HPLC Analysis of Total Polysaccharide in Fruit Juice and Wine

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A simple and fast high performance liquid chromatography method for the analysis of polysaccharides in fruit juice and wine is presented. The direct injection of sample and the effect of sample clean-up by membrane dialysis and solid-phase extraction was verified.

Introduction

Polysaccharides are natural macromolecules located in the primary cell walls of fruit, and may be released under the action of specific endogenous and exogenous enzymes during fruit juice and wine processing.[1–3] Complex polysaccharides obtained from plants and microbes are finding increased application in the food industry as additives to improve functional properties of processed foods. Polysaccharide analysis is of great importance because these macromolecules are responsible for several types of physicochemical and sensorial phenomena, including crispness, juiciness and mealiness of fruits[4], prevention of protein haze in wine[5], and a detrimental role in the microfiltration process.[6]

Total polysaccharide determination using spectrophotometric methods is time consuming and does not permit automation. Also, the use of different recovery and analysis procedures does not allow a direct comparison of literature data.[7–10] Thus, there is an ongoing interest in the development of reliable, rapid and accurate methods to analyse the polysaccharide content of foods, for both routine analysis and quality control purposes.

This study reports a fast and simple method based on the direct determination of total polysaccharide content in fruit juice and wine by high performance liquid chromatography (HPLC) using refractive index (RI) detection and a calcium sulfonated divinyl benzene-styrene copolymer based column.

Materials and Methods

Standards of apple pectin and poligalacturonic acid were purchased from a commercial source (Sigma, Milan, Italy). Samples of fruit juice (apple and apricot) and white wine were obtained from local markets. Three HPLC procedures were compared: (1) direct injection, (2) sample clean-up with dialysis membrane at 1000 and 10 000 Daltons MWCO (Spectra/Por, Spectrum, Milan, Italy), and (3) sample clean-up with Bond Elute SPE C18 cartridges (Varian Inc., Harbour City, California, USA), respectively. The SPE cartridge (5 cc/500 mg) was previously activated with 5 mL of methanol followed by 5 mL of milli–Q water. Subsequently, the sample (5 mL) was passed through the cartridge and two fractions were eluted with 0.1 N HCl (pH = 2), and methanol, respectively. Fraction 1 was later dialysed against water for 24 hr to remove the HCl. The sample was diluted 10-fold and filtered through a 0.45 µm cellulose–acetate membrane (F-0139, Sigma) before injection into the HPLC system.

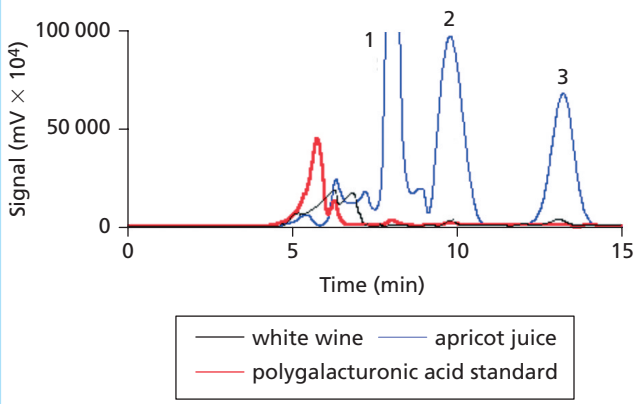
The instrument used was a Jasco HPLC system (Jasco Inc., Tokyo, Japan) equipped with a pump (PU980) and connected to an RI detector (RI830). Samples were injected with a 20 µL loop using a 7125 valve (Rheodyne Inc., Cotati, California, USA). The separation was performed with an Aminex HPX-87-Ca column (300 × 7.8 mm) protected with a precolumn (30 × 4.6 mm) filled with the same stationary phase (Bio-Rad Laboratories, Hercules, California, USA). The conditions of analysis were as follows: column temperature = 80 °C, flow = 0.5 mL/min, eluent = HPLC-grade water. Data acquisition and peak processing were performed with Borwin 5.0 software (JMBS Developments, Grenoble, France).

Results and Discussion

The Aminex HPX-87-Ca column is a calcium sulfonated divinyl benzene-styrene copolymer based column normally used for the analysis of simple carbohydrates. The ability of this

column to analyse polysaccharides was verified in this study. Poligalacturonic acid was used as external standard in the range 10–500 mg/L, and its linearity of response, expressed in terms of coefficient of determination (r^2) was $r^2 \geq 0.998$, implying an excellent suitability for the HPLC system. Pectin was used for the repeatability test (12 run); its coefficients of variation being 2.0 and 0.2 for area and retention time, respectively. Figure 1 shows the chromatograms of pectin, apricot juice and white wine. Analysis of apple juice gave a similar result (data not shown). As expected, apricot juice showed the presence of simple carbohydrates such as sucrose, glucose and fructose. In both fruit juice and wine analysis the presence of additional early eluting peaks starting at ~5 min were also detected. These peaks overlapped with both apple pectin (Figure 1) and poligalacturonic acid standards (not shown), suggesting the presence of polysaccharides in these samples — their content being in the range 414–1190 mg/L. Samples analysed by direct injection and after membrane dialysis at 1000 MWCO Daltons gave similar results, implying a lack of interference compounds (e.g., salts) in fruit juice and wine. A blank test with water demonstrated that during dialysis the cellulose acetate membrane may release a small amount of unidentified compound (probably cellulose). For this reason, if sample clean-up is required an alternative method based on dextran gel selectivity might be considered. As expected, dialysis at 10 000 Daltons partly removed macromolecules from the sample, resulting in the lowest peak area. The presence of polysaccharides in samples was verified by treatment with ethanol containing 1 N HCl. After treatment of the sample with ethanol/HCl the polysaccharides were precipitated and removed, and both precipitate and supernatant were analysed. The peak of polysaccharides was present in the precipitate; moreover, in wine supernatant a residual peak (~6% of total) was still detected. This result confirmed the presence of polysaccharides in the samples, and the residual peak found in wine was tentatively attributed to polyphenolic compounds eventually combined with polysaccharides. To verify this hypothesis, the clean-up of supernatant with SPE C18 was performed. This procedure allowed the preliminary separation of neutral and acidic fractions, and the later elution of polyphenols with methanol. The fraction eluted with 0.1 N HCl and methanol accounted for 53%, and 47%, respectively. Fraction 2 was well represented ($47 \times 6 = 2.8\%$ of the total), thus confirming the presence of polyphenolic compounds. At this stage, the combination of polyphenols and polysaccharides can only be supposed. The contribution of proteins in terms of peak area was negligible; in fact, gelatin, a protein added during red wine processing, was analysed and did not generate an RI signal at the concentration normally used in wine.

Figure 1: Chromatograms of the polygalacturonic acid standard, apricot juice and white wine. Chromatographic conditions described in the text.



Peaks: 1 = sucrose, 2 = glucose, 3 = fructose.

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Conclusion

This HPLC method offers a rapid and flexible means to analyse total soluble polysaccharides in fruit juice and wine, and might be extended to other products. The quality control and the processing change in food science and biotechnology are among the areas in which this method can have important application.

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