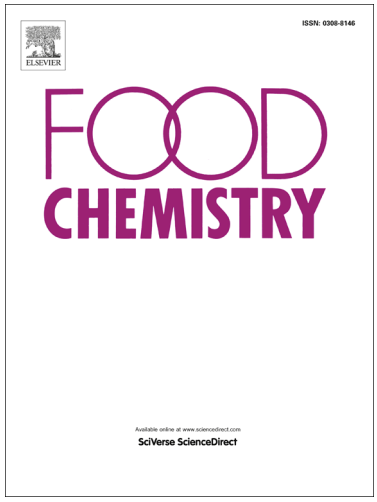

Great diversity among commercial inactive dry-yeast based products

Remigio López-Solís, Celeste Duarte-Venegas, Millaray Meza-Candia, Rubén del Barrio Galán, Álvaro Peña-Neira, Marcela Medel-Marabolí, Elías Obreque-Slier

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Great diversity among commercial inactive dry-yeast based products

Remigio López-Solís[b], Celeste Duarte-Venegas[a], Millaray Meza-Candia[a], Rubén del Barrio Galán[a], Álvaro Peña-Neira[a], Marcela Medel-Marabolí[a] and Elías Obreque-Slier [a*]

[a] *Department of Agro–Industry and Enology, Faculty of Agronomical Sciences, University of Chile, P.O. Box 1004, Santiago, Chile.*

[b] *Program of Cellular and Molecular Biology, Faculty of Medicine–ICBM, University of Chile, Independencia 1027, Santiago, Chile.*

Corresponding author. Tel.: +56 2 29785730, *E–mail address*: eobreque@uchile.cl (Elías Obreque-Slier)

ABSTRACT

Commercial inactive dry-yeast based (IDYB) products have been shown to impact positively in different ways on the winemaking process, including sensory enhancement. Despite their relevance little information about physicochemical characteristics of individual IDYB products is available. This study aimed to physicochemically characterize a group of ten commercial IDYB products. Organic, protein and carbohydrate contents by spectrophotometric methods, protein diffusion on cellulose membranes and electrophoretic protein profiles were assessed. Interaction of a IDYB product (CP10) with either salivary protein or a proanthocyanidin-rich extract (binary mixtures) or with both of them (ternary mixtures) was also assessed. Marked physicochemical differences were observed among all ten products. CP10 was found to interact with seed extract and salivary protein. Also, as part of CP10-SE complexes, CP10 interacted with the salivary protein to form ternary complexes. Due to their huge diversity, physicochemical characterization of IDYB products before use in winemaking is recommended.

Key words: Inactive dry yeast product; wine; polysaccharides; astringency; mannoprotein; winemaking process

1. Introduction

In the past few years, the wine industry has been markedly influenced by technological innovation and introduction of new enological supplies (Benavente, 2006). Among the latter ones, use of inactive dry-yeast based (IDYB) products has spread widely (Gonzalez-Royo et al., 2013). IDYB products are mostly derived from cultured *Saccharomyces cerevisiae* strains that are subsequently subjected to thermal or enzymatic inactivation (Pozo-Bayon, Andujar-Ortiz Moreno-Arribas & 2009). However, a diversity of preparative technological procedures has led to a wide variety of IDYB products, including yeast autolysates, yeast cell walls, yeast soluble extracts and inactive yeast cells, among others (Pozo-Bayon et al., 2009; Masino, Montevecchi, Arfelli& Antonelli, 2008). In spite of such diversity, most of IDYB products are composed of inactive yeasts, free cell metabolites that are released after yeast lysis (aminoacids, peptides and proteins, polysaccharides, nucleotides and fatty acids), yeast cell walls, vitamins and minerals (Pozo-Bayon et al., 2009; Caridi, 2006; Vuchot, Vidal, Riou, Bajard-Sparrow, Fauveau & Pellerin, 2008). Mannoprotein, a main component of *S. cerevisiae* cell wall (25-50% dry weight), comprising roughly 70-80% polysaccharide and 20-30% protein, may represent as much as 35% of wine polysaccharides (Vidal, Williams, Doco, Moutounet & Pellerin, 2003; Ribéreau-Gayon, Dubourdieu, Donéche & Lonvaud, 2000; Guadalupe & Ayestarán, 2008). Accordingly, commercial IDYB products have been shown to impact positively on the winemaking process. Thus, IDYB products have been featured as boosters in alcoholic and malolactic fermentations (Feuillat, 2001), protective agents for yeast rehydration (Dulau, Ortiz-Julien & Trioli, 2004), color stabilizers (Guadalupe, Palacios & Ayestarán, 2007) and sensory enhancers (Caridi, 2007; Comuzzo, Tat, Tonizzo & Battistutta, 2006; Pozo-Bayon et al., 2009). In this last regard, IDYB

products have been widely used for modulating astringency, a drying, roughing and puckering sensation occurring in different areas of the mouth following their contact with some foods or drinks (Lee & Lawless, 1991). In the case of wine, such sensation has been associated with physicochemical interactions between salivary protein components and proanthocyanidins or tannins (Prinz & Lucas, 2000). Several reports have shown that some polysaccharides, including mannoproteins, would decrease astringency either by interacting with wine phenolics, by reducing the levels of free reactive proanthocyanidins or by increasing salivary viscosity (Caridi, 2006; Guadalupe & Ayestarán, 2008). Unfortunately, despite their relevance to astringency modulation in particular and to wine quality in general, little information about the physicochemical characteristics of IDYB products is available, other than that provided by the corresponding supplying companies. Given the diversity of IDYB products, their overall characterization would be useful to orient winemakers towards a more focused use of these products. This study was aimed to physicochemically characterize a group of commonly available IDYB products. The effect of one of those commercial IDYB products on the interaction between a grape seed proanthocyanidin-rich extract and the salivary protein fraction was also assessed.

2. Materials and methods

2.1. Materials

Eight inactive dry-yeast based (IDYB) commercially available products were acquired from Lallemand (Quebec, Canadá), namely, Red Style (CP1), Booster Rouge (CP2), Opti Red (CP3), Opti White (CP4), Booster Blanc (CP5), Noblesse (CP6), White Style (CP7) and Vin' Style (CP8). In addition, Biolees (CP9) and Surli Velvet (CP10) were purchased from Laffort (Bordeaux, France) and Enartis Vinquiry (Windsor, CA, USA), respectively. All reagents, polysaccharide standards (pectins from Citrus and *Leuconostoc mesenteroides* dextrans), protein standards and electrophoresis reagents were acquired from Sigma-Aldrich (St. Louis, Missouri, USA). Protein dyes (Coomassie Blue R-250) were obtained from Merck-Millipore Corporation (Darmstadt, Germany).

2.2. Aqueous solubility assay of IDYB products

Following supplier guidelines, each IDYB product was dissolved (10 mg/mL) in distilled water at 35°C with constant mechanical shaking for 10 min. 15 µL aliquots of each solution were placed sharply on a point of a horizontally positioned cellulose membrane (Whatman 1) and allowed to diffuse freely at room temperature. Once the cellulose membrane was dry, it was processed for protein detection, as previously described (López, Castillo, Traipe & López, 2007; Obreque-Slier, Mateluna, Peña-Neira & López-Solís, 2010a; Obreque-Slier, Peña-Neira & López-Solís, 2010b). Briefly, cellulose membranes were sequentially immersed with continuous

rocker shaking in 5% trichloroacetic acid (5 min), 80% ethanol (5 min), 0.25% Coomassie blue in 15% isopropanol/10% acetic acid (20 min),7% acetic acid (3 x 3 mins) and water (30 sec). In a parallel assay, each one of the solutions of the IDYB products was centrifuged at 600 g x 5 min right before placing 15-µL aliquots of the corresponding supernatants on the cellulose membrane. The rest of the procedure for detecting protein on the cellulose membrane was the same. Digital images of the processed cellulose membranes were obtained and the areas of protein distribution were measured by using 1.45S Image J software.

2.3. Spectrophotometric analyses

Solid IDYB products were dissolved in distilled water (1 mg/mL). For the assessment of organic matter, those solutions were diluted in water until absorbance readings at 280 nm reached the linear range (Stoscheck, 1990). Protein content was determined by the Bradford method (1976). Briefly, 50 µL of the water solutions were mixed with 1 mL of the Bradford reagent and after 3 min at room temperature absorbances were read at 595 nm. Bovine serum albumin was used as standard. Polysaccharide content was determined by the method of Dubois, Gilles, Hamilton, Rebers & Smith (1956). To this end, 200µL of a 1.5 mg/mL aqueous solution of each IDYB product were mixed with 200 µL of 5% phenol and 1 mL sulfuric acid. After vigorous mixing the tubes were incubated at 100°C for 5 min, immersed in ice-water for 1 min and allowed to stand at room temperature for 5 min just before measuring the optical density at 490 nm. Mannose was used as standard.

2.4. Electrophoresis of IDYB products

One-dimensional electrophoresis in slab SDS-polyacrylamide gels (12%) and the discontinuous buffer system described by Laemmli (1970) was used. 20 µL of fresh water solutions (10 mg/mL) of the IDYB products CP1, CP2 and CP3 were mixed with sample buffer (1:1 v/v), boiled for 5 min, loaded onto 14 x 12.5 x 0.15 (cm) gels and run at constant 25 mA per gel for the concentrating stage and constant 35 mA for the resolving stage (Laemmli, 1970). After the run, gels were fixed in 15% isopropanol/10% acetic acid and stained in 0.25% Coomassie blue dissolved in 40% isopropanol/10% acetic acid. On the other hand, fresh water solutions of the IDYB products CP4 through CP10 were dissolved in water (10 mg/mL) as indicated above and 1 mL aliquots were concentrated by mixing them with cold acetone (1:1 by volume) and centrifugation at 600 g x 5 min. Sediments were dissolved in 20 µL of water, mixed with sample buffer and subjected to electrophoretic analysis as indicated above. Digital images of gels were obtained.

2.5. Polysaccharide extraction and analysis

10 mL aliquots of each IDYB product (10 mg/mL) were centrifuged (4855 g x 10 min) and the supernatants were vacuum-evaporated to a final volume of 2 mL. Polysaccharides were precipitated by adding 10 mL of 0.3M HCl in absolute ethanol and allowed to stand at 0-4°C for 24 h. After centrifugation (4855 g x 15 min), the sediments were washed three times with absolute ethanol. Finally, the sediments were dissolved in 1 mL of ultrapure water, freeze-dried and stored at -80°C until analysis. Samples were resuspended in 1 mL of 50 mM ammonium formate and filtered through a 0.22 µm cellulose filter. Aliquots (100 µL) of the solution were subjected to high-resolution size exclusion chromatography (HR-SEC) using a 1260 Infinity Series System HPLC (Agilent Technologies, Santa Clara, CA) consisting of degasser (G1322A), quaternary pump

(G1311A), autosampler (G1329A), column oven (G1316A), and a refractive index detector (RID) (G1362A). Separation was carried out at 20°C using serially connected Shodex gel permeation HPLC columns OHpack SB-803 HQ and SB-804 HQ (300 mm x 8 mm i.d.; Showa Denko, Tokio, Japan). The mobile phase consisted of constant flow (0.6 mL/min) of 50 mM ammonium formate for 60 min. RID detection was carried out at 35°C. Eluates were contrasted against profiles of molecular weight commercial standards (mixes of dextrans and pectines) over the range 0-2 g/L (Del Barrio-Galán, Medel-Marabolí & Peña-Neira, 2015).

2.6. Grape seed extract (SE)

Grape seeds of *Vitis vinifera* L cv Carménère were collected and processed for polyphenol extraction as detailed elsewhere (Obreque-Slier, Peña-Neira, López-Solís, Zamora, Da Silva & Laureano, 2010c). Full characterization of the seed extract used in the study is shown in an independent report (Obreque-Slier, López-Solís & Peña-Neira, 2012).

2.7. Saliva collection

Right before the experiments and always between 9.00 and 11.00 AM**,** saliva accumulated passively (without using sialogogues) for 1 minute in the mouth of a single subject (about 1 mL) was expectorated into a sterile glass vessel and maintained in ice throughout the experiment (Obreque-Slier et al., 2010b).

2.8. Interaction of a IDYB product (CP10) with a proanthocyanidin-rich extract and the salivary protein fraction

Diffusion and precipitation assays using cellulose membranes were conducted as described in detail elsewhere (Obreque-Slier et al., 2010a, 2010b). Briefly, for assays with binary mixtures variable volumes (0-400 µL) of a 1 mg/mL solution of the IDYB product CP10 were mixed with either 100 µL of saliva (S) or 1000 µL of the seed extract (SE) and completed to 1500 µL with distilled water. For assays with ternary mixtures, the above indicated volumes of CP10 and SE were thoroughly mixed 5 min before the addition of 100 µL of saliva. 20 µL of the binary or ternary mixtures were placed sharply on a point of a horizontally positioned cellulose membrane (Whatman 1). After total unrestricted radial diffusion of each experimental mixture, the circular moistened areas on the membrane were demarcated at the cardinal points with graphite and the membrane was allowed to dry spontaneously. After protein staining on the membrane as detailed above, the area of protein diffusion for each experimental condition was measured by using Image J software (diffusion assay).The rest of the mixture was centrifuged at 600 g x 5 min and a 20-µL aliquot of the supernatant was spotted on the cellulose membrane to assess salivary protein precipitation (precipitation assay) (Obreque-Slier et al., 2010a, 2010b).

2.9. Statistics

ANOVA analysis with Minitab 16® Statistical Software was initially used. In case of significant differences between samples, a Tukey's multiple range test at the 0.05 level of significance was conducted.

3. Results

3.1. Spectrophotometric analyses

As shown in Table 1, absorbance at 280 nm displayed by equivalent solutions (10 mg/mL) of the IDYB products CP1, CP2 and CP3 were about three times higher than those shown by all the other IDYB products, with the exception of CP10. Likewise, protein contents in CP1, CP2 and CP3 were about 2-5 times as high as those of the other IDYB products. By contrast, polysaccharide contents of the whole group of IDYB products showed a completely different profile over the range from 16.72 to 53.53 g of mannose equivalents/kg of product. Thus, IDYB products CP4, CP6, CP7 and CP8 showed the lowest polysaccharide contents (about 18.50 g mannose equivalents/kg) whereas CP10 showed the highest one (almost twice as high as CP1, CP2 and CP3).

3.2. Polysaccharide fractions of IDYB products

Table 1 shows the contents of polysaccharide fractions in the IDYB products under study as identified by HPLC fractionation. Polysaccharide fraction III (65-48 kDa) was observed in all ten commercial IDYB products. However, the content of this fraction in CP10 almost tripled the corresponding contents in all the rest of the IDYB products. By contrast, the polysaccharide fraction IV (<10 kDa) displayed a roughly similar distribution in most of the IDYB products, with the exception of CP10 that lacked of it. On the other hand, polysaccharide fraction I (1460-1320 kDa) was found only in CP9 whereas polysaccharide fraction II was fully undetectable in the whole group of IDYB products.

3.3. Presence and diffusion of IDYB-product protein on cellulose membranes

Aliquots of the IDYB products in solution were placed on cellulose membranes and processed for staining with the selective protein dye Coomassie blue. As shown in Fig. 1, different Coomassie blue staining patterns were observed when spots on the cellulose membranes of all ten IDYB products were compared. Thus, CP1 through CP8 displayed biphasic modes of diffusion, that is, Coomassie blue was distributed into two distinctive zones in the spots, namely a diffusible fraction (DF) and a non diffusible fraction (NDF). NDF was represented by the centermost dark blue area close to the point where the sample was originally placed whereas DF represented the clearer outer band surrounding the corresponding NDF. In addition, the DFs of the spots corresponding to the IDYB products CP1, CP2 and CP3 were markedly more intense than the DFs of all the other IDYB products. Strikingly, IDYB products CP9 and CP10 showed no reactivity at all with Coomassie blue. On the other hand, when solutions of all ten IDYB products were centrifuged at low speed (600 g x 5 min) before spotting 20-µL aliquots from each supernatant on the cellulose membrane, with no exception the NDFs fully disappeared (Fig. 1). However, staining intensities of the resulting homogeneous blue spots differed markedly from each other so that the supernatants corresponding to CP1, CP2 and CP3 exhibited the highest staining intensities. Areas of diffusion of the aliquots from all ten supernatants were identical.

3.4. Electrophoretic fractionation of IDYB products followed by protein staining with Coomassie blue

All commercial IDYB products, together with human saliva as positive control, were subjected to a conventional electrophoretic fractionation in SDS-polyacrylamide denaturing gels (Fig. 1). In

contrast with the characteristic presence of the α-amylase duplet at around 55kDa in the electrophoretogram of human saliva, gel lanes corresponding to several IDYB products showed only some hardly visible bands. Thus, the IDYB products CP5, CP6 and CP8 showed some weakly stained bands at sizes over 55 kDa whereas CP4 showed weakly stained bands along most of the electrophoretic lane. Surprisingly, the IDYB products CP1, CP2 and CP3 showed only a diffuse staining without any evident electrophoretic band. However, a more commonly observed feature in this assay, as in the case of IDYB products CP1, CP2, CP3 and CP9, was the presence of a marked staining at and close to the dye front, which is highly suggestive of protein degradation.

3.5. Interaction of the IDYB product CP10 with either the seed extract or saliva

As expected, Coomassie blue staining revealed a biphasic mode of diffusion of the protein fraction of saliva on a horizontally positioned cellulose membrane (Fig. 2F). On the other hand, neither the seed extract (Fig. 2 K) nor growing amounts of CP10 (Fig. 2 B-C) placed on those membranes became stained by the protein dye. On these grounds, growing amounts of CP10 in the range of 0 and 400 µL (1 mg/mL) were mixed with either saliva (Fig. 2 G-J) or grape seed extract (Fig. 2 L-O) and the corresponding areas of protein diffusion on a cellulose membrane were measured (Table 2). The observed area of diffusion of the protein fraction being part of mixtures of saliva and growing amounts of CP10 was progressively reduced to as much as 65% of the whole area of diffusion displayed by the salivary protein alone (Fig. 2 G-J and Table 2). Likewise, when growing amounts of CP10 were mixed with a constant amount of the seed extract (SE), we observed a drastic decrease in the distribution area together with a progressive increase

in the staining intensity of the blue spots, here representing CP10/proanthocyanidin complexes (Fig. 2 K-O and Table 2).On the other hand, when both series of reaction mixtures (CP10-saliva and CP10-SE) were centrifuged (600 g x 5 min) to separate eventual precipitates and aliquots from the corresponding supernatants were spotted on a cellulose membrane, both decrease or even full loss in the stained intensity of the blue spots was evidenced (Fig. 2, U-Y and Z-D'). In addition, diffusion area of the salivary protein fraction remaining in the supernatant was reduced as the amount of CP10 mixed with saliva was increased thus pointing to the occurrence of soluble CP10-salivary protein complexes (Fig. 2 U-Y, Table 2). By contrast, supernatants produced by centrifuging mixtures of SE and growing amounts of CP10 resulted in full loss of Coomassie blue staining (Fig. 2 A'-D' *versus* Fig. 2 L-O) thus suggesting full precipitation.

3.6. Interaction of the IDYB product CP10 with both the seed extract and saliva

Altogether, CP10, SE and saliva in the reaction tube make up a ternary system. Considering that CP10 interacts separately with either a proanthocyanidin-rich extract or the salivary protein fraction, experiments were also conducted to test whether saliva was reactive against the CP10-seed extract binary system. In the assay, SE was thoroughly mixed with varying amounts of CP10 and 5 minutes later saliva was added to the reaction tube. Under the conditions of the experiment, in the absence of CP10, saliva mixed with SE displayed an intensely stained and well-defined biphasic mode of diffusion (Figure 3A). By contrast, the diffusible fraction of the tripartite mixtures was reduced in direct correspondence with the amount of CP10 in the mixture so that it almost disappeared in presence of the highest amount of CP10 in the study (Fig. 3 A-E and Table 3). When those tripartite mixtures were centrifuged just before placing aliquots of the corresponding supernatants on the cellulose membrane, the non diffusible component fully disap-

peared and the Coomassie blue-stained diffusible component showed also a marked reduction in direct correspondence with the amount of CP10 in the mixture (Fig. 3 F-J and Table 3). Such observation suggested an active role of the IDYB product, probably being part of binary complexes with SE, in the interaction with saliva.

4. Discussion

Introduction of IDYB products in modern winemaking industry has been made due to their ability to enhance fermentation, act as protective agents during yeast rehydration, increase color stability and attenuate astringency perception (Feuillat, 2001; Dulauet al., 2004; Caridi, 2007; Comuzzo et al., 2006; Pozo-Bayonet al., 2009). Despite the undisputed relevance of IDYB products, there is not much information about their composition or the mechanisms underlying their effects. In this study ten commercial IDYB products were compared using a set of physicochemical parameters. In addition, a selected IDYB product was characterized for its effect on interactions between a proanthocyanidin-rich extract and the protein fraction of saliva, a well-known physicochemical condition underlying astringency.

Physicochemical characterization of the IDYB products showed striking differences among them. Thus, the IDYB products CP1, CP2 and CP3 showed the highest contents of 280 nm-absorbing organic material, a finding correspondingly paralleled by their highest Bradford protein contents. By the same token, the IDYB product CP10 showed the highest content of polysaccharides and an intermediate content of protein, thus suggesting a higher content of glycosylated components compared with most of the IDYB products, such as CP1, CP2 and CP3. Also, marked differences were observed in the affinities of IDYB products for the selective protein-binding dye Coomassie blue R-250 (CB). In effect, CP1, CP2 and CP3 showed a high affinity for CB, whereas five IDYB products (CP4 thorough CP8) showed an intermediate reactivity and, strikingly, two displayed a nil reactivity towards that colorant. In addition, IDYB products in the study displayed marked differences in the mode of diffusion on cellulose membranes. Accordingly, based on this feature three subsets of IDYB products were identified, namely, the one displaying a biphasic mode of diffusion (CP1, CP2, CP3) comprising equally abundant diffusible and non-diffusible

fractions, those exhibiting a highly predominant non-diffusible component (CP4, CP5, CP6, CP7, CP8) and, finally, the two fully unstainable products (CP9, CP10). Interestingly, the non-diffusible fractions of the first two groups of IDYB products represented a water-insoluble proteinaceous component that was fully sedimented after a single low speed centrifugation. In the same regard, only CP1, CP2 and CP3 contain a significant fraction of water-soluble constituents. Again, such characteristic of these three IDYB products fully coincides with their highest absorbances at 280 nm and highest protein contents, as defined by independent analytical approaches.

Further characterization of the IDYB products was achieved by conventional one-dimensional electrophoretic fractionation in SDS-containing 12% polyacrylamide gels and staining with the protein-binding dye Coomassie blue R-250. By this method, protein mobility depends basically on the molecular weight of the protein. Under the conditions of the fractionation, protein bands over the size range from 15 to 200 kDa were expected to be revealed. However, a marked scarcity or even a full absence of sharp polypeptide bands together with an intense staining of the dye front were two highly common observations in the electrophoretograms of most of the IDYB products in the study. Both features are consistent with a small size of the components in the samples under fractionation. Quite likely, proteinaceous components of the IDYB products were degraded to small protein fragments at some stage of the preparation of the commercial products either because of the action of endogenous or exogenous proteases, or by any other proteolytic agent acting on inactive yeasts or yeast components. Nevertheless, it cannot be fully discounted that the absence of medium sized polypeptide bands in the electrophoresis gels is due to their lack of reactivity towards Coomassie blue as a consequence of a high degree of glycosylation. This would be very unlikely because the marked differences in the carbohydrate to protein mass ratios

among the IDYB products in the study would suggest the presence of scarcely glycosylated proteins, and thence reactive to Coomassie blue, at least in some of the products.

Following the physicochemical characterization of the IDYB products we assessed the effect of one of them on the interaction between a proanthocyanidin-rich grape seed extract and the protein fraction of saliva. Protein diffusion and precipitation assays on cellulose membranes were conducted (Obreque-Slier et al., 2010a; 2010b). The IDYB product CP10 was selected based on its lack of reactivity to Coomassie blue, which would facilitate monitoring of the salivary protein on the cellulose membrane. A number of previous studies have documented cross-reactivity between proanthocyanidins and the salivary protein fraction as well as the high stability of the complexes they form (Prinz & Lucas, 2000). Such complexation has been closely associated with the astringency produced by proanthocyanidin-rich foods and drinks (Bacon & Rhodes, 2000). Preliminary assays in the study showed that on the cellulose membrane the Coomassie blue dye was either unreactive or marginally reactive to the seed extract. By contrast, a 1:14 dilution of saliva in water displayed a typical biphasic mode of diffusion of the protein fraction as revealed by Coomassie blue (López et al., 2007; Obreque-Slier et al., 2010b). On these grounds, distribution of the salivary protein on the cellulose membrane can be monitored without interference by polyphenols (Obreque-Slier et al., 2010a). Binary mixes comprising growing amounts of CP10, on the one hand, and a constant amount of the proanthocyanidin-rich seed extract, on the other, resulted in a progressive reduction of the distribution area of the mix on a cellulose membrane, thus supporting the postulate that polyphenols cross-react with mannoproteins (Cadiri, 2006; Guadalupe & Ayestarán, 2008). On the other hand, binary mixes of saliva and the IDYB product CP10 also resulted in a marked diminution in the diffusion area of the salivary protein, which is a clear indication of CP10/salivary protein complexes displaying a lower ability to diffuse on the cellulose membrane. To further substantiate this interpretation we also conducted precipitation assays.

Thus, binary mixes between either the seed extract or saliva and the CP10 product resulted in no staining or faint staining of the corresponding supernatants placed on cellulose membranes and stained with Coomassie blue, which is fully consistent with precipitation of CP10/tannin and CP10/salivary protein complexes (Siebert, Carrasco & Lynn, 1996; Saucier, Glories & Roux, 2000; Guadalupe & Ayestarán, 2008; Guadalupe et al., 2007). Given the observed reactivity of the IDYB product CP10 with either the proanthocyanidin-rich extract or the protein fraction of saliva and bearing in mind that astringency has been closely associated with tannin-salivary protein interactions, experiments with tertiary mixes (tannin, saliva and CP10) were conducted. Thus, considering that the mixing order may affect cross-interaction of components in a multipartite complex system, the selected protocol for producing the experimental mixture was the one mimicking the condition occurring during wine tasting, that is, polyphenols mixed with mannoproteins (as in red wine) were put in contact with saliva (as in a tasting event). Under these conditions, both a remarkable decrease in the diffusion area of the salivary protein (diffusion assay) and a marked disappearance of the protein from the corresponding supernatants (precipitation assay) were observed in direct correspondence with the amount of CP10 in the ternary mixture. Thus, both results strongly suggested that CP10-tannin complexation is not sufficient to prevent interactions between their components, or between the complexes themselves, with the salivary protein. Such an observation, which should be additionally tested as part of more complex matrices (model hydroalcoholic solutions and wines) supplemented with IDYB products, is in conflict with data from various laboratories indicating that mannoproteins (or polysaccharides) and proanthocyanidins form stable major supramolecular structures that are unreactive to the salivary protein, thus decreasing astringency perception (Escot, Feuillat, Dulau & Charpentier, 2001; Guadalupe & Ayestarán, 2008). Nevertheless, as shown in this study, different commercial IDYB enological products may have highly diverse physicochemical properties that may account

for some functional peculiarities. That is why, characterization of the IDYB products may be critical, not only because most of them are marketed worldwide under a generic name and usually with a weak technical data support, but because they may influence unforeseeably sensory properties of wines, such as astringency.

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Figure captions

Figure 1. Diffusion pattern of IDYB products on cellulose membrane and profiles of IDYB products subjected to electrophoretic fractionation. A. *Upper panel*: Twenty-µL-aliquots of IDYB products were spotted on a cellulose membrane, fixed and stained for protein with Coomassie blue. *Lower panel*: The assay is the same as above except that the cellulose membrane was spotted with aliquots of supernatants obtained by centrifugating the IDYB solutions. **B.** Aliquots of each of the IDYB products in the study were fractionated in polyacrylamide gels under denaturing conditions. A fresh sample of human saliva (Sal) was run as a positive control for protein banding.

Figure 2. Interaction of the IDYB product CP10 with either saliva or a proanthocyanidin-rich extract: diffusion and precipitation assays. Growing amounts of CP10 (0-400 µL) were mixed with either saliva (saliva-CP10) or proanthocyaniodin-rich extract (SE-CP10) and the final volume of the mixture was taken to 1.4 mL with water. Blanks were prepared by diluting 0-400 µL of CP10 to 1.4 mL with water (CP10-water). *Top panel*: Aliquots of each mixture (F-J and K-O) or blank (A-E) were spotted sharply on points of a cellulose membrane and allowed to diffuse. The *bottom panel* shows the same assay as above except that the cellulose membrane was spotted with aliquots taken from the supernatants of saliva-CP10 mixtures (U-Y), seed extract-CP10 mixtures (Z-D') and CP10-water blanks (P-T). Numbers on top of the figure represent the amount of CP10 in the mixtures and blanks.

Figure 3. Interactions among components of the ternary system CP10/SE/saliva: diffusion and precipitation assays on cellulose membranes. Growing amounts of CP10 (0-400 µl) were mixed with water up to a final volume of 400 µL and then with 1000 µL of proanthocyaniodin-rich extract (SE). After 5 min, 100 µL of saliva was added to each tube (A through J). Aliquots of the mixtures A-E were spotted on cellulose membranes and allowed to diffuse (diffusion test). On the other hand, the mixtures F-J were centrifuged and aliquots from the resulting supernatants were spotted on cellulose membranes (precipitation test). Numbers on top of the figure represent the amount of CP10 in the ternary mixture, including blanks A and F (no CP10).

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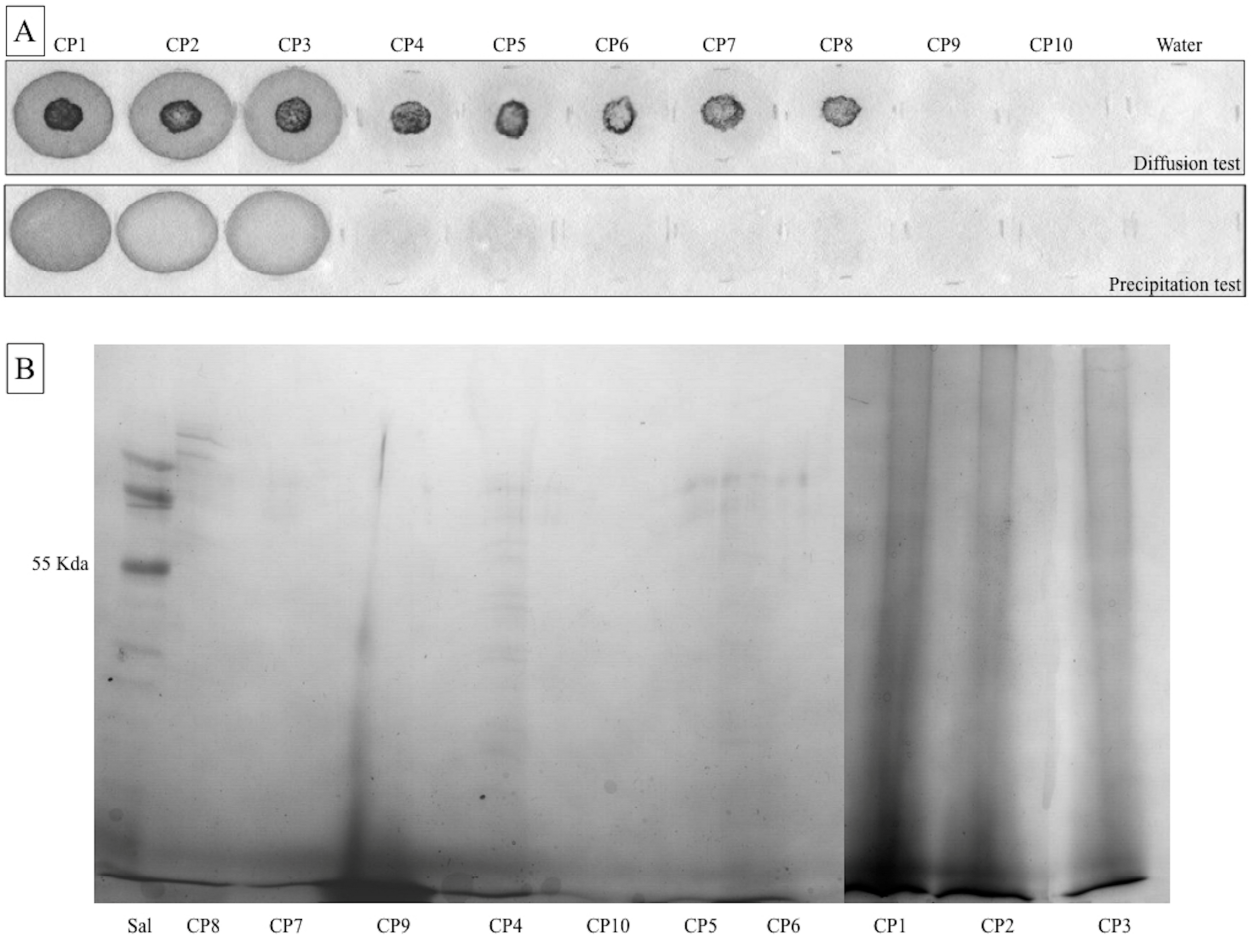

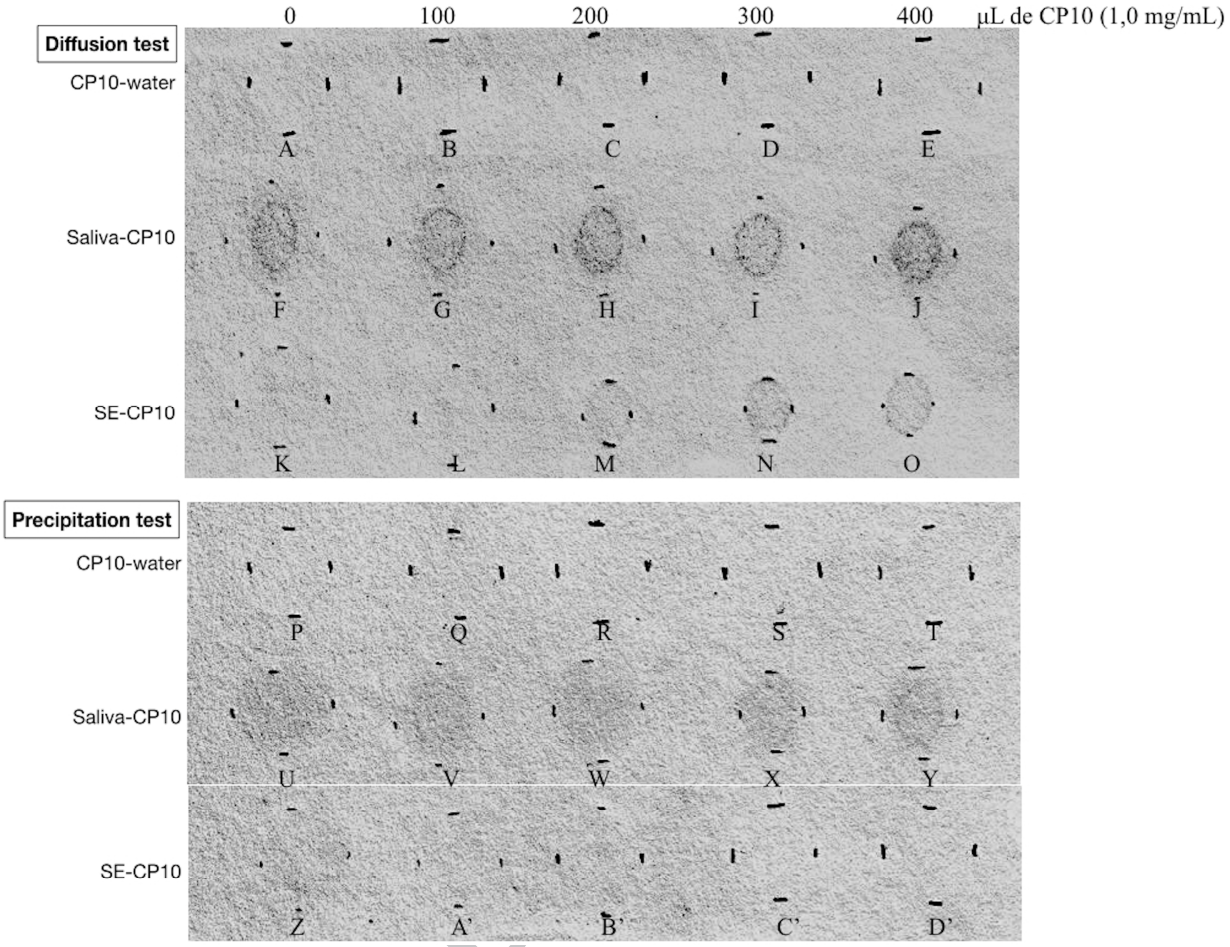

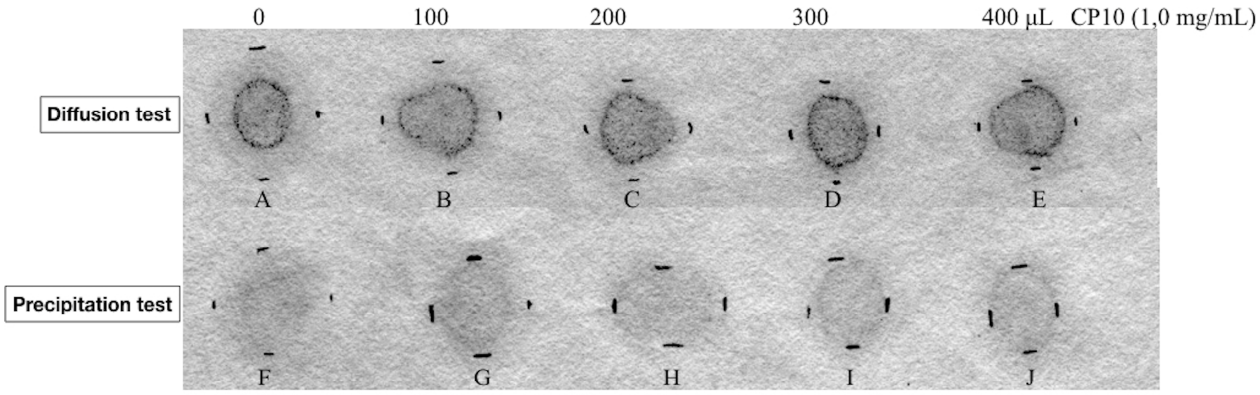

Table 1. Organic matter, protein and polysaccharide content in ten commercial IDYB products in the study

Product	Absorbance at 280 nm*	Protein content **	Polysaccharide content ***
CP 1	0.67 ± 0.01 a	13.30 ± 0.02 a	39.80 ± 3.53 b
CP 2	0.63 ± 0.03 a	13.15 ± 0.02 a	37.38 ± 0.62 b
CP 3	0.64 ± 0.02 a	11.70 ± 0.01 b	23.61 ± 0.92 c
CP 4	0.20 ± 0.01 cd	5.12 ± 0.01 cd	16.72 ± 0.93 d
CP 5	0.19 ± 0.04 cd	3.83 ± 0.02 de	41.80 ± 1.12 b
CP 6	0.15 ± 0.03 d	2.38 ± 0.01 f	18.46 ± 0.73 cd
CP 7	0.20 ± 0.04 cd	5.39 ± 0.01 c	19.25 ± 2.51 cd
CP 8	0.23 ± 0.04 c	2.43 ± 0.01 ef	22.07 ± 1.81 cd
CP 9	0.23 ± 0.02 c	4.70 ± 0.02 cd	37.03 ± 3.22 b
CP 10	0.48 ± 0.01 b	5.08 ± 0.01 cd	53.53 ± 0.72 a

Products	Fraction I****	Fraction III****	Fraction IV****
CP 1	N.D.	44.27 ±0.28 bc	3.18 ±0.74 cd
CP 2	N.D.	45.46 ±3.28 bc	10.33 ±3.46 ab
CP 3	N.D.	52.08 ±2.47 bc	1.68 ±1.07 d
CP 4	N.D.	39.21 ±3.52 bc	11.63 ±1.07 ab
CP 5	N.D.	49.56 ±2.86 bc	15.16 ±1.03 a
CP 6	N.D.	28.36 ±1.36 c	14.15 ±1.98 ab
CP 7	N.D.	42.50 ±2.37 bc	4.89 ±0.63 cd
CP 8	N.D.	39.03 ±0.38 bc	11.28 ±1.91 ab
CP 9	2.64 ±0.53	59.82 ±8.31 b	7.31 ±2.13 bc
CP 10	N.D.	177.20 ±20.66 a	N.D.

Mean ± standard deviation (triplicates). *corresponding to 10 mg/mL solutions; ** expressed as mg BSA/g (Bradford, 1976); ***expressed as mg mannose/g (Dubois et al., 1956). ****Polysaccharide fractions by HPLC (mg/g) (Del Barrio-Galán et al, 2015). ND, Not detected. Different lower case letters next to values stand for statistically significant differences among mean values in a column (Tukey test, $p \leq 0.05$).

Table 2. Interaction of the IDYB product CP10 with either saliva or a proanthocyanidin-rich seed extract: Morphometric estimation of diffusion areas of binary mixtures on cellulose membranes

µL CP10	**0**	**100**	**200**	**300**	**400**
Whole mixture					
CP10/water	1.8 ± 0.1 a	1.8 ± 0.1 a	1.9 ± 0.1 a	1.9 ± 0.0 a	1.9 ± 0.0 a
Saliva/CP10	1.4 ± 0.2 ab	1.4 ± 0.0 a	1.2 ± 0.0 b	1.2 ± 0.1 b	0.9 ± 0.0 c
SE/CP10	1.7 ± 0.1 a	1.6 ± 0.1 a	0.6 ± 0.0 b	0.6 ± 0.0 b	0.6 ± 0.0 b
Supernatant					
CP10/water	1.8 ± 0.0 a	1.9 ± 0.1 a	2.0 ± 0.2 a	1.9 ± 0.1 a	1.9 ± 0.2 a
Saliva/CP10	1.8 ± 0.2 ab	1.7 ± 0.2 ab	1.9 ± 0.1 a	1.0 ± 0.1 c	1.2 ± 0.1 c
SE/CP10	1.6 ± 0.1 a	1.4 ± 0.2 a	1.6 ± 0.1 a	1.6 ± 0.1 a	1.5 ± 0.1 a

Data corresponds to areas produced by 20-uL aliquots in the experiment shown in Figure 2. Values (cm^2) represent mean ± standard deviation of triplicates. Numbers on the upper row of the table (0-400) represent the amount of CP10 in the binary mixture, including blanks (CP10=0). Different lower-case letters next to values stand for statistically significant differences among values in a row (Tukey test, $p \leq 0.05$).

Table 3. Interactions among components of the ternary system CP10/saliva/SE: Morphometric estimation of diffusion areas of ternary mixtures on cellulose membranes

µL CP 10	0			100			200			300			400		
Whole mixture															
SE-CP10 plus Sal (NDF)	0.6 ± 0.1	a		0.7 ± 0.1	a		0.6 ± 0.0	a		0.6 ± 0.1	a		0.6 ± 0.1	a	
SE-CP10 plus Sal (DF)	1.5 ± 0.2	a		1.0 ± 0.1	b		0.8 ± 0.1	bc		0.7 ± 0.0	c		0.5 ± 0.1	c	
SE-Sal plus CP10(NDF)	0.5 ± 0.0	a		0.6 ± 0.0	a		0.6 ± 0.0	a		0.6 ± 0.1	a		0.5 ± 0.0	a	
SE-Sal plus CP10 (DF)	1.4 ± 0.1	a		1.3 ± 0.1	ab		1.4 ± 0.0	a		1.1 ± 0.0	c		1.2 ± 0.1	bc	
Supernatant															
SE-CP10 plus Sal (DF)	1.5 ± 0.0	a		1.3 ± 0.1	b		1.3 ± 0.1	b		1.0 ± 0.1	c		0.9 ± 0.1	c	
SE-Sal plus CP10(DF)	1.5 ± 0.1	a		1.4 ± 0.1	a		1.5 ± 0.1	a		1.4 ± 0.1	a		1.5 ± 0.1	a	

Data corresponds to areas produced by 20-uL aliquots in the experiment shown in Figure 3. Values (cm^2) represent mean ± standard deviation of triplicates. Numbers on the upper row of the table (0-400) represent the amount of CP10 in the ternary mixture, including blanks (CP10 = 0). DF and NDF stand for Diffusible fraction and Non diffusible fraction, respectively. Different lower-case letters next to values stand for statistically significant differences among values in a row (Tukey test, $p \leq 0.05$).

HIGHLIGHTS

Commercial inactive dry-yeast based (IDYB) products impact on the winemaking process.

>Marked physicochemical differences were observed among all ten commercial IDYB products.

>Commercial product 10 (CP10) was found to interact with the seed extract and the salivary protein.

>CP10 interacted with the salivary protein to form ternary complexes.