Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 1008–1018
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of variety and harvest date on pectin extracted from chicory roots (*Cichorium intybus* L.)

Christelle Robert [a,*], Thomas Happi Emaga [a,b], Bernard Wathelet [a], Michel Paquot [a]

[a] *Unité de Chimie Biologique Industrielle, Faculté Universitaire des Sciences Agronomiques, Passage des Déportés, 2, B-5030 Gembloux, Belgium*
[b] *Centre Africain de Recherche sur Bananier et Plantain (CARBAP), BP 832 Douala-Cameroon*

Received 27 June 2007; received in revised form 16 October 2007; accepted 6 December 2007

Abstract

The influence of harvest date on pectin quality and yield of five varieties of chicory is investigated in this work. The pectin is extracted from the raw material with an acidic treatment at 85 °C for an hour after inulin extraction. Main changes due to harvest date are observed in terms of neutral sugar content, average molecular weight, esterification degree and extraction yield. The galacturonic acid content remains relatively constant throughout the harvest period. While no statistically significant differences ($p < 0.05$) in terms of galacturonic acid content and neutral sugar content are observed between the five cultivars studied, average molecular weight, esterification degree and extraction yield are significantly affected by the cultivar type. Depending on the harvest date, a broad range of pectins can easily be extracted from chicory pulps.

Keywords: Pectins; Extraction; Chicory roots; Variety; Harvest date; Characterisation

1. Introduction

Chicory roots (*Cichorium intybus* L.) are cultivated in Europe for the production of inulin, an unbranched polymer consisting of fructose units with a terminal glucose residue, and its hydrolysis product. The chain length of inulin is reported to be influenced by climate, date of sowing, harvest date and variety (Amaducci & Pritoni, 1998; Baert, 1997).

Chicory roots pulps are an important by-product of the inulin processing industries and are usually used in animal feed. However, other applications can be found for these materials since we showed in our previous research that the extraction of chicory pulps yields high levels of pectin, a polysaccharide extensively used in food as a gelling agent, thickening agent and stabilizer.

Pectins are complex carbohydrate polymers consisting of homogalacturonan (HG), a linear chain of α-(1 → 4)-linked-D-galactopyranosyluronic acid residues partially methylesterified. This HG region is interrupted by a type I rhamnogalacturonan (RG-I) consisting of the repeating disaccharide unit: (1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-GalA*p* (Renard, Crépeau, & Thibault, 1995). Side chains, mainly consisting of arabinan and/or galactan, are attached to the RG-I backbone at the C-4 position of the rhamnose residues (Schols & Voragen, 1996) forming hairy blocks of the RG-I. Another rhamnogalacturonan of type II (RG-II) is constituted by a homogalacturonan backbone that can be substituted at O-2 and/or O-3 of galactopyranosyl acid residues, by side chains containing rare sugars, in addition to the galactose and arabinose.

Carpita and Gibeaut (1993) have reported that the biosynthesis and the degradation of cell wall components such as cellulose microfibrils, hemicelluloses and pectic polysaccharides take place at the same time in the cell wall in order to achieve equilibrium. Plant development involves a coordinated series of biochemical processes that, among other

* Corresponding author. Tel.: +32 (0)81 62 22 32; fax: +32 (0)81 62 22 31.
E-mail address: robert.c@fsagx.ac.be (C. Robert).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.12.013

things, result in the biosynthesis and degradation of cell wall components.

Pectic polymers are highly complex and in consequence have significant potential for structural modification both during biosynthesis and within the cell wall (Ridley, O'Neill, & Mohnen, 2001). These changes are due to the action of pectinase (e.g. polygalacturonase, pectinesterase) which are mainly involved in pectin degradation. The activities of enzymes differ widely between species resulting in changes in depolymerisation and pectin solubilisation.

In the case of chicory, it is well known that inulin undergoes changes in yield and in chain length during harvest. However up to now no information is available on the evolution of pectin in chicory roots with harvest date.

This paper is in keeping with our preliminary works which allowed us to determine the best way to extract pectins (Robert, Devillers, Wathelet, Van Herck, & Paquot, 2006). Its aim is to evaluate the influence of harvest date on the chemical composition and pectin yield (13 different stages) and of five different chicory varieties grown in Belgium.

2. Experimental

2.1. Raw material

In 2002, five chicory (*C. intybus* L.) varieties (Nausica, Melci, Arancha, Madona and Vivace) were sown (6th April) and grown on a light sandy silt soil in Pottes (Hainaut, Belgium). The choice of these varieties was done since they give rise to differences in inulin yield and polymerisation degree.

The roots were harvested manually at 13 different dates (15 day intervals) between July and January 2003. Maximum and minimum air temperatures were recorded continuously during the growing period at a nearby weather station of the Royal Meteorological Institute. Cold temperatures (<5 °C) were observed from October and negative temperatures were only recorded in December (Fig. 1).

The trial fields were arranged in an experimental design replicated three times for Nausica and Melci varieties and one time for the 3 others chicory varieties. Each plot consisted of three rows of 11.80 m. Distance between plants in a row was 10 cm and between two adjacent rows was approximately 50 cm. About 20 roots of the central row were harvested. The external rows served as a protection border. The roots were washed with distilled water, weighed, cut in a stainless steel Dito Sama cutter (TRS21) with a grating disc J7 and stored at −18 °C before analysis.

2.2. Extraction of pectins

Our previous results have shown that neither hot water nor chelating agent were able to extract pectin. Thus, an acidic extraction procedure was designed to minimise pectin degradation and solubilise pectic polymers in a form as close as possible to their native form.

Before performing pectin extraction, the chicory roots were immersed in hot water for an hour at 85 °C under continuous shaking in order to isolate the inulin. The 'deinulinized' pulps were isolated by filtration and placed into hot water (85 °C) adjusted to pH 1.5 with H_2SO_4 in order to release the pectic polysaccharide. After 1 h of immersion, the slurry was filtrated under vacuum on a 20 μm nylon membrane and was rapidly cooled to room temperature in a water/ice bath. The whole volume of rich pectin extract was recorded.

Two aliquots of 50 ml were sampled, precipitated with four volumes of denatured ethanol and centrifuged at 10,000 rpm for 20 min. Gelatinous pectin was washed twice with ethanol 50%, centrifuged at 6000 rpm for 20 min and

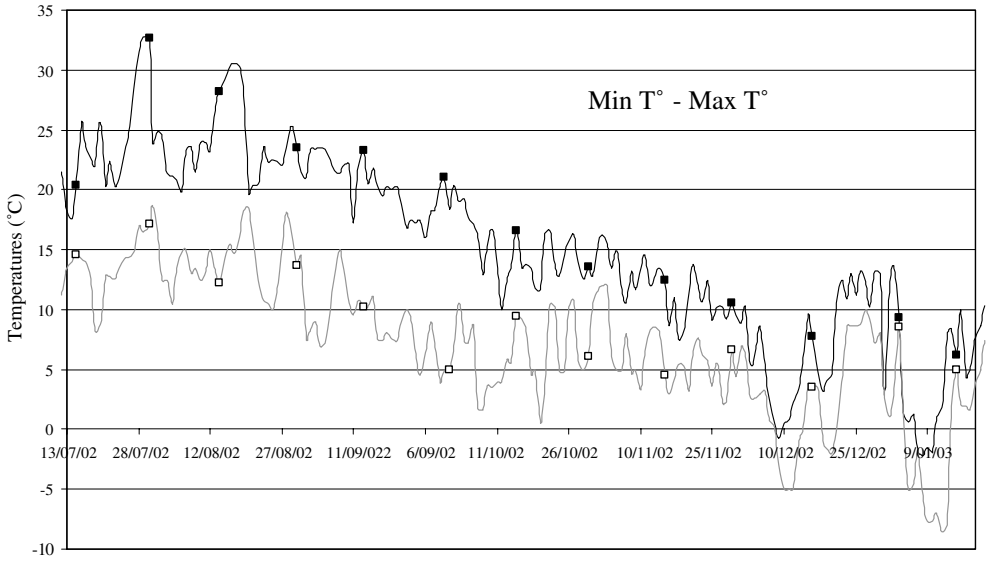

Fig. 1. Maximum and minimum air temperature (°C) in 2002–2003 growing season.

finally, the precipitate was collected, dispersed in 50 ml of distilled water before freeze drying in order to determine the yield of extraction.

The pH of the remaining pectic solution was adjusted to 3.5 at room temperature with 1 M K_3PO_4 and concentrated four times by ultrafiltration using an hollow fibre tangential flow filtration membrane (polysulfone, 0.5 mm id, 615 cm^2 sa) of 50 kDa molecular weight cut-off. The concentrate extract was treated with EtOH (4 /1; v/v) and the resulting precipitate was recovered by centrifugation (10,000 rpm/min, for 20 min). Gelatinous pectin was washed twice with ethanol 50%, centrifuged at 6000 rpm for 20 min and finally, the precipitate was collected, rinsed with ethanol 96% and air-dried at room temperature for 48 h.

The extraction yield was calculated, based on dried matter (g/100 g). Each extraction was conducted in duplicate. The dry matter content of both pulps and pectic solution was determined by drying a known fresh weight of samples at 105 °C until constant weight was obtained. After cooling, the samples were weighed. Dry matter and water content were calculated from the difference.

2.3. Molecular weight

The average molecular weight of pectic polysaccharides was obtained by high-performance size exclusion chromatography (HPSEC) method on a 2690 Waters HPLC chipped with a TSK PW_{XL} column (7.8 × 300 mm) (Tosohaas, Japan) coupled on-line with three detectors. The system consisted of a 2410 Waters differential refractive index detector (RI) and a dual system T-50A composed of a RALLS (right angle laser light scattering) detector and a differential viscosimeter (Viscotek, T-50A). In order to calculate the molecular weight by the TriSEC software (Viscotek), the specific refractive index increment (dn/dc) or the concentration of the pectic solution were required. The exact concentration was determined by drying at 106 °C for 24 h a known and precise volume of this solution filtered through a 0.45 μm membrane filters (Millipore). The elution was performed at a 0.7 ml/min flow rate with 50 mM $NaNO_3$ containing 0.05% NaN_3 as preservative.

2.4. Galacturonic acid content

The galacturonic acid content was determined according to the procedure of Garna, Mabon, Nott, Wathelet, and Paquot (2006) by high-performance anion exchange chromatography coupled with a pulsed amperometry detector (HPAEC-PAD) after enzymatic degradation with a pectinase (Viscozym L9, Novozym) for 2 h at 50 °C in 50 mM acetate buffer (pH 4.6). The analytical column was a Dionex Carbopac PA-100 (250 × 4 mm) with a PA-100 guard column (50 × 4 mm). The flow rate was set at 1 ml/min and the separation occurred with NaOH 100 mM and NaOAc 150 mM. Glucuronic acid was used as the internal standard.

2.5. Neutral sugars content

Neutral sugars were analysed after sulphuric acid hydrolysis and after their conversion to alditol acetates according to Blakeney, Harris, Henry, and Stone (1983) modified by Garna, Mabon, Wathelet, and Paquot (2004). These derivatives were analysed by gas chromatography using a FID detector and fitted with a HP-1 capillary column (30 m × 0.5 mm id) with a film thickness of 0.30 μm. The injection and detector temperature was 200 and 250, respectively. The column temperature was set to 120 °C when injected, then increased at 4 °C/min to 180 °C followed by 20 °C/min to 240 °C. The amount of neutral sugars was calculated against a 2-desoxyglucose internal standard.

2.6. Methyl and acetylesterification

Degrees of methylation and acetylation of pectic polysaccharides were determined after saponification of 100 mg of polymer with a 0.8 M NaOH/isopropanol (50/50; v/v) mixture according to Voragen, Schols, and Pilnik (1986). The methanol and acetic acid were separated on an ion exclusion column HPX87H (Biorad) and quantification by refractometry using succinic acid as the internal standard. Elution was carried out with 4 mM H_2SO_4 at 0.7 ml/min.

2.7. Statistical analysis

Data collected for each result (variety and harvest date) were analysed in duplicate and the figures were then averaged. The statistical software used to evaluate the experimental design results was Minitab version 13.31. Data were assessed by analysis of variance. Two-way ANOVA (variety and harvest date) was applied in order to know the incidence of these factors at a confidence level of 95%.

3. Results and discussion

In this work, 13 different development stages of five varieties of chicory roots are analysed in terms of yield, average molecular weight and chemical composition of acid soluble pectins. The main experimental results are presented in histograms to visualize the differences between varieties at the different stages of growth.

The kinetic of growth in terms of roots fresh weight during a culture cycle is shown in Fig. 2. An exponential phase occurred between the 1th and 7th harvest date and was followed by a stationary phase. The fresh root weight reached its peak on October which corresponds to the maximum root development and the maximum inulin polymerisation degree after which DP began to decrease.

3.1. Pectin extraction

The acidic procedure used in this work to extract pectic polymers from chicory roots leads to the solubilisation of

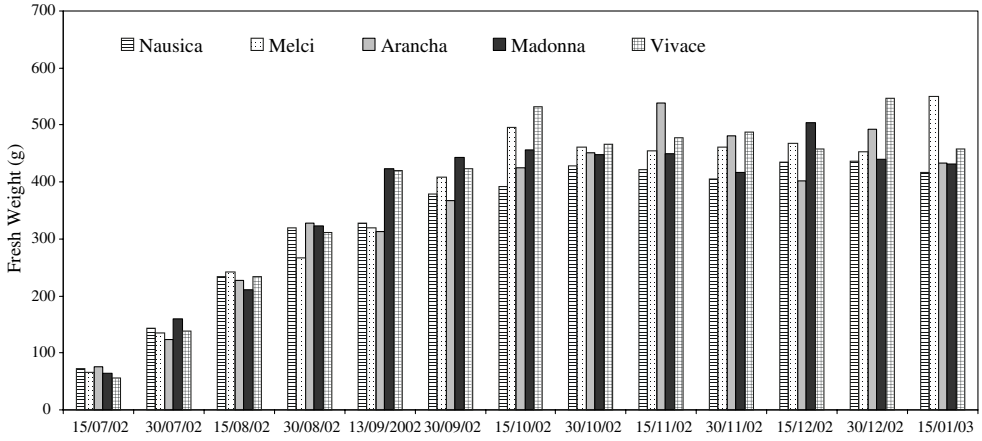

Fig. 2. Root fresh weight (g) during development of chicory.

covalently bound pectin. The pectin yield of each cultivar at each development stage was expressed as the percentage of alcohol insoluble residue (AIR) of the dry matter weight of chicory roots. Fig. 3 shows changes in pectin yield as a function of the harvest date and plots of the main effects for harvest date are shown in Fig. 4.1. The mean yield obtained for each chicory cultivar varies between 2.8% and 4.7% of AIR found in the dry matter of chicory roots. Comparable values of extraction yield were reported for acid soluble pectins from sugar beet pulp (Michel, Thibault, Mercier, Heitz, & Pouillaude, 1985), carrots (Massiot, Rouau, & Thibault, 1988) and chicory roots (Robert et al., 2006). Nevertheless, our results were lower than those obtained in the studies of Panouillé, Thibault, and Bonnin (2006) and Pilnik and Voragen (1992), respectively, on chicory roots and on dried sugar beet pulps. Higher yields obtained by others are probably due to the use of harsher extraction conditions.

The maximum pectin yield is produced by the varieties Melci and Nausica with 4.65% and 4.62%, respectively. Difference between highest and lowest yield of extraction is maximum for Nausica while Madona showed the most regular yield of pectin.

The two-way statistical analysis using "variety" and "harvest date" as factors shows that pectin levels vary significantly with variety ($p = 0.049$) and highly significantly with the harvest date ($p = 0.000$). For the first four harvest dates, reduction in yield is observed for all varieties and a minimum pectin yield is obtained during October as shown by Fig. 4.1. Increase in pectin yield is found at the end of harvest except for Arancha. This variety produces its highest yield end of November before exhibiting a new decrease in pectin yield.

In this study, the values of yield are not expressed for overall pectin yield, but only for pectin released after an hour by acidic treatment. Our results must thus be discussed in term of extractable pectin.

The increase in acid soluble pectin may be due to the "de novo" synthesis of pectic molecules or may indicate changes in bonding of the various cell wall polymers to each others.

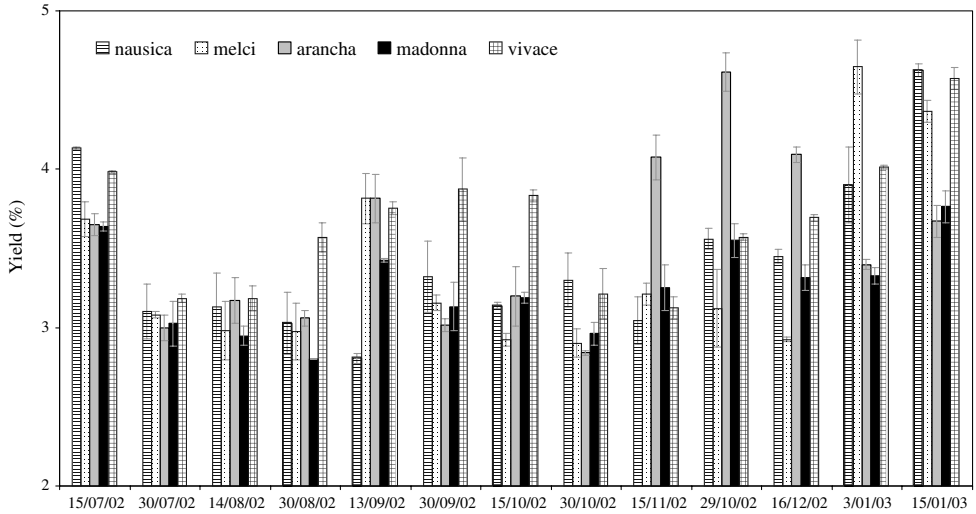

Fig. 3. Changes in chicory pectin yield of chicory pectins (% dry weight) as a function of harvest time and varieties.

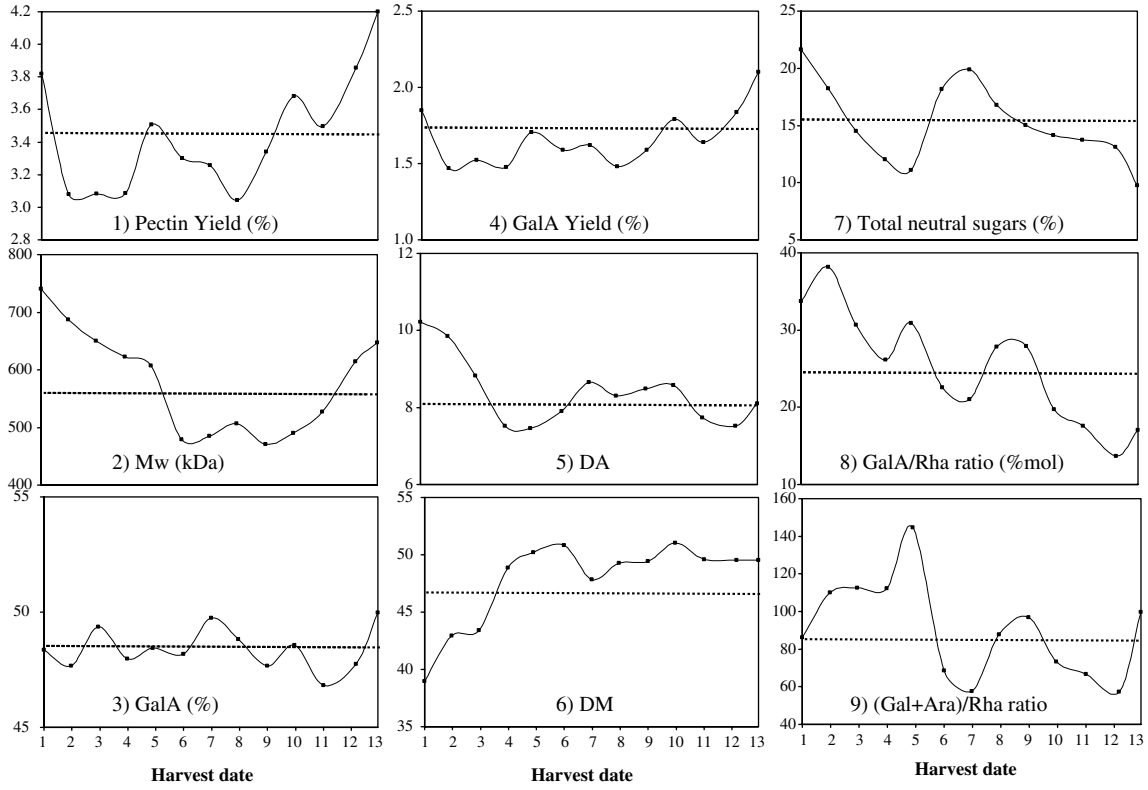

Fig. 4. Main-effect plot of harvest date on yield and chemical composition of chicory roots pectin.

It is well known that pectin polymers become less tightly bonded in the cell wall during ripening and that a gradual solubilisation of protopectin occurs in the cell walls. The increase in acid soluble pectin could also be the consequence of a solubilisation of esterified pectin by enzymes of the cell walls.

It was also postulated that pectin solubilisation could be associated with a gradual degradation of galactane and/or arabinane side chains. Architecturally, pectins and other components of the primary wall matrix are stabilized owing to the presence of inter- as well as intra-molecular cross-links. These pectic arabinogalactan side chains probably contribute to the formation of inter-polymeric cross-links. Therefore, side chain hydrolysis may affect the cell wall integrity and consequently may influence pectin solubility and yield.

3.2. Molecular weight

The changes in molecular weight of pectin during development were determined by high pressure size exclusion chromatography performed on a TSK PWxl column. Fig. 5 shows that for the different chicory varieties used in this study the average molecular weight (M_w) ranges from 300 to 890 kDa. Values are much higher than those found for sugar beet (Levigne, Ralet, & Thibault, 2002; Rombouts & Thibault, 1986; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007) but quite similar to those obtained spent hop (Oosterveld, Voragen, & Schols, 2001) and chicory roots (Panouillé et al. 2006) which were also isolated in acidic media.

As is apparent from the main-effects plot in Fig. 4.2, a highly significant marked decrease ($p < 0.05$) in M_w for all varieties is observed between the 1st and 6th or 7th harvest date which corresponds to the end of the roots growth: from 890 kDa at the beginning, it declined approximately by half to reach less than 400 kDa. The decline in M_w of pectins is comparable to results found in many fruits for which CDTA-soluble pectins underwent some depolymerisation during ripening.

Surprisingly, this decrease in M_w observed at the end of the roots growth is followed by an M_w increase for three chicory varieties: Nausica, Madona and Arancha. Vivace and Melci roots differ from the other varieties in showing a rather constant evolution of M_w when their growth is done.

These results are similar to those reported in previous studies on flax, where some higher molecular weight pectin extracted with HCl were synthesised during the ageing stems (Bédouet, Denys, Courtois, & Courtois, 2006).

The effect of the varieties on M_w is also significantly different. M_w of all varieties falls during the root development and, in particular for Vivace and Arancha which show the fastest and biggest decrease. Between the 1st and 6th stage there is a release of acid-soluble pectin and an associated increase in smaller pectic fragments. It has been showed that inulin exhibit a maximum chain length in October and a decrease in length at the latest harvest (Koch,

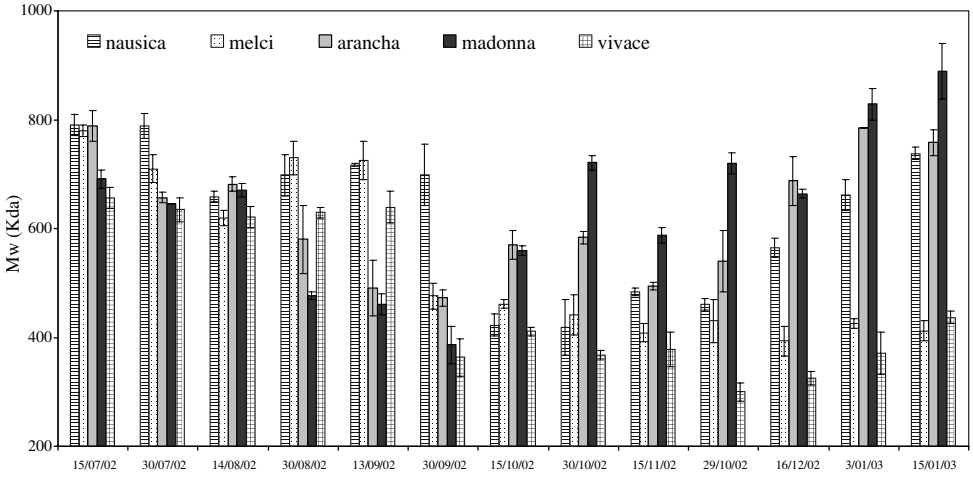

Fig. 5. Changes in average molecular weight (kDa) of chicory pectins as a function of harvest time and varieties.

Andersson, Rydberg, & Åman, 1999) when nights gets cooler, indicating a impact of the temperature on the inulin DP. Depolymerisation in fructans are triggered by the occurrence of the first frost in the fall (Van den Ende, Mintiens, Speleers, Onuoha, & Van Laere, 1996). After the first frost, the activity of fructan 1-exohydrolase increases and leads to the breakdown of fructans in chicory roots. Similarly, cold temperature may also have an impact on pectin by affecting enzymes in the cell walls.

In fact at the same period, pectin molecular weight shows the lowest values. A competition between synthesis of storage polysaccharide "inulin" and synthesis of structural pectic polysaccharides could occur during the growth of the chicory roots. This hypothesis was supported by the paper of Reiter and Vanzin (2001) who shows the interconversion between the activated sugars and thus the potential competition between UDP-Glc and UDP-GalA.

The varieties Madona, Arancha and Nausica exhibit a significant increase in M_w when their root growth is ended. The recovering of high molecular weight pectins during the latest sampling dates can be due to the absence of polygalacturonase (PG) which is responsible for pectin degradation. The absence of PG activity in chicory roots was reported for different varieties and in particular for Arancha and Nausica (Thonar, unpublished results).

3.3. Galacturonic acid content

Fig. 6 shows that the galacturonic acid content of pectins varies between 43% and 53%. However, the observed values do not differ significantly during development ($p = 0.484$) and according to the varieties ($p = 0.350$). These values are similar than those obtained by Panouillé, Thibault and Bonnin on chicory roots (~53.1%) and for carrot pectin (~54.7) (Massiot et al., 1988).

The same results were found for total sunflower pectin isolated from different cultivars (Lin, Humbert, & Sosulski, 1976). GalA content remains also constant during linear elongation of cotyledon tissues of soybean (Koch, Horbowicz, & Obendorf, 1999).

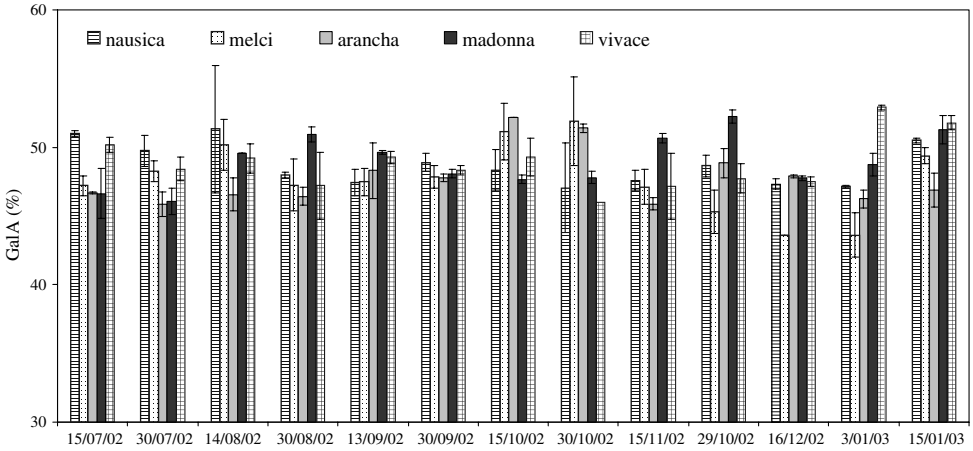

Fig. 6. Changes in galacturonic acid content (% dry weight) of chicory pectins as a function of harvest time and varieties.

However, total uronic acid content obtained by taking into account the yield and the GalA content of pectins is significantly influenced by harvest date ($p = 0.000$) (Fig. 7).

3.4. Neutral sugar content

The neutral saccharides composition was analysed in the pectic extract by gas chromatography. Significative difference ($p = 0.000$) in the total neutral sugar content is observed according to harvest date (Fig. 8). Nausica exhibits the highest levels of monosaccharide for six of the 13 harvest dates but no significative differences are found for chicory varieties ($p = 0.162$). The neutral sugars content ranges from 9.5% to 22.7%. These values are similar to the results reported for chicory roots (Panouillé et al., 2006), for carrots (Massiot et al., 1988) and sugar beet pectin (Oosterveld, Beldman, Searle-van Leeuwen, & Voragen, 2000) and substantially higher compared to commercial pectins. This indicates that chicory pectins are rich in hairy regions. Galactose and arabinose commonly found in pectin side chains are obviously most abundant neutral sugars (Table 1). Contrary to the results of Panouillé et al. (2006) for which arabinose is the most abundant neutral sugar (around 13%), our results shows that galactose is the main neutral sugar. Other sugars are present in relatively low amount (less than 1%). Traces of glucose and xylose could be due to a contamination from hemicellulose or from xylogalacturonan (Vincken et al., 2003).

The change in level of rhamnose was the most important from 0.3 to on average 0.8 for the 5th and 7th harvest date

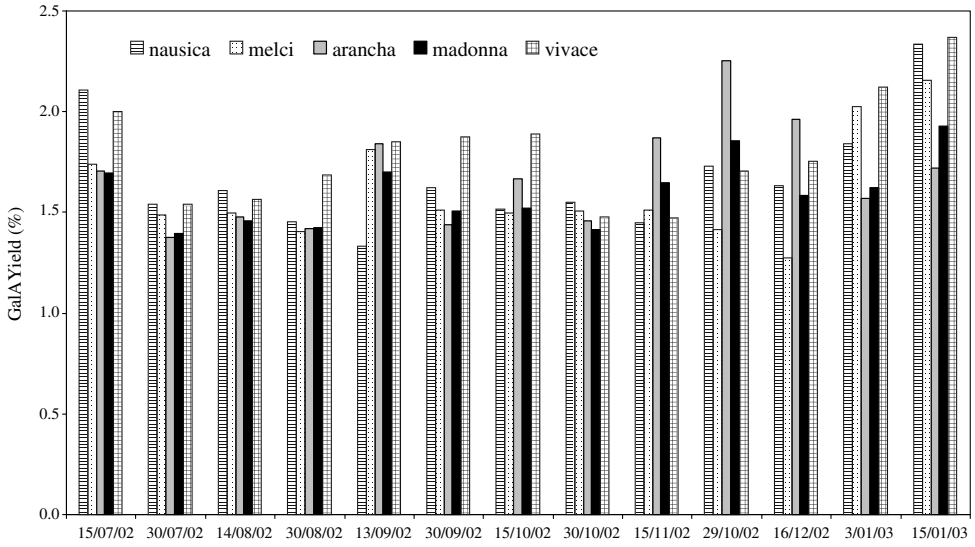

Fig. 7. Changes in galacturonic acid yield (% dry weight) of chicory pectins as a function of harvest time and varieties.

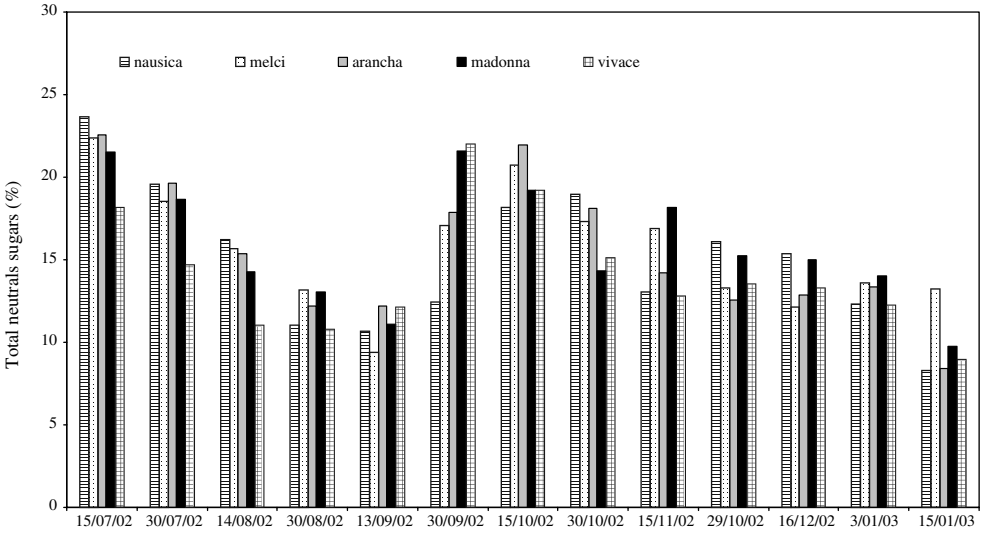

Fig. 8. Changes in total neutral sugar content (% dry weight) of chicory pectins as a function of harvest time and varieties.

Table 1
Changes in neutral sugars content (% of dry matter of pectin) of chicory root pectins as a function of harvest date and variety

		Harvest date												
		15/07/02	30/07/02	14/08/02	30/08/02	13/09/02	30/09/02	15/10/02	30/10/02	15/11/02	29/11/02	16/12/02	3/01/03	15/01/03
Rha	Nausica	0.6	0.4	0.5	0.4	0.3	0.4	0.8	0.7	0.4	0.5	0.7	0.7	0.5
	Melci	0.6	0.5	0.4	0.4	0.3	0.7	0.9	0.6	0.5	0.4	0.7	0.8	0.6
	Arancha	0.5	0.4	0.3	0.4	0.3	0.7	0.8	0.5	0.4	0.8	0.6	0.8	0.5
	Madonna	0.5	0.4	0.5	0.6	0.3	0.8	0.9	0.5	0.5	0.8	0.7	0.9	0.4
	Vivace	0.5	0.4	0.4	0.3	0.3	0.8	0.7	0.4	0.4	0.7	0.7	0.7	0.4
Ara	Nausica	4.6	3.6	3.2	2.7	2.1	2.6	3.9	4.5	2.6	3.6	3.7	1.6	1.2
	Melci	5.4	3.2	3.1	2.4	1.3	3.9	5.2	4.5	4.3	2.9	3.0	3.5	3.3
	Arancha	3.7	3.2	2.8	2.3	3.0	5.1	6.5	4.6	3.5	2.8	4.0	2.3	1.8
	Madonna	3.6	3.6	2.6	2.4	2.0	5.5	4.8	3.3	5.5	4.4	4.6	4.3	1.6
	Vivace	3.5	2.6	2.6	2.4	1.3	6.9	3.6	3.7	2.6	3.0	3.2	2.7	1.7
Xyl	Nausica	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.2
	Melci	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.2	0.2	0.3	0.2
	Arancha	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.1	0.1	0.2	0.2	0.2	0.2
	Madonna	0.1	0.2	0.1	0.1	0.1	0.3	0.2	0.1	0.2	0.2	0.3	0.2	0.2
	Vivace	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.2	0.2	0.2	0.2	0.2
Man	Nausica	0.8	0.6	0.6	0.3	0.4	0.5	0.6	0.7	0.6	0.6	0.7	0.5	0.4
	Melci	0.7	0.6	0.6	0.4	0.6	0.8	1.1	0.8	0.7	0.6	0.4	0.6	0.6
	Arancha	1.1	0.8	0.5	0.4	0.5	0.8	1.0	0.7	0.6	0.6	0.4	0.5	0.4
	Madonna	0.7	0.6	0.4	0.2	0.4	0.9	0.7	0.6	0.7	0.5	0.7	0.6	0.4
	Vivace	0.6	0.5	0.3	0.4	0.4	0.9	0.8	0.6	0.7	0.6	0.5	0.8	0.4
Glu	Nausica	2.4	1.1	1.0	0.5	0.6	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.5
	Melci	2.2	1.8	0.9	0.8	0.7	0.9	1.0	0.8	0.8	0.6	0.7	0.7	0.6
	Arancha	2.8	2.4	1.0	0.6	0.6	0.8	1.0	0.6	0.6	0.8	0.5	0.6	0.5
	Madonna	2.7	1.4	0.7	0.4	0.5	1.0	0.8	0.6	0.6	0.5	0.7	0.7	0.4
	Vivace	1.6	1.0	0.7	0.7	0.5	1.0	0.9	0.6	0.8	0.6	1.0	0.8	0.4
Gal	Nausica	15.1	13.7	10.8	7.1	7.2	8.2	12.0	12.2	8.7	10.5	9.4	8.5	5.5
	Melci	13.4	12.3	10.5	9.0	6.3	10.6	12.1	10.5	10.3	8.5	7.1	7.7	7.9
	Arancha	14.3	12.7	10.7	8.4	7.7	10.1	12.3	11.5	8.9	7.3	7.3	8.8	5.0
	Madonna	13.9	12.6	10.0	9.4	7.7	13.0	11.7	9.2	10.7	8.9	8.0	7.4	6.7
	Vivace	11.8	10.0	6.9	6.9	9.6	12.1	13.1	9.6	8.1	8.3	7.7	7.0	5.8

For clarity, the variance is not shown but is less than 6.4%.

respectively. Changes in rhamnose content were also observed for pectins from flax stems. A synthesis of hairy regions after the growth period seemed to occur.

While GalA content remains constant throughout the whole development period (Fig. 6), the neutral sugar level shows some fluctuation during the chicory growth. Examining the details of the main effect plots (Fig. 4.7.) reveals that it decreases until the 5th harvest date, then steadily increases to reach a maximum, and finally undergoes a second decline. Such variation of neutral sugar content can be attributed to changes in specific activities of glycosidases during the development.

The galactan and arabinan side chains of the pectic polysaccharides are regulated in relation to the proliferation and differentiation during cell development of the carrots (Willats, Steele-King, Marcus, & Knox, 1999). As suggested earlier, the loss of neutral sugars from side chains of pectins may increase its solubility and therefore affect yield.

It is also important to analyse the GalA/Rha molar ratio since it correlates with the degree of pectin ramification and consequently allows an estimation of the association with other polymers. Calculation of the molar ratio GalA to Rha (45/1–160/1) shows that chicory pectin is very rich in homogalacturonan. Since GalA content remains rather constant throughout the harvest season, GalA/Rha ratio exhibits the same behaviour as Rha content. Consequently, the ratio is significantly influenced by harvest date (Fig. 9).

It can be seen from the main-effect plots (Fig. 4.9.) and from Fig. 10 that the ratio (Gal + Ara)/Rha ratio consistently decreased during exponential growth, possibly indicative of a reduction of the length of the side chains and to a lesser extent in the degree of substitution of pectin backbone with side chains. There is a first decrease associated with a decrease of the M_w. Thereafter, there is a concomitant increase in degree of substitution and in M_w. After this period, a release of side chains occurs during the stationary phase. That supports the assumption that inulin and pectin biosynthesis are competing.

3.5. Methyl and acetylesterification

The degree of methylation (DM) of acid soluble pectins range from 35% to 56% and is slightly lower than those reported for sugar beet (Levigne et al., 2002) but are in

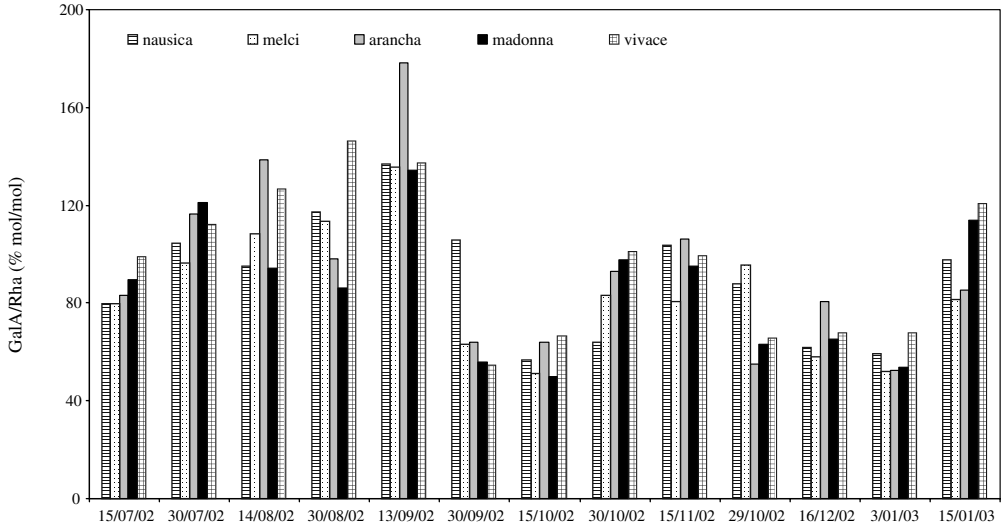

Fig. 9. Changes in the molar ratio of rhamnose to GalA (Rha/GalA) of chicory pectins as a function of harvest time and varieties.

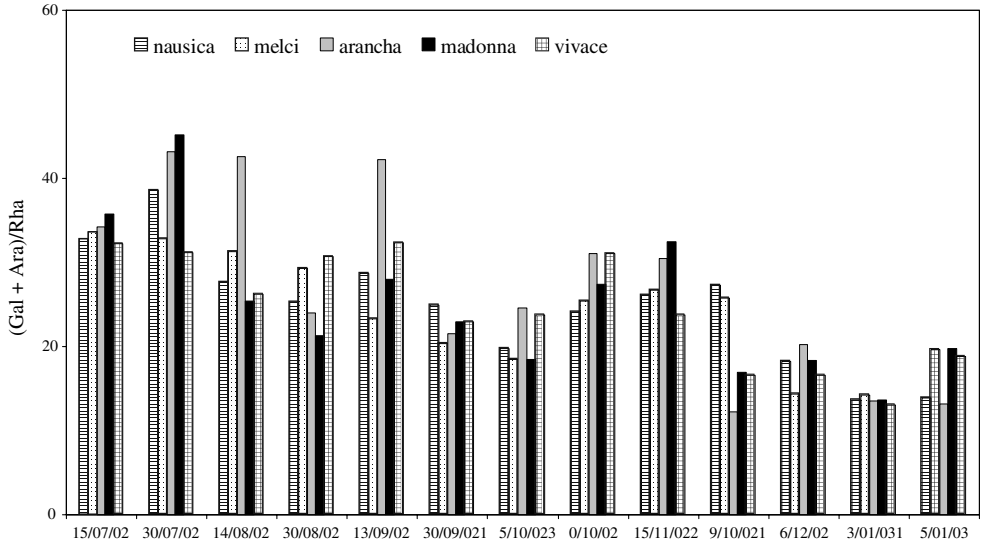

Fig. 10. Changes in the ratio of Gal + Ara to Rha (Gal + Ara/Rha) of chicory pectins as a function of harvest time and varieties.

agreement with previous results on carrots and on chicory roots (Massiot et al., 1988; Panouillé et al., 2006) (Fig. 11). Therefore, all isolated pectins are medium to low methoxyl-pectin.

The DM significantly differs with harvest date (Fig. 4.6) but not with chicory variety. Our data show that DM remains quite constant at a low value at the first and the last harvest dates but increased significantly during the mid harvest. Generally, the end of the growth period is accompanied by a very important decrease in pectin methylesterification but this phenomenon did not occur in our study.

Our data support observations from Bédouet et al. (2006) which reported that the degree of methylesterification of flax pectins only causes a limited decrease after the growth period.

Increase in methylation degree observed at the end of the roots growth may be due to the novo synthesis of methylated residues during development, to a loss of homogalacturonan fragments of lower methylesterification and/or to the resistance of methylated pectin to degradation by endogenous pectinmethylesterase that were detected for the same chicory roots as those used in our study (Thonar, Liners, & Van Cutsem, 2006).

The degree of acetylation (DA) observed in our study varied from around 6% to 11% and is comparable to the one of acid pectin extracted from chicory roots (Panouillé et al., 2006) or carrots pectins (Massiot et al., 1988) (Figs. 12 and 4.5). DA values are much higher than commercial pectin but smaller than sugar beet pectins.

The DA also significantly varies with harvest date and variety but pectin remains quite acetylated whatever the variety or the harvest date.

Significant differences in DA are found between the variety Vivace and the four other chicories. Indeed, Vivace exhibits significantly lower DA values compared to the other varieties.

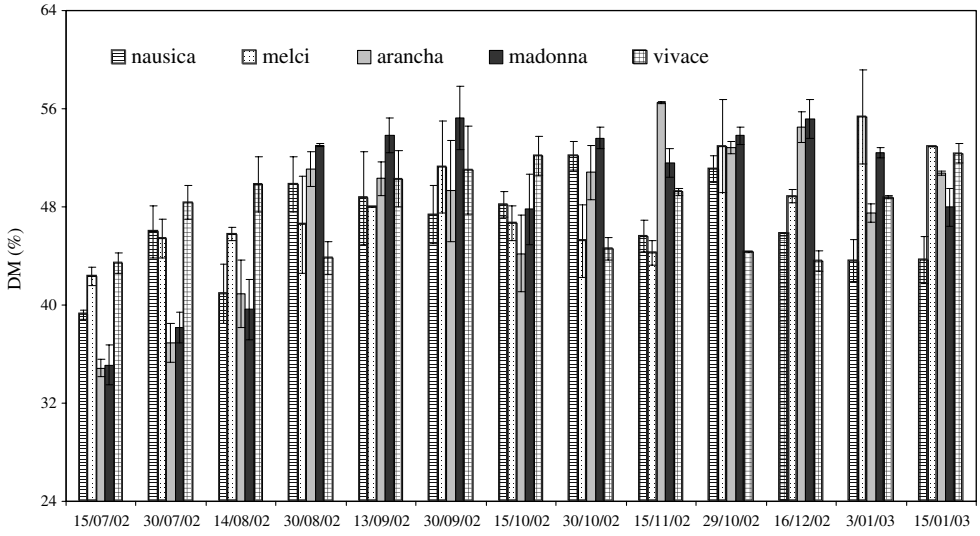

Fig. 11. Changes in the degree of methylation of chicory pectins as a function of harvest time and varieties.

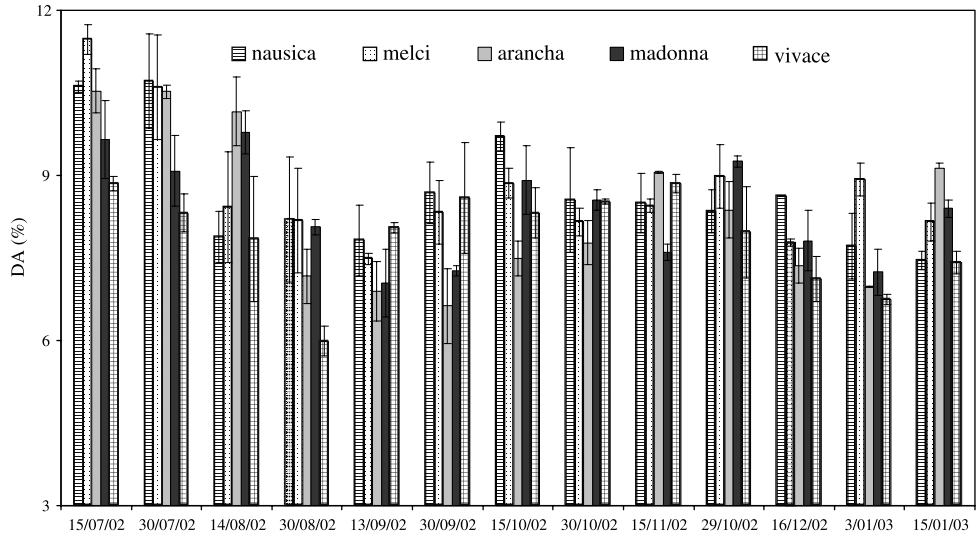

Fig. 12. Changes in the degree of acetylation of chicory pectins as a function of harvest time and varieties.

A decrease in DA for the first harvest dates is noted and rises again. But as a whole, the degree of acetylation decreases probably owing to the presence of pectinacetylesterase activity.

Presence of acetyl esters groups clearly inhibits rhamnogalacturonase activity and limits depolymerisation of RG-I polysaccharides (Schols, Geraeds, Searle-van Leeuwen, Kormelink, & Voragen, 1990). Moreover, pectin esterase activity is hindered by the presence of acetyl groups in both rhamnogalacturonans and homogalacturonans regions. Combination of these parameters with the absence of PG activities can therefore explain the relatively high M_w values.

4. Conclusions

There are obvious quantitative and qualitative changes in pectin extracted from chicory pulps with harvest date and cultivar even if throughout root development the uronic acid content relatively remains constant. The modification rate in composition and in yield during development markedly differed with the variety; Nausica and Arancha exhibiting the fastest and the slowest rates, respectively. A key point in the change in yield and pectin quality appears to be at the 6th harvest date when temperature dropped below 5 °C. Therefore, according to the harvest date, it will possible to produce naturally a broad range of pectin with various physicochemical properties.

Finally, in order to understand the modification process, it would be interesting to correlate pectin evolution with in situ enzymatic activities. Exposure to cold stress may be also studied.

Acknowledgement

The financial support from the Walloon Region of Belgium is acknowledged.

References

Amaducci, S., & Pritoni, G. (1998). Effect of harvest date and cultivar on *Cichorium intybus* yield components in north Italy. *Industrial Crops and Products, 7*, 345–349.

Baert, J. R. A. (1997). The effect of sowing and harvest date and cultivar on inulin yield and composition of chicory (*Cichorium intybus* L.) roots. *Industrial Crops and Products, 6*, 195–199.

Bédouet, L., Denys, E., Courtois, B., & Courtois, J. (2006). Changes in esterified pectins during development in the flax stems and leaves. *Carbohydrate Polymers, 65*, 165–173.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research, 113*, 291–299.

Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal, 3*, 1–30.

Garna, H., Mabon, N., Nott, K., Wathelet, B., & Paquot, M. (2006). Kinetic of the hydrolysis of pectin galacturonic acid chains and quantification by ionic chromatography. *Food Chemistry, 96*(3), 477–484.

Garna, H., Mabon, N., Wathelet, B., & Paquot, M. (2004). New method for a two-step hydrolysis and chromatographic analysis of pectin neutral sugar chains. *Journal of Agricultural and Food Chemistry, 52*, 4652–4659.

Koch, K., Andersson, R., Rydberg, I., & Åman, P. (1999). Influence of harvest date on inulin chain length distribution and sugar profile for six chicory (*Cichorium intybus* L.) cultivars. *Journal of the Science of Food and Agriculture, 79*(11), 1503–1506.

Koch, J. L., Horbowicz, M., & Obendorf, R. L. (1999). Methanol, pectin and pectinesterase changes during soybean seed maturation. *Seed Science Research, 9*, 311–320.

Levigne, S., Ralet, M.-C., & Thibault, J.-F. (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydrate Polymers, 49*, 145–153.

Lin, M. J. Y., Humbert, E. S., & Sosulski, F. W. (1976). Extraction of pectins from sunflower heads. *Canadian Institute of Food Science and Technology Journal, 9*, 70–74.

Massiot, P., Rouau, X., & Thibault, J.-F. (1988). Characterisation of the extractable pectins and hemicelluloses of the cell wall of carrot. *Carbohydrate Research, 172*, 229–242.

Michel, F., Thibault, J.-F., Mercier, C., Heitz, F., & Pouillaude, F. (1985). Extraction and characterisation of pectins from sugar beet pulp. *Journal of Food Science, 50*, 1499–1502.

Oosterveld, A., Beldman, G., Searle-van Leeuwen, M. J. F., & Voragen, A. G. J. (2000). Effect of enzymatic deacetylation on gelation of sugar beet pectin in the presence of calcium. *Carbohydrate Polymers, 43*, 249–256.

Oosterveld, A., Voragen, A. G. J., & Schols, H. A. (2001). Characterization of hop pectins shows the presence of an arabinogalactan-protein. *Carbohydrate Polymers, 49*, 407–413.

Panouillé, M., Thibault, J.-F., & Bonnin, E. (2006). Cellulase and protease preparations can extract pectins from various plants byproducts. *Journal of Agricultural and Food Chemistry, 54*, 8926–8935.

Pilnik, W., & Voragen, A. G. J. (1992). Advances in plant cell biochemistry and biotechnology 1. *JAI*, 19–270.

Reiter, W.-D., & Vanzin, G. F. (2001). Molecular genetics of nucleotide interconversion pathways in plants. *Plant Molecular Biology, 47*, 95–113.

Renard, C. M. G. C., Crépeau, M. J., & Thibault, J.-F. (1995). Structure of the repeating units in the rhamnogalacturonic backbone of apple, beet and citrus pectins. *Carbohydrate Research, 275*, 155–165.

Ridley, B. L., O'Neill, M. A., & Mohnen, D. (2001). Pectins: Structure, biosynthesis, and oligogalacturonide-related signalling. *Phytochemistry, 57*, 929–967.

Robert, C., Devillers, T., Wathelet, B., Van Herck, J.-C., & Paquot, M. (2006). Use of a Plackett–Burman experimental design to examine the impact of extraction parameters on yields and compositions of pectins extracted from chicory roots (*Chicorium intybus* L.). *Journal of Agricultural and Food Chemistry, 54*, 5167–5174.

Rombouts, F. M., & Thibault, J.-F. (1986). Sugar beet pectins: Chemical structure and gelation through oxidative coupling. In M. L. Fishman & J. J. Jen (Eds.), *Chemistry and functions of pectins* (pp. 49–60). Washington, DC: American Chemical Society.

Schols, H. A., & Voragen, A. G. J. (1996). Complex pectins. In J. Visser & A. G. J. Voragen (Eds.), *Pectin and pectinase* (pp. 3–19). Amsterdam: Elsevier Science.

Schols, H. A., Geraeds, C. C. J. M., Searle-van Leeuwen, M. F., Kormelink, F. J. M., & Voragen, A. G. J. (1990). Rhamnogalacturonase: A novel enzyme that degrades the hairy regions of pectins. *Carbohydrate Research, 206*, 105–115.

Thonar, C., Liners, F., & Van Cutsem, P. (2006). Polymorphism and modulation of cell wall esterase enzyme activities in the chicory root during the growing season. *Journal of Experimental Botany, 57*(1), 81–89.

Van den Ende, W., Mintiens, A., Speleers, H., Onuoha, A. A., & Van Laere, A. (1996). The metabolism of fructans in roots of *Cichorium intybus* during growth, storage and forcing. *New Phytologist, 132*, 555.

Vincken, J.-P., Schols, H. A., Oomen, R. J. F. J., Beldman, G., Visser, R. G. F., & Voragen, A. G. J. (2003). Pectin – The hairy thing. In A. G. J. Voragen, H. Schols, & R. Visser (Eds.), *Advances in pectin and pectinase research* (pp. 47–59). Dordrecht: Kluwer Academic Publishers.

Voragen, A. G. J., Schols, H. A., & Pilnik, W. (1986). Determination of the degree of methylation and acetylation of pectin by HPLC. *Food Hydrocolloids, 1*, 65–70.

Willats, W. G. T., Steele-King, C. G., Marcus, S. E., & Knox, J. P. (1999). Side chains of pectic polysaccharides are regulated in relation to cell proliferation and cell differentiation. *The Plant Journal, 20*(6), 619–628.

Yapo, B. M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin. *Food Chemistry, 100*(4), 1356–1364.