LWT - Food Science and Technology 50 (2013) 193–203

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Blanching of red beet (*Beta vulgaris* L. var. conditiva) root. Effect of hot water or microwave radiation on cell wall characteristics

Maria Emilia Latorre [a,b], Marina Francisca de Escalada Plá [a,b], Ana Maria Rojas [a,b], Lía Noemí Gerschenson [a,b,*]

[a] Industry Department, Natural and Exact Sciences School, University of Buenos Aires (UBA), Ciudad Universitaria, Intendente Güiraldes 2620, (1428) Ciudad Autónoma de Buenos Aires, Argentina
[b] National Scientific and Technical Research Council of Argentina (CONICET), Argentina

ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form
30 May 2012
Accepted 1 June 2012

Keywords:
Red beet
Blanching
Microwave
Cell wall
Microstructure

ABSTRACT

The effect of enzymes inactivation through traditional (water, 90 °C) and microwave blanching (constant power of 350 W and changing power with values higher than 900 W) on cell wall polysaccharides as well as on microstructure of beet root tissue was studied with the goal of attaining a better understanding of the changes produced by treatment and its consequences on the functional properties of cell wall polymers. Powers higher than 900 W produced greater tissue microstructural damage due to alteration of the cell wall network. The contact between neighboring cells persisted after microwave treatment at 350 W while traditional blanching or blanching at higher microwave powers (90MW) produced separation of the middle lamella at different points and also cell separation was observed for the last mentioned treatment. The formation of entanglements of pectin-in-extensin in 350 W-treated beet root and the higher content of calcium and diferulated cross linked pectins in the case of traditional treatment may account for the better mechanical performance observed for these tissues. The microwaving at 350 W modified the cell wall polymers in such a manner that produced an increase in their hydrophilicity.

1. Introduction

Blanching is an important step in the industrial processing of vegetables and fruits. It is a thermal treatment applied before freezing, frying, drying and canning to inactivate deleterious enzymes and to reduce the microbial load. The efficiency of blanching process is usually based on the inactivation of heat resistant enzymes like peroxidase (POX) and polyphenoloxidase (PPO) (Dorantes-Alvares & Parada-Dorantes, 2005; Gökmen, Savas-Bahceci, Serpen, & Acar, 2005). However some adverse effects have been reported such as tissue softening, pigment modifications and nutrient losses (Goncalves, Pinheiro, Abre, Brandao, & Silva, 2007; Kidmose & Martens, 1999).

Microwave heating takes place in dielectric materials such as vegetables tissues due to the polarization effect of electromagnetic radiation at frequencies between 300 MHz and 300 GHz. According to Chen, Collins, Mc.Carty, and Johnston (1971), microwave energy may be used to blanch vegetable tissues as an alternative to conventional steam and water blanching.

The polysaccharides composition influences the structure of the cell wall and, consequently, the texture of vegetables and fruits (Ng, Harvey, Parker, Smith, & Waldron, 1998; Ratnayake, Melton, & Hurst, 2003). Kidmose and Martens (1999) studied carrot slices during blanching and observed that the thermal processing produced many changes in the cell wall structure and, consequently, the tissue suffered softening. These changes produced the cell membrane disruption and changes in cell wall polymers. On the other side, the cell wall is the source of dietary fiber, acquiring as such a nutritional importance (Dongowski & Ehwald, 1999; Ou, Kwok, Li, & Fu, 2001) and changes of polysaccharides during processing can affect fiber functionality.

According to Kratchanova, Pavlova, and Panchev (2004), microwave pretreatment leads to destructive changes in the plant tissue. These changes modify the capillary-porous characteristic and water sorption capacity of the plant material. The intermolecular friction produced by microwave heating may cause internal cell pressure leading to rupture resulting in a loss of cell contents

* Corresponding author. Industry Department, Natural and Exact Sciences School, University of Buenos Aires (UBA), Ciudad Universitaria, Intendente Güiraldes 2620, (1428) Ciudad Autónoma de Buenos Aires, Argentina. Tel.: +54 11 4576 3366/3397; fax: +54 11 4576 3366.
E-mail address: lia@di.fcen.uba.ar (L.N. Gerschenson).

0023-6438/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2012.06.004

List of acronyms

AIR	alcohol-insoluble residue
Ara	arabinose
CDTA	trans-1,2-cyclohexanediaminetetraacetic acid
DA	degree of acetylation
db	dry basis
dm	dry matter
DM	degree of methylation
DRW	dried residue weight
FT-IR	Fourier transform infrared spectroscopy
HG	homogalacturonan
HRGPs	pectin-extensin complexes
HRW	hydrated residue weight
IR	Infrared
NS	neutral sugars
POX	peroxidase
PPO	polyphenoloxidase
RG-I	rhamnogalacturonan I
Rha	rhamnose
ROS	reactive oxygen species
SW	swelling capacity
WHC	water holding capacity
WRC	water retention capacity
90C	traditional treatment in water at 90 °C
350W	treatment with constant microwave powers of 350 W
90MW	treatment with microwave powers higher than 900 W

and organization. The knowledge of the structure and chemical composition may help to highlight the modifications that occur during processing (Femenia, Sanchez, Simal, & Rossello, 1998). For that reason it is necessary to perform specific studies for different tissues, trying to state a systematic background of information that allows to find the optimum conditions (i.e.: time, power level) that permit to attain the goal of the process with minor nutritional and organoleptical damage.

Red beet (*Beta vulgaris* L. var. conditiva) is a traditional and popular vegetable in many parts of the world. It is especially rich in fibers as well as in sugars but with moderate caloric value. It has an important content of B-vitamins (B1, B2, B3 and B6) as well as folic acid. Red beet roots are consumed either fresh or after thermal processing or fermentation. The soluble and cell wall associated phenolics as well as betalains, the main pigments in red beet responsible for its reddish-purple hue, are bioactive compounds (Schwartz & von Elbe, 1980), being their antioxidant capacity beneficial for human health (Kanner, Harel, & Granit, 2001). Latorre, Bonelli, Rojas, and Gerschenson (2012) evaluated the effect of microwave (constant powers of 250, 350, 450 W or powers higher than 900 W) and water traditional blanching at 90 °C on peroxidase (POX) and polyphenoloxidase (PPO) inactivation, texture and color changes as well as time efficiency. The inactivation of POX and PPO was strongly influenced by temperature observing, in general, that higher temperatures resulted in more rapid inactivation by microwave treatment. The shortest time for enzyme inactivation was shown by a microwave treatment performed most of the time at powers higher than 900 W (called '90MW') and with a similar temperature profile to traditional blanching (treatment at 90 °C, called '90C'). Concerning textural characteristics, all treatments reduced the elastic characteristics of the tissue being this specially marked for 90MW treatment while treatment 90C produced the smaller changes. Latorre (2012) informed that the specific energy consumption was lower for microwave treatments, being 350W, 450W and 90MW those with the lower values.

The object of the present research was to evaluate the chemical and microstructural characteristics of red beet tissues submitted to blanching with water at 90 °C or with microwaves at powers higher than 900 W and at constant power of 350 W, with the goal of attaining a better understanding of the changes produced by treatment and its consequences on the functional properties of cell wall polymers.

2. Materials and methods

2.1. Chemicals

They were of analytical grade unless stated. In general, chemicals were provided by MERCK Argentina (Buenos Aires, Argentina). Ethanol was provided by SAPORITI S.A.C.I.F.I.A. (Buenos Aires, Argentina); gallic acid by Anedra S.A. (Buenos Aires, Argentina); D-galacturonic acid, glutaraldehyde, OsO_4 and Durcupan resin by SIGMA-Aldrich (St Louis, MO).

2.2. Sample preparation

Red beet (*B. vulgaris* L. var. conditiva) roots harvested in Argentina were obtained at the local market. They were carefully cleaned, peeled and cut into 10 mm-thick slices perpendicular to their longitudinal axis. Cylindrical samples of 15 mm diameter were then obtained from each slice by using a cork borer. This sample geometry was chosen by considering its adequacy for mechanical assays.

2.3. Microwave blanching

Batch microwave processing was carried out in a microwave system ETHOS Plus (Milestone SRL, Sorisole, Italy) with a magnetron of 2450 MHz. The microwave used the ATC-400 system for continuous monitoring and control of the internal temperature. The optical sensor used was fitted in a Teflon coated ceramic thermowell. The samples were distributed in six glass vessels and placed on a polypropylene container (cylindrical shape; 27.9 cm diameter and 19.7 cm height.) according to Latorre et al. (2012). A 360° alternate movement was programmed to avoid bending of the sensor connection during experiments while assuring a more homogeneous treatment of the samples. The container was covered with a lid that served as supporter of the ceramic coated optical fiber. For each batch, the thermal sensor was placed in the center of one of the tissue cylindrical samples placed into one of the vessels, and the temperature profiles were recorded during microwave processing.

Red beet root tissue cylinders (36 g) were immersed in a 300 ml-total volume of deionized water distributed among the mentioned vessels for performing the experience. After each treatment, samples were immersed rapidly in an ice/water bath (during 5 min) to stop the blanching effect. Non-treated cylinders (raw material) were used as control samples.

The following microwave treatments were performed:

a) Constant output powers of 350 W (350W).
b) The equipment was programmed so as to obtain, in the center of the tissue cylinders, a similar temperature profile to the one observed in water blanching at 90 °C-constant temperature (90MW).

In both cases, temperature profiles of sample cylinders and water bath, and the output power evolution were recorded during

the time required for achieving an enzyme activity reduction of, at least, 90%. In the case a), the treatment was stopped after 5 min and 1 min of venting was performed. In the case b), the treatment was stopped after 2 min and 1 min of venting was performed. The times proposed for each treatment were those previously defined (Latorre et al., 2012) as determining at least 90% enzymes activity reduction.

Eight replicates were performed for obtaining enough material for microscopical and chemical studies.

2.4. Water blanching

Conventional thermal treatment was performed by immersion of tissue cylinders into a water bath at 90 °C-constant temperature (90C). During heating, temperatures were simultaneously recorded every 10 s for the water bath and for the central point of each tissue cylinder, by using thin-wire copper-constantan thermocouples. These determinations were performed during 7 min, the time required for achieving an enzyme activity reduction of, at least, 90% (Latorre et al., 2012). The relation of tissue mass to water was the same as the one used for microwave treatments. The assays were carried out eight times for having enough material for microscopical and chemical studies.

2.5. Chemical analyses

The samples (control and blanched ones) were (a) directly used to evaluate microstructural characteristics or (b) submitted to extraction of the alcohol-insoluble residue (AIR). Unless stated, the analyses were performed in duplicate. All chemical solutions used in the following analyses were prepared by using deionized water (Milli-Q, USA).

2.5.1. Alcohol insoluble residue (AIR)

An amount of ≈300.00 g of raw (control) or blanched tissue was suspended in a solution containing 95 ml of ethanol per 100 ml (1:4 ratio) for separation of its insoluble residue (AIR) (Martin-Cabrejas, Waldron, & Selvendran, 1994), homogenized with a mechanical device and then boiled for 10 min under stirring. The obtained residue was then extracted for 10 min with a boiling solution containing 65 ml of ethanol per 100 ml. The insoluble residue was separated and washed with the same ethanol solution. Between each ethanol treatment, the suspension was filtered (Whatman filter: GF/C, UK) and the supernatant was discarded. The cell wall material (AIR) obtained was left overnight under a lab hood to eliminate the remaining ethanol, and then freeze dried (Stokes freeze-drier, Stokes Company, Philadelphia, MA, USA) after freezing with liquid nitrogen. The product was then milled and the resulting powder was distributed in plastic tubes and stored at −18 °C till usage. The AIR was used for chemical analyses: (a) lignin, cellulose and non-cellulosic carbohydrates content, (b) uronic acids, degree of methylation (DM) and degree of acetylation (DA), (c) total phenolics,(d) FT-IR spectroscopy, (e) moisture content, (f) sequential extraction of cell wall polymers, (g) evaluation of hydration properties: swelling capacity (SW), water holding capacity (WHC) and water retention capacity (WRC).

2.5.2. Lignin, cellulose, non-cellulosic carbohydrates, methanol and acetyl contents

The AIR obtained was used to determine uronic acid, total non-cellulosic carbohydrates, cellulose and lignin contents. Hydrolysis of cellulose and non-cellulosic polysaccharides of AIR was performed according to Ng, Parr, Ingham, Rigby, and Waldron (1998) by dispersion of ≈0.3000 g of sample product into 2080 μl of sulfuric acid solution (72 ml sulfuric acid per 100 ml of solution) for 3 h at room temperature. This dispersion was diluted with enough deionized water (until 25.00 ml-final volume) till attaining a concentration of 1 mol of sulfuric acid per liter and each sample was heated at 100 °C for 2.5 h in a water-bath. After this, all dispersions were cooled, centrifuged at 12,000× *g* for 10 min and the supernatant was separated. The residue was washed three times with deionized water, centrifuged at 12,000× *g* for 10 min and, finally, freeze-dried. The residue obtained was weighed and reported as lignin.

A second procedure was carried out dispersing other portion of ≈0.3000 g of each sample into 2080 μl of sulfuric acid solution (72 ml sulfuric acid per 100 ml of solution) and water was immediately added to dilute the system till attaining a concentration of 1 mol of sulfuric acid per liter, followed by 2.5 h of heating at 100 °C. The final residue corresponded to cellulose + lignin. The carbohydrate content of the supernatant was constituted by non-cellulosic polysaccharides and it was determined by phenol-sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), using D-galacturonic acid for the standard curve.

The third hydrolysis-procedure was performed with a new portion of each sample following the technique applied for the first procedure, but 1 h of heating at 100 °C in a water bath was applied in this case. Only the supernatant was separated for the quantification of the uronic acid content by the method reported by Filisetti-Cozzi and Carpita (1991).

Methanol content was determined through saponification of AIR with NaOH solution (0.5 mil equivalent/L) for 1 h at room temperature, followed by acidification with sulfuric acid. Methanol quantification was performed through the spectrophotometric method of Wood and Siddiqui (1971). Acetyl groups in the AIR of each fiber fraction were determined according to the method of Naumenko and Phillipov (1992). Degree of methylation (DM) was calculated as the percent ratio between moles of methanol and galacturonic acid in a given sample. Degree of acetylation (DA) was calculated in the same manner (Fissore, Ponce, Stortz, Rojas, & Gerschenson, 2007). Protein content was determined according to Lowry, Rosebrough, and Randall (1951).

All determinations were performed in triplicate.

2.5.3. Cell wall phenolic contents

Total phenolics (ferulic and non-ferulic) determination was carried out on each AIR sample according to Bunzel, Ralph, Marita, and Stainhart (2000). An amount of ≈0.9000 g of AIR was mixed with 50.0 ml of NaOH aqueous solution (1 mol/L) under vacuum and also protected from light, at room temperature, during 18 h. The sample was acidified with 9.5 ml of HCl (pH < 2) and then centrifuged for 15 min at 12,000× *g* (6 °C). The obtained supernatant was used to evaluate total phenolics using the Folin-Ciocalteau technique reported by Shui and Leong (2006). Gallic acid (Anedra, Buenos Aires, Argentina) was used as standard and the results were expressed as gallic acid equivalent (GAE), which means mg of gallic acid per 100 g of AIR sample (dried base).

Non-ferulic phenolic compounds were determined according to Budini, Tonelli, and Girotti (1980) and Parr, Ng, and Waldron (1997). A sample of ≈0.9000 g of AIR was mixed with HCl solution (2 mol/L) in a 1 g: 50 ml ratio and the system was heated for 30 min in a 95 °C water-bath. Centrifugation was performed at 6 °C and 12,000× *g* for 15 min. The supernatant obtained was used to evaluate non ferulic phenolic content by using the Folin-Ciocalteau technique above indicated.

2.5.4. FTIR spectroscopy

Transmission spectra of the samples were recorded from KBr (1% w/w) pellets in the range 4500–525 cm^{-1} with a Spectrum BX FT-IR (Perkin Elmer, Massachusetts, USA) spectrometer. Each

spectrum was obtained by recording transmittance (%) through 64 scans performed with a resolution of 4 cm^{-1}. Spectra were analyzed through the Perkin Elmer Spectrum One Procedures Software (Microsoft® Visual Basic 4.0, Massachusetts, USA).

2.5.5. Moisture

Moisture content was determined in $a \approx 1.0000$ g of AIRs, using an IR moisture analyzer (OHAUS, Pine Brook, NJ, USA), working at 90 °C till constant weight. This determination was performed in triplicate.

2.5.6. Sequential extraction of the cell wall polymers from AIR

It was made according to Ng and Waldron (1997) using deionized (Milli-QTM) water. AIR (0.5000 g) was suspended in deionized water (50 ml) and stirred for 2 h at 18–20 °C. The water-insoluble residue was further extracted in NaCl (50 ml, 0.136 mol/L; pH 6.5) for 2 h at 18–20 °C. Residues were successively extracted in CDTA (55 ml; 0.050 mol/L, pH 6.8) for 5 h at 18–20 °C; Na_2CO_3 (20 ml; 0.050 mol/L) for 16 h at 4 °C and Na_2CO_3 (20 ml; 0.050 mol/L) for 2 h at 20 °C (Marry et al., 2006); NaOH (20 ml; 0.1 mol/L) for 2 h at 18–20 °C (Peña & Carpita, 2004); KOH (20 ml; 1 mol/L) for 2 h at 18–20 °C and finally were extracted in KOH (20 ml; 4 mol/L) for 2 h at 18–20 °C (Coimbra, Waldron, Delgadillo, & Selvendran, 1996).

2.6. Hydration properties

The following hydration properties of the cell wall polymers isolated (AIR) were measured according to de Escalada Pla, Ponce, Stortz, Gerschenson, and Rojas (2007).

2.6.1. Swelling capacity (SC)

An AIR sample accurately weighed (≈0.2000 g) was placed in a graduated conical tube. Around 10 ml of water was added and it was hydrated for 18 h at 25 °C of temperature. After this time, the final volume attained by the fiber-product was measured (Raghavendra, Rastogi, Raghavarao, and Tharanathan, 2004; Robertson, Monredon, Dysseler, Guillon, & Amado, 2000). This assay was performed three times for each fraction. SC was calculated:

$$\mathrm{SC\,[ml/g]} = \mathrm{Volume\ occupied\ by\ sample/Original\ sample\ weight}$$

2.6.2. Water-holding capacity (WHC)

An AIR sample accurately weighed (≈1.000 g) was hydrated in a graduated conical tube with 30 ml of water for 18 h at 25 °C. The supernatant was removed and the residue was weighed. The weight of the hydrated residue was recorded (HRW). After freeze-drying, the weight of the dried residue was also recorded (DRW). WHC was determined three times for each fraction and it was calculated as:

$$\mathrm{WHC\,[g/g]} = (\mathrm{HRW} - \mathrm{DRW})/\mathrm{DRW}$$

2.6.3. Water retention capacity (WRC)

WRC was determined by hydration at 25 °C (18 h) of an accurately weighed sample (≈1.0000 g) with 30 ml of water, into a graduated conical tube. Centrifugation for 30 min at 2000× g was then performed into the same tube. The supernatant was separated and the wet residue ($R + W_2$) was weighed and submitted to freeze-drying. The freeze dried residue was also weighed (R) and WRC was calculated:

$$\mathrm{WRC\,[g/g]} = W_2/R$$

being W_2, the water still retained after centrifugation.

The assay was performed three times for each fraction.

2.7. Microstructural analyses

Samples of about 4 mm^3 were cut from raw or blanched beet root tissue cylinders. They were fixed in 2.5 g/100 g-glutaraldehyde solution buffered by phosphate solution (0.2 mol/L; pH = 7.2) solution and postfixed in OsO_4 solution (1.5 g/100 g) into the same buffer solution, followed by fixation and contrast in uranyl acetate solution in distilled water (5 g/100 g). Dehydration was performed in a graded acetone buffer series prior to embedding in Durcupan (epoxi) resin. Ultrathin sections of 0.5 microns were first performed in a Reichert Jung Ultracut E (Leica, Germany) microtome and stained with toluidin blue for observation in the optical microscopy (Axioplan, Japan). After this work, ultrathin sections of 70–90 nm were obtained from the uranyl acetate stained pieces and examined in an EM 10 C Zeiss transmission electron microscope (Karl Zeiss, Berlin, Germany).

2.8. Statistical analysis

The results were reported as the average and standard deviation. Results were analyzed through ANOVA (α: 0.05) followed by pair multiple comparisons evaluated by Tukey's significant difference test, using the Statgraphic package (Statgraphic Plus for Windows, version 5.0, 2001, Manugistic Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Cell wall composition

Multicellular plants depend for their integrity on effective adhesion between their component cells. This adhesion depends upon various cross-links, ionic, covalent or weak interactions between the macromolecules of the adjacent cell walls. To explore this aspect, the cell wall components were isolated from raw (control) tissues as well as from treated tissues at the instant of reaching at least a 90% of POX and PPO inactivation. The extracted alcohol insoluble residues (AIR) are constituted by the cell wall polymers. The yield of AIR obtained from raw tissue was 3.6% and this value changed only in a 5% for treated root tissues (Table 1). Greve et al. (1994) observed important cell wall polysaccharide changes during blanching of carrot tissue by immersion in boiling water. Hence, the walls of beet root cells were considerable stable and resistant to thermal or microwave processing concerning polymer loss, at least during the treatment period assayed. Pectins, which in part can be more water soluble than the other cell wall polymers, did not seem to suffer significant depolymerization by β-elimination (Table 1) of their highly methoxylated (HG) chains, as expectable from thermal treatments (van Buggenhout, Sila, Duvetter, van Loey, & Hendrickx, 2009). Or probably, beet root pectins were able to persist anchored in the cell wall network beyond some degree of degradation. Fissore et al. (2010) found an important proportion of arabinan in the rhamnogalacturonan I (RG-I) side chains (arabinose to rhamnose molar ratio, Ara/Rha = 17) of red beet root pectins. These authors observed that this proportion of Ara at RG-I might anchor these pectins to the cell wall by entanglements with cellulose network (Fry, 1986), protecting them better from acid hydrolysis at pH 2.0 with an aqueous solution of citric acid.

As can be observed in Table 1, cell wall polymers were mainly constituted by carbohydrates (≈85%). Cellulose content was between 30 and 37 g/100 g AIR dry basis (db) whereas non-cellulosic carbohydrates content was ≈50 g/100 g AIR (db). The latter involved 35 g/100 g AIR (db) of neutral sugars (NS) and ≈15 g/100 g AIR (db) of uronic acids. At least 50% of the NS content is arabinose (Fissore et al., 2010). On the other hand, cell wall proteins (12 g/

Table 1
Yield and composition[d] of the cell walls (alcohol insoluble residues, AIR) extracted from raw (control) and treated red beet root tissues which were submitted either to traditional (90C) or microwave blanching at 350 W (350W) and at powers higher than 900 W (90MW) for the time needed to reach 90% of enzyme (PPO and POX) inactivation.

	Control	350W	90C	90MW
g AIR/100 g tissue	3.6	3.4	3.4	3.8
Moisture content g water/100 g AIR	6.6 ± 0.6[a]	6.9 ± 0.1[a]	6.27 ± 0.0[a]	8.1 ± 0.2[b]
Protein[e] g/100 g AIR (db)	11.9 ± 0.9[a]	11.5 ± 0.2[a]	11.6 ± 0.4[a]	11.9 ± 0.2[a]
Lignin[e] g/100 g AIR (db)	1.9 ± 0.3[a]	2.5 ± 0.1[a]	2.0 ± 0.2[a]	1.60 ± 0.01[a]
Cellulose[e] g/100 g AIR (db)	37 ± 3[a]	35 ± 1[a]	30 ± 3[b]	31 ± 1[b]
Non-cellulosic carbohydrates[e,f] g/100 g AIR (db)	49 ± 3[a]	49 ± 3[a]	56 ± 4[a]	50 ± 1[a]
Uronic acid[e] g/100 g AIR (db)	16 ± 1[a]	14.6 ± 0.3[a]	15.9 ± 0.1[a]	17.0 ± 0.5[a]
Degree of methylation (DM)[g] (molar %)	53.3 ± 0.5	54.4 ± 0.9	55.3 ± 8.2	54.0 ± 1.0
Degree of acetylation (DA)[h] (molar %)	84 ± 6[a]	96 ± 5[b]	87 ± 2[ab]	88 ± 2[ab]
Total phenolic content[i] g GA/100 g AIR (db)	1.179 ± 0,003[a]	1.37 ± 0.07[b]	1.12 ± 0.04[c]	1.22 ± 0.03[a]
Non-ferulic-phenolics[i] g GA/100 g AIR (db)	1.04 ± 0,01[a]	0.84 ± 0.02[b]	0.93 ± 0.02[c]	1.09 ± 0.02[a]
Ferulic compounds[j] g GA/100 g AIR (db)	0.14 ± 0.02[a]	0.49 ± 0.08[b]	0.19 ± 0.06[a]	0.13 ± 0.04[a]

[d] Mean and standard deviation are shown ($n = 3$). Different letters in the same row indicate significant differences ($p < 0.05$).
[e] Components of AIR db: dry basis.
[f] Non-cellulosic carbohydrates were calculated from the standard curve made with D-glucose.
[g] DM was calculated as percent ration between moles of methanol and moles of uronic acids per 100 g AIR.
[h] DA was calculated as percent ration between moles of acetyl and moles of uronic acids per 100 g AIR.
[i] Phenolic contents are indicated as g gallic acid (GA) per 100 g of AIR.
[j] Ferulic compounds are indicated as the mathematical differences between total and non-ferulic phenolics.

100 g AIR on dried basis) and lignin (1.9–2.5/100 g AIR on dried basis) were also found. The cell wall composition was similar for all (raw and treated) tissues. Also, the moisture content as well as the degree of acetylation and methylation of uronic acids did not change with processing. The DA and DM indicated highly acetylated and methoxylated pectins, expectable from beet root.

Some significantly ($p < 0.05$) higher lignin content was determined in the AIR obtained from 350W-treated tissue whereas a trend to a lower, but non significant, proportion of lignin was found in the AIR extracted from 90MW tissue. Some tendency to a higher content of total cell wall phenolics was observed for 350W-treated tissue, which accounted, probably, for the highest content of ferulic compounds. Higher lignin and ferulic compounds in 350W treated tissue, can be indicative of POX activation as a response to the thermal and non thermal stresses of microwaves. At this condition, it could be observed (Fig. 1) that there is a lag time of ≈4 min in POX inactivation during which, the enzyme could catalyze lignin formation and accumulation as well as ferulic production (Latorre et al., 2012). The latter may also be involved in some degree of pectin cross-linking catalyzed by POX (Passardi, Cosio, Penel, & Dunand, 2004) in the cell walls of 350W-treated tissue. Fig. 1 shows the kinetics of POX inactivation at each condition of tissue treatment herein studied. A high activity of POX remained up to 4 min in 350W-treated tissues before the beginning of the activity decay, which could have determined the increase in the previously cited chemical parameters (Table 1). Exposure of living organisms to

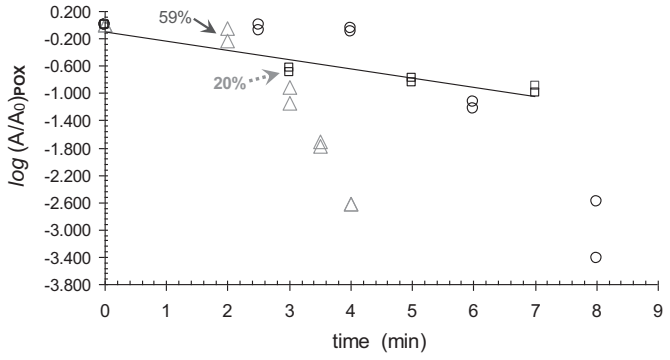

Fig. 1. Residual peroxidase (POX) activity (A/A_0) plotted against time of processing for traditional blanching (90C) and microwaving (350W or 90MW). Tissue was soaked in water while microwaving. ○ 350W; △ 90MW; □ 90C.

ionizing and non-ionizing irradiation constitutes a major exogenous source of reactive oxygen species (ROS). Radiation that has enough energy to move atoms in a molecule around or cause them to vibrate, but not enough to remove electrons, is referred to as "non-ionizing" radiation (EPA, 2011). Microwave is an example of non-ionizing radiation. Yamaguchi et al. (2001) determined that the radical-scavenging activity and ascorbic acid content of vegetables cooked in a microwave in the absence of water during 1, 3 and 5 min were generally higher than in those cooked by boiling water. In the present work, POX could catalyze the reduction of H_2O_2, which can derive as a tissue response to microwave injury at 350 W, by taking electrons from donors like phenols giving origin to ferulics and lignin precursors, probably to accomplish some lignification. Cross-linking of the arabinogalactan side chains by feruloyl ester (covalent) bridges in beet root pectins could also occurred. Actually, phenolic synthesis can increase *in vivo* as a consequence of the enzymatic pathway stimulation as a response to the increased requirements for cross-linking of cell wall biopolymers.

3.1.1. Sequential extraction

In order to analyze the cross-linking of the polymers involved in the cell wall network and matrix and their change with treatment, a sequential extraction was also carried out from the AIRs isolated from control and treated red beet root tissues at the instant of getting at least 90% inactivation of POX and PPO. The extractive sequence began with water at 18 °C (Brett & Waldron, 1996). Some of the pectin in plant tissue is soluble in cold water, indicating little or no binding to the cell wall (Fry, 1986). Water was able to extract ≈25% of the cell wall polymers present in the AIR of untreated (control), 350W- and traditionally blanched (90C) tissues (Fig. 2). Red beet root submitted to the highest microwave power (90MW) showed significantly ($p < 0.05$) higher proportion of water extractable cell wall polymers (≈31%). This suggested that the cell walls of 90MW-tissue were partially degraded (Coimbra et al., 1996) or their networks could have been disorganized. The composition of supernatants was determined. The polymers dissolved in cold water were mostly non cellulosic carbohydrates (≈80 g/100 g AIR dissolved by water, dry basis) which included higher proportion of NS than of uronic acids (11–24%). Lower content of uronic acids were found in the water soluble fractions obtained from 350W and 90C AIRs (14 and 11%, respectively). No difference was observed between the uronic acid contents determined in the water soluble fractions of 90MW and control AIRs

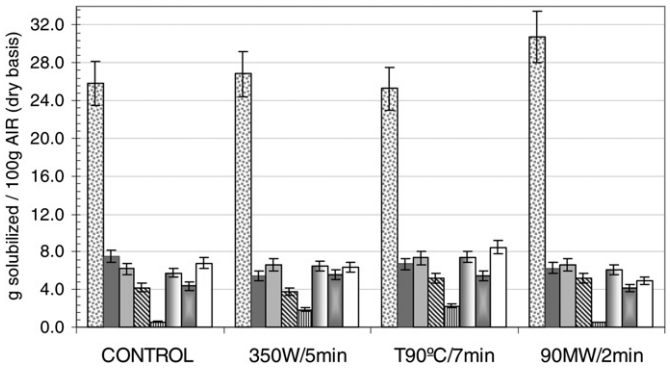

Fig. 2. Mass of cell wall polymers (alcohol insoluble residue, AIR) sequentially dissolved by water or by the reactive solutions: water, NaCl, CDTA, Na_2CO_3 (16 h, 4 °C), Na_2CO_3 (2 h, 20 °C), NaOH, KOH (1 mol/L), KOH (4 mol/L). Unless specifically indicated, extractions were performed at 18 °C. Bars inform the standard deviation for $n = 3$.

(24–21%). All these compositions indicated that water extracted some polymers of pectic origin. Also, 3.8–4.7 g of protein/100 g AIR (dry basis) was extracted by water at 18 °C.

Pectin polymers ionically related with extensin, a hidroxiproline rich protein of the cell wall, are extracted by the subsequent 0.136 M-NaCl aqueous solution at 18 °C (Brett & Waldron, 1996; Fry, 1986). Significantly lower proportions of cell wall polymers were dissolved from the residue by the following extractive solutions (Fig. 2). The extracted amount (5.5 g/100 g AIR, dry basis) from 350W-residue was significantly ($p < 0.05$) lower than the one extracted from the other residues. Some extensin, mainly the newly laid down molecules, can be extracted from the cell walls with salts. Lower extractability of polymers with respect to the AIR solubilized from raw tissue (7.5 g AIR/100 g AIR, on dry basis) means that the ionically related extensin-pectin could be in part transformed in some covalently related form. It is considered that POX plays an important role in processes such as insolubilization of pectin-extensin (HRGPs) complexes (Jackson & Armstrong, 1999; Passardi et al., 2004). That complex can derive in part from the oxidative coupling between extensin chains through isodityrosine (ether) bridges. This reaction is catalyzed by the extracellular or cell wall POX, which consumes H_2O_2 (Fry, 1986; Jackson and Armstrong, 1999). From this oxidative cross-linking, extensin loops are formed which produces the physical constrain of the HG chains of pectins, which would be unable to get out of the noose because HG is flanked by highly branched RG-I domains (Fry, 1986).

The subsequent CDTA extraction during 6 h at 18 °C dissolved those pectins which were held in the cell walls through Ca^{2+} (electrostatic) bridges. In the case of polysaccharides present in the cell wall of raw tissue, they accounted for 6.2 g/100 g AIR (dry basis). In general, tissue treatment did not affect CDTA extractability but the content of Ca-cross linked pectins tended to increase when beet root tissue was submitted to traditional blanching (90C) by immersion in water at constant 90 °C (Fig. 2). Hence, this blanching process seemed to contribute to higher cell–cell adhesion through the increment in calcium-cross linking at the middle lamellae region (Vincken et al., 2003). Since Bartolome, and Hoff's work (1972) about the firming effect on potatoes, it has been extensively reported that the initial increment in tissue temperature of many vegetables during traditional blanching leads to an initial increase in pectinmethyl esterase activity, which hydrolyzes the methoxylated groups of cell wall pectins. After this, the carboxyl groups can interact with the endogenous Ca^{2+}, Mg^{2+} and similar cations, with evidences of tissue strengthening (Ni, Lin, and Barrett, 2005).

Marry et al. (2006) observed that not only calcium-chelated homogalacturonan-pectin regions were important in cell–cell adhesion in the case of sugar beet tissue but also feruloyl ester (covalent) bridges in the arabinogalactan side chains of pectins, which are usually removed through successive Na_2CO_3 treatments at 4° and 20 °C. The following extraction was performed at 4 °C with a de-esterifying agent such as 50 mM Na_2CO_3 aqueous solution. A trend to higher polysaccharide extractability was observed for 90C and 90MW cell wall residues. But at 20 °C, Na_2CO_3 extracted significantly higher proportions of pectins from 350W and 90C-AIR residues. As a global result, Na_2CO_3 extracted in total more pectins covalently related from 90C-blanched tissue. POX activity was then inferred that occurred more significantly not only in 350W- but also in 90C-treated tissue (Fig. 2) promoting the increase of the content of covalently related pectins through diferulic cross-linking. Also, pectins can be cross linked to other components of the cell wall matrix by alkali labile esters such as *O*-D-galacturonoyl esters (Brown & Fry, 1993; Marry et al., 2006) which could also be extracted by Na_2CO_3. van Buggenhout et al. (2009) reported that red beet root, which is, like sugar beet, in the family of *B. vulgaris* var. vulgaris, contains the same type of phenolics present in sugar beet. However, only 10% of ferulic acid in beet root is found in the dehydrodimer form, which can explain the relatively rapid increase in the ability of the beet root cells to separate and the softening observed after 30 min of cooking, in contrast to that observed in sugar beet where 20% of the ferulic acid content is in the dimeric form (8-O-4′-diferulic acid and 8,5′-diferulic acid benzofuran form). Ng, Harvey et al. (1998) determined that incubating beet root (*B. vulgaris* var. Detroit 2 crimson) tissue in H_2O_2 changed the mechanical properties of the cell walls and the rate of thermal softening after boiling in water, from 130 min to 650 min. This was accompanied by a large decrease in esterified cis- and trans-ferulic acid and a 2-fold increase in 5,5′-, 8-*O*-4′- and 5,8′-diferulic acid moieties (benzofuran form), and thus an increase in the pectin cross linking in the cell walls of beet root. van Buggenhout et al. (2009) suggested that incubation with H_2O_2 could be a strategy for texture engineering of thermally processed vegetables and fruits, which can be better managed through POX and/or laccase activities.

Significantly higher extraction of covalently related pectins was observed for 90C-cell wall residue than for raw (control) and 90MW residues through 0.1 M NaOH solution (Fig. 2). However, the difference was not significant between 90C- and 350W-cell wall residues. The 0.1 M NaOH solution can remove other polymers possibly esterified to the wall matrix (Carpita et al., 2001) including *O*-D-galacturonoyl remaining esters of pectin to cellulose or ester bonds between uronic acids and neutral sugars (Fry, 1986).

Hemicelluloses constitute a diverse group of β-(1 → 4)-linked backbone polysaccharides. Xyloglucans, the major hemicellulose of the growing dicotyledon cell wall, have in particular the ability to cross-link cellulose through H-bonds and may play a role in keeping cellulose fibrils apart (McCann, Wells, & Roberts, 1990; Peña & Carpita, 2004). Other comprise xylans (glucuronoxylan, arabinoxylan and glucuronoarabinoxylan), and mannans (glucomannan, galactomannan and galactoglucomannan). As hemicelluloses bind tightly to the surface of cellulose through hydrogen bonds they can only be extracted from plant cell walls by strong alkali (Fry, 1986). The following extractions were then sequentially performed with 1 M and 4 M KOH aqueous solutions to produce hemicellulosic extracts (Fry, 1986). Slightly higher extraction of polysaccharides was observed in 350W- and 90C- than in control-AIR residue. Conversely, lower amount of material was solubilized from 90MW. The later result coincided with a trend to minor amounts of the cellulose residues finally obtained, in comparison to control sample (Table 1). Cardoso et al. (2009) found similar results

for table olives processed according to the Italian traditional "Ferrandina" method which includes an initial blanching step of black Cassanese olives, followed by salting and oven-drying. The authors found that pectic and hemicellulosic polysaccharides of these processed olive fruits were more soluble in the aqueous solutions of immersion, possibly due to degradation promoted by the blanching step. On the other hand, it is generally observed that in contrast with pectin, hemicellulose and cellulose show minimal structural changes upon heating of plant-based foods (Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011).

It has been recently observed in tomato tissue that hemicellulose polymers are present at cell junctions, developing a role in cell adhesion (Ordaz-Ortiz, Marcus, & Knox, 2009). The decrease in the proportion of hemicelluloses extracted from 90MW residue may be ascribed to the loss of these polymers as a consequence of the alteration of the hemicellulose-cellulose network after microwave treatment at very high powers (>900 W) with simultaneous water soaking (Fig. 2). This can be the result of the thermal and non thermal effects of microwaves (Latorre et al., 2012; Perreux & Loupy, 2001). Chemical characteristics of the last residue obtained after the 4 M KOH extractive step performed on AIRs were analyzed through FTIR spectroscopy. The spectra obtained from all of them indicate the only presence of cellulose (Fig. 3A). Rests of the long arabinan side chains of pectins anchored to the cellulose network of beet root (Fissore et al., 2010) were not detected. As above mentioned, lignin was found in the AIR (Table 2) and, hence, it is expectable to be found in the last residue of the sequential extractions. However, lignin was not detectable in the FTIR spectra probably due to its low proportion. Hence, the typical 1520 cm^{-1} band that was observed in the FTIR spectra of AIRs obtained from control, 350W and 90C beet root tissues (Fig. 3B) can be ascribed to the presence of feruloylated pectins (Synytsya, Copikova, & Jankovska, 2003) into the cell walls. This characteristic band was absent in the spectrum recorded from the 90MW-beet root AIR. This suggested the alteration of cell wall network integrity after microwave blanching through the highest powers (Fig. 4). This fact was not well detected from differences in extractability through sequential extraction of cell wall polymers with Na_2CO_3 solutions (Fig. 2). Other relevant difference observed with respect to raw (control) AIR was the lowest level of hemicelluloses extracted through KOH in the case of 90MW-cell walls (Fig. 2). Somani, Choudhari, Rao, and Mirajkar (2003) mentioned the possibility of the rupture of hydrogen bonds after microwave treatments, which might proceed as a consequence of the decrease in the energy barrier of reactions or of the pre-exponential related factor of Arrhenius' equation (Bohr & Bohr, 2000; Perreux and Loupy, 2001). Removal of some hemicelluloses from the cell walls as a consequence of 90MW microwave treatment may allow, for example, lateral association of cellulose microfibrils (McCann et al., 1990; Wang et al., 2010) with alteration in the mechanical response of cell walls (Chanliaud, Burrows, Jeronimidis, & Gidley, 2002). It is also remarkable the highest water extractability of 90MW-AIR (Fig. 2) which may in part explain the lowest rheological performance evaluated through the force relaxation assay performed at a 10% constant deformation and reported by Latorre et al. (2012). These authors recorded relaxation data from non fractured tissue samples along 10 min ($F(t)$) and performed its normalization through the division by the initial relaxation force (F_0). All treated tissues (350W, 90MW and 90C) showed significantly lower residual elasticity (F_∞/F_0) than raw tissue, trend that was associated to turgor loss. Furthermore, tissues submitted to treatment 90MW showed the lowest residual elasticity (F_∞/F_0) and the highest proportion of the total stress applied lost through flow. On the other hand, higher conversion of ionically related pectin-extensin complexes to entanglements of pectin-in-extensin in 350W beet root tissue or some higher content in calcium and diferulated cross linked pectins in 90C tissue, may explain the better relaxation response observed by Latorre et al. (2012) for these tissues with respect to 90MW tissue.

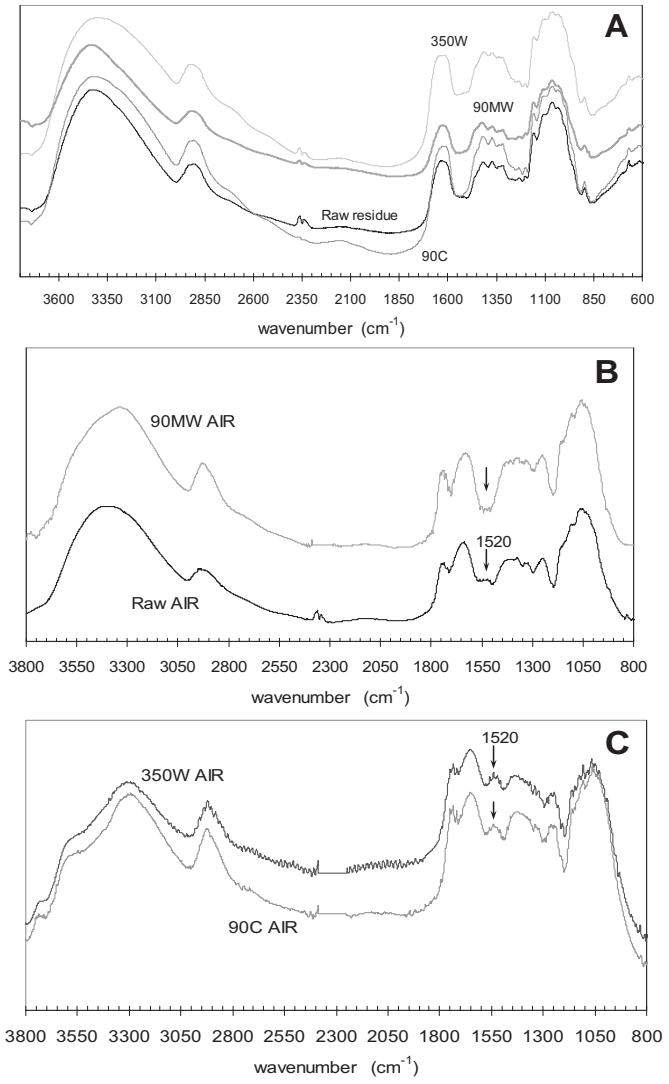

Fig. 3. (A) FTIR spectra obtained from the final cell wall residue remaining after the sequential extractions performed on the AIR extracted from raw (control) or processed beet root tissues either by traditional blanching (90C) or by microwaving (treatments 350W or 90MW). (B) FTIR spectra obtained from the alcohol-insoluble residues (AIR) isolated from raw or 90MW processed tissues. (C) FTIR spectra obtained from the AIR isolated from 90C or 350W processed tissues.

Table 2
Hydration properties[d] of the alcohol insoluble residue (AIR) extracted from raw (control) or blanched (350W, 90MW or 90C) red beet root tissues which were processed for the time necessary to accomplish 90% enzyme inactivation. All microwave (350W and 90MW) treatments included 1 min of venting in the processing time reported.

Sample	Swelling capacity (SC) (mL water/g AIR *dm*)	Water holding capacity (WHC) (g water/g AIR *dm*)	Water retention capacity (WRC) (g water/g AIR *dm*)
Control	44.1 ± 0.4^a	32.0 ± 0.4^a	41 ± 2^a
350W/5 min	48.9 ± 0.6^b	32.9 ± 0.3^a	38 ± 1^a
90C/7 min	48.6 ± 0.8^b	$30.2 \pm 0.6^{a\ b}$	40 ± 4^a
90MW/2 min	38.3 ± 0.8^c	28 ± 1^b	41.4 ± 0.5^a

dm means "dry matter".
The same letter in a column indicates non significant differences ($p < 0.05$) between the values.
[d] Mean and standard deviation for $n = 3$ are reported.

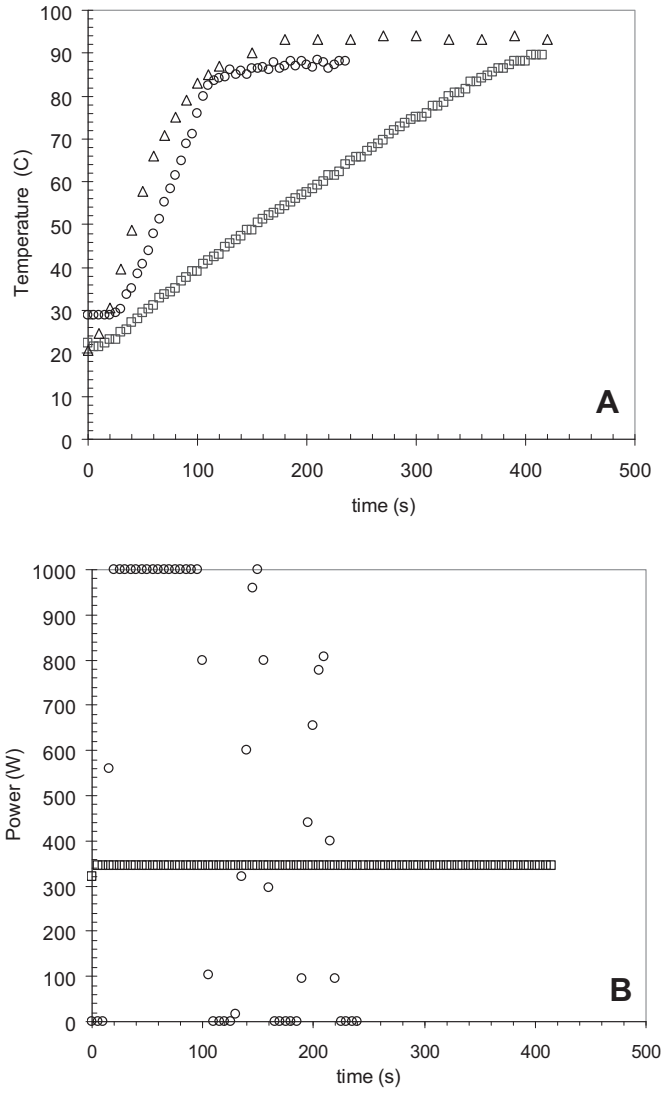

Fig. 4. Thermal (A) and power (B) time profiles recorded during microwave treatments (350W or 90MW). The temperature profile of treatment 90C is also shown in panel (A). □ 350W; ○ 90MW; △ 90C.

3.1.2. FTIR analysis

The FTIR spectra of AIRs obtained from raw (control) and 90 MW (Fig. 3B), 350W and 90C treated tissues (Fig. 3C) are dominated by pectins. It can be observed that pectins dominate in the AIR composition. The main difference between spectra is the absence of the feruloyl (aromatic) signal of pectins at 1520 cm^{-1} in 90MW-AIR, as above mentioned. Between 1020 and 1170 cm^{-1}, cellulose and hemicelluloses partly masked some typical pectin bands like the characteristic peaks of the polygalacturonic backbone in the fingerprint zone (1200 to 900–850 cm^{-1}) of pectins at 1020 and 1105 cm^{-1}, though other pectin peaks at 1142, 1082 and 1062 cm^{-1} can be distinguished (McCann, Hammouri, Wilson, Belton, & Roberts, 1992). Between 1440 and 1230 cm^{-1} there are not any difference with respect to typical pectin profiles. The water adsorbed in the cell wall pectins led to a sharp peak at 1630 cm^{-1} (Wilson et al., 2000), which masked the broad band usually manifested between 1615 and 1650 cm^{-1} by the carbonyl stretching of the carboxylic (non-esterified) group of galacturonic and glucuronic, in the case of red beet (Strasser & Amado, 2001) residues of pectins. The band in the zone attributed to the characteristic C=O stretching of the esterified carboxyl group was observed at 1730 cm^{-1}, as a single signal. The broad OH-stretch band at a frequency of ≈3310 cm^{-1}, reported as characteristic of polysaccharides from cell walls (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1999), can be seen. It can be also observed the shorter band at 2905 cm^{-1}, which corresponds to the OH-stretching in the carboxylic group of pectins and to the C–H stretching in the polysaccharide backbone. Pectins, cellulose and other polysaccharides of the cell wall contribute to the bands at wavenumbers above 2800 cm^{-1}. It is well evident an important shoulder at ≈3550 cm^{-1}, with a sharp maximum at 3275 cm^{-1} for 350 W and 90C AIRs (Fig. 3C) whereas control and 90MW AIRs showed the typical broad band at 3322 and 3383 cm^{-1}, respectively (Fig. 3B). This is the result of the hydrogen bonding of –OH groups.

FTIR spectra of the residues finally obtained after the sequential extraction of AIRs did not reveal differences among them and indicated that residues are only constituted by cellulose (Fig. 3A). Typical peaks of this polysaccharide at 1643, 1430, 1374, 1322 and 1280 cm^{-1}, as well as those peaks in the fingerprint zone of cellulose (1165, 1115, 1062, 1037 cm^{-1}) can be observed. Other peaks at 899 and 670 cm^{-1} are also present (Oh, Yoo, Shin, & Seo, 2005). The broad OH-stretch band reported as characteristic of polysaccharides from cell walls (Coimbra et al., 1999) was found at ≈3460 cm^{-1} as well as the shorter band at 2905 cm^{-1}.

3.2. Hydration properties

The hydration properties of the cell wall polysaccharides (AIR) extracted from raw (control) and blanched (350W, 90MW and 90C) beet root tissues were determined as an additional tool for detecting differences in the properties of cell wall polymers developed as a consequence of the beet root processing.

The maximal amount of water that the cell wall polysaccharides can hold is a function of its chemical, physical and microstructural characteristics (Brett and Waldron, 1996; Raghavendra et al., 2004). SC is defined as the ratio of the volume occupied by sample after immersion in excess of water and equilibration to the actual weight (Raghavendra et al., 2004). Hence, this parameter indicates how much the polymeric matrix swells as water is absorbed. Loosely associated water is also being considered in this assay. As can be observed in Table 2, the SC was the hydration test that permitted to find out differences due to tissue processing. The cell wall polymers extracted from 350W and 90C treated tissues showed the highest SC values whereas those isolated from 90MW tissue presented lower SC than control AIR. Also, 90MW cell wall polymers presented lower WHC than control and 350W AIR. This parameter also includes the proportion of water loosely associated to the polymers. No difference was detected between cell wall polymers through the WRC assay.

Blanching of tissue samples was performed for enough time to accomplish 90% enzymes inactivation. The processing carried out by microwaving at 350 W modified the cell wall polymers in such a manner that produced an increase in their hydrophilicity and macromolecular mobility. On the other hand, microwaving at powers higher than 900 W (90MW treatment) decreased the hydrophilicity of cell wall polymers of beet root.

3.3. Microstructure

As can be seen in Fig. 5A, optical microscopy image of raw (control) beet root cells cut at different levels show a continuum of thick cell walls that surrounds uniformly stained cellular contents. The latter can be ascribed to the tonoplast vacuoles responsible for

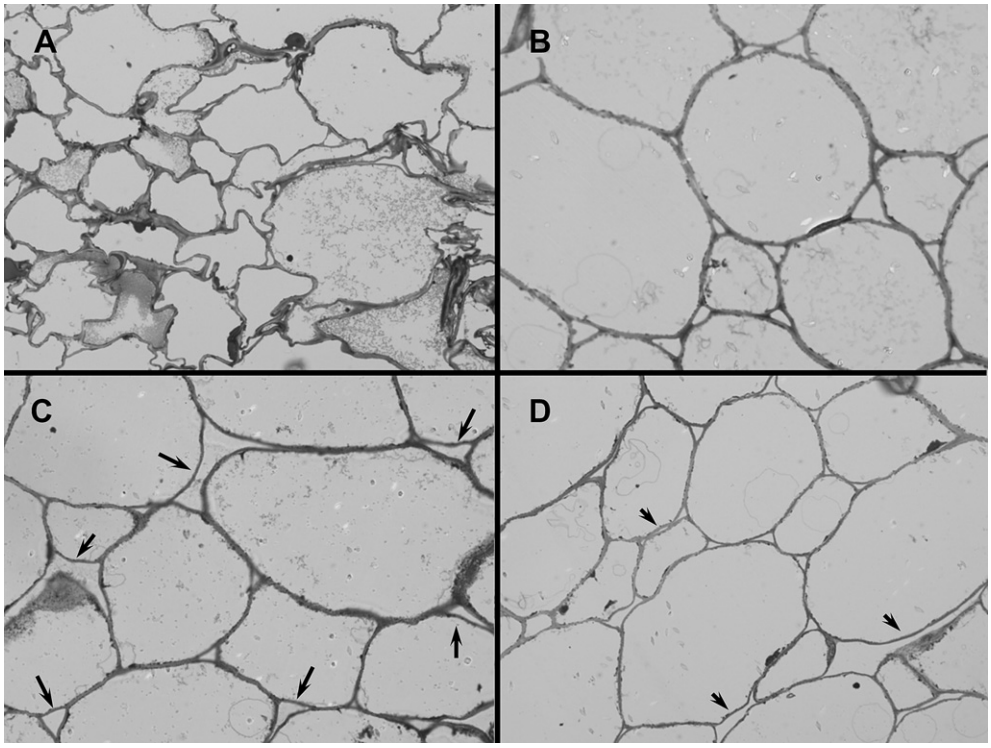

Fig. 5. Optical microscopy images obtained from raw (A) and 350W-processed beet root tissues (B); tissues submitted to treatment 90C (C); tissues submitted to treatment 90MW (D). Arrows indicate points of cell–cell separation. Bar = 10 μm.

the cell wall pressure and, hence, for the turgor pressure development. The middle lamellae can be observed without separation along the cell wall continuum and appears thickened at the tricellular junction corners. Specimens cut from treated tissues generally presented round cells (Fig. 5B, C, D). Beet root cells of 350W-treated tissue show separation of the middle lamellae mainly at the corners (Fig. 5B). Hence, contacts between all neighboring cells persisted after this microwave treatment and are clearly observed. Traditional blanching of red beet root tissue (90C) by soaking in water at 90 °C produced a great impact mainly at the cell corners with clear separation of the middle lamella from them, as indicated in the micrograph (arrows; Fig. 5C). High microwave powers (>900 W) produced separation of the middle lamella at different points between adjacent cells (90MW tissue) and also cell separation is observed (Fig. 5D).

4. Conclusions

The cell wall composition was similar for all (raw and treated) tissues showing that walls of beet root cells were considerable stable and resistant to traditional or microwave processing concerning polymer loss, at least during the treatment period assayed (time necessary for getting at least 90% inactivation of POX and PPO). Concerning microstructure, contacts between neighboring cells persisted after microwave treatment 350 W while traditional blanching (90C) and higher microwave powers (90MW) produced separation of the middle lamella at different points and also cell separation was observed in the case of 90MW.

Some significantly higher lignin content was determined in the alcohol insoluble residue (AIR. cell wall polymers) obtained from 350W-treated tissue whereas a trend to a lower, but non significant, proportion of lignin was found in the AIR extracted from 90MW tissue. Some tendency to a higher content of total cell wall phenolics was observed for 350W-treated tissue, which accounted, probably, for the highest content of ferulic compounds. All these can be indicative of POX activation as a response to the stresses produced by microwaves.

The decrease in the proportion of hemicelluloses extracted from 90MW residue may be ascribed to the loss of these polymers as a consequence of the alteration of the hemicellulose-cellulose network after microwave treatment at very high powers (>900 W).

Red beet root submitted to the highest microwave power (90MW) showed significantly higher proportion of water extractable cell wall polymers. Some extension could be extracted from the cell walls with NaCl. The amount extracted from 350W-residue was significantly lower than the one extracted from the other residues showing that the ionically related extensin-pectin could be in part transformed in some covalently related form probably mediated by POX. Ca-cross linked pectins tended to increase when beet root tissue was submitted to traditional blanching; this blanching process seemed to contribute to higher cell–cell adhesion through the increment in calcium-cross linking at the middle lamellae region. The 350W and 90C treated beet root tissues showed the presence of feruloylated pectins into the cell walls.

The highest water extractability of 90MW-treated tissue may explain the lowest rheological performance previously evaluated by the authors. Higher conversion of ionically related pectin-extensin complexes to entanglements of pectin-in-extensin in 350W-treated beet root as well as some higher content in calcium and diferulated cross linked pectins in 90C-treated tissue may account for the better mechanical performance reported. The microwaving at 350 W modified the cell wall polymers in such a manner that produced an increase in their hydrophilicity and macromolecular mobility.

Blanching at constant 350W powers with simultaneous soaking of beet root cylinders produced better results with reference to cell wall integrity and hydration properties. It can be concluded that

processing at constant 350W with simultaneous soaking of beet root cylinders in water can be a useful alternative to traditional blanching at 90 °C.

Acknowledgments

The authors acknowledge the financial support from University of Buenos Aires (UBACyT EX-089; UBACyT 20020100100726), National Agency of Scientific and Technological Promotion of Argentina (ANPCyT-PICT 38239) and National Scientific and Technical Research Council of Argentina (CONICET-PIP 11220090100531).

References

Bartolome, L. G., & Hoff, J. E. (1972). Firming of potatoes: biochemical effects of preheating. *Journal of Agricultural and Food Chemistry, 20*(2), 266–270.

Bohr, H., & Bohr, J. (2000). Microwave enhanced kinetics observed in ORD studies of a protein. *Bioelectromagnetics, 21*, 68–72.

Brett, C., & Waldron, K. (1996). *Physiology and biochemistry of plant cell wall*. London, UK: Chapman and Hall.

Brown, J. A., & Fry, S. C. (1993). Nove1 O-D-galacturonyl esters in the pectic polysaccharides of suspension-cultured plant cells. *Plant Physiology, 103*, 993–999.

Budini, R., Tonelli, D., & Girotti, S. (1980). Analysis of total phenols using Prussian blue method. *Journal of Agricultural and Food Chemistry, 26*(6), 1236–1238.

van Buggenhout, S., Sila, D. N., Duvetter, T., van Loey, A., & Hendrickx, M. (2009). Pectins in processed fruits and vegetables: part III-texture engineering. *Comprehensive Reviews in Food Science and Food Safety, 8*(2), 105–117.

Bunzel, M., Ralph, J., Marita, J., & Stainhart, H. (2000). Identification of 4-O-5′coupled diferulic acid from insoluble cereal fiber. *Journal of Agricultural and Food Chemistry, 48*, 3166–3169.

Cardoso, S. M., Mafra, I., Reis, A., Barros, A. S., Nunes, C., Georget, D. M. R., et al. (2009). Traditional and industrial oven-dry processing of olive fruits: influence on textural properties, cell wall polysaccharide composition, and enzymatic activity. *European Food Research and Technology, 229*, 415–425.

Carpita, N., Defernez, M., Findlay, K., Wells, B., Shouel, D. A., Catchpole, G., et al. (2001). Cell wall architecture of the elongating maize coleoptile. *Plant Physiology, 127*, 551–565.

Chanliaud, E., Burrows, K. M., Jeronimidis, G., & Gidley, M. J. (2002). Mechanical properties of primary plant cell wall analogues. *Planta, 215*, 989–996.

Chen, S. C., Collins, J. L., Mc.Carty, I. E., & Johnston, M. R. (1971). Blanching of white potatoes by microwave energy followed by boiling water. *Journal of Food Science, 36*, 742–743.

Coimbra, M. A., Barros, A., Barros, M., Rutledge, D. N., & Delgadillo, I. (1999). FTIR spectroscopy as a tool for analysis of olive pulp cell wall polysaccharides extracts. *Carbohydrate Research, 317*, 145–154.

Coimbra, M. A., Waldron, K. W., Delgadillo, I., & Selvendran, R. R. (1996). Effect of processing on cell wall polysaccharides of green table olives. *Journal of Agricultural and Food Chemistry, 44*, 2394–2401.

Dongowski, G., & Ehwald, R. (1999). Binding of water oil and bile acids to dietary fibers of the cellan type. *Biotechnology Progress, 15*, 250–258.

Dorantes-Alvares, L., & Parada-Dorantes, L. (2005). Blanching using microwave processing. In H. Schubert, & M. Regier (Eds.), *The microwave processing of foods*. Boca Raton, Fl: CRC Press, (Part 2. Chapter 9).

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

de Escalada Pla, M. F., Ponce, N., Stortz, C. A., Gerschenson, L. N., & Rojas, A. M. (2007). Composition and functional properties of enriched fiber products obtained from pumpkin (*Cucurbita moschata* Duchesne ex Poiret). *LWT – Food Science and Technology, 40*, 1176–1185.

Femenia, A., Sanchez, E. S., Simal, S., & Rossello, C. (1998). Effects of drying pretreatments on the cell wall composition of grape tissues. *Journal of Agricultural and Food Chemistry, 46*, 271–276.

Filisetti-Cozzi, T. M., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry, 197*, 157–162.

Fissore, E. N., Ponce, N. M. A., de Escalada Pla, M., Stortz, C. A., Rojas, A. M., & Gerschenson, L. N. (2010). Characterization of acid-extracted pectin-enriched products obtained from red beet (*Beta vulgaris* L. var. *conditiva*) and butternut (*Cucurbita moschata* Duch ex Poiret). *Journal of Agricultural and Food Chemistry, 58*(6), 3793–3800.

Fissore, E. N., Ponce, N. M. A., Stortz, C. A., Rojas, A. M., & Gerschenson, L. N. (2007). Characterization of fiber obtained from pumpkin (*Cucumis moschata*, Duch.) mesocarp through enzymatic treatment. *Food Science and Technology International, 16*(1), 1–7.

Fry, S. C. (1986). Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annual Review of Plant Physiology, 37*, 165–186.

Gökmen, V., Savas-Bahceci, K., Serpen, A., & Acar, J. (2005). Study of lipoxygenase and peroxidase as blanching indicator enzymes in peas: change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. *LWT – Food Science and Technology, 38*(8), 903–908.

Goncalves, E. M., Pinheiro, J., Abre, M., Brandao, T. R. S., & Silva, C. L. M. (2007). Modelling the kinetics of peroxidase inactivations, color and texture changes of pumpking (*Curcubita maxima* L.) during blanching. *Journal of Food Engineering, 81*(4), 693–701.

Greve, C., Shackel, A. K., Ahmadi, H., McArdle, R. N., Gohlke, J. R., & Labavitch, J. M. (1994). Impact of heating on carrot firmness: contribution of cellular turgor. *Journal of Agricultural and Food Chemistry, 42*, 2896–2899.

Houben, K., Jolie, R. P., Fraeye, I., Van Loey, A. M., & Hendrickx, M. E. (2011). Comparative study of the cell wall composition of broccoli, carrot, and tomato: structural characterization of the extractable pectins and hemicelluloses. *Carbohydrate Research, 346*(9), 1105–1111.

Jackson, M. B., & Armstrong, W. (1999). Formation of parenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology, 1*, 274–287.

Kanner, J., Harel, S., & Granit, R. (2001). Betalains. A new class of dietary cationized antioxidants. *Journal of Agricultural and Food Chemistry, 49*, 5178–5185.

Kidmose, U., & Martens, H. J. (1999). Changes in texture, microstructure and nutritional quality of carrot slices during blanching and freezing. *Journal of the Science of Food and Agriculture, 79*, 1747–1753.

Kratchanova, M., Pavlova, E., & Panchev, I. (2004). The effects of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. *Carbohydrate Polymers, 56*, 181–185.

Latorre, M. E. (2012). *Influencia de las radiaciones gamma y de microondas en las características químicas, bioquímicas y mecánicas de tejidos vegetales con especial referencia a Beta vulgaris L. var. conditiva*. Ph. D. thesis, Buenos Aires University.

Latorre, M. E., Bonelli, P., Rojas, A. M., & Gerschenson, L. N. (2012). Microwave inactivation of red beet (*Beta vulgaris* L. var. conditiva) peroxidase and polyphenoloxidase and the effect of radiation on vegetable tissue quality. *Journal of Food Engineering, 109*, 676–684.

Lowry, O. H., Rosebrough, N. J., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Marry, M., Roberts, K., Jopson, S. J., Huxham, I. M., Jarvis, M. C., Corsar, J., et al. (2006). Cell-cell adhesion in fresh sugar-beet root parenchyma requires both pectin esters and calcium cross-links. *Physiology Plantarum, 126*(2), 243–256.

Martin-Cabrejas, M. A. M., Waldron, K. W., & Selvendran, R. R. (1994). Cell wall changes in Spanish pear during ripening. *Journal of Plant Physiology, 144*, 541–548.

McCann, M. C., Hammouri, M., Wilson, R., Belton, P., & Roberts, K. (1992). Fourier Transform Infrared Microspectroscopy is a new way to look at plant cell walls. *Plant Physiology, 100*, 1940–1947.

McCann, M. C., Wells, B., & Roberts, K. (1990). Direct visualisation of cross-links in the primary plant cell wall. *Journal of Cell Science, 96*, 323–334.

Naumenko, I. V., & Phillipov, M. P. (1992). Colorimetric method for determination of acetyl groups in pectic substances. *Akademi Nauk Republi Moldova Biologi i Khimie Nauki, 1*, 56–59.

Ng, A., Harvey, A. J., Parker, M. L., Smith, A. C., & Waldron, K. W. (1998). Effect of oxidative coupling on the thermal stability of texture and cell wall chemistry of beet root (*Beta vulgaris*). *Journal of Agricultural and Food Chemistry, 46*, 3365–3370.

Ng, A., Parr, A. J., Ingham, L. M., Rigby, N. M., & Waldron, K. W. (1998). Cell wall chemistry of carrots (*Daucus carota* Cv. Amstrong) during maturation and storage. *Journal of Agricultural and Food Chemistry, 46*, 2933–2939.

Ng, A., & Waldron, K. W. (1997). Effect of steaming on the cell wall chemistry of potatoes (*Solanum tuberosum* Cv. Bintje) in relation to firmness. *Journal of Agricultural and Food Chemistry, 45*, 3411–3418.

Ni, L., Lin, D., & Barrett, D. M. (2005). Pectin methylesterase catalyzed firming effects on low temperature blanched vegetables. *Journal of Food Engineering, 70*, 546–556.

Oh, S. Y., Yoo, D. I., Shin, Y., & Seo, G. (2005). FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide. *Carbohydrate Research, 340*, 417–428.

Ordaz-Ortiz, J. J., Marcus, S. E., & Knox, J. P. (2009). Cell wall microstructure analysis implicates hemicellulose polysaccharides in cell adhesion in tomato fruit pericarp parenchyma. *Molecular Plantarum, 2*, 910–921.

Ou, S., Kwok, K., Li, Y., & Fu, L. (2001). In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agricultural and Food Chemistry, 49*, 1026–1029.

Parr, A. J., Ng, A., & Waldron, K. W. (1997). Ester-linked phenolic components of carrot cell walls. *Journal of Agricultural and Food Chemistry, 45*, 2468–2471.

Passardi, F., Cosio, C., Penel, C., & Dunand, C. (2004). Peroxidases have more functions than a Swiss army knife. *Plant Cell Reports, 24*(5), 255–265.

Peña, M. J., & Carpita, N. C. (2004). Loss of highly branched arabinans and debrenching of rhamnogalactonan I accompany loss of firm texture and cell separation during prolonged storage of apple. *Plant Physiology, 155*, 1–9.

Perreux, L., & Loupy, A. (2001). A tentative rationalization of microwave effects in organic synthesis according to the reaction medium and mechanistic considerations. *Tetrahedron, 57*, 9199–9223.

Raghavendra, S. N., Rastogi, N. K., Raghavarao, K. S. M. S., & Tharanathan, R. N. (2004). Dietary fiber from coconut residue: effects of different treatments and particle size on the hydration properties. *European Food Research and Technology, 218*, 563–567.

Ratnayake, R. M. S., Melton, L. D., & Hurst, P. L. (2003). Influence of cultivar, cooking, and storage on cell-wall polysaccharide composition of winter squash (*Cucurbita maxima*). *Journal of Agricultural and Food Chemistry, 51*, 1904–1913.

Robertson, J. A., Monredon, F. D., Dysseler, P., Guillon, F., & Amado, R. (2000). Hydration properties of dietary fiber and resistant starch: a European collaborative study. *LWT – Food Science and Technology, 33*, 72–79.

Schwartz, S. J., & von Elbe, J. H. (1980). Quantitative determination of individual betacyanin pigments by high performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 28*, 540–543.

Shui, G., & Leong, L. P. (2006). Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. *Food Chemistry, 97*, 277–284.

Somani, O. G., Choudhari, A.,L., Rao, B. S., & Mirajkar, S. P. (2003). Enhancement of crystallization rate by microwave radiation: synthesis of ZSM-5. *Materials Chemistry and Physics, 82*(3), 538–545.

Strasser, G. R., & Amado, R. (2001). Pectic substances from red beet (*Beta vulgaris* conditiva). Part I. Structural analysis of rhamnogalacturonan I using enzymic degradation and methylation analysis. *Carbohydrate Polymers, 44*, 63–70.

Synytsya, A., Copikova, J., & Jankovska, P. (2003). Spectroscopic estimation of feruloyl groups in sugar beet pulp and pectin. *International Sugar Journal, 105*, 481–488.

United States Environmental Protection Agency, EPA. (2011). http://www.epa.gov/radiation/understand/ionize_nonionize.html#nonionizing Accessed 24.03.11.

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. A. Schols, & R. Visser (Eds.), *Advances in pectin and pectinase research* (pp. 47–59). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Wang, A., Sun, D., Ren, N., Liu, C., Liu, W., Logan, B. E., et al. (2010). A rapid selection strategy for an anodophilic consortium for microbial fuel cells. *Bioresource Technology, 101*, 5733–5735.

Wilson, R. H., Smith, A. C., Kacuraková, M., Saunder, s P. K., Weller, N., & Waldron, K. W. (2000). The mechanical properties and molecular dynamics of plant cell wall polysaccharides studies by Fourier-transform infrared spectroscopy. *Plant Physiology, 124*, 397–406.

Wood, P. J., & Siddiqui, I. R. (1971). Determination of methanol and its application for measurement of pectin ester content and pectin methyl esterase activity. *Analytical Biochemistry, 39*, 418–428.

Yamaguchi, T., Mizobuchi, T., Kajikawa, R., Kawashima, H., Miyabe, F., Terao, J., et al. (2001). Radical-scavenging activity of vegetables and the effect of cooking on their activity. *Food Science and Technology Research, 7*, 250–257.