Journal of Chromatography A, 1216 (2009) 7296–7300

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Identification of free disaccharides and other glycosides in wine

Ana Isabel Ruiz-Matute [a], M. Luz Sanz [a], M. Victoria Moreno-Arribas [b], Isabel Martínez-Castro [a,*]

[a] Instituto de Química Orgánica General (CSIC), C/Juan de la Cierva, 3, 28006, Madrid, Spain
[b] Instituto de Fermentaciones Industriales (CSIC), C/Juan de la Cierva, 3, 28006, Madrid, Spain

ARTICLE INFO

Article history:
Available online 6 September 2009

Keywords:
Wine
Disaccharides
Ethyl-glucoside
Glyceryl-glycosides
GC–MS

ABSTRACT

Free soluble carbohydrates of different wine samples were analyzed by GC–MS as their trimethylsilyloximes using a methylsilicone column. Besides α,α-trehalose, several β-glucosylglucoses such as cellobiose, sophorose, laminaribiose and gentiobiose were the main disaccharides identified. With the exception of gentiobiose, these disaccharides are now reported for the first time in wine. Lactose (4-O-β-D-galactopyranosyl-D-glucose), previously described in this product, was also tentatively identified. Several free glycosides: β-ethyl-glucoside and seven glyceryl-glycosides (including glucosides and galactosides) were also identified for the first time in wine. On the contrary, disaccharides in grape juice were mainly constituted of fructose derivatives, including sucrose, and no glycosides were detected. Although the total amount of disaccharides was different in white wines (<50 mg/L) from those in rosé and red wines (80–130 mg/L), the chromatographic profile was noticeably similar in all wine samples. The method here reported allows the identification of several carbohydrates which have not been previously detected in wines and could contribute to increase the understanding of enzymatic activity during winemaking.

1. Introduction

Free carbohydrates in wine are mainly constituted of monosaccharides, sugar alcohols, sugar acids and disaccharides. Several of them are natural constituents of musts, while others are formed as a result of fermentation. Wine monosaccharides have been studied in detail [1–4], the presence of glucose, galactose, fructose, mannose, arabinose, rhamnose, ribose and xylose have been reported.

Wine sugar alcohols consist of linear polyalcohols and cyclitols. Erythritol, threitol, ribitol, arabitol, xylitol, sorbitol, mannitol, and traces of galactitol have been reported in different wines and sherries [1,5,6]. Regarding cyclitols, *myo*-inositol, *scyllo*-inositol [5] and *chiro*-inositol [7] have been reported in different wines, whereas the presence of quercitol (1,3,4/2,5-cyclohexanepentol) has been only found in wines aged in contact with oak wood (barrels or chips) but not in wines aged in bottles [7].

Sugar acids have special relevance in those wines affected by the action of "noble rot" (*Botrytis cinerea*) [8,9] that attacks grapes in humid climate conditions, causing the production of higher sugar and acid contents. Gluconic [5] and galacturonic acids [8] are those sugar acids found in greater amounts in these wines.

α,α-Trehalose has been reported to be the main disaccharide in wine [10] formed as a result of the metabolic activity of yeasts. Its level has been reported within 0–611 mg/L in wines [10] and within 0–53 mg/L in sherries [11]. In *Saccharomyces cerevisiae*, under normal growth conditions, trehalose accumulates after cells enter the stationary phase and also acts as a protectant that contributes to survival during stress conditions [12]. Small amounts of other disaccharides have been reported: sucrose within 20–120 mg/L [1]; other disaccharides (isomaltose, lactose and turanose) within 5 and 50 mg/L, and possible traces of melibiose and gentiobiose [10]. However, there are currently still a number of minor carbohydrates in wines without a conclusive identification.

Although analytical methods used for the characterization of wine carbohydrates have been mainly based on LC [2,3], GC–MS with capillary columns have been also utilized, since it affords the high resolution necessary to analyze such a complex mixture. Trimethylsilyl ethers (TMS) [10,13] and TMS oximes (TMSO) [1] have been used as derivatives for GC analysis.

In the present work, a method based on GC–MS of TMSO has allowed the identification of the main disaccharides of wine and other simple glycosides; their formation process is also discussed.

2. Materials and methods

2.1. Standards

Cellobiose (4-O-β-D-glucopyranosyl-D-glucose), isomaltose (6-O-α-D-glucopyranosyl-D-glucose), gentiobiose (6-O-β-D-glucopyranosyl-D-glucose), lactose (4-O-β-D-galactopyranosyl-D-glucose), laminaribiose (3-O-β-D-glucopyranosyl-D-glucose), β-phenyl-D-glucoside and sucrose (α-D-glucopyranosyl-β-D-

* Corresponding author. Tel.: +34 915622900x212; fax: +34 915644853.
E-mail address: iqomc16@iqog.csic.es (I. Martínez-Castro).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.08.086

Table 1
Description of the wine and must samples.

Samples	Type	Grape variety	Origin
Red wines	Young	Tempranillo	Ribera de Duero cellar, Spain
	Young	Tempranillo	Ribera de Duero cellar, Spain
	Young	Tempranillo	Ribera de Duero cellar, Spain
	Young	Cabernet-Sauvignon	Rioja cellar, Spain
	Young	Cabernet-Sauvignon	Rioja cellar, Spain
	Oak aged	Tempranillo + Cabernet-Sauvignon + Merlot	Peñafiel, Valladolid, Spain Bottled
	Oak aged/dry "Amarone"	Corvina + Rondinella + Molinara	Valpolicella, Verona, Italy Bottled
White wines	Young	Verdejo	Rueda cellar, Spain
	Young	Verdejo	Rueda cellar, Spain
	Young	Albariño	Rias Baixas cellar, Spain
	Young/medium-sweet	Airén	Tomelloso (La Mancha), Spain; bottled
Rosé wines	Young	Garnacha	Navarra cellar, Spain
	Young	Garnacha	Navarra cellar, Spain
Musts	Fresh	Verdejo	Rueda cellar, Spain
	Pasteurised	Muscat	Bottled grape juice
	Fresh	Muscat	Obtained in the laboratory

fructose) were obtained from Sigma Chemical Co. (St. Louis, US). Melibiose (6-O-α-D-galactopyranosyl-D-glucose), α,α-trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) and turanose (3-O-α-D-glucopyranosyl-D-fructose) were purchased from Fluka (Buchs, Ch). Sophorose (2-O-β-D-glucopyranosyl-D-glucose) was acquired from Sarsynthèse (Merignac, France).

Retention data and mass spectra for those compounds whose standards were not commercially available were obtained from different sources: α-glucosides (glyceryl-glucosides and ethyl-glucoside) from sake (Kuromatsu-hakushika, Tatsuuma-honke brewing Co., Ltd., Japan) as reported by [14] as provenient from the action of α-glucosidase from *Aspergillus oryzae* on glucose and glycerol, β-glyceryl-glucosides from leaves of *Lilium* spp. [15], α-glyceryl-galactosides from alga nori sheets (Blue dragon, G Costa & Co. Ltd., UK) [16] and β-glyceryl-galactosides from sugar mixtures obtained by transglycosidation with β-galactosidase [17]. This last product was kindly gifted by Dr. Montilla (CSIC, Spain). Extracts were centrifuged for 15 min at 5000 × *g* and immediately refrigerated until analysis.

2.2. Samples

Different types of industrially manufactured still wines including red, white and rosé were provided by cellars from different Spanish wine making areas. These wines were manufactured in 10,000 L stainless-steel tanks according to traditional practices without any storage or ageing in oak barrels. Samples were taken after clarification and stabilization procedures and before bottling. For comparison, three bottled still wines were purchased in different markets: a Spanish medium-sweet white, a Spanish red aged in oak cask and an Italian dry red from partially desiccated grapes (Amarone) also aged in oak. Three different samples of must were examined: one from the tank of a cellar, a commercial grape juice bottled and pasteurised, and the juice of 100 g fresh white grapes, pressed, filtered and centrifuged in the laboratory. Table 1 summarizes the description of these samples.

Wine and must samples were collected, centrifuged for 15 min at 5000 × *g* and immediately refrigerated until analysis. Each analytical assay was performed at least in duplicate.

2.3. Analysis of free carbohydrates

2.3.1. Derivatization

0.5 mL of sample or 1 mL standard (1 mg mL^{-1} in methanol:water 30:70, v/v) was mixed with 0.125 mL or 1 mL of a 70% ethanolic solution of β-phenyl-D-glucoside (1 mg/mL), which was used as internal standard. After drying the samples under vacuum, 350 μL of 2.5% hydroxylamine chloride in pyridine were added and heated at 75 °C for 30 min. Silylation reaction was carried out with 350 μL of hexamethyldisilazane (HMDS) and 35 μL of trifluoroacetic acid (TFA) at 45 °C for 30 min [18]. Derivatized samples were centrifuged and 1 μL of supernatant was injected into the injection port of the gas chromatograph. While non-reducing carbohydrates gave only one chromatographic peak, reducing carbohydrates presented two isomers corresponding to *syn* and *anti*, or *E* and *Z* isomers.

2.3.2. GC–MS analysis

GC–MS analysis was carried out using a Hewlett-Packard 7890A gas chromatograph coupled to a 5975C quadrupole mass detector operating in electronic impact (EI) mode at 70 eV (both from Agilent, Palo Alto, CA, USA). Analyses were carried out in split mode (1:40) on a 30 m × 0.25 mm i.d. × 0.25 μm film thickness TRB-1 column (Teknokroma, Barcelona, Spain). The oven was heated at 200 °C for 15 min, then programmed to 270 °C at a heating rate of 15 °C min^{-1}, then programmed to 290 °C at 1 °C min^{-1} and held for 30 min. Injector temperature was 300 °C and the transfer line was thermostatised at 280 °C. Helium at ∼1 mL min^{-1} was used as carrier gas. Acquisition was done using a HPChem Station software (Hewlett-Packard, Palo Alto, CA, USA).

Linear retention indices (I^T) were calculated from the retention times of TMSO disaccharides and suitable *n*-alkanes.

Wine disaccharides were identified by comparison of their retention times and experimental spectra with those of standards run in the laboratory under identical operation conditions.

2.3.3. GC-FID analyses

Samples were also injected in a HP 7890A gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with a flame ionization detector (FID) for quantitative purposes using nitrogen as carrier gas and the same column and temperature program described above. Carbohydrate quantitative data were obtained from FID peak areas using the response factor (RF) relative to β-phenyl-D-glucoside (internal standard) calculated over the expected range. In those cases where no standards were available, RF were estimated as 1. Detection (LOD) and quantification limits (LOQ) were 0.84 μg L^{-1} and 2.8 μg L^{-1}, respectively. Reproducibility was evaluated by the quintuplicate analysis of a red wine sample being the relative standard deviation lower than 10% for the target compounds (disaccharides and glyceryl-glycosides).