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A chemical method for the assessment of grapes according to their seed content [1])

U. MERIN, I. ROSENTHAL and U. LAVI

Une méthode chimique pour la détermination des raisins selon leur teneur en pépins

Résumé. — Une analyse chimique pour la détermination de l'absence de grains de raisins est décrite. La méthode est basée sur la détermination colorimétrique de composés phénoliques dans les raisins.

Introduction

Commercially used grapes are divided in wine grapes, table grapes, raisin grapes and a minor category of canning grapes. There is a steadily increasing demand for seedless raisin and table grape varieties, and the expanding production of canned grapes is also due to the use of seedless varieties (WINKLER *et al.* 1974). For these reasons, the aim of research efforts is to develop new seedless cultivars. Obviously, this research requires a simple, convenient and reliable test for the determination of seedlessness. The assessment as is carried out today is highly subjective and unreliable. A sample of 10 berries is usually cut into halves, the seeds are counted, and the berries are tasted for seed traces or hard seed coats.

In this work, we suggest an objective and reliable chemical method based on the polyphenol content of the berries, in order to assess grape varieties for their seeds content.

Materials and methods

Grapes of known cultivars as well as progenies from controlled crosses were obtained from the vineyard at The Volcani Center of the Agriculture Research Organization. A sample of 10 average-sized berries was taken from each cluster. The berries were carefully detached from the stems in order to leave the receptacle inside the berry. The berries were peeled, collected in 50 ml plastic tubes and weighed. Three consecutive extractions were carried out by distintegrating the berries for 1 min with 5 ml of EtOH, using an Ultra-Turrax (Janke & Kunkel KG, Switzerland), equipped with an 18 mm shaft. The supernatant of the three extractions was collected after centrifugation in a Sorvall centrifuge at 5,000 rpm for 5 min, and filtered through Whatman No. 1 filter paper. 0.5 ml of the supernatant was added to a 50 ml volumetric flask with 30 ml of water. Then, 5 ml of Folin-Ciocalteu reagent and 5 ml of saturated Na_2CO_3 solution were added and the volume was made up to 50 ml with water. Absorbance at 765 nm was measured after 30 min using a Spectronic 21 UVD spectrophotometer (Bausch &

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Lomb Inc., Rochester, NY) with a standard 1 cm glass cell. The recorded result was divided by the sample weight in g. The procedure was basically similar to that described in AOAC (HORWITZ 1975), but instead of the Folin-Deniss reagent we used the Folin-Ciocalteu reagent (E. Merck AG, Darmstadt, W. Germany), as suggested by SINGLETON and ROSSI (1965). A standard curve was determined using tannin (E. Merck) representing the relation of tannin concentration to absorbance: 0.1 mg tannin equaling 0.1 absorbance. The results of the test are expressed in terms of tannin equivalents × 1,000 per g of sample (t. e./g) in order to express phenol content according to SINGLETON and ROSSI (1965) and SINGLETON and ESAU (1969). All chemicals used were of analytical reagent grade.

Results and discussion

The present "taste" method for the classification of grapes according to their seed content is not satisfactory. Its two main disadvantages are: 1. The method is highly subjective and gives different rating of the very same sample analysed by different assessors, and 2. it does not allow to place the sample into a distinct class or to quantitatively determine the seed content.

The chemical method, on the other hand, is based on an objective criterium which is the content of "tannin" of wine including all of the polyphenolic compounds (SINGLETON and DRAPER 1964). The data presented hereby imply that polyphenol compounds represent the seed content, which is easily determined in a quantitative manner and, therefore, it has the potential of drawing a border line between seeded and seedless grapes.

The distribution of phenols in grape berries — seed, pulp and skin — is given in Table 1. The results show that the phenols are mainly located in the skin and seeds and only a small fraction of them is found in the pulp. These results and similar results presented by SINGLETON and ESAU (1969) show that, if the skin is removed before the test, the phenol content represents the seed content of the berry. Grape berries may contain normally up to four seeds. Their number may be less because of the absence or abortion of one or several ovules. In seedless varieties usually aborted seeds are present. Irrigation, soil and climatic factors, as well as genotype, can influence the number of aborted seeds (WINKLER *et al.* 1974). At different seedless varieties seed abortion occurs

Table 1

Distribution of phenols in grapes

Distribution des phénols dans les raisins

Variety	Type	Weight of sample (g)	Color	t.e./g		
				Seed	Pulp	Skin
Perlette	Seedless	19.91	White	10.1	8.1	36.5
Golden City	Seeded	41.79	White	71.8	8.8	79.0
Female	Seeded					
Queen of the Vineyards × Flame Seedless	Seeded	20.00	Red	77.5	14.8	74.5
Alphonse × Sultanina	Seeded	24.86	Red	42.4	9.8	53.7

Table 2

The "content" of phenols per g in grape berries of known cultivars classified as seedless

La «teneur» en phénols par g dans les raisins des cultivars connus classifiés comme sans pépins

Variety	Weight of sample (g)	Color	t. e./g
Flame Seedless	43.02	Red	2.0
Thompson Seedless	37.51	White	4.9
Delight	20.21	White	5.3
Ruby Seedless	40.27	Red	7.2
Emperor Seedless	33.53	Red	7.3
Calmeria Seedless	22.36	White	9.2
Sultanina	22.54	White	12.2
Perlette	20.44	White	17.7

at different stages. This could together with the environmental factors mentioned above result in the wide range of polyphenol content in both the seeded and the seedless varieties.

It is quite clear from Tables 2 and 3 that the content of polyphenol compounds is markedly different in the seedless varieties compared to the seeded ones. A statistic "t" test for a two-sample comparison was performed. The hypothesis that the mean population value for the seedless varieties (μ_1) is equal to the mean of the seeded ones (μ_2) was rejected, since the calculated $t = 3.971$ is greater than the tabular t value for 12 degrees of freedom. This implies that the two means of the populations are different at $\alpha = 0.005$ (99.5 %).

Since the natural situation is the lack of a sharp border line between seedless and seeded varieties, it seems to us much more feasible to assess grapes for seedlessness by an objective chemical method, rather than by a subjective "taste" method. The color of the grapes did not interfere with this test, as seen from Tables 2 and 3. Both seeded and seedless varieties, wether red or white, fell within the above-mentioned range regardless of color. A comparison of fresh and frozen samples, after 3 months of storage at

Table 3

The "content" of phenols per g in grape berries of known cultivars classified as seeded

La «teneur» en phénols par g dans les raisins des cultivars connus classifiés comme avec pépins

Variety	Weight of sample (g)	Color	t. e./g
Alphonse	45.56	Red	20.7
Dabuki	45.43	White	22.5
Muscat Hamburg	46.49	Red	32.7
Queen of the Vineyards	21.24	White	64.0
Italia	32.95	White	66.3
Cardinal	21.75	Red	95.7

Table 4

The "content" of phenols per g in grape berries in hybrids of controlled crosses between seeded and seedless varieties

La «teneur» en phénols par g dans les fruits de raisins dans les hybrides des croix controllées entre cultivars avec pépins et sans pépins

Cross	Plant no.	Color	t.e./g[1])				Taste[2])
Queen of the Vineyards × Perlette	1	White	22.6	27.7	28.1	28.3	B
Queen of the Vineyards × Perlette	2	White	2.3	5.2			S
Cardinal × Perlette	1	Red	14.2	15.9	18.3		S
Cardinal × Perlette	2	White	22.7	54.1			B
Cardinal × Perlette	3	Red	24.6				S
Cardinal × Perlette	4	White	40.3	43.0			B
Cardinal × Perlette	5	Red	43.7	69.2			B
Cardinal × Perlette	6	White	76.6	89.3			B
Dabuki × Emerald Seedless	1	White	30.2	32.9			B
Queen of the Vineyards × Sultanina	1	White	36.7	45.0			B
Queen of the Vineyards × Flame Seedless	1	Red	111.3				B
Alphonse × Sultanina	1	Red	19.0	21.3			B
Cardinal × Flame Seedless	1	Red	5.6				S

[1]) The values represent different measures taken at different times.
[2]) Olmo and Baris (1973). This test was carried out only on a single sample. B = seeds detectable S = not detectable.

– 20 °C, shows identical values for our chemical assay. This would enable the collection of samples during the season and their preservation in the frozen state for later examination. Results for 13 hybrids from different progenies in the Bet Dagan grape-breeding program using this method, are presented in Table 4. The correlations between the results obtained by the chemical method (t. e./g) and those obtained by tasting seem satisfactory. However, it must be emphasized that an inter-cluster variability in the presence of seeds does exist. Such a variation is the reason for the different t. e./g values obtained for the same hybrid. Some of these hybrids, and especially Cardinal × Perlette plant no. 2, show a high degree of variability in their berries. A t. e./g score of 22.7 and one of 54.1 represent two extreme samples of the same plant (the "taste" test was carried out only on a single sample). In order to overcome this problem, better sampling methods should be developed. These methods are of course independent of the classification method described here.

A value of 22—25 t. e./g could be the border line between the detectable (B) and the non-detectable (S) seed content (Olmo and Baris 1973). A more precise determination will emerge if more analyses will be performed with known and new varieties.

In conclusion, the chemical method based on the polyphenol content compared to assessment by taste is more reliable, quantitative and relatively easy to perform. Hopefully, it will assist breeders in detecting satisfactory seedless varieties and will be helpful in genetic analysis of the inheritance of the trait.

Summary

Polyphenolic compounds, which are unevenly distributed between seed, pulp and skin, are the basis of a chemical method for the assessment of seedlessness in grapes.

Known seedless and seeded grapes were evaluated using this method and a statistic test showed a significant difference between them. The color of the grapes did not interfere with this method. Frozen storage of samples at −20 °C for 3 months did not alter the results of the test. Hybrids of known progenies which were evaluated gave results which generally agree with those of the taste test.

This chemical method, compared to assessment by taste, is more reliable, quantitative and relatively easy to perform.

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Dr. U. MERIN
Dr. I. ROSENTHAL
Department of Food Technology

Dr. U. LAVI
Department of Fruit Breeding and Genetics

Agricultural Research Organization
The Volcani Center
P. O. Box 6
Bet Dagan 50250
Israel