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Characterization of a neutral polysaccharide with antioxidant capacity from red wine

M. J. Aguirre [a], M. Isaacs [b], B. Matsuhiro [a,*], L. Mendoza [a], E. A. Zúñiga [a]

[a] Facultad de Química y Biología, Universidad de Santiago de Chile, Avenida Libertador Bernardo O'Higgins 3363, Santiago, Chile
[b] Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Santiago, Chile

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ABSTRACT

A neutral fraction (PS-SI) (0.3 g/L) with MW of 74 kDa, which contained galactose, arabinose, mannose, and glucose in the molar ratio of 1.0:0.6:0.4:0.2 was obtained by treatment of the whole polysaccharide extracted from red wine with cetrimide, followed by gel permeation chromatography. Spectroscopic and methylation analyses indicated that PS-SI is a mixture of neutral polysaccharides, consisting mainly of β (1→3)-linked galactopyranosyl residues, with side chains of galactopyranosyl residues at positions *O*-6. Arabinofuranosyl residues linked α (1→5), α-mannopyranosyl and glucosyl residues appear to be components of different polysaccharides. The in vitro antioxidant capacity of fractions of wine polysaccharide was studied by hydroxyl radical scavenging and ORAC assays. Fraction PS-SI presented the strongest effect on hydroxyl radicals (IC_{50} = 0.21).

1. Introduction

Polysaccharides in red wine originate either in grape berries (*Vitis vinifera*) or from yeast during fermentation. They comprise arabinogalactan proteins and rhamnogalacturonans from grape, and yeast-derived mannoproteins.[1–5] The presence and concentration of polysaccharides in wine depend on the grape variety, maturity, and climate, among numerous factors.[6,7] Wine polysaccharides play several roles such as controlling wine stability, and increasing organoleptic properties.[8–10] The antioxidant activity of red wines is well known and it has been related to the presence of phenolic compounds.[11–14]

It has been found that some polysaccharides from yeast, seaweeds, and fungi possess in vitro antioxidant activities.[15–20] Recently, Luo and Fang[21] reported the antioxidant properties of neutral glucans from ginseng. In another report, Zou et al. found that sulfation of neutral (1→3)-linked D-galactans from lac tree afforded derivatives with good antioxidant capacities.[22] As far as we know, the antioxidant capacity of wine polysaccharides has not yet been studied. In this work, we report the characterization of Cabernet Sauvignon red wine polysaccharides and the study in vitro of their antioxidant properties.

2. Results and discussion

Liquid–liquid extraction of Chilean Cabernet Sauvignon red wine followed by ethanol precipitation, gave a beige solid (PS) at a concentration of 1.6 g/L. The chemical composition (Table 1) suggested the presence of mannans, arabinogalactans, and pectin type polysaccharides. The FT-IR spectrum of the polysaccharide shows a shoulder around 1750 cm^{-1}, which was assigned to the C=O stretching vibration of an ester function, and two strong bands at 1617 and at 1067 cm^{-1}, the latter flanked by shoulders (Fig. 1A). Better characterization was achieved from the second-derivative FT-IR spectrum. Second-derivative FT-IR spectra give more information than the normal spectra, and they have been used for the characterization of polysaccharides and biomolecules present in organisms such as red seaweeds, fungi, and bacteria.[23–26] In the second-derivative spectrum of the crude polysaccharide (Fig. 1B), it can be seen that the band at 1617 cm^{-1} in the normal spectrum is resolved into three bands, two of them could be assigned to amide I and to the carboxylate group vibrations (1660.4 cm^{-1} and 1601.7 cm^{-1}, respectively).[27] Furthermore, the broad band centered around 1067 cm^{-1} in the normal spectrum is resolved into a band at 1153.1 cm^{-1}, assigned according to Kačuráková et al.[28] to arabinogalactan, and bands at 1071.7 and 976.0 cm^{-1} assigned to rhamnogalacturonan.[29] Absorptions at 881.0 and 839.2 cm^{-1} indicate the presence of β- and α-linked galactopyranosyl residues, respectively.[30] The polysaccharide was shown by gel permeation chromatography (GPC) to be composed of at least six fractions with molecular weight distribution between 70 kDa and 700 kDa (Fig. 2).

* Corresponding author. Tel.: +56 2 7181159; fax: +56 2 6812108.
E-mail address: betty.matsuhiro@usach.cl (B. Matsuhiro).

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Table 1
Chemical composition (%) of wine polysaccharides

Components	PS	PS-S	PS-SI	PS-P
Neutral Sugar[a] (%)	46.5	79.6	63.6	38.7
Galactose	1	1	1	1
Rhamnose	tr[b]	tr	tr	0.4
Arabinose	0.6	0.1	0.6	1.1
Mannose	0.6	2	0.4	0.8
Xylose	tr	tr	tr	0.1
Glucose	tr	0.5	0.2	0.1
Uronic acids (%)	32.5[c]	nd[d]	nd[d]	43.1[c]
Proteins (%)	6.1	9.9	0.5	2.1

PS—whole polysaccharide, PS-S—fraction soluble in cetrimide, PS-SI—homogeneous fraction from PS-S, PS-P—fraction insoluble in cetrimide.
[a] By GC analysis.
[b] tr = traces.
[c] By the method of Blumenkrantz and Asboe-Hansen (1973).
[d] nd = non-detected by the methods of Blumenkrantz and Asboe-Hansen (1973), and Filisetti-Cozzi and Carpita (1991).

Treatment of the whole polysaccharide with cetrimide gave a soluble fraction (PS-S) (0.9 g/L) containing galactose, arabinose, mannose, and glucose in the molar ratio 1.0:0.1:2.0:0.5 (Table 1). The main peak in its FT-IR spectrum appeared at 1074 cm^{-1}, flanked by two shoulders while in the second-derivative spectrum it was resolved into several bands that could be ascribed to arabinogalactans and mannans.[4,28] In this region C–OH deformation, CCH bending, C–O and C–C stretching vibrations occurred; each band may be due to contributions of two or more kinds of motions.[31] Furthermore, the second-derivative spectrum (Fig. 3A) shows the bands assigned to amide function of proteins at 1652.1, 1558.1, and 1419.3 cm^{-1}. Gel permeation chromatographic analysis of the cetrimide soluble fraction (PS-S) indicated the presence of at least five fractions. Only one fraction could be separated by preparative GPC, which by freeze-drying gave a white solid (PS-SI, yield 0.3 g/L). It was shown to be homogeneous by GPC (Fig. 2) with a MW of 74 kDa. The molecular weight determined by the reducing-end method corroborated the value deduced by GPC. It contained galactose, arabinose, mannose, and glucose in the molar ratio 1.0:0.6:0.4:0.2. Proteins were present in low proportions and it was devoid of uronic acids (Table 1). Its UV spectrum did not show any absorbance in the range 230–600 nm.

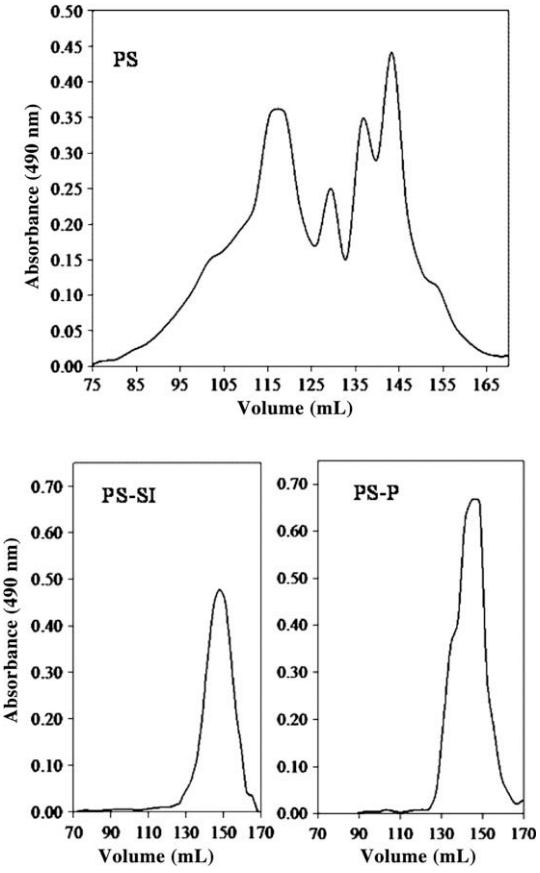

Figure 2. GPC on Sephadex 4B-CL of wine polysaccharides. PS—wine polysaccharide, PS-SI— neutral fraction from fraction soluble in cetrimide (PS-S), PS-P—fraction insoluble in cetrimide.

According to these results, the second-derivative FT-IR spectrum of PS-SI shows a weak signal assigned to amide groups and no absorption attributed to carboxyl group is present (Fig. 3B). The band at 1069.6 cm^{-1} in the normal FT-IR spectrum is resolved in the second-derivative spectrum into four signals, the peak at

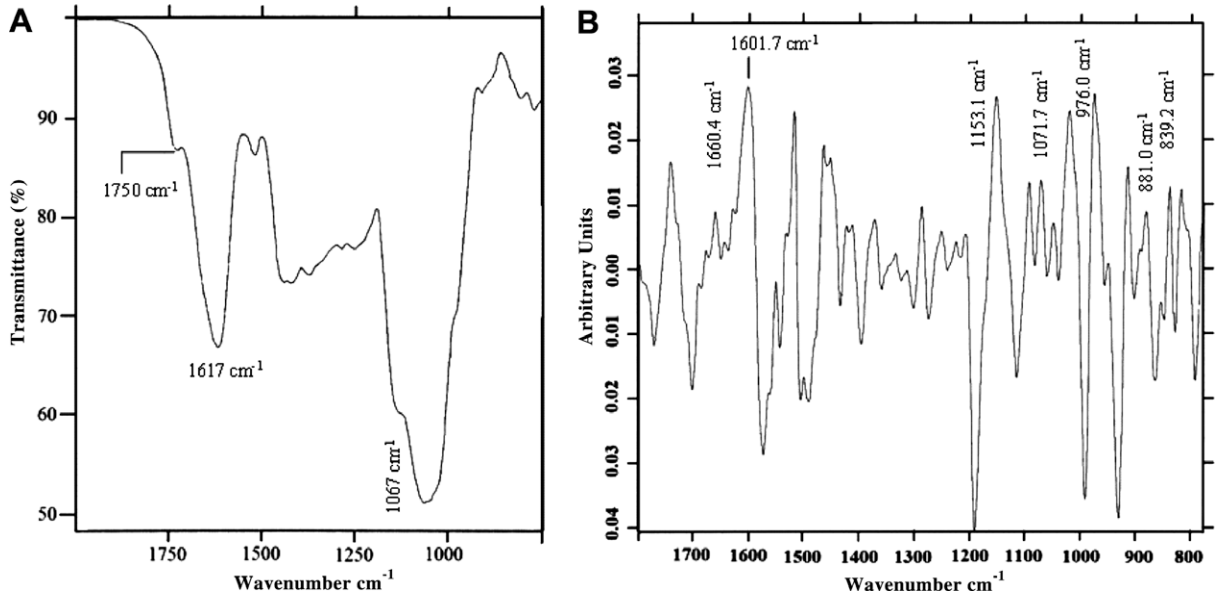

Figure 1. FT-IR spectra of the wine polysaccharide (PS), A—Normal spectrum, B—Second-derivative spectrum.