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Prediction of perceived astringency induced by phenolic compounds

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Abstract

A method which can be used to estimate perceived astringency due to polyphenolic compounds is presented here. Thirty subjects were selected on the basis of them having similar salivary flows and they were trained to rate the perceived astringency of tannic acid and grape seed extract solutions. A scale of phenolic compound concentrations ranging from 0 to 3.2 g/L was selected in order to obtain an experimental curve describing the perceived intensity of the sensation. The same astringent solutions were added to a mucin solution in conditions resembling those present in the oral cavity. The formation of polyphenol–protein complexes was measured on the basis of the increasing turbidity of the reaction mixture and was expressed in terms of nephelometric turbidity units (NTU). Experimental curves describing NTU vs polyphenol concentration were obtained. Predictive models of astringency intensity vs NTU were produced. The predictive capacity of the models was checked by comparing the measured and predicted intensities of a set of samples prepared at phenolic compound concentration level varying from 0.94 to 2.13 g/L.

Keywords: Polyphenols; Proteins; Mucin; Sensory evaluation; Astringency; Nephelometry

1. Introduction

The sensation of astringency on the human palate has been defined as a complex group of sensations involving dryness of the oral surface and tightening and puckering sensations of the mucosa and muscles around the mouth (Gawell, Oberholster, & Francis, 2000; Lee & Lawless, 1991). It is generally accepted that astringent compounds form complexes with salivary proteins, resulting in their aggregation and/or precipitation and the loss of their lubricating properties (Kallithraka, Bakker, & Clifford, 1998). These interactions have led to the theory that astringency is, at least initially, due to de-lubrication via the removal of slippery coating on the oral surface. Mechanoreceptors stimulation might contribute to this sensation (Thorngate & Noble, 1995). It has also been suggested that other factors such as the presence of precipitate on the tongue and on the soft palate might contribute to the sensation. Moreover, traces of astringent substance remaining in solution might interact with taste receptors and might stimulate the classical taste pathways and contribute to the overall sensation (Critchley & Rolls, 1996). The formation of soluble complexes of polyphenol–protein able to modulate salivary viscosity has also been proposed as a factor affecting astringency perception (Clifford, 1997). The reintroduction of various sort of lubricants such as gums, polysaccharides and proteins alleviates astringency after it has been elicited (Brannan, Setser, & Kemp, 2001; Colonna, Adams, & Noble, 2004).

A recent study pointed out the importance of individual saliva flow and composition variations in influencing the intensity of the perceived sensation (Horne, Hayes, & Lawless, 2002).

The intensity of perceived astringency plays a key role in determining the acceptability of various food products and is produced by a variety of oral-chemical stimuli such as aluminium salts, acids and polyphenols. Several studies have been performed to mimic the polyphenol–protein reaction which takes place in the oral cavity in order to produce chemical–physical responses that can be correlated to the astringency inducing capability of compounds under consideration. The in

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vitro reactivity of polyphenols with various proteins has often been used for this purpose (Bacon & Rhodes, 1998; de Freitas & Mateus, 2001a; Edelmann & Lendl, 2002; Glories, 1978; Sarni-Manchado, Cheynier, & Moutounet, 1999). The formation of complexes between polyphenols and purified salivary proteins (Horne et al., 2002; Kallithraka, Bakker, & Clifford, 2000) as well as gelatin (Siebert, Troukhanova, & Lynn, 1996), bovine serum albumine (de Freitas & Mateus, 2001b; de Freitas, Carvalho, & Mateus, 2003; Naczk, Amarowicz, Zadernowski, & Shahidi, 2001) and other plant proteins (Asquit et al., 1987; Luck et al., 1994), can lead to haze and sediment development in the reaction mixture. The curvilinear relationship found between both protein and polyphenol concentrations and haze formation produced a polyphenol–protein model of reactivity analogous to the antigen–antibody model in which each polyphenol molecule is viewed as having a fixed number of binding ends and each reactive protein is seen as having a fixed number of polyphenol binding sites (Siebert et al., 1996). The stages occurring in the binding and precipitation of polyphenol–protein complexes are schematised in Fig. 1: proteins and polyphenols combine to form soluble complexes and these can grow to colloidal size at which point they scatter light and become larger still which can lead to sediment formation (Charlton et al., 2002).

Experimental relationships were found between turbidity development in human saliva and tannic acid mixtures prepared over a range of concentrations known to elicit astringent sensations (Horne et al., 2002; Kallithraka et al., 1998; Kallithraka, Bakker, Clifford, & Vallis, 2001; Lawless, Hartono, Horne, & Siebert, 1999).

Based on the literature cited above, it is possible to assert that turbidity measurement has proved to be a very sensitive and simple technique in measuring the reactivity of polyphenol–protein in order to determine to what extent single phenolic compounds are able to bind both a selected model protein or human salivary proteins.

The aim of this study is to provide an in vitro assay which can correlate turbidity measurements with astringency sensory response in order to obtain a prediction of perceived astringency induced by specific phenolic compounds. This assay should find its application in the possibility of estimating the potential contribution to astringency of different phenolic classes in food products, with particular reference to red wine.

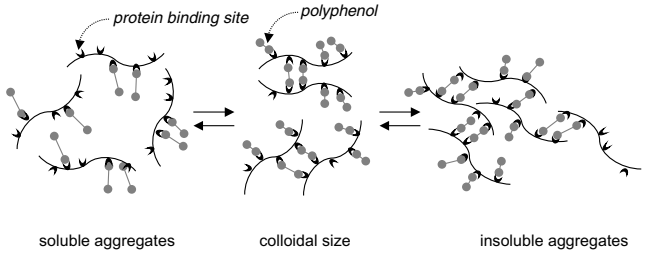

Fig. 1. Schematic representation of stages occurring in binding an precipitation of polyphenols by proteins. Polyphenols are represented as bi-dentate linkers and reactive proteins as having a fixed number of binding sites.

For this purpose the relationship between astringency induced by polyphenol solutions and their reactivity with a model protein has been systematically investigated.

The experimental plan consisted of four stages:

1. A first stage to study the perceived astringency intensity in relation to selected polyphenol solution concentrations (training set sample selection).
2. A second stage to study the reactivity of selected polyphenol solutions with a model protein by nephelometric measurement of the resulting turbidity (setting up an in vitro assay).
3. A third stage to build up an astringency predictive model based on the relationship between the perceived intensity of the selected stimuli and the turbidity development in the in vitro assay (building up a predictive model).
4. A fourth stage to validate the predictive capacity of the model by comparing predicted and measured astringency intensities in test set samples (predictive model validation).

2. Materials and methods

2.1. Samples

Tannic acid (TA, Sigma-Aldrich) and Grape Seeds extract (GSE, Inntec, Verona, Italy) were selected as phenolic compounds for astringency stimulation. The amount of phenolics in samples was determined according to the Folin–Ciocalteau method (Off. J. Eur. Communitie, 1992) and expressed as catechin (g/L). TA and GSE contain 0.85 and 0.75 g of phenolics per gram of material, respectively. In the present work, sample concentration (g/L) of phenolic compounds was always expressed in terms of phenolic content.

Sample solutions were prepared by dissolving TA and GSE in 1% ethanol. All samples were prepared approximately 2 h prior to testing.

Training set solutions were prepared at the following concentrations: TA—0, 0.43, 0.60, 0.83, 1.16, 1.63, 2.28, 3.20 g/L; GSE—0, 0.36, 0.50, 0.70, 0.97, 1.36, 1.90, 2.67 g/L.

These concentrations were chosen, following earlier tests, by experienced laboratory personnel to provide the same wide range of astringency for both polyphenol compounds. Test set solutions were prepared at the following concentrations: TA—0.5, 0.94, 1.53, 2.13 g/L; GSE—0.4, 0.78, 1.35, 1.88 g/L.

2.2. Subjects

Thirty volunteers 16 females and 14 males from the University of Basilicata were selected on the basis of their salivary flow so that individual saliva flow would not act as a source of variation in the intensity of perceived astringency. Subject selection was performed according to the procedure described by Peleg, Gacon, Schlich, and Noble (1999). Judges ingested 15 mL of an aqueous solution of citric acid (4 g/L) which was expectorated at 10 s. The subjects then spat stimulated saliva into a weighed container for 1 min. The saliva collected in duplicate was weighted on an analytical balance. The salivary flow of selected subjects ranged from 1.5 to 2.8 g/min with a mean value of 2.09 g/min.

Prior to their participation in the experiments, the subjects were trained to recognize and rate the perceived intensity of the following different sensations: sourness, bitterness and astringency using the following standard solutions: citric acid—0.25, 0.38, 0.50 g/L; quinine monohydrochloride dihydrate—0.025, 0.037, 0.050 g/L; aluminium potassium sulphate—0.3, 0.6, 0.9 g/L.

Then subjects were trained to rate the perceived astringency of TA solutions with concentration values of 0.55, 1.2 and 2.32 g/L, and GSE solutions with concentration values of 0.50, 0.98 and 1.92 g/L. During training sessions the subjects were asked to rate the perceived astringency on a 7 point category scale (not astringent; very weak; weak; moderate; strong; very strong; extremely strong). The different concentrations were not anchored to specific intensity categories. Subjects participated in a total of four training sessions.

2.2.1. Training sets sample evaluation

Subjects took part in the experiment which consisted of two repetitions for both TA and GSE solutions and participated in a total of four sessions. During the first two sessions the set TA solution was evaluated. In each session eight samples were presented. There was a 30-min interval between the evaluation of two subsets each consisting of four samples. For each sample, subjects received a glass containing 15 mL of solution. Samples were presented at room temperature. In order to evaluate the target stimuli, subjects were asked to take each sample in their mouth for 8 s, spit it out and rate the intensity of astringency on a 7 point category scale named: not astringent, very weak, weak, moderate, strong, very strong, extremely strong. Between the evaluation of two samples, subjects were asked to rinse their mouths with distilled water for 45 s, to have some plain crackers for 30 s and finally to rinse their mouths with water for a further 45 s. The evaluations were performed in individual booths under red lights in order to eliminate any visual clues. The data were collected using the FIZZ computer system ver. 1.31 (Biosystemes, Couternon, France).

Within each session the presentation order of samples was balanced for first order and carry over effects.

2.2.2. Test set sample evaluation

Three weeks after the last session of the training sets sample evaluation, subjects participated in a further test session. They were presented eight samples and asked to rate the perceived astringency intensity using a 7 point category scale (from not astringent to extremely astringent). Two subsets of four samples were evaluated at 30-min intervals. Half of the subjects were presented the four AT test set samples first; whereas the other half were presented GSE samples first. Within each subset the presentation order of samples was balanced for first order and carry over effect. Samples were evaluated under the same conditions with regards to: amount of sample; sample temperature and presentation; break between samples; rinsing procedure; data acquisition as described above. Test set sample evaluations were not replicated.

2.3. Polyphenol–mucin reactivity assay

Mucin from bovine sub-maxillary glands (Sigma-Aldrich, Lot no. 06 4H7170) was used as a model protein.

Polyphenol solutions (8 mL) in 1% ethanol were mixed with 2 mL of mucin solution 0.2% in citrate phosphate buffer pH 3.5. The protein concentration was chosen taking into consideration the amount used in the artificial saliva formulations (van Ruth & Roozen, 2000). Two milliliters of mucin solution mixed with 1% (8 mL) ethanol and (8 mL) astringent solutions mixed with 2 mL citrate phosphate buffer were used as reference samples. All the reagents were thermo-stated at 37 °C corresponding to the average temperature in the oral cavity.

The turbidity values of all the three mixes were measured by HACH 2001N Laboratory Turbidimeter (Hach Co., Loveland, CO) in nephelometric turbidity unit (NTU). The optical system was fitted with a tungsten-filament lamp with three detectors: a 90° scattered light detector, a forward-scatter light detector and a transmitted light detector. The instrument was calibrated prior to the experiment with Formazin primary standards prepared from a 4000 NTU stock solution (Hach Co.) and HPLC grade deionised water. The instrument calibration was periodically verified during the experiments using Gelex secondary turbidimetry standards (Hach Co.). Each phenol solution was prepared and tested in six replicates. For each tube turbidity was measured after 1, 60 and 180 min. For both reference samples NTU values did not vary over time.

The polyphenol–mucin reactivity was expressed in terms of nephelometric turbidity units by subtracting the NTU values measured for the mucin reference sample (NTU_M) plus the polyphenol reference sample (NTU_A)

from the NTU value of the polyphenol–mucin sample after 1 min of reaction (NTU_S):

$$NTU = NTU_S - (NTU_M + NTU_A)$$

2.4. Data analysis

2.4.1. Sensory data regarding training sets samples

Astringency intensity ratings from TA and GSE solutions were treated independently from each other in ANOVA mixed effect models, assuming subjects as random effect, in order to estimate both polyphenol concentration and replicate effects.

The effect of individual differences in salivary flow amongst subjects was tested. For the purpose subjects were separated into high flow and low flow with reference to a median stimulated salivary flow rate as proposed by other authors (Horne et al., 2002). A three-way ANOVA model was performed assuming flow rate group, polyphenol compound, concentration level and their interactions as factors. Replications were treated as two observations per cell.

2.4.2. Sensory data regarding test set samples

Astringency intensity ratings from TA and GSE solutions were treated independently from each other in one-way ANOVA models. Within each test set sample series, significant differences between mean astringency ratings were tested by means the least significant difference post hoc test at a level of significance of 95%.

2.4.3. NTU data

The NTU values from TA and GSE solutions were treated independently from each other in two-way ANOVA models, assuming concentration and replicate as factors.

3. Results and discussion

3.1. Sensory evaluation regarding training set samples

The results from mixed ANOVA model performed on astringency intensity ratings from TA and GSE solutions (Table 1) showed a significant effect of increasing concentration on the astringency intensity ratings. The replicate showed no significant effect ($p \gg 0.05$). The intensity of the perceived sensation steeply increases with the concentration of tannic acid and grape seed extracts in a range from 0.32 to 1.63 g/L (Fig. 2). These results bear our findings of previous scientific reports (Arnold, Noble, & Singleton, 1980). At higher levels of concentration the strength of the perceived sensation was less affected by increasing astringent levels and both curves form a plateau.

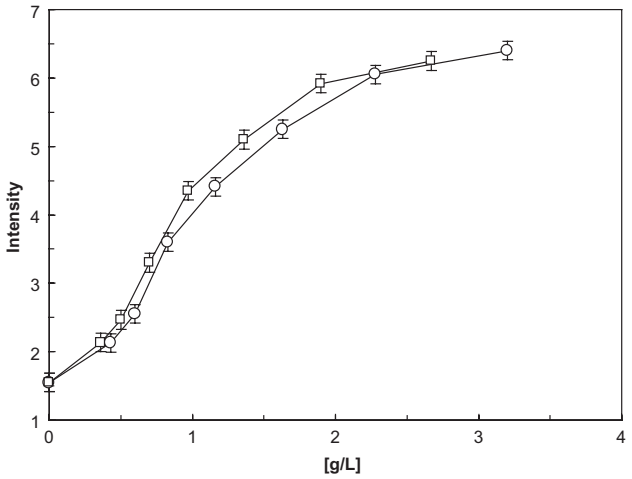

Fig. 2. Intensity of perceived astringency as a function of tannic acid (○) and grape seed extract (□) samples concentration ($n = 60$). Bars represent standard error.

For each level of concentration, differences between mean intensity ratings of both TA and GSE solutions were tested by means of a *t*-test. There were no significant differences between any of the pairs assumed to have the same stimulus gradation.

Individual differences in salivary flow amongst selected subjects (from 1.5 to 2.8 g/min) did not affect mean astringency ratings of training sample sets. Results from the three-way ANOVA carried out on astringency ratings from the training set samples indicate that no significant effects ($p \gg 0.05$) were found for all the tested factors except for the concentration factor which was expected to have a significant effect ($p = 0.000$). Thus, in this study, a relevant source of variation of astringency perception was kept under control. The results confirm the effectiveness of the procedure described by Peleg et al. (1999) in grouping subjects in relation to their salivary flow.

Table 1
Analysis of variance performed on training set samples sensory data: mixed ANOVA model (panelists random effect); TA and GSE concentration effect and replication effect

Factor	df	Training set samples			
		Tannic acid		Grape seed extract	
		F-value	*p*-Value	*F*-value	*p*-Value
Concentration	7	138	0.000	126	0.000
Replication	1	0.11	0.745	0.10	0.740

3.2. Test set sample sensory evaluation

Results from the one-way analysis of variance carried out on the astringency ratings from the TA and GSE test set sample solutions showed a significant and positive effect of the factor "concentration" (Table 2). Mean intensity ratings and their standard errors are reported in Table 3. The results of the LSD post hoc test showed that, in both AT and GSE sample sets, the mean of the intensity ratings of the two samples prepared at lower concentration levels, were not significantly different ($p > 0.05$).

As expected the intensity of perceived sensation increases with the concentration of phenolic compounds. Mean intensity ratings of AT samples ranged from 2.30 to 5.76, indicating that the perceived strength of sensation varied, on average, from very weak to very strong on the used 7 point category scale. Similarly, the mean intensities of GSE samples ranged from 3.32 to 5.52 indicating that the perceived strength of the sensation varied, on average, from weak to very strong on the used scale. Although both test set sample series were composed of four solutions only, the mean intensity ratings, within each sample series, covered a wide range of the strength of the sensation rated on a 7 point category scale. Thus the number of test set samples was assumed to be sufficient in order to validate the proposed predictive model.

3.3. In vitro assay setting up

Mucin from bovine sub-maxillary glands was chosen as model protein taking into consideration that it represents the major glycoprotein synthesized and secreted by mucous cells and serves to lubricate the oral cavity and to protect it from the external environment. Moreover, mucin serves as a major protein component in artificial saliva formulations (Friel & Taylor, 2001; Hutteau & Mathlouthi, 1998; van Ruth & Roozen, 2000) and it has already been reported to form complexes with tannins (Asquit et al., 1987).

The turbidity of mucin/polyphenol mixes developed almost instantaneously and then increased slightly over incubation time reaching equilibrium in 1 h (Fig. 3). The

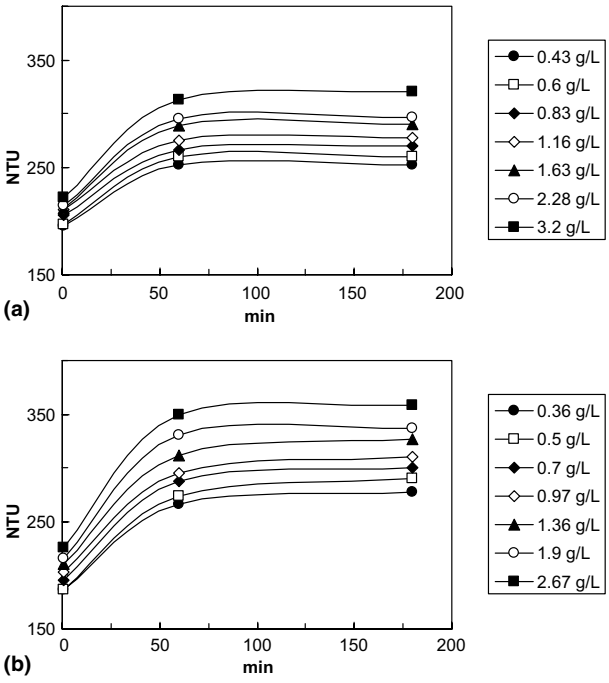

Fig. 3. Change in turbidity, measured as NTU values, as a function of incubation time of mucin solutions with increasing concentration of tannic acid (a) or grape seed extracts (b) samples ($n = 6$).

Table 2
Analysis of variance performed on test set samples sensory data: one way ANOVA model; TA and GSE concentration effect

Factor	df	Test set samples			
		Tannic acid		Grape seed extract	
		F-value	*p*-Value	*F*-value	*p*-Value
Concentration	3	26.00	0.000	13.66	0.000

Table 3
Test set samples sensory evaluation: astringency mean intensity ratings and their standard deviation

Test set samples					
Tannic acid ($n = 30$)			Grape seed extract ($n = 30$)		
Concentration*	Mean	Standard error	Concentration*	Mean	Standard error
0.50	2.30	0.29	0.40	3.46	0.32
0.94	2.91	0.27	0.78	3.32	0.27
1.53	4.89	0.37	1.35	4.58	0.30
2.13	5.76	0.38	1.88	5.59	0.27

* Phenolic content (g/L).

results confirm previous data on the high reaction rate between both bovine serum albumin and salivary proteins with polyphenol solutions (de Freitas & Mateus, 2001b; Horne et al., 2002). The formation of insoluble complexes has been described as a spontaneous phase separation which starts when two polyphenol-coated protein complexes are bridged together and the doubling in size renders the complex insoluble (Charlton et al., 2002). The precipitate forms in a time-dependent way depending on medium characteristics (pH, composition, protein–polyphenols relative concentrations). Dynamic laser light scattering measurements showed the formation of particles of various sizes depending on the polyphenol concentration of the medium (Charlton et al., 2002). On the other hand, nephelometry requires ideal conditions in which all the particles are small and identical. On this basis a short reaction time was adopted (1 min) after which the colloidal aggregates might just have formed and are likely to have small differences in size.

Turbidity of reference samples with mucin only ranged from 85 to 105 NTU and the one with polyphenols only ranged from 1 to 8. The turbidity values of both reference samples did not change over the reaction time thus confirming that the turbidity development in the reaction mixes was related to specific polyphenols–mucin interactions.

The results of the two-way ANOVA models carried out on the NTU values from TA and GSE solutions showed a significant effect ($p = 0.000$) of increasing concentration on the NTU values ($F_{6;30} = 547$ for TA; $F_{6;30} = 765$ for GSE). In both ANOVA models, the effect of replicates was not significant at a 95% level of significance ($F_{5;30} = 1.53$, $p = 0.21$ for TA; $F_{5;30} = 1.39$, $p = 0.26$ for GSE).

The turbidity of mucin/polyphenol mixes as a function of the concentration of tannic acid and grape seed extract samples is reported in Fig. 4. In the initial part of the curves the turbidity increased with the phenol concentration, then NTU values were less affected by increasing astringent levels and both curves tend to a plateau. The shape of these curves reproduce those relevant to the intensity of perceived astringency vs tannic acid and grape seed extract concentrations.

In a range of concentration varying from 0.36 to 0.83 g/L the turbidity responses of TA and GSE samples describe two superimposed curves. At a phenolic compound concentration above 0.83 g/L the two curves are clearly separated. This difference in NTU readings is probably due to the different effect of the concentration ratio phenols/mucin on the light scattering capability of mucin–AT and mucin–GSE complexes. At low concentration levels of phenolic compounds it can be supposed that both TA and GSE samples form relatively small mucin–phenol complexes, similar in size, and thus

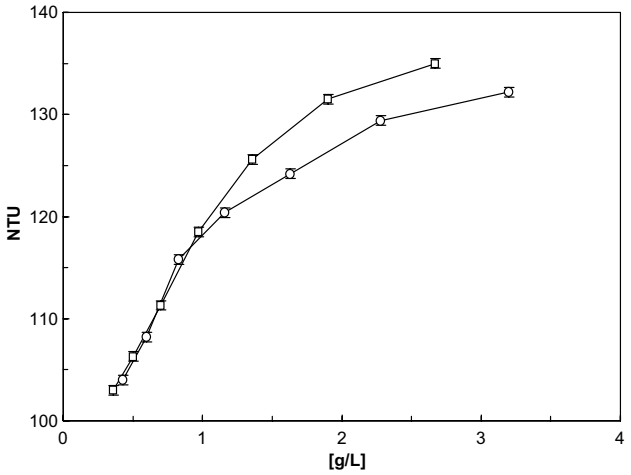

Fig. 4. NTU values measured after 1 min of reaction with mucin as a function of tannic acid (○) and grape seed extract (□) concentration ($n = 6$ for both compounds). Bars represent standard errors.

with the same light scattering capacity. At higher concentration levels of phenols (over than 0.83 g/L), mucin concentration became a limiting factor in mucin–phenol complex formation. In these conditions any phenols in excess can bridge together two or more small mucin–phenol complexes and relatively large aggregates are likely to be formed. It can be supposed that, due to their chemical composition, AT and GSE determine the formation of large aggregates differing in size and thus in light scattering capability.

The turbidity responses from AT and GSE training set samples suggest that the relationship between NTU values and perceived astringency is phenolic-sample-specific.

3.4. Building up predictive models

The relationship between the intensity of perceived astringency and NTU was studied. Linear correlations were obtained by relating mean intensity ratings of astringency and mean NTU values induced either by training set solutions of tannic acid or of grape seed extract. Two predictive models were obtained. Fig. 5 shows the results of the linear regressions in a concentration range from 0.43 to 3.20 g/L for TA and from 0.36 to 2.67 g/L for GSE. Standard errors associated with predictive models for tannic acid and grape seed extract were 0.27 and 0.10 respectively.

The goodness of fit was estimated too. Residual values were never higher than 0.20 (absolute value) for either TA or GSE predictive model. The residuals for TA and GSE, respectively ranged from −0.01 to 0.16 and from −0.20 to 0.18.

TA residual distribution around the zero value indicates that the predictions have the same level of accuracy over the whole range of variation of predicted

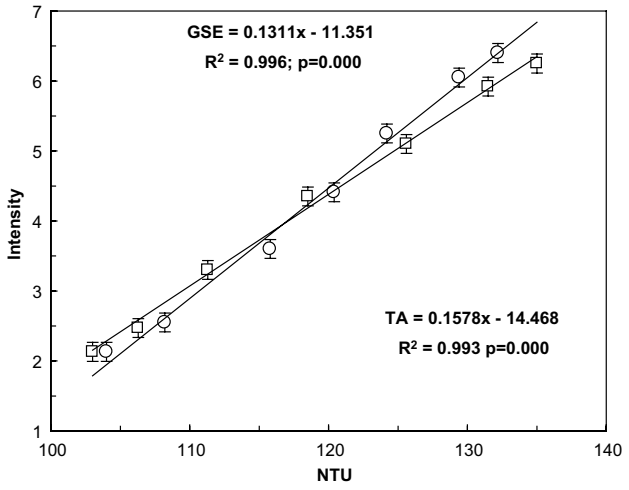

Fig. 5. Predictive model: relationship between astringency mean intensity ratings (bars represent standard errors; $n = 60$) and mean NTU values ($n = 6$) induced by tannic acid (○) or by grape seed extract (□) training set solutions: linear regressions.

astringency intensity. Within a small range of variability, the absolute values of residuals associated to the low concentration GSE samples (from 0.36 to 0.7 g/L) tend to be higher than those associated to the high concentration samples (from 1.36 to 2.37 g/L). Thus, over or under estimations of predicted astringency intensities are expected to be relatively higher at low rather than high concentration level of GSE samples.

On the basis of these results it is possible to assert that the response of the proposed in vitro assay can be linearly correlated with the astringency intensity experienced by a panel of trained subjects, selected in relation to their salivary flow. It must be underlined that the obtained linear regressions cannot be used to predict perceived astringency of a normal random-sample of subjects with different salivary flow rates. Thus, they do not represent a unique prediction model of astringency. Nevertheless the method could find an application in predicting astringency induced by specific phenolic compounds whenever the main purpose of the investigation is of practical importance and aimed at finding ways of optimising food production. It is very well known that the intensity of perceived astringency plays a key role in determining the acceptability of various food products and beverages therefore the availability of an in vitro assay to estimate the strength of the sensation induced by different phenolic compounds or extracts becomes crucial in order to optimise the food processing conditions in relation to this important determiner of food acceptability.

Taking into account the practical applications of the proposed method, on the basis of the linear regressions, the 7 point category scale used in rating the perceived intensity of astringency could be related to different NTU ranges (Table 4). Since low astringency intensities (from not astringent to weak on the used 7 point category scale) are not likely to affect food acceptability, the risk of a small over or under estimation of the predicted astringency induced by GSE sample at a low concentration level was assumed to be not relevant.

Table 4
Predictive models: relationship between 7 point category scale and NTU ranges

Category	Acid tannic predictive model	Grape seed extract predictive model
Not astringent	NTU < 104	NTU < 102
Very weak	104 ⩽ NTU < 111	102 ⩽ NTU < 109
Weak	111 ⩽ NTU < 117	109 ⩽ NTU < 117
Moderate	117 ⩽ NTU < 123	117 ⩽ NTU < 125
Strong	123 ⩽ NTU < 130	125 ⩽ NTU < 132
Very strong	130 ⩽ NTU < 136	132 ⩽ NTU < 140
Extremely strong	NTU ⩾ 136	NTU ⩾ 140

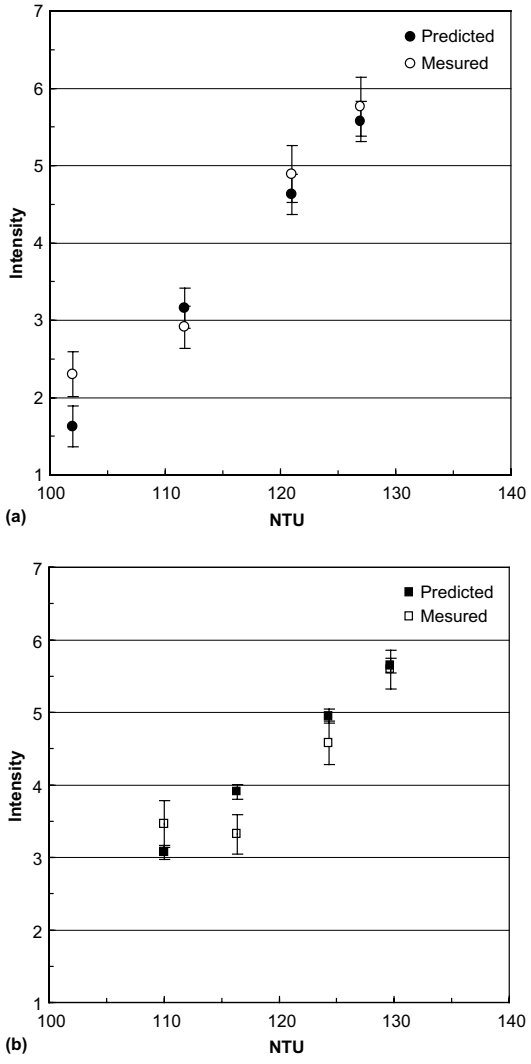

Fig. 6. Predictive models validation: measured and predicted astringency intensities in test set samples of tannic acid (a) and grape seed extracts (b). Mean values ($n = 30$ for both compounds) and standard errors.

3.5. Predictive model validation

The predictive capacity of the models was tested by comparing measured and predicted astringency intensity in the test set sample. Results are reported in Fig. 6(a) and (b).

The predicted intensity scores were not significantly different from the measured mean scores, except for two observations. However, in one of these two cases, the predicted values of the astringency intensity fell within the same category range as the measured values.

4. Conclusions

Despite extensive research the available experimental data are not yet sufficient to explain the entire mechanism of astringency. However, when polyphenolic compounds are involved, it seems likely that the formation of salivary protein–polyphenol complexes is the first step in the development of astringency. Moreover, it is evident that individual factors are of great importance in the modulation of the perceived intensity of this sensation. The simplification of complex physiological phenomena in suitable in vitro assays has already proved to be an useful approach (Garret, Failla, & Sarama, 1999) whenever the main purpose of research is of practical importance and aimed at finding ways of optimising food production.

In the present study, the reactivity of polyphenol compounds with a model salivary protein was assumed to be an index of their capability of inducing astringency. The reaction conditions were standardized in order to mimic the complex oral cavity environment and turbidity measurements, in terms of NTU, were used to estimate the extent of protein–polyphenol interaction. The response of the proposed in vitro assay proved to be linearly correlated with the astringency intensity experienced by a panel of trained subjects. On the basis of these results a predictive model of astringency has been proposed in which the capability of polyphenolic extracts to induce astringency can be estimated on the basis of their ability to develop turbidity in the in vitro assay. Seven ranges of NTU values have been defined which predict the strength of astringency measured on a 7 point category scale.

Further studies will be performed to verify the possibility of extending the present model to different classes of polyphenolic compounds.

The proposed method may have a practical application in predicting the astringency of different fractions of both grape, wine and other plant polyphenolic extracts in order to optimise the food processing conditions in relation to this important driver of food acceptability.

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