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The Vineland Grape Flavor Index — a new objective method for the accelerated screening of grape seedlings on the basis of flavor character

by

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Le «Vineland Grape Flavor Index» — une nouvelle méthode objective pour le triage précoce de plants de vigne basée sur l'arôme

Sommaire. — Nous avons mesuré les esters volatiles totaux et l'anthranylate de méthyle dans le jus de raisins de certains cépages sélectionnés et de jeunes plants issus de croisement. Les cépages étaient soit des hybrides nouveaux provenant de Vineland, de Geneva et de France, soit des hybrides nord-américains, soit des *Vitis vinifera* L. Les résultats ont montré que les raisins possédant l'arôme «foxé» typique des labruscas contiennent un taux élevé de l'un ou des deux composés chimiques. De plus, faites sur les premiers raisins issus d'un croisement, ces analyses permettent de déceler les variétés foxées. Nous proposons l'indice suivant: Vineland Grape Flavor Index (VGFI) = anthranylate de méthyle (ppm) × 100 + esters volatiles totaux (ppm). Toute valeur supérieure à 14 est généralement associée à un arôme «foxé» pouvant être perçu par olfaction. A part son objectivité, la méthode permet d'économiser temps et travail.

Introduction

Grape breeding, using interspecific hybridization, started at the Horticultural Research Institute of Ontario (H.R.I.O.) in 1913. A major objective is the combination of favorable viticultural characteristics (e.g. cold hardiness, disease resistance, vigor) of the North American *Vitis* species with the desirable flavor characteristics of the best *Vitis vinifera* L. cultivars. The flavor quality of the wine produced from new selections has become particularly important with the recent shift in consumer preference from dessert to table wines.

Many native North American cultivars are well suited to the environmental conditions of the Niagara peninsula. Cultivars with *V. labrusca* L. parentage were used extensively in the Institute's breeding program in the early years. A high proportion of the seedlings from such crosses have labrusca flavor character. As table wines become more important in the Canadian market, early elimination of seedlings with labrusca flavor character became highly desirable. Since it takes at least 6 years from the original cross to attain a level of productivity where sufficient quantity of fruit is available for microvinification, testing the flavor potential of seedlings through sensory analysis of the wine is a lengthy process. An objective test for screening of grape seedlings for labrusca flavor character would be a distinct advantage. Such screening would allow the grape breeder to discard those seedlings which would produce wine with labrusca flavor character as soon as the seedlings fruited. The screening method would have to fulfill the following criteria:

1. Reliable, with good correlation between the objective tests with the grapes and sensory evaluation for labrusca flavor character of the wine.

2. Requiring small sample size to permit early screening of grape seedlings.
3. Simple and rapid methodology to allow the screening of large numbers of samples.

Some sensory qualities of wine can be predicted from the chemical composition of grapes. Acid and tannin content of grapes will give an indication of the tartness and astringency of a wine. The total anthocyanin content affects color and color stability of the wine. However, it is not feasible to measure labrusca flavor character directly because the compounds responsible for it are not known. The flavor composition of North American grape cultivars has been extensively studied (9, 10, 11, 14). Large numbers of volatile flavor compounds were identified but none was responsible for the "foxy" or labrusca flavor of these wines.

During a survey of the Institute's grape cultivar collection for methyl anthranilate (MA) and total volatile esters (TVE), it was noticed that those with labrusca flavor character were high in one or both of these flavor components. Cultivars free of labrusca flavor contained either zero or only traces of MA and TVE. These results suggested that MA and TVE content could be used as measures of labrusca flavor character. The development of a new objective flavor index based on MA and TVE analyses of grapes is described in this paper.

Materials and methods

With the exception of *V. rotundifolia* MICH., grape samples were obtained from the Grape Research Station at the H.R.I.O. Analysed grape cultivars, selections and seedlings were classified on the basis of origin into the following seven groups:

1. Vinifera: Cultivars and selections belonging to the species *V. vinifera* L. or European grape.
2. Other species: Cultivars and selections which belong to a pure species other than *V. vinifera*.
3. French hybrid: Cultivars and selections from France arising from crosses of *V. vinifera* with certain wild American species or North American cultivars. *V. labrusca* L. the "fox" grape played a minor role in the development of these cultivars (7).
4. Vineland hybrid: Cultivars, selections and seedlings developed at the H.R.I.O., Vineland Station, Ontario. A French hybrid or a pure vinifera cultivar was used as one of the parents in many cases (1, 2).
5. Geneva hybrid: Cultivars and selections from the grape breeding program of the New York State Agricultural Experiment Station, Geneva, New York. Their newer introductions carry a high percentage of vinifera (15).
6. North American cultivar: Cultivars developed by private grape breeders in the U.S.A. and Canada prior to 1908, the date of publication of HEDRICK's monograph, "The grapes of New York" (8). Possibly none of them are pure *V. labrusca* L. but rather interspecific hybrids.
7. Miscellaneous hybrid: Interspecific hybrids originating from North America and Europe which do not fit into any of the above hybrid groups.

Sample preparation

It is more convenient to carry out MA and TVE analyses on juice rather than on whole grapes. The juicing process gives homogeneous subsamples by eliminating the variation found among berries. Also, the juice can be stored in a freezer at higher temperatures and for longer periods of time than whole grapes without any loss of MA or TVE. Because skin is richer in MA and TVE than flesh, the method of juice extraction

will greatly influence the final results. Since we were interested in the total MA and TVE content of grapes, the recommendation of CLORE *et al.* (4) was followed using whole grapes rather than juice.

A 5 kg sample was harvested at maturity from each cultivar. Grapes were washed, removed from stems and frozen at −40 °C. Analyses for MA and TVE were carried out soon after freezing.

To obtain representative subsamples for analyses, frozen grape samples were ground without breaking the seeds in a Model F juice extractor (Chisholm-Ryder Co. Inc., Niagara Falls, N.Y.) using the No. 5 Monell alloy screen assembly with 5 mm diameter holes and thoroughly mixed. The extractor was kept in a cold room (2 °C) to prevent thawing. The ground, frozen material was thoroughly mixed and two 50 g aliquots were removed for steam distillation.

Steam distillation

Samples were placed in an all-glass steam distillation apparatus (Cat. No. JD-2115, SGA Sci. Bloomfield, N.J.). 100 ml distillate was collected in 30 min. Where no MA could be detected at this dilution, the distillation was repeated on duplicate 50 g samples collecting 50 ml in 20 min. The distillate was used to determine both the MA and TVE. Glass-distilled water was steam distilled under the same conditions as the grapes, to produce blanks for the MA and TVE analyses.

Determination of MA

A highly sensitive fluorometric method was used (3). The fluorometer reading was related to the MA concentration by using a standard curve. To eliminate interference from fluorescent volatile substances other than MA and ethyl anthranilate, the reading taken on a vinifera cultivar corresponding to few ppb MA was subtracted from each measurement.

Determination of TVE

A modification of THOMPSON's method (17) was used. The reaction of esters with hydroxylamine in aqueous alkaline solution forms hydroxamic acids, which react with the ferric ion to form red ferric hydroxamate complexes.

Fresh alkaline hydroxylamine solution was prepared by mixing equal volumes of 6 M hydroxylamine hydrochloride and 10.5 N sodium hydroxide. 2 ml of this solution and 20 ml of the distillate were added to a 25 ml volumetric flask. These were mixed thoroughly and allowed to stand for 5 min. Then 1 ml of conc. hydrochloric acid was mixed in, followed by 1 ml of 1.11 M ferric chloride. The flask was filled to volume with 0.046 M ferric chloride solution and the color was measured on a Zeiss DMR 21 spectrophotometer (C. Zeiss, Oberkochen, Germany), at 540 nm. A standard curve, prepared by using various concentrations of ethyl acetate, was used to determine the concentration of TVE in the sample.

Sensory evaluation

In 1975 and 1976 wine was produced from a number of surveyed grape cultivars and selections. Young wines were evaluated by 5 or 6 members of the Expert Wine Taste Panel of the H.R.I.O. for the presence of labrusca flavor character. The number of samples presented at a session never exceeded 10. A cultivar was classified as

"labrusca" when more than 20 % of the tasters found the wine to have labrusca flavor character.

Fresh grapes brought into the laboratory for MA and TVE analyses from 1975 to 1980 were tasted by the author. The strength of labrusca flavor was scored on a 0 to 4 scale. Typical labrusca type cultivars with strong flavor such as Concord and Niagara were rated as 4 for labrusca flavor intensity.

Other flavor components present in grapes and wine can mask mild labrusca flavor. Di- and tri-terpenes are responsible for muscat aroma (5, 13, 16, 18). The MA and TVE analyses are not affected by terpenes but their strong aroma could confuse the taster. To avoid this problem results of grape and wine tastings on cultivars with muscat flavor character were not included in the comparison between objective and sensory evaluation.

Results and discussion

The MA and TVE content of a great many grape cultivars, selections and seedlings (cultivars in the following) has been determined in this laboratory. When the distribution of MA and TVE content for the seven groups of grape cultivars was tabulated, it was found that low MA and TVE content were characteristic of those groups where the cultivars are free of labrusca flavor (Vinifera and French hybrid). This suggested that either of these measurements could be used as an indicator of labrusca flavor character. However, a number of the North American cultivars, most of which have labrusca flavor character were also low in MA or TVE. To create a better index for labrusca flavor character than either MA or TVE content alone, the two measurements were combined as follows:

$$\text{Vineland Grape Flavor Index (VGFI)} = \text{MA (ppm)} \times 100 + \text{TVE (ppm)}$$

The distribution of VGFI for the seven groups of grape cultivars is presented in Table 1. It shows that all the Vinifera and French hybrid cultivars but only 2 of the

Table 1

Distribution of grape cultivars based on their Vineland Grape Flavor Index

Distribution des cultivars sur la base du «Vineland Grape Flavor Index»

Cultivar group	Vineland Grape Flavor Index					
	0—7	8—14	15—25	26—50	51—100	>100
Vinifera	22	—	—	—	—	—
Other species	2	—	1	—	1	1
French hybrid	52	—	—	—	—	—
Vineland hybrid	351	44	40	53	49	44
Geneva hybrid	45	5	3	4	4	11
North American	2	—	3	4	5	4
Miscellaneous hybrid	16	—	1	—	6	3
Total	490	49	48	61	65	63

Table 2

Relationship between Vineland Grape Flavor Index (VGFI) and labrusca flavor intensity of grapes

Rapport entre le «Vineland Grape Flavor Index» et l'intensité de l'arôme labrusca dans les raisins

Cultivar group	0—14 VGFI					> 14 VGFI					Discrimination[2])
	Labrusca flavour strength[1])										
	0[3])	1	2	3	4	0	1[3])	2[3])	3[3])	4[3])	%
Vineland hybrid	132	4	1	—	—	5	18	12	2	—	94.3
Geneva hybrid	19	1	—	—	—	1	1	2	—	—	91.7
Miscellaneous hybrid	13	—	—	—	—	1	1	1	5	2	95.7
Total	164	5	1	—	—	7	20	15	7	2	94.1

[1]) The strength of labrusca flavor was scored on a 0 to 4 scale where 0 denotes the absence of this flavor character.
[2]) Proportion of grape selections where objective and sensory data are in agreement.
[3]) Objective and sensory data are in agreement.

18 North American cultivars examined had low VGFI. The 2 North American cultivars were Dutchess and Lomanto which are also free of labrusca flavor character.

Since VGFI is intended primarily as a screening tool for grape breeders, it was necessary to establish a value, above which the probability of having labrusca flavor character becomes sufficiently large to warrant rejection of the seedling. Viniferas and all examined French hybrids were free of labrusca flavor character. Their VGFI was below 8 (Table 1). Therefore, this value could serve as a limit value for the plant breeder. However, such a low value would result in the rejection of an appreciable number of seedlings which could be free of labrusca flavor character. A listing of VGFI values for established grape cultivars revealed that the limit value could be set as high as 14 VGFI, without an appreciable number of seedlings with labrusca flavor character being retained. Accordingly, a VGFI of 14 was suggested as a limit value above which the chance of a grape seedling having labrusca flavor character is sufficiently great to warrant rejection. This was then tested against sensory data.

Table 2 illustrates relationship between VGFI and labrusca flavor intensity of the grape. The table does not include data on any cultivars where the names might have prejudiced the taster. In most cases, sensory data were available for more than 1 year. Ideally, grapes with VGFI or 14 or less should be free of labrusca flavor. In the majority of cases, there was agreement between VGFI and sensory evaluation.

The VGFI value of the grapes was compared to the results of tastings for labrusca flavor character in the wine (Table 3). Since wines were presented to tasters under numercial code, data for all cultivars tasted and for which VGFI values were available were included in Table 3. Most cultivars were tasted in both years. Results showed that there was good agreement between VGFI and sensory evaluation (86.8 %).

A comparison of the data in Table 2 with that in Table 3 shows that grape tasting gave better agreement between VGFI and sensory data than wine tasting. Limiting the comparison to only those cultivars where both the wine and the grape were tasted still gave better results with the grape tasting. The flavor of wine is more complex than that of grapes which makes wine tasting a more difficult task. Faulty fermentation and

spoilage can produce off-flavors which, at low levels, could be mistaken for labrusca flavor by some tasters. These factors might explain the better agreement between objective and sensory data obtained with grapes.

The discrepancies between VGFI and sensory evaluation results fell into two categories:

I. Labrusca flavor detected by the tasters when VGFI 14 or less: The grape breeder using VGFI for screening should not be overly concerned with this error because these seedlings could be eliminated later when the wines are tasted.

II. Labrusca flavor not detected by the tasters when VGFI above 14: This error is of

Table 3

Relationship between Vineland Grape Flavor Index and the presence of labrusca flavor character in 159 wines

Rapport entre le «Vineland Grape Flavor Index» et la présence de l'arôme labrusca dans 159 vins

Sensory evaluation[1])	Vineland Grape Flavor Index			
	0—14		> 14	
	No.	%	No.	%
Non-labrusca	111[2])	69.8	6	3.8
Labrusca	15	9.4	27[2])	17.0

[1]) The cultivar is classified as "labrusca" or "non-labrusca" on basis of sensory evaluation of the wine.

[2]) Objective and sensory data are in agreement.

Table 4

Reliability of Vineland Grape Flavor Index (VGFI) values from year to year

Degré de confiance dans les valeurs du «Vineland Grape Flavor Index» obtenues au cours des années

No. of years analysed	Number of cultivars		Reliability
	Consistent[1])	Inconsistent[2])	%
2	144	1	99
3	62	3	95
4	18	3	86
5	7	1	88
6	4	1	80
7	—	—	—
8	2	—	100
Total	237	9	96

[1]) The VGFI was either below or above the borderline value (14) every year the cultivar was analysed.

[2]) The VGFI crossed over the 14 limit value from one year to the other.

Table 5

Methyl anthranilate and total volatile esters content of *Vitis* species

Anthranylate de méthyl et esters volatiles totaux chez diverses espèces de *Vitis*

Species	No. of cultivars analysed	Methyl anthranilate[1]) ppm	Total volatile esters[1]) ppm
V. amurensis RUPR.	1	0.00	1
V. labrusca L.	1	3.40	160
V. riparia MICH.	1	0.00	2
V. rotundifolia MICH.	2	0.00	58
V. vinifera L.	22	0.00	2

[1]) The mean is given when more than one cultivar was analysed.

greater concern because these seedlings would be discarded. However, the number of cultivars in this category was small (7 out of 221 and 6 out of 159 for grape and wine tasting, respectively).

The annual variation of VGFI values was fairly large in cultivars with high VGFI. However, the VGFI for any one cultivar rarely crossed over the 14 VGFI limit value (Table 4). Such variation occurred with a few cultivars which had average VGFI values of slightly over 14. Since the results showed high consistency as far as the 14 VGFI limit value is concerned, the proposed method could be used for reliable screening in grape breeding.

The MA and TVE contents of 5 *Vitis* species were determined. The results presented in Table 5 show that *V. labrusca* L. is the only species where MA is present. Interestingly, high TVE content was typical of both species with "foxy" flavor (*V. labrusca* and *V. rotundifolia*). This indicated that the compounds responsible for this flavor character were among those determined as TVE, but not the anthranilic acid esters. Furthermore, this suggests that TVE content could be used by grape breeders working with *V. rotundifolia* MICH. in a similar manner as VGFI used with grapes having *V. labrusca* ancestry.

Although VGFI was developed for screening grape seedlings, it is being used to aid in the identification of grape cultivars. To help such efforts, a list of VGFI values for 62 commercial grape cultivars is presented in Table 6. Since MA and TVE content are inherited characteristics (6, 12), this list should also help grape breeders in their selection of parental material.

The proposed objective flavor index is a reliable tool for the screening of grape seedlings for labrusca flavor character. In case a more simplified method is required, TVE determination with a limit value of 10 ppm could serve such purpose. For screening of grape breeding material it is sufficient to analyse cold pressed grape juice instead of whole grapes.

The advantages of using VGFI for screening of grape breeding material are as follows:

1. It permits early screening of grape seedlings based on flavor character.
2. Requires less labor than experimental wine making and tasting.
3. It is an objective method were biases encountered in sensory evaluation are eliminated.

Table 6

Vineland Grape Flavor Index (VGFI) values for some commercially important grape cultivars

Valeurs du «Vineland Grape Flavor Index» (VGFI) dans les raisins de quelques cultivars commerciaux

Cultivar	Type[1])	No. years analysed	Av. °Brix	Av. VGFI
Agawam	A	3	18.0	59
Alden	G	2	15.6	5
Aurore	F	2	19.1	3
Baco Noir	F	3	18.4	3
Bath	G	3	18.0	40
Buffalo	G	2	19.2	5
Canada Muscat	G	3	18.1	23
Cascade	F	2	19.8	3
Catawba	A	4	18.0	67
Cayuga White	G	3	17.8	2
Chambourcin	F	3	19.3	2
Chancellor	F	2	18.1	3
Chardonnay	E	3	19.8	4
Chelois	F	3	17.9	3
Colobel	F	2	17.5	3
Concord	A	8	17.0	416
De Chaunac	F	5	19.7	4
Delaware	A	5	19.9	34
Diamond	A	3	17.1	204
Diana	A	3	17.7	54
Dutchess	A	3	16.9	6
Elvira	A	4	15.5	25
Festivee	V	3	18.8	3
Florental	F	3	17.3	2
Fredonia	G	5	14.2	89
Gamay Beaujolais	E	4	18.5	3
Gewürztraminer	E	1	18.7	2
Herbert	A	2	17.4	97
Himrod	G	2	18.1	2
Ives	A	2	16.6	338
Le Colonel	F	2	18.0	2
Le Pourpré	F	1	18.7	2
Le Commandant	F	4	15.6	3
Le Général	F	2	16.9	2
Léon Millot	F	2	21.4	3
Maréchal Foch	F	2	20.1	3
Muscat du Moulin	F	2	19.4	3
New York Muscat	G	4	19.3	7
Niagara	A	5	15.8	391
Noah	A	3	17.5	71

[1]) A = North American; E = *V. vinifera;* F = French hybrid; G = Geneva hybrid; M = Miscellaneous hybrid; V = Vineland hybrid.

Table 6
(continued)

Cultivar	Type[1])	No. years analysed	Av. °Brix	VFGI
Okanagan Riesling	M	2	19.2	4
Ontario	G	2	18.9	133
Othello	A	3	17.2	46
Pougette Musquée	F	3	16.9	2
Rayon d'Or	F	3	19.2	2
Rosette	F	2	18.3	3
Rougeon	F	2	18.2	4
Seneca	G	2	20.0	10
Seyval	F	2	19.8	3
Valérien	F	2	17.3	1
Van Buren	G	3	15.5	107
Veeblanc	V	2	15.9	13
Veeport	V	4	16.1	30
Ventura	V	3	18.4	3
Verdelet	F	3	18.9	3
Vidal Blanc	F	2	20.6	4
Vignoles	F	2	20.9	2
Villard Blanc	F	3	17.5	2
Villard Noir	F	3	18.0	4
Vincent	V	5	16.9	2
Vinered	V	4	18.4	15
White Riesling	E	4	18.0	3

Summary

The total volatile exters (TVE) and methyl anthranilate (MA) content of grape cultivars, selections and seedlings including Vineland, Geneva and French hybrids, North American and *Vitis vinifera* L. cultivars were determined. The results showed that grapes which had labrusca flavor character were high in one or both of these flavor components. Based on these findings, TVE and MA analyses were proposed for the early screening of grape seedlings for labrusca flavor character. The following index was developed: Vineland Grape Flavor Index = MA (ppm) × 100 + TVE (ppm). Seedlings with an index of over 14 would likely have labrusca flavor character. The advantages of the proposed method are objectivity and efficiency of time and labor.

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