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Presence of rhamnogalacturonan II in the juices produced by enzymatic liquefaction of Agave pulquero stem (*Agave mapisaga*)

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ABSTRACT

Rhamnogalacturonan II (RG-II), a small complex pectic polysaccharide is released from Agave pulquero stems (*Agave mapisaga*), after the production period of aguamiel. RG-II was obtained by treatment with two commercial liquefying enzyme preparations, it was isolated by size-exclusion chromatography and characterized. RG-IIs contains diagnostic sugars such as apiose, 2-*O*-methyl-L-fucose, 2-*O*-methyl-D-xylose, aceric acid, Kdo and Dha. Glycosyl-linkage compositions of the Agave pulquero RG-II like structures are similar to the theoretical model described through sycamore RG-II structure. The presence of 3′-linked apiose indicates that the obtained juice from Agave pulquero plant contains the free RG-II dimer. Thus, when pectinolytic enzyme preparations are used to process Agave pulquero (*A. mapisaga*), RG-II is released as one of the main soluble polysaccharide fraction.

1. Introduction

Agaves, which belong to the family of Agavaceae, are monocotyledoneous and monocarpic plants. They have played an important role for Mexican inhabitants in the past and they still have important ecological and economic roles in this part of the world. First, they protect the soil against both water and wind erosion. The Agave plants are also cultivated for their capacities to grow under arid or semi arid conditions and stop land turning to desert. Besides many species of Agave have been used for diverse purposes: the production of spirits (e.g., tequila and pulque), forage, food, drinks, drugs, construction, weaving, paper (Granados-Sánchez, 1993). Pulque, which is prepared from the stem of several species of maguey such as *Agave mapisaga, Agave atrovensis* and *Agave americana*, is a non-distilled traditional alcoholic beverage. It is produced by the fermentation of the sap known as aguamiel (Escalante et al., 2004). This beverage is currently produced and consumed mainly in the central states of Mexico. However, as Mexicans showed a real preference for beer, pulque consumption has dramatically decreased since the Sixties. As a result, the culture of the Agave pulquero plant has been discouraged since it did not ensure an enough income to the producers.

In order to maintain the culture of Agave plants in Mexico, the Mexican government has thus encouraged research programs related to the development of high-added-value products extracted from Agave pulquero. In a particular research, conducted at the Technological University of Tecamachalco (UTT) since 2002 has led to the emergence of new products and new outlets for the Agave pulquero plants as syrup, drinks, cookies and sweets elaborated from aguamiel and the stem (Badillo de la Concha, Salas-Dorado, & Ortiz-Basurto, 2007; Ortiz-Basurto, Torrestiana-Sanchez, Pourcelly, & Calderon-Cervantes, 2005). The production of "pectin" from Agave plant must be considered as one of such products. Pectins are a polysaccharide family of covalent linked galacturonic acids mainly, including homogalacturonan, rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), xylogalacturonan (XGA) and apiogalacturonan (AP) (Albersheim, Darvill, O'Neill, Schols, & Voragen, 1996; De Vries, 1988; Mohnen, 2008) which constitute the major part of the plant cell wall.

RG-II is a small structural complex polysaccharide that contains 12 different glycosyl-residues connected via 20 different linkages (Pérez, Rodríguez-Carvajal, & Doco, 2003) as shown in Fig. 1 (Mohnen, 2008). Its backbone is composed of a 1,4-linked α-D-galacturonosyl residues (Darvill, McNeill, & Albersheim, 1978; Melton, McNeill, Darvill, & Albersheim, 1986; Vidal et al., 2000) in which four structurally well-defined oligosaccharide side chains are attached (Darvill et al., 1978; O'Neill, Albersheim, & Darvill, 1990; O'Neill, Ishii, Albersheim, & Darvill, 2004). These side chains contain several rare "diagnostic" monosaccharides such as apiose (Darvill et al., 1978), 2-*O*-methyl-L-Fuc (Darvill et al., 1978), 2-*O*-methyl-D-Xyl (Darvill et al., 1978), aceric acid

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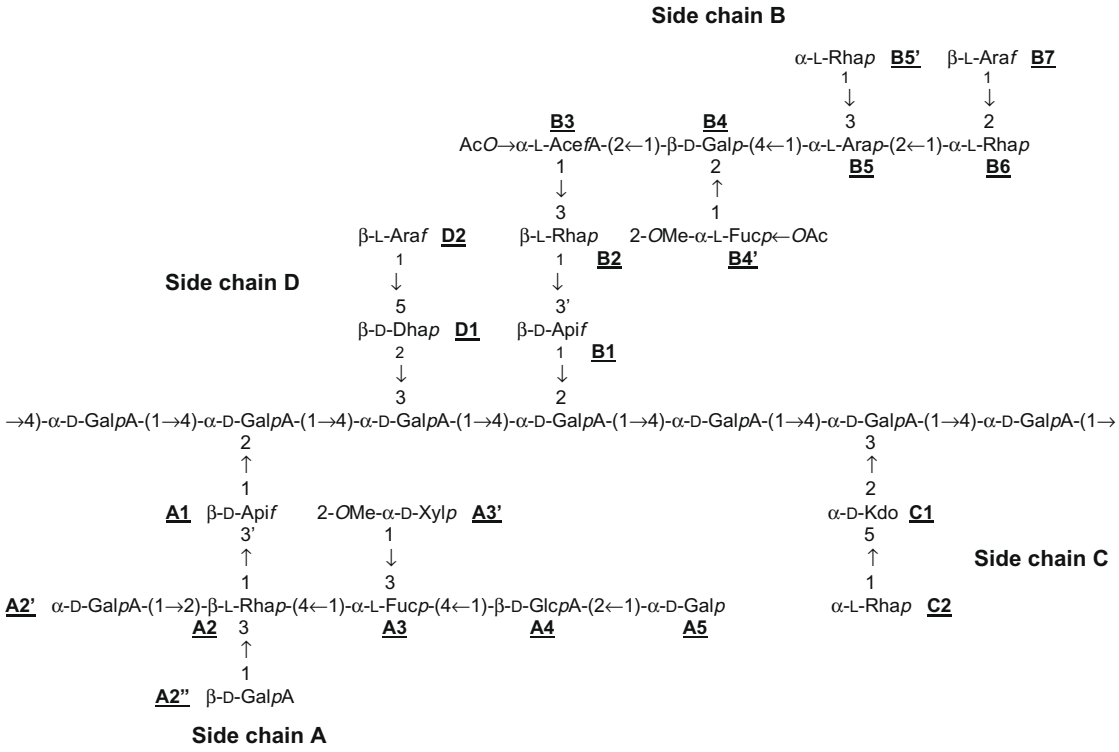

Fig. 1. Schematic representation of the primary structure of RG-II (Mohnen, 2008). The galacturonic acids backbone is substituted by four structurally different glycosyl side chain, called side chain A–D whose monosaccharide residues are numbered according to the model sequence (Pellerin et al., 1996). Oac, *O*-acetyl ester; Ome, *O*-methyl ether.

(3-*C*-carboxy-5-deoxy-L-Xyl) (Spellman, McNeill, Darvill, Albersheim, & Hendrick, 1983), Kdo (3-deoxy-D-*manno*-octulosonic acid) (Stevenson, Darvill, & Albersheim, 1988) and Dha (3-deoxy-D-*lyxo*-heptulosaric acid) (York, Darvill, McNeill, & Albersheim, 1985). Kobayashi, Matoh, and Azuma (1996)), O'Neill et al. (1996) have shown that RG-II is present predominantly as a dimer in plant cell walls. This dimer is cross-linked by borate-diol diesters located on the apiosyl residue in side chain A (2-*O*-methyl xylose containing side chain) (Ishii et al., 1999; Matoh, Ishigaki, Ohno, & Azuma, 1993; Mazeau & Perez, 1998; O'Neill et al., 1996), and seems to play an important role in the structure and functions of pectic polysaccharides in primary cell walls (O'Neill et al., 2004).

RG-II can be released from pectins by enzymatic degradation with pectinases and in particular with *endo*-polygalacturonase (Darvill et al., 1978; Ishii & Matsunaga, 2001) present in some commercial enzyme preparations which is generally used for plant/fruit processing industry to enhance yields, to improve liquefaction, clarification and filterability of juices, maceration and extraction of plant tissues (Benen, Voragen, & Visser, 2003). It was shown that RG-II was the dominant polysaccharide in apple, carrot, tomato, bilberry and black currant juices obtained by enzymic liquefaction of fruits and vegetables (Doco, Williams, Vidal, & Pellerin, 1997; Hilz, Williams, Doco, Schols, & Voragen, 2006). During vinification RG-II is released from berry cell wall, and characterized as one of the major components of wine polysaccharides. (Ayestaran, Guadalupe, & Leon, 2004; Doco & Brillouet, 1993; Doco, Quellec, Moutounet, & Pellerin, 1999; Pellerin et al., 1996; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003). If RG-II is present in primary cell walls, it is expected to be present in juice produced with the help of enzymes since pectolytic enzymes cannot degrade it (Ayestaran et al., 2004; Doco et al., 1997; Hilz et al., 2006). In 1997 Stewart et al. suggested that a pectic polysaccharide similar to RG-II's was present in fiber of *Agave sisalana*, a sisal plant of the Agave family (Stewart, Azzini, Hall, & Morrison, 1997).

In this paper, we report the purification, identification and quantification of RG-II like structure in juices obtained by enzymatic liquefaction of Agave pulquero stems (*A. mapisaga*).

2. Experimental

2.1. Vegetable materials

A stem of *A. mapisaga,* after 6 months of aguamiel production period was bought on the local market in the community of Lomas de Romero, Tecamachalco, Puebla México (18° 53′ N, 97° 44′ W); altitude of 2020 m. The stem was frozen and stored at −20 °C in order to avoid microbial growth and enzymatic activity before being transported to France.

2.2. Liquefaction

The Agave stem (100 g) was sliced, crushed in a Waring blender (2 min) in the presence of 9 mM ascorbic acid solution and treated at 40 °C between 24 h with 0.1% of both Rapidase LIQ® (DSM/Gist Brocades, The Netherlands) and Pectinex® Ultra SP-L (Novozymes, Denmark). The suspension was centrifuged (14,000*g*, 30 min, 10 °C). After enzyme inactivation (100 °C, 10 min), 50 ml of supernatant were dialyzed against water (M_w cut off 8000–10,000 Da) 48 h, 4 °C and submitted to a second enzymatic degradation carried on in the same conditions. The final supernatant was concentrated five times under reduced pressure at 40 °C, centrifuged (15,000*g*, 10 min, 10 °C), dialyzed, concentrated and finally freeze–dried to give Total Enzyme Solubilised Polysaccharide (TESP).

2.3. Isolation of rhamnogalacturonan II fractions

First, the RG-II like structures were purified from Total Enzyme Solubilised Polysaccharide (TESP) by size-exclusion chromatogra-

phy on a Superdex 30 HR column (1.6 × 60 cm; Pharmacia) equilibrated at 1.0 ml/min in 30 mM ammonium formiate buffer pH 5.8. The fractions collected between 50 and 60 min, (which eluted at the same volume as an isolated red wine RG-II (Pellerin et al., 1996) were then injected on a Superdex-75 HR column (1.3 × 30 cm; Pharmacia) equilibrated at 0.6 ml/min in 30 mM ammonium formiate buffer, pH 5.8.

2.4. Neutral sugar composition as alditol acetates

Neutral sugars were determined as alditol acetates after TFA hydrolysis by GLC (Harris, Henri, Blakeney, & Stone, 1984). Separation was carried out on a DB225 column (30 m × 0.32 mm ID; 0.25 μm film; J&W Scientific) with hydrogen as carried gas (0.6 bar inlet pressure). Allose was used as internal standard (Hilz et al., 2006).

2.5. Sugar composition as trimethylsilyl derivatives

The neutral and rare acidic sugar composition was determined after solvolysis with anhydrous MeOH containing 0.5 M HCl (80 °C, 6 h), by GC of their per-*O*-trimethylsilylated methyl glycoside derivatives. The TMS derivatives were separated on a DB-1 (temperature programming 120–200 °C at 1.5 °C/min) capillary columns (30 m × 0.32 mm i.d., 0.25 μm film), coupled to a single injector inlet through a two-holed ferrule, with H_2 as the carrier gas on a Hewlett–Packard Model 5890 gas chromatograph (Doco, O'Neill, & Pellerin, 2001).

2.6. Glycosyl-linkage determination

The glycosyl-linkages composition were determined by GC–MS of the partially methylated alditol acetates. One milligram of polysaccharides in 0.5 ml dimethylsulfoxide was methylated using methyl sulfinyl carbanion and methyl iodide (Hakomori, 1964). The methylated materials were then treated with 2 M TFA (1 h at 120 °C). Finally the released monosaccharides were converted to their corresponding alditols by treatment with $NaBH_4$ and then acetylated (Harris, 1984). Partially methylated alditol acetates were analyzed by GC-EI-MS using a DB-1 capillary column (30 m × 0.32 mm i.d., 0.25 μm film); temperature programming 135 °C for 10 min, then 1.2 °C/min to 180 °C, coupled to a HP5973 MSD (Vidal, Williams, O'Neill, & Pellerin, 2001).

2.7. Analysis of RG-II backbone fragments

Homogalacturonan backbone fragments were obtained by partial acid hydrolysis using 0.1 M trifluoroacetic acid for 16 h at 80 °C. The resulting fragments were separated by HPAEC with a Dionex system as described by Whitcombe, O'Neill, Steffan, Albersheim, and Darvill (1995) and by Pellerin et al. (1996). The elution was performed at 1 ml/min with a gradient of sodium acetate in 100 mM NaOH:100 mM sodium acetate (0–5 min); a linear gradient up to 500 mM sodium acetate (5–25 min) and finally, a linear gradient up to 600 mM sodium acetate (25–50 min). The column was equilibrated under initial condition for 10 min prior to sample injection. The degrees of polymerization (dp) of the homogalacturonan fragments were directly determined by comparison with the elution times of 1,4-linked α-D-oligogalacturonide acids generated by treating a solution of polygalacturonic acid at 0.2% in 0.1 M sodium acetate, pH 4.8, with a purified endopolygalacturonase (Megazyme, Australia, 4 nkat/ml, 40 °C, 16 h), following the description reported by Vidal et al. (2000).

3. Results and discussion

In this study, the purification, identification and quantification of RG-II-like structure in Agave pulquero juice is presented. First the Total Enzyme Solubilised Polysaccharide (TESP) of Agave pulquero stem obtained thanks to enzymatic liquefaction with Rapidase LIQ® and Pectinex® Ultra-SPL were separated by high resolution size-exclusion chromatography on a Superdex 30 HR column. Then, the fraction eluted at the same retention time than RG-II isolated from wine (Pellerin et al., 1996) were collected and injected on a Superdex-75 HR column. The obtained chromatographic profile is presented on Fig. 2. Compared to TESP (Fig. 2 A), this purified fraction of Superdex 30 was enriched in a fraction eluted between 19 and 21 min (Fig. 2B). This fraction was then collected and injected on a Superdex-75 HR (see Fig. 2C). It corresponds to the Agave pulquero RG-II.

Glycosyl-residue composition of this isolated fraction was compared to those of a red wine RG-II (Table 1). First, we can notice that this fraction presents the characteristic composition of RG-II structure including diagnostic sugar such as apiose, 2-*O*-methyl-L-Fuc, 2-*O*-methyl-D-Xyl, aceric acid (3-*C*-carboxy-5-deoxy-l-Xyl), Kdo (3-deoxy-D-*manno*-octulosonic acid) and Dha (3-deoxy-D-*lyxo*-heptulosaric acid). GalA, Ara, Rha and Gal were found to be

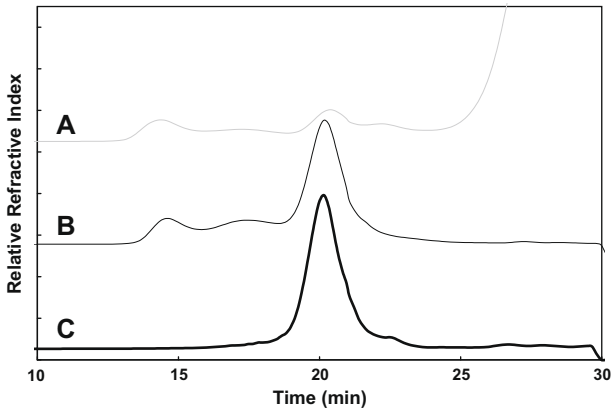

Fig. 2. Purification by high resolution size-exclusion chromatography of RG-II on Superdex-75HR column: (A) Total Enzyme Solubilised Polysaccharide (TESP) obtained from Agave pulquero after enzymic liquefaction with 0.1% of Rapidase LIQ® and Pectinex® Ultra-SPL. (B) RG-II rich fraction purified on a Superdex 30 HR column. (C) Agave pulquero RG-II obtained after Superdex 30 and Superdex-75 HR chromatography.

Table 1
Glycosyl composition (mole percentage) of RG-II like structures isolated from Agave pulquero heart (*Agave mapisaga* "Blanco") and wine RG-II.

	Agave pulquero	Red wine[a]
Rha[b]	12.1	16.9
2-*O*-Me-Fuc	4.0	4.2
Fuc	1.7	3.6
Api	4.5	7.2
Ara	9.6	9.7
2-*O*-Me-Xyl	4.0	3.1
Gal	8.7	5.0
Glc	4.9	–
Man	4.2	–
Gal A	32.4	37.2
Glc A	3.4	3.4
Aceric acid	6.4	2.2
Kdo	2.1	5.0
Dha	1.4	2.5

[a] According to Pellerin et al. (1996).
[b] Rha, rhamnose; 2-OMeFuc, 2-O-CH3-fucose; Fuc, fucose; Api, apiose; Ara, arabinose; 2-OmeXyl, 2-O-CH3-xylose; Gal, galactose; Glc, glucose; Man, mannose;Gal A, galacturonic acid; Glc A, glucuronic acid; Aceric acid, 3-*C*-carboxy-5-deoxy-L-xylose; Kdo, 3-deoxy-D-*manno*-octulosonic acid; Dha, 3-deoxy-D-*lyxo*-heptulosaric acid.

Table 2
Glycosyl-linkage composition (mole percentage) of RG-II fractions isolated from Agave pulquero stems obtained by the liquefaction process.

Glycosyl residue	Linkage	Residue[a]	Pulquero	Wine RG-II[a]
2,3,4-Rhamnose[b]	Terminal	B6, C2	6.9	12.2
3,4-Rhamnose	2-Linked	B6	7.6	10.7
2,4-Rhamnose	3-Linked	B2	7.5	8.3
4-Rhamnose	2,3-Linked		1.4	–
3-Rhamnose	2,4-Linked	Unknown	–	1.4
Rhamnose	2,3,4-Linked	A2	3.4	8.5
2,3,4-Fucose	Terminal	B4′	4.4	5.6
2-Fucose	3,4-Linked	A3	0.7	6.3
2,3,5-Arabinose	Terminal	B7, D2	10.1	11.6
3,4-Arabinose	2-Linked	B5	–	1.4
2,5-Arabinose	3-Linked		1.5	–
3,5-Arabinose	2-Linked		3.9	–
2,4-Arabinose	3-Linked		–	–
2,3-Arabinose	5-Linked		1.0	–
4-Arabinose	2,3-Linked	B5	3.6	6.1
2,3-Apiose	3′-Linked	A1, B1	7.2	11.0
Apiose	2,3,3′-Linked	A1 or B1	3.7	3.6
2,3,4-Xylose	Terminal	A3′	5.6	4.9
2,3,4,6-Galactose	Terminal	A5	1.9	2.2
2,3,4-Galactose	6-Linked		2.0	–
2,4,6-Galactose	3-Linked		2.3	–
2,6-Galactose	3,4-Linked	Unknown	2.9	2.2
3,6-Galactose	2,4-Linked	B4	4.9	7.0
2,4-Galactose	3,6-linked		3.9	–
2,3,4,6-Glucose	Terminal		1.1	–
2,3,6-Glucose	4-Linked		1.0	–
2,3,4,6-Mannose	Terminal		3.8	–
3,4,6-Mannose	2-Linked		2.2	–
2,4,6-Mannose	3-Linked		1.6	–

[a] According to Pellerin et al. (1996).
[b] 2,3,4-Rhamnose is 1.5 di-*O*-acetyl-2,3,4-tri-*O*-methyl rhamnitol, etc.

the major monosaccharides and in a molar ratio of 32.4, 9.6, 12.1 and 8.7, respectively. Actually molar ratios of most of glycosyl-residue found are very close to the values reported for isolated wine RG-II (Table 2) (Doco & Brillouet, 1993; Pellerin et al., 1996). Kdo and Dha contents are lower than the values expected due to their acid-lability and for their uneasy quantitative determination (Doco et al., 2001; Stevenson et al., 1988). Anyway, all these results suggest that the polysaccharide fraction isolated from Agave pulquero stem is composed of at least 90% of RG-II presented as a dimer. Actually, from high resolution size-exclusion chromatography results (Fig. 2C), the molecular weight (M_W) of Agave pulquero RG-II is estimated to be at 9500 Da corresponding to the dimer form (Aboughe-Angone et al., 2008; Doco et al., 1997; O'Neill et al., 1996; Pellerin et al., 1996).

In our conditions of enzymatic liquefaction, the concentration of RG-II in Agave pulquero juice is equal to 270 mg/L which corresponds to a concentration of 293 mg of RG-II per kg of fresh Agave stem. This value is very similar to the concentration found in apple juice (350 mg/L) with the same enzymatic process (Doco et al., 1997).

The glycosyl-linkage compositions of Agave pulquero RG-II are shown in Table 2 and compared to those of wine RG-II. Most methyl ethers obtained from Agave pulquero RG-II correspond to known glycosyl residues of RG-II (Doco & Brillouet, 1993; Doco et al., 1997;Pellerin et al., 1996; Puvanesarajah, Darvill, & Albersheim, 1991; Strasser & Amado, 2002; Thomas, Darvill, & Albersheim, 1989), and are similar to the theoretical model previously proposed (Mohnen, 2008; Pellerin et al., 1996) and reported on Fig. 1. The galacturonic acid backbone is substituted by four structural different glycosyl side chains, called side chain A–D and monosaccharide residues are numbered according to the model sequence (Pellerin et al., 1996). 2,3,4-Linked rhamnosyl, 3,4-linked fucosyl, terminal 2-*O*-methylxylosyl and terminal galactose expect to be a part of side chain A, found in the glycosyl linkage of the Agave pulquero RG-II. Furthermore, terminal 2-*O*-methyl-fucosyl, 2,4-linked galactosyl, 2-linked rhamnosyl and 2-linked arabinosyl residues are characteristic glycosyl residues of the side chain B, the so-called aceric acid-oligosaccharide (Vidal et al., 2000). In addition, apiose is presented as 3′-linked and 2,3,3′-linked residues which correspond to residues B1 and A1, respectively. This result, in particular the presence of 2,3,3′-linked apiose confirms that RG-II isolated by enzymatic liquefaction of Agave stem pectin, was released as dimer form mainly. Actually, the apiose residue A1 in side chain A is esterified with boric acid in the RG-II dimer (Ishii et al., 1999; Matoh et al., 1993; Mazeau & Perez, 1998; O'Neill et al., 1996). Dimeric RG-II is linked to pectin cell wall and can be released by enzymatic degradation of homogalacturonan (Darvill et al., 1978; Fleischer, O'Neill, & Ehwald, 1999; O'Neill et al.,

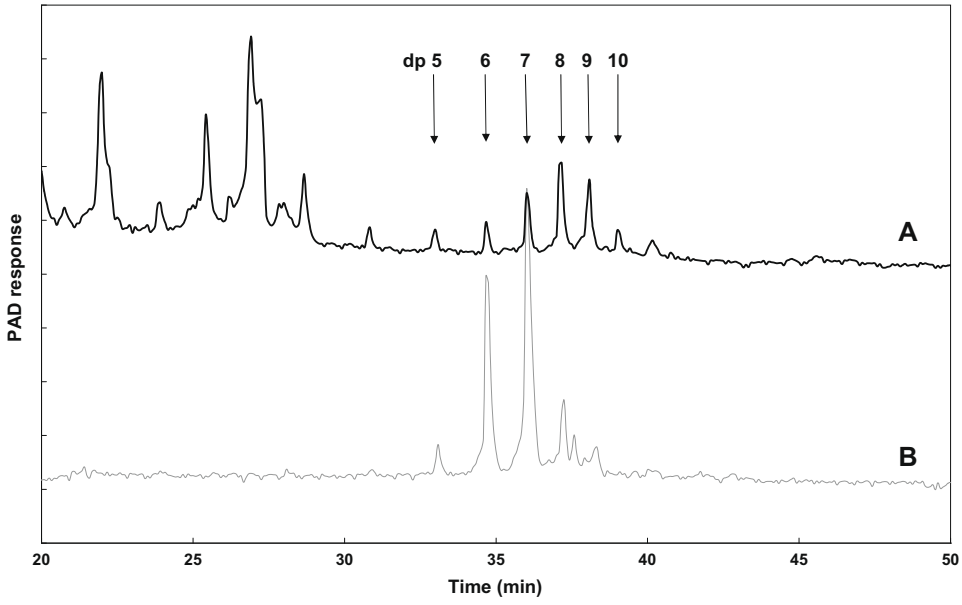

Fig. 3. HPAEC analysis of the Agave pulquero RG-II backbone fragments released by partial mild acid hydrolysis (A). Elution times of dp 5–9 of 1,4-linked a-D-galacturonic acids are shown in profile (B).

1996). Glucose (terminal and 4-linked glucosyl) and mannose (terminal, 2-linked and 3-linked mannosyl) are detected in the glycosyl linkage compositions of Agave pulquero RG-II, but they are not thought to be components of RG-II.

The degree of polymerization of the galacturonic acid backbone of the Agave pulquero RG-II was determined by HPAEC after mild acid hydrolysis of the fraction isolated from juice. The hydrolysate contains a series of oligosaccharides co-eluted with standard of 1,4-linked α-D-galacturonides obtained by treatment with an endopolygalacturonase (Fig. 3). According to this figure, Agave pulquero RG-II has an average dp between 8 and 9.

In summary, a RG-II like structure can be isolated in juices obtained by enzymatic liquefaction of Agave pulquero stem. The glycosyl residue and glycosyl-linkage compositions of Agave RG-II and wine RG-II are very similar. It confirms that Agave RG-II corresponds to the theoretical model described for sycamore and wine RG-II structure. It was shown that the presence of RG-II is likely to have an incidence on the physico-chemical properties and thus on the quality and the organoleptic properties of the juice. It was demonstrated that, RG-II fraction decreased significantly the astringengy given by a grape anthocyanin fraction added to a wine model solution (Vidal et al., 2004). In wines, RG-IIs are involved in many oenological phenomena. Wine RG-IIs may interact and aggregate with polyphenols (Riou, Vernhet, Doco, & Moutounet, 2002), they play a role on tartaric stability (Gerbaud, Gabas, Blouin, Pellerin, & Moutounet, 1997; Gerbaud et al., 1996) and also make complex divalent cations (O'Neill et al., 1996; Pellerin et al., 1997).

The possible role of RG-II in Agave juice has not been investigated yet, but the production of a "special" RG-II enriched juice from Agave pulquero stem which can be considered as valorisation way of Agave pulquero plants and may provide an economic answer to the problems of aguamiel producer.

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References

Aboughe-Angone, S., Nguema-Ona, E., Ghosh, P., Lerouge, P., Ishii, T., Ray, B., et al. (2008). Cell wall carbohydrates from fruit pulp of *Argania spinosa*: Structural analysis of pectin and xyloglucan polysaccharides. *Carbohydrate Research, 343*, 67–72.

Albersheim, P., Darvill, A. G., O'Neill, M. A., Schols, H. A., & Voragen, A. G. J. (1996). An hypothesis: The same six polysaccharides are components of the primary cell walls of all higher plants. In J. Visser & A. G. J. Voragen (Eds.), *Pectins and Pectinases* (pp. 47–53). Amsterdam: Elsevier Science.

Ayestaran, B., Guadalupe, Z., & Leon, D. (2004). Quantification of major grape polysaccharides (*Tempranillo* v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta, 513*(1), 29–39.

Badillo de la Concha, A., Salas-Dorado, A., & Ortiz-Basurto, R.I. (2007). XXXI Premio nacional en ciencia y tecnología de alimentos. Desarrollo de un néctar de tuna endulzado con jarabe de aguamiel obtenido por tecnología de membrana como fuente de inulina que no aporte más del 6% de glucosa para su consumo para diabéticos. http://www.pncta.com.mx/pages/pncta_investigaciones_07h.asp?page=07e3.

Benen, J. A. E., Voragen, A. G. J., & Visser, J. (2003). Pectic enzymes. In J. R. Whitaker, A. G. J. Voragen, & D. W. S. Wong (Eds.), *Handbook of food enzymology* (pp. 169–188). New York, Basel: Marcel Dekker, Inc..

Darvill, A. G., McNeill, M., & Albersheim, P. (1978). The structure of plant cell walls: VIII. A new pectic polysaccharide. *Plant Physiology, 62*, 418–422.

De Vries, J. A. (1988). Repeating units in the structure of pectin. In G. O. Philips, D. J. Wedlock, & P.A. WIlliams (Eds.), Gums and stabilisers from the food industry, Vol. 4 (pp. 25–29). Oxford: IRL Press.

Doco, T., & Brillouet, J.-M. (1993). Isolation and characterization of a rhamnogalacturonan II from red wine. *Carbohydrate Research, 243*, 333–343.

Doco, T., O'Neill, M. A., & Pellerin, P. (2001). Determination of the neutral and acidic glycosyl residue compositions of plant cell polysaccharides by GC-EI-MS analysis of the trimethylsilyl methyl glucoside derivatives. *Carbohydrate Polymers, 46*, 249–259.

Doco, T., Quellec, N., Moutounet, M., & Pellerin, P. (1999). Polysaccharide patterns during the aging of Carignan noir red wines. *American Journal of Enology and Viticulture, 50*(1), 25–32.

Doco, T., Williams, P., Vidal, S., & Pellerin, P. (1997). Rhamnogalacturonan II, a dominant polysaccharide in juices produced by enzymic liquefaction of fruits and vegetables. *Carbohydrate Research, 297*, 181–186.

Escalante, A., Flores, M. E., Martínez, A., López-Munguía, A., Bolívar, F., & Gosset, G. (2004). Characterization of bacterial diversity in pulque, a traditional Mexican alcoholic fermented beverage, as determined by 16S rDNA analysis. *FEMS Microbiology Letters, 235*, 273–279.

Fleischer, A., O'Neill, M. A., & Ehwald, R. (1999). The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. *Plant Physiology, 121*(3), 829–838.

Gerbaud, V., Gabas, N., Blouin, J., Pellerin, P., & Moutounet, M. (1997). Influence of wine polysaccharides and polyphénols on the crystallization of potassium hydrogen tartrate. *Journal International des Sciences de la Vigne et du Vin, 31*(2), 65–83.

Gerbaud, V., Gabas, N., Laguerie, C., Blouin, J., Vidal, S., Moutounet, M., et al. (1996). Effect of wine polysaccharides on the nucleation of potassium hydrogen tartrate in model solutions. *Transaction of Institution of Chemical Engineers, 74*, 782–790.

Granados-Sanchez, D. (1993). Cultura y utilzation del maguey. Los agaves en México. Chapingo, México, Universidad Autónoma de Chapingo, pp. 11–22.

Hakomori, S. I. (1964). A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *Journal of Biochemistry (Tokyo), 55*, 205–208.

Harris, P. J., Henri, R. J., Blakeney, A. B., & Stone, B. A. (1984). An improved method for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydrate Research, 127*(1), 59–73.

Hilz, H., Williams, P., Doco, T., Schols, H. A., & Voragen, A. G. J. (2006). The pectic polysaccharide rhamnogalacturonan II is present as a dimer in pectic populations of bilberries and black currants in muro and in juice. *Carbohydrate Polymers, 65*(4), 521–528.

Ishii, T., & Matsunaga, T. (2001). Pectic polysaccharide rhamnogalacturonan II is covalently linked to homogalacturonan. *Phytochemistry, 57*(6), 674–696.

Ishii, T., Matsunaga, T., Pellerin, P., O'Neill, M. A., Darvill, A., & Albersheim, P. (1999). The plant cell wall polysaccharide rhamnogalacturonan II self-assembles into a covalently cross-linked dimmer. *Journal of Biological chemistry, 274*(19), 13098–13104.

Kobayashi, M., Matoh, T., & Azuma, J.-L. (1996). Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. *Plant Physiology, 110*, 1017–1020.

Matoh, T., Ishigaki, K., Ohno, K., & Azuma, J. I. (1993). Isolation and characterization of a boron–polysaccharide complex from radish roots. *Plant and Cell Physiology, 34*, 639–642.

Mazeau, K., & Perez, S. (1998). The preferred conformations of the four oligomeric fragments of rhamnogalacturonan II. *Carbohydrate Research, 311*, 203–217.

Melton, L. D., McNeill, M., Darvill, A. G., & Albersheim, P. (1986). Structural characterization of oligosaccharides isolated from the pectic polysaccharide rhamnogalacturonan II. *Carbohydrate Research, 146*, 279–305.

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology, 11*(3), 266–277.

O'Neill, M. A., Albersheim, P., & Darvill, A. G. (1990). The pectic polysaccharides of primary cell walls. In P. M. Dey (Ed.), *Methods in plant biochemistry, Vol. 2* (pp. 415–441). London: Academic Press.

O'Neill, M. A., Ishii, T., Albersheim, P., & Darvill, A. G. (2004). Rhamnogalacturonan II: Structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annual Revue of Plant Biology, 55*, 109–139.

O'Neill, M. A., Warrenfeltz, D., Kates, H., Pellerin, P., Doco, T., Darvill, A. G., et al. (1996). Rhamnogalacturonan II, a pectic polysaccharide in the walls of growing plant cell, forms a dimer that is covalently cross-linked by a borate ester: In vitro conditions for the formation and hydrolysis of the dimmer. *Journal of Biological chemistry, 271*(37), 22923–22930.

Ortiz-Basurto, R. I., Torrestiana-Sanchez, B., Pourcelly, G., & Calderon-Cervantes, F. (2005). *Desarrollo de bioprocesos para la elaboración de alimentos funcionales y aditivos biotecnológicos a partir de Agave pulquéro*. Puebla México: Project report to Produce Puebla Foundation.

Pellerin, P., Doco, T., Vidal, S., Williams, P., Brillouet, J. M., & O'Neill, M. A. (1996). Structural characterization of red wine rhamnogalacturonan II. *Carbohydrate Research, 290*, 183–197.

Pellerin, P., O'Neill, M. A., Pierre, C., Cabanis, M. T., Darvill, A., Albersheim, P., et al. (1997). Lead present in wines is bound to the dimers of rhamnogalacturonan II, a pectic polysaccharide from the grape berry cell walls. *Journal International des Sciences de la Vigne et du Vin*, 33–41.

Pérez, S., Rodríguez-Carvajal, M. A., & Doco, T. (2003). A complex plant cell wall polysaccharide: Rhamnogalacturonan II. A structure in quest of a function. *Biochimie, 85*(1–2), 109–121.

Puvanesarajah, V., Darvill, A. G., & Albersheim, P. (1991). Structural characterization of two oligosaccharide fragments formed by the selective cleavage of Rhamnogalacturonan II: Evidence for the anomeric configuration and attachment sites of apiose and 3-deoxy-2 heptolosaric acid. *Carbohydrate Research, 218*, 211–222.

Riou, V., Vernhet, A., Doco, T., & Moutounet, M. (2002). Aggregation of grape seed tannins in model wine-effect of wine polysaccharides. *Food Hydrocolloid, 16*, 17–23.

Spellman, M. W., McNeil, M., Darvill, A. G., & Albersheim, P. (1983). Characterization of a structurally complex heptasaccharide isolated from the pectic polysaccharide rhamnogalacturonan II. *Carbohydrate Research, 122*, 131–153.

Stevenson, T. T., Darvill, A. G., & Albersheim, P. (1988). 3-Deoxy-D-lyxo-2heptulosaric acid, a component of the plant cell wall polysaccharide rhamnogalacturonan-II. *Carbohydrate Research, 179*, 269–288.

Stewart, D., Azzini, A., Hall, A-T., & Morrison, I-M. (1997). Sisal fibres and their constituent non-cellulosic polymers. *Industrial Crops and Products, 6*(1), 17–26.

Strasser, G. R., & Amado, R. (2002). Pectic substances from red beet (*Beta vulgaris* L. var. *conditiva*). Part II. Structure characterisation of rhamnogalacturonan II. *Carbohydrate Polymers, 48*, 263–269.

Thomas, J. R., Darvill, A. G., & Albersheim, P. (1989). Isolation and structural characterization of the pectic polysaccharide rhamnogalacturonan II from walls of suspension-cultured rice cells. *Carbohydrate Research, 185*, 261–277.

Vidal, S., Doco, T., Williams, P., Pellerin, P., York, W. S., O'Neill, M. A., et al. (2000). Structural characterization of the pectic polysaccharide rhamnogalacturonan II: Evidence for the backbone location of the aceric acid-containing oligoglycosyl side chain. *Carbohydrate Research, 326*, 277–294.

Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V., et al. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry, 85*, 519–525.

Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003). The polysaccharides of red wine: Total fractionation and characterisation. *Carbohydrate Polymers, 54*, 439–447.

Vidal, S., Williams, P., O'Neill, M. A., & Pellerin, P. (2001). Polysaccharides from grape berry cell walls. Part I: Tissue distribution and structural characterization of the pectic polysaccharides. *Carbohydrate Polymers, 45*, 315–323.

Whitcombe, A. J., O'Neill, M. A., Steffan, W., Albersheim, P., & Darvill, A. G. (1995). Structural characterization of the pectic polysaccharide, rhamnogalacturonan-II. *Carbohydrate Research, 271*, 15–29.

York, W. S., Darvill, A. G., McNeil, M., & Albersheim, P. (1985). 3-Deoxy-D-manno-2-octulosonic acid (KDO) is a component of rhamnogalacturonan II, a pectic polysaccharide in the primary cell of plants. *Carbohydrate Research, 138*, 109–126.