ELSEVIER

Journal of Chromatography A, 888 (2000) 121–127

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

High-performance liquid chromatographic determination of the riboflavin concentration in white wines for predicting their resistance to light

F. Mattivi[a,*], A. Monetti[a], U. Vrhovšek[a,b], D. Tonon[a], C. Andrés-Lacueva[c]

[a]Istituto Agrario di S. Michele, Dip. Laboratorio Analisi e Ricerche, Via E. Mach 1, 38010 S. Michele all'Adige, Italy

[b]Agricultural Institute of Slovenia, Hacquetova 17, Ljubljana, Slovenia

[c]Nutrició i Bromatologia, CeRTA, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XIII s/n, 08028 Barcelona, Spain

Received 3 February 2000; received in revised form 29 April 2000; accepted 9 May 2000

Abstract

This research paper describes a new application in the field of quality control of white wine. A high-performance liquid chromatography analysis of riboflavin was used together with a simple sensorial test to produce a classification of wines, rating their susceptibility to exposure to light. Based on a wide survey covering 85 commercial white wines of different varieties in three countries (Italy, Spain and Slovenia), an average value of riboflavin of 98.63 μg/l with a rather high standard deviation of 41.91 μg/l, and a normal distribution was obtained. Our statistical study of the frequency distribution of the sensorial scores of light-exposed wines by means of the Expectation–Maximization algorithm demonstrated that a large majority (71%) of these products were susceptible to the light, 31% of them belonging to the most severely affected group. Content of riboflavin was correlated with severity of appearance of the off-flavor in light-exposed white wines. These methods are directly applicable in the quality control of wine, being a valuable aid for oenologists in choosing appropriate fining protocols to reduce the appearance of the undesired ‘‘sunlight flavor’’ in bottled products.

Keywords: Wine; Food analysis; Riboflavins; Vitamins

1. Introduction

The principal forms of riboflavin (vitamin B_2) found in nature are flavin mononucleotide (FMN) and flavin–adenine dinucleotide (FAD). Free riboflavin (RF) is also naturally present in raw and processed fruits and fermented beverages. FAD and sometimes FMN are present together with RF in significant amounts in fruit juices and in beers, while RF is the only form of riboflavin present in important amounts in wine [1].

RF, FMN and FAD are the main agents responsible for the off-flavor that white wine and other beverages can develop when they are exposed to light. This highly undesired, detrimental effect of light on the aroma of white wines is due to the generation of sulfur compounds that produce an onion/garlic odor, for which RF is required.

The photogeneration of thiols (methylmercaptan, H_2S) and dimethyldisulfide in wine exposed to light

*Corresponding author. Tel.: +39-0461-615-255; fax: +39-0461-615-288.

E-mail address: fulvio.mattivi@ismaa.it (F. Mattivi).

PII: S0021-9673(00)00561-6

of wavelengths below 450 nm is caused by photodegradation of methionine and cysteine in the presence of RF, which acts as a photosensitizer and oxidating agent in wine [2–5].

Attempts to control of the ‘‘sunlight flavor’’ in wine include the use of cupric cation, dithionite anion and tannins [4,5], while satisfactory results have recently been obtained by fining with bentonite [6]. Pichler [6] also proposed a simple standardized method for inducing the sunlight flavor through exposure of 100 ml of wine to direct light, which can identify wines that are likely to develop the defect when not stored under proper conditions.

Display practices such as bottling in colored glass containers and protecting from bright light do have a fundamental protective role [7]. It has been reported that RF concentration can be a good index of the likely degree of deterioration in the flavor of still and sparkling wines on exposure of bottles to light [8]. Since marketing strategies require the sale of some wines in clear (or green) glass bottles, it is important to develop methods to evaluate susceptibility of this beverage to light. Concentration of RF appears to be a key factor in assessing the risk of the appearance of a sunlight flavor and should be applied for routine analysis of wine [1].

This article reports the first application of a high-performance liquid chromatography (HPLC)–fluorescence method for quantification of RF levels in a wide sample of still and sparkling white wines produced in Italy, Slovenia and Spain. The RF data were correlated with results of a simple sensorial test, in order to develop new criteria to be applied in quality control of white wines for the assessment of their susceptibility to develop the sunlight flavor.

2. Experimental

2.1. Wine samples

The sampling was focused mainly on still white wines produced with grape varieties Chardonnay and Pinot gris, which are widely cultivated and are highly susceptible to the appearance of the sunlight flavor. Other samples were chosen among different still and sparkling non aromatic (neutral) varieties.

Eighty-five samples of commercial white wines were sampled in three countries. Nineteen Trentino DOC Chardonnay and 13 Trentino DOC Pinot gris wines where sampled in Trentino, NE Italy. Fifteen Chardonnay and eight Pinot gris wines were obtained in Slovenia. They were all classified as of the highest quality in Slovenia (‘‘vrhunsko vino’’). Seventeen still (D.O. Penedes) and 13 sparkling wines (D.O. Cava), were sampled in the region of Penedes, Spain. The still wines were produced with the grape varieties Chardonnay, Macabeo, Xarel.lo, Parellada, Riesling, Muscat and Gewürztraminer.

2.2. Chemical analysis

RF, FMN and FAD in wine were analyzed by HPLC with fluorescence detection [1]. A Hewlett-Packard (HP) 1100 gradient liquid chromatograph with a HP G1312A quaternary pump, a HP 1046A fluorescence detector and with a HP G1396A autosampler was used (Hewlett-Packard, Waldbronn, Germany). The detector was operating at 265/525 nm ($\lambda_{excitation}/\lambda_{emission}$) using a 500-nm cut-off filter, with a 2×2 mm slit on the excitation side and two 4×4 mm slits on the emission side. Samples were passed through Millex-GV[13], 0.22-μm filter (Millipore), and 20-μl aliquots were directly injected without sample preparation into a Hypersil column ODS C_{18}, 200 mm×2.1 mm I.D., with a 5-μm particle size (Hewlett-Packard) at room temperature, ca. 22°C, with a pre-column (20×2.1 mm) containing the same stationary phase.

The following solvents were used: solvent A, 0.05 *M* buffer NaH_2PO_4 at pH 3.0 with H_3PO_4 and solvent B, acetonitrile. The mobile phase was passed through a membrane (0.45 μm) and degassed with He. The linear gradient elution profile was as follows: 0 min, A–B (95:5); min 8, A–B (75:25), min 12, A–B (95:5), with a post-time of 3 min and with a constant flow-rate of 0.6 ml/min. Quantification was obtained with the external standard method [1], repeating daily the calibration with a fresh standard of RF. The limit of quantification for RF, calculated by repeated injections of diluted solutions, at a signal-to-noise ratio of 10, was 1.72 μg/l.

Total polyphenols reactive to Folin–Ciocalteau after clean-up on a C_{18} cartridge were assessed with the method of Di Stefano and Guidoni [9]. The white wine was diluted twice with 0.5 *M* H_2SO_4 and processed under the conditions described by Rigo et al. [10].

Copper and iron were estimated by atomic absorption according to the literature [11] with a Varian SpectrAA-10 Plus spectrophotometer. The data of absorbance at 320 nm, which in white wines is proportional to the concentration of the hydroxycinnamates, was recorded in a 1-mm quartz cell with a Hitachi U-2000 spectrophotometer.

2.3. Sensorial analysis

The average intensity of the ‘‘sunlight flavor’’ was estimated with the method of Pichler [6] by a panel of eight tasters (researchers and technicians). Each one of the 85 wine samples was prepared in a pair, with and without the addition of RF (1 mg/l), and the two sets of samples were subsequently exposed to light. A 100-ml volume of sample was poured into a glass column and exposed to direct artificial light (150 W clear tungsten bulb E27-ES, distance from the source 30 cm, for 48 h). The two glass columns in each pair presented to the panelists were of identical shape. Panelists were trained beforehand to perceive the ‘‘off-flavor’’ aroma. Panelists rated the intensity of the off-flavor aroma on a three-point category scale (0=not present; 1=perceptible; 2=intense). The average of the evaluations of the eight tasters gave a numerical value variable from 0 to 2, proportional to the intensity of the sunlight flavor [1].

2.4. Statistical treatment

The statistical study of the finite mixture distributions which originate the frequency distribution of the sensorial scores of natural (no RF added) light-exposed wines was obtained by means of the Expectation–Maximization algorithm of Monetti and Dalpiaz [12], implemented in SAS IML [13]. Linear regressions were obtained with the software Microsoft Excel 97.

3. Results and discussion

3.1. Levels of riboflavin in wines

Fig. 1 shows the results of chromatographic analysis of a standard solution and a white wine. In agreement with previous literature [1,8] the FAD and FMN were always below the limit of quantitation

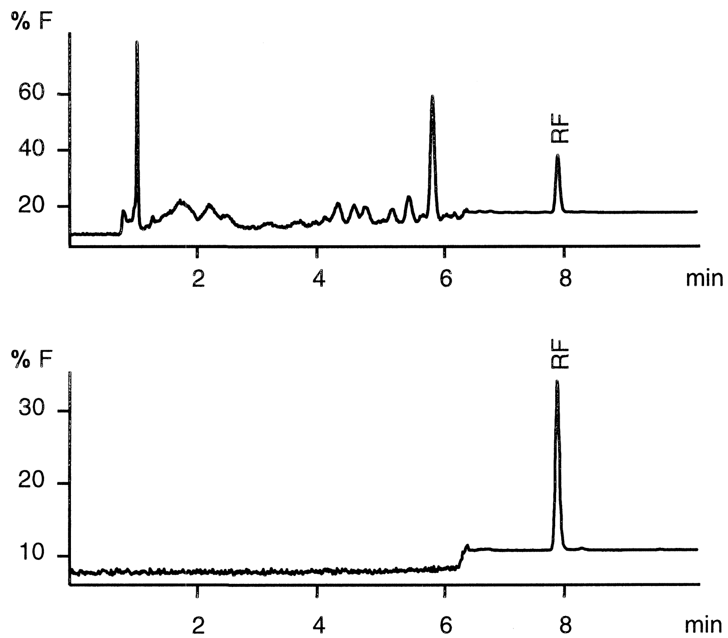

Fig. 1. Chromatographic analysis of a standard solution (bottom) and a white wine (top). RF=Riboflavin. Time scale in min. F=Fluorescence.

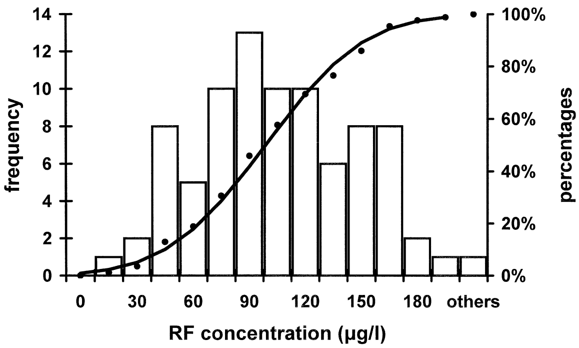

Fig. 2. Frequency distribution of RF in the sample of white wines (bars). The continuous line shows the percentage cumulative frequency for the normal distribution and the symbol (·) gives the experimental percentage cumulative frequency.

(LOQ) of the method, therefore these two substances are not an important cause of sunlight flavor in wine. Vitamin B_2 was present in white wines of our study only in the free form (RF), with a minimal value of 8.2 μg/l and maximal values up to 200.3 μg/l, with normal distribution (Fig. 2), an average value of 98.63 and a rather high standard deviation of 41.91 μg/l. The average values in the three countries were increasing from the Spanish (82.1 μg/l) to the Italian (100.3 μg/l) and to the Slovenian wines (117.8 μg/l) but the differences between the means were not statistically significant, due to the high variability of the RF contents of the wines. Standard deviations of the sample sets from each country were comparable to that of the whole sample.

In spite of the long contact time with yeast during the secondary fermentation which should increase the levels of RF [14,15], the average RF contents of the still and sparkling wines from Spain were almost the same (Table 1). The Italian and Slovenian wines of the variety Chardonnay showed similar contents of RF, while for the Pinot gris the average concentration of RF was lower for Trentino in comparison to Slovenia.

3.2. Sensorial value

Sensorial tests on wine samples after exposure to direct artificial light were carried out on wines without and with addition of excess RF (Table 1). From the comparison of the sensorial scores, it was observed that the intensity of the off-flavor was greatly enhanced from the addition of extra RF, thus confirming that the concentration of the vitamin B_2 is an important enhancing factor for the appearance of this off-flavor. Only two out of 85 samples were not able to develop the sunlight flavor after addition of excess RF. Therefore, control of RF concentration is an important goal for the stability of wine, in particular when bottled in clear glass.

Observing frequency distribution of the sensorial scores of natural (no RF added) light-exposed wines it was observed that it greatly differed from normal distribution. The same was true for each separate country and for the total sample. For a precise understanding of the sensorial score it was advisable to study this distribution.

Finite mixture distributions (FMDs) arise naturally

Table 1
Mean concentration of RF (μg/l) and mean sensorial values of the sunlight flavor in different groups of commercial white wines[a]

Country	Wine	No. of samples	Mean RF (SD)	Mean sensorial values (SD)	
				Control	Sample+RF
Italy	Chardonnay	19	111.77 (33.31)	0.79 (0.38)	1.65 (0.21)
	Pinot gris	13	83.60 (54.33)	0.57 (0.37)	1.48 (0.25)
Slovenia	Chardonnay	15	116.06 (38.89)	0.65 (0.32)	1.42 (0.31)
	Pinot gris	8	121.12 (44.73)	0.75 (0.49)	1.38 (0.47)
Spain	Penedes (still)	17	79.65 (27.38)	0.45 (0.36)	1.49 (0.37)
	Cava (sparkling)	13	85.34 (44.32)	0.68 (0.19)	1.48 (0.25)
All	Various	85	98.63 (41.91)	0.64 (0.36)	1.50 (0.31)

[a] Samples were divided by country and variety. The sensorial values were measured after exposure to light, without and with RF added.

when a statistical population is a mixture of k component populations. In this case f, the probability density function of the variables under examination, does not depend on the global mean and dispersion but on these parameters in each population and the proportions of components of the mixture [16]. Many different estimation methods of the FMDs exist.

The maximum likelihood approach, under very general conditions, has some useful properties like consistent estimators, which converge in probability to the true parameter values, and asymptotic normality [17].

At the same time, the complex dependence of the likelihood function on the parameters to be estimated originates computational difficulties which cannot be explicitly solved and need an approximate solution through an iterative procedure [18] like the Expectation–Maximization (EM) algorithm [19]. A detailed description of this approach has been given by Flury [19], while an example of its application in the beverage analysis has been discussed by Monetti et al. [20].

The application of this algorithm allowed one to estimate three different groups, which were characterized by their respective parameters of average, standard deviation and percentage weight in the total sample (Fig. 3). On the basis of the average values of the sensorial scores, the three groups could be defined with respect to the increasing sunlight flavor as group I (not present), II (perceptible), and III (intense). The percentage weights of the three groups in the total sample were, respectively, 29, 40 and 31%.

In order to define the sensorial threshold for the sunlight flavor, our attention should be focused on the groups where the off-flavor was perceived. In light of the normal distribution of data, and with reference to the single-tailed z distribution, it was possible to calculate the sensorial score defining the lower limit associated with the 95% probability of classification into the group II as:

$$\text{lower limit (group II)} = \text{average sensorial value (group II)} - 1.65 \times \text{s.d.}$$

which corresponds to $0.6485 - 1.65 \cdot 0.1179 = 0.454$. In the case of the most severely affected group III, the corresponding lower limit was computed as: $1.0387 - 1.65 \cdot 0.2256 = 0.666$.

In conclusion, below the sensorial value of 0.454 were all the wines belonging to the group resistant to the light, while between 0.454 and 0.666 were the samples attributed to group II, where the sunlight

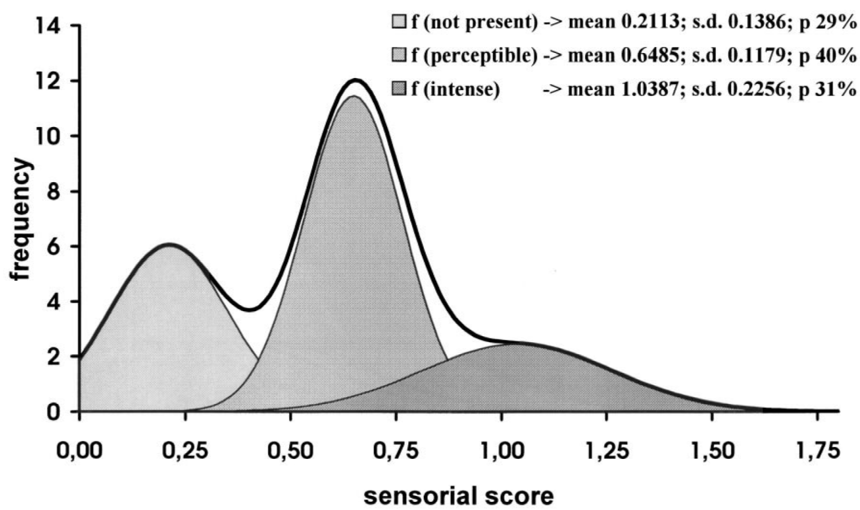

Fig. 3. Probability density functions (f) of the values of the sensorial scores for the sunlight flavor in light-exposed white wines, calculated by the Expectation–Maximization algorithm. Three different groups are characterized by their respective parameters of mean, standard deviation and percentage weight in the total sample.

flavor can be perceived, without being dominant. Finally, the samples having the highest probably to develop intense sunlight flavor were associated with sensorial values higher than 0.666.

The classification into the three groups of the wine samples produced in Italy (25/28/47%) and Slovenia (22/30/48%) were almost identical, while slightly more resistant were the Spanish wines (40/20/40%). The sensorial test applied on a highly representative sample of commercial products demonstrated that the formation of the sunlight flavor in light-exposed wines does not represent a rare event; on the contrary, it is an highly probable condition very likely to appear in over two thirds of commercial products when exposed to excessive irradiation without adequate protection.

3.3. Inverse correlation between riboflavin and resistance to light

In order to verify if lower levels of riboflavin were associated with increased resistance of the wine to the light, the correlations between RF concentration in wine and the sensorial values of the sunlight flavor after exposure to light were studied. These correlations were verified for the whole sample and for the subsets divided by country or by grape variety (Table 2).

Taking into consideration the whole sample, a highly significant ($P<0.001$) correlation between development of sunlight flavor (y) and the initial content of RF (x) was evident ($y=0.0031x+0.3409$). The general trend is certainly that the highest frequency and the highest intensity of the off-flavor is associated with the wines containing the highest values of RF. On the other hand, the correlation coefficient ($r=0.3544$) was clearly too low to allow the use of the RF values to predict the intensity of formation of the sunlight flavor.

In conclusion, the general trend is in good agreement with the theory, confirming the role of RF as an enhancing factor of the formation of the sunlight flavor, but with exceptions. A considerable number of samples with high values of RF were rather resistant to the light, and, most problematic from the point of view of quality control, a few wines exhibited the formation of sunlight flavor in spite of the content of RF being quite below the average. This observation demonstrates that it is not advisable to define a security level for the RF below which the wine could be considered resistant. More different compounds could also affect the light stability of wine, such as those playing a role in redox reactions, like the transition metals and many polyphenols, and the precursors of the off-flavor, the amino acids containing sulfur. The last hypothesis could only be true for the few wines which do not develop the sunlight flavor when excess riboflavin is added.

Different groups of samples showed variable correlation between RF and sensorial values (Table 2). A particularly high correlation was found for the Spanish still wines from Penedes area, which contained both the main white grape varieties of this region (Macabeo, Xarel.lo and Parellada), Chardonnay and aromatic varieties. Still significant were the levels obtained for the Cava and Pinot gris. No

Table 2
Linear regression between the sunlight flavor in light-exposed white wines (y, sensorial score) and the RF concentration (x, μg/l) for the subsets of commercial wines divided by country or by grape variety[a]

Wine	No. of samples	r	Intercept	Slope	F significance
Chardonnay	39	0.1189	0.5479**	0.0013	0.471
Pinot gris	21	0.4086	0.3257	0.0032	0.066
Penedes (still)	12	0.7769	−0.4588	0.0131**	0.003
Cava (sparkling)	13	0.5671	0.480**	0.0024*	0.043
Slovenia	23	0.4342	0.1994	0.0041*	0.038
Italy	32	0.1815	0.5415**	0.0016	0.320
Spain	30	0.4538	0.2065	0.0042*	0.012
All	85	0.3544	0.3409**	0.0031**	0.001

[a] * = Significant ($P<0.05$); ** = significant ($P<0.01$).

correlation between RF and sensorial value was found for the Chardonnay wines, thus suggesting the other factors as particularly important for these wines.

A subset of 14 Chardonnay wines including seven samples having relatively low RF (mean = 98.2; SD = 21.5) and high sunlight flavor (mean = 1.13; SD = 0.23) and seven samples having high RF (mean = 148.3; SD = 22.1) and low sunlight flavor (mean = 0.36; SD = 0.26) were further analyzed to identify possible co-factors. The latter group of wines was not lacking the precursors because all the samples were able to develop intense sunlight flavor in the sensorial test with RF added (mean = 1.52; SD = 0.33). The two subsets did not significantly differ in the average values of iron, copper, total polyphenols, hydroxycinnamates and pH.

4. Conclusions

This survey confirmed the role of RF as important enhancing factor in the formation of sunlight flavor in light-exposed white wines. It must be emphasized that the complex mechanism inducing the formation of this off-flavor cannot be entirely explained with the presence of this photosensitizer. As a consequence, the removal of excessive RF can certainly be an important factor to improve the resistance to light of white wines. In some cases of wines having homogeneous origin and winemaking technique, the RF concentration could also be a good predicting factor for their resistance to light. On the other hand, other and to-date unknown factors can modify the susceptibility to light, either increasing or decreasing it. Our joint use of HPLC analysis of RF together with simple sensorial tests has been demonstrated to be a powerful tool for the quality control of white wines, useful to measure different levels of resistance to light of white wines, and to identify the wine lots requiring appropriate fining protocols to remove excessive RF levels before bottling in clear glass bottles.

References

[1] C. Andrés-Lacueva, F. Mattivi, D. Tonon, J. Chromatogr. A 823 (1998) 355.

[2] B. Haye, J. Maujean, C. Jacquemin, M. Feuillat, Connaissance Vigne Vin 11 (1977) 243.

[3] A. Maujean, M. Haye, M. Feuillat, Connaissance Vigne Vin 12 (1978) 277.

[4] A. Maujean, N. Seguin, Sci. Aliments 3 (1983) 589.

[5] A. Maujean, N. Seguin, Sci. Aliments 3 (1983) 603.

[6] U. Pichler, Enotecnico 32 (3) (1996) 57.

[7] O.A. Ajayii, G.O. Oladapo, Qualitas Plantarum – Plant Foods Hum. Nutr. 36 (1986) 197.

[8] N. Moll, G. Charalambous, in: Development in Food Science, Chalkidiki, Greece, Elsevier, Amsterdam, 1989, p. 753.

[9] R. Di Stefano, S. Guidoni, Vignevini 16 (1–2) (1989) 47.

[10] A. Rigo, F. Vianello, G. Clementi, M. Rossetto, M. Scarpa, U. Vrhovsek, F. Mattivi, J. Agric. Food Chem. 48 (6) (2000) 1996.

[11] B.W. Zoecklein, K.C. Fugelsang, B.H. Gump, F.S. Nury, Wine Analysis and Production, Chapman and Hall, New York, 1994.

[12] A. Monetti, G. Dalpiaz, It. J. Appl. Statistics 9 (4) (1997) 489.

[13] SAS Institute, SAS/IML Software: Usage and Reference, SAS Institute, Cary, NC, 1989.

[14] A. Ournac, M. Decor, Ann. Technol. Agric. 16 (1967) 309.

[15] A. Ournac, Ann. Technol. Agric. 17 (1968) 67.

[16] B.S. Everitt, D.J. Hand, Finite Mixture Distributions, Chapman and Hall, London, 1981.

[17] B.S. Everitt, S. Kotz, N.L. Johnson, C.B. Read, in: Encyclopedia of Statistical Sciences, Vol. 5, Wiley, New York, 1985, p. 559.

[18] R.A. Redner, H.F. Walker, SIAM Rev. 26 (1984) 195.

[19] B. Flury, A First Course in Multivariate Statistics, Springer, New York, 1997.

[20] A. Monetti, G. Versini, G. Dalpiaz, F. Reniero, J. Agric. Food Chem. 44 (1996) 2194.