

Chemical and instrumental characterization of pectin from dried pomace of eleven apple cultivars

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ABSTRACT. Pectic substances from eleven samples of dried apple pomace were extracted using an acid procedure (100 mM HNO₃, 10 min., 80°C), dried, ground and stored in a P₂O₅ atmosphere. The degree of esterification was determined by the classical titrimetric method and confirmed by the instrumental FTIR method. The samples showed an average DE of 73% and the typical fingerprint in IR spectrogram. The steric exclusion chromatograms obtained with the multi-angle laser light scattering (MALLS) and refraction index (RI) detectors showed that all samples had a high molar fraction eluted at 36-38 min., another large polysaccharide family had an elution time around 43 min, and some samples showed yet another low molecular oligosaccharide that appeared at 58-60 min. Gas chromatography of alditol acetate allowed estimates of the proportion of the neutral sugar in the samples. The ratio of galacturonic acid and rhamnose may give the average size of the galacturonan fraction. These results showed that yields of pectin extracted by the same acid procedure were slightly different. However, the degree of esterification of pectin was similar, with both factors pointing to the best sources in this group of varieties.

Keywords: apple pomace, dietary fibers, pectin, degree of esterification.

RESUMO. Caracterização química e instrumental de pectinas isoladas de bagaço de 11 cultivares de maçã. Substâncias pécicas de 11 amostras de bagaço desidratado de maçã foram extraídas usando um procedimento ácido (HNO₃ 100 Mm, 10 min., 80°C), desidratadas, moídas e armazenadas em atmosfera de P₂O₅. O grau de esterificação das amostras foi determinado pelo método titulométrico, confirmado pelo instrumental FTIR. As amostras mostraram DE médio de 73% e o *fingerprint* característico no espectrograma em infravermelho. Os cromatogramas de exclusão estérica, obtidos com o detector para espalhamento multiangular de luz a laser (MALLS) e com o índice de refração (RI), indicaram que todas as amostras apresentam fração de elevada massa molar, eluída a 36-38 min. e outra grande família de polissacarídeos com tempo de eluição de 43 min. Outros oligossacarídeos de baixo peso molecular aparecem aos 58-60 min em algumas das amostras. A cromatografia gasosa dos alditóis derivatizados permitiu estimar as proporções de açúcares neutros das amostras. A razão entre os açúcares ácidos com a ramnose pode ser usada com parcimônia para a determinação do tamanho médio das cadeias de ácido poligalacturônico.

Palavras-chave: bagaço de maçã, fibras dietéticas, pectina, grau de esterificação.

Introduction

Pectin has been used in jelly manufacture for more than 200 years (WILLATS et al., 2006), but, *in praxis*, only two traditional sources have been economically important, citrus albedo and apple pomace, despite research into new raw materials (IGLESIAS; LOZANO, 2004; LEVIGNE et al., 2002). Apple pectin shows a similar rheological quality to that of citrus pectin, but due to the presence of phenol compounds, the latter is preferred for use in translucent or transparent foods.

The process of producing a colorless acidic polysaccharide from apple induces the separation of pectin from phenolic compounds by ion exchange chromatography (SCHIEBER et al., 2003). Closely related to cellulose and hemicelluloses, soluble pectin may be removed by water showing non-covalent bonds (THAKUR et al., 1997), and insoluble pectin, more strongly associated with cellular structure and known as protopectin, may become soluble after a controlled acid hydrolysis procedure (BERK, 1976).

Pectins are acid polysaccharides, and their composition depends on source, processing and

environmental factors (IGLESIAS; LOZANO, 2004). The major backbone is a polymer of D-galacturonic acid with $\alpha(1 \rightarrow 4)$ bonds between anhydrogalacturonic residues with varying amounts of methyl esterified carboxyl groups (PÉREZ et al., 2003). According to Renard et al. (1995), the insertion of deoxy-hexose into the main chain occurs as chained dimers [$\text{GalA } \alpha(1 \rightarrow 2)\text{-Rha}$] that may join together approximately 20 monomers. Usually, pectin contains 65% galacturonic acid and may show three kinds of forms: the linear homopolygalacturonan and the ramified, rhamnogalacturonan I and II (WILLATS et al., 2006). According to Novosel'Skaya et al. (2000), several authors reported a galacturonic acid:rhamnose ratio from 25 to 200. In the case of a citrus source, the ratio was 40, but only 15% of the rhamnose was in the linear form, and 85% was in the ramified fraction. In apple pectin (RENARD et al., 1995), 22% of the rhamnose is in the linear form, and 78% is in the ramified fraction. The hairy fraction has two different structural features. The first contains several GalA-Rham dimers in the main chain where the side neutral oligosaccharides composed of arabinose and galactose (RG I) are attached and the second, where the side neutral and rare monosaccharides are directly linked to the main chain in a locus containing approximately seven Galacturonic acid residues (RG II).

Pectin quality and acid extraction are related to many factors, including the type of fruit (THAKUR et al., 1997), and, concerning apple pomace, the variety and process (COSTENLA et al., 2002), including extraction, purification and drying. The type and concentration of acid (FERTONANI et al., 2006) and the time and temperature of reaction (FERTONANI et al., 2006; GARNA et al., 2007; SCABIO et al., 2007) play a fundamental role in the balance of pectin extraction and degradation.

In this work, dried pomace from eleven apple cultivars harvested during the 2006–2007 season in the agricultural region of Caçador (9 cultivars) and São Joaquim (2 cultivars) were used as raw material. The main objective of this study was to investigate the influence of variety on the yield and chemical characteristics of pectin.

Material and methods

Material

Samples of apple from eleven cultivars given by the Experimental Stations of Caçador and São Joaquim, which belong to the Empresa de Pesquisa Agrícola e de Extensão Rural de Santa Catarina – Epagri, were codified as (1) Catarina, (2) Joaquina, (3) M-11/00, (4) M-11/01, (5) M-1/01, (6) M-12/00,

(7) M-13/00, (8) M-2/00, (9) M-8/01 and (11) MRC. Pectinolytic enzymes, produced by Novo, were donated by LNF Ltda., from Bento Gonçalves, Rio Grande do Sul State. Chemical products were all *pro analysis*.

Methods

Extraction of pectin. The pomace obtained from premium apple juice production was removed from the hydraulic press, washed with water (1:1) for five minutes at 22°C, centrifuged at 860 g until total liquid drainage and dried overnight in an air-circulated oven at 70°C. The dried pomace was ground through 60 MESH size and stored in polyethylene bags in a gel silica atmosphere at room temperature. Pectin from apple pomace was extracted according to the experimental conditions reported by Fertoni et al. (2006), with a nitric acid (HNO_3) solution (solid-liquid ratio 1:40) adjusted to 100 mM, at the boiling point for 10 min. Pectin was isolated after cooling to 4°C by ethanol 66% (v/v) precipitation, air-dried in a ventilated oven at 50°C and stored under a P_2O_5 atmosphere until use. The gravimetric yield was calculated as the ratio (%) of pectin obtained from the apple pomace raw material.

Analysis. All results were calculated on a dry matter basis. The moisture and mineral content were determined by gravimetric losses after 4h at 104 and 550°C, respectively (TANNER; BRUNNER, 1985). Total fat content was determined by Soxhlet extraction with n-hexane and the protein content ($\text{N} \times 6.25$) by the Kjeldahl procedure (IAL, 2005). Reducing and total reducing sugar were determined by the classical method of Somogyi (1945) as modified by Nelson (1944). Glucose was determined by a glucose oxidase (GOD) kit, fructose by the difference between reducing sugar and glucose, and sucrose by the difference between total reducing sugar and reducing sugar. Total acidity was determined by titration with 0.1 N NaOH using the factor 0.64 to express the results as malic acid, in $\text{g } 100 \text{ g}^{-1}$ (TANNER; BRUNNER, 1985). Total dietary fiber was calculated using the enzymatic-gravimetric method (AOAC, 2000).

Pectic Substances. Pectic substances were characterized by titration of acidic functions of galacturonic acid before and after saponification. The total content of anhydrogalacturonic acid (AUA) and the number of methoxyl groups (MeO) were calculated by specific equations, shown as percentages (BOCHEK et al., 2001; FERTONANI et al., 2006). The sum of these fractions represents the acid polysaccharides – namely, pectinic acid – and the

neutral are the remainder. Pectic substances were also characterized according to their fingerprint from the Fourier transform infrared (FTIR) method in the IR region 4000 cm^{-1} – 400 cm^{-1} , with 4 cm^{-1} resolution. The FTIR spectra analyzed were obtained using the corrected peak areas at 1639.4 (COO-asymmetric stretching) and at 1747 cm^{-1} (C=O esterified), using the following expression $DE(\%) = 100 \cdot (A_{1747}) / (A_{1747} + A_{1639})$ according to the literature in Gnanasambandam and Proctor (2000), Monsoor et al. (2001) and Faravash and Ashtiani (2008).

Individual neutral sugars were analyzed as their alditol acetates by gas-liquid chromatography (GLC) using an HP 5890 SII with FID system, equipped with a capillary column (025 mm \varnothing x 30 m, model B-210, with film $0.25\ \mu\text{m}$) (WOLFROM; THOMPSON, 1963a and b).

High performance size-exclusion chromatography (HPSEC) was carried out on the pectin solutions using multidetection equipment with a Waters 2410 differential refractometer (RI) and an on-line adapted Wyatt Technology Dawn F multi-angle laser light scattering (MALLS) detector. Four Waters Ultrahydrogel 2000/500/250/120 columns were connected in series and coupled to the multidetection equipment. A 0.1 M NaNO_2 solution containing NaN_3 (0.5 g L^{-1}) was used as eluent. The samples were previously filtered by a cellulose acetate membrane ($0.22\ \mu\text{m}$; Millipore) and injected at 1.5 mg mL^{-1} . HPSEC data were collected and analyzed by a Wyatt Technology ASTRA program (HOKPUTSA et al., 2004).

Results and discussion

The raw material

The results of the simple statistical descriptive analysis of pectin are shown in Table 1. There was less variation in the ash and protein contents, with dispersion lower than 10%, and the sugar and malic acid contents were more dispersive. Total dietary fiber was more constant and less susceptible to the selected treatments, although the variation coefficient was 12.83%. Sudha et al. (2007) reported that the samples of commercial pomace showed contents of ash ($0.50\text{ g }100\text{ g}^{-1}$), total fat ($2.70\text{ g }100\text{ g}^{-1}$) and protein ($2.06\text{ g }100\text{ g}^{-1}$), which represents 24.63, 156.06 and 66.88%, respectively, compared to our results. The average contents described by Paganini et al. (2005) were $1.52\text{ g }100\text{ g}^{-1}$ of ash and $0.09\text{ g }100\text{ g}^{-1}$ of acid to BelGolden, Gala and Fuji pomace apples, after first pressing to juice production. Cho and Hwang (2000) had the following contents from dried apple pomace produced in South Korea after juice manufacture: 11.4% moisture content, 3.7% protein, 4.5% fat, 1.8% ash and 70.9% carbohydrate.

Table 1. Approximate composition average of eleven dried pomace apple cultivars (dry basis)*.

Components	Average	Standard Deviation	Variation (%)	Confidence	
				-95%	+95%
Ash	2.03	0.13	6.40	1.95	2.12
Total Fat	1.73	0.40	23.12	1.46	1.99
Protein	3.08	0.21	6.82	2.94	3.23
Malic acid	1.16	0.20	17.24	1.02	1.29
Glucose	12.56	3.55	28.26	10.18	14.95
Fructose	17.93	2.90	16.17	15.98	19.88
Sucrose	7.10	4.81	67.78	3.87	10.33
Reducing sugar	30.34	4.03	13.28	27.45	33.22
Total sugar	39.13	8.05	20.57	33.72	44.53
Total dietary fibers	43.71	5.61	12.83	39.94	47.48
Soluble fibers	15.04	1.54	10.24	14.01	16.08
Insoluble fibers	28.67	5.69	9.85	24.85	32.49

Source: Sato et al. (2010).

Fiber fraction

In Table 2, the results of total dietary fiber are shown, both soluble and insoluble. Total dietary fiber (TDF) presented a total average of $43.71\text{ g }100\text{ g}^{-1}$ with $15.02\text{ g }100\text{ g}^{-1}$ (34%) from soluble fiber or pectic substances and $28.69\text{ g }100\text{ g}^{-1}$ (66%) from insoluble fiber, mainly cellulose and hemicellulose. The results reported by Sudha et al. (2007) showed 14.60% soluble fiber and 36.50% insoluble fiber, similar if the standard deviations were considered. On the other hand, the values were lower than the contents of TDF reported by Figuerola et al. (2005) from apple fiber concentrate of Royal Gala, Granny Smith and Liberty varieties. These results were 78.2, 60.7 and 89.8%, respectively.

The yield of pectic substances had a variation coefficient around 10%, which indicated potential varieties of interest to be used for pectin extraction [coded (1), (3) and (5)].

Table 2. Fraction of alimentary fibers.

Sample	Fiber ($\text{g }100\text{ g}^{-1}$)		
	Total	Soluble	Insoluble
(1)	44.50	17.65	26.85
(2)	43.77	12.26	31.51
(3)	45.95	16.09	29.86
(4)	48.48	13.26	35.22
(5)	46.05	17.11	28.94
(6)	40.89	14.48	26.41
(7)	46.52	15.07	31.45
(8)	33.4	14.79	18.61
(9)	51.85	14.90	36.95
(10)	34.19	14.66	19.53
(11)	45.24	15.22	30.02
Average	43.71	15.04	28.67
Std Dev.	5.61	1.54	5.69
Variation	12.84	10.23	19.85

Therefore, phenolic compounds are retained in the extracted pectin of apple pomace and can contribute to the brown color after oxidation (SCHIEBER et al., 2003).

Titrimetric evaluation of pectic substances

In Table 3, titrimetric features of pectin are shown.

Table 3. Physicochemical characteristics of isolated pectin.

Cultivar	AUA (%)	MEO (%)	Acidic fraction (%)	Neutral fraction (%)	Degree of esterification (%)
(1)	46.90 ± 4.90	5.88 ± 0.59	52.79 ± 5.48	47.21 ± 5.48	71.24 ± 0.67
(2)	51.21 ± 2.97	6.49 ± 0.21	57.70 ± 3.15	42.30 ± 3.15	72.05 ± 2.63
(3)	50.86 ± 1.88	6.34 ± 0.27	57.19 ± 1.71	42.81 ± 1.71	70.86 ± 5.36
(4)	51.57 ± 4.53	6.67 ± 0.54	58.25 ± 4.95	41.75 ± 4.95	73.59 ± 4.46
(5)	51.57 ± 4.06	6.70 ± 0.24	58.27 ± 4.29	41.73 ± 4.29	74.01 ± 3.57
(6)	51.31 ± 4.34	6.66 ± 0.43	57.97 ± 4.73	42.03 ± 4.73	73.82 ± 3.03
(7)	53.38 ± 3.89	6.93 ± 0.24	60.32 ± 4.11	39.68 ± 4.11	73.90 ± 3.22
(8)	50.25 ± 2.41	6.35 ± 0.24	56.60 ± 2.59	43.40 ± 2.59	71.82 ± 2.47
(9)	50.41 ± 1.45	6.30 ± 0.49	56.72 ± 1.69	43.28 ± 1.69	70.99 ± 5.23
(10)	48.68 ± 1.54	6.06 ± 0.52	54.74 ± 2.04	45.26 ± 2.04	70.59 ± 3.88
(11)	52.85 ± 3.92	6.72 ± 0.28	59.57 ± 4.15	40.43 ± 4.15	72.33 ± 3.58
Average	50.82	6.46	57.28	42.72	72.29

The quantification of total uronic acid indicates the amount of free carboxyl and methoxylated total (AUA, %). The group of eleven genotypes had an average value of 50%, with a minimum of 48% and a maximum of 53% and the average value of methoxyl (MEO) is 6.46%, varying from 5.88 to 6.93%. Both results (AUA+MEO) represent the acidic fraction of pectin (pectinic acid), with an average value of 57.28%.

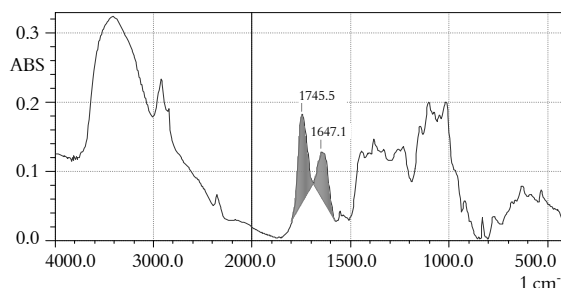
The neutral fraction, also named ballast (BERK, 1976), is composed of neutral sugars as arabinose and galactose attached to the rhamnose in the main chain as polymers (RG I) or directly to the GalA in the main chain (RG II) (WILLATS et al., 2006). The average value was 42.72%. The degree of esterification is also shown in Table 3, and the values found are similar, identifying the homogeneous group of high methoxyl pectin with an average DE of 72.29%. Constenla et al. (2002) extracted pectin from Granny Smith apple pomace, dried at 70°C. They found an average anhydrogalacturonic content of 58.8% and a degree of esterification of 73.9%.

FTIR analysis

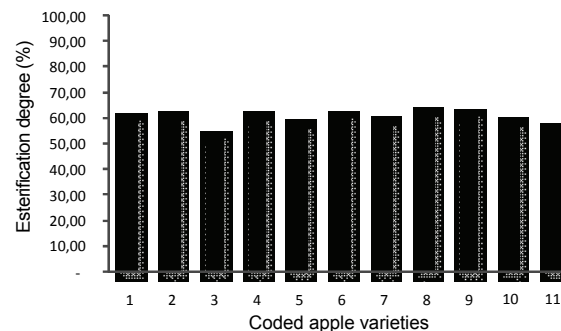
In Figure 1, a typical spectrogram obtained from the analyzed pectin samples is shown. It shows the fingerprint of high methoxyl pectin, with a degree of esterification higher than 50%. However, the values found by this methodology were lower than those obtained by classical titrimetry. Such an approach is effective to confirm the pectin functional identity from the peaks concerned with esterified carboxyl groups (1747 cm⁻¹) and related to free carboxyl groups (1639.4 cm⁻¹), which is consistent with the literature (MARCON et al., 2005; FERTONANI et al., 2006).

In this study, the calibration procedure was not used to fit the results obtained from the titrimetric method to those from FTIR. The determination of the esterification degree by instrumental methods underestimated the values of DE (Figure 2). The

calibration technique introduces the effect of stretching single C = O at 2900 cm⁻¹, and the results fit with a correlation R² > 0.98 (SCABIO et al., 2007). Another procedure to calculate DE by FTIR introduces the transmittance at 1820 cm⁻¹ into the equation, giving still lower values (LEGENTIL et al., 1995).

**Figure 1.** Spectrogram of a pectin sample showing the two wavelengths.

According to Gnanasambandam and Proctor (2000), a small difference in the structure and composition of a molecule can result in significant changes in the absorption peaks. Hence, samples from the same source/origin can be expected to have lower FTIR spectral variations as observed in this work.

**Figure 2.** Esterification degree as determined by IR spectroscopy.

Neutral sugars composition

The sugar composition of the neutral fraction from all samples is shown as the results of simple descriptive

statistical analysis. The results do not conflict with the literature, and even the high amounts of glucose observed are explained by Legentil et al. (1995) as being released from a glucan because all free sugars were removed during the alcohol extraction.

In Table 4, the proportional composition of the sugars from acid and neutral fractions is shown, including the methoxylated group. Such composition was calculated with respect to the amount of acidic and neutral fractions reported in Table 3, corresponding to AUA+MeO (pectinic acids) and the identified neutral sugar, respectively. Pectinic acids were quite homogeneous, with a variation lower than 4% and 5% for both components, also as seen in Table 3. However, all other components from the neutral fraction show a high dispersion, from 17% (glucose) to 72% (fucose). Rhamnose content was also very heterogeneous, but arabinose, xylose and galactose showed less dispersion among the samples, with a variation coefficient of approximately 24-27%. With these differences in neutral sugar composition, it is reasonable to accept differences in pectin structure as well.

Table 4. Proportional composition of the sugars from acid and neutral fractions.

Cultivar	Fraction (%)								
	AUA	MeO	Rham	Fuc	Ara	Xyl	Man	Gal	Glc
1	46.90	5.88	0.65	0.12	3.33	1.31	2.23	2.51	37.3
2	51.20	6.49	0.85	0.31	4.70	3.21	0.05	4.67	28.9
3	50.90	6.34	0.82	0.32	4.70	2.46	4.10	2.94	27.5
4	51.60	6.67	0.01	0.01	3.48	3.55	9.55	5.11	24.7
5	51.60	6.70	0.96	0.23	4.23	2.50	2.99	2.37	28.5
6	51.30	6.66	1.35	0.54	5.05	3.90	4.31	3.46	23.4
7	53.40	6.93	1.08	0.33	4.88	3.00	3.25	4.38	22.8
8	50.30	6.35	2.09	0.74	8.54	3.78	3.95	3.14	21.2
9	50.40	6.30	0.34	0.00	4.79	3.86	3.43	4.31	25.4
10	48.70	6.06	0.91	0.20	3.76	2.00	2.78	3.27	32.4
11	52.90	6.72	1.08	0.35	5.03	2.90	3.96	4.68	22.4
Average	50.80	6.46	0.92	0.29	4.77	2.95	3.69	3.71	26.8
Standard Deviation	1.72	0.30	0.51	0.21	1.33	0.79	2.17	0.91	4.60
Coefficient of Variation (%)	3.39	4.62	55.57	72.00	27.86	26.82	58.92	24.44	17.21

The composition of neutral sugars of apple pectin from Poland were 22 $\mu\text{g mg}^{-1}$ of Rha, 10 $\mu\text{g mg}^{-1}$ of Ara, 14 $\mu\text{g mg}^{-1}$ of Xyl, 26 $\mu\text{g mg}^{-1}$ of Gal and 36 $\mu\text{g mg}^{-1}$ of glucose in a total sugars of 10.8% (ZALESKA et al., 2000). Apple pomace from France presented similar composition of neutral sugar to some sugars but was significantly different in the contents of Ara (higher at 7.4%) and Man (lower at 1.5%) (RENARD et al., 1996). In Table 5, the ratios related to HG (GalA/Rham), to RGI ((Gal+Ara)/(Rham)) and to RGII ((Gal+Ara)/(GalA)) are shown. The mean value found for the ratio of uronic acid:rhamnose was 67.81 ± 35.71 . Even with a high variation coefficient of 52.66%, these values are in agreement with the literature, where different authors reported values between 25 and 200 (NOVOSEL'SKAYA et al., 2000). Legentil et al. (1995) found the same value, although they expressed

it as rhamnose:uronic acid ($1/67.81 = 0.014$). Values represent the total amounts found in each sample of pectin and are supposed to be dispersed among three types of structures of the macromolecule. Indeed, as stated previously, in apple pectin, 22% of the rhamnose is in the linear form, and 78% is in the ramified fraction (RENARD et al., 1995). Therefore, the figures of galacturonan could increase to an average of 308 units of galacturonic residue to 1 unit of rhamnose. Also, the hairy structure would rise from 9.96 to 12.77 units of neutral sugar to 1 unit of rhamnose.

Table 5. Usual neutral sugars in pectin and their relationships.

Cultivar	GalA:	Gal+Ara:	Gal+Ara:
	Rham	Rham	GalA
1	81.20	8.93	0.12
2	67.87	11.06	0.18
3	69.80	9.32	0.15
4	58.27	Nd	0.17
5	60.73	6.88	0.13
6	42.93	6.31	0.17
7	55.86	8.57	0.17
8	27.11	5.59	0.23
9	166.76	26.47	0.18
10	60.18	7.77	0.14
11	55.20	8.96	0.18
Average	67.81 ± 35.71	9.96 ± 5.70	0.17 ± 0.03

Molecular mass dispersion

The steric exclusion chromatography coupled to the interferometric refractometry detector and to the multi-angle light laser scattering detector was effective for characterizing the homogeneity and molecular mass dispersion of this batch of pectin. The first sensor gives a response according to the concentration of macromolecules and the second to the size and concentration, providing information about the molecular weight and distribution. In Figure 3, a chromatogram of the pectin isolated from one apple pomace (coded 1) is shown, whose profile suggests the presence of four groups of polysaccharides. In the first set, minor components with the highest molecular weight are detected in a small volume at 38 min of elution by a MALLS sensor with high light scattering. The next set is the largest predominant and shows an elution peak volume at 43 min, followed by another set of low molecular weight in low concentration and ultimately, one final set of large oligosaccharides.

The results obtained by Scabio et al. (2007) were similar, showing the same retention times and polysaccharide profiles. Similar results were shown by Hokputsa et al. (2004) in their report regarding molecular dispersion of hydrosoluble acid polysaccharides from *Felinus gilvus*. Indeed, the mixture of these polysaccharides allows the gel formation.

Figure 4 shows all polysaccharide fractions of all apple pomace pectins identified by an RI detector. All

samples have the high molecular weight fractions and the next low molecular weight fraction, but only some have the third polysaccharide fraction with still lower molecular weight.

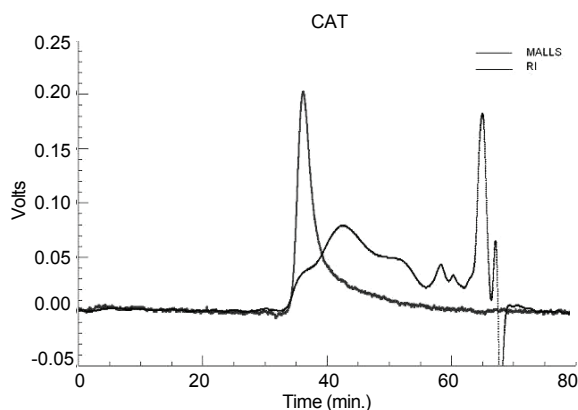


Figure 3. Molecular mass profile provided by multi-angle light laser scattering and by interferometric refractometry index detector in the high performance steric extrusion chromatography of a sample of pectin extracted from apple pomace of cv. Catarina.

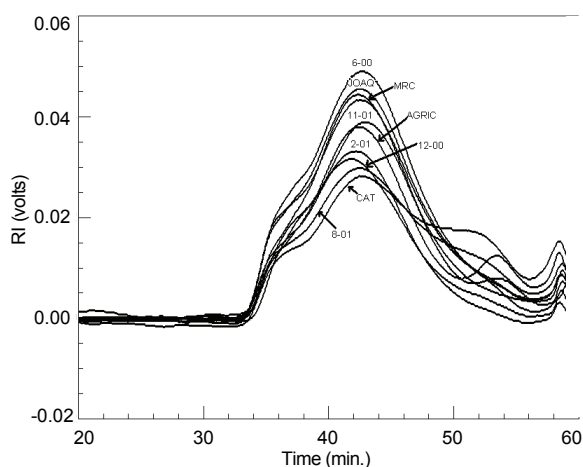


Figure 4. Molar mass distribution profiles of the eleven samples of pectin detected by refractometry index.

Conclusion

Acid-soluble polysaccharide extracted with an average yield of $15.04\% \pm 1.53$ (variation = 10%) reflects the small difference of hydrocolloids in the raw material, and the average esterification degree of 72.29% points to a good processing. The mean value of the pectic fraction was 57.28%, and the neutral sugar from the hairy region (ballast) was 42.72%. The high glucose content suggests simultaneous extraction of cellulose or other glucan. The titrimetric, spectrometric and chromatographic characterizations indirectly provide structural information about the homogalacturonan and hairy regions. According to HPSEC, there is a group of very high molecular weight detected by the MALLS sensor at 38 min. of elution and a major set of lower molecular weight

detected by the RI sensor at 43 min. The 3rd polysaccharide group was not observed in all samples, nor were the peaks corresponding to large oligosaccharides of around 58-60 min. of elution. The analysis by FTIR confirms the identity of pectic substances by the similarity with the pectin fingerprint, showing peaks at 1747 and 1639 cm^{-1} related to methoxylated and free carboxyl groups, respectively.

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