

# Polysaccharide content of extracts obtained from unfermented skins from red varieties

María Curiel-Fernández<sup>1</sup>, Belén Ayestarán<sup>2</sup>, Zenaida Guadalupe<sup>2</sup>, and Silvia Pérez-Magariño<sup>1</sup>

<sup>1</sup>Instituto Tecnológico Agrario de Castilla y León, Ctra Burgos Km 119, 47071 Valladolid, Spain

<sup>2</sup>Instituto de Ciencias de la Vid y el Vino (Universidad de La Rioja, Gobierno de La Rioja, CSIC), Finca de La Grajera, Ctra. Burgos 6, 26007 Logroño, Spain

**Abstract.** Grape pomace is one of the main by-products generated by wine industry and contains several bioactive compounds such as polysaccharides. They are contained in the cell walls of the grape skins. The variety can be an important factor influencing the polysaccharide content of grapes. Actually, there is great interest in the reevaluation of by-products and their incorporation into the production process. Therefore, the aim of this work was to evaluate the polysaccharide content of different extracts obtained from unfermented pomaces of different red grape varieties from Castilla y León. Eight different grape varieties were studied. The polysaccharides from grape pomace was obtained following a flash extraction process and the total polysaccharide content and the molecular weight distribution were estimated by High-Performance Size-Exclusion Chromatography with a Refractive Index Detector. Statistically significant differences were found in the content of total polysaccharides by grape variety varying between 112 and 200 mg/g. Only high and low molecular weight polysaccharide fractions were detected. All varietal extracts presented higher percentage of low molecular weight polysaccharides (50.8-64.3%) than of high molecular weight (35.7-49.2%).

## 1 Introduction

Wine industry generates different wastes throughout the winemaking process [1]. Grape pomace is one of the main by-products generated and consists of grape skins, seeds and stems pieces [2]. There are several bioactive compounds contained in grape pomace such as fiber, proteins, phenolic compounds, polysaccharides, minerals and oil seed [3]. Currently, there is a great interest in the reevaluation of wine by-products and their application in production processes. Polysaccharides from grapes are contained in the cell walls of the grape skin and represent 43-47% of its dry weight [4] and they have an essential function in the structure of cell wall [5]. Therefore, the skins can be an important source for obtaining extracts rich in polysaccharides.

Grape polysaccharides are divided in different groups due to their composition: polysaccharides rich in arabinose and galactose (PRAG), rhamnogalacturonan of type I and II (RG-I and II), homogalacturonans (HG) and non pectic polysaccharides (NPP) [5-7].

The content of polysaccharides can vary depending on the maturation degree, the terroir and the grape variety [8].

Therefore, the objective of this work was to evaluate the polysaccharide content of different extracts obtained from unfermented pomaces of different red grape varieties from Castilla y León.

## 2 Materials and methods

### 2.1 Grape pomace materials and enological parameters

All red grape varieties came from Castilla y León, a region in northern Spain and were: Tempranillo, Tinta del País and Cabernet Sauvignon from the Denomination of Origin (D.O.) Ribera del Duero, Tinta de Toro from the D.O. Toro, Prieto Picudo from the D.O. Valles de Benavente, Juan García from the D.O. Arribes, Rufete from the D.O. Sierra de Salamanca and two samples of Garnacha with different degrees of maturation from the D.O. Cebreros.

The grapes were destemmed, crushed and pressed, and the skins were frozen in airtight bags at -15 °C until their extraction.

Classical oenological parameters of the grapes were determined according to the official methods of OIV [9].

## 2.2 Extraction of the polysaccharides from grape pomace

The extraction of the polysaccharides from grape pomace was carried out following the method developed by Canalejo et al. [10] with slight modifications. Grape pomace was defrosted and homogenized with the Ultra Turrax. Then, an acidic solution of tartaric acid at pH 1 was added to the homogenized grape pomace and put in the ultrasonic bath for 30 minutes. Subsequently the samples were stirred in an orbital shaker for 18 hours. After that, the samples were centrifugated and the supernatant was concentrated in a rotary evaporator. The polysaccharides were precipitated with four volumes of cold acidified alcohol for 24 h at 4 °C [6]. The precipitate was centrifuged and the pellet was freeze-dried. All the extractions were carried out in triplicate.

## 2.3 Total polysaccharide content and molecular weight distribution

The estimation of the total polysaccharide content and the molecular weight distribution in the extracts obtained from grape pomace were determined by High-Performance Size-Exclusion Chromatography with a Refractive Index Detector (HPSEC-RID, Agilent Technologies 1200 Series), following the chromatographic conditions described in Guadalupe et al. [6]. Seven analytical standards of dextran from *Leuconostoc mesenteroides* were used for the molecular weight calibration. Dextran with a 270 kDa molecular weight and one pectin (esterified potassium salt from citrus fruit) were used as external standards for quantification.

## 2.4 Statistical analysis

The data obtained were analysed by analysis of variance (ANOVA) and the least significant difference (LSD) test with a confidence level of 95% using the software Statgraphics Centurion XVIII.

# 3 Results and Discussion

## 3.1 Oenological parameters

Table 1 shows the oenological parameters determined in the samples studied. Titratable acidity (TA) was evaluated by an accredited method by ISO 17025 Norm and the uncertainty has also been calculated according to this Norm. TA was expressed in g/L of tartaric acid.

Differences can be observed in the maturation degree. JG was the variety which have the less Brix degree. On the other hand, the TM and TP have the highest Brix degree values.

JG, CS and PP are the three varieties with a higher content in titratable acidity and TM are the variety with less content.

**Table 1.** Varieties studied with their origin and the Brix degree of each variety.

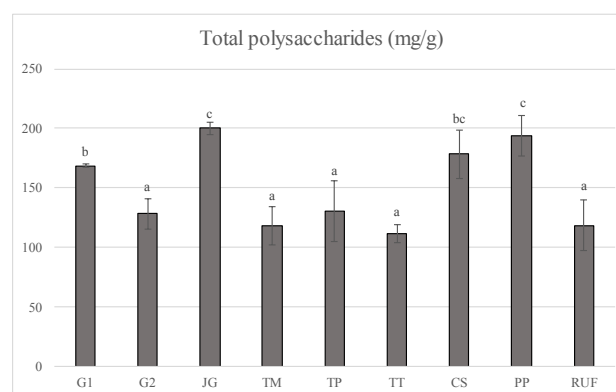
| Grape variety           | Denomination of Origin | °Brix | TA (g/L) |
|-------------------------|------------------------|-------|----------|
| Garnacha (G1)           | Cebreros               | 24.4  | 4.5±0.2  |
| Garnacha (G2)           | Cebreros               | 22.8  | 5.0±0.2  |
| Juan García (JG)        | Arribes                | 18.2  | 6.4±0.2  |
| Tempranillo (TM)        | Ribera del Duero       | 25.4  | 3.8±0.2  |
| Tinta del País (TP)     | Ribera del Duero       | 24.6  | 5.0±0.2  |
| Tinta de Toro (TT)      | Toro                   | 24.4  | 5.1±0.2  |
| Cabernet Sauvignon (CS) | Ribera del Duero       | 22.7  | 6.4±0.2  |
| Prieto Picudo (PP)      | Valles de Benavente    | 23.5  | 6.3±0.2  |
| Rufete (RUF)            | Sierra de Salamanca    | 23.3  | 4.1±0.2  |

## 3.2 Total polysaccharide content

The results show statistically significant differences in the content of total polysaccharides extracted from grape pomace of the different red grape varieties studied (Fig. 1). The JG, PP and CS varieties presented the highest contents of total polysaccharides with 200, 194 and 178 mg/g extract, respectively.

On the contrary, the varieties with the lowest total polysaccharide content were TT, TM, RUF, G2 and TP with a content of total polysaccharides of 112, 118, 127, 128 and 131 mg/g extract.

The Garnacha with high maturation degree has a higher content of total polysaccharides than the Garnacha with have a less maturation degree, with a difference in their content of 40.3 mg/g extract.

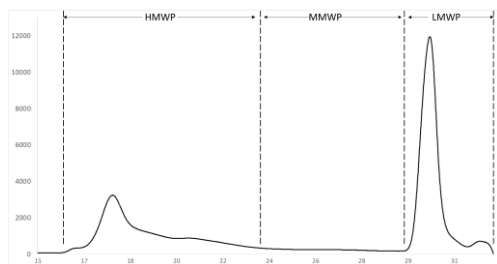


**Figure 1.** Total polysaccharide content estimated of the extracts obtained by grape variety. Values with different letters indicate statistically significant differences ( $p < 0.05$ ).

## 3.3 Molecular weight distribution

Three different fractions of polysaccharides were estimated according to their molecular weight: high

molecular weight (HMWP, 1000 – 30 kDa), medium molecular weight (MMWP, 30 – 5 kDa) and low molecular weight polysaccharides (LMWP, < 5 kDa).



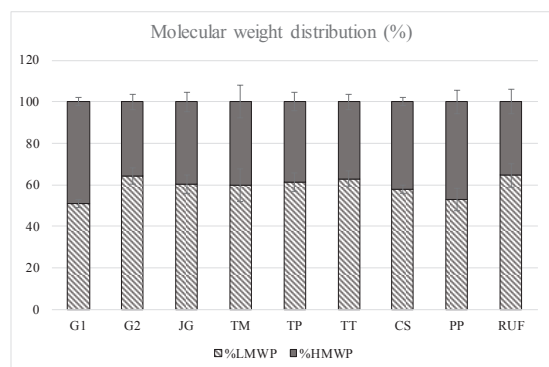
**Figure 2.** Chromatogram of the Cabernet Sauvignon variety with the different fractions of molecular weights: HMWP, MMWP and LMWP.

The extracts studied of different grape varieties only presented HMWP and LMWP, and all of them showed statistically significant differences between varieties (Table 2). G1 presented similar content of HMWP and LMWP. The remain varieties showed more content of LMWP than HMWP, with the JG, CS and PP varieties showing the highest content in LMWP.

**Table 2.** Content estimated of the different polysaccharide fractions per variety (mg polysaccharide/g). Values with different letters indicate statistically significant differences ( $p < 0.05$ ).

| Grape variety | HMWP <sup>a</sup> | LMWP <sup>b</sup>  |
|---------------|-------------------|--------------------|
| G1            | 82.8 <sup>b</sup> | 85.5 <sup>ab</sup> |
| G2            | 45.6 <sup>a</sup> | 82.4 <sup>ab</sup> |
| JG            | 79.4 <sup>b</sup> | 121 <sup>c</sup>   |
| TM            | 48.2 <sup>a</sup> | 70.2 <sup>a</sup>  |
| TP            | 50.7 <sup>a</sup> | 79.9 <sup>ab</sup> |
| TT            | 41.5 <sup>a</sup> | 70.0 <sup>a</sup>  |
| CS            | 75.2 <sup>b</sup> | 103 <sup>b</sup>   |
| PP            | 92.0 <sup>b</sup> | 102 <sup>b</sup>   |
| RUF           | 42.3 <sup>a</sup> | 76.1 <sup>ab</sup> |

Figure 3 shows the molecular weight distribution of polysaccharides.



**Figure 3.** Molecular weight distribution of polysaccharides in percentage of each varietal extract.

All varietal extracts presented higher percentage of low molecular weight polysaccharides (50.8-64.3%) than of high molecular weight (35.7-49.2%). The extracts obtained from the grape pomace of PP and Garnacha varieties with a higher degree of maturation have shown a higher content of high molecular weight polysaccharides (47.2% and 49.2%, respectively), while the RUF and Garnacha varieties with a lower degree of maturation were richer in low molecular weight polysaccharides (64.0% and 64.3%, respectively).

## 4 Conclusion

These results show the influence of the grape variety on the total polysaccharide content and on the molecular weight distribution of the extracts from unfermented red skins. Therefore, it is necessary to carry out a more complete characterization of these extracts in order to determine the types of polysaccharides that are extracted.

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