Food Hydrocolloids 60 (2016) 598–605

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Influence of polysaccharides and glycerol on proanthocyanidin precipitation by protein fining agents

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ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form
25 March 2016
Accepted 25 April 2016
Available online 29 April 2016

Keywords:
Wine
Protein
Polysaccharides
Glycerol
Complex
Tannin

ABSTRACT

Fining treatment is an important step in winemaking but its mechanism is still not well known. To have a better understanding of the protein fining process, the influence of polysaccharides and glycerol, major compounds in wine, on proanthocyanidin precipitation was studied. A wine and a model wine obtained from its polyphenol fraction were analyzed before and after fining by a gelatin and a plant protein, a hydrolyzed gluten HG. The results showed that polysaccharides and glycerol modify quantitatively proanthocyanidin precipitation, but not much the nature of the precipitated proanthocyanidins. The nature of the remaining proanthocyanidins in the treated model wines after protein fining was not much modified neither. All proanthocyanidins were not recovered after the fining treatments when polysaccharides and glycerol were added to the model wines. These results suggested the creation of soluble complexes between proanthocyanidins, fining proteins and polysaccharides. The efficiency of the fining treatment could be thus modified by the content of the wines.

1. Introduction

Protein fining treatment is commonly used to clarify wine and to reduce astringency (Ribereau-Gayon, Dubourdieu, Doneche, & Lonvaud, 1998). In this fining process, proanthocyanidins (i.e. condensed tannins) that contribute to astringency (Haslam & Lilley, 1988; Preys et al. 2006) and haze in beverages such as wine and beer, interact with fining proteins (e.g. gelatin (Ribereau-Gayon et al., 1998)) and are precipitated by them (Gambuti, Rinaldi, & Moio, 2012; Maury, Sarni-Manchado, Lefebvre, Cheynier, & Moutounet, 2001). Previous research has shown that the extent and the nature of precipitated proanthocyanidins depend directly on the chemical characteristics of the protein (e.g. molecular mass, amino acid composition) used for the treatment (Maury et al., 2001, 2003).

There is considerable evidence that polysaccharides influence the interaction between proanthocyanidins and proteins and astringency perception. Tannins (proanthocyanidins) are perceived less astringent in the presence of polysaccharides (Vidal, Doco, Moutounet, & Pellerin, 2000). Moreover, recent studies confirmed that polysaccharides reduce wine astringency (Boulet et al., 2016; Quijada-Morin, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014). Two mechanisms have been proposed to explain the reduction in astringency response induced by polysaccharides (Le Bourvellec & Renard, 2012; Scollary, Pásti, Kállay, Blackman, & Clark, 2012; de Freitas & Mateus, 2012): the formation of ternary complexes involving polysaccharides, tannins, and proteins or polysaccharide encapsulation of tannins interfering with the tannin-protein interaction (Mateus, Carvalho, Luis, & de Freitas, 2004). There is experimental evidence that partially supports both mechanisms (see Scollary et al., 2012) and more recent work on tannins and polysaccharides (Bautista-Ortin, Cano-Lechuga, Ruiz-García, & Gómez-Plaza, 2014; Ruiz-Garcia, Smith, & Bindon, 2014; Watrelot, Le Bourvellec, Imberty, & Renard, 2014) or tannins and proteins (Iturmendi & Marin-Arroyo, 2012; Poncet-Legrand, Gautier, Cheynier, & Imberty, 2007; Gonzalez-Neves, Favre, & Gil, 2014) has not resolved the issue.

Importantly for this study, there has been essentially no specific work on the influence of wine polysaccharides on the interaction

Abbreviations used: mWi, model wine; Wi, wine; Da, Dalton; WPF, wine polysaccharide fraction; mDP, mean degree of polymerization; % gall, percentage of galloylation; EGC, epigallocatechin unit; SDS, sodium dodecyl sulfamide; HG, hydrolyzed gluten; G, glycerol.

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http://dx.doi.org/10.1016/j.foodhyd.2016.04.034
0268-005X/

between wine proanthocyanidins and fining proteins.

Glycerol, the third most abundant compound in wines (Flanzy, 1998), exerts a major role in the sweet character of wines (Noble & Bursick, 1984) and thus contributes to sensory perception in mouth even if is not involved in the aroma perception (Lubbers, Verret, & Voilley, 2001). There is tentative evidence from a study on Riesling that glycerol influences the viscosity mouthfeel effect (Gawel, Sluyter, & Waters, 2007). The potential for viscosity effects influencing the polysaccharide/proanthocyanidins/fining protein interactions has not been examined.

To provide a better understanding of the interactions occurring during fining treatments, this study was designed to examine the influence of wine polysaccharides and glycerol on wine proanthocyanidin precipitation. Two experimental strategies were undertaken. First, a Syrah wine and a model wine prepared from a polyphenol fraction of the same Syrah wine were fined with a gelatin fraction. Second, the fining experiments were repeated on the model wine in the presence of known amounts of wine polysaccharides and glycerol, identified as the major compounds removed in the preparation of the model wine with potential impact on protein-proanthocyanidin interactions and fining. The latter experiments were also performed with a hydrolyzed wheat gluten. Analysis of the wine and model wine before and after fining was performed to determine the influence of the wine components on the fining process.

2. Materials and methods

2.1. Materials

Organic solvents and phenylmethanethiol (PubChem CID:7509) were purchased from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland) respectively. Sodium dodecyl sulfate (SDS) (PubChem CID: 3423265) was supplied by Sigma Chemical Co. (Poole, Dorset, U.K.).

2.2. Model wine (mWi)

The initial wine (Wi) was an unfiltered eight-month old wine from *Vitis Vinifera* var. Syrah (Nîmes, Gard, France). It was analyzed and/or fined as soon as the bottle was opened.

A model wine was obtained by resolubilization of a polyphenolic fraction prepared from wine Wi. This polyphenolic fraction was prepared at the INRA Unité Expérimentale de Pech Rouge (Gruissan, Aude, France) by the method previously described (Maury et al., 2003).

Briefly, the wine (100L) was loaded onto a vinyldivinylbenzene column. After washing with water (92L), wine polyphenols were eluted with methanol (60 L). The methanol fractions obtained from two batches of wine (100L each) were pooled, concentrated to 4.5 L and atomized, generating 746 g of powder from 200 L of wine. The model wine (mWi) was prepared by dissolving 3.73 g/L of powder in an aqueous ethanol solution (12.3% v/v) containing potassium hydrogen tartrate (0.015 mol/L, pH 3.7). Analysis of the wine and of the polyphenol fraction showed that the fractionation process induced larges losses of glycerol (100%), amino acids and proteins (over 90%), and glycosyl residues (66%), as well as some losses of phenolic acids (40%) and flavonols (27%) while anthocyanins and proanthocyanidins were totally recovered in the polyphenol fraction.

A wine polysaccharide fraction (WPF) was prepared from red wines after the precipitation of the total colloids by cold ethanol. After a dialyze, the polysaccharides of this salt-free colloid solution were then separated from the other compounds though a cation-exchange column as previously described (Pellerin et al., 1996).

Table 1
Composition of the initial wine (Wi) and the model wine (Wi). Means with different letters in a line differ significantly ($p < 0.05$). Results ± standard deviation are average of three analyses.

Compounds	Wi (T0 = 8 months)	mWi
Anthocyanins (mg/L)	264.4 ± 45.4[a]	259.0 ± 13.2[a]
Phenolic acids (mg/L)	168.8 ± 45.2[a]	104.1 ± 29.4[b]
Flavonols (mg/L)	19.6 ± 4.4[a]	14.3 ± 1.2[b]
Proanthocyanidins (g/L)	1.05 ± 0.11[a]	1.06 ± 0.06[a]
mDP	9.5 ± 0.6[a]	8.5 ± 0.2[b]
% Galloylation	5.0 ± 0.29[a]	4.9 ± 0.2[a]
% EGC	17.7 ± 1.0[a]	17.9 ± 1.1[a]
Free amino acids (mg/L)	822 ± 45[a]	8 ± 1[b]
Protein amino acids (mg/L)	30 ± 2[a]	2 ± 2[b]
Polysaccharides (mg/L)	235 ± 16[a]	80 ± 39[b]
Glycerol (g/L)	8.2 ± 0.4[a]	0.0 ± 0.1[b]

The % of the major polysaccharides are presented in Table 2. The composition of the polysaccharides of the initial wine was in accordance with the literature (Vidal, Williams, Doco, Moutounet, & Pellerin, 2003). The addition of WPF to the model wine allowed it to get a polysaccharide concentration close to that of the initial wine.

This wine polysaccharide fraction (WPF) or glycerol was added to the model wine in amounts to obtain approximately the concentrations of the initial wine that is to say addition of 160 mg/L of WPF and 8 g/L of glycerol: (a) addition of WPF (b) addition of glycerol and (c) addition of WPF and glycerol together.

2.3. Fining agents

2.3.1. Plant proteins

Commercial plant proteins (HG) were provided by Martin Vialatte Œnologie (Epernay, France). HG was a powder preparation isolated from enzymatically hydrolyzed wheat gluten. HG is mainly composed by 10–16 kDa proteins as well as some larger, in particular 50 kDa proteins (see electrophoresis in Maury et al., 2003). On the basis of amino acid determination, HG contained 88% of proteins (Maury et al., 2003). The plant protein preparation was suspended in water to obtain a final concentration of 10 g of protein per liter. The sampling of this HG suspension was achieved under agitation.

2.3.2. Gelatin

A gelatin fraction (G16) was obtained from a liquid commercial product (Gelisol) by different ammonium sulfate precipitations (Maury et al., 2001). Proteins of this preparation had a MW centered on 16 kDa (mainly from 10 to 25 kDa, see the protein distribution in Maury et al., 2001). Kjeldahl analysis showed that G16 contained 72% of proteins, with a concentration of 35 g of proteins per liter (Maury et al., 2001).

2.4. Fining experiments

Fining experiments were performed by adding solutions of plant proteins or gelatin to 23 mL of sample (wine or model wine complemented with WPF and/or glycerol; see Supporting Information Fig. S1) to obtain a final protein concentration of 0.1 g/L. The mixture was kept at 25 °C for 48 h and then centrifuged at 4 °C at 1900 *g* for 10 min (SORVALL® Ultraspeed Centrifuge RC5 B, Dupont de Nemours, Les Ullis, France; rotor SA-600) giving a pellet and a supernatant (treated wine). All experiments were carried out in triplicate, and one analysis was performed per sample.