JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

New Method for Evaluating Astringency in Red Wine

MARÍA C. LLAUDY, ROSER CANALS, JOAN-MIQUEL CANALS, NICOLAS ROZÉS, LLUÍS AROLA, AND FERNANDO ZAMORA*

Departament de Bioquímica i Biotecnologia, Facultat d'Enologia de Tarragona, (CeRTA), Universitat Rovira i Virgili, C/Ramón y Cajal 70, 43005 Tarragona, Spain

Astringency is an important sensory attribute of red wine. It is usually estimated by tasting and is subject to a certain subjectivity. It can also be estimated by using the gelatin index. This procedure is not very reproducible because there are many gelatins on the market with a heterogeneous composition. Furthermore, the gelatin index determines procyanidin concentration by acid hydrolysis that gives only an approximate result. This paper proposes a new and reproducible method that determines astringency by using ovalbumin as the precipitation agent and tannic acid solutions as standards. Statistical analysis of the results indicates that this method is more reproducible (RSD = 5%) than the gelatin index (RSD = 12%) and correlates better with sensorial analysis.

KEYWORDS: Astringency; tannin; red wines

INTRODUCTION

The color of red wine is due to the phenolic compounds fraction and, in particular, to anthocyanins. However, procyanidins, also known as condensed tannins, contribute to color stability by combining with anthocyanins (*1*). These combinations of anthocyanins and procyanidins seem to be responsible for the red color of aged wines (*2*). In fact, winemakers usually say that only tannic wines can age. Besides, tannins are also associated with such texture sensations as body and astringency (*3*).

Astringency is probably one of the most important sensory attributes of red wines, and it is caused by the capacity of some phenolic compounds to bind salivary proteins, producing drying and puckering sensations in the mouth (*4*). Naturally occurring procyanidins are mainly responsible for astringency (*5*, *6*). However, aging wine in oak barrels and using enological tannins can provide a certain amount of gallotannins and ellagitannins, which can also contribute to wine astringency (*7*, *8*).

The interactions between tannin and salivary proteins depend on the pH and the characteristics of the protein and the procyanidins (*9*, *10*). Salivary proteins with a high proline and hydroxyproline proportion (PRP) seem to be the major target for the procyanidin reaction (*11*, *12*). On the other hand, the size of the procyanidin molecule size seems to be related to the sensation of astringency. The greater the degree of polymerization, the greater is the sensation of astringency (*13*). Nevertheless, combination between anthocyanins and procyanidins might reduce the capacity of tannins to react with salivary proteins and, therefore, decrease astringency (*14*).

Nowadays, deeply colored and full-bodied wines are highly valued by the market, which is why winemakers try to make these wines, which are necessarily very tannic. However, excessive phenolic compound extraction may sometimes take place during winemaking, making the wine more astringent and affecting its quality (*15*).

The astringency of red wine is usually estimated by tasting. This method, however, needs a group of expert wine tasters and is always subject to a certain subjectivity (*16*). Because astringency is a major factor in wine quality, winemakers are interested in an analytical and objective method to evaluate it.

In the literature there are many studies about protein−tannin interactions that use different strategies (*17*−*24*). Recently, a predictive model for astringency estimation was published that is based on phenolic compounds and color analysis (*25*). However, to our knowledge, the gelatin index is still the only analytical method for estimating astringency in red wine (*26*). Nevertheless, this procedure requires procyanidin concentration be determined before and after precipitation with an excess of gelatin. Because total procyanidin estimation in wines by means of acid hydrolysis (*27*) gives only an approximate result (*28*), this analytical method also does. Besides, gelatin is a heterogeneous mixture of proteins, and its composition may change among the different commercial products. This may also be an important source of variability and imprecision.

Evidently, the most suitable proteins for evaluating astringency are the salivary proline-rich proteins (PRP). However, it is very difficult to obtain enough PRP as their purification is highly complicated (*29*). A possible alternative is the use of ovalbumin as a precipitation agent. Ovalbumin is one of the most used proteins for fining red wine because of its ability to bind and precipitate tannins. Besides, ovalbumin is a single protein and not a heterogeneous mixture of proteins like gelatin.

This paper proposes a new and reproducible method using ovalbumin as a precipitation agent that makes it possible to determine astringency alternatively.

* Author to whom correspondence should be addressed (fax 34 97 72 50347; e-mail fzm@astor.urv.es).

10.1021/jf034795f CCC: $27.50 © 2004 American Chemical Society
Published on Web 01/22/2004

MATERIALS AND METHODS

Reagents. All of the products were of high purity. Gelatin (type B, Sigma) and hydrochloric acid (Panreac, Barcelona, Spain) were used for gelatin index estimation. Tannic acid and ovalbumin solutions were prepared in a synthetic solution similar to wine: 4 g/L of tartaric acid (Panreacn), 95 g/L of ethanol (Panreac), adjusted to a pH of 3.5 with sodium hydroxide (Panreac). Solutions of tannic acid (ACS reagent, Sigma) at concentrations of 0, 0.2, 0.4, 0.6, and 0.8 g/L were used as standards. Ovalbumin solutions (grade V, Sigma) at concentrations of 0.0, 0.4, 0.8, 1,6, 2,4, 3,2, and 4.0 g/L were used as the protein to precipitate astringent tannins.

Wines. Ten red wines from different origins were used to validate the method. These wines were selected in a previous sensorial session in order to cover the entire scale of astringencies. The concentration of phenolic compounds was determined by measuring the absorbance value at 280 nm (*28*).

Tannin Assay. Tannin concentration was measured according to the method of Ribéreau-Gayon and Stonestreet (*27*). Two tubes with 4 mL of previously diluted (1:50) wine, 2 mL of distilled water, and 6 mL of HCl (12 N) were prepared and hermetically sealed. One of them was heated to 100 °C in a water bath, and the other was maintained at room temperature. After 30 min, 1 mL of ethanol (95%) was added to both tubes. After stirring, absorbance at 550 nm was measured. The tannin concentration was obtained by multiplying 19.33 by the difference between absorbances.

Gelatin Index. The gelatin index of the different wines was measured using the methodology described by Glories (*26*). To two Erlenmeyer flasks with 50 mL of wine was added 5 mL of distilled water or 5 mL of gelatin solution (70 g/L). After 3 days, the samples were centrifuged at 11700*g* for 10 min (Sorval RC5C). The supernatants were assayed to determine the tannin concentration (*27*). The results were expressed as astringency intensity and as a percentage. Astringency intensity was calculated as the difference between the total wine tannin concentration and the concentration after gelatin precipitation. The percentage was calculated by referring this astringency intensity to the total tannin concentration.

New Astringency Estimation Method. All of the experiments were carried out at room temperature (20 ± 2 °C). For each tannic acid concentration or wine astringency analysis, 12 tubes were prepared that contained 1 mL of solutions with increasing concentrations of ovalbumin (0.0−4.0 g/L). To each tube was added 1 mL of corresponding tannic acid solution or wine. The tubes were stirred, and 10 min after, the samples were centrifuged at 11700*g* for 10 min (Sorval RC5C).

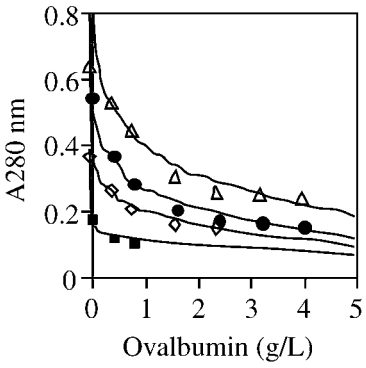
The supernatants were diluted 1/50 with distilled water. Absorbances were measured immediately at 280 nm (Ultrospec 5100 pro, Amersham Pharmacia Biotech) in a quartz bucket with an optical path of 10 mm.

Sensory Analysis. All of the wines were tasted by a panel of 10 expert enologists from the Rovira i Virgili University. Each expert evaluated the astringency of each wine on a scale from 1 to 100. Two previous training sessions of tasting were carried out to standardize criteria among the panelists. During these training sessions, panelists were required to agree by consensus on the score of three previously selected wines. Wine 1 was selected because it was very soft and was qualified with 30 points. Wine 2 was a medium-bodied wine with an agreeable sensation of astringency and was qualified with 50 points. Finally, wine 3 was a very astringent press wine and was qualified with 85 points.

Statistics. All of the data are expressed as the arithmetic average ± standard deviation from five replicates. Linear and logarithmic regressions as well as Fisher's correlation analysis were carried out using Statview (software for Macintosh). Relative standard deviation (RSD) was calculated as the quotient between the standard deviation and its corresponding mean value expressed as a percentage.

RESULTS AND DISCUSSION

Figure 1 shows the graph of the absorbance at 280 nm versus the amount of ovalbumin added for the different tannic acid solutions. As expected, adding ovalbumin precipitated tannic acid and clearly decreased the supernatant absorbance at



$$A_{280} = a \times \log [\text{ovalbumin}] + b$$

[tannic acid]	Symbol	a	b	r^2
0.2 g/L	■	0.0475	0.0875	0.9998
0.4 g/L	◇	0.1525	0.1508	0.9762
0.6 g/L	●	0.218	0.2017	0.9668
0.8 g/L	△	0.3081	0.3042	0.9664

Figure 1. Influence of ovalbumin additions on A_{280nm} from different tannic acid solutions.

280 nm. This decrease in A_{280nm} depends on the amount of ovalbumin added, and the behavior is clearly logarithmic. In fact, the curves fitted reasonably well to logarithmic equations. This behavior was probably due to the following reasons. When a small amount of ovalbumin was added to a tannic acid solution, a protein−tannic acid complex was formed that had the appearance of a cloudy precipitate. Initially the tannic acid concentration was higher than that of ovalbumin and so all of the protein precipitated together with a certain amount of tannic acid. This is why A_{280nm} decreased so drastically initially. However, as more ovalbumin was added, the tannic acid concentration became increasingly lower until no tannic acid remained in the solution. At this point, A_{280nm} began to increase because the excess of ovalbumin did not precipitate.

All of the tannic acid concentrations were found to behave in a similar way. Nevertheless, a relationship between tannic acid concentration and the initial slope of the curves was detected: the slope was greater when the tannic acid solution was higher. **Figure 2** shows the relationship between the fitted logarithmic equation slope and the initial tannic acid concentration. The slopes of the logarithmic equations obtained fitted perfectly to the tannic acid concentration of the different solutions.

Tannic acid is very reactive with proteins and may therefore reproduce the behavior of the astringent phenolic compounds in wine. Our results indicate that there is a close relationship between tannic acid concentration and the corresponding slopes of logarithmic equations. For this reason, we have considered applying this methodology to estimate the astringency of red wines.

Table 1 compares the gelatin index, expressed as a percentage and as astringency intensity, with the proposed method for 10 different wines. Both analytical methodologies are compared with astringency sensorial analysis. The table also shows the absorbance of the 10 wines at 280 nm as an indicator of their phenolic concentration. These wines were chosen because of

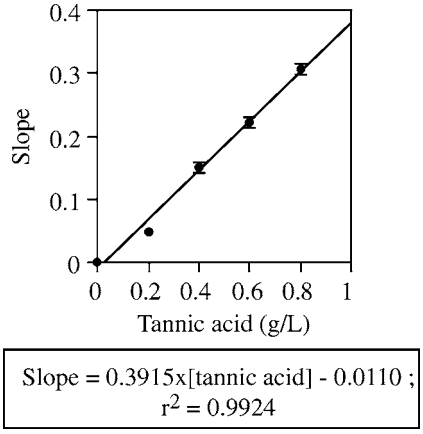


Figure 2. Relationship between logarithmic equation slope versus initial tannic acid concentration.

Table 1. Comparison of the Astringency of the Different Wines Estimated by Sensorial Analysis, the Gelatin Index, and the Proposed Method

	sensorial		gelatin index		proposed method
wine	astringency	A_{280nm}	%	AI[a] (g/L)	TA[b] (g/L)
1	27.3 ± 10.1	37.3 ± 0.4	38.6 ± 9.4	0.41 ± 0.15	0.132 ± 0.016
2	38.8 ± 13.6	39.3 ± 0.2	54.3 ± 7.4	0.79 ± 0.14	0.150 ± 0.008
3	47.7 ±16.7	46.2 ± 0.1	50.2 ± 5.4	0.66 ± 0.08	0.125 ± 0.011
4	48.5 ± 10.8	40.8 ± 0.3	56.3 ± 5.5	0.84 ± 0.09	0.112 ± 0.007
5	51.4 ± 9.7	54.9 ± 0.3	62.8 ± 3.6	1.02 ± 0.04	0.190 ± 0.015
6	53.2 ± 15.8	57.1 ± 0.6	33.3 ± 5.4	0.67 ± 0.12	0.291 ± 0.011
7	58.3 ± 13.2	65.1 ± 0.5	58.1 ± 4.1	1.74 ± 0.19	0.322 ± 0.011
8	64.6 ± 14.1	65.8 ± 0.3	65.7 ± 3.9	1.75 ± 0.18	0.371 ± 0.012
9	67.8 ± 13.8	58.1 ± 0.2	78.9 ± 2.7	2.12 ± 0.13	0.332 ± 0.002
10	78.8 ± 12.1	80.5 ± 0.4	69.4 ± 2.7	1.71 ± 0.05	0.566 ± 0.003

[a] Astringency intensity. [b] Tannic acid.

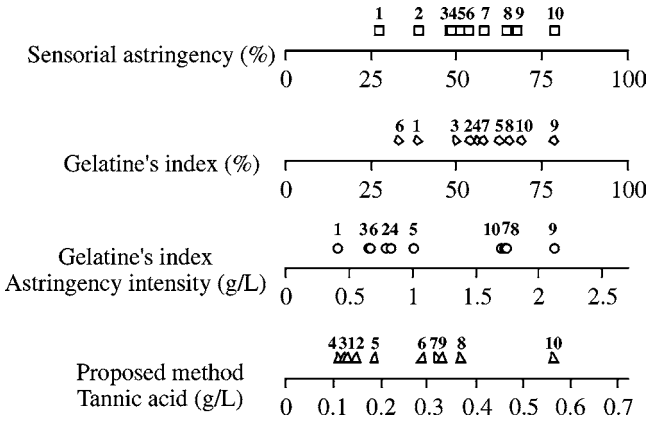


Figure 3. Wines in increasing order of astringency (relative comparison among the different methodologies).

their different phenolic compositions and sensorial astringency levels so that the real performances of both methods could be verified.

In general terms, the gelatin index and the proposed method seem to have the same tendency as sensorial astringency, which shows that it can be useful for analytical astringency estimation. However, if the wines were tested in order of increasing astringency, some important divergences between the different methodologies were detected (see **Figure 3**). The gelatin index gave a classification that depends on the unit of expression, which points out the necessity of comparing astringency estimation systems of expression to obtain the best optimized system closed to sensorial perception.

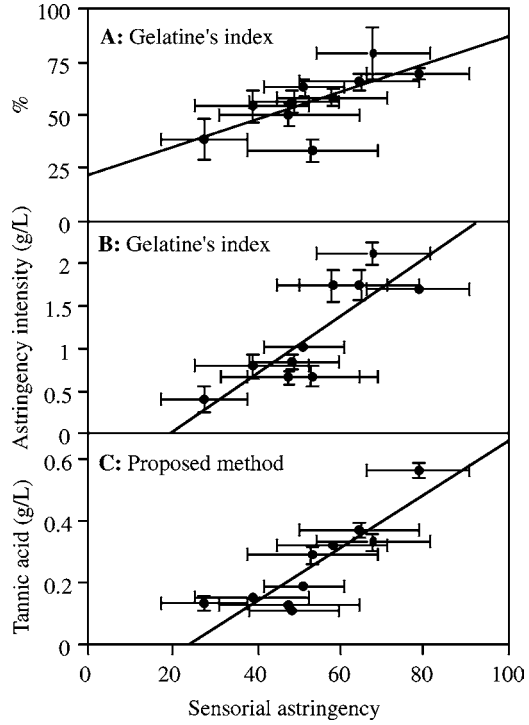


Figure 4. Comparison of sensorial astringency with the different analytical methods. Horizontal lines indicate the standard deviation for sensorial astringency estimation. Vertical lines indicate the standard deviation for the corresponding analytical method.

When the gelatin index was expressed as a percentage, considerable differences were found in sensorial estimation. In particular, the gelatin index considered wine 6 to be the least astringent, whereas sensorial analysis placed it in sixth position. On the other hand, sensorial analysis considered wine 2 to be the second least astringent, whereas the gelatin index put it in fourth position.

When the gelatin index was expressed as astringency intensity, the order of the wines seemed to be better than when it was expressed as a percentage. However, wines 6 and 10 were still a long way from their sensorial locations.

The proposed method seemed to be closer to the sensorial astringency estimation. Wines 5−10 were arranged in nearly the same order by the two methods. Only wines 8 and 9 changed their positions. However, the proposed method placed wine 10 relatively further away than sensorial analysis. On the other hand, the proposed method indicated that wines 1−4 had very similar astringencies, whereas sensorial analysis found certain differences.

Figure 4 compares sensorial astringency and the various analytical methods for the 10 wines. The graphs confirm the clear relationship between both analytical methodologies and sensorial analysis. However, some points should be made. Sensorial astringency estimation (horizontal lines) gave very high standard deviations. In general terms, the standard deviations (vertical lines) of all analytical astringency methods were lower than those of sensorial analysis. However, the standard deviations of the gelatin index were higher than those of the proposed method.

Sensorial astringency estimation has an average relative standard deviation of 25.8%. This value may be high because wine sensorial analysis is always subject to a certain subjectivity. Even well-trained wine tasters can confuse bitterness and astringency or have difficulty distinguishing between them (*30*). Furthermore, differences between the experts' salivary flow and

Table 2. Linear Regression Coefficients, Fisher's Correlation Coefficients, and *p* Values between Sensorial Astringency and the Different Analytical Methods

	linear regression coeff	Fisher's correl coeff	*p* value
gelatin index (%) vs sens anal.	0.5014	0.708	0.0191
gelatin index (AI[a]) vs sens anal.	0.7127	0.844	0.0011
proposed method (TA[b]) vs sens anal.	0.7737	0.879	0.0003

[a] Astringency intensity. [b] Tannic acid.

composition, as well as between oral gustatory and tactile sensitivities, may produce certain divergences (*31*). It has been reported that the intensity and duration of an astringent sensation increases with repeated ingestion (*32*). This may be of particular importance in our case, inasmuch as experts had to taste 10 wines consecutively.

The gelatin index had an average relative standard deviation of 11.3% when it was expressed as a percentage and 12.9% when it was expressed as astringency intensity. These values were lower than those for sensorial analysis, which indicate that this method was more reproducible. Nevertheless, the major analytical problem of the gelatin index is that it requires procyanidin concentration to be analyzed before and after precipitation with an excess of gelatin. Procyanidins are usually analyzed according to the method described by Ribéreau-Gayon and Stonestreet (*27*). Although this method is highly reproducible, it gives only an approximate result because it does not take into account the effect of the various structures present in wine or their degrees of polymerization or the other components in wine that interfere with the assay (*28*).

Furthermore, the gelatin index obviously needs to use gelatin. As gelatin is produced by the hydrolysis of collagen from different animal species, there are many gelatins on the market with a heterogeneous composition (*33*, *34*). It has been reported that the reactivity of procyanidins depends on the composition of the gelatin (*34*). In our case, all of the analyses were made with exactly the same gelatin from the same solution. It is logical to imagine that the variability among the compositions of commercial gelatins is also a source of variability and imprecision.

The proposed method presents an average relative standard deviation of 5.2%. This value is low and indicates the high reproducibility of the method, which is probably due to the fact that ovalbumin is a single protein and not a heterogeneous mixture of proteins, making the conditions of this assay more reproducible.

In general terms, the reproducibility of both of the analytical methods for estimating astringency seems to be higher than that of sensorial analysis. However, sensorial analysis must be used as the control reference for astringency estimation and any analytical method must be compared with it. **Table 2** shows the linear regression coefficient, Fisher's correlation coefficient, and the statistical significance (*p* value) between sensorial analysis and the analytical methods.

In all cases, there is a statistically significant correlation between the sensorial and analytical methods. However, Fisher's correlation coefficient was high and the *p* value was low for the proposed method. The gelatin index gives better results when it is expressed in terms of astringency intensity.

Although the linear regression coefficients indicate that the linear behavior of the analytical methods is not close to that of sensorial analysis, the proposed method does show the highest value.

As stated in the Introduction, astringency perception is associated with the ability of tannins to bind salivary proteins in the buccal cavity (*11*, *12*). However, current knowledge does not allow us to establish which proteins, tannins, and/or tannin combination are responsible for this phenomenon (*35*). Evidently, no method can substitute completely for sensorial analysis, but the proposed method is a reproducible index that correlates quite well with it.

CONCLUSIONS

All of the analytical astringency estimation methods studied in this paper present a statistically significant correlation with sensorial astringency. The proposed method, which uses ovalbumin as precipitation agent and tannic acid solutions as standards, has the lowest relative errors, the highest linear regression coefficient, the highest Fisher correlation coefficient, and the lowest *p* value. These results indicate that this method is more reproducible than the gelatin index and correlates better with sensorial analysis.

LITERATURE CITED

(1) Mazza, G. Anthocyanins in grapes and grapes products. *Crit. Rev. Food Sci. Nutr*. **1995**, *35*, 341−371.
(2) Bakowska, A.; Kucharska, A. Z.; Oszmianski, J. The effect of heating, UV irradiation and storage on stability of the anthocyanin-polyphenol copigment complex. *Food Chem*. **2003**, *81*, 349−355.
(3) Lea, A.; Arnold, G. M. The phenolics of ciders bitter and astringency. *J. Sci. Food Agric*. **1978**, *29*, 478−483.
(4) ATSM. Standard definitions of terms relating to sensory evaluation of materials and products. *Annual Book of ATSM*; American Society of Testing and Materials: Philadelphia, PA, 1989.
(5) Hagerman, A. E.; Butler, L. G. The specificity of proanthocyanidin−protein interactions. *J. Biol. Chem*. **1981**, *256*, 4494−4497.
(6) Zamora, M. C.; Guirao, M. Analysing the contribution of orally perceived attributes to the flavor of wine. *Food Qual. Pref*. **2002**, *13*, 275−283.
(7) Zamora, F. *Elaboración y Crianza del Vino Tinto; Aspectos Científicos y Prácticos*; A. Madrid Vicente Ediciones: Madrid, Spain, 2003.
(8) Fernández de Simon, B.; Hernández, T.; Cadahía, E.; Dueñas, M.; Estrella, I. Phenolic compounds in a spanish red wine aged in barrels made of Spanish, French and American oak wood. *Eur. Food Res. Technol*. **2003**, *216*, 150−156.
(9) McManus, J. P.; Davis, K. G.; Lilley, T. H.; Haslam, E. The association of protein with polyphenols. *J. Chem. Soc., Chem. Commun.* **1981**, 309−311.
(10) Minaguchi, K.; Bennick, A. Genetic human salivary proteins. *J. Dent. Res*. **1989**, *68*, 2−15.
(11) Baxter, N.; Lilley, T. H.; Haslam, E.; Williamson, M. P. Multiple interactions between polyphenols and salivary proline-rich proteins repeat result in complexation and precipitation. *Biochemistry* **1997**, *36*, 5566−5577.
(12) Prinz, J. F.; Lucas, P. W. Saliva tannin interactions. *J. Oral Rehabil*. **2000**, *27*, 991−994.
(13) Vivas, N; Glories, Y. Role of oak wood ellagitannins in the oxidation process of red wines during aging. *Am. J. Enol. Vitic*. **1996**, *47*, 103−107.
(14) Vidal, S.; Cartalade, D.; Souquet, J. M.; Fulcrand, H.; Cheynier, V. Changes in praonthocyanidin chain length in winelike model solutions. *J. Agric. Food Chem*. **2002**, *50*, 2261−2266.
(15) Bertuccioli, M.; Zini, S.; Siliani, A.; Picchi, M. Valutazione sensoriales dei risultati di tecniche di vinificazione. *Ind. Bevande* **2002**, 350−356.

(16) Valentovà, H.; Skrovánková, S.; Panovská, Z.; Pokorn×c6, J. Time-intensiy studies of astringent taste. *Food Chem*. **2002**, *78*, 29−37.
(17) Edelmann, A.; Lendl, B. Toward the optical tongue: Flow-through sensing of tannin-protein interactions based on FTIR spectroscopy. *J. Am. Chem. Soc*. **2002**, *124*, 14741−14747.
(18) Hagerman, A.; Butler, L. Determination of protein in tannin−protein precipitates. *J. Agric. Food Chem*. **1980**, *28*, 944−947.
(19) Bacon, J. R.; Rhodes, M. J. C. Development of a competition assay for the evaluation of the binding of human paratid salivary proteins to dietary complex phenols and tannins using peroxidase-labeled tannin. *J. Agric. Food Chem*. **1998**, *46*, 5083−5088.
(20) Chapon, L. Nephelometry as a method for studying the relations between polyphenols and proteins. *J. Inst. Brew*. **1993**, *99*, 49−56.
(21) Serafini, M.; Maiani, G.; Ferro-Luzzi, A. Effect of ethanol on red wines tannin−protein (BSA) interactions. *J. Agric. Food Chem*. **1997**, *45*, 3148−3151.
(22) Sarni-Manchado, P.; Cheynier, V.; Moutounet, M. Interaction of grape seed tannins with salivary proteins. *J. Agric. Food Chem*. **1999**, *47*, 42−47.
(23) De Freitas, V.; Mateus, N. Nephelometric study of salivary protein−tannin aggregates. *J. Sci. Food Agric*. **2001**, *82*, 113−119.
(24) Charlton, A. J.; Haslam, E.; Williamson, M. P. Multiple conformations of the proline-rich protein/epigallocatechin gallat complex determined by time-averaged nuclear overhauser effects. *J. Am. Chem. Soc*. **2002**, *124*, 9899−9905.
(25) Cliff, M.; Brau, N.; King, M. C.; Mazza, G. Development of predictive models for astringency from anthocyanin, phenolic and color analyses of British Columbia red wines. *J. Int. Vigne Vin* **2002**, *36*, 21−30.
(26) Glories, Y. La coleur des vins rouges, 2emè Partier. Mesure, origine et interpretation. *Connaiss. Vigne Vin* **1984**, *18*, 253−271.
(27) Ribéreau-Gayon, P.; Stonestreet, E. Le dosage des tanins du vin rouge et determination de leur structure. *Chem. Anal*. **1966**, *48*, 188−192.
(28) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. *Handbook of Enology*. *Vol. 2. The Chemistry of Wine Stabilisation and Treatments*; Wiley: Chichester, U.K., 2000.
(29) Oho, T.; Rahemtulla, F.; Mansson-Rahemtulla, B.; Hjerpe, A. Purification and characterization of a glycosylated proline-rich protein from huma parotid saliva. *Int. J. Biochem*. **1992**, *24*, 1159−1169.
(30) Kallithraka, S.; Bakker, J.; Clifford, M. N. Interaction of (+) cathechin, (−) epicathechin, procyanidin B_2 and procyanidin C, with pooled human saliva in vitro. *J. Sci. Food Agric*. **2000**, *81*, 261−268.
(31) Guinard, J. X.; Zoumas-Morse, C.; Walchak, C. Relation between parotid saliva flow and composition and the perception of gustatory and trigeminal stimuli in foods. *Physiol. Behav*. **1998**, *63*, 109−118.
(32) Lee, C. B.; Lawless, H. T. Time-course of astringent sensation. *Chem. Senses* **1991**, *16*, 225−238.
(33) Lagune, L.; Glories, Y. Les gélatines oenologiques: matière première, fabrication. *Rev. Fr. Enol*. **1996**, *157*, 35−38.
(34) Poinsaut, P.; Scotti, B. Clarification of wines with gelatin: science and tradition agree. *Enotecnico* **1998**, *34*, 89−96.
(35) Maury, C.; Sarni-Manchado, P.; Lefebvre, S.; Cheynier, V.; Moutounet, M. Influence of fining with different molecular weight gelatins on proanthocyanidin composition and perception of wines. *Am. J. Enol. Vitic*. **2001**, *52*, 140−145.

Received for review July 17, 2003. Revised manuscript received December 15, 2003. Accepted December 19, 2003. We thank CICYT (AGL 2001-0716) for financial support.

JF034795F