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Published on Web 11/13/2002

Toward the Optical Tongue: Flow-Through Sensing of Tannin−Protein Interactions Based on FTIR Spectroscopy

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Received March 25, 2002

Abstract: The interaction of polyphenols (tannins) with proline-rich proteins (gelatin) has been studied using an automated flow injection system with Fourier transform infrared spectroscopic detection to gain insight into chemical aspects related to astringency. In the perception of astringency, a major taste property in red wines and other beverages such as beer, tea, or fruit juices, an interaction between proline-rich salivary proteins and tannins present in the sample takes place. To study this interaction, agarose beads carrying gelatin (a proline-rich protein) were placed in the IR flow cell in such a way that the beads were probed by the IR beam. Using an automated flow system, we injected samples in a carrier stream and flushed over the proteins in a highly reproducible manner. Simultaneously, any retardation due to tannin−protein interactions taking place inside the flow cell was monitored by infrared spectroscopy. Tannins of different sources (grapes, wooden barrels, formulations used in wine making) were investigated, and their flow-through behavior was characterized. Significant differences in their affinity toward gelatin could be observed. Furthermore, because of small but characteristic differences in the IR spectrum, it is possible to distinguish condensed from hydrolyzable tannins. Nonastringent substances such as alcohols, sugars, and acids did not show retention on gelatin. The selectivity of the flow-through sensor was also demonstrated on the example of red and white wine. In contrast to white wine, where no interaction could be observed, in red wine a major interaction of the red wine tannins was found.

Introduction

Mimicking the human olfaction and taste system, the nose and the palate, is a scientific challenge with important application potential in the food and beverage industry. To do so, attributes such as smell and taste and consequently their quality and intensity need to be measured. In the case of smell analysis, electronic noses consisting of gas sensor arrays have been developed. The sensors of the array differ in their chemical selectivity toward gases. The response of the whole sensor array is evaluated by pattern recognition and multivariate chemometric methods. So far, electronic noses have already proven their applicability for, for example, food quality control,[1] environmental monitoring,[2] and medical purposes.[3] Electronic noses are by far more elaborated than their counterparts for taste measurement (electronic tongues or taste sensors); however, recently some concepts for liquid analysis have been described[4−7] in the literature. The goal to develop biologically relevant taste sensors resulted in the development of analysis systems providing information related to the five tasting senses: sour, salty, sweet, bitter, and umami. These taste sensors are focused on giving qualitative answers, rather then determining the concentration and identity of single analytes. The sensor arrays used for liquid sensing are mainly based on electrochemical methods such as potentiometry and voltammetry and read out similarly to electronic noses. However, recently also spectroscopic methods have been applied,[8,9] where a sensor array based on UV and fluorescence spectrometry was developed for multianalyte detection including sugars, acids, and metal cations.

The sensation of astringency on the human palate is not a taste itself; it has rather been defined as the complex of oral sensations due to shrinking, drawing, or puckering of the epithelium as a result of exposure to substances such as tannins.[10] In the perception of astringency, saliva plays a dominant role. The salivary proteins chemically interact with many tastants in foods. In particular, proteins rich in proline

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and hydroxyproline (PRPs) have been shown to interact with tannins in food and beverages.[11,12] For example, the taste threshold of tannic acid in mice is directly dependent on the ability of PRPs to bind tannic acid.[13] The sensation of astringency on the human palate is rather complex and not necessarily experienced in isolation. Even well-trained panelists might confuse bitterness and astringency or have difficulties distinguishing between them.[14] Therefore, an objective analytical tool to measure the quality of astringency is of greatest importance not only to assess the quality of foodstuffs but also to elucidate the nature and binding of astringent substances. Different approaches to assess astringency have been published during the past years. Protein−tannin interactions are commonly measured using assays based on the competition of a test protein with a standard labeled protein to bind and coprecipitate with the tannin.[15,16] Also, precipitation assays, where proteins and tannins are mixed and incubated, can be found in the literature.[17−19] However, although proteins and tannins form complexes, such interactions do not necessarily lead to precipitation.[20] Because protein−tannin interactions related to astringency may not only involve insoluble complexes,[19] precipitation assays may not always be appropriate.[21] In a recent study, it could be shown that the amount of precipitates could not be compared with the relative astringency reported from the taste panelists.[14] Therefore, a method, which does not only rely on precipitation of the protein, may be of great interest to elucidate the problem of astringency. Immobilization of the protein on a solid inert matrix is definitely an interesting approach, because soluble complexes can also be evaluated. Hoff and Singleton[22] presented a method where small columns of BSA immobilized on Sepharose were utilized to achieve separation between tannins and other nontannin compounds.

As the papilla grooves on the human tongue are narrow and deep, slow diffusion cannot explain the rapid entry of tastants; therefore, a pumping action during tissue movement is necessary.[23] The focused tasting of food as it is the case in wine tasting is not necessarily a continuous process; rather a portion of substance is introduced into the mouth and sensed by the taste and tactile receptors. In principle, the tasting process can be compared with the concept of the flow-through sensor, where the sample retention and detection are built into one single unit.[24] The analyte is retained on the active material at the place of measurement and removed after analysis, as this is the case on the human palate. In protein binding competition experiments,[15,21] it could be shown that after rat parotid proline-rich protein, gelatin had the highest affinities to sorghum tannin and tannic acid among the proteins tested. Therefore, the PRP gelatin (proline content typically 16%) was selected in the present work, to mimic the parotid salivary proteins. In the present study, we combined the concept of flow-through sensors with the concept of protein−tannin interactions to develop a tool for the simulation of the sensation of astringency. We recently showed that FTIR spectroscopic analysis of the total phenol content of red wines by FTIR spectroscopy is a rapid method for discrimination of wine varieties.[25] Because tannins as they occur in nature are very complex in their composition, FTIR spectroscopy was chosen also here as a detector to obtain information on the kinetics of the interaction between tannins and gelatins and to be able to distinguish between the different tannins interacting with the proteins.

Experimental Section

A. Reagents. Agarose beads (Gelatin Sepharose 4B) were purchased from Pharmacia Amersham Biotech (Uppsala, Sweden). The commercial tannin preparations were obtained from Laffort Œnologie (Bordeaux, France) and Esseco (San Martino Trecate, Italy). Grape seed tannin was from Tunisian origin. The wine samples were provided by wineries located in Carnuntum, Austria.

B. Apparatus. Flow-Through Cell. A standard Perkin-Elmer flow cell with 2 mm thick CaF_2 windows equipped with a 50 μm Teflon spacer was modified by placing a small amount (10 μL) of agarose beads suspension between the windows. The agarose beads have a size of approximately 100 μm. They form small gellike disks when squeezed between the windows and are held securely in place. More details on the preparation of flow-through cells can be found in previous publications.[24,26]

FTIR Spectrometer and Automated Flow Injection (FI) Manifold. For all measurements, a Bruker IFS 88 FTIR spectrometer (Bruker, Karlsruhe, Germany) equipped with a mercury cadmium telluride (MCT) detector was used. For the control of the spectrometer, the Software package OPUS 3.0/IR (Bruker) was used. All spectra were recorded at a resolution of 8 cm^{-1} from 1900 to 900 cm^{-1} (128 scans, apodization function: Blackman-Harris-3-term). A low pass filter with a 5% cut on at 1900 cm^{-1} was employed to increase the signal-to-noise ratio in the spectral range of interest (1900−900 cm^{-1}).[27] During the course of analysis, spectra were recorded by means of the incorporated OPUS-chromatography-IR-software package. The FI-manifold was set up with a CAVRO (Sunnyvale, CA) XP 3000 syringe pump (syringe size: 2.5 mL) and a Valco (Houston, TX) 14-port selection valve equipped with an electric micro actuator. Polytetrafluoroethane (PFTE) tubing (i.d. 0.75 mm) and fittings were obtained from Global FIA (Gig Harbor, WA). The whole setup including triggering the data acquisition was controlled with the AnalySIA software package (Center for Biotechnology, Åbo Akademi University, Turku, Finland). Automated spectra acquisition was obtained by a custom-made macro (OPUS Macro Language software of the spectrometer), which was triggered by TTL-signals from the AnalySIA control software.

C. Procedure. The FI-manifold (Figure 1) enables automated variation of analysis parameters such as flow rate and injection volumes of the reagents. All of the measurements were performed using an injection volume of 500 μL and an injection speed of 250 μL/min. The procedure included conditioning of the beads, sample aspiration, and injection followed by washing with 2000 μL of water (removal of nonbinding components). After analysis, the beads were washed with

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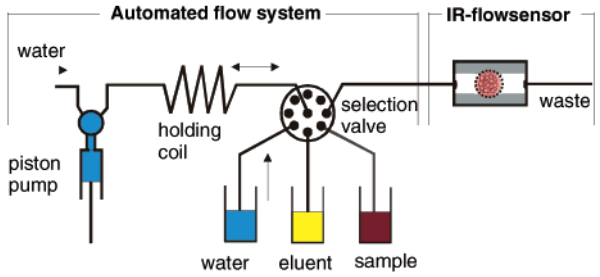

Figure 1. Scheme of the experimental setup consisting of an automated flow system and a IR-flow-through sensor containing gelatin immobilized on agarose beads.

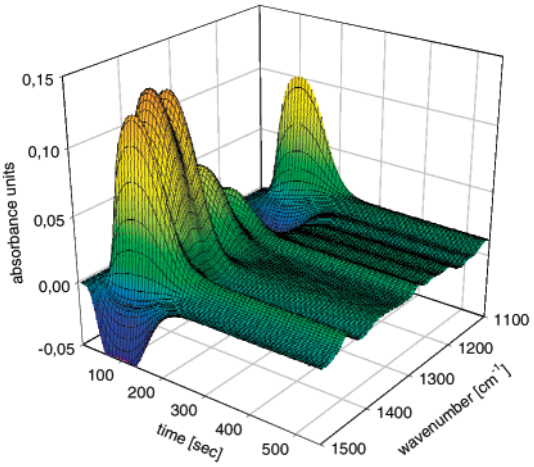

Figure 2. 3-D plot of red wine response during the course of analysis. Both spectral and time information can be obtained.

500 μL of dimethylformamide and reconditioned with 2000 μL of water. The background spectrum was recorded during water flow immediately before injection of the sample. IR spectra were continuously recorded during the injection and the subsequent washing step. Thus, two-dimensional data are obtained, where both spectral and time information are accessible (Figure 2). The spectral region between 1560 and 960 cm^{-1} with a baseline point at 1800 cm^{-1} was integrated to obtain an overall trace of the experiment. All spectra shown below were taken at 500 s, where all nonbinding components such as sugars, organic acids, and alcohols were removed.

Results and Discussion

A. Development of a Mid-IR-Flow-Through Sensor for Tannins. Applicability of the Support Agarose. Agarose beads are widely used in various chromatographic applications. Their inert nature makes them an ideal support for immobilization of ligands,[22] and unspecific binding is generally negligible. However, the support was tested for its applicability to this specific application. Sepharose 4B was prepared as described above and subjected to grape seed tannins (1.5 g/L) dissolved in 10% ethanol. As shown in Figure 3, in the case of pure agarose beads no grape seed tannin was present in the flow-cell 500 s after sample injection, whereas in the case of gelatin agarose beads a significant retention of the tannins could be observed at this time.

This experiment proved that agarose could be used as an inert support matrix for gelatin.

Tannin Binding Properties and Recovery of Gelatin. Tannic acid is a strong astringent, and it was found to have the highest affinity to gelatin and salivary proteins in competition

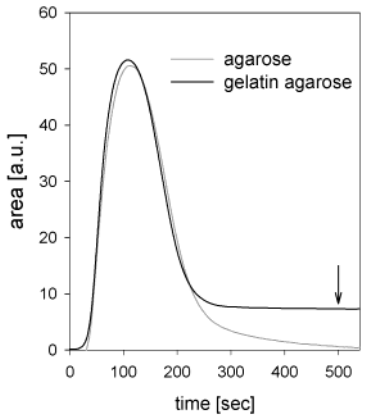

Figure 3. Comparison of the flow-through recordings obtained for a standard (1.5 g/L of grape seed tannin in 10% ethanolic solution) using gelatin-containing and pure agarose beads as measured with the flow-through sensor. The traces were calculated from the recorded IR spectra by integration of the recorded intensities between 1560 and 960 cm^{-1} with a baseline point at 1800 cm^{-1}. The arrow indicates the time at which IR spectra were taken for data evaluation.

assays.[21,28] Because of its high affinity toward PRPs, tannic acid was utilized as the standard tannin in the experiments described below. To prove the species bound to the sensor, spectra of the bound tannin were compared with spectra of tannin recorded in the same flow-through cell but without beads. The same concentration, injection volume, and flow rate were used in both experiments. The spectral features of both spectra were almost identical, proving that tannic acid was actually retained on the protein. Certainly the applicability of the new sensor is mainly dependent on the feasibility of regeneration and multiple use of the flow sensor. Quantitative elution and recovery of tannins from BSA-Sepharose were obtained with solvents containing dimethylformamide.[22] Also, Siebert et al. suggested dissolving protein−tannin haze with dimethylformamide.[29] In our experiments, 500 μL of the pure solvent followed by 2 mL of distilled water were sufficient for quantitative elution of all tannins investigated. Complete removal of the tannins was checked by comparing the spectra of the sensor before injection of the tannins to spectra taken after the elution and washing step. The sensitivity of the flow-through sensor over time was checked by comparing the spectral intensities obtained upon multiple analysis of tannic acid. The results showed that there was no loss in sensitivity during 30 repetitive analyses.

B. Tannins and Their IR Spectra. Two distinct phenol classes occur in grapes and wines, the flavonoid-derived condensed and the nonflavonoid-derived hydrolyzable tannins.[30] Flavonoids may exist as monomers or polymerized (procyanidins) and are primarily derived from the seeds and skins of the fruit.[30] In red wines, they commonly constitute more than 85% of the phenol content.[30] Hydrolyzable tannins in wine are mainly polymers of ellagic (stable dilactone of hexahydroxydiphenic acid) or gallic and ellagic acids with glucose[30] and are extracted during aging in oak barrels. Structural information of both tannin classes together with their main building blocks is given in Figure 4.

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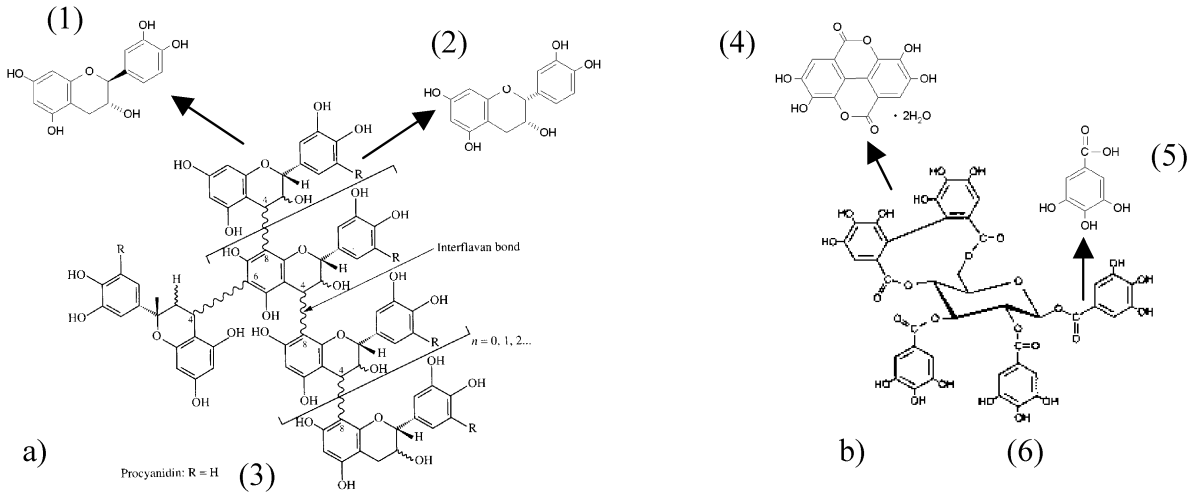

Figure 4. Structures of the two classes of tannins found in wine. (a) Flavonoids or condensed tannins: Structure of procyanidins (3) and their building blocks catechin (1) and epicatechin (2). (b) Ellagi-(Gallo)-tannins or hydrolyzable tannins: Example of monomeric ellagitannin (6) and its building blocks ellagic acid (4) and gallic acid (5).

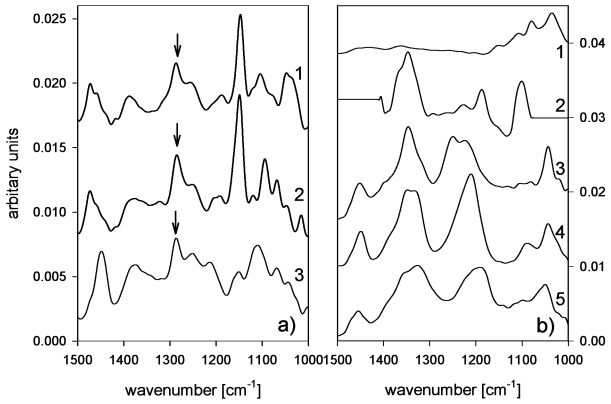

Figure 5. Comparison of the two classes of tannins recorded in aqueous solution. (a) Spectra of grape seed tannin (3) and the corresponding monomers catechin (1) and epicatechin (2). (b) Spectra of ellagitannins (5), tannic acid (4), gallic acid (3), ellagic acid (2), and glucose (1). Ellagic acid recorded in methanol (because of the high absorbance of the solvent methanol above 1400 cm^{-1} and below 1100 cm^{-1}, the noise in the spectrum of ellagic acid was erased and substituted with flat lines for better visualization). The arrow shows the marker band at 1285 cm^{-1} for the flavonoids.

It was recently shown[31] that the chemical and physical properties of hydrolyzable tannin−protein complexes differ from those of condensed tannin−protein complexes. While pentagalloyl glucose (hydrolyzable tannin) precipitates by forming a hydrophobic coat around the protein, epicatechin$_{16}$ (procyanidin) was suggested to form hydrogen-bonded cross-links between protein molecules. Consequently, different classes of tannins might also differ in their astringent qualities. In this context, Gawel et al.,[32] who developed − in analogy to the already well-known aroma wheel − a mouthfeel wheel to categorize different subqualities of astringency, speculated that the category "graininess" might describe hydrolyzable ellagitannins. Spectra of both types of tannins together with their main building blocks are given in Figure 5. By means of IR spectroscopy, the structural origin of different tannins can be clearly distinguished. This is particularly true for the procyanidins, where both the monomers catechin and epicatechin and the polymers (oligomers) exhibit the same characteristic spectral features (Figure 5a). In particular, the peak around 1285 cm^{-1} is very strong and indicates a characteristic feature for the flavonoid-based tannins. This peak was assigned to the ethereal C−O stretching vibration[33] arising from the pyran-derived ring structure of this class of tannins. By focusing on this characteristic marker band, which is absent in the spectra of hydrolyzable tannins, it was possible to distinguish between the two classes of tannins. In Figure 5b, the class of hydrolyzable tannins is illustrated and compared with their building blocks. As can be seen, the peak at 1285 cm^{-1} found in the flavan-3-ols and procyanidins is not present in any of the spectra. Generally, two broader peaks at around 1350 cm^{-1} and between 1290 and 1150 cm^{-1} can be observed in the spectra of gallic acid, tannic acid (gallotannin), and in the ellagitannin preparation with shifts in exact peak position and deviations in peak shape. Both peaks can be assigned to the combination of C−O stretching and OH deformation vibrations.[33] It should be mentioned that, instead of hexahydroxydiphenic acid, its stable dilactone ellagic acid had to be used here. Because of insufficient solubility in water, the spectrum of ellagic acid was recorded in methanolic solution. Spectra of all substances shown in Figure 5 but recorded in methanolic solution are available in the Supporting Information (Figure S1). As illustrated by the similarities of the IR spectra of tannic acid (mainly pentagalloyl glucose) and its building blocks gallic acid and glucose, we can conclude that the class of hydrolyzable tannins can be identified on the basis of their IR spectra. As already briefly discussed by Gawel et al.,[32] different sensory properties of the two tannin classes might be explained by their different interaction mechanism with the protein, which results from their different chemical compositions. The differences in chemical composition, however, can also be seen directly in their IR spectra as shown above. Therefore, we propose that there is a correlation between the sensory properties of the tannins and their IR spectra.

C. Application of the Developed Sensor. Nonastringent Substances. Red wine has a rather complex composition. In addition to polyphenolic compounds, red wine contains organic

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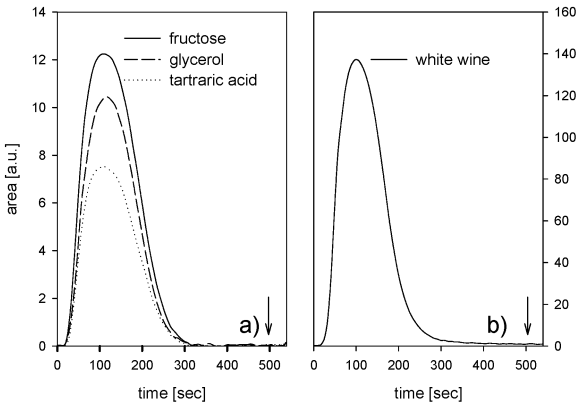

Figure 6. (a) Nonastringent substances on the example of fructose, glycerol, and tartaric acid subjected to the sensor. No interaction between the gelatin and major components of wine such as sugars, organic acids, and alcohols was observed. (b) White wine. No components of the wine matrix were retained. Integration limits for calculation traces for (a) and (b) were 1560−960 cm^{-1} with a baseline point at 1800 cm^{-1}. The arrows indicate the time at which IR spectra were taken for data evaluation.

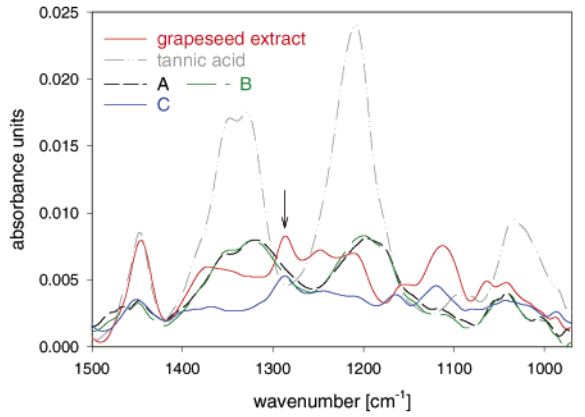

Figure 7. Spectra of tannin samples (0.5 g/L) as retained on the immobilized protein after 500 s. The arrow shows the marker band at 1285 cm^{-1} for the flavonoid-derived tannins.

acids such as tartaric, lactic, and acetic acid, residual sugars (fructose and glucose), and alcohols such as ethanol and glycerol as further major and minor constituents. It is commonly accepted that these substances do not exhibit astringent properties. The retention characteristics of single wine compounds tartaric acid, fructose, and glycerol, but also of white wine, were investigated. As illustrated in Figure 6, no retention of the tested compounds and white wine could be observed, which is in excellent agreement with their gustatory perception.

Tannin Samples. Commercially available enological tannin preparations (0.5 g/L) from different origins were compared with tannic acid and grape seed extract with respect to their affinity toward gelatin. The spectra obtained 500 s after sample injection showed that tannins adsorbed on the beads (Figure 7). As can be seen from the spectra, both classes of tannins are present. Samples A and B can be clearly assigned to the class of hydrolyzable tannins, whereas sample C shows the characteristic marker band at 1285 cm^{-1} arising from the flavonoid-derived tannins. This result is in agreement with the claims of the producers who referred to samples A and B as oak tannins and to sample C as predominantly procyanidins. For the evaluation of the affinity toward the protein, the peak areas of the whole spectral region between 1500 and 1080 cm^{-1} were calculated. To compensate for varying extinction coefficients, reference

Table 1. Degree of Retention of Different Tannin Preparations[a]

	I_P[b]	I_R[b]	DR	S(DR)[c]	DR/DR_{TA}
tannic acid (TA)	2.70	1.63	1.65	0.06	1.00
grape seed extract	1.33	1.26	1.06	0.04	0.64
A	1.24	1.47	0.84	0.04	0.51
B	1.31	1.55	0.85	0.02	0.51
C	0.50	0.97	0.51	0.02	0.31

[a] I_P: Integration results of spectra from tannins as retained on the protein 500 s after injection. I_R: Integration results of the reference spectra. $DR = I_P/I_R$: Degree of retention obtained by rating I_P to I_R. DR/DR_{TA}: Degree of retention relative to highest DR (tannic acid). [b] Integration limits: 1500−1080 cm^{-1}. [c] Standard deviation.

spectra of all tannin samples were recorded with a conventional transmission cell at the same optical path (50 μm) and were integrated using the same integration parameters. By dividing the corresponding peak areas, it was possible to normalize the integration results obtained with the flow-through sensor and to determine the degree of retention for each sample. Significant differences in the degree of retention and thus in the protein binding properties of the samples were obtained (see Table 1). It was found that tannic acid as revealed by the highest degree of retention was the most reactive tannin. To be able to compare the relative values, the degree of retention of each sample was divided by the value found for tannic acid. All samples were analyzed in triplicate, and in all cases the relative standard deviation was less than 5%. The degree of retention of the tannins relative to tannic acid decreased as follows: tannic acid $\gg$ grape seed extract $>$ A $=$ B $>$ C (see Table 1). It was remarkable that samples A and B exhibited the same affinity toward the protein, although they were obtained from different producers. We propose to correlate the measured degree of retention with the sensory property astringency, as the underlying chemical process for both parameters is practically the same. The obtained ranking of the sample in terms of astringency was confirmed by sensory studies performed by a panel of six judges using solutions of 50 mg/L of tannins.

The enological tannin preparations A and B were described as drying, furry, and coating the tongue (characteristic indications of astringency), A also as bitter, whereas sample C was perceived bitter and rather sour. However, according to the tasting panel, the long duration of bitterness on the palate interfered with the perception of astringency. In other sensory studies where the intensity of astringency of grape seed tannin was compared with tannic acid, it was found that grape seed tannin exhibits approximately 70% of the intensity of tannic acid at a concentration of 0.5 g/L in aqueous solution, which is also in good agreement with our findings.[34]

Flavan-3-ols Catechin and Epicatechin. The reactivity of the monomers (+) catechin and (−) epicatechin toward proteins and the oral sensation of them regarding astringency are rather controversial in the literature, because the human palate might confuse astringency and bitterness. The sensory properties of (+) catechin and (−) epicatechin were studied in ethanolic solution, where both monomers were found to exhibit bitterness and astringency.[35] Other recent sensory studies suggest that the flavan-3-ols are more bitter than astringent.[36] However, it is

(34) Naish, M.; Clifford, M. N.; Birch, G. G. *J. Sci. Food Agric.* **1993**, *61*, 57−64.
(35) Thorngate, J. H., III; Noble, A. C. *J. Sci. Food Agric.* **1995**, *67*, 531−535.
(36) Kielhorn, S.; Thorngate, J. H., III. *Food Quality and Preference* **1999**, *10*, 109−116.

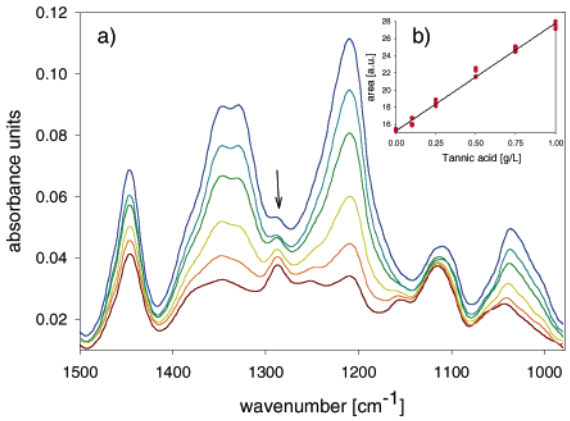

Figure 8. (a) Red wine spiked with tannic acid (concentrations: 0, 0.1, 0.25, 0.5, 0.75, 1 g/L. As indicated by the arrow, the characteristic band for the flavonoid-derived tannins is present in all spectra. (b) Linear correlation between the concentration of tannic acid and sensor signal. The integration results (areas) were calculated using the integration limits from 1560 to 960 cm^{-1} with a baseline point at 1800 cm^{-1}.

generally accepted that the astringent properties increase with increasing molecular weight. The monomeric flavan-3-ols have molecular weights less than 500 and do not precipitate proteins; therefore, they do not belong to the class of tannins. However, with the newly developed sensor, affinities toward proteins without the need of precipitation can be studied, which is highly relevant in the case of the nonprecipitating monomers. In our experiments, 1 g/L of substance in 10% ethanolic solution was investigated. No retention of flavan-3-ols on the protein could be obtained, suggesting that the monomers cannot exhibit significant sensory astringency.

Real Red Wines. The newly developed sensor was also applied to real red wines. First, the dynamic range of the sensor was investigated by analyzing red wines spiked with tannic acid (Figure 8). As can be seen, the increase in tannic acid concentration is reflected in the recorded intensities with good linear correlation ($r^2 = 0.991$). Similar experiments were performed using grape seed tannins for standard addition (0−2 g/L). Also, in this case a linear correlation was found ($r^2 = 0.997$). Typical phenolic contents of Austrian red wines can be found between 0.5 and 3 g/L; thus, the whole range of phenolic concentrations present in wines could be covered. It is important to note that the findings above are in agreement with sensory data. Robichaud and Noble studied the correlation of astringency and concentration of grape seed tannin and tannic acid in white wine matrix,[37] where they found that the astringency of both tannins increased as a linear function of their concentration (concentrations studied: 0−1.5 g/L).

In Figure 9, the result spectra of red wines originating from different grape varieties and aging regimes together with grape seed tannin in 10% ethanolic solution are displayed. As already shown in the example of white wine, the nonastringent components of red wine including organic acids, carbohydrates, and alcohols did not retain on the sensing protein. Red wine components of polyphenolic origin, however, interacted with the sensing protein as illustrated in Figure 9. As the sample volume was constant in these experiments, the spectral intensities give information on the amount of tannins and hence the astringency of the wine.

(37) Robichaud, J. L.; Noble, A. C. *J. Sci. Food Agric.* **1990**, *53*, 343−353.

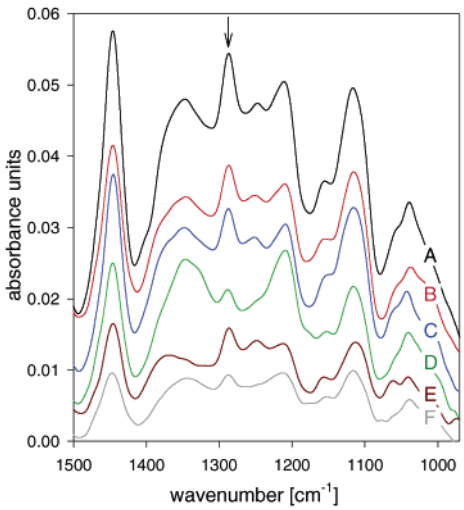

Figure 9. Spectra of the protein reactive fraction of wines taken at 500 s after injection: (A) *Cabernet Sauvignon* (1 month in new oak), (B) *Merlot* table wine (2 years), (C) *Zweigelt* during fementation, (D) *Zweigelt* (9 month aged in new oak and tannin treatment), (E) grape seed tannin 0.5 g/L in 10% ethanolic solution, (F) *Blauer Portugieser* (9 month, tannin treatment, already bottled). For better visualization, an appropriate offset was used.

The spectra of the wines generally agree with the standard grape seed tannin; spectral differences will be discussed in detail below. The wines showed significant differences in the quantity of the retained tannins. The young, tannin-rich, somewhat harsh *Cabernet Sauvignon* showed the highest amount of tannin retained on the protein of the sensor, and the *Blauer Portugieser*, a very supple table wine with little and soft tannins, showed the smallest amount of tannins retained on the protein. The tannin reactivity of the *Zweigelts* and the *Merlot* could be found in between. These findings are consistent with our expectations after we tasted the wines. However, as already mentioned above, not only differences in overall intensities but also in the spectral features can be observed. The *Zweigelt* (sampled during fermentation) and the *Merlot* table wine showed very similar characteristics, as well in the spectral features as in the tannin quantity, which retained on the beads. Both wines were neither aged in oak, nor treated with enological oak tannins. It is very interesting to note that the spectra of these wines correlated best with the spectra of the grape seed tannins. The wines, which are aged in oak and/or treated with enological oak tannins, additionally showed characteristics of the hydrolyzable tannins in their spectra. As can be seen in Figure 8, where red wine was spiked with increasing amounts of tannic acid, the spectral features of the hydrolyzable tannins manifest themselves by shifting peak positions as it is obtained in the wines subjected to oak tannins. This is particularly true for the *Zweigelt* sample D, where significant contributions of hydrolyzable tannins can be found both in the spectra and in the sensory characteristics when tasted. Because of these results, it can be concluded that the newly developed sensor can be applied not only to standard samples but also to real wines. Additionally, different styles of wines are directly reflected in their IR spectra.

Conclusions

The sensor for assessing the main sensory property of tannins, astringency, was developed on the basis of the underlying chemical reaction between the tannins and the proline-rich proteins in the saliva. It could be shown that different tannins

exhibit different affinities toward the immobilized protein. Tannic acid was shown to be the strongest astringent, which is in excellent agreement with sensory studies. Differences in the chemical composition of the tannins as reflected by their IR spectra may also lead to differences in the sensory perception of astringency. Therefore, it can be assumed that there is a correlation between the sensory properties of the tannins and their IR spectra. The sensor was also applied to real red wines. It could be shown that red wine showed similar spectral features as the grape seed tannins, which demonstrates the selectivity of the sensor in a rather complex matrix. Additionally, sensory perceptible practices during wine aging, such as the use of oak, were directly accessible in the IR spectra. Nonastringent substances such as alcohols, acids, and carbohydrates as demonstrated on the example of white wine did not interact with the sensing protein.

Acknowledgment. The authors thank Dr. Erich Leitner and the tasting panel for the sensory analysis of the enological tannin samples. Financial support of this work by the FWF Austrian Science Fund within the projects P13868 and P13350 is gratefully acknowledged.

Supporting Information Available: Experimental spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA026309V