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Effect of egg albumin fining, progressive clarification and cross-flow microfiltration on the polysaccharide and proanthocyanidin composition of red varietal wines

Leticia Martínez-Lapuente *, Zenaida Guadalupe, Belén Ayestarán *

Instituto de Ciencias de la Vid y del Vino (Universidad de la Rioja, Gobierno de La Rioja y CSIC), Ctra. Burgos Km. 6, 26007 Logroño, La Rioja, Spain

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ABSTRACT

The effect of egg albumin fining, progressive clarification and cross-flow microfiltration on the polysaccharide and proanthocyanidin composition of four red varietal wines was studied in this work. Discriminant analyses were applied to achieve a possible differentiation of the wines according to treatment or grape variety. Egg albumin fining did no produce a significant decrease in the content of wine polysaccharides. Progressive clarification caused a significant reduction of mannoproteins, homogalacturonans and polysaccharides rich in arabinose and galactose in Graciano wines. However, both treatments reduced the total content of proanthocyanidins in all varietal wines. Cross-flow microfiltration produced the highest retention of polysaccharides and proanthocyanidins in all the wines, mainly polysaccharides rich in arabinose and galactose, yeast mannoproteins and highly polymerized phenols. Polysaccharides rich in arabinose and mannoproteins were more retained on the ceramic membrane than polysaccharides rich in galactose and proanthocyanidins. Discriminant analyses allowed a clear differentiation of cross-flow microfiltrated wines from the rest of the wines.

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1. Introduction

Polysaccharides and polymerized phenolic compounds are the main compounds of colloidal nature in red wines. The content of these compounds in wines depends mainly on the grape variety, their concentration in grape, the winemaking technology used and their transformation during the wine ageing process (Spranger et al., 2004; Sun & Spranger, 2005; Sun, Spranger, Roque-do-Vale, Leandro, & Belchior, 2001). Both phenolic compounds and polysaccharides play an important role in the sensory properties of wines. In particular, phenolic compounds are directly related not only to color, astringency, bitterness and oxidative level of red wine, but also to well-known health beneficial effects as antioxidants. On the other hand, polysaccharides are known as protective colloids and play an important role on a number of technological and quality properties of wines.

Natural wine colloids together with some particles like yeasts, bacterial strains and cell debris cause turbidity in the crude red wine after alcoholic and malolactic fermentation. This is not well accepted by the consumer and it is perceived as a sign of product deterioration.

Indeed, limpidity and wine color is the first visual quality observed by consumers. Limpidity must be maintained during all the storage period (even in tank or bottle) and storage condition (aeration, lighting, temperature, etc.) (El Rayess et al., 2011). Filtration is made to provide limpidity and microbiological stabilization of wines. However, this cleaning operation does not guarantee the physico-chemical stabilization of wine, thus it does not prevent the formation of organic and inorganic hazes and deposits after packaging. Wine stability is defined as a state or a condition such as the wine will not, for some definite period, exhibit undesirable physical, chemical, or organoleptic changes (Thoukis, 1974). The undesirable changes that denote wine instability include: browning or other color deterioration, haziness or very slight cloudiness, cloudiness, deposits and undesirable taste or odor (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006; Thoukis, 1974).

Nowadays, enologists enhance the stabilization and limpidity of crude wines by using technologies such as centrifugation, dead-end filtration (filter presses, filtration on sheets and on cellulose, diatomaceous earth filtration) and the use of fining agents. Enologists subject the crude wines to a progressive clarification by using several cleaning techniques such as natural clarification by gravity, clarification with fining agents, or several filtration steps on diatomaceous or on cellulose, prior to the final microbial stabilization obtained by dead end filtration on sheets or membranes (Hidalgo Togores, 2003; Lüdemann, 1987). The

* Corresponding authors.
E-mail addresses: leticia.martinez@unirioja.es (L. Martínez-Lapuente), belen.ayestaran@unirioja.es (B. Ayestarán).

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cross-flow microfiltration is a relatively new technique that involves a one-step procedure, and can substitute the conventional processes of progressive clarification. Cross-flow microfiltration offers additional advantages compared to conventional processes such as elimination of filter aids use and its associated environmental problems, and the combination of clarification, microbial stabilization and sterile filtration in one single continuous (El Rayess et al., 2016). However, many researchers have demonstrated the negative effects of wine polysaccharides and polyphenols on the permeation flux, especially by adsorption of these molecules on membrane materials (Belleville, Brillouet, Tarodo de la Fuente, & Moutounet, 1992; Ulbricht, Ansorge, Danielzik, Konig, & Schuster, 2009; Vernhet & Moutounet, 2002). Others have also provided evidence that some membrane materials exhibit various levels of adsorption of typical foulants in wine such as polysaccharides and polyphenols (Arriagada-Carrazana, Saez-Navarrete, & Brodeu, 2005; Ulbricht et al., 2009; Vernhet & Moutounet, 2002).

Finning agents are used to provide limpidity and stabilization of wines but also to improve some organoleptic properties of wines. They are used to eliminate or reduce some phenolic compounds of colloidal nature implicated on oxidation phenomena or wine astringency. Studies have shown that the use PVPP, gelatin, egg albumin and casein reduced phenolic levels and modified the color in some wines (Castillo-Sánchez, Mejuto, Garrido, & García-Falcón, 2006; Gomez-Plaza, Gil-Muñoz, López-Roca, & Martinez, 2000; Maury, Sarni-Manchado, Lefebvre, Cheynier, & Moutounet, 2001). Moreover, Oberholster, Carstens, and du Toit (2013) also observed that gelatin, egg albumin and cross-flow microfiltration removed the highly polymerized phenols (tannins) of Pinotage wines. The present paper aims to increase the knowledge of these studies by analyzing other winemaking techniques such as progressive clarification and other wine compounds such as polysaccharides.

Wines in this paper were submitted to different clarification processes: (i) clarification with egg albumin; (ii) progressive clarification (clarification with egg albumin followed by filtration plates on cellulose); and (iii) cross-flow microfiltration. Different monovarietal wines from Merlot, Tempranillo, Garnacha and Graciano were used. The effect of all these techniques on the proanthocyanidin and polysaccharide composition of these wines is described for the first time in this paper.

2. Materials and methods

2.1. Chemicals

All reagents were analytical grade unless otherwise stated. D-(+)-fucose, L-rhamnose, 2-*O*-methyl-D-xylose, L-(+)-arabinose, D-(+)-galactose, D-(+)-mannose, Kdo (2-keto-3-deoxyoctonate ammonium salt) and D-apiose solution were supplied by Sigma-Aldrich (Beerse, Belgium), D-(+)-galacturonic acid, D-glucuronic acid, myo-inositol and trifluoroacetic acid were obtained from Fluka (Buchs, Switzerland), and (+)-catechin and (−)-epicatechin were purchased from Extrasynthèse (Lyon, France). Ethanol 96% (v/v), acetone, HPLC grade methanol, acetic acid glacial, sodium acetate and acetyl chloride were supplied by Scharlab (Barcelona, Spain), hydrochloric acid 37% was purchased from Carlo Erba (Rodano, Milan, Italy) and ascorbic acid was obtained from Panreac (Barcelona, Spain). Hexane, dried methanol, pyridine, hexamethyldisilazane and trimethylchlorosilane were obtained from Sigma-Aldrich (Beerse, Belgium). Toyopearl gel HW-50F was obtained from Tosoh Corporation (Tokyo, Japan).

2.2. Vinifications and samples

Vinifications were carried out in the wine cellar of the University of La Rioja using the red grapes *Vitis vinifera* cv. Merlot, Tempranillo, Graciano and Garnacha. Grapes were harvested on 2015 vintage at commercial maturity, and monovarietal wines were prepared using traditional wine technology. Grapes were destemmed and crushed and distributed into 1000 L stainless steel tanks, sulphited with 3 g/HL SO_2 and inoculated with 25 g/HL *Saccharomyces cerevisiae* yeast (Lalvin ICV-D254, Lallemand, Canada). The prefermentation process went on for 6 h at 12 ± 1 °C, the fermentation–maceration process was carried out at a maximum temperature of 28 ± 2 °C and lasted 10 days. After racking the pomace was pressed and resulted in wine added to the main wine. Wines were then inoculated with 1 g/HL of a commercial preparation of *Oenococcus oeni* (Viniflora, CH16, CHR Hansen, Denmark) to induce malolactic fermentation, carried out at 18.5 ± 1 °C. After malolactic fermentation, all the wines were racked.

The different clarification processes were then applied: (i) one hundred liters of each wine was not submitted to any clarification process, and was employed as crude (C) or unfined wine; (ii) other 200 L of each wine were distributed into 100 L stainless-steel tanks and clarified with egg albumin as fresh egg whites (10 egg whites/HL for egg albumin (EA)); (iii) other 200 L of each wine were distributed into 100 L stainless-steel tanks and submitted to a progressive clarification (PC) with 10 egg whites/HL for egg albumin and filtration over a plate filter (PC) with a MINUS 20 × 20 (Fratelli Marchisio, Italy) with GS-100 cellulose filter sheets (PBFiltracion, Barcelona, Spain); (iv) other 200 L of each wine were subjected to cross-flow microfiltration (CFMF) using a cross-flow Millipore filter (Millipore, Molsheim, France), with an α-alumina ceramic membrane and a pore diameter of 0.2 μm. Samples for analysis were taken from crude wines (C), wines clarified with egg albumin (EA), wines submitted to progressive clarification (PC), and wines submitted to cross-flow microfiltration (CFMF).

2.3. Standard enological parameters

Standard enological parameters (pH, titratable acidity, alcohol content, volatile acidity, color intensity, hue and total polyphenol index) were measured using an Infrared Analyser FTIR and UV–Vis (Bacchus 3 Multispec, Thermo Fisher Scientific Inc., Langenselbold, Germany). A turbidimeter (model 2100N, Hach Instruments Inc., USA) was used to determine the turbidity of the wines.

2.4. Precipitation of total soluble wine polysaccharides

Soluble wine polysaccharides were recovered by precipitation after ethanolic dehydration as previously described (Guadalupe, Martínez-Pinilla, Garrido, Carrillo, & Ayestarán, 2012). Samples were homogenized and centrifuged using a Sorvall Lynx 4000 refrigerated centrifuge (Thermo Fisher Scientific Inc., Langenselbold, Germany), and 2 mL of the supernatants were taken and introduced into 15 mL falcon-tubes to be concentrated to dryness in a miVac Duo system (Genevac, Ipswich, United Kingdom). Polysaccharides were then precipitated by adding 2 mL of cold ethanol/acid (ethanol 96% containing 0.3 M HCl) and kept for 24 h at 4 °C. Thereafter, the samples were centrifuged, the supernatants discarded and the pellets washed several times with 96% ethanol to remove the interference materials. The pellet, which corresponded to total soluble polysaccharides (TSP), was finally freeze-dried using a ScanVac Coolsafe TM freeze-drying apparatus (LaboGene, Lynge, Denmark). The polysaccharide extraction was performed in triplicate in each sample.

2.5. Identification and quantification of monosaccharides by GC–MS

The monosaccharide composition of the TSP precipitates was determined by GC–MS of their trimethylsilyl-ester *O*-methyl glycosyl residues obtained after acidic methanolysis and derivatization as previously described (Guadalupe et al., 2012). GC was made on an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector (MS), equipped with a 7653B automatic injector, and controlled by the ChemStation software. Samples were injected in duplicate. The content

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