir de l'analyse de l'hétérogénéité de maturation sur un cep. Bull. OIV 679-690.

Cayla, L.; Cottereau, P.; Renard, R.; 2002: Estimation de la maturité phénolique des raisins rouges par la méthode I.T.V. standard. Rev. Française Oenol. **193**, 10-16.

Huglin, P.; Julliard, B.; 1955: Etude sur la morphologie, la phénologie et la productivité des principaux cépages de *V. vinifera* L. cultivés en Alsace. Ann. Amélior. Plantes, INRA **I**, 5.

Huglin, P.; Julliard, B.; 1959: Le contrôle de maturation du raisin par prélèvement de baies. Progrès Agric. Vitic. **76**, 37-41

Mpelasoka, B. S.; Schachtman, D. P.; Treeby, M. T.; Thomas, M.; 2003: A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape Wine Res. 9, 154-168.

Possner, D. R. E.; Kliewer, W. M.; 1985: The localisation of acids, sugars, potassium and calcium in developing grape berries. Vitis **24**, 229-240.

Rogiers, S. Y.; Coetzee, Z. A.; Walker, R. R.; Deloire A.; Tyerman, S. D.; 2017: Potassium in the grape (*Vitis vinifera* L.) berry: transport and function. Front. Plant Sci. **8**, 1629.

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