International Journal of Biological Macromolecules 52 (2013) 148–156

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Characterization of sugar beet pectic-derived oligosaccharides obtained by enzymatic hydrolysis

Agnan Marie Michel Combo[a,*], Mario Aguedo[a], Nicolas Quiévy[b], Sabine Danthine[c], Dorothée Goffin[a], Nicolas Jacquet[a,c], Christophe Blecker[c], Jacques Devaux[b], Michel Paquot[a]

[a] University of Liège, Gembloux Agro-Bio Tech, Department of Industrial Biological Chemistry, Passage des déportés 2, B-5030 Gembloux, Belgium
[b] Université catholique de Louvain, Institute of Condensed Matter and Nanosciences (IMCN), Research Division: Bio- and Soft Matter (BSMA), Croix du Sud 1, B-1348 Louvain-la-Neuve, Belgium
[c] University of Liège, Gembloux Agro-Bio Tech, Department of Bioresources Valorisation, Passage des déportés 2, 5030 Gembloux, Belgium

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 1 September 2012
Accepted 9 September 2012
Available online 14 September 2012

Keywords:
Pectic oligosaccharides
Sugar beet
HPAEC-PAD
Thermal analysis
X-ray
FTIR

ABSTRACT

Three pectic oligosaccharides (POS) obtained by enzymatic hydrolysis of sugar beet pectin by combining endopolygalacturonase and pectinmethylesterase, were characterized using high performance liquid chromatography, thermogravimetric analysis, Fourier transform infrared spectroscopy, differential scanning calorimetry and X-ray diffraction. According to chromatographic analyses, POS are composed of mixture of polymers with different molecular weights and different galacturonic acid contents. The thermal analysis showed no major variation in thermal behavior regarding POS composition but showed that POS were more sensitive to thermal degradation than the parent pectin as well as the deesterified pectin. No change in composition of the gaseous products was obtained through TGA-FTIR analysis. The X-ray pattern of POS clearly indicated a considerable decrease in crystallinity when compared to the native pectin. Thus, thermal characterization of POS may have practical repercussions if the formulation in which POS is incorporated is submitted to a high temperature treatment.

1. Introduction

Pectins are natural carbohydrates defined as a mixture of heteropolysaccharides mainly found in higher plant middle lamellae and primary cell walls [1]. They may be constituted by different associated structural elements, such as homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan type I (RGI), rhamnogalacturonan type II (RGII), arabinan and arabinogalactan [2]. The dominant feature of pectin is a linear chain of (1–4) linked α-D-galacturonic acid residues that are often methyl-esterified at O-6 and sometimes acetyl-esterified at O-2 or O-3 [3]. Pectins are characterized by their high water absorption and gelation properties which allow their use in the food industry, medicine, pharmacy and cosmetics [4–6]. Pectins are also classified as dietary fiber and are reported to exert a beneficial effect on the gastrointestinal tract of the host [7]. Thus the oligosaccharides derived from pectins may have similar or additional functionalities.

In recent years, oligosaccharides have found applications in various fields, notably because of their specific biological activities. The potential of plant cell wall polysaccharides as sources of novel high value-added bio-products has received special attention. Oligosaccharides derived from pectin have been found to exhibit various biological activities, such as immuno-modulation [8], anti-ulcer and anti-cancer [9]. Moreover, the prebiotic potential of pectic oligosaccharides (POS) has been reported, since they selectively increase the populations of beneficial bacteria in human gastrointestinal tract such as bifidobacteria and *Eubacterium rectale* [10,11]. Moreover, the composition and the structure of POS depend on the plant source and the production process. POS can be obtained by chemical, physical or enzymatic processes, leading to products with a defined range of degree of polymerization (DP) and specific properties [12]. As the use of POS is in constant increase in food, medicine, pharmacy and cosmetics industry, these oligosaccharides need to be characterized.

It is well known that molecular structure modifications have a considerable impact on the thermal behavior of pectins as mentioned by Einhorn-Stoll et al. [13]. To date, there is little or no information available on the physical characterization of POS, whereas some studies have been done on oligosaccharides such as inulin and galactomannan and guar gum [14–16]. The molecular

* Corresponding author. Tel.: +32 0 81 62 22 32; fax: +32 0 81 62 22 31.
E-mail addresses: ammcombo@student.ulg.ac.be, comboagnan@yahoo.fr (A.M.M. Combo).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.09.006

structure of these products was notably related to thermal characterizations through thermogravimetric and calorimetric analyses.

In the present study, the thermal characterization of three different POS preparations produced by enzymatic depolymerization of sugar beet pectin was compared with that of sugar beet pectin and deesterified sugar beet pectin. Thermogravimetric analysis coupled to Fourrier transform infrared spectrometry (TGA-FTIR), differential scanning calorimetry (DSC) and X-ray diffraction analysis (XRD) were used to investigate the influence of pectin's mean DP on the thermal profile; in particular to evaluate the effects of POS composition on the thermal properties.

2. Materials and methods

2.1. Materials

All chemicals used were the purest available. Rhamnose (Rha) monohydrate, galactose (Gal), glucose (Glu), Arabinose (Ara), xylose (Xyl), mannose (Man), D-galacturonic acid monohydrate (GalA), digalacturonic acid (DiGalA) and trigalacturonic acid (TriGalA) were from Sigma–Aldrich Chemical Co. (St. Louis, Mo, USA). Two commercial food grade pectinases Endopolygalacturonase-M2 (EPG-M2) from Megazyme International Ireland Ltd and Rapidase Smart® (pectinmethylesterase) from DSM were used to digest sugar beet pectin (SPB).

2.2. Pectin extraction

Dried SBP was provided by Warcoing Sugar Industry (Warcoing, Belgium). A solid–liquid ratio of 1:29 (w/v) was suspended in an aqueous solution adjusted to pH 1.5 with concentrated HCl, heated to 80 °C and stirred at 250 rpm for 1 h. The macerate was rapidly cooled to room temperature in an ice bath and then filtered through two stacked-up layers of nylon cloth (100 and 20 μm). The initial pH of clarified crude extract was measured before adjusting to pH 3.5 with 0.2 M KOH. The extract was dispersed into four volumes of 96% ethanol for 1 h at room temperature. Pectin gel was washed twice with 70% ethanol, hand-squeezed in nylon cloth to eliminate ethanol remnant, dried at room temperature and finely ground in an IKA-A10 mill (IKA GmbH Labortechnik, Staufen, Germany). Homogenous pectin powders were stored at room temperature in a desiccator before used.

2.3. Demethoxylation

Pectin samples were also chemically treated for demethoxylation. Powders were dissolved in water at ambient temperature and pH was brought to 11.0 with 1 M sodium hydroxide under stirring. The solution was maintained at constant pH using an automatic pH-stat (718 STAT titrino, Metrohm) with 1 M of a sodium hydroxide as the titrant. After 7 h, the pH was acidified to pH 3–4 by 1 M chlorhydric acid and the pectin was precipitated by three volumes of 96% ethanol. The precipitate was separated and air-dried.

2.4. Characterization of sugar beet pectin and alkali modified sugar beet pectin

All analyses were performed in duplicate. Moisture content of the pectin was determined as the weight loss after oven drying at 105 °C for 24 h. GalA content was determined by a high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The chromatographic system was a Dionex ICS-3000 model (Sunnyvale, CA, USA) equipped with an ED-3000 electrochemical detector, and a SP gradient pump. The column was a Dionex CarboPac PA-100 (250 mm × 4 mm i.d.) coupled to a CarboPac guard column (40 mm × 4 mm i.d.). The mobile phase consisted of 100 mM sodium hydroxide (eluent A), 600 mM sodium acetate in 100 mM sodium hydroxide (eluent B) and 500 mM sodium hydroxide (eluent C). The flow-rate was 1 ml/min and the injection volume was 25 μl.

Neutral sugars were analyzed by gas chromatography after hydrolysis with 1 M H_2SO_4 at 100 °C for 3 h and conversion to alditol acetates [17]. Alditol acetate derivatives were separated and quantified by the chromatograph (HP 6890 GC with a flame ionization detector). The column was a HP1-methylsiloxane (30 m × 0.32 mm, 0.25 μm film thickness, Scientific Glass Engineering, Melbourne, Australia). 2-deoxy-D-glucose (purity > 99.5%, Sigma Chemical Co., St Louis MO) was used as internal standard.

Methoxy and acetyl groups were released from pectin by saponification with 0.2 M NaOH in 50% isopropanol for 2 h at 4 °C and quantified by high performance liquid chromatography (HPLC) on an Aminex HPX-87 column (300 mm × 7.8 mm, Biorad, Hercules, CA) [18]. Elution was carried out with 5 mM H_2SO_4 solution at a flow rate of 0.6 mL/min. Succinic acid was used as internal standard. Degree of methylation (DM) and degree of acetylation (DA) were expressed as the percent molar ratio of methanol (MeOH) or acetic acid (HAc) to the GalA content (quantified by HPAEC-PAD).

Phenolic compounds were analyzed by HPLC after saponification and extraction. Pectin samples (500 mg) were saponified in 30 ml of 2 M NaOH under nitrogen in the dark for 2 h at 30 °C, after which 1 ml of cinnamic acid solution (5 g/l in 0.2 M NaOH) was added as an internal standard and they were neutralized to pH 2 with 3 M HCl. Phenolic compounds were extracted into diethyl ether (~30 ml) after centrifugation (5000 rpm for 5 min) and recovery of the organic phase. This was done twice. Organic phase was combined and evaporated in a rotavap at 40 °C. Residue was dissolved into 10 ml of methanol 50%. 25 μl of this material was injected into a 2690 HPLC system (Waters Inc., Milford, MA, USA) equipped with a C18 reversed phase column (250 mm × 25 mm, Vydac, Hesperia, CA, USA) and coupled on line with a waters 996 photodiode array detector (DAD). Elution was carried out with 75% solvent A (Milli-Q water + 0.05% trifluoroacetic acid (TFA)) and 25% solvent B (acetonitrile + 0.05% TFA) at constant temperature of 35 °C and at a flow rate of 1 ml/min. Phenolic compounds were detected at 320 nm. Ferulic acid (purity > 99%, Sigma–Aldrich Chemie GmbH., Steinheim, Germany) was used as an external standard.

2.5. Preparation of pectin derived oligosaccharides

Three depolymerised pectin samples were prepared enzymatically by treating the SBP with EPG-M2 and Rapidase Smart®. Pectin was diluted to a final concentration of 0.5% (w/v) and the pH was adjusted to 5.0 in order to be in the optimal working range of the enzymes. The solution was placed in a water bath set at 50 °C and allowed to equilibrate. A volume of 20 μl of each enzyme was added simultaneously, corresponding to 9.55 U/ml of EPG-M2 and 0.52 U/ml of Rapidase Smart. This mixture was hydrolyzed at determined time points (2, 5 and 15 min). At the end of hydrolysis, the samples were heated at 100 °C for 15 min to inactivate the enzymes. After cooling, samples were filtered through a 0.45 μm membrane filter and aliquots were taken for HPLC analyses. The remaining liquid fraction was concentrated using a rotavap and freeze dried. Samples were preserved at room temperature in a desiccator before any analysis.

2.6. High-performance size exclusion chromatography of pectin and POS

The molecular weight distribution of the samples was analyzed by HPSEC on a Waters 2690-HPLC system (Waters Inc., Milford, MA) equipped with TSKgel GPWXL column (300 mm × 7.8 mm, TosoHaas Co. Ltd., Tokyo, Japan) and coupled on-line with three

detectors: a Waters 2410 differential Refractometer that measures the refractive index (RI), a right angle laser light scattering (RALLS) and a differential viscometer detector (Model T-50A, Viscotek, Houston, TX). Elution was performed with 50 mM sodium nitrate solution containing 0.05% sodium azide at a flow rate of 0.7 ml/min at 10 °C and monitored with a refractive index detector. The system was calibrated using dextran standards from Fluka and galacturonic acid.

2.7. Depolymerization profile of POS

The three samples of depolymerized pectin obtained after enzymatic treatment were characterized by HPAEC-PAD. The chromatographic system was a Dionex ICS-3000 model (Sunnyvale, CA, USA) equipped with an ED-3000 electrochemical detector, and a SP gradient pump. The column was a Dionex CarboPac PA-100 (250 mm × 4 mm i.d.) coupled to a CarboPac guard column (40 mm × 4 mm i.d.). The mobile phase consisted of 100 mM sodium hydroxide (eluent A), 600 mM sodium acetate in 100 mM sodium hydroxide (eluent B) and 500 mM sodium hydroxide (eluent C). The flow-rate was 1 ml/min and the injection volume was 25 μl. GalA, DiGalA, TriGalA and oligomers above DP3 obtained by autohydrolysis of polygalacturonic acid according to the method of Bonnin et al. [19] were used as external standards. Data were collected and analyzed with Dionex Chromeleon 6.80 SP3 Build 2345 software.

2.8. Thermal analysis

The couple thermogravimetric/infrared spectrometry analysis was performed using TGA/SDTA 851e (Mettler Toledo) thermogravimetric analyzer that was interfaced to the Nicolet Nexus 870 FTIR (ThermoElectron Corporation) spectrometer with an analysis cell for the volatile products. The experiments were performed under a nitrogen flow of 50 ml/min. Sample weights of approximately 6 mg were heated from 25 to 600 °C at a heating rate of 5 °C/min.

The powders were also analyzed by using a differential scanning calorimeter (DSC) (Mettler Toledo DSC 821e) with 40 μl Al crucibles. The top of the crucibles was pierced twice to let the volatiles escape. Samples were stabilized during 5 min at 25 °C and then heated from 25 to 400 °C at a heating rate of 5 °C/min. Analyses were performed in duplicate.

2.9. X-ray diffraction

Samples were analyzed by XRD. Measurements were carried out using a D8 Advance diffractometer (40 kV, 30 mA) (Bruker, Germany) (λ Cu = 1.54178 Å) equipped with a Lynxeye detector (Bruker, Germany). Analyses were performed isothermally at room temperature from 6 to 33° 2θ (step size 0.02° 2θ, time per step: 5 s). All the X-ray experiments were done in triplicate.

3. Results and discussion

3.1. Chemical characterization of pectin samples

The chemical composition of SBP and deesterified SBP is shown in Table 1. Galactose was found to be the most abundant neutral sugar, followed by arabinose and rhamnose. Other neutral sugars, such as xylose, mannose and glucose were present, but in low amounts. Galacturonic acid amounts (59.5%) were close to data already published [20].

Previous studies reported that DM and DA of pectins from SBP were generally around 50–60 and 30% respectively [21,22]. In this work, DM and DA of pectin were generally within this range even if a low DM value was obtained. Ferulic acid (FA) content of pectins (0.43%) was consistent with those reported by Rombouts and Thibault. [21].

Table 1
Physico-chemical composition (%w/w) of the pectin samples. SBP: sugar beet pectin; Al SBP: alkali modified sugar beet pectin; DM: degree of methylation; DA: degree of acetylation; FA: ferulic acid; Mw: molecular weight.

Compositions	SBP	Al SBP
Rha	4.01 ± 0.03	2.1 ± 0.06
Ara	4.5 ± 0.06	3.7 ± 0.31
Xyl	0.21 ± 0.00	0.21 ± 0.05
Man	0.08 ± 0.02	0.07 ± 0.04
Gal	10.13 ± 0.07	6.02 ± 0.65
Glu	0.53 ± 0.02	0.2 ± 0.00
Total neutral sugars	19.5	12.3
GalA	59.5 ± 0.50	54.2 ± 0.70
DM	44 ± 2.60	1.42 ± 0.37
DA	35.8 ± 1.70	8.8 ± 0.03
FA	0.43 ± 0.01	0.4 ± 0.00
Mw (kDa)	263.0	222.4

Alkaline treatment of pectin showed a decrease in the methyl and acetyl content and the length of the main chain of galacturonic acid which occurs by β-elimination, but a low removal of arabinosyl, rhamnosyl and galactosyl residues was observed (Table 1).

3.2. Molecular size distribution

Molecular distribution of SBP and POS was analyzed by HPSEC. This technique allowed thus to follow the hydrolysis of pectin. The molecular weight (Mw) distributions before and after enzymatic treatment are shown in Fig. 1. The Mw of SBP decreased with time, while at the same time, the Mw distribution broadened. A broad distribution was indicative of the molecular heterogeneity of the product obtained by the action of the enzymes. As shown in Fig. 1, the elution profiles of the polymers can be subdivided into three fractions after 2 min and an additional fraction for further hydrolysis times (5 and 15 min). On the other hand, enzymatic degradation of SBP with the combination of the EPG-M2 and Rapidase Smart® resulted in low release of galacturonic acid. Molecular fraction distribution presented in Table 2, shows that Mws are affected by the hydrolysis time. Mw of each fraction decreased with hydrolysis time. Production of POS with low DP was possible, as observed notably for the fraction F3 and F4 of SBP at 2 min and SBP at 5–15 min respectively.

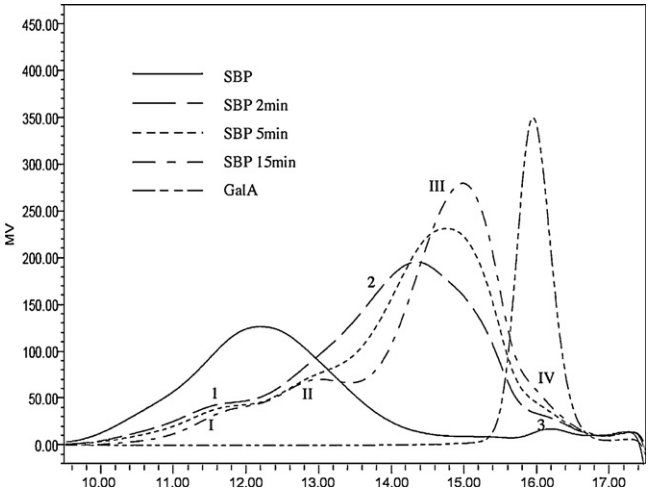

Fig. 1. HPSEC elution profiles of POS obtained during enzymatic hydrolysis of SBP.

Table 2
Evolution of molecular weight at peak in kDa (Mw_p), average degree of polymerization (at peak) (DP_p) and proportions (%w/w) (P) of each molecular fraction during hydrolysis.

	SBP 2 min			SBP 5 min			SBP 15 min		
	Mw_p	DP_p	P	Mw_p	DP_p	P	Mw_p	DP_p	P
F1	422	2398	6.1	408	2318	4.6	404	2293	3.4
F2	14	80	76.3	98.1	558	8.6	84.3	479	14.9
F3	1.8	10	2.3	8.1	46	71.1	6	34	65.8
F4				1.9	11	3.1	1.6	9	3.2

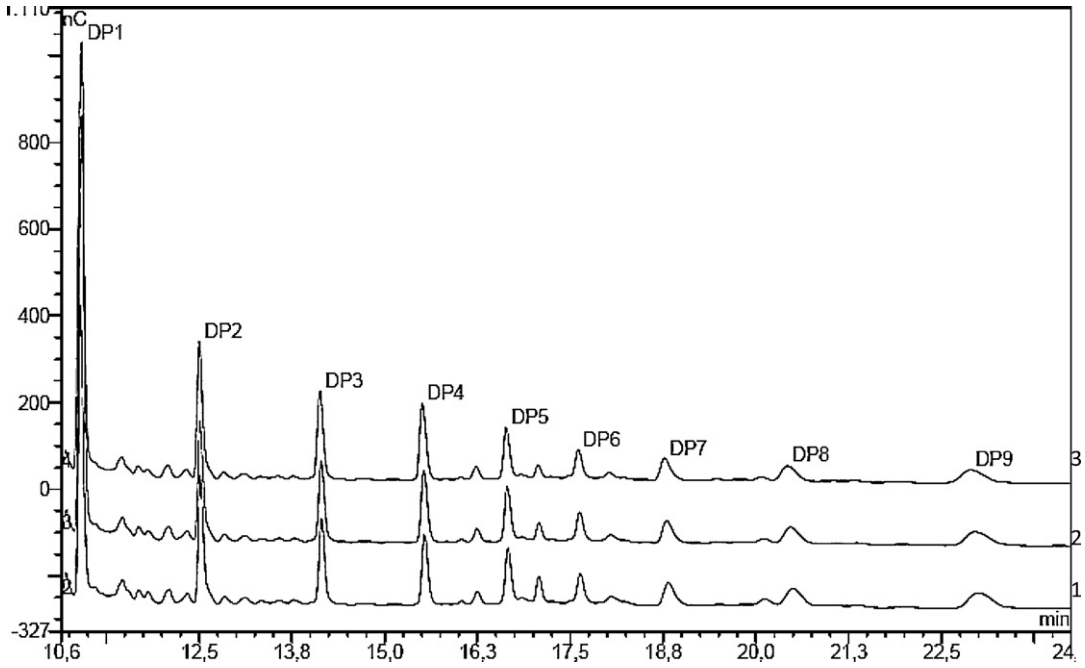

Fig. 2. HPAEC-PAD profiles of oligogalacturonides released by the action of commercial EPG-M2 and Rapidase Smart® from sugar beet pectin for different incubation times: (1) 2 min; (2) 5 min; (3) 15 min.

Table 3
Degrees of polymerizations DP1 to DP9 area in the fractions during hydrolysis of SBP.

	Time (min)	DP1	DP2	DP3	DP4	DP5	DP6	DP7	DP8	DP9
SBP	2	50.25	25.93	18.00	15.37	11.39	6.65	8.91	8.78	12.20
	5	75.10	24.29	17.24	15.93	11.42	6.61	8.53	8.11	11.55
	15	87.62	27.96	19.24	17.43	10.74	7.71	8.22	7.69	10.82

3.3. Anion-exchange chromatographic profiles of POS

To investigate POS formation, hydrolysates were analyzed by HPAEC-PAD. Elution was performed at alkali pH in order to selectively separate galacturonic acid and oligomers. The oligomers above DP3 were identified by comparison of their retention times with a preparation of oligoGalA (DP 4–9) obtained by polygalacturonic acid autohydrolysis. Elution profiles of POS are presented in Fig. 2. After only 2 min of digestion, commercial pectinases produced a mixture of oligoGalA from SBP, showing the feasibility of the enzymatically catalyzed production of POS. The pattern of oligoGalA produced at the beginning of the degradation of SBP with the enzymes indicated that these enzymes act by a mechanism of random cleavage of SBP. Indeed, as it can be seen in Table 3, during extended hydrolysis, a change in the peak area of oligoGalA was observed; higher DPs were hydrolyzed into smaller DPs. Due to the unavailability of commercial standards higher than DP3, only DP1 to DP3 were quantified here. The concentrations were calculated from the HPAEC chromatograms and reported as a percentage of the total pectin mass. Quantification of compounds up to DP3 released during the hydrolysis is summarized in Table 4. The composition changed slightly between 2 and 15 min. The ratio DP3/DP1 decreased, evidencing a partial hydrolysis of DP3 into DP1.

3.4. Thermogravimetric and infrared analysis

TGA and FTIR analyses were performed in order to understand the thermal behavior of POS and how this behavior can be influenced by the polysaccharide structure. The TGA and the first derivative TGA (DTGA) curves are shown in Fig. 3a and b respectively. Based on this analysis, it is possible to observe and compare the thermal behavior of the native SBP sample with the

Table 4
Percentages (%, w/w) of galacturonic acid oligomers released during hydrolysis.

Hydrolysis time (min)	GalA	DiGalA	TriGalA
2	1.8	2.6	3.2
5	2.5	2.8	3.5
15	2.7	3.1	3.7

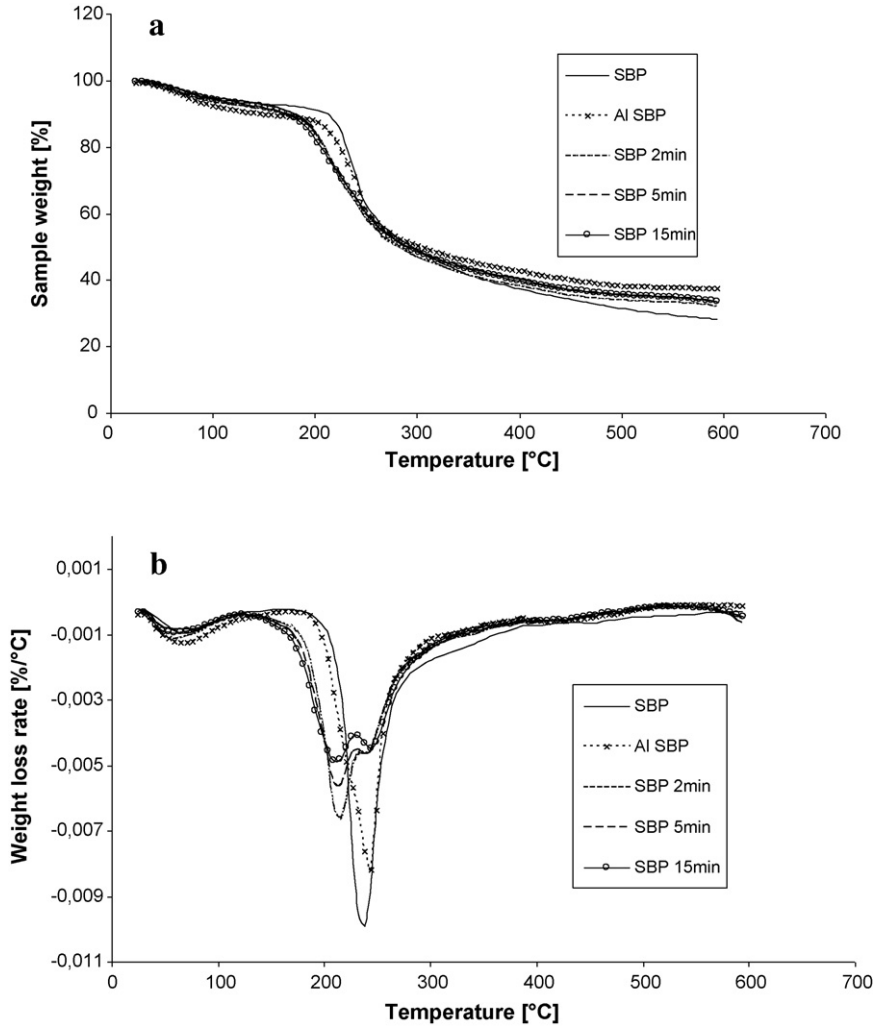

Fig. 3. TGA (a) and DTGA (b) curves of pectins and POS samples obtained at a heating rate of 5 °C/min under nitrogen atmosphere.

enzymatically produced POS. The TGA curves show three regions at 25–150, 150–350 and 350–600 °C. Such a division was also reported in other works [23–25]. The first region (25–150 °C) is attributed to the water loss during the temperature rise. It allows the determination of the water content of the samples by a weight loss calculation from the TGA signal. It was found that water content was between 7 and 10% in all five samples. Such water contents were observed in previous studies [25], when native and modified polysaccharides were analyzed. However, this loss of weight was greater for modified pectin samples than for native pectin. This suggests that, as demethylation favors the unmasking of the carboxylic functions, a higher amount of hydroxyl groups may interact with the water molecules. The second region, between 150 and 350 °C corresponds to a rapid mass loss (43–47%) due to the polysaccharide decomposition, when the galacturonic acid chains start to undergo extensive thermal degradation, with the evolution of various gaseous products and the formation of solid char [24,25]. The third region (350–600 °C) shows the slow mass loss after volatilization of water and other compounds, i.e. the thermal decomposition of char [25]. In the DTGA curve, besides the peaks at about 100 °C resulting from free water release, POS samples exhibit two distinct mass losses. The three POS showed similar profile patterns, being only different in the intensity. The first DTGA peak temperature appears at 215 °C and the second at 244 °C which shows the double-stage decomposition for these samples. For SBP and desesterified SBP, only one peak appears, at 238 and 244 °C respectively (Fig. 3b). POS thermal degradation thus started before that of SBP samples which may indicate that they have a lower thermostability. It was also observed that POS displayed larger peaks compared to pectin samples. This may be correlated to the heterogeneity of POS which are constituted of oligosaccharides with different range of thermal stability according to Einhorn-Stoll and Kunzek [26]. The lower DM may also explain a lower thermostability; indeed demethylation leads to the unmasking of carboxylic functions in pectin which allow the formation of inter- and intra-molecular hydrogen bonds, favoring the thermal degradation of modified pectins [13,26]. Moreover, TGA-FTIR was used to evaluate the degradation products, which provides a representation of the actual combustion products during TGA weight loss step. Fig. 4 shows the 3D FTIR spectra (absorbance–wavenumber–minutes) of the gaseous products during the samples pyrolysis by TGA-FTIR. Several absorption regions were observed, with the strongest absorption band appearing at the carbon dioxide region (2419–2240 cm^{-1}). The other detected species were water (3990–3400 cm^{-1}), hydrocarbons (3100–2600 cm^{-1}), carbon monoxide (2240–2040 cm^{-1}), carboxyl and carbonyl compounds C=O (1999–1600 cm^{-1}) and C—O bonds (1120–920 cm^{-1}) from alcohols, ethers and phenols. These products were also reported on pyrolysis of citrus pectin [25]. The pyrolysis product profiles for samples are showed in Fig. 5. Generally, all samples showed the same trends, excepted for CO in the case of SBP. As seen in Fig. 5, at approximately 20 min (or 100 °C) only few H_2O and carbonyl were emitted. These first emissions at

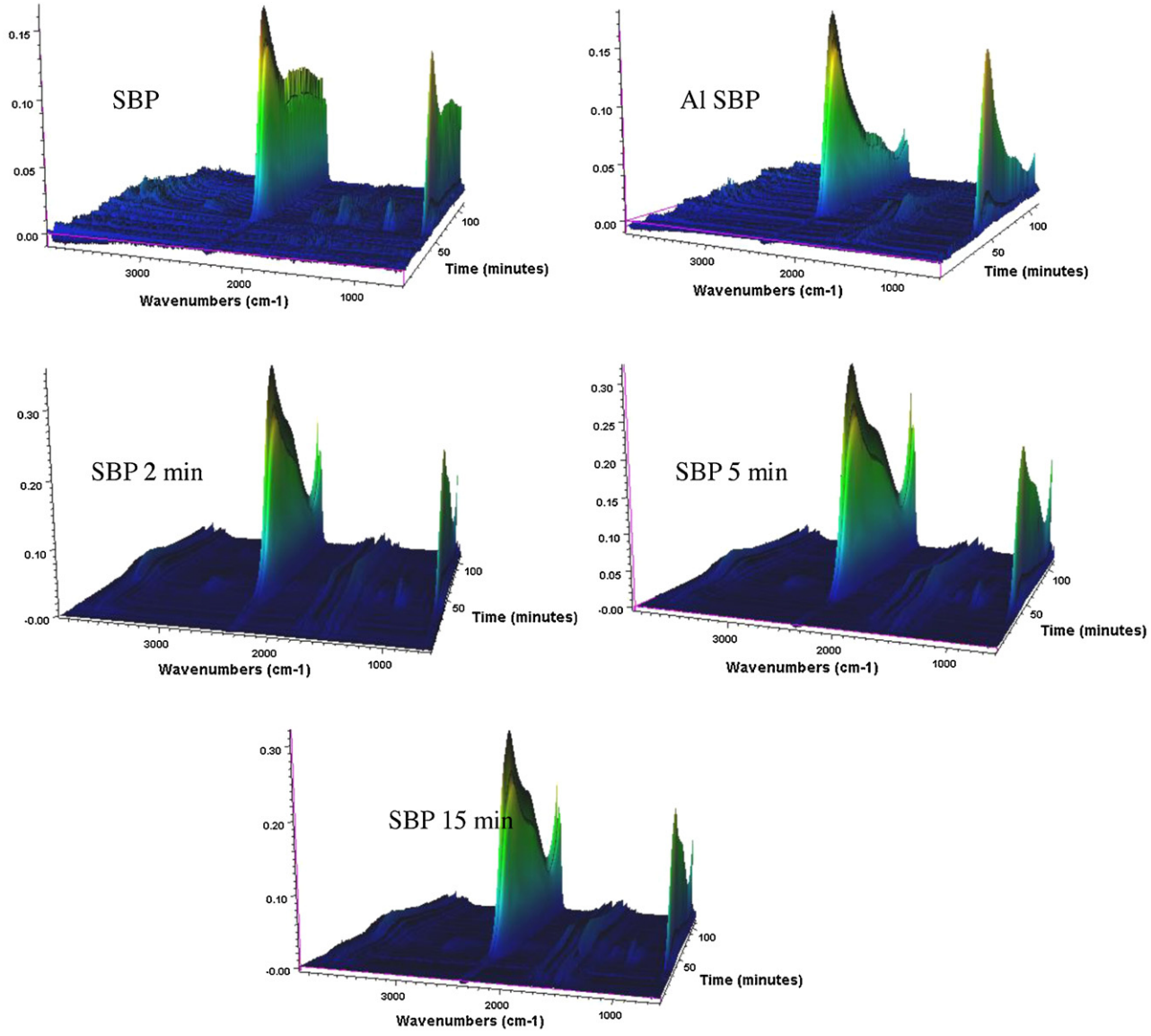

Fig. 4. 3D graph for the FTIR spectra of the evolved gases produced by the pyrolysis of pectin and POS samples.

low temperature were due to the release of the absorbed water by evaporation and the emission of carbonyl results from thermal degradation of galacturonic rings [27]. Beyond 20 min, all the main gases mentioned above are emitted, CO_2 being the most abundant. Hajaligol et al. [28] reported that in the range of 200–300 °C the emission of CO_2 is due to the cleavage of carboxyl in the lateral chains, and after 300 °C CO_2 is generated by the cracking of carboxylic group in the cyclic structure of pectin. The second emission of water is due to the cleavage of hydroxyl groups in the lateral chains [29]. Moreover, the second emission of carbonyl is caused by the extensive thermal degradation of galacturonic rings and the secondary pyrolysis of primary compounds [27]. The CO emission is due to the cleavage of C–O bonds in the ether bridges joining the SBP sub-units [30]. The methanol emission is almost nonexistent in Al SBP due to the demethylation, since the methoxyl groups in the pectin structure are the most probable contributors to the formation of methanol [31]. The hydrocarbon formation is caused by the thermal decomposition of methanol and by the cleavage of methyl groups in the lateral chains of pectin. However, the release of hydrocarbon is observed here after 70 min (350 °C) without any methanol emission. This indicates that these emissions are only due to the methyl groups in the lateral chains of pectin. As can be seen for these two latter gases, POS reach their maximum emission at 45 min (225 °C) while maximum of SBP samples is reached at 50 min (250 °C). However, it must be highlighted that all these gas emissions come from the second stage of decomposition (150–350 °C) which corresponds to a drastic drop in weight of samples. The pyrolysis products of the different samples mainly vary in amounts but not in species and the possible pathways of thermal degradation are decarboxylation, deesterification, dehydration, depolymerization and dehydroxylation [26]. These results confirm that there are no major functional group transformations during enzymatic hydrolysis of SBP.

3.5. Calorimetric profiles of samples

DSC was used for studying thermal transitions occurring in the course of heating under an inert atmosphere. Typical DSC thermograms (Fig. 6) showed a broad endothermic peak for all samples clearly observed between 25 and 150 °C. The transitions associated with loss of water correspond to the hydrophilic nature of functional groups of the samples. Also, no glass transition

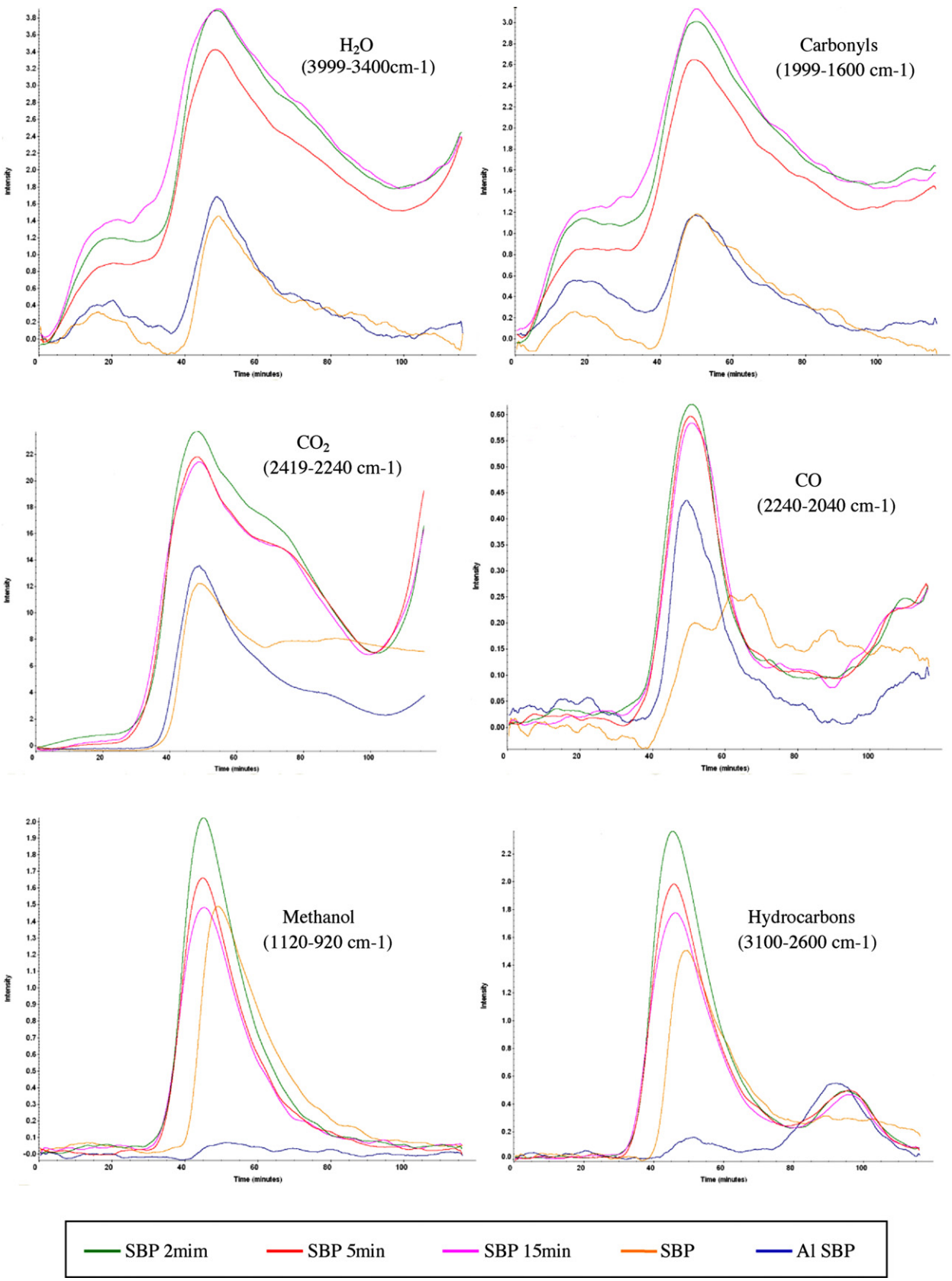

Fig. 5. Evolution of different gaseous products during the pyrolysis of pectin and POS samples.

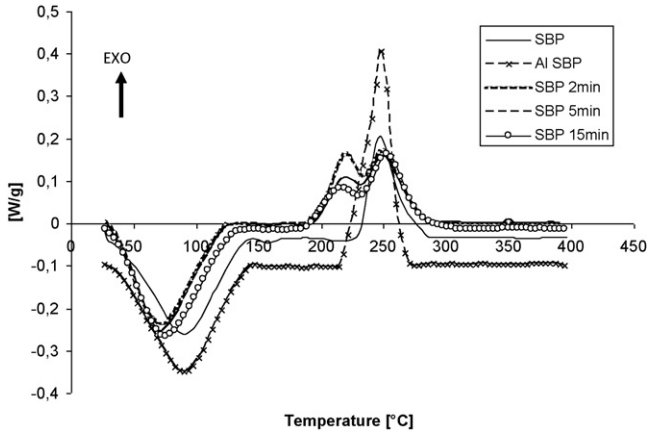

Fig. 6. DSC thermograms of pectins and POS samples obtained at a heating rate of 5 °C/min under nitrogen atmosphere.

temperature (T_g) was observed in the scanned range. The reason may be attributed to interference of T_g with the moisture endothermic peak, or structural reorganization during the rise in temperature. Another hypothesis is that the complexity of the system might have masked the T_g. Above 150 °C, exothermic reactions occur, corresponding to the degradation and the elimination of volatile products of the polymer [13]. POS exhibited two exothermic peaks centered at 220 and 250 °C which are also seen in DTGA curve (Fig. 3b), whereas pectin samples show only one exothermic peak (centered at 248 °C), which is more pronounced in the case of deesterified SBP. The wide decomposition peak of SBP samples and POS obtained from DTGA were corroborated by the DSC curves. From thermal analyses, it is clear that enzymatic hydrolysis of SBP led to decrease in the decomposition temperature. Thus, modification of the molecular structure (different substituents or decreasing molecular weight) led to a decrease in temperature of the thermal decomposition. In view of these thermal analyses, these results are an important starting point for the characterization of these POS in order to a future development of value-added natural food products. Pectins and oligosaccharides derived from pectins are classified as dietary fiber and as such, they could be added to food products that can be subjected to higher temperature, like bread, cakes and pastries for example; so it is of interest to assess the thermal stability of these products.

3.6. X-ray diffraction analysis

The analysis by X-ray diffraction allowed to study structural organization before and after enzymatic treatment. In general, crystalline material shows a series of sharp peaks, while amorphous product produces a broad background pattern. The X-ray powder diffraction patterns of SBP, Al SBP and POS are presented in Fig. 7. The curves have been offset for clarity. In addition to the two peaks (14° and 21°) generally observed with pectin in the literature [32], two others peaks appeared in the diffractogram of SBP and deesterified SBP at scattering angles 2θ values of respectively 26.4° and 32°. This indicates the semi-crystalline behavior of pectin samples and it is clear that pectin has a mixture of two types of molecular network structures. POS X-ray diffractograms indicate that the hydrolysates have lost much of their ordered structure and have become more amorphous than pectin samples. Hilden and Morris [33] describe that the most common techniques for producing an amorphous product are among other lyophilization or spray-drying. Lyophilization or freeze-drying consists in a rapid cooling that leads to the occurrence of an extremely viscous state before the solute molecules have time to rearrange and orient into a crystalline structure, and the system remains amorphous [34]. Thus we can assume that amorphous POS obtained here are a consequence of lyophilization. Another possibility would be that the enzymatic treatment leads to a destabilization and/or destroys crystallites of the network structure of SBP. The amorphous POS obtained could be applied during the drying process (spray-drying, drum-drying and freeze-dring) instead of for example maltodextrins which are used as spray-drying aids for flavors and seasonings, as carrier agents to produce food powders (sauce powder, fruit juice powder), as fat replacers, and as bulking agents due to their physical properties, such as high solubility in water, viscosity and stability [35].

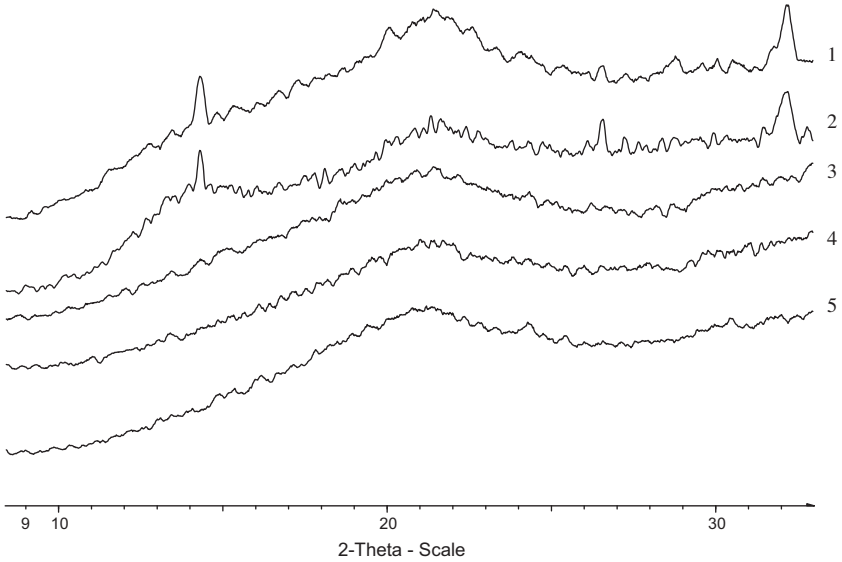

Fig. 7. XRD patterns of SBP (1), Al SBP (2), SBP 2 min (3), SBP 5 min (4) and SBP 15 min (5).

4. Conclusion

In the present study, the structural and thermal properties of three pectin-derived oligosaccharides prepared by partial hydrolysis of SBP using enzymes were examined and compared with native SBP and deesterified SBP. The HPLC profiles of POS revealed the presence of four populations and no difference was seen in terms of the composition of oligosaccharides. Thermal analyses showed that the composition and Mw range of POS influence their thermal behavior. POS showed lower thermal stability than pectin samples as revealed by DTGA and DSC analyses. The gases analysis by TGA–FTIR showed no major change in functional groups after enzymatic hydrolysis of SBP. On the other hand, X-ray analysis showed that there was a considerable decrease in crystallinity of SBP after enzymatic treatment. POS obtained from the hydrolysis of pectin were found to be heterogeneous in composition, with lower Mw and lower intrinsic viscosities, influencing their functional properties. Their low Mw makes them possibly more soluble than the native pectin. The anionic property of partially demethylated POS offers an opportunity to create electrostatic interactions with other cationic saccharides such as chitosan oligosaccharide to form films in a number of applications, including medical applications (production of patches, biodegradable pouches or bags, textile for the treatment of wounds, . . .). The lower viscosity of POS makes them more amenable to be incorporated into foodstuffs: for instance the texture of yogurt may be improved by POS added to the milk. Low viscosity POS can also be used as humectants. Oligosaccharides are less sweet than sucrose and therefore POS could be used to obtain different sweetness profiles with low caloric densities.

Acknowledgements

The research reported in this paper was supported by the French community of Belgium within the program WALEO Hydrasanté. The authors are grateful to Mrs Lynn Doran and Mr Sandrino Filloco for technical assistance.

References

[1] P. Albersheim, A.G. Darvill, M.A. O'Neill, H.A. Schols, A.G.J. Voragen, Progress in Biotechnology 14 (1996) 47–55.
[2] G.J. Coenen, E.J. Bakx, R.P. Verhoef, H.A. Schols, A.G.J. Voragen, Carbohydrate Polymers 70 (2007) 224–235.
[3] P. Perrone, C.M. Hewage, A.R. Thomson, K. Bailey, I.H. Salder, S.C. Fry, Phytochemistry 60 (2002) 67–77.
[4] M. Ashford, J. Fell, D. Attwood, H. Sharma, P. Woodhead, Journal of Controlled Release 30 (1994) 225–232.
[5] L. Tuoping, L. Suhong, N. Wang, L. Jinfu, European Food Research and Technology 227 (2008) 1035–1041.
[6] K.N.P. Humblet-hua, G. Scheltens, E. Van Der Linden, L.M.C. Sagis, Food Hydrocolloids 25 (2011) 307–314.
[7] M. Sanaka, T. Yamamoto, H. Anjiki, K. Nagasawa, Y. Kuyama, Clinical and Experimental Pharmacology and Physiology 34 (2007) 1151–1155.
[8] T. Matsumoto, M. Moriya, M.H. Sakurai, H. Kiyohara, Y. Tabuchi, H. Yamada, International Immunopharmacology 8 (2008) 581–588.
[9] C.L. Jackson, T.M. Dreaden, L.K. Theobald, N.M. Tran, T.L. Beal, M. Eid, M.Y. Gao, R.B. Shirley, M.T. Stoffel, M.V. Kumar, D. Mohnen, Glycobiology 17 (8) (2007) 805–819.
[10] E. Olano-Martin, G.R. Gibson, R.A. Rastall, Journal of Applied Microbiology 93 (2002) 505–511.
[11] K. Manderson, M. Pinart, K.M. Tuohy, W.E. Grace, A.T. Hotchkiss, W. Widmer, M.P. Yadhav, G.R. Gibson, R.A. Rastall, Applied and Environment Microbiology 71 (12) (2005) 8383–8389.
[12] J. Courtois, Current Opinion in Microbiology 12 (2009) 261–273.
[13] U. Einhorn-Stoll, H. Kunzek, G. Dongowski, Food Hydrocolloids 21 (2007) 1101–1112.
[14] C. Blecker, J.-P. Chevalier, C. Fougnies, J.-C. Van Herck, C. Deroanne, M. Paquot, Journal of Thermal Analysis and Calorimetry 71 (2003) 215–224.
[15] M.A. Cerqueira, B.W.S. Souza, J. Simões, J.A. Teixeira, M.R.M. Domingues, M.A. Coimbra, A.A. Vicente, Carbohydrate Polymers 83 (2011) 179–185.
[16] D. Mudgil, S. Barak, B.S. Khatkar, International Journal of Biological Macromolecules 50 (2012) 1035–1039.
[17] H. Garna, N. Mabon, B. Wathelet, M. Paquot, Journal of Agricultural and Food Chemistry 52 (2004) 4652–4659.
[18] A.G.J. Voragen, H.A. Schols, W. Pilnik, Food Hydrocolloids 1 (1986) 65–70.
[19] E. Bonnin, K. Clavurier, S. Daniel, S. Kauppinen, J.D.M. Mikkelsen, J.-F Thibault, Carbohydrate Polymers 74 (2008) 411–418.
[20] M.A.V. Axelos, J.-F Thibault, International Journal of Biological Macromolecules 13 (1991) 77–82.
[21] F.M. Rombouts, J.-F Thibault, in: M.L. Fishman, J.J. Jen (Eds.), Chemistry and Function of Pectins, American Chemical Society, Washington, DC, 1986, pp. 49–60.
[22] F. Guillon, J.-F Thibault, Lebensmittel-Wissenschaft & Technologie 21 (1988) 198–205.
[23] P. Mangiacapra, G. Gorrasi, A. Sorrentino, V. Vittoria, Carbohydrate Polymers 64 (2006) 516–523.
[24] C. Osorio, J.G. Carriazo, H. Barbosa, Quimica Nova 34 (2011) 636–640.
[25] S. Zhou, Y. Xu, C. Wang, Z. Tian, Journal of Analytical and Applied Pyrolysis 91 (2011) 232–240.
[26] U. Einhorn-Stoll, H. Kunzek, Food Hydrocolloids 23 (2009) 40–52.
[27] D.K. Shen, S. Gu, Bioresource Technology 100 (2009) 6496–6504.
[28] M.R. Hajaligol, B. Waymack, D. Kellogg, Preprint Papers – American Chemical Society, Division of Fuel Chemistry 44 (1999) 251–255.
[29] Q. Liu, S. Wang, Y. Zheng, Z. Luo, K. Cen, Journal of Analytical and Applied Pyrolysis 82 (2008) 170–177.
[30] C. Amen-Chen, H. Pakdel, C. Roy, Bioresource Technology 79 (2001) 277–299.
[31] B.S. Souza, A.P.D. Moreira, A.M.R.F. Teixeira, Journal of Thermal Analysis and Calorimetry 97 (2009) 637–642.
[32] R.M. Gohil, Journal of Applied Polymer Science 120 (2011) 2324–2336.
[33] L.R. Hilden, K.R. Morris, Journal of Pharmaceutical Sciences 93 (2004) 3–12.
[34] P. Gabarra, R.W. Hartel, Journal of Food Science 63 (1998) 523–528.
[35] L.M. Marchal, B.B. Beeftink, J. Tramper, Trends in Food Science and Technology 10 (1999) 345–355.