Food Chemistry 113 (2009) 325–330

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Prediction of grape polyphenol astringency by means of a fluorimetric micro-plate assay

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ARTICLE INFO

Article history:
Received 11 April 2008
Received in revised form 20 July 2008
Accepted 21 July 2008

Keywords:
Turbidity
Mucin
Sensory evaluations
Saliva characteristic
pH

ABSTRACT

Previous works have demonstrated that astringency can be predicted on the basis of turbidity developed by polyphenol/mucin mixes. In the present study, a micro-plate assay, based on the ability of haze particles to screen the radiation emitted by a fluorescent compound (I%), is proposed for polyphenol/mucin reactivity estimation. Grape seed extract (GSE) solutions prepared in a range of physiochemical active concentrations (0.0–2.9 g/L) were reacted with mucin. A significant linear relationship ($r = 0.99$; $p = 0.000$) was found between turbidity (NTU) and I% values of GSE/mucin samples. Within the experimental range of phenolic samples, the CV value of I% determinations was always lower than 10%. The relationship between sensory and instrumental responses, as a function of phenolic solution characteristics, was investigated. Variations in phenol concentration and pH induce similar modifications of both the intensity of perceived astringency and I% values. A significant linear relationship ($r = 0.98$; $p = 0.000$) was found by relating sensory ratings to the relevant I% values.

1. Introduction

Astringency is a tactile sensation perceived as a diffuse stimulus in the mouth and commonly described as a puckering, roughing and drying of the oral surface (Lee & Lawless, 1991). Dietary tannins form complexes with salivary proteins resulting in their aggregation and/or precipitation and the loss of their lubricating properties (Kallithraka, Bakker, Clifford, & Vallis, 2001). A friction-based mechanism for astringency development, due to precipitation of salivary proteins, has been suggested (de Wijk & Prinz, 2006; Prinz & Lucas, 2000). Different chemical stimuli may induce the astringency sensation, and among them are grape and wine condensed tannins (Herderich & Smith, 2005; Kennedy, Saucier, & Glories, 2006; Vidal, Francis, Noble, Chenier, & Waters, 2004). Sensations perceived in the act of tasting are important determinants of a consumer's response to wine. Astringency descriptors account for more than half of the total terms in the mouth-feel wheel of red wine (Gawel, Oberholster, & Francis, 2000). Studies addressing acceptance of astringency indicate that this is perceived as a negative attribute and is cited as a reason for food rejection (Drenowski & Gomez-Carneros, 2000; Lesschaeve & Noble, 2005). On the other hand, this sensation enhances the complexity and palate-length of high quality red wines. The possibility to predict the strength of astringency induced by polyphenols could help optimize processing conditions and thus improve the quality of red wine. With this aim, several studies have been performed in which the reactivity of phenols with different proteins was considered as an index of phenolic compound astringency (Edelmann & Lendl, 2002; Hofmann et al., 2006; Kennedy, Ferrier, Harbertson, & Peyrot des Gachons, 2006; Poncet-Legrande, Gautier, Cheynier, & Imberty, 2007; Wang, Ho, & Haung, 2007). Among several techniques that have been used to investigate the interaction between dietary polyphenols and protein, nephelometry has proved to be very useful (Carvalho, Povoas, Mateus, & de Freitas, 2006; de Freitas, Carvalho, & Mateus, 2003; de Freitas & Mateus, 2002). This simple technique has resulted sensitive enough to determine the reactivity of protein with polyphenolic samples in a range of physiologically active phenol concentrations (Horne, Hayes, & Lawless, 2002). Recently, the Astringency Mucin Index (AMI) was defined (Condelli, Dinnella, Cerone, Monteleone, & Bertuccioli, 2006; Monteleone, Condelli, Dinnella, & Bertuccioli, 2004). This predictive index is based on the relationship between astringency intensity and nephelometric turbidity units (NTU) developed by the reaction of grape and wine polyphenols with mucin.

In the present study a micro-plate assay, based on the ability of haze particles to screen the radiation emitted by a fluorescent compound for routine estimation of polyphenol/mucin reactivity, is proposed.

The experimental plan consisted of two phases:

1. Development of a fluorimetric assay for turbidity evaluation using both formazin turbidity standards and polyphenol-mucin samples;

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doi:10.1016/j.foodchem.2008.07.058

2. Study of the relationships between fluorimetric assay response and sensory data considering phenol concentration and pH effects.

The first part of the experiment aimed to compare the fluorimetric assay response with a nephelometric index which had already proved to be capable of predicting astringency induced by grape and wine phenolic compounds. The predictive capacity of fluorimetric response with respect to astringency induced by samples with different phenolic concentrations and pH values was tested in the second part of the experimental plan.

2. Materials and methods

2.1. Chemical

Formazin stock solution (Hach Co, Loveland, USA) at 4000 Nephelometric Turbidity Units (NTU) was used to prepare turbidity standards. 5(6)-Carboxyfluorescein mixed isomers from Sigma–Aldrich were used as fluorescent compound. Grape Seed Extract (GSE) from Intec (Verona, Italy) was used to prepare phenolic samples. Mucin from bovine sub-maxillary glands from Sigma–Aldrich (Lot no. 06 4H7170) was used as a salivary protein model.

2.2. Equipment

A Gel Documentation System (Gel Doc™ 2000, Bio-Rad, Hercules, CA, USA) was used. The whole apparatus was composed of a darkroom cabinet equipped with a camera for capturing images, a transilluminator with a 302 nm ultraviolet source and a computer. The images were acquired with Gel Doc™ 2000 and analysed by Quantity One v. 4.3.0 (Bio–Rad) software. The fluorescent intensity (FI) of the image-selected area was calculated and expressed as intensity × mm^2.

2.3. Phenolic samples

Phenolic solutions were prepared, approximately 2 h prior to testing, by dissolving GSE in 1% ethanol. The amount of phenolic compounds in GSE samples were determined according to the Folin–Ciocalteau Method (Off. J. Eur. Communitie, 1992). GSE contained 0.75 g of phenols per gram of material. Sample concentration was always expressed in terms of phenolic content as catechin (g/L).

Two sample sets were prepared. Sample set 1 consisted of eight GSE solutions at the following concentrations: 0.0, 0.4, 0.5, 0.76, 1.1, 1.5, 2.1 and 2.9 g/L. These concentrations were chosen to provide a wide range of perceived astringency from extremely weak to extremely strong (Monteleone et al., 2004). Set 1 samples showed a pH value of 5.0. Sample set 2 consisted of five GSE solutions (0.8 g/L) at the following pH values: 2.8, 3.0, 3.4, 3.8 and 4.0.

2.4. Fluorimetric assay for evaluation of turbidity

2.4.1. Formazin turbidity standard

The assay was carried out using two black 96-well microtitre plates (A and B) with flat transparent bottom (Greiner, Labortechnik, USA). Formazin stock solution was diluted in deionised water to prepare 11 samples with turbidity ranging from 650 to 10 NTU. Sample solutions and deionised water, used as reference, were loaded in plate A wells (150 µl/well). Plate B wells were filled with 150 µl of 1 mM 5(6)-carboxyfluorescein solution in citrate phosphate buffer, pH 7.0. Plate A was superimposed on plate B and, after 30 s of UV light exposure, the image was acquired and analysed.

The Fluorescence Intensity Inhibition percentage (I%) was calculated using the following formula:

$$I\% = 100 - (\mathrm{FIs} \times 100/\mathrm{FIr})$$

FI_s = fluorescence intensity detected in sample-containing wells. FI_r = fluorescence intensity detected in water-containing wells.

Formazin samples were prepared and tested in six replicates. A schematic representation of the system is reported in Fig. 1.

2.4.2. Polyphenol–mucin sample

The polyphenol–mucin reaction was carried out as described by Monteleone et al. (2004). Sample set 1 solutions (20 mL) were mixed with 5 mL of 0.2% mucin in citrate phosphate buffer 0.5 M, pH 3.5. The final pH of sample set 1 reaction mixture was 3.6. Sample set 2 solutions (20 mL) were mixed with 0.2% mucin in citrate phosphate buffer 0.5 M with the same pH value of the relevant phenolic solution. The final pH of sample set 2 reaction mixtures was 2.8, 3.0, 3.4, 3.8 and 4.0, respectively. Reaction mixtures were kept for 1 min at 37 °C. GSE–mucin reactivity was evaluated by nephelometry in terms of NTU. Polyphenol–mucin reactivity was also evaluated by the fluorimetric assay. Reaction mixtures and water reference samples were loaded in plate A wells (150 µl/well). Plate B wells were filled with 150 µl of 1 mM 5(6)-carboxyfluorescein solution. The fluorescent image was acquired and ana-

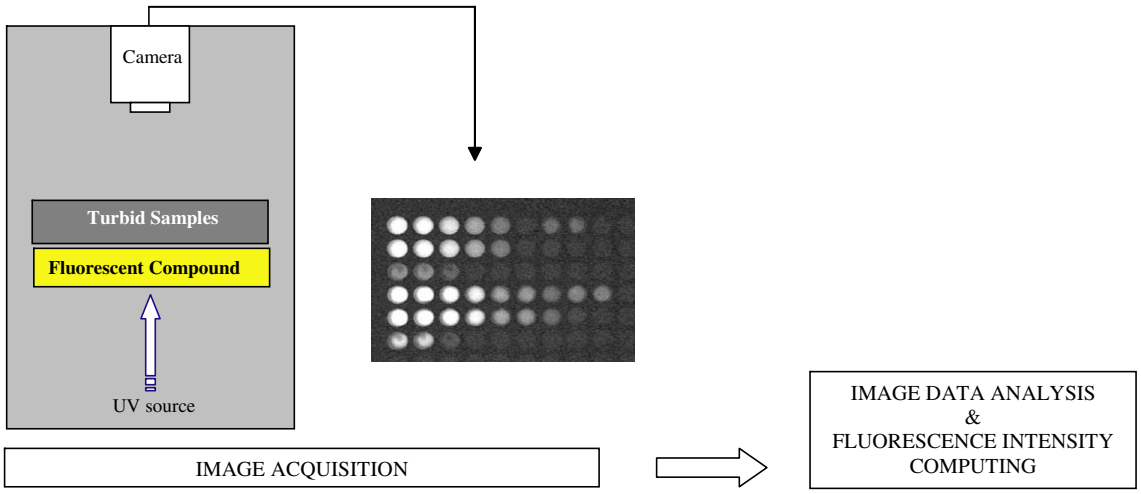

Fig. 1. Scheme of the system used for turbidity evaluation by fluorimetric assay.